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Vienna International Centre, P.O. Box 300, 1400 Vienna, Austria
Tel: (+43-1) 26026-0 · www.unido.org · unido@unido.org

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Workshop on the Development of Advanced Pharmaceutical Formulations

University of Strathclyde
Glasgow, UK, 22-27 November 1992
XP/RLA/92/109

REPORT*

VOLUME I

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* This document has not been edited.

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TABLE OF CONTENTS

		PAGES
I.	Polymeric Inserts and Implants for the Controlled Release of Drugs	1 - 34
	Controlled Drug Delivery Systems	35 - 39
	Professor Neil B. Graham	
II.	Transdermal Drug Delivery : Problems and Possibilities	40 - 62
	Transdermal Delivery	63 - 101
	Professor Jonathan Hadgraft	
III.	Multiple w/o/w Emulsions as Drug Vehicles	102 - 122
	Microspheres and Microencapsulation for Drug Delivery	123 - 150
	Biodegradable Polymers for Drug Delivery	151 - 175
	Dr. Tony L. Whateley	
IV.	Liposomes as Drug Delivery Devices	176 - 189
	Drug Delivery to the Lung	190 - 209
	Professor Ian W. Kellaway	
V.	Biological Dispersion and the Design of Site-Specific Protein Therapeutic Systems	210 - 228
	Theory and Practice of Site-Specific Drug Delivery	229 - 336
	Impact of the New Biologies on the Medical and Pharmaceutical Sciences	337 - 349
	Professor Eric Tomlinson	

INSTRUCTIONAL STAFF

Course Organisers:

Professor Neil B. Graham	Department of Pure & Applied Chemistry University of Strathclyde, Glasgow
Dr. Tony L. Whateley	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow

Lecturers:

Professor Jonathan Hadgraft	The Welsh School of Pharmacy University of Wales College of Cardiff, Cardiff, Wales
Professor Ian W. Kellaway	Welsh School of Pharmacy University of Wales College of Cardiff, Cardiff, Wales
Dr. Russell Paterson	Department of Chemistry University of Glasgow, Glasgow
Professor Eric Tomlinson	GeneMedicine Inc, Houston, Texas
Mr. Robert F. Weir	Controlled Therapeutics (Scotland) Ltd East Kilbride, Glasgow
Professor Clive G. Wilson	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow

UNIDO WORKSHOP - PROGRAMME

"THE DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS"

22nd - 27th November 1992
Venue
Strathclyde Graduate Business School
199 Cathedral Street, Glasgow, G4 OG2, Scotland

Leaders:	Professor Neil B. Graham	Department of Pure and Applied Chemistry
	Dr. Tony L. Whateley	Department of Pharmaceutical Sciences
Secretary:	Mrs. Carole Britton	Department of Pure and Applied Chemistry

Sunday 22nd November 18.30 - 20.00pm	Arrival - Cocktails + Buffet Supper
Monday 23rd November 09.00 - 09.15	Fundamental Principles of Controlled Drug Delivery Professor Neil B. Graham. Welcome
09.15 - 10.15	Professor Neil B. Graham "An Introduction to Controlled Release"
10.15 - 11.10	Dr. Russell Paterson "Principles of Diffusion and Molecular Transport I"
11.10 - 11.30	Tea Break
11.30 - 12.30	Professor Neil B. Graham "The Applications of the Dosage of Practical Programmed Delivery Systems"
12.30 - 13.30	Lunch
14.00 - 15.00	Dr. Russell Paterson "Principles of Diffusion and Molecular Transport II"
15.30 - 17.30	Practical Laboratories
17.30 - 18.30	Group Discussion with Course Staff
Tuesday 24th November 09.00 - 10.00	Transdermal Delivery and Biodegradables Professor Jonathan Hadgraft "Transdermal Delivery I"
10.00 - 11.00	Professor Jonathan Hadgraft "Transdermal Delivery II"
11.00 - 11.30	Tea Break
11.30 - 12.30	Dr. Tony L. Whateley "Biodegradables"
12.30 - 13.30	Lunch
14.00 - 15.00	Group Discussion with Course Staff
15.00 - 17.00	Practical Laboratories

<u>Wednesday 25th November</u> 09.00 - 10.00	Professor Ian W. Kellaway "Liposomes"
10.00 - 11.00	Professor Ian W. Kellaway "Nasal and Lung Delivery"
11.00 - 11.30	Tea Break
11.30 - 12.30	Mr. R. F. Weir "Regulatory Considerations"
12.30 - 13.30	Lunch
14.00 - 16.00	Practical Laboratories
16.00 - 17.00	Group Discussion with Course Staff
<u>Thursday 26th November</u> 09.00 - 10.00	Dr. Tony L. Whateley "Microspheres and Microencapsulation"
10.00 - 11.00	Professor Clive G. Wilson "GI Tract Transport and Delivery"
11.00 - 11.30	Tea Break
11.30 - 12.30	Dr. Tony L. Whateley "Multiple Emulsions"
12.30 - 13.30	Lunch
14.00 - 16.00	Practical Laboratories
16.00 - 17.00	Group Discussion with Course Staff
<u>Friday 27th November</u> 09.00 - 10.00	Professor Eric Tomlinson "Site Specific Drug Delivery"
10.00 - 10.30	Tea Break
10.30 - 11.30	Professor Eric Tomlinson "Molecular Biological Approaches to Drug Delivery"
11.30 - 13.00	Forum to discuss specific problems of delegates
13.00	Lunch
14.30	Coach to Ross Priory on Loch Lomondside and Workshop Closing Dinner

<u>Practical Sessions</u>

1. Preparations of liposomes - TLW
2. Preparation of microspheres (biodegradable) - TLW
3. Characteristics of release from hydrogels using USP Dissolution Apparatus - NBG
4. Particle sizing of nasal aerosol delivery systems - TLW
6. Computer simulations of diffusion and pharmacokinetics - CW, RP, NBG AND TLW

POLYMERIC INSERTS AND IMPLANTS FOR THE CONTROLLED RELEASE OF DRUGS

Neil B. Graham and David A. Wood

TABLE OF CONTENTS

I. Introduction

II. Physical Forms of Drug-Polymer Composites as Inserts and Implants

III. Release Mechanisms and Prediction of Release Profiles
- A. Membrane Devices
- B. Release from Monoliths with Dissolved Drug
- C. Release from Matrices with Dispersed Drug
- D. Release from Devices with Capillaries and Pores
- E. Release from Hydrogels
- F. Boundary-Layer Controlled Release
- G. Release from Erodible Devices

IV. Fundamentals of Polymer Chemistry for the Controlled Release of Drug from Polymeric Membranes and Matrices
- A. Physical States and Structure of Polymers
- B. The Swelling of Cross-Linked Polymers
- C. Diffusivity of Drugs in Polymers

V. Hydrophobic Nondegradable Devices
- A. Silicones
- B. Substituted Polyethylene

VI. Hydrogel Systems
- A. The Polymers Used in Hydrogels

VII. Biodegradable and Bioerodible Systems

VIII. Routes of Administration and Targeting

IX. Polymer-Tissue Interactions and Biologically Active Polymers

References

1. INTRODUCTION

Polymers are intrinsic to all living sytems, and life as we know it could not exist without the presence of organized large molecules. They fill a number of roles of which three may be clearly identified as follows.

1. Contributing mechanical strength to skeletal structures exemplified by bones, sinews, and muscles in humans and to cell membranes in all life at the cellular level
2. Providing very specific chemical action, as for example, their function as enzymes, RNA, DNA, and certain hormones
3. Controlling the concentration of small molecules in intra- and extracellular fluids by acting as membranes with precise diffusion characteristics or as adsorption centers to maintain a low, precisely controlled level of dissolved low molecular weight species

The increasing basic knowledge of the chemistry and biochemistry of cellular and body functions combined with an expanding analysis of the engineering operation of body parts has led in the past decade to an explosion of research into three areas related to (1) prosthetic devices, (2) polymeric drugs, and (3) drug carriers and devices for the controlled release of drugs. This chapter will discuss the latter class (3) with some additional brief reference to (2) as in reality overlap occurs between these classifications.

The principles of controlled release of biologically active compounds are found in a multitude of applications as varied as preventing barnacles from adhering to ships' bottoms, promoting the growth of trees, keeping flies out of houses and fleas off pets, to the particular focus of this work, that of the prophylaxis and treatment of human diseases. The usual method for drug administration is by taking tablets at intervals during the day. This technique is far from ideal as it provides wide variations in plasma levels at different times as shown in Figure 1 for a patient taking four tablets per day. For every drug there is a so-called therapeutic index which measures the ratio of the plasma level of the drug causing toxic side effects in a defined proportion of subjects to the level which is therapeutically ineffective. Obviously, drugs with high therapeutic indexes are the most desirable and those with low values will be suspect because of the probability of plasma levels becoming either too high or too low with undesirable consequences in both cases. A controlled release dosage form should achieve the "desired level" providing more consistent protection without toxic effects. The removal of the necessity for a patient to comply to a prescribed dosage regimen is also a major advantage of such systems.

Certain drugs, such as the prostaglandins, are rapidly metabolized to inactive compounds and effectively eliminated from the body. In this way the organism protects itself against continuation of the often dramatic effects induced by minute quantities of such active materials. For their application in therapy, a continuous adminstration is often necessary and a simple controlled release device provides an attractive alternative to an i.v. infusion.

Yet another benefit of controlled release is in reducing the patient exposure to a massive excess of drug over that required at the desired site of action. Normal therapy involves a gross overkill technique in which the entire body is subjected to drug when only a small local concentration is required.

The benefits of controlled release devices can thus be summarized as follows:

1. To improve the control of systemic blood levels of drug
2. To localize drug action at a particular site
3. To eliminate patient compliance problems

Major research effort is currently being made to apply the principles of physical chemistry,

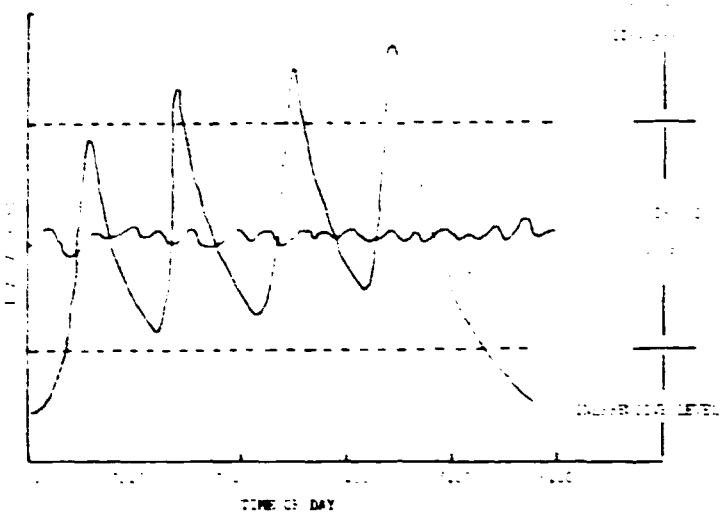


FIGURE 1. Hypothetical plasma level of drug with conventional multidosing schedule (e.g., four tablets daily) compared with controlled release device.

polymer science, and engineering to the design of controlled release devices for human and veterinary application. The pace of advance is rapid, and it is perhaps surprising the precision that has already been achieved in the release of drugs from quite complex polymeric devices and a high degree of precision promises to be obtained from even simpler polymer systems.

Conventional hydrophobic polymers, as rate controlling membranes or matrices, often will not provide a high enough release rate for short-term applications — particularly when water-soluble drugs are being used. They also have a significant disadvantage in that for surgical implantation (rather than insertion) in general two minor operations are required (i.e., for both implantation and removal of the device). Water-swollen matrices, commonly called hydrogels, will provide high permeation rates for water-soluble drugs while biodegradable polymer matrices offer a major potential improvement in the design of controlled release devices for implantation as they may decompose in vivo to nontoxic products which are readily eliminated from the body.

These sophisticated delivery devices can be conceived in many different shapes and sizes. Such devices could be utilized without surgery as inserts into accessible body cavities such as the conjunctival sac of the eye, the ear, mouth, GI tract, rectum, uterus, and vagina or merely placed in contact with the skin as in a transdermal therapeutic system.

An understanding of the physical and organic chemistry of the polymers used for these devices is most important if they are to be well-designed and this will be emphasized for the various types of controlled release devices. The principles involved are reasonably well-defined, but the precise predictions of the physical chemist can often be complicated or even confounded by the added complexity encountered when the device is placed in living tissue which may encapsulate, degrade, calcify, or swell it. An interdisciplinary approach is required for successful research in this field.

II. PHYSICAL FORMS OF DRUG-POLYMER COMPOSITES AS INSERTS AND IMPLANTS

All physically controlled drug release devices can be classified into two distinct types as shown in Figure 2: (1) matrix devices in which the drug is dispersed throughout the polymer or can in some cases be completely soluble in the polymer and (2) membrane devices in which a core of drug is enveloped by a polymeric membrane. These two fundamental types form the basis for systems with a variety of geometrical configurations and topography.

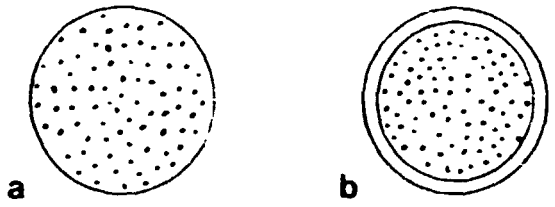


FIGURE 2. Schematic representation of controlled release devices. (a) Matrix device with dispersed drug; (b) membrane device with reservoir of drug.

Matrices are composed of solid polymer in which the drug is dissolved or dispersed. In most cases the loading of the drug exceeds its solubility in the polymer and the drug is present as a homogeneous dispersion of fine particles. These types are also referred to as monolithic devices and can be prepared by a variety of methods. With thermoplastic polymers, such as poly(lactic acid), a drug is dispersed in the melt which can be transfer molded as beads or extruded into rods.[1] Films can be prepared by casting from a common solvent system[1,2] and spray-drying from a solvent produces injectable powders.[3] When the polymer cannot be processed in this way, the polymerization reaction is often carried out in the presence of drug. Using this method, reactive liquids are polymerized by thermal curing[4] or irradiation[5] in molds to produce highly cross-linked matrices. In the case of hydrogels, it may be more convenient to prepare the polymeric device in a desired form prior to loading with a solution of the drug.

The membrane devices consist of a reservoir of drug, often dispersed in solvent, enveloped by a polymeric membrane which controls the rate of release of the drug to the surrounding medium. Microscopic devices take the form of microcapsules[6,7] or nanocapsules[8] while macroscopic devices can take a variety of shapes with the hollow cylinder being the most easily fabricated and most widely studied.[9,10] In general, a hollow tube of polymer is cut to the desired length and sealed at one end; the lumen is then filled with drug and the other end is sealed.

This chapter concentrates on the above types of formulation which involve the polymer as a "physical carrier". However, it should be noted that polymers are increasingly being used as "chemical carriers" in controlled release applications. In this case the drug molecules are linked to a polymeric backbone,[11,12] perhaps by spacer groups, or can be monomers or comonomers used to prepare polymeric molecules.[13,14] These polymers may be either soluble or insoluble in the body fluids at the time of administration. These approaches to drug therapy introduce the fields of polymeric drugs,[15,16] affinity labeled drugs,[17,18] and biologically active polymers.[19,20]

III. RELEASE MECHANISMS AND PREDICTION OF RELEASE PROFILES

The release of drug from any of the different forms of drug-polymer composites, described above, must be predictable and often a constant release rate (zero order) is desired. For many years a considerable amount of mathematical analysis of the theoretical rates of diffusional[21,22] release from the various fixed geometrical configurations has been reported and correlated with experimental results. Three other basic rate determining mechanisms can control the release profile of drug, these being found in swelling, boundary-layer controlled, and erodible devices. The basic concepts involved in the treatment of each of these mechanisms are given below and are followed by a discussion of how these and polymer structure are related.

A. Membrane Devices

Diffusion of a solute, such as a drug molecule, through a polymer occurs as random

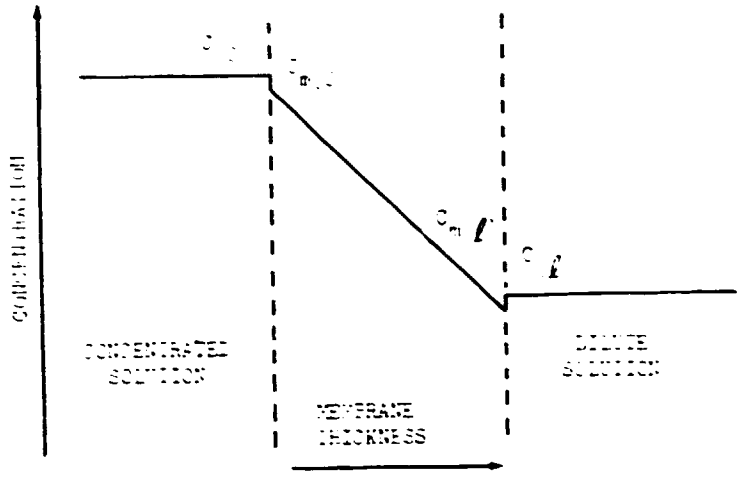


FIGURE 3. Diffusion of solute across a polymeric membrane showing concentration differences.

molecular movement and because a concentration gradient exists between the polymer phase and the external phase the drug tends to diffuse down the concentration gradient. It is generally assumed that the processes involved obey Fick's first law which for diffusion in a nonporous solid polymer membrane can be expressed as:

$$J = -D \frac{dC_m}{dx} \tag{1}$$

Where J is flux ($g\ cm^{-2}\ sec^{-1}$)
C_m is the concentration of the permeant in the membrane ($g\ cm^{-3}$)
$\frac{dC_m}{dx}$ is the concentration gradient, and
D is the diffusion coefficient ($cm^2\ sec^{-1}$)

The negative sign reflects that the direction of flow is down the gradient.

Figure 3 shows the arrangement found for a membrane which separates two solutions of different concentration. It is assumed that the concentration of permeant in the surface layer is in equilibrium with either side. Therefore, if K is the partition (or distribution) coefficient of the solute between the two phases;

$$C_{m(0)} = K\,C_0 \text{ upstream } x = 0$$

$$C_{m(\ell)} = K\,C_\ell \text{ downstream } x = \ell$$

In the steady state

$$J = \frac{D\,(C_{m(0)} - C_{m(\ell)})}{\ell} \tag{2}$$

$$= \frac{D\,\Delta C_m}{\ell} \tag{3}$$

where ℓ is membrane thickness. Usually the concentration in the membrane is not known and ΔC is measured as the concentration difference between the two sides so that:

$$J = \frac{D\,K\,\Delta C}{\ell} \tag{4}$$

The terms D and K are not always easily measured and frequently values of permeability (P) are quoted where

$$P = D\,K \tag{5}$$

Therefore for a membrane device with constant activity, such as one with a saturated solution of the permeant over a large excess of insoluble material, the steady state release rate is expressed by:

$$\frac{dM_t}{dt} = \frac{A\,D\,K\,\Delta C}{\ell} \tag{6}$$

where M_t is the mass released and A is the surface area of the device. When the concentration of drug in the surrounding body fluids is maintained at an extremely low level (i.e., sink conditions), the term ΔC in Equations (4) and (6) can be replaced by C_s, the saturated concentration of drug in the reservoir.

In membrane devices, particularly seen with steroid-silicone systems, "burst" and "lag" effects are found. Newly prepared devices require a period of time to establish a concentration gradient within the membrane and a "lag" effect is found if drug release from the device is assessed. When devices of this type have been stored for some time, the membrane becomes saturated with drug and a "burst" effect, observed as a higher release rate, is found until the concentration gradient associated with the steady state is established. Burst or lag effects are commonly encountered in most types of devices.[23] Inert[24] and biodegradable[25] matrices may often exhibit burst effects due to the presence of crystals of the drug at the surface[26] which readily dissolve when the device is placed in an aqueous environment.

B. Release from Monoliths with Dissolved Drug

The exact solutions for the desorption of solutes from a medium of given simple geometry (e.g., sphere, cylinder, and slab) are available in standard works.[27,28] However, these equations are clumsy for routine use and several approximate solutions can be used for early time and late time release (Table 1). An even simpler unifying equation for the simple geometric forms can be used if less rigourous treatment is acceptable; in this the fraction released at time, t, is given by:

$$\frac{M_t}{M_\infty} = 2\frac{S}{V}\left(\frac{Dt}{\pi}\right)^{1/2} \tag{7}$$

where M_t is the amount of drug released after time, t, M_∞ is the amount present initially, and S and V are the surface area and volume of the device. Practically "blocks", short cylinders, and disks are met more frequently and solutions for these are available in three dimensions where "end-effects" and "edge-effects" require to be taken into account.

C. Release from Matrices with Dispersed Drug

In many cases, the solubility of a particular drug in a given polymer is much lower than that required to provide an adequate amount of drug in a device of limited size. When this situation is encountered, the drug is assumed to be homogeneously dispersed as small particles throughout the polymer. The release kinetics of this type of system were derived by Higuchi,[29] using a model which assumes that the solid drug dissolves from the surface layer and that

Table 1
APPROXIMATE SOLUTIONS FOR DIFFUSIONAL RELEASE OF SOLUTES FROM SLABS, CYLINDERS AND SPHERES (SEE TEXT FOR DEFINITIONS OF SYMBOLS)

Geometry	Early time	Late time
Slab r = thickness	$\frac{M_t}{M_\infty} = 4\left(\frac{D\,t}{\pi\,r^2}\right)^{1/2}$	$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\exp\left(\frac{-\pi^2\,D\,t}{r^2}\right)$
Cylinder r = radius	$\frac{M_t}{M_\infty} = 4\left(\frac{D\,t}{\pi\,r^2}\right)^{1/2} - \frac{D\,t}{r^2}$	$\frac{M_t}{M_\infty} = 1 - \frac{4}{(2.405)^2}\exp\left(\frac{-(2.405)^2\,D\,t}{r^2}\right)$
Sphere r = radius	$\frac{M_t}{M_\infty} = 6\left(\frac{D\,t}{\pi\,r^2}\right)^{1/2} - 3\,\frac{D\,t}{r^2}$	$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\exp\left(\frac{-\pi^2\,D\,t}{r^2}\right)$

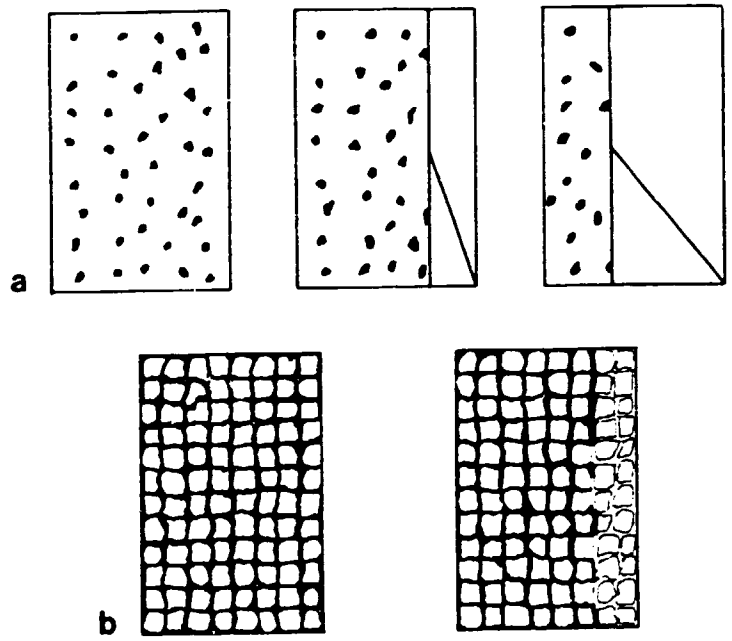


FIGURE 4. Models of drug release from polymeric devices. (a) Drug dispersed in continuous polymer; (b) drug dispersed in capillary channels of a porous polymer matrix.

this layer becomes exhausted of dispersed drug particles, as shown in Figure 4a. From the figure the dependence of concentration gradient with time is apparent. The validity of this matrix-controlled model has been demonstrated in several experimental studies.[30,31] In particular, the visual appearance of cylinders containing dispersed particles of steroid has shown a gradual depletion of the steroid from the surface layer which progressed towards the core with time.[32] The derivation of the Higuchi Equation (8) relies on Fick's first law and assumes:

1. A Pseudosteady state exists.
2. The drug particles are small compared to the average distance of diffusion in the device.
3. The diffusion coefficient is constant.
4. Perfect sink conditions exist in the external medium.

$$Q = (C_s\,D\,(2A - C_s)\,t)^{1/2} \qquad (8)$$

and when

$$A \gg C_s \qquad Q = (2 C_s D_m A t)^{1/2} \tag{9}$$

where Q is the amount of drug released at time, t. C_s is the solubility of drug in the polymer, and A is the amount of drug present initially.

The most important point to note from these equations is that the amount of drug released is related to the square root of time ($Q\text{-}t^{1/2}$ relationship) essentially throughout the lifetime of the device. Whereas with monoliths with dissolved drug a $Q\text{-}t^{1/2}$ relationship is found initially and the second half of the release decays exponentially (see Table 1). Higuchi suggested that the Equation (8) would be valid only for systems in which A is greater than C_s by a factor of three or four. More recently, the model has been developed and the newer equations can be used with more accuracy over a wider range of conditions.[33,34]

D. Release from Devices with Capillaries and Pores

All of the above models were based on simple diffusion of a permeant molecule in a homogeneous polymer phase. If, however, the polymer phase is discontinuous, such as in the situation where a considerable volume fraction of drug has been compressed into an infinite slab with a polymer powder, the situation is more complex. The small extent of diffusion through the polymer is neglected and it is assumed that diffusion occurs predominantly in the capillaries between polymer particles and through the pores left behind as drug particles dissolve and are replaced by the permeating liquid (Figure 4b). Account must be taken of the increase in the length of the diffusional path around the inert polymer particles and the effective cross-section of diffusion must be reduced to allow for the fact that the cross-section available for diffusion is that of the permeant-filled holes left behind by the drug particles after dissolution and not the total volume of the inert polymer-drug-permeant combination.

Higuchi[35] developed a similar equation to that above Equation (8) for release of drug from one face of an inert granular matrix containing a dispersion of drug. The increased path length in the capillaries using a tortuosity factor ($\tau = 3$) and the cross section of pores left by the dissolved drug by a porosity factor (ϵ) were taken into account. This provides an equation,

$$Q = \left(C_s D_s \frac{\epsilon}{\tau} (2A - \epsilon C_s)\, t\right)^{1/2} \tag{10}$$

where C_s and D_s are the solubility and diffusion coefficient of the drug in the permeating fluid. The terms Q, A, and t have similar definitions as in Equation 8 and again a $Q\text{-}t^{1/2}$ relationship is predicted. Higuchi deduced a related equation for the release from spheres of the same composite structure. More recently, the basic Higuchi models have been developed for more practical shapes — disks, biconvex tablets, and cylinders.[36-38]

Since it was first introduced, the Higuchi Equation (10) has frequently been applied to experimental data, particularly inert granular matrix tablets of the sustained-action type for oral administration. In some cases, the fundamental parameters have been studied in great detail. Values for C_s and D_s for a particular drug can be determined readily using conventional techniques. Desai et al.[39-42] studied the release of various drugs and model compounds from polyethylene and poly(vinyl chloride) matrices and described the components involved in the porosity term, ϵ, and how to evaluate them. When the matrix tablets were subjected to vacuum treatment prior to study, tortuosity values obtained experimentally for poly(vinyl chloride) were in the range 1.5 to 4, which is in good agreement with theoretical values.[42] Similar studies with polyethylene matrices produced tortuosity values in the range 7 to 10

when a surfactant was added to the elution medium to wet the polymer.[41] Both types of polymer required some pretreatment before ideal release behavior was found and the differences in tortuosity values was attributed to the different particle size and density of the polymer powders. Apparent tortuosity values of the order of a thousand were found with polyethylene matrices when surfactant was not used to aid the removal of air from within the pores. In further extensions of these studies, the theoretical equations for the release of two noninteracting[43] and two interacting drugs[44] were presented and compared with experimental data.

Increasingly, other polymers (generally hydrophilic) are being included as additives to formulations in an attempt to modify the release rate from the major polymer component. In this way, the addition of gelatin or sodium alginate to silicone cylinders was found to increase the release rate of morphine sulfate (hydrophilic drug.)[45] When 20% alginate was added to the formulation, the devices swelled with water forming microscopic pores and channels and about 90% of the drug was released after 10 days compared to 9% from devices without alginate. The release of morphine sulfate was found to follow second-order kinetics and a loss of alginate was observed throughout most of the period of drug release. The release of macromolecules, such as proteins, from ethylene-vinyl acetate copolymers[46] may occur by a similar mechanism involving the uptake of water by osmotic pressure resulting in the formation of microchannels.

E. Release from Hydrogels

With hydrogels a considerable proportion of the device is composed of water which may be several times the dry weight of the polymer. When the hydrogel is initially swollen and contains water-soluble drug, the release equations given previously (i.e., Table 1) can be applied. Davis[47] deduced the following empirical expression to calculate the apparent diffusion coefficient of any soluble drug in any hydrogel,

$$D_p = D_0 \exp -(0.05 + 10^{-6}M)P \qquad (11)$$

where D_p is the diffusion coefficient of the solute in the swollen polymer gel containing P% (by weight) of polymer; M is the molecular weight of the solute; and D_0 is the diffusion coefficient of the solute in water. The study involved both cross-linked poly(acrylamide) and poly(vinylpyrrolidone) hydrogels and solutes with a wide range of molecular weight (125,000 to 150,000) and included radio-labeled rabbit immunoglobulin, bovine serum albumin, insulin, a prostaglandin, and sodium iodide.

Various forms of the Higuchi Equations (8) and (10) have been used to describe the release of drugs from hydrogels,[48] and in a study by Chien and Lau,[49] the diffusion coefficient in the gel D_m ($D_m = D_{0f}^{\phi}$) was related to the degree of cross-linking.

The release kinetics from initially dry hydrogels, as would be expected, are complicated by the added consideration of the diffusion of solvent into the polymer. Good[50] has derived equations where the diffusion coefficient is a time dependent variable and fitted experimental data based on the release of a water-soluble drug (tripelenamine·HCl) from a poly(2-hydroxyethyl methacrylate) hydrogel. This hydrogel had a low degree of swelling and the water uptake was approximately balanced by the loss of drug, and the dimensions of the device were assumed not to change during release. Recently, the much more complex situation of diffusion from a device which is simultaneously swelling,[51,52] and undergoing dimensional change, has been analyzed.

F. Boundary-Layer Controlled Release

With drugs which are very poorly water soluble, the external medium in contact with the polymer phase may become saturated with drug and the release from the device is effectively

stopped until drug has diffused out of the unstirred boundary layer. As an example of this phenomenon, also termed partition controlled release, Chien et al.[53] studied the release of estradiol diacetate from silicone devices and found a desirable zero-order (Q-t relationship) release when the steroid was poorly soluble in the eluting medium (water). However, when increasing proportions of poly(ethylene glycol) were added to increase the solubility of the steroid in the eluting medium, the release profile changed to a typical matrix-controlled type ($Q\text{-}t^{1/2}$ relationship).

The boundary-layer effect has considerable implications in vivo[54] where the composition and the movement of the eluting medium may vary from site to site. The effect should be considered when in vitro release kinetics are compared with in vivo release[49,55-57] and the in vitro methodology designed to favor reasonable correlation. If the release characteristics of a device change with the rate of stirring in an in vitro test, it is a strong indication of some measure of boundary control.

G. Release from Erodible Devices

Hopfenberg[58] considered controlled release from erodible slabs, cylinders, and spheres. Where a single zero-order process controls erosion, the theoretical equations can be rearranged in the form

$$\frac{M_t}{M_\infty} = 1 - \left(1 - \frac{k_0 t}{C_0 a}\right)^n \qquad (12)$$

where k_0 is the single zero-order rate constant for the erosion process, C_0 is the uniform initial concentration of drug, M_t is the amount of drug released at time, t, and M_∞ is the total amount of drug present initially. For the infinite slab, n = 1 and a is the half-thickness; for the cylinder, n = 2 and a is the radius; for the sphere, n = 3 and a is the radius. From the analysis, it is evident that zero order is only obtained from the infinite slab and that delivery rates from cylinders and spheres should decrease with time. It is implicit in the overall concept that a boundary exists between unaffected polymer and the previously degraded material and that the rate determining step occurs at the boundary . Specifically, water sorption by the polymer and the diffusion of the drug out of the matrix have negligible effect on release rate. Cooney[59] discussed the effect of geometry on the dissolution of pharmaceutical tablets presenting the common shapes and suggesting that spheres and cylinders with internal bores cross and clover leaf-shapes may offer better release profiles.

Devices which undergo surface erosion have been reported,[60] however, at this time, most biodegradable systems have release profiles complicated by water sorption, degradation in the bulk, and diffusion of the drug from bulk polymer.

IV. FUNDAMENTALS OF POLYMER CHEMISTRY FOR THE CONTROLLED RELEASE OF DRUG FROM POLYMERIC MEMBRANES AND MATRICES

In controlled release applications, the drug molecule is chosen for its biological action in a particular therapy and therefore is generally not considered to be a variable. Furthermore, in any proposed application the amount (dosage level) released over a chosen time scale from a particular dosage form are the desired objectives at the outset.

The physicochemical properties of the drug can have significant effect on its release from any polymeric system and molecular size, concentration, and solubility both in body fluids and the polymer are of extreme importance.

Perhaps the most important parameter in any controlled release system is the diffusion coefficient of the drug in the polymeric device. The rate of diffusion of one molecule through a medium depends mainly upon:

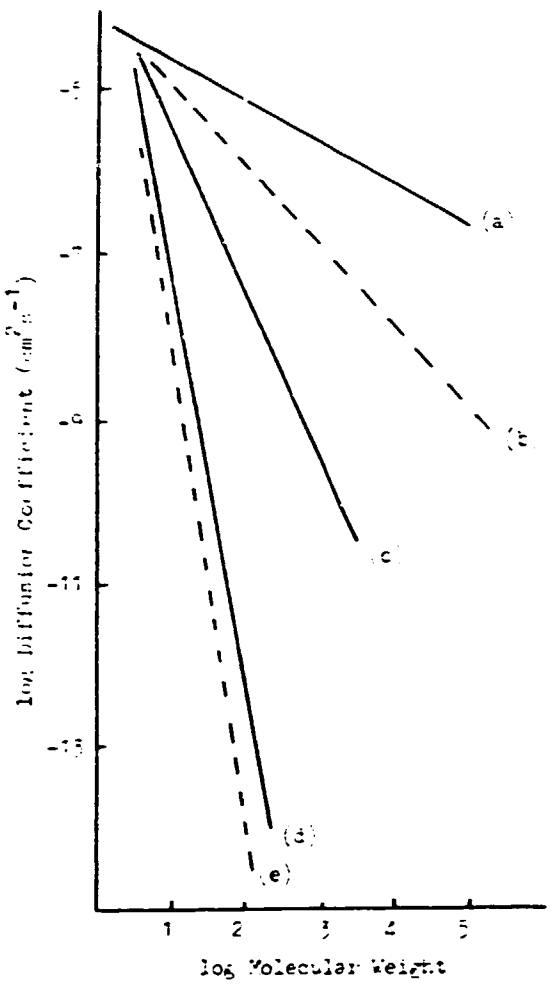


FIGURE 5. Relationship between log molecular weight of drug and its diffusion coefficient in different media. (a) Aqueous solution; (b) possible plot for a hydrogel; (c) a natural rubber; (d) an organic glass (polystyrene); and (e) possible plot for a cross-linked glassy polymer (some data from Reference 23).

1. The thermodynamic driving force which can approximate to a function of a concentration difference
2. The size of the diffusing molecule
3. The resistance to molecular movement presented by the medium

A. Physical States and Structure of Polymers

The density of a polymer, indeed any particular material, will usually decrease in the order crystalline state, glassy state, rubbery state. This change in density is related to the molecular holes or free volume present in each state. This free volume increases from lowest in the crystalline state to highest in the rubbery state. As the free volume represents the holes into which diffusing molecules jump as they move down a concentration (or chemical potential) gradient, it is easy to postulate in general terms how diffusion coefficients increase as one goes from the crystalline to the glassy and then to the rubbery state. This is illustrated in Figure 5 in which the difference in both diffusion coefficient in different states can be seen and also how the different states show markedly different changes in their response to the diffusion of larger molecules. This plot demonstrates clearly why water-swollen hydrogels provide such appropriate systems for the short-term release of water-soluble drugs by providing the highest available diffusion coefficients amongst polymer matrices.

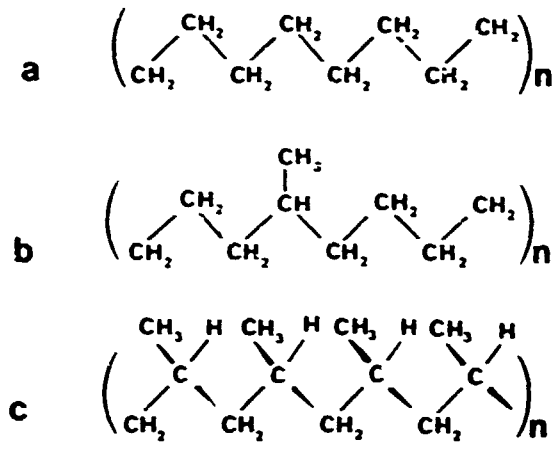


FIGURE 6. Polyethylene and ethylene/propylene structures producing rubber or crystalline polymers. (a) Crystalline; (b) rubbery; and (c) crystalline.

In the glassy state, polymers are hard, rigid, and often brittle.[61] There is a low level of molecular movement and the rate of diffusion of large molecules is very low. On heating at a defined rate, the glassy solid changes to a more flexible rubbery solid over a given small temperature range. Above this so-called glass transition temperature (T_g), the polymer is in the rubbery state and, if not constrained by some molecular interaction or bonding, will exhibit fluid flow. The fluid flow is prevented in many useful plastics by the presence of phase separation, crystallites, or cross-linking.

Crystallites can form in polymers when long sequences of the polymer chain have a stereoregular structure. The simplest example of this is polyethylene as shown in Figure 6. An approximate relationship T_g = (0.5 to 0.66) T_c is found to hold for many polymers (where T_c in degrees Kelvin is the temperature at which the crystallites melt). The main point to note is that $T_c > T_g$ and as polymers are never 100% crystalline such polymers above their T_g contain rubbery portions bound together with varying proportions of crystalline domains through which many chains pass.

Block or graft copolymers as represented in Figure 7 are comprised of long sequences of two or more different polymers. As high molecular weight chains rarely dissolve in each other such block or graft copolymers form a mixture of domains in which polymer separation has occurred and the units of each species have aggregated. If one type of these separate domains is glassy or crystalline while the other is rubbery, the effect is to prevent flow of the chains and hence bulk polymer.

The need for polymers with a range of precise requirements, as in controlled release applications, has led increasingly to the study and use of copolymers. Three broad groups of polymers used in controlled release can be divided into (1) inert hydrophobic, (2) inert hydrophilic, and (3) bioerodible. Examples of these three classes are given later. However, it should be noted that classification is far from simple and many of the devices which have been studied to date have been relatively complex. Classification of groups of polymers should be treated with caution because, for example, the "acrylates" can be

1. Inert hydrophobic, e.g., poly(methyl methacrylate)
2. Inert hydrophilic, e.g., poly(hydroxyethyl methacrylate)
3. Bioerodible, e.g., poly(methyl 2-cyanoacrylate).

B. The Swelling of Cross-Linked Polymers

The ability of a polymer to swell with a given solvent is governed by the free energy of mixing of the solvent with the polymer and by the density of the cross-linking.[62,63] The

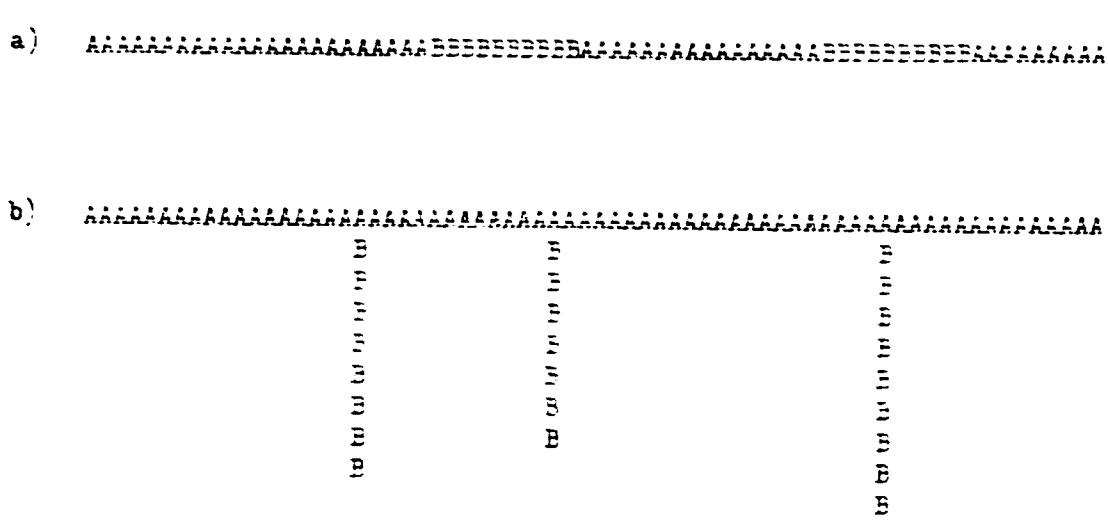


FIGURE 7. Simple representation of (a) block and (b) graft copolymers, where A and B represent units derived from different monomers.

Table 2
DIFFUSION OF ^{3}H-LABELED PROGESTERONE THROUGH VARIOUS POLYMER MEMBRANES

Polymer	Progesterone diffused (%)
Polydimethylsiloxane (Silastic®)	100
Polyamide (nylon)	1.0
Acetylcellulose (cellophane)	0.1
Fluorethylene	0.1
Polyester (Mylar®)	0.1
Polycarbonate (Lexan®)	0.1
Polyethylene	0.1
Polystyrene copolymer (Cr 37)	0.1

From Kincl, F. A., Benagiano, G., and Angee, I., *Steroids*, 11, 673, 1968. With permission.

theoretical analysis of the swelling is complex and will not be discussed here. The combination of a polymer with a swelling solvent which might be a plasticizer in the case of a commonly used membrane such as ethylene/vinyl acetate copolymers or water in the case of hydrogels is commonly used to moderate and control the diffusion of drugs through polymers.

The degree of swelling is increased by reducing the cross-linking density. As diffusion coefficients also increase with increasing swelling this can be used as a means of controlling the rate of release of incorporated soluble drugs.

C. Diffusivity of Drugs in Polymers

Table 2 shows the relative permeabilities of ^{3}H-labeled progesterone in a range of different polymeric membranes[64] and the difference between silicone rubber, with its very flexible backbone, and the other common polymers is readily apparent. The effect of soaking membranes in plasma prior to diffusion experiments with steroid was examined in another study.[65]

In addition to molecular size of the diffusing molecule other physicochemical properties are extremely important. The permeation of a molecule through a membrane is not only dependent upon its diffusion coefficient, but also its solubility in the polymer and its dis-

Table 3
AVERAGE DIFFUSION RATE OF VARIOUS STEROIDS ACROSS SILICONE MEMBRANES

Steroid	Diffusion rate*
19-Norprogesterone	1353
Progesterone	469
Testosterone	317
Megestrol acetate	236
Norethisterone	73
Estradiol	61
Mestranol	43
Corticosterone	21
Cortisol	6

* μg (100 mm²) (0.1 mm) (24 hr).

From Kincl, F. A., Benagiano, G., and Angee, L., *Steroids*, 11, 673, 1968. With permission.

tribution coefficient between the polymer and the external medium. The effect of small changes in the structure of steroid molecules on their release from silicone[64] is shown in Table 3. Detailed studies[65-67] have since been carried out to relate the effect of these slight changes which have negligible effect on molecular size, but considerable effect on solubility. Similar studies have been reported for the release of steroid from hydrogels.[68]

Other additives apart from the drug may produce significant effects and can be used to modify the release profile. The effects of fillers[67,69] and plasticizers have been extensively studied while copermeant enhancement[70] and the addition of hydrophilic polymers[45] to produce a heterogeneous polymer matrix with microchannels appear to add further design variables. In the latter approach, as discussed previously, high molecular weight water-soluble materials (e.g., proteins) can be released from a hydrophobic polymer by the formation of microchannels.

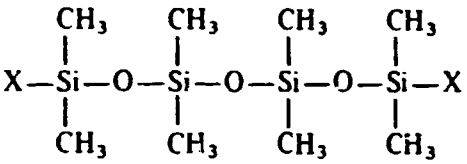
V. HYDROPHOBIC NONDEGRADABLE DEVICES

The release of biologically active compounds from "inert" synthetic polymers has been the subject of a considerable amount of research for many years and probably most classes of polymer have been screened in some controlled release application or another. Table 2 showed some which were studied as potential membranes for the release of steroids. Inert synthetic polymers have also been investigated as the wall material of microcapsules and many processes and applications have been reported. It is clear that in many cases the release rate of drug from simple macroscopic devices would be inadequate for most applications. However, two groups of polymers which showed some potential, the silicones and the substituted polyethylenes discussed in some detail below, have been the subject of concentrated study and their advantages and limitations have become apparent.

A. Silicones

Silicone rubber[71] has been used in a variety of biomedical applications due to its inertness and good biocompatibility. In 1964, Folkman and Long[72] reported the use of this polymer in sustained release formulations.[73]

The backbone of the silicone polymer is made up of alternating silicon and oxygen atoms and the most widely used member of the family is poly(dimethylsiloxane). This material can be prepared as a fluid elastomer or a resin and is available commercially as Silastic®.



Silicone polymers end-blocked (group denoted X above) with CH_3, are unreactive and generally used as fluids. Alternatively, chains can have reactive hydroxyl end groups which may be cross-linked by curing at room temperature and these are used to prepare molded or cast devices. This type of polymer has been widely studied in implants and inserts for the controlled release of steroids,[66,67,74] chloroquine,[75] pyrimethamine, indomethacin,[76] atropine, and histamine.[77] The devices are generally macroscopic implants of both the membrane and the matrix type. Chien and Lambert[78] developed a system with microsealed compartments (10 to 200 μm) containing drug in a hydrophilic solvent enclosed within a matrix of silicone polymer. By careful choice of the composition of the hydrophilic solvent [e.g., aqueous solutions of poly(ethylene glycol)], the release rates of various drugs could be controlled over a wide range. A microsealed device of this type which delivered deoxycorticosterone acetate, was used to study the production of metacorticoid hypertension in rats.[79]

While the flexibility of the backbone of the silicone polymer in combination with the saturated solubility level of hydrophobic drugs in the polymer allows adequate release rates to be obtained, the release of hydrophilic drugs will be considerably slower due to their lower solubility. Attempts to increase the permeability of the silicone polymer to hydrophilic drugs employed the addition of a considerable proportion of hydrophilic polymer (e.g., sodium alginate) to produce microchannels, as described above. Devices modified in this way have been used to study morphine induced tolerance and physical dependence in animals.[80]

B. Substituted Polyethylene

High density polyethylene is used in a variety of biomedical applications and the low density material has been used in the fabrication of a contraceptive intrauterine device which also releases progesterone.[81] The local release of a steroid, such as progesterone, has a synergistic effect on the contraceptive action of the device which is provided by its physical presence. This local direct drug action in the uterus reduces systemic side effects to undetectable levels and normal menstrual cycles occur. For controlled release devices, it is usual to add other monomers to provide a range of copolymers with the desired characteristics (see Figure 8). Thus, the Progestasert® device (Alza Corportion) uses an ethylene-vinyl acetate copolymer (9% vinyl acetate) as a rate controlling membrane. In this way a constant release of 65 μg/day of progesterone is obtained for a period of 1 year.[82,83] Another device developed by the Alza Corporation, and approved by the FDA in the U.S. is the Ocusert Pilo. The device, composed of two membranes of ethylene-vinyl acetate copolymer, is inserted into the cul-de-sac of the eye and provides a constant release of pilocaprine for the reduction of intraocular pressure in glaucoma. The two devices available, Pilo 20 and Pilo 40, release at a rate of 20 or 40 μg/hr for 1 week and offer much better control than eye drops instilled several times daily and remove problems with patient noncompliance.

The effect of the comonomer ratio of ethylene and vinyl acetate on the in vitro release of hydrocortisone from molded devices was studied by Fu et al.[84] It was found that increasing the proportion of vinyl acetate, thereby changing crystallinity, increased the release rate. In another study, an ethylene-vinyl acetate copolymer (40% vinyl acetate) was used to prepare

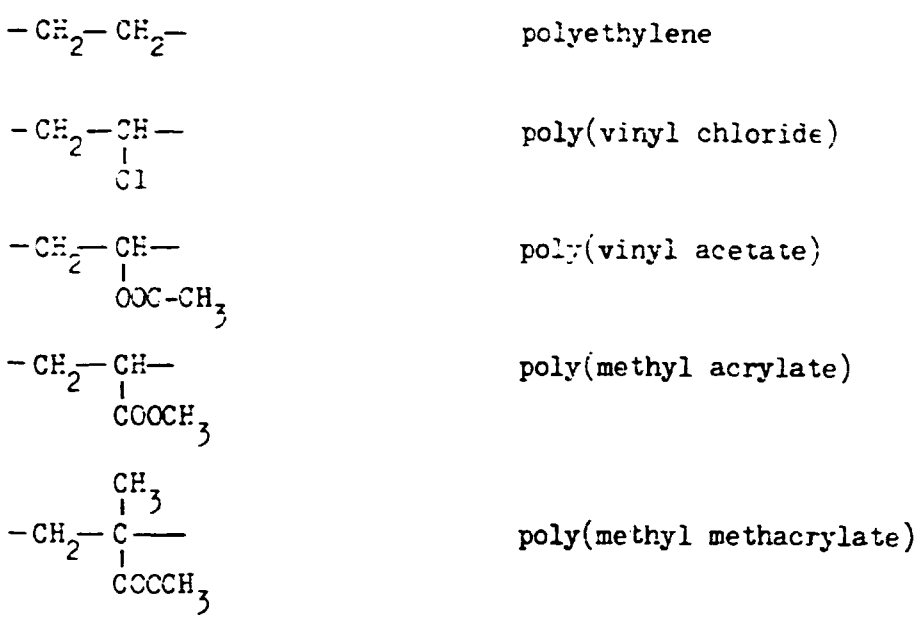


FIGURE 8. Some examples of the repeating units of substituted polyethylene polymers which have been used in controlled release devices.

pellets which released biologically active macromolecules (e.g., soy bean trypsin inhibitor, lysozyme, alkaline phosphatase, catalase, insulin, heparin, and DNA) for times in excess of 100 days at relatively constant rates depending on the particular preparation.[46] It was suggested that copolymers of this type could be used to prepare carriers for a wide range of enzymes and other biologically active macromolecules which would release at desired rates.[85] While it seems likely (see before) that the hydrophilic macromolecules would draw water into the matrix and swell it with the formation of microchannels, it was noted in that study that once the macromolecule had been released the membrane was not permeable to molecules of similar size.

Both polyethylene and poly(vinyl chloride) have been used to prepare matrix tablets by compression of the powdered polymer with hydrophilic drug and under certain conditions were found to obey the Higuchi Equation (10). Similar studies have been carried out with copolymers of methyl acrylate and methyl methacrylate.[86,87] Recently, methyl methacrylate membranes have been applied to a core of poly(hydroxyethyl methacrylate) and the resulting trilaminate device has provided zero order release of tetracycline for periods in excess of 100 days.[88] The trilaminate devices, when implanted intraperitoneally in rats, were used in a pharmacokinetic study of tetracycline.[89]

Surgeons using methyl methacrylate as a bone cement in total replacement of joints, especially hip joints, have found that the addition of antibiotics to the cement prior to use significantly reduced the incidence of infection. Several studies have been carried out[90,91] and the effect of several antibiotics on the mechanical properties has been reported.[92,93] The concept led to the evaluation of implantable beads of poly(methyl methacrylate) containing gentamycin for the treatment of chronic bone infections.[94] Such beads have been found in vitro and in vivo to provide adequate release locally for several months while serum concentrations were low thereby minimizing the risk of problems with side effects.

Devices composed of cross-linked diethylene glycol dimethacrylate have been prepared by γ-irradiation of the frozen monomer at $-78°C$.[95,96] Mixtures of the monomer with a variety of anticancer drugs were molded, frozen, and irradiated.[5] The effect of the incorporation of other polymers (e.g., poly(ethylene glycol), poly(methyl methacrylate), and poly(vinyl acetate) at 10% of the total weight) on the release profiles of the drugs were briefly discussed. It was found that the rate of release could be greatly enhanced by some of these incompatible additives which formed microchannels in the glassy matrix.

It is readily evident that a very diverse range of devices can be prepared from simple polymers, such as the two groups discussed above. Various constraints are placed by the solubility of the drug and the desired duration of action and in general each case has to be studied in some detail.

VI. HYDROGEL SYSTEMS

Hydrogels comprise a large group of polymers which swell to a considerable degree with water. They have attracted considerable interest in prosthetic applications such as contact lenses or promising nonthrombogenic surfaces and in the context of this chapter as matrices for the controlled release of drugs. Their most interesting attribute is their high permeability to water-soluble drugs which is in contrast to the low permeability of these materials often obtained with hydrophobic polymers such as silicones. Their physical strength and durability in the dry and swollen states are important factors in their practical application and some indications of how these can be optimized are discussed later. They obey the release equations described earlier and can be obtained in monolithic slab, cylinder, or spherical configurations and also in envelope or matrix forms, all with the kinetics predicted by the well-developed mathematical models. Powders of hydrogels can provide injectable formulations which may be further developed.[97] The kinetics of release from devices which are changing their dimensions during their effective lifetime have recently been modeled, while other studies on systems which both lose crystallinity and swell simultaneously have been shown to provide constant rates of drug release from a monolithic device — something which is very useful, but not to date predicted by theory. Polymers can be designed which are hydrophobic at one pH and change to hydrophilic hydrogels at another. These can be used to design matrices which only release their contained drug at a particular pH. These points of design will be discussed in more detail below.

A. The Polymers Used in Hydrogels

Any water-soluble polymer which can be rendered insoluble by cross-linking or incorporation into a block or graft copolymer (Figure 7) in which the other component is a hydrophobic unit can in principle be used as a hydrogel for the delayed or controlled diffusion of molecules which may or may not be drugs. Probably the best-known and most widely used hydrogel for such purposes is cross-linked collagen which is used as the basis of photographic films as well as in the pharmaceutical industry. While cross-linked collagen has been used for microencapsulation, it is not a very suitable polymer for preparing monolithic hydrogel devices with consistent performance properties especially if low degrees of swelling are desired. For this consistency with ease of preparation, research has favored the synthetic polymers of hydroxyethylmethacrylate,[50,98] acrylamide[47] and its *N*-sugar substituted derivatives,[99] *N*-vinylpyrrolidone[47,48] and poly(ethylene oxides),[100-104] as shown in Figure 9. Poly(glutamic acid)[102] and cross-linked dextrans and starches[97] have also been used. The most desirable hydrogels should be very strong and tough, but not brittle in the dry state, swell to a reproducible degree in water, buffer, or plasma, and be strong in the swollen state. Practically, the drug/polymer composite should be readily obtained in the desired geometry by a process capable of scaling up to production. It should not contain any toxic residues of monomer, initiators, stabilizer, or drug modified by the polymerization process. Although polyacrylamide has been the focus of some academic study, the risk of residual carcinogenic monomer might well rule it out in practice. The largest amount of work has been done on hydroxyethylmethacrylate which is unfortunately brittle as a homopolymer and has a relatively low degree of swelling (42%). In many studies it is polymerized with a cross-linking agent in aqueous solution containing drug and the residual initiation fragments and monomer are not removed.

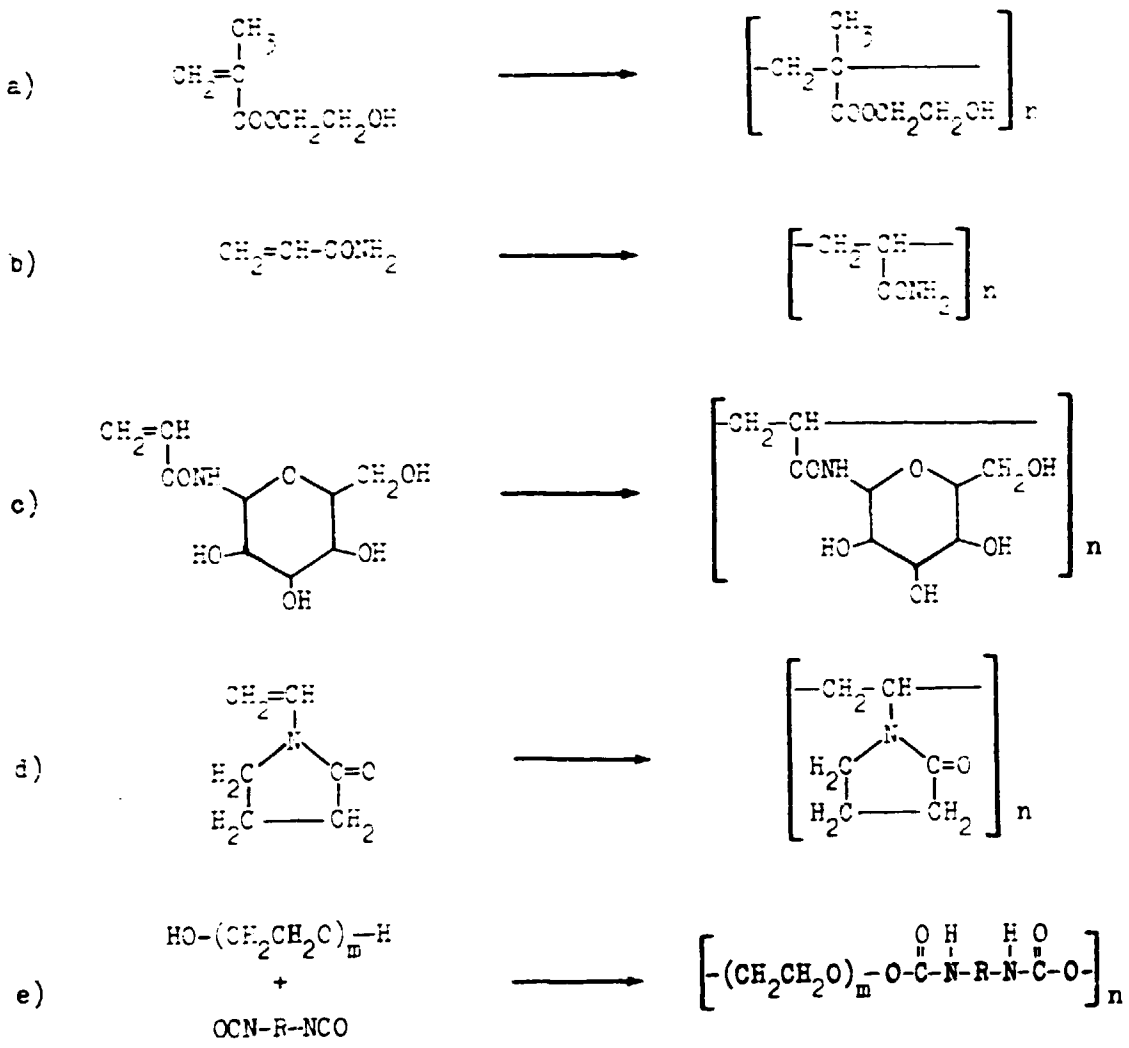


FIGURE 9. Structures of some commonly used hydrogels. (a) Poly(2-hydroxyethyl methacrylate) (HEMA); (b) polyacrylamide; (c) *N*-substituted derivative of polyacrylamide; (d) poly(vinyl-*N*-pyrrolid-2-one); (e) polyurethane prepared from poly(ethylene glycol) and a diisocyanate.

There is little difference in the release characteristics normally attributable to the chemical nature of the hydrogel for basically amorphous polymers.[47] The big differences arise from control of physical parameters such as the water content, degree of cross-linking, crystallinity, and morphology, i.e., if two or more phases are present. Thus, much recent research has been concerned with obtaining stronger hydrogels from what are essentially block or graft copolymers of either a thermoplastic or thermoset nature. Ciba-Geigy[101] has obtained cross-linked hydrogels of poly(ethylene oxide) capped with double bonds and subsequently co-polymerized with hydrophobic monomers to obtain polyphase systems which are claimed to have improved physical strength. ICI[104] achieved a similar multiphase system which also has the distinct advantage of retained polymer solubility allowing washing and purification of the polymer combined with the thermo-forming properties of a linear block copolymer. This allowed thin films to be obtained by the application of pressure and heat.

Another promising development along these lines by Graham et al.[103] involves cross-linked polymers of poly(ethylene oxide) using diisocyanates and polyols to provide cross-linking. If poly(ethylene glycols) of molecular weights above 2000 were used dry gels containing up to 50% crystalinity could be obtained. The crystallites act to reinforce an already quite perfect polymer network to provide a tough polymer akin in its physical properties to low density polyethylene. By control of the degree of cross-linking, hydrogels

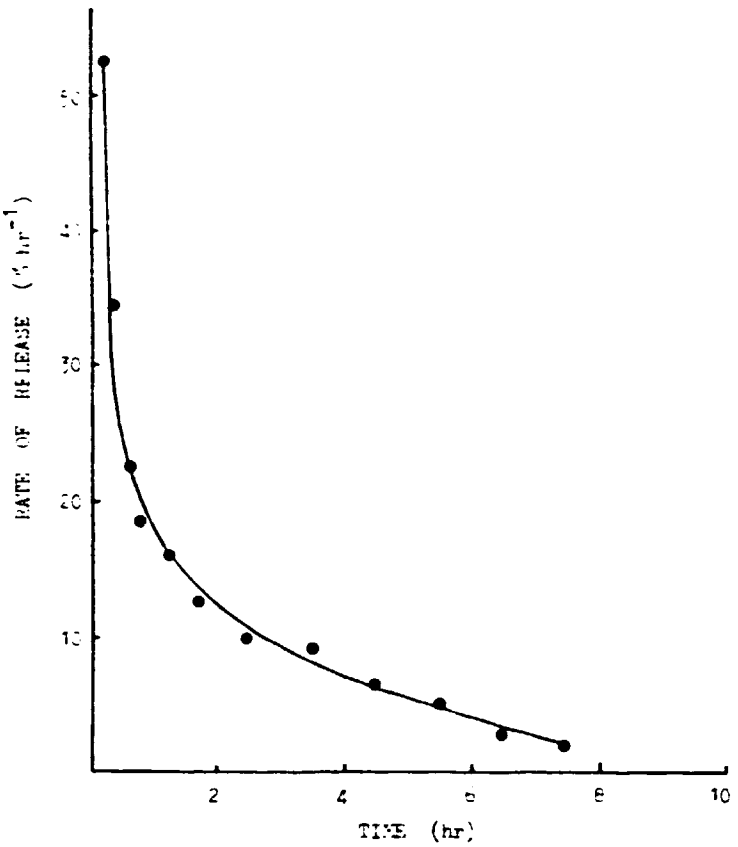


FIGURE 10. Release of prostaglandin E_2 from an initially fully swollen hydrogel based on poly(ethylene glycol) at 37°C and pH 7.4 (rate $\propto t^{-1/2}$ relationship).

capable of swelling with water to typically five times their dry weight could be made. In the fully swollen state these polymers behaved like any other hydrogel, releasing their contained drug at a rate proportional to $(\text{time})^{-0.5}$ (Figure 10), but in the dry state slices of the polymer released a considerable proportion of the contained drug at a constant rate (Figure 11). This is because of a balance struck between decreasing drug release from an unswollen crystalline core and a physically coupled increasing permeability of the swelling outer layer. This constant release from a monolithic hydrogel is of obvious clinical importance and its first use has been in aiding the performance in delivery of pregnant women suffering from a so-called unripened cervix at full term.[105]

The above polymer in the form of a small vaginal pessary containing between 5 and 15 mg of prostaglandin E_2, depending on the assessed requirements of the individual patient, was inserted into the vaginal vault on the eve of the predicted delivery date. The uniform release of the prostaglandin E_2 over 12 to 24 hr induced cervical ripening and significantly reduced the need for a Caesarian operation as well as inducing normal and shortened labor with reduced pain in many patients. A major benefit of this device is that the prostaglandin E_2 which is normally quite unstable on storage was shown to be largely unchanged after 14 months storage at 4°C. This points to these drug-in-dry-hydrogel devices having enhanced practical application because of this ability to protect the contained drug from degradation or isomerization. This ability of hydrogels to stabilize prostaglandin E_2 has been confirmed in another briefly reported study using dry cross-linked starch gels.[106]

Other attempts to obtain constant rates of release from either fully swollen[88] or initially dried down[50] hydrogels have been reported in various studies. Lee et al.,[107] for example, have taken a drug containing monolithic cylinder prepared from derivatives of poly(methacrylate) and adsorbed and polymerized additional cross-linking monomers into

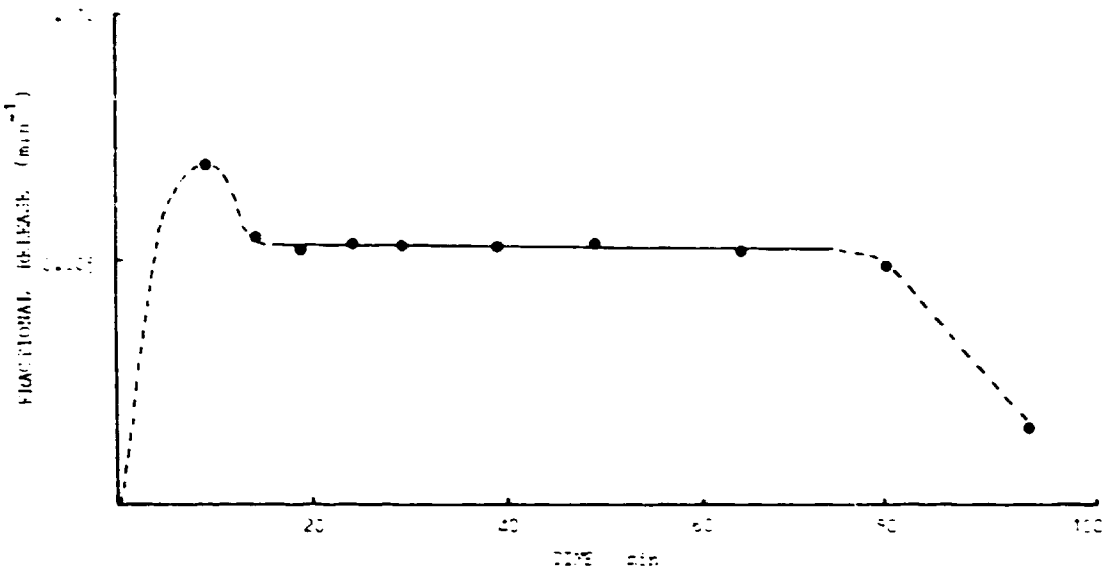


FIGURE 11. Release of promethazine hydrochloride from an initially dry, crystalline hydrogel based on poly(ethylene glycol) (rate × t relationship for 37% of total release).

the external surface. This produces a skin around the device which has a lower degree of swelling than the core and as a result has a lower permeability to the contained drug. This skin becomes the rate controlling step of drug diffusion out of the device which is effectively converted into an "envelope" configuration for which the rate is predicted and found to be constant with time. Other related devices for the release of fluoride to teeth have also been reported.[108]

Cores of drug coated with rate controlling membranes have been used in even more sophisticated ways. Thus, by the incorporation of covalently bound carboxyl groups it is possible to obtain hydrogels which swell under basic conditions,[98] while swelling under acidic conditions can be obtained by the covalent incorporation of basic groups such as amine. Such groups have been incorporated into both vinyl and urethane polymers. An elegant example of such a use has been reported by Fildes of ICI,[109] who encapsulated a core tablet of quinoxaline di-*N*-oxide with a nylon copolymer containing basic groups in its backbone made by interfacial or condensation polymerization (Figure 12). These coatings were essentially unswollen at a pH of 7, but became highly swollen hydrogels at pH 4 and thus, having a high permeability to water-soluble drugs at this latter pH, released the contained drug in 4 hr. This arrangement was made for the purpose of protecting the drug in the rumen of cattle where the pH is around 7 and allowing release of the drug in the abomasum where the pH is around 4.

An example of systems of low swelling under neutral conditions, but releasing at high pH, is provided by the urethane polymers containing glucuronolactone.[110] The lactone group is not involved in the formation of a network by reaction of the hydrogens in this molecule with isocyanate. In high pH conditions the lactone ring opens and allows the polymer to dissolve if it is not cross-linked or to swell if it is cross-linked. These fundamental ideas were well-described in an early patent to the Czech Academy of Sciences[98] on the controlled release from vinyl hydrogels.

An even greater degree of sophistication has been developed by the Alza Corporation in their Oros® devices. In these, a solid tablet is coated with a hydrogel into which coating a very fine hole is introduced by a laser beam. In its simplest form, an elementary osmotic pump,[111] such as the Oros® device, consists of a membrane with an orifice in it (Figure 13a). In this type of device the membrane allows the passage of water, following an osmotic gradient, into the core which contains the drug which acts as an osmotic driving force for

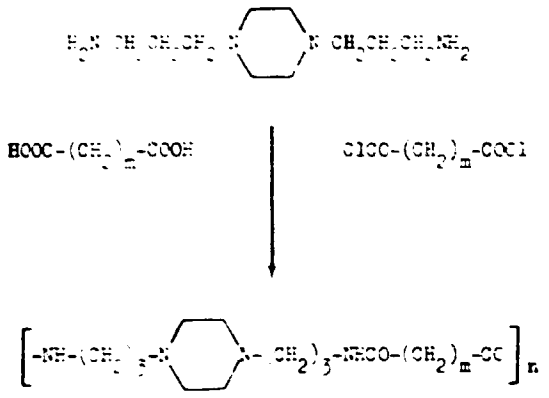


FIGURE 12. Structure of a polyamide polymer which responds to the pH of its environment (i.e., swells at about pH 4 while remaining unswollen at pH 7).

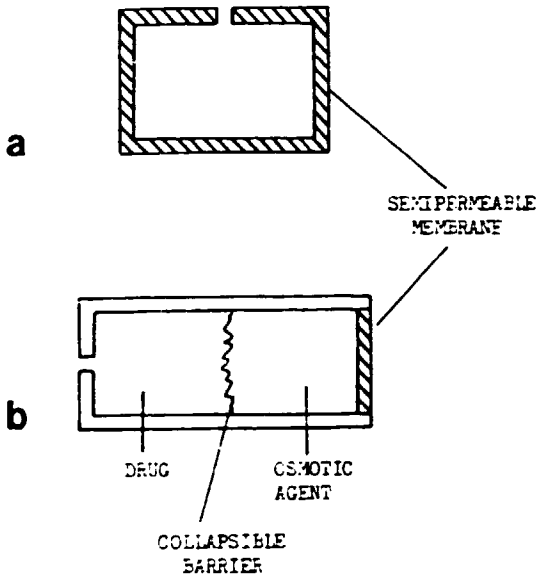


FIGURE 13. Schematic representation of osmotic pumps. (a) Elementary type; (b) pump with collapsible membrane separating drug compartment from osmotic agent.

water transport. The ingress of water is balanced by a saturated solution of drug being pumped out through the orifice. More sophisticated variations are available[112,113] (e.g., Figure 13b) in which the drug compartment is separated, by a movable barrier, from another compartment which contains an osmotic agent.

In principle there is no reason why any of the above devices cannot be utilized for use as inserts or implants in addition to their use orally. There has been a considerable amount of work done on devices for the controlled administration of drugs which come under a heading of bioengineering and are beyond the scope of this chapter. One should be mentioned, however, in the context of hydrogels as it is a commercial and very useful research tool for the study of the pharmacology and pharmacokinetics of drugs.[114] This is the Alzet® Osmotic Minipump. The implantable Alzet® device provides a typical delivery rate of 1 $\mu\ell$/hr for 1 week and is small enough for implantation in animals as tiny as mice. Before implantation the pump is primed by injecting the desired drug solution into the orifice. Several water-soluble drugs have been investigated using these devices, and poorly water-soluble steroids have been delivered using polyethylene glycol as solvent.[115]

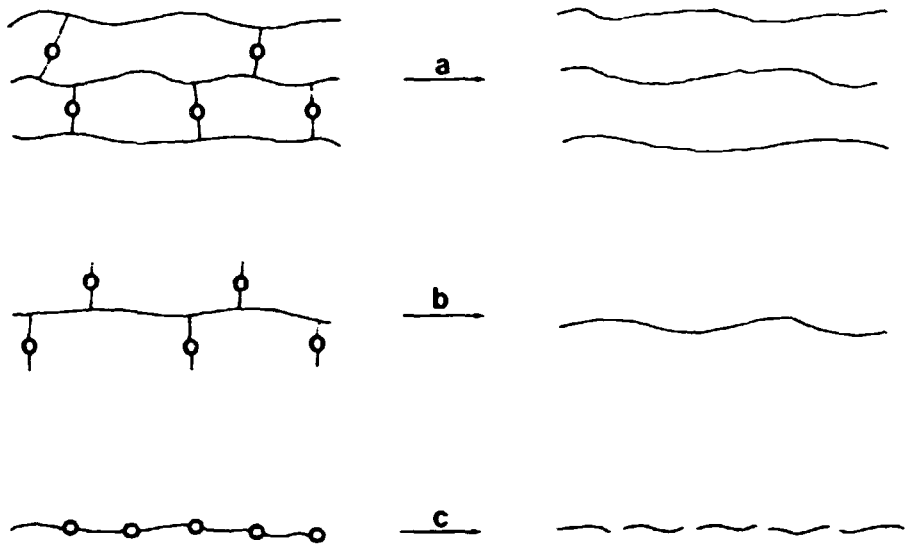


FIGURE 14. Schematic representation of three basic types of bioerodible polymers where -○- denotes a hydrolyzable linkage (see text for description).

VII. BIODEGRADABLE AND BIOERODIBLE SYSTEMS

The use of biodegradable polymers in drug delivery systems[116] allows the implantation of the device without the need for its removal after the drug has been released. The terms biodegradable, bioadsorbable, and bioerodible are frequently used interchangeably. The term bioabsorbable has long been associated with surgical sutures which gradually dissolve in the body. Bioerodible polymers may erode by slow dissolution as a result of side-group hydrolysis or ionization while the polymer backbone remains undegraded — frequently the erosion occurs as a surface phenomenon. Biodegradable is a more general term which is applied to many different materials other than polymers[117] including drugs,[118] surfactants, insecticides, and synthetic compounds in general.[119,120]

Three basic classes of polymer are generally recognized in biodegradable systems (Figure 14):

1. Water-soluble polymers which are insolubilized by hydrolytically unstable cross-links
2. Linear polymers which are initially water insoluble which become solubilized by hydrolysis, ionization, or protonation of pendant groups, but which do not undergo backbone cleavage
3. Polymers which are water soluble and degrade to small soluble products by backbone cleavage

In general, biodegradation takes place by a hydrolysis reaction when the device is placed in the aqueous environment of the body. In some cases enzymes have been found to accelerate the degradation reaction considerably.[121,122] Perhaps the most important consideration with biodegradable implants is whether the degradation products are nontoxic and readily eliminated from the body. Standards have been proposed to allow a comparison between the cytotoxicity of novel biodegradable polymers and established ones.[123] Polymers which undergo erosion of the type (2) above would probably not be suitable for implantation because a high molecular weight polymer is not readily excreted from the body. However, this can be put to advantage by using surface eroding polymers as inserts, as opposed to implants, the degradation products of which would be less liable to be absorbed due to their high molecular weight. Heller and Trescony[124] have used partial esters of maleic anhydride copolymers to prepare devices which release drug by dissolution of the polymer. The rate

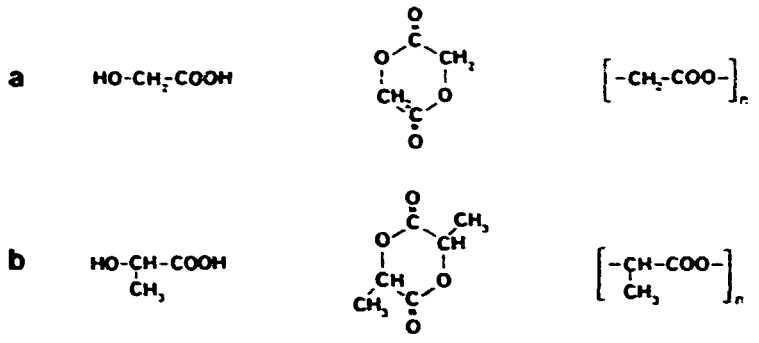


FIGURE 15. Structures of (a) glycolic acid (b) lactic acid, their cyclic dimers (glycolide and lactide), and homopolymers.

of surface erosion is controlled by the particular ester group and the pH of the environment. In a development of this system, the polymeric devices were coated with a hydrogel containing urease. The presence of urea resulted in the production of ammonium ions which accelerated the erosion, and the drug release, by a reversible mechanism. The Alza Corporation has patented an intrauterine device which bioerodes, and thereby releases drug, in the uterus.[125] A particular advantage of this type of device is that its shape can be designed for maximum retention in the uterus.

Polymers which undergo surface erosion are, almost by definition, hydrophobic while containing readily hydrolyzable groups. Workers at Alza have developed several novel polymers of this type which also undergo backbone cleavage to low molecular weight molecules which would therefore be of potential application in biodegradable implants.[126-128] The polymers have been given the general name Chronomer® and include hydrophobic poly(orthoesters) and poly(orthocarbonates). Heller,[129] one of the inventors, reviewed bioerodible drug delivery systems from a general point of view and included these as a possible group of polymers which could be used as implants. Choi,[130] another of the inventors, has described the development of these polymers for use in biodegradable systems. The Alza bioerodible polymers have been studied for controlled release of narcotic antagonists for the treatment of narcotic addiction[131] and of steroidal contraceptives for fertility control.[132]

Studies with polymers which undergo backbone cleavage have been restricted mainly to copolymers of lactic and glycolic acid[133,134] (Figure 15). Biodegradable drug delivery systems based on these polymers have shown the potential of this form of administration and have been reviewed by Wise et al.[135] The four main areas of application have used steroidal contraceptives,[25,136,137] narcotic antagonists,[1,138-140] antimalarials,[3,141,142] and anticancer drugs.[143,144] The forms in which these drug-polymer composites have been studied in vivo and in vitro include implantable cylinders,[136] spheres,[141] and films[139] and injectable microcapsules[137] and powdered formulations.[139] The polymers are generally prepared from the cyclic dimers of lactic and glycolic acid (i.e., lactide and glycolide) (Figure 15). Lactic acid has an asymmetric carbon atom, and therefore exists as two optical isomers. The different isomers and the racemic mixture produce polymers with significantly different properties. Poly(L-lactic acid), having steroregular sequences in the chain, has about 37% crystallinity while poly(DL-lactic acid) is totally amorphous.

Water uptake by a series of copolymers of L-lactic and glycolic acid has been studied recently by Gilding and Reed.[145] Poly(L-lactic acid) is the most hydrophobic and crystalline of the series and has the lowest equilibrium water content. With increasing the glycolic acid content, the degree of crystallinity and hydrophobicity decrease resulting in an increase in the equilibrium water content. A maximum of about 30% water uptake is found with the 30:70 copolymer (lactide to glycolide), thereafter with the onset of crystallinity the water content decreases.

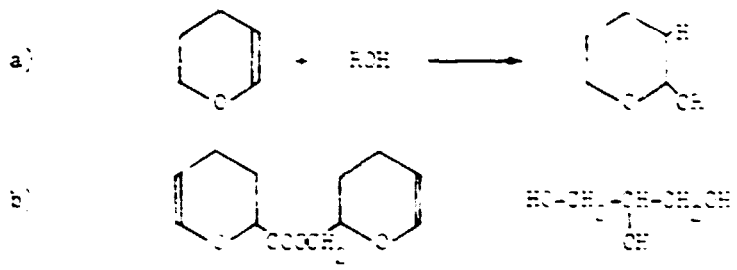


FIGURE 16. Biodegradable polymers based on dihydropyrans. (a) Reaction of dihydropyran with an alcohol; (b) typical monomers used to prepare cross-linked biodegradable polymers for drug delivery systems.

The presence of water in this type of polymer allows degradation to occur in the bulk amorphous region of the polymer matrix. Moiseev et al.[146] have considered the role of water, trace elements, and enzymes in the macrokinetics of polymer degradation in general and discussed the hydrolysis of poly(glycolic acid) in some detail. While the degradation of these polymers in the bulk complicates the prediction of the release profiles, it can have the fortuitous advantage of compensating for the decrease in release rate found with cylindrical or spherical devices which undergo surface erosion. Thus, a crudely constant release of ^{14}C-norgestrel from cylinders of poly(lactic/glycolic acid), in the rat, has been followed for 2 years.[136] Attempts to correlate release with the molecular weight of the polymer have shown that these systems can be difficult to design. Woodland et al.[138] found no significant differences in the release rate of cyclazocine from composites prepared from poly(lactic acid) with molecular weight of 45,000 and 70,000. However, when poly(lactic acid) with molecular weight of about 150,000 and 450,000 was used to prepare implantable beads containing sulfadiazine, the amount of drug released after 90 days was about 40% from beads prepared from the higher molecular weight material compared to 80% with the lower weight material.[141] Further, small changes in the structure of the drug molecule may have profound effects on release rates. The in vivo release from particles of poly(lactic acid) of naltrexone, cyclazocine, and naloxone after 60 days was 68, 38, and 26%, respectively, while in vitro release studies showed considerably faster rates.[139]

Sterilization of poly(lactic/glycolic acid) copolymers by irradiation is known to affect their properties and is found to alter the molecular weight distribution.[145] Ethylene oxide sterilization has been used prior to in vivo studies with these devices.[147] Closely related polyesters may offer modified properties and poly(dioxanone) has been evaluated as a bioabsorbable suture material which can be sterilized by irradiation without significant loss of physical properties.[148] Poly(caprolactone) has recently been studied as a potential biodegradable polymer for controlled release of contraceptive steroids.[2,10]

Graham et al.[149] have developed a series of biodegradable polymers which possess both ester and glycosidic linkages and can be prepared using the well-known reaction of 3,4-dihydro-2H-pyran with alcohols to give a tetrahydropyranyl ether (Figure 16a). Monomers which contain two such dihydropyran groups, linked by an ester group, can form linear polymers with diols or can be used to prepare cross-linked matrices when multifunctional alcohols are used as comonomers[4] (Figure 16b). Drugs can be incorporated into the reaction mixture prior to molding and curing, and the resulting thermosetting polymers provide release over prolonged periods of time. Such formulations have been found to release ^{14}C-labeled norethisterone in baboons for over 10 months. Related systems prepared by mixing preformed polymer with the antimalarial drug pyrimethanime have protected mice against *Plasmodium berghei* infection for at least 3 months. The range of possible monomers in addition to formulation variables has shown that devices have the potential to release drug over periods ranging from a few hours to in excess of 1 year.[149]

The identification of suitable biodegradable polymers for drug delivery systems has been relatively difficult, due to the number of properties required of the material for this application, and it is perhaps not surprising that polymers which have already been studied, and gained some acceptance, in other biomedical applications are attractive candidates. One such class is the poly(amino acids) and their derivatives which can be prepared with a wide range of properties and have been studied in several different disciplines. The in vivo degradation rate of copolymers of L-leucine and L-aspartic acid has been found to be dependent upon the degree of hydrophilicity of the polymer.[150] The resorption of sutures prepared from poly(glutamic acid) is delayed by partial esterification with lower alcohols,[151] while copolymers of glutamic acid and leucine form a biodegradable matrix which is well tolerated in rats.[152] Several studies have shown the potential of poly(glutamic acid) as a drug carrier in cancer chemotherapy where *p*-phenylene mustard[153] and cyclophosphamide[154] have been bound to the polymer.

Another class of biomedical polymer with potential use in biodegradable drug delivery systems is the poly (alkyl 2-cyanoacrylates). The alkyl 2-cyanoacrylate monomers have been used as biodegradable tissue adhesives in a variety of applications.[155] The butyl monomer is regarded as the most suitable due to a combination of its spreadability on biological fluids, rate of polymerization,[156] and its low toxicity.[157] The ability of the monomers to polymerize in the presence of water was exploited by Florence et al. to prepare microcapsules containing aqueous solutions of protein by *in situ* interfacial polymerization of butyl 2-cyanoacrylate in water-in-oil emulsions.[158] Microcapsules containing enzymes and other proteins have a variety of potential biomedical applications as demonstrated in many studies by Chang[159] and by other groups.[160] The possibility of using biodegradable polymers as the membranes of these "artificial cells" may encourage further study of these systems which have shown potential in therapy of substrate-dependent tumors and enzyme-deficiency diseases when nondegradable polymers have been used. Couvreur et al.[161,162] have prepared nanoparticles (200-nm diameter) from methyl and ethyl 2-cyanoacrylate monomers and studied the adsorption of several antineoplastic drugs. By virtue of their size, structure, and drug sorptive properties these nanoparticles have considerable potential as biodegradable lysosomotropic carriers and their tissue distribution, after i.v. injection in the rat, has shown their targeting potential.[163]

VIII. ROUTES OF ADMINISTRATION AND TARGETING

As mentioned previously, polymeric inserts and implants can provide both prolonged release and a localized release of a drug at a particular site. With inserts the localized action can be obtained by placing the device in the vicinity of the receptor organ; thus a direct action on, for example, the eye or uterus is possible. Recently the Alza Corporation has described ocular inserts which can direct the release of drug to a particular region of the eye.[164] Other body cavities may favor a sustained release for systemic action. These more traditional routes, particularly the GI tract, are associated with a shorter duration of action with a time scale of hours, and a zero order release may not be optimal if the drug is absorbed from a particular region of the GI tract.[165] The vaginal and rectal routes may also have limited potential as sites for sustained release of certain drugs which are absorbed into the systemic circulation.[166,167] The use of transdermal delivery systems is rather new because the skin has traditionally been viewed as a barrier, but development in this area is probable. Synthetic polymers are also being investigated as wound dressings which deliver topical antimicrobial drugs.[168]

Traditionally, implants and depot injections have been designed to provide a prolonged release with a time scale of days, weeks, or even months. Considerable effort is being made in this area to extend the duration of action and to improve reproducibility of release. Implants

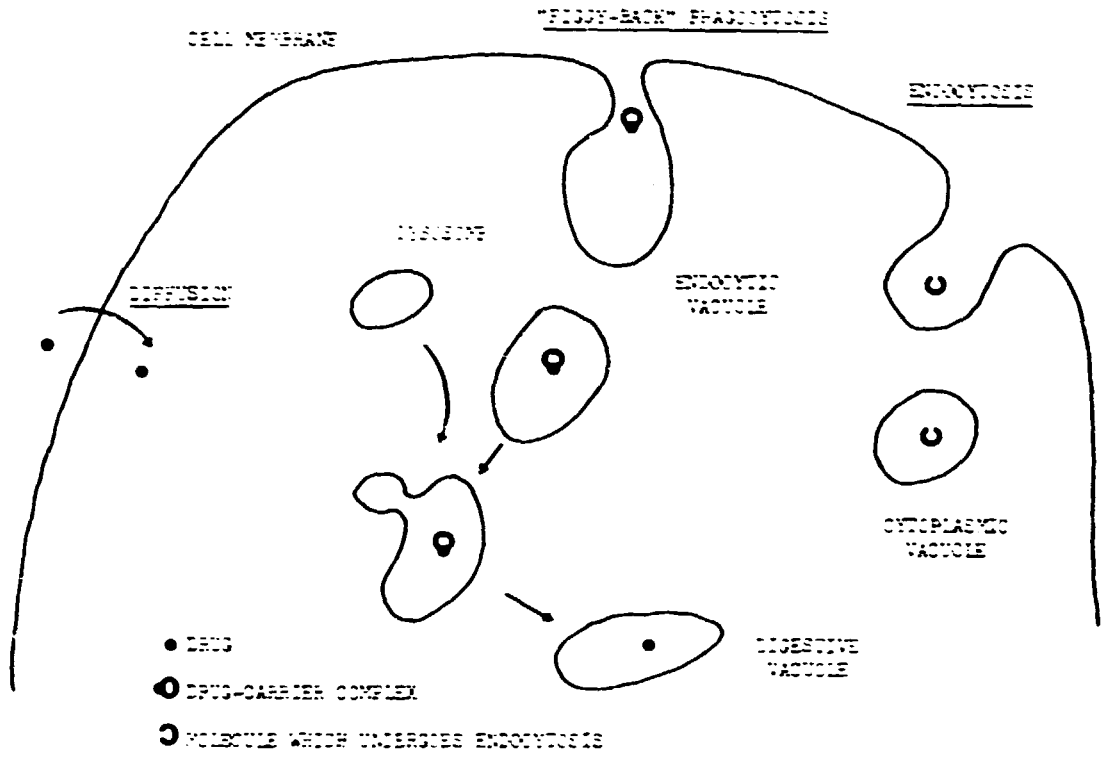


FIGURE 17. Diagramatic representation of entry of biologically active molecule into phagocytic cell.

also have some potential to localize action, as for example, the implantation of a device inside bone cavities for the treatment of chronic bone infections as described previously.[94]

More recently there has been considerable interest in the use of drug carriers in targeting therapy.[169] Many experimental approaches have been investigated particularly by the use of liposomes[170,171] and polymers, both natural and synthetic, as carriers. In many systems the targeting is accomplished by the selective uptake of the drug-carrier system by phagocytic cells following injection (Figure 17).

The macrophage[172] is a type of cell which is present throughout the body, in some cases as specialized cells (e.g., Kupffer cells of the liver). Characteristically, macrophages have the ability to take up large amounts of particulate matter of relatively large size (e.g., bacteria and protozoa) and have a highly developed ability to discriminate between particles of different types. These cells ingest solid or fluid matter by interiorizing part of their plasma membrane.[173,174] Where the material ingested is a particulate solid, the process is termed phagocytosis. The term pinocytosis is used where liquid droplets are engulfed, and the general term endocytosis encompases both cases. "Piggy-back" phagocytosis describes the way in which a substance, not normally ingested, gains entry to the cell by being phagocytosed along with a particle which is selectively taken up by the macrophage.[175] There is considerable interest in this type of approach for cancer chemotherapy and antimicrobial therapy for diseases in which the causative organism is localized within fixed macrophages.

Many attempts have been made to bind drugs and antibodies to a polymeric carrier which ideally will bind with, for example, tumor cells and subsequently release the active drug molecule. Poly(glutamic acid) was used as a biodegradable carrier for *p*-phenylene mustard and an antibody and the conjugate was found to suppress tumor growth in mice.[153] Dextran has been used as a carrier of daunomycin.[18] Unfortunately, a high degree of specificity for the target cells has not been achieved in most studies and systemic side effects still occur. In an attempt to avoid the use of biochemical differences between the target cell and normal cells to obtain specificity, the use of magnetic microspheres has been studied.[176,177] In this

method magnetic particles are incorporated into the drug-carrier system and by the application of a magnetic field can be localized at a particular site in the body.

IX. POLYMER-TISSUE INTERACTIONS AND BIOLOGICALLY ACTIVE POLYMERS

The possible interactions between implanted polymers (such as drug delivery systems) and body tissues are manifold, and over recent years a tremendous amount of research interest has revealed the complexities and provided fundamental knowledge. The complexities of these potential interactions are readily appreciated if one considers that living tissues consist of a wide variety of soluble low and high molecular weight compounds and insoluble structural components in dynamic equilibrium. Similarly, drug delivery systems can have present both low and high molecular weight material which is either soluble or insoluble. At present, little detailed information is available on the effects that these interactions may have on the release of drugs from delivery systems. "Medical grade" polymers and the control of additives have removed most of the problems associated with the leaching of toxic low molecular weight compounds. However, the uptake of lipid-soluble compounds from the body into poly(dimethylsiloxane) devices has been reported.[178]

Previously it was thought that "inert" polymers could be implanted without problem and bland or minimal responses with a wide range of polymers have been reported. However, the physical presence of an implanted polymer can cause foreign body tumorigenesis[179] and the shape and size of polymeric implants can produce different tissue responses.[180] Microporous hydrogels are encapsulated while the same polymer with a macroporous structure allows ingrowth of capillaries[181] or calcification.[182]

The adsorption of plasma proteins onto synthetic surfaces has been, and continues to be, the subject of a tremendous amount of research. The adsorption behavior of the particular proteins is dependent upon the hydrophilic/hydrophobic character of the surface. This field, in which the material in question is generally insoluble in the body fluids, still requires to be reviewed in terms of the "current understanding"[183] and our understanding of the interactions with soluble polymers is perhaps less clear.

Soluble polyelectrolytes of diverse nature have been found to exhibit biological activity. Synthetic heparinoid polymers have been designed and studied,[184] but many polymers with no apparent resemblance to natural macromolecules have been found to possess significant activity. The Pyran copolymer (divinyl ether-maleic anhydride copolymer, DIVEMA) has been the subject of many studies,[20,185] while its detailed structure is still uncertain.[186] DIVEMA has been found to exhibit antitumor, antiviral, antibacterial, antifungal, anticoagulant, and antiarthritic activity in addition to being an inducer of interferon and a macrophage activator. Both the biological activity and the toxicity of the material are related to its molecular weight. DIVEMA is by no means unique and other anionic polyelectrolytes display some biological activity. The antiviral activity of poly(acrylic acid) is related both to its molecular weight and tacticity.[187] However, atactic poly(methacrylic acid) has been reported to be devoid of antiviral action while the activity of the isotactic form is just detectable.

Even simple neutral water-soluble polymers can interact with living systems to a significant degree. Block copolymers of poly(oxyethylene)-poly(oxypropylene) (e.g., Pluronic®-F68) have been used in priming solutions for cardiopulmonary bypass and several advantages were found including an antisludging effect and a decrease in hemolysis.[188] However, poly(ethylene glycol) has been found to cause cell fusion, the mechanism of action being believed to be related to the reduction of the surface potential of the cells when the polymer adsorbed onto their surfaces.[189] Another study demonstrated that linking poly(ethylene glycol) to the enzyme catalase produced a conjugate which was nonimmunogenic, while experimental animals remained immune competent to the unmodified enzyme.[190] Poly(ethylene glycol)

has also been used to study intestinal permeability in man because it is presumed to be nontoxic and nondegradable.[141]

Nevertheless, implanted polymeric prosthetic devices and controlled release implants and inserts have now been safely used in humans for many years. Increasingly, as the factors involved at the implant-tissue site are studied and the fact that even "inert" polymers have physicochemical properties, the trend is to design systems which interact with the living environment in a desired manner. With insight and imagination the complexities are becoming better understood. The current increase in cost of getting new drug entities to the market will guarantee that significant developments of controlled release systems will improve drug therapy for the treatment and prophylaxis of disease.

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PHARMACEUTICALS

Controlled Drug Delivery Systems

NEIL B GRAHAM

Drug delivery systems can play a significant part in the therapeutic efficacy of the agent being formulated. Professor Graham looks at the past developments which led to the delivery profiles currently in commercial use. He also looks to the future of biodegradable implants, and targeted, responsive and pulsed drug delivery systems. New therapies may result from either a new or an older drug in a precise programmed delivery form: the field is challenging, socially beneficial, and potentially very profitable.

The past half century has seen the growth of the pharmaceutical industry based on new chemical entities which have done so much to improve health. From simple analgesics such as aspirin, through antibiotics like the penicillins and cephalosporins, and on to the myriad of agents to treat every condition from head to toe, the past 50 years have been a credit to the skills of the synthetic organic chemists and microbiologists, who have produced this large variety of chemical entities by either synthetic or fermentation methods. The recipients of these powerful products have taken them in various forms, such as tablets, liquids, capsules and other less common units. These delivery systems can play a significant part in the therapeutic efficacy of the agent being formulated.

The therapeutic agent is a combination of drug and delivery system

The therapeutic agent is thus a combination of a drug and a delivery system and it is almost impossible to separate the two. Initially, delivery systems were for instant delivery of the agent and as such were to some extent taken for granted. The problems of instant delivery were well known. The peaks and troughs of plasma levels lead to excessive concentrations and undesirable side effects, and to inadequate concentrations which provide insufficient cover for the condition being treated (see Fig 1). This led to the concept of a therapeutic index,[1] defining the ratio of plasma levels of agent which provides effective therapy to that providing freedom from undesirable reactions in 50 per cent of the test population.

The introduction of a time dimension into controlled drug delivery leads to the idea that the active agent, the drug, should be released at specified rates over defined time periods. The precise control of the release in such a programmed manner can lead not only to more convenient dosage forms but also to an improved therapy, though it must be clearly understood that it is only the first of a number of steps towards obtaining the appropriate plasma levels. This review will provide a selection of past developments leading to the delivery profiles in commercial use today and, with a knowledge of some of the ideas being currently evaluated, try to project forward into the future.

Polymers in controlled delivery

Polymers can be made in an enormous range of compositions which can exist in three major states: a crystalline solid; an amorphous hard glass; or an amorphous rubber which can be, if desired, plasticised by a suitable lower molecular weight liquid. The diffusion coefficients for a given diffusate will normally increase as one passes from a glass to a rubber to a plasticised rubber and as the free volume also increases. The free volume may be thought of as the volume fraction of molecular size holes which must be available for diffusion to occur. The change in the diffusion coefficient in going from a glass to a solvent-swollen polymer can be as much as ten decades (billion), depending on the molecular weight of the diffusate.

The general form of the relationship between the logarithm of the diffusion coefficient and that of the molecular weight of the diffusate is given in Figure 2. In general there is a need to provide more data on the prediction of the diffusion coefficients for high molecular weight diffusates, as the delivery of high molecular weight peptides and proteins arising from the biotechnology revolution is becoming of considerable importance. This will be referred to later. However, as the diffusion coefficient clearly depends on the polymer selected for use, the ready availability of such a wide variety of these materials clearly provides a very versatile tool for the design and construction of controlled delivery systems.

The equation governing normal Fickian diffusion from an infinite flat slab configuration of material for the first 60 per cent of the release is given by:

$$M_t/M_0 = -4[Dt/\pi l^2]^{1/2}$$

where M_t is the change in the amount of the diffusate at time 't'. M_0 is the amount of diffusate present at time zero. D is the diffusion coefficient and 'l' is the thickness of the slab. π has its normal meaning.

It can be readily seen that the variation of the thickness provides a further control factor which can be used. Rate controlling films can be from a few microns to millimetres, thus providing a further four decades of possible time control. By means of these two simple but powerful parameters the resistance to diffusion can, in principle, be varied over a range of some 14 decades. As polymers can be synthesised and fabricated relatively readily they are very useful for accessing this potential.

The earliest use of this principle was in the production of granules coated with wax or shellac, in which the coating provided some considerable prolongation in the release of agents such as antihistamines and decongestants. The 'tiny time pills' of Smith Kline and French were born in the 1950s and a major new treatment for hayfever, sinus problems and colds opened up what is still a major market today. Microencapsulation for obtaining sustained delivery of up to 24 hours is being used on an increasing scale for many drugs even though it is now quite old technology.

Chemists and chemical engineers

The principles of mass transport and diffusion are the familiar domain of chemists and chemical engineers, so it is perhaps not surprising that the field of controlled delivery has been increasingly invaded by scientists with these skills. Pharmacists tended to utilise techniques and materials which have provided well proven safety and reproducibility. This is highly commendable from the points of view of quality, safety and efficacy, but was a somewhat inward looking approach from which radical new departures were perhaps less likely. Today many of the major innovations in controlled and programmed delivery are being generated by non-pharmacists.

The Alza Corporation (Palo Alto, California) took the simple principle of using thin polymer layers as precise rate controlling membranes and developed a number of elegant delivery systems. These were for the sustained and constant delivery of very active agents which needed guaranteed and precise control for microgram to milligram quantities per day. Thus the *Ocusert* for the treatment of glaucoma, delivering pilocarpine from a small device inserted in the conjunctival sac of the eye, was very elegant but not commercially very successful.[2] It is shown diagrammatically in Figure 3. The *Progestasert*, delivering milligram quantities per day of progesterone from an interuterine contraceptive device (IUD), was very effective for periods of years providing reduced bleeding when compared with a non-medicated IUD.[3]

Transdermal delivery, utilising a rate controlling membrane in the device to overcome the variability of human skin, initiated a market which rapidly grew into hundreds of millions of dollars. The first devices were developed for astronauts who became sick in space. The precise delivery of scopolamine via a little patch placed behind the ear made a significant contribution to the relief of this problem and has resulted in the product *Transderm-Scop* for the relief of travel sickness.[4] Then fol-

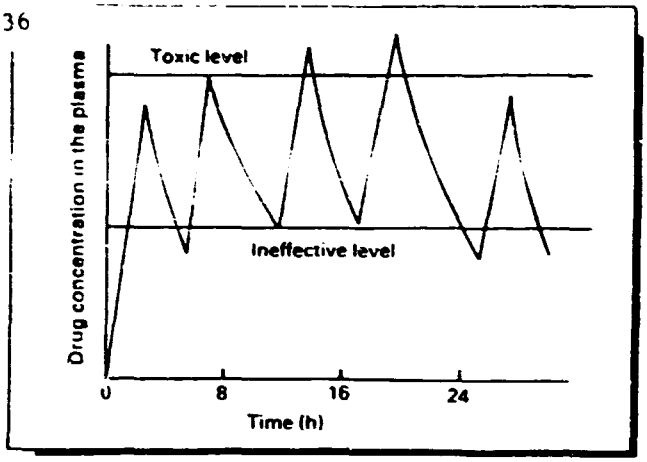


Fig 1 The fluctuations in plasma levels which can result from taking instant delivery pills four times daily

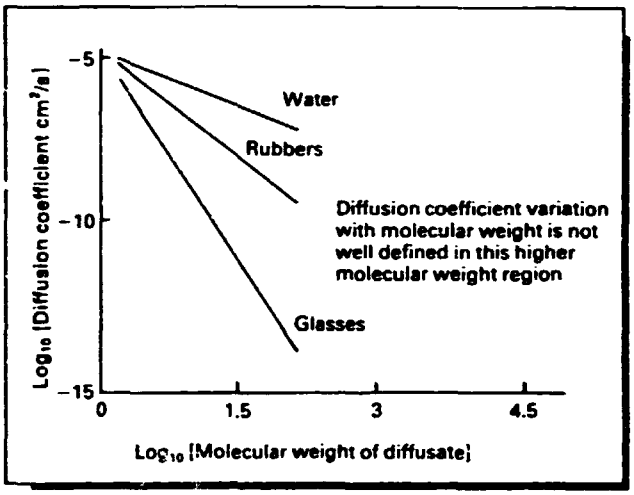


Fig 2 The change in $\log_{10}$ [diffusion coefficient] with $\log_{10}$ [molecular weight of diffusate] in water, rubbers and glasses

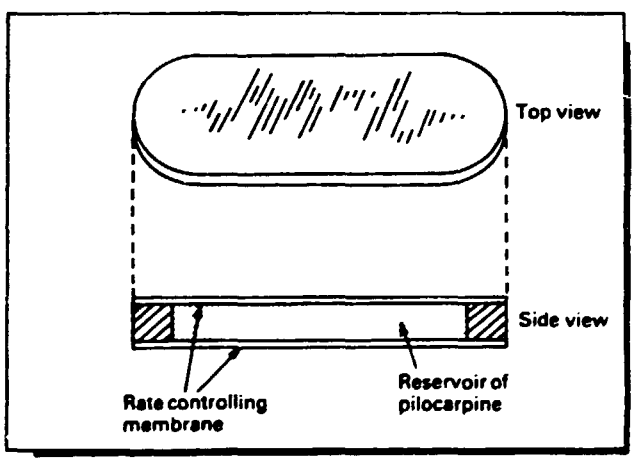


Fig 3 The Ocusert *ocular sustained delivery device for pilocarpine*

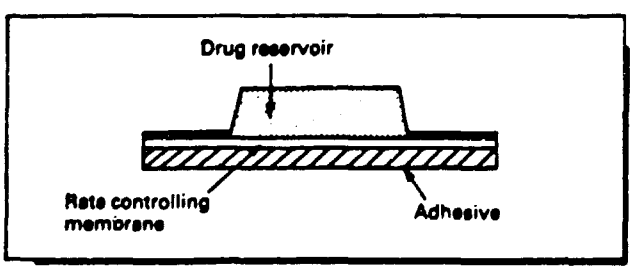
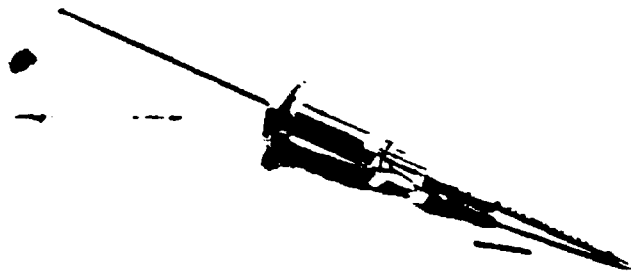


Fig 4 A transdermal delivery system to be placed on the skin for delivery of the agent over several days

lowed a scramble for the market for the transdermal delivery of glyceryl trinitrate from a variety of patches attached to the skin. A number of other therapeutic agents, including clonidine, have been delivered from these patches. A recent example is delivery of estradiol and/or progesterone for post-menopausal hormone replacement therapy. It is not by choice that these systems are utilised only for agents active in very small quantities, for the permeability of the skin is so small that only very low fluxes of relatively low molecular weight compounds are possible and the agents able to be delivered in this manner are severely restricted. The design of a typical skin patch is shown in Figure 4.



ICI's Zoladex: *fastest-growing product in its class*

Alza developed an ingenious and novel approach to obtaining constant drug delivery from a tablet by the use of a semi-permeable membrane coating on a tablet of water-soluble drug with an agent which would develop an osmotic pressure. When immersed in water or aqueous fluid the semi-permeable membrane would allow water to be imbibed and the resulting solution developed an osmotic pressure within the tablet. By incorporating a tiny drilled or laser-punched hole in the semi-permeable membrane the internal pressure caused a constant flow of the contained solution to be squirted out of the hole. So long as the inflow through the semi-permeable membrane was greater than, or equal to, the outflow through the hole a pressure would be maintained and a constant flow of drug solution produced. This device, in its simple form, has been licensed for use with a number of active agents. A recent example is *Volmax*, a Glaxo product for the sustained release of salbutamol sulphate for treating asthma. A more sophisticated two compartment system has been developed for use with relatively water-insoluble drugs. Utilising this system Pfizer has developed and obtained a product license for the sustained oral delivery of *Nifedipine*, an insoluble calcium channel blocker for the treatment of hypertension. The construction of both the simple and more complex osmotic devices are shown in Figure 5.

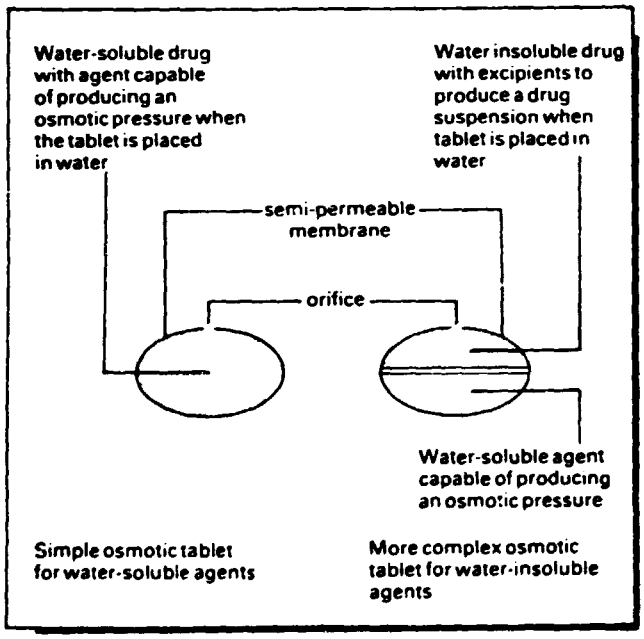


***Fig 5** Osmotic tablets for drugs of different water solubilities*

Most polymeric membranes are hydrophobic and water-soluble drugs often have a very low solubility in the membrane thus providing an extremely low flux. It is possible to crosslink water-soluble polymers and obtain a product membrane which can be highly swollen with water and in which the water-soluble drugs will have a much higher solubility and will permit a potentially high flux. These materials are called hydrogels in the water swollen state and xerogels in the dry state.[5-8]

Hydrogels

Common hydrogels in use for biomedical and pharmaceutical applications include those based on poly(hydroxyethylmethacrylate), poly(vinylpyrrolidone), poly(vinylalcohol), poly(ethyleneoxide) and dextrans. Many natural hydrogels such as gelatin, cellulosic derivatives, alginates and others are also commonly encountered.

Among these hydrogels those based on poly(ethyleneoxide) have several unique features which make them an attractive group of materials. They are particularly biocompatible and they can be partially crystalline. They appear not to be recognised by the normal body defence mechanisms so that they can disguise and protect such agents as enzymes circulating in the blood,[9] or they can provide particularly blood-compatible materials which not only show a low level of non-thrombogenicity but also a very low level of activation of complement.[10] Water-swollen hydrogels can provide fluxes of as high as grams per day of water-soluble agents and they thus are in a different class than other continuous polymeric membranes.

A major feature discovered in the study of hydrogels was that the use of a xerogel impregnated with a drug could provide practically constant release for a significant fraction of the contained drug. This was contrary to the expectations of normal Fickian diffusion and is in fact what is referred to as Case II diffusion. By use of this discovery it is possible to formulate

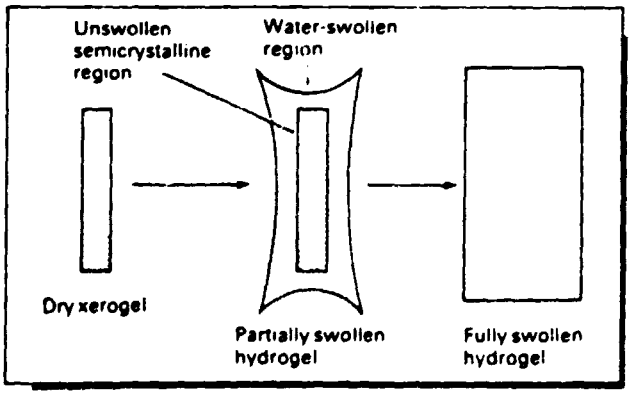


***Fig 6** The swelling of a semi-crystalline xerogel of poly(ethyleneoxide) in water*

very safe devices for, unlike the membrane coating technique, there is not a core reservoir of drug to rupture and potentially cause some untoward incident by the sudden drug dumping. The drugs to be used in single membrane devices should not be life threatening if the total dose is dumped. In a monolithic hydrogel device it is not possible to dump the dose even if the device is cut up into pieces! The added feature of controllable crystallinity in the hydrogels based on poly(ethyleneoxide) provides a toughening mechanism for the materials which aids their fabrication into devices but also gives an extra measure of control over the release profile.[11]

The problem encountered with the use of monoliths, that of obtaining reasonably constant rates of release, has been largely solved by utilising the dried down xerogels. The precise release pattern cannot yet be analysed and predicted mathematically, as the solution involves a complex set of moving boundaries and changing concentrations and dimensions. The mechanism of obtaining the pseudo-constant rates of release can however be readily understood by a simple qualitative explanation.

A xerogel slice of a crosslinked but partially crystalline poly(ethylene oxide) swells on the exterior but remains dry in the interior for a long time. The exact length of time is determined by the thickness of the slice. During swelling the slice takes up a configuration as shown in Figure 6. In this the exterior is swollen to some degree less than the equilibrium value

Transdermal delivery - a space age spin-off

and the degree of swelling decreases as the core is approached. The total swelling is slowed by the crystallinity of the core which physically constrains the swelling until all of the crystallites have finally been melted by interaction with water. The result, insofar as the release of a contained drug is concerned, is to flatten the release profile. The release pattern which would pertain without the swelling would be a rapidly decreasing rate with progressing time. The swelling of the exterior however provides a steadily increasing diffusion coefficient which counteracts this decreasing rate. If an appropriate balance can be struck an effectively constant rate can be obtained up to the point of complete swelling, usually to about half of the contained drug (see Fig 7).[12]

An example of the application of hydrogel technology is a

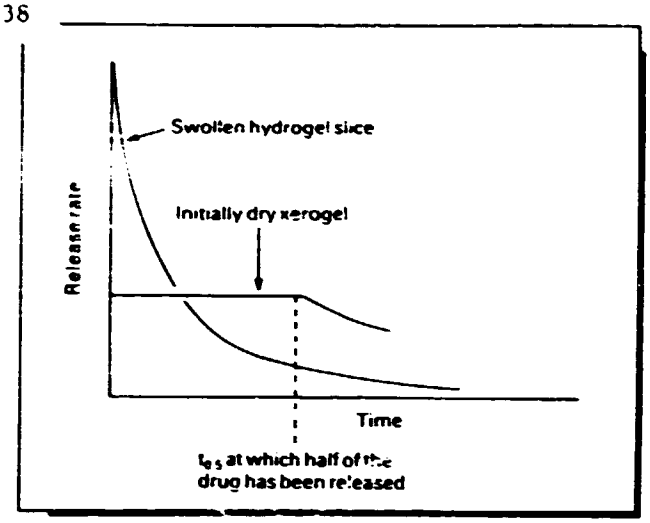


Fig 7 Comparison of typical drug release profiles from a fully-swollen hydrogel and a dried xerogel

delivery system for prostaglandin E_2 for the ripening of the cervix in women at full term in labour.[13,14] This product has now been granted a product licence in the UK and Ireland and is marketed under the name *Propess* by Roussell. The principle will be applied to other drugs to produce new therapies.

Biodegradable delivery systems

Simplistically it might be thought that an ideal delivery system would utilise a polymer which would be biodegraded by the body and, after presentation orally or by some other method such as implantation, would be absorbed harmlessly. Prolonged delivery and treatment over periods of weeks or months from a single implant might thus be envisaged without the necessity of removing the implant empty shell at some subsequent time. The problems of proving the toxicological safety of such novel materials is however very large and for the past decade this approach fell somewhat into disfavour with the opinion developing that the difficulties were too great. However with the advent of the sudden explosion in active agents from the combination of genetic engineering, biotechnology and peptide synthesis there has been a resurgence of interest in biodegradable matrices which were seen as a possible solution to the urgent need for delivery systems for these products. The product licenses awarded, for example, for delivery of a peptide analogue of luteinising hormone-releasing hormone under the

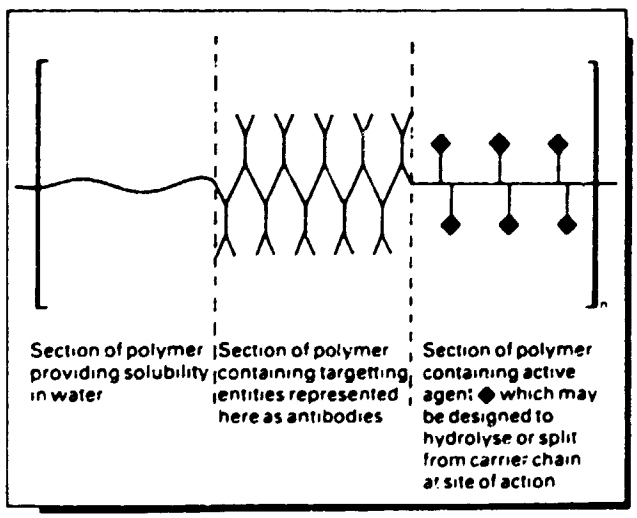


Fig 8 Proposed design of a polymer which is in principle capable of delivery and targeting a drug to a particular receptor site in the body

trade names *Zoladex* and *Decapeptyl – CR* provided a watershed. These products are indicated for the treatment of male prostatic cancer and the hormone is presented as an implant or microcapsules of a lactic/glycolic acid copolymer which releases the hormone over a month. *Zoladex* is said in ICI's 1986 annual report to be the fastest growing product in its class. There is now, as a result, a resurgence of interest in biodegradable delivery systems and other product licences will be granted in the future.

The future

As it can take from seven to 15 years for a new delivery system to progress from conception to sale, the major products to reach the marketplace over the next ten years must already be much more than gleams in an inventor's eye. The four novel areas which seem most promising are those involving biodegradable implants, targeted delivery, responsive delivery and pulsed delivery.

The concept of targeted delivery includes any technique which allows a drug to locate at a particular body site where it is needed for its physiological effect. When used in this manner a smaller quantity of drug can often be used to the same or greater therapeutic effect. The therapeutic index is increased and the potential side effects are reduced. The targeting can be, in its simplest form, by containing the drug in particles which are sieved by part of the circulatory system. This has been used as an approach to the treatment of tumours.[16] At the other extreme the targeting is in theory able to be attained by the attachment of moieties such as antibodies to a water-soluble polymer containing the covalently attached drug.[17] If the antibody is specific for a receptor on the particular cell to be treated, then the antibody-containing complex might well act as the designer 'magic bullet'. The design of such a molecule is shown in Figure 8. In the Figure the polymer is represented as a block copolymer for convenience. It could also be a random or graft copolymer.

'Responsive' or 'intelligent' polymers are already known. Thus membranes have been devised which will release insulin in response to the presence of glucose[18,19] and release naloxone in response to morphine.[3] These are as yet far from practical but represent a promising beginning for delivery systems which begin to mimic the responsive nature of the glands in the body.

Finally, pulsed delivery in which a dosage form delays the release of a contained active agent has already been demonstrated. Thus the possibilities of delivery during the sleeping period become possible and the developing knowledge of the requirements of chronobiology can begin to be addressed.

There is a major current activity in research and development in the field of controlled and programmed delivery, driven both by the desire to produce new and improved therapy, and by the potentially large financial rewards. The field is challenging in the extreme and most satisfying because of the clear benefits to all society should the efforts be blessed with success. There will be many such successes over the next decade and beyond.

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Professor Graham is at the Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL

TRANSDERMAL DRUG DELIVERY: PROBLEMS AND POSSIBILITIES

Authors: **Victoria M. Knepp**
Department of Pharmaceutical Chemistry
University of California at San Francisco
San Francisco, California

Jonathan Hadgraft
The Welsh School of Pharmacy
University of Wales Institute of Science
and Technology
Cardiff, Wales

Richard H. Guy
School of Pharmacy
University of California at San Francisco
San Francisco, California; and
Department of Dermatology
School of Medicine
University of California at San Francisco
San Francisco, California

Referee: Gary W. Cleary
Cygnus Research
Redwood City, California

I. INTRODUCTION

A primary function of human skin is to provide a barrier to the ingress of foreign compounds into the body.[1] It must be stated that, on the whole, given the enormous range of chemicals with which man comes into contact, the skin performs this role with considerable success. Thus, an initial reaction of surprise to the concept of transdermal drug delivery for systemic effect is not unreasonable. One might ask, "Why administer compounds across a membrane which has been designed rather specifically to inhibit such transport?" Some answers to this question are attempted in this review.

The concept of a small topically administered bandage containing, and capable of delivering, sufficient medicine for a day or longer is elegant and attractive. An alternative to oral administration is, in many cases and for large numbers of individuals, an important option that is infrequently available. The control of drug input, which is potentially available with transdermal delivery (Figure 1), is also alluring. The saw-tooth profile of drug concentration in the biophase vs. time, characteristic of conventional dosing regimens, can be damped by administration via the skin. A clear advantage for drugs of narrow therapeutic index is thereby implicated.[2]

Enthusiasm for topical drug input to elicit systemic effect must be tempered, however, by recognition of the fact that skin represents one of the most formidable barriers of biology. It is also a tissue which registers insult in a manner designed to discourage repeat events. Hence, transdermal delivery is presently limited to potent drugs which elicit no, or minimal, local irritating effects.[3] A few simple calculations serve as an initial guide to the feasibility of transdermal delivery for some representative "candidate" compounds. Assume, for the purpose of illustration, that a transdermal device exists which is capable of providing zero-

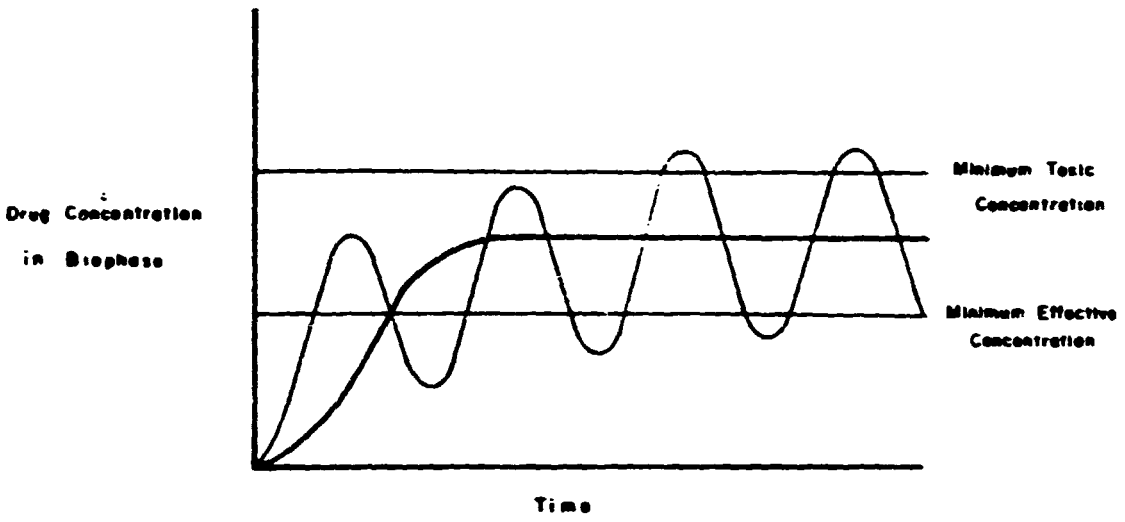

FIGURE 1. Schematic representations of drug levels in the biophase as a function of time following conventional (e.g., oral) multiple-dosing (oscillating line) and sustained (e.g., transdermal) drug delivery (horizontal line)

order delivery to the skin surface at a rate (k_o μg/cm²/hr) slightly less than the maximum flux (J_m μg/cm²/hr) of a model drug across the stratum corneum. Taking J_m = 35 μg/cm² hr.then a value of k_o = 25 μg/cm²/hr is appropriate.[4] If if the target plasma concentration of the drug is C_T μg/mℓ, it follows that Equation 1 must hold at steady state:

$$A \cdot k_o = Cl \cdot C_T \quad (1)$$

where A cm² is the area of the patch and Cl cm³/hr is the drug clearance. Setting k_o less than J_M is desirable to retain drug input control within the delivery system and to avoid variability associated with differential skin permeabilities within a patient population.[4] Given this constraint, Equation 1 contains only A as a manipulative parameter. In other words, if inherent skin permeability cannot be increased in some way, then the input function can be maneuvered only within the confines of $k_o < J_m$ and that A be "reasonable".

The limitations can be emphasized with reference to Table 1 in which an initial pharmacokinetic feasibility assessment of transdermal delivery is performed for an arbitrary selection of compounds. Clearances and target therapeutic plasma levels were obtained from the literature;[5] k_o was fixed at 25 μg/cm²/hr; and the value of A required by solution of Equation 1 was calculated for each drug. The results clearly indicate the nonfeasible candidates (aspirin, acetominophen, cimetidine, indomethacin) for which A is unacceptably large. In the case of aspirin, for example, the calculated A is approximately nine times that of a normal adult's skin surface area! For economic and practical reasons, a delivery system area of 50 cm² is a reasonable upper limit. The remaining drugs pass the initial screen, although it should be emphasized that the theophylline calculation is for pediatric purposes only.[6] For clonidine, digoxin, estradiol, and scopolamine, A is very small and a reduction in k_o is indicated so that a patch of manageable dimensions can be fabricated. This is exactly the strategy that has been followed in the development of the marketed clonidine, estradiol, and scopolamine systems (see below).

However, this simplistic approach leaves a number of questions unanswered. Is a percutaneous flux of 25 μg/cm²/hr possible for all compounds? Probably not, and some degree of input control may therefore need to be sacrificed. How long will be required for the attainment of C_T following application of the patch? The half-life of the drug can be of some use here, but if absorption through the skin is slow (the usual case), an unacceptably long approach to the target level may be apparent. Which of the "feasible" candidates will

Table 1
FEASIBILITY SCREEN FOR REPRESENTATIVE TRANSDERMAL DELIVERY "CANDIDATES"

Drug	Cl (cm³/hr)[a]	C^{ss} (μg/cm³)	A^{ss} (cm²)[b]
Acetaminophen	23.076	15	13.846
Aspirin	29.085	150	174.510
Cimetidine	48.510	1	1.940
Clonazepam	4.032	0.025	4.0
Clonidine	12.054	0.001	0.48
Digoxin	6.800	0.002	0.54
Estradiol	66.859	0.0001	0.27
Indomethacin	9.049	0.5	181
Isosorbide dinitrate	174.636	0.001	7.0
Nitroglycerin	4,213.440	0.0001	17
Propranolol	48.594	0.02	39
Scopolamine	43.330	0.0002	0.35
Theophylline	138	5	28

[a] Calculated for a 70 kg adult *except* in the case of theophylline, the assessment of which is for a 2.5 kg preterm infant.
[b] The total body surface area of a 70 kg, 1.83-m-tall adult is approximately 19,000 cm². The values in this column are calculated using Equation 1 with k_a = 25 μg/cm²/hr.

elicit a local irritating effect on the skin? There are no simple nor reliable methods to predict skin irritation from a compound's structure and properties unless information pertinent to a closely related homolog or analog is known.[7]

It should be clear, therefore, that transdermal drug delivery presents nontrivial challenges (independent of formulation itself) while offering certain unique opportunities. The goal of this review is to place these difficulties and attributes in perspective and to indicate strategies which can evaluate the likelihood of successful transdermal delivery for different drug moieties. The organization of the paper is as follows: (1) the advantages and disadvantages of transdermal drug delivery are enumerated; (2) the delivery systems developed for the transdermal route are described and compared; (3) pharmacokinetic and pharmacodynamic observations following transdermal delivery are reviewed for several drugs and the efficiency of the administration route is evaluated; (4) approaches to the selection of transdermal drugs are considered and a kinetic model for delivery and percutaneous absorption is reviewed; (5) the potential application of penetration enhancers to expand the range of possible transdermal drug candidates is discussed; and (6) conclusions and prognoses for the future are offered.

II. ADVANTAGES AND DISADVANTAGES OF TRANSDERMAL DRUG DELIVERY

Drug delivery via the skin to elicit systemic effect offers several advantages over more conventional methods of administration including the following:[3]

1. Steady-state drug concentrations within the therapeutic window can be maintained. The "peaks and valleys" associated with conventional multiple-dosing regimens are avoided (Figure 1). This precise control over plasma drug concentration enables the selectivity of drug action to be enhanced and decreases unwanted side effects.
2. Gastrointestinal tract variables such as erratic or incomplete absorption are circum-

vented. Hepatic first-pass metabolism, which can severely limit the systemic availability of a drug, is elimated.

3. An alternative route to oral administration is provided for those situations in which patient variables (e.g., geriatric or pediatric cases; nausea and vomiting symptoms) preclude conventional dosing.
4. A substitute parenteral form of therapy is possible without the inconvenience and anxiety associated with IV infusions, boluses, or IM injections.
5. Transdermal therapeutic systems are able to extend significantly the duration of action of many drugs, thereby reducing the frequency of drug dosing necessary with conventional dosage forms. This reduction may lead to enhanced patient compliance and, consequently, more effective therapy.
6. When medical needs demand, therapy can be terminated quickly and simply by removing the system.

These important attributes of transdermal drug delivery are counterbalanced by a number of major drawbacks, any one (or more) of which may be sufficient to preclude its use:

1. The skin is an excellent barrier to chemical penetration into the body.[1] The stratum corneum is a tough, resilient, hydrophobic membrane through which drug diffusion is slow. In order to gain systemic access, a transporting molecule must breach this layer and then partition into the much more aqueous in nature viable epidermis and dermis. Balanced physicochemical properties (i.e., reasonable solubility in oil and in water; moderate or low molecular weight; conservative lipid-water partitioning characteristics) are prerequisite for a successful penetrant, therefore.
2. As percutaneous absorption is slow, the drug must be pharmacologically potent because the concentration of active species in the biophase will be low. Currently, very few drugs whose effective plasma concentrations exceed 1 to 10 ng/mℓ are seriously considered for delivery transdermally.
3. In addition to the above pharmacokinetic limitation, there may exist a (potentially) more restrictive pharmacodynamic disadvantage. The excellent barrier nature of the skin means that continuous transdermal delivery will produce rather steady drug concentrations in the biophase. Such a situation is not necessarily optimal from a pharmacological point of view and may exacerbate the potential for tolerance development (see below).
4. Local, unwanted biological effects may also occur. The drug and/or contact system of the transdermal device must not elicit irritant or allergic reactions within the skin at the site of application. Cutaneous binding and metabolism remain, at this time, unknowns, the significance of which await clearer demarcation and quantitation.
5. Transdermal delivery systems are relatively expensive compared to conventional dosage forms. They may contain large amounts of drug, of which only a small percentage may be used during the application period.

III. TRANSDERMAL THERAPEUTIC SYSTEMS

The objective of a transdermal therapeutic system is to deliver drug into the body at a controlled, efficacious rate such that inter- and intrapatient variations in skin permeability are overcome. Thus, the rate-limiting step in transdermal drug absorption is ideally provided by the delivery system and not the skin. At this time, a number of transdermal delivery systems have been described. They may be classified broadly into three general categories:

A. Membrane Moderated

A reservoir containing the drug is enclosed on all sides, bar that through which drug is

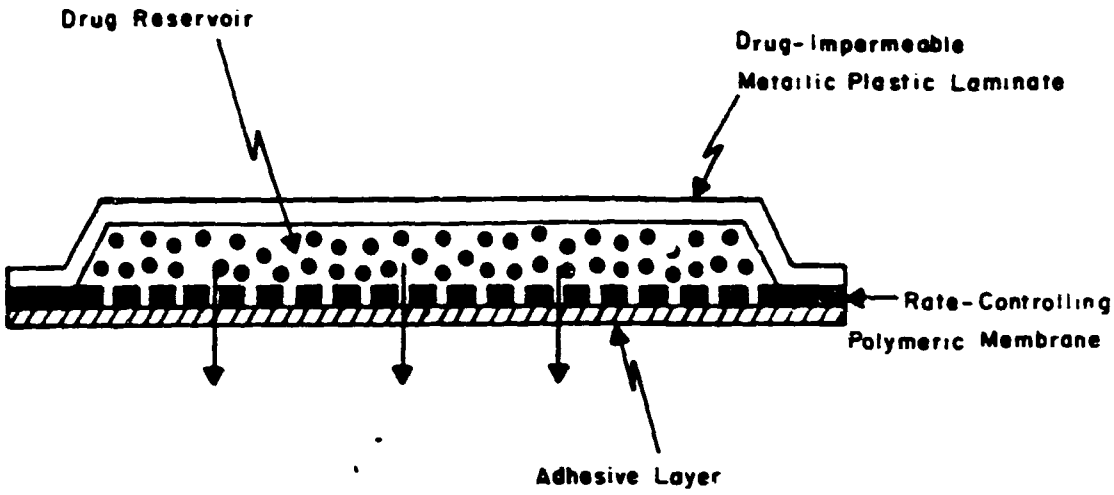

FIGURE 2. Diagram of a membrane-moderated transdermal drug delivery system.

released, by an impermeable laminate (Figure 2). The releasing face of the reservoir is covered by a rate-controlling polymeric membrane. Different release rates are achieved by variation of the polymer composition and the thickness of the membrane. The devices of this type, which have been described, include the following:

1. Transderm Scop (CIBA-GEIGY). This system consists of a backing of aluminized polyester film; a reservoir of mineral oil and polyisobutylene containing 1.5 mg of scopolamine; a rate-controlling membrane of microporous polypropylene; and an adhesive layer of mineral oil, polyisobutylene, and scopolamine. The system is programed to deliver 0.5 mg of scopolamine at a constant rate to the systemic circulation for approximately 3 days. In vitro, there is an initial burst of drug which has been liberated from the adhesive, followed by zero-order release from the reservoir into an aqueous sink of approximately 3.8 $\mu g/cm^2\ hr^{-1}$.
2. Transderm Nitro (CIBA-GEIGY). The components of this system are an impermeable backing of aluminized plastic; a reservoir of nitroglycerin adsorbed to lactose and dispersed in a colloidal suspension of silicone dioxide and silicone medical fluid; and a rate-controlling membrane of ethylene/vinyl acetate copolymer. The system adheres to the skin by a hypoallergenic silicone adhesive. It is available in 5, 10, 20, and 30 cm^2 areas delivering 2.5, 5.0, 10.0, and 15.0 mg of nitroglycerin into an aqueous receptor phase at around 40 $\mu g/cm^2/hr$ between 2 and 24 hr following an initial rapid burst effect.[9]
3. Catapres TTS (BOEHRINGER-INGELHEIM). In this system, the components are a backing material of pigmented polyester; a reservoir of clonidine, mineral oil, polyisobutylene, and colloidal silicone dioxide; and a rate-controlling membrane of microporous polypropylene. An adhesive formula of clonidine, mineral oil, polyisobutylene, and colloidal silicone dioxide affixes the system to the skin. It is available in sizes of 3.5, 7.0, and 10.5 cm^2 with delivery rates of 100, 200, and 300 μg of clonidine per day. The in vitro release rates into an aqueous solution from these devices are between 1.5 and 2.0 $\mu g/cm^2/hr$.
4. Estraderm (CIBA-GEIGY) This system is similar to that of the Transderm Nitro and Catapres TTS systems described above. The reservoir in this case consists of estradiol solubilized in ethanol. Because this system is currently awaiting FDA approval,* little information as to its construction has been published.
5. The Hercon company has developed a nitroglycerin transdermal patch which recently

Now approved.

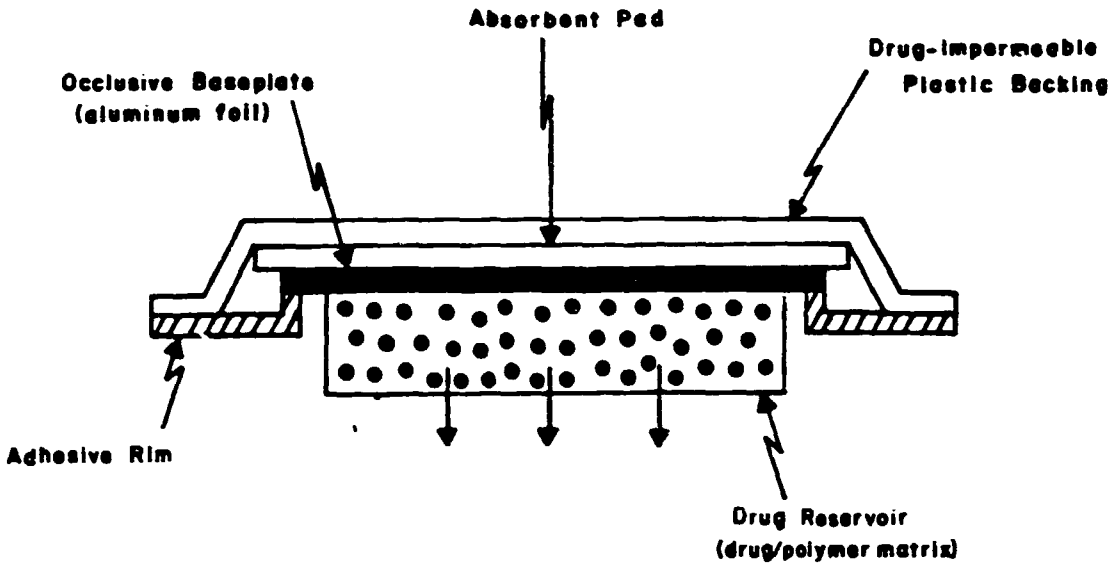

FIGURE 3 Diagram of a matrix dispersion-type transdermal drug delivery system.

has been granted FDA approval. This system consists of an outer layer impervious to nitroglycerin; a reservoir containing the drug; and a rate-controlling membrane in contact with the skin. In vitro, a representative 1 in.^{2} system released approximately 18 mg of nitroglycerin in 8 hr.[10]

B. Matrix Diffusion Controlled

The reservoir is manufactured by homogeneously dispersing the drug in a polymer matrix which is then molded into a disc with a defined surface area and thickness (Figure 3). Drug release from the device into the body is controlled by diffusion through the matrix reservoir material. Devices of this type include the following:

1. The Nitrodur (KEY/SCHERING-PLOUGH) system, which consists of a nonpermeable aluminum foil backing; a gel-like matrix of glycerin, water, lactose, polyvinyl alcohol, povidone, sodium citrate, and nitroglycerin; and a medical-grade microporous tape to adhere the system to the skin. It is available in dosage strengths which deliver 2.5 mg (5 cm^2), 5 mg (10 cm^2), 7.5 mg (15 cm^2), 10 mg (20 cm^2), and 15 mg (30 cm^2) in 24 hr.
2. The Nitrodur II (KEY/SCHERING-PLOUGH) system, a more elegant formulation of the Nitrodur system, contains nitroglycerin in an acrylic-based polymer adhesive with a resinous cross-linking agent. The system is available in 5, 10, 15, 20, and 30 cm^2 sizes, delivering 2.5, 5.0, 7.5, 10.0, and 15.0 mg in 24 hr, respectively.
3. The Deponit TTS (PHARMA-SCHWARZ), a device currently available only in Europe. It consists of a flexible carrier foil about 20 μm thick which is impermeable to nitroglycerin; an adhesive film about 300 μm thick of polyisobutylene resin which is charged with nitroglycerin; and a protective foil approximately 100 μm thick which is impermeable to nitroglycerin and is peeled off before use. A 16 cm^2 system delivers 5 mg in 24 hr in vivo. In vitro, the system releases 7.1 mg in 24 hr.[11]

C. Microsealed

The microsealed category is represented by the Nitro-Disc (SEARLE) device (Figure 4). In this system, the reservoir is formed by dispersing nitroglycerin adsorbed to lactose in a hydrophilic solvent system of 10 to 30% (v/v) polyethylene glycol in distilled water, which is subsequently distributed in a silicone elastomer by mechanical force to form thousands

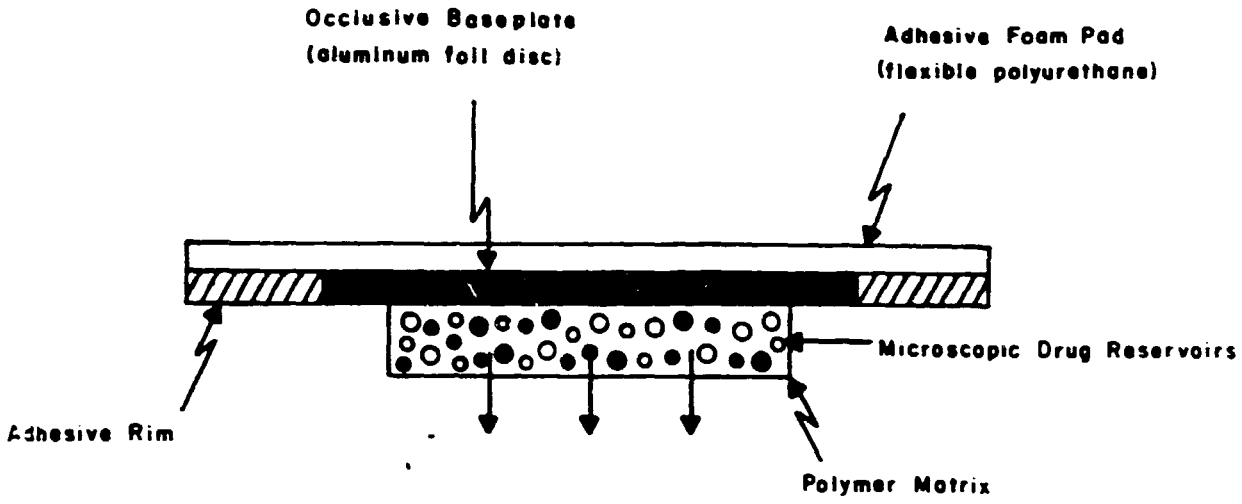

FIGURE 4. Diagram of the microsealed transdermal drug delivery system.

of microscopic drug compartments. Drug release is controlled by diffusion through the polymeric matrix. It is available in 8 cm^2 (Nitrodisc 5) and 16 cm^2 (Nitrodisc 10) sizes delivering 5 and 10 mg in 24 hr, respectively.

IV. PHARMACOKINETICS AND PHARMACODYNAMICS OF TRANSDERMALLY DELIVERED DRUGS

A. Estradiol

in preventing or reversing these symptoms.[15] However, orally administered estradiol undergoes massive first-pass liver and gut metabolism to estrone,[16,17] and leads to the induction of several liver proteins.[18,19] Delivery of estrogen by this route also maintains the plasma concentration ratio of estradiol (E2) to estrone (E1) at the postmenopausal level of 0.2 to 0.3, rather than achieving the ideal premenopausal level of 1 or more.[20] Estrogen replacement therapy via transdermal delivery has been proposed, therefore, as a way in which (1) the total daily dose of estrogen, which is required to achieve plasma levels comparable to those of a premenopausal woman, can be reduced; (2) the extensive liver metabolism experienced by oral forms of the drug can be avoided, and the E2/E1 ratio thereby returned to the premenopausal level; and (3) the induction of liver proteins can be reduced or eliminated.

To determine whether nonorally administered estradiol would provide effective physiologic replacement without altering hepatic function, Laufer et al.[21] studied 20 postmenopausal women before and after 3 weeks of treatment with either prototypical estrogen transdermal systems or placebo. In the treatment group, the subjects of which replaced their systems every 3 days for 21 days, the mean values of estradiol and estrone rose significantly from baselines of 7 ± 1 and 16 ± 1 pg/mℓ, respectively, to 72 ± 6 and 37 ± 3 pg/mℓ. When these end-of-treatment values were compared to premenopausal control subjects, it was found that the mean estradiol values were not significantly different, whereas mean estrone levels were significantly lowered. The corresponding values in the placebo group did not change from baseline (Figure 5A). No significant changes were noted in the concentrations of the hepatic proteins renin substrate and thyroxine-binding globulin, nor were there any alterations in the binding capacities of cortisol-binding globulin and sex hormone-binding globulin in either population, indicating that when delivered transdermally, estradiol has limited effect on hepatic function.

The decline in ovarian function and subsequent loss of endogenous estrogen at the menopause are associated with many symptoms including hot flashes, atrophic vaginitis, and osteoporosis.[12-14] Estrogen replacement therapy has been shown to be at least partially successful

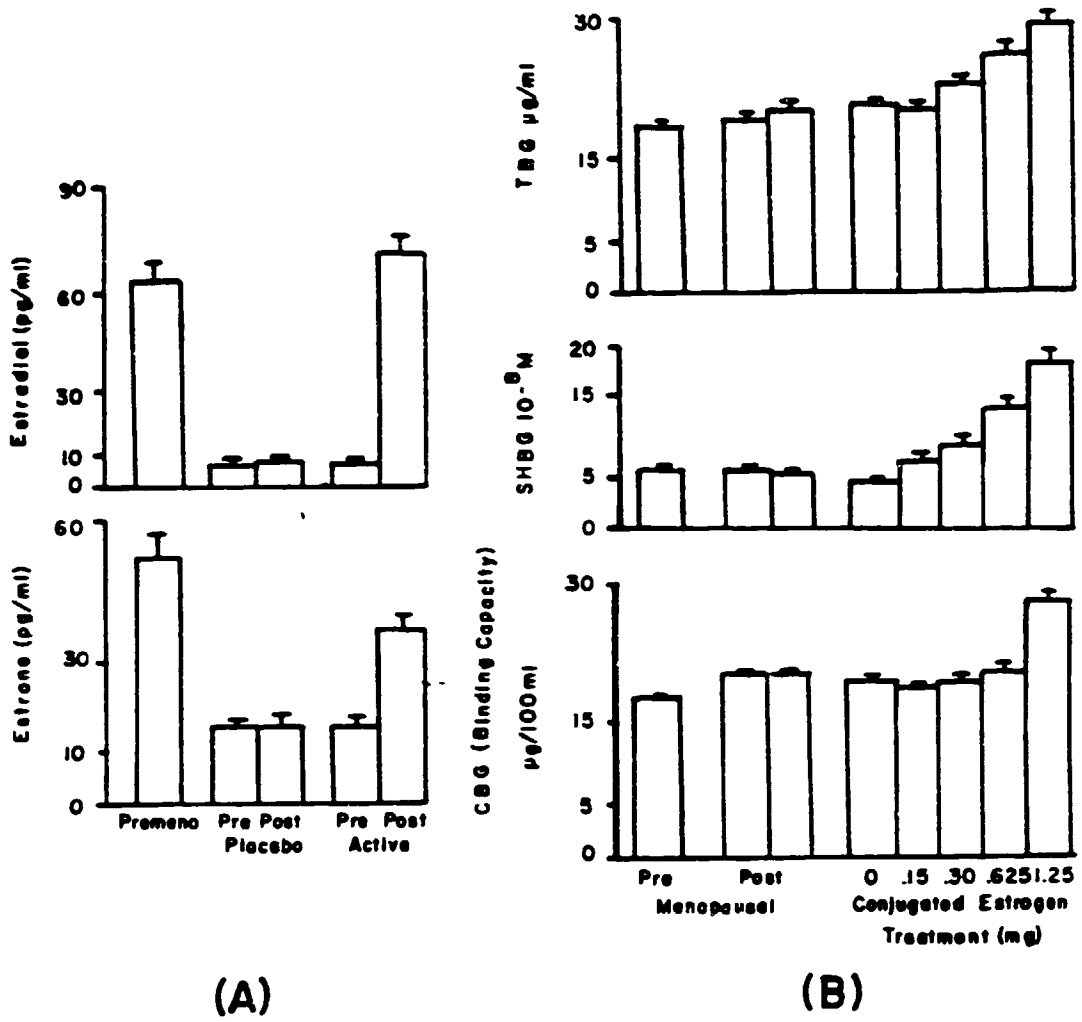

FIGURE 5. (A) Plasma concentrations of estradiol and estrone in premenopausal subjects, postmenopausal subjects both before and after 3 weeks of treatment with placebo patches, and postmenopausal subjects both before and after 3 weeks of treatment with active patches.[21] (B) Plasma concentrations of corticosteroid-binding globulin, sex hormone-binding globulin, and thyroxin-binding globulin in postmenopausal women both before and after 6 weeks of treatment with transdermal estradiol, and after 6 weeks of orally administered conjugated estrogens.[18]

In a more recent study, Powers et al.[22] compared the pharmacokinetics of transdermally delivered vs. orally administered forms of estradiol to 14 postmenopausal women. The mean baseline pretreatment levels of estradiol and estrone were 7.4 and 32.2 pg/mℓ, respectively. The average steady-state values during the application period of 3 different Estraderm systems (delivering 0.025, 0.05, and 0.1 mg/day) were 23, 39, and 74 pg/mℓ, respectively, for estradiol, and 33, 41, and 59 pg/mℓ for estrone. Oral administration of Estrace (micronized estrogens, 2 mg/day) and Premarin (conjugated estrogens, 1.25 mg/day) resulted in serum estradiol levels of 66 and 31 pg/mℓ, respectively, and estrone levels of 334 and 152 pg/mℓ (mean steady-state values, measured 24 hr after the third dose). Again, no significant elevation of hepatic proteins was found following transdermal administration of the drug (Figure 5B).

This system demonstrates quite elegantly the advantages that can be gained through rate-controlled transdermal drug delivery. In essence, this system allows for a more physiologically correct estrogen replacement pattern by:

1. Maintaining the correct estradiol to estrone ratio, thus avoiding excessive tissue accumulation of estrogens
2. Avoiding the induction of hepatic proteins

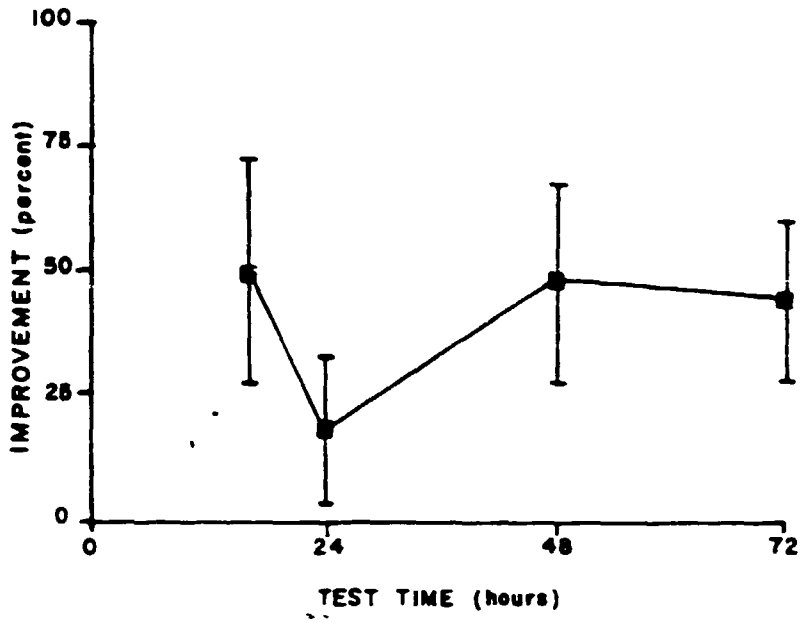

FIGURE 6. Percent improvement (± SEM; n = 9), as a function of time, in the prevention of motion sickness (as measured by the number of stressful head movements made in a rotating chair) following administration of transdermal scopolamine.[26]

3. Bypassing first-pass hepatic metabolism, thereby reducing the total daily dose of estradiol required

Studies have also shown that there is a quick return to baseline urinary excretion of endogenous estrogen compounds upon removal of the system.[20] This will allow the physician to provide cyclical therapy, further improving the ability of this system to mimic the state of the premenopausal woman.

B. Scopolamine

Of the wide range of antiemetic agents with which to treat motion sickness, scopolamine has been shown to be superior.[23,24] However, its use is limited due to the short half-life of the drug, and the appearance of dose-related side effects such as drowsiness, dry mouth, blurred vision, and, in cases of higher dosage, mental confusion and hallucinations. The aim of transdermally delivered scopolamine is to eliminate the relatively high plasma concentrations of drug which normally follow oral or intramuscular administration, and, hence, to minimize side effects and attain a superior pharmacokinetic profile. Schmitt et al.[25] determined that during the steady-state phase of scopolamine administration, the transdermal system was functionally equivalent to an intravenous infusion.

Homick et al.[26] evaluated the time course of effectiveness of transdermally administered scopolamine in the prevention of motion sickness induced by exposure to coriolis stimulation in a rotating chair. They observed a highly variable response, with an overall 40% improvement ($p < 0.05$) in test scores 16 to 72 hr after application of Transderm-Scop systems to 11 subjects (Figure 6). Such variability was also noted in a study performed by Graybiel et al.,[27] where the efficacy of transdermal scopolamine was compared at 12 and 72 hr postadministration. In 6 subjects, after 12 and 72 hr, the number of beneficial responses were 4 and 0, respectively. On repeating the test in the same subject population, the corresponding figures at the same time points were 4 and 3.

McCauley et al.[28] compared the efficacy of transdermally administered scopolamine to orally administered dimehydramine and either orally or transdermally administered placebo

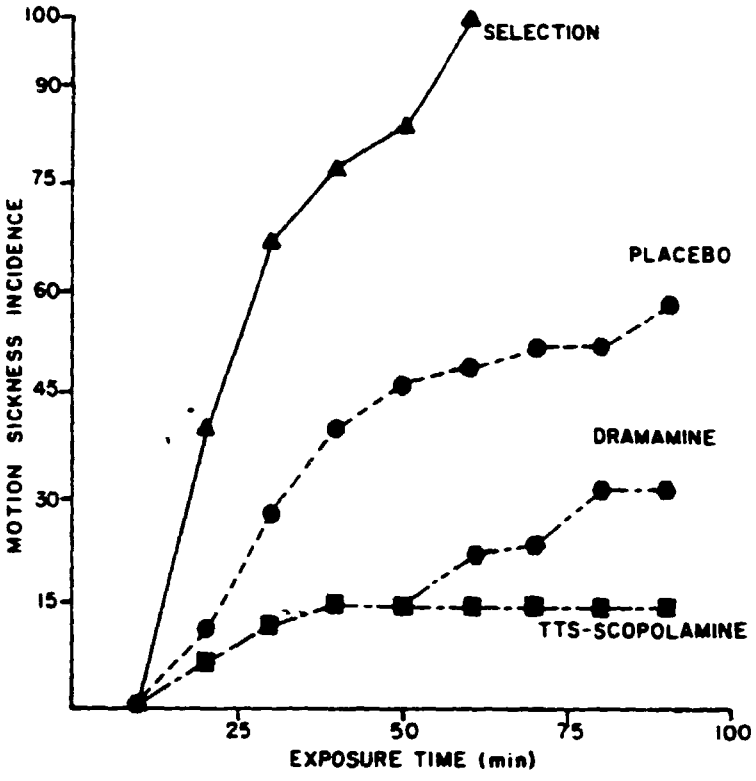

FIGURE 7. Reduction in motion sickness incidence as a function of exposure time to vertical oscillation and treatment modality (no treatment, placebo treatment, oral dramamine treatment, and transdermal scopolamine treatment).[28]

in the prevention of motion-induced nausea in a vertical oscillator. Thirty-five subjects were utilized in a double-blind crossover study. It was found that a placebo effect reduced the motion sickness incidence [(MSI); the percent of subjects vomiting within 90 min] from 100 to 59%, whereas dimenhydramine reduced the MSI to 32%. Scopolamine further reduced the MSI to 16% (Figure 7). Other investigators[29,30] reported similar results when comparing the same treatment modalities aboard vessels at sea.

The occurrence of side effects following transdermal scopolamine was minimal in all these studies. However, Homick et al.[26] noted that one or more side effects (including dry mouth, drowsiness, blurred vision, and irritation at the site of application) were reported during 60% of test trials when the topical system was used. It should be noted, however, that all of these symptoms except blurred vision were also reported with placebo treatment.

C. Clonidine

Hypertension is associated with an increased risk of premature cardiovascular complications. Antihypertensive therapy decreases the incidence of these events, but effective results hinge on patient compliance which is increased when side effects are few and administration is convenient. Thus, a transdermal therapeutic system, Catapres TTS, has been developed to provide rate-controlled, continuous release of the antihypertensive drug clonidine for a 7-day period. Such a lengthening of the dosage interval should increase patient compliance as well as minimize the dose-dependent side effects, such as dry mouth and sedation, which occur with oral dosage forms of the drug.

A crossover clinical study[9] compared Catapres TTS with oral Catapres. Seventeen subjects were randomly assigned to one of two regimens: either two Catapres TTS devices (5 cm^2 total area) for 7 days on the upper outer arm, or 0.1 mg of oral Catapres every 12 hr for 4 days. With the transdermal system, plasma concentrations of clonidine gradually increased.

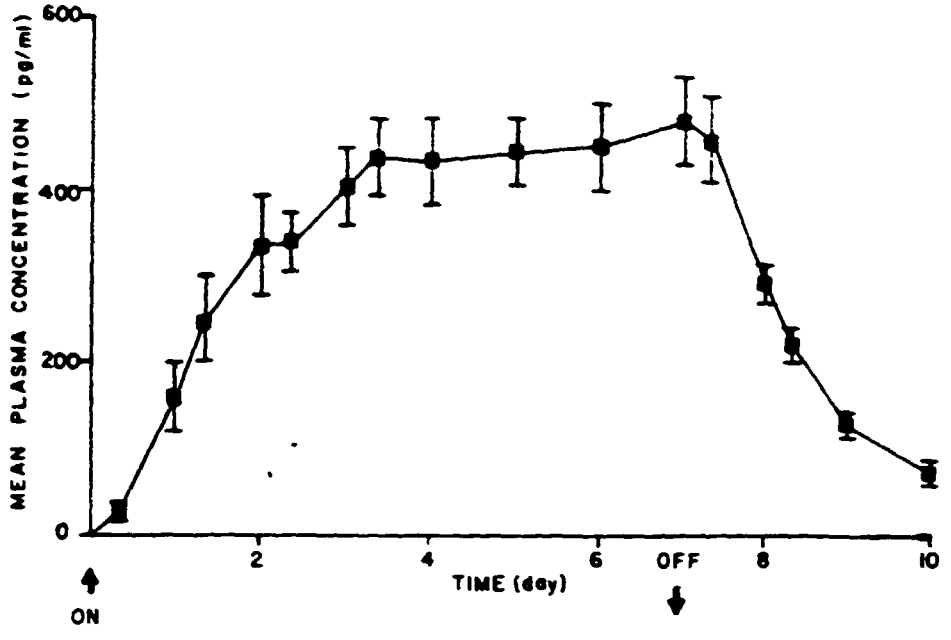

FIGURE 8. Plasma concentrations of transdermally administered clonidine (5 cm^2 system) as a function of time (± SEM; n = 17). Arrows indicate application and removal of the system.*

attaining an average steady-state value of 400 pg/mℓ on day 3 of the study (Figure 8). This value remained constant during the remainder of the application period. With oral administration, plasma levels of clonidine peaked at an average value of 800 pg/mℓ on day 4 of the study. Plasma "trough" concentrations averaged approximately 400 pg/mℓ by day 3 of the study.

MacGregor et al.[31] found mean steady-state plasma clonidine concentrations of 0.39, 0.84, and 1.12 ng/mℓ with 3.5, 7.0, and 10.5 cm^2 devices, respectively, with no significant differences when the site of application was rotated. Diastolic blood pressure fell by at least 10% in 37 patients, and was normalized (i.e., less than 90 mmHg) in 64% of the patients tested (total number of patients was 85).

Weber et al.[32] compared Catapres TTS (3.5 cm^2) patches to placebo patches in their ability to reduce diastolic blood pressure in 20 patients with essential hypertension. Blood pressure was reduced to less than 90 mmHg in 12 of 20 patients wearing the active patches. When these 12 responders were placed on placebo patches, blood pressure rose to its pretreatment value. Plasma concentrations were similar to those observed in other studies.

D. Nitroglycerin

Nitroglycerin (GTN) has been used in the treatment of angina pectoris for over 100 years. It is frequently prescribed in the sublingual form, a route which results in the immediate relief of anginal pain. Unfortunately, the duration of action of sublingual GTN is extremely short because of the drug's rapid elimination from the body; hence, it cannot be given via this route as prophylaxis against further angina attacks. Oral preparations, although longer acting, also provide relatively brief therapeutic effect due to extensive first-pass hepatic metabolism; hence, orally, GTN must be administered four to six times per day. This extreme sensitivity of GTN to metabolism, and its corresponding short biological half-life, suggest the drug as an excellent candidate for transdermal delivery.

Transdermal nitroglycerin in the form of an ointment has been available for over 30 years, and has been shown effective in the prophylaxis of angina attacks.[33] However, the use of the conventional topical dosage form is limited by:

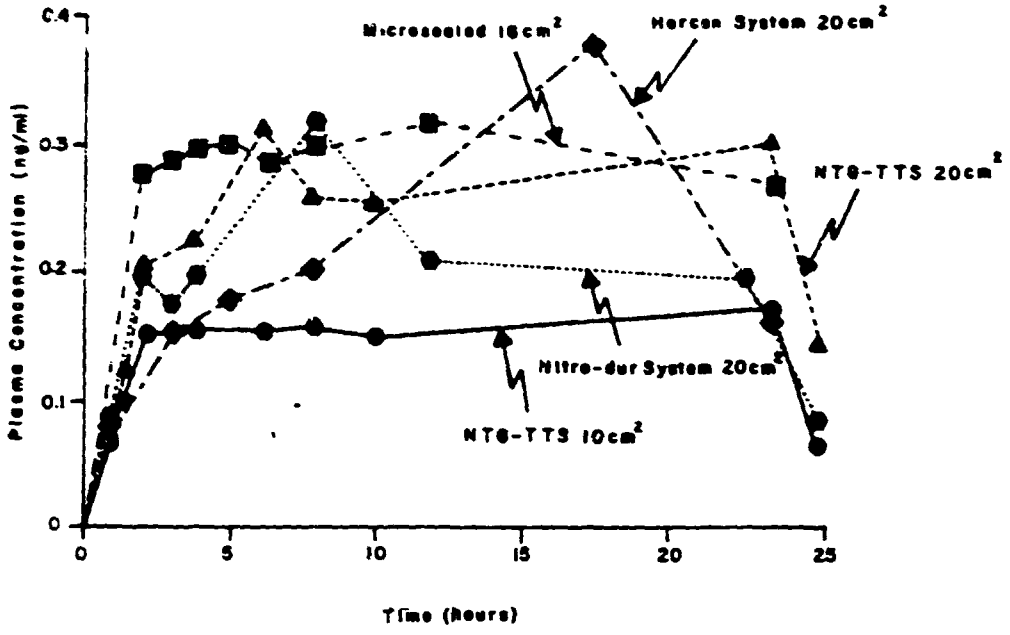

FIGURE 9 Plasma GTN concentrations as a function of time following administration of the various GTN transdermal systems.[10,35,38]

1. The difficulty in obtaining reproducible dosage, in terms of the amount of drug applied and the area of application.
2. The release of GTN from these ointment bases is rather rapid and, as a result, the rate of drug input into the systemic circulation is controlled by the skin. Therefore, GTN plasma levels may vary considerably both within and between patients because of inter- and intraindividual variations in skin permeability.
3. Metabolism of GTN by skin microflora[34] may be greater with this formulation than with the newer transdermal devices in which most of the drug is physically protected within the device.
4. Patient compliance: the ointment should be spread as evenly as possible over a rather large surface area, and then covered with an occlusive material (such as Saran® wrap) to minimize loss of drug to the patient's clothing,etc. The dosage interval of the ointment is every 8 hr.

The need to provide a dosage formulation of GTN which would overcome the above problems and give prophylactic protection against angina pain led to the development of transdermal delivery systems of GTN. To date, four systems — Nitrodisc, Nitrodur, Transderm Nitro, and Hercon — have been conditionally approved by the FDA for once-daily dosing of GTN.

Although the fabrication and the principles of release control of different nitroglycerin transdermal systems differ, remarkably similar plasma levels are found in vivo following their administration (Figure 9). For example, when 16 cm^2 Nitrodisc systems were applied to 12 healthy volunteers, plasma concentrations remained constant over the 32-hr study at 280 pg/mℓ.[35] The mean plasma concentrations of 12 volunteers wearing the Transderm Nitro system ranged from 175 to 300 pg/mℓ over 28 hr;[36] in another study, volunteers wearing the 10 and 20 cm^2 systems achieved steady-state plasma concentrations of 160 and 250 pg/mℓ, respectively, between 2 and 24 hr.[37] The Hercon system resulted in mean steady-state plasma concentrations of 176 ± 79 pg/mℓ over 72 hr when applied to 16 healthy male volunteers.[38] Finally, application of the Nitrodur system to 6 healthy human volunteers resulted in average steady-state plasma concentrations of 201 pg/mℓ.[38] The obvious implication of these data

is that GTN input to the systemic circulation is probably controlled in large part by the skin rather than by the devices themselves.

Although now used extensively on a daily-dosing basis, controversy exists as to the efficacy of the transdermal GTN devices. Several studies indicate that nitrate tolerance can occur as soon as 24 hr after onset of prolonged steady-state plasma levels of GTN.

Reichek et al.[39] studied the effect of high and low dose transdermal GTN compared to placebo on exercise tolerance (bicycle protocol, NIH). At 4 hr, the bicycle exercise time increased by 7% with low dose therapy (9.4 mg/day Nitrodisc); however, this was not significantly different than the response seen in placebo-treated subjects. On the other hand, improvements of 29 and 39% ($p < 0.01$) were achieved during high dose treatment with the 24 mg/day Nitrodisc and 22 mg/day Transderm Nitro systems, respectively. Increases in exercise time were indistinguishable from placebo at 24 hr for all forms of therapy, and even at high doses the peak effect on exercise tolerance was 50% of that obtained with sublingual GTN. Crean et al.[40] compared the 10 cm^2 Transderm Nitro system to placebo in 10 patients in a randomized, double-blind, double-crossover trial of 4 1-week periods. Efficacy was evaluated by exercise testing, ST segment recording, and the number of angina attacks experienced by each patient. The net result was that the number of anginal attacks was not decreased over placebo, nor was the time to angina different between therapies; there was no reduction in consumption of sublingual nitrates, and the time to 1 mm ST segment depression was the same as for placebo. A subsequent[41] study tested the Nitrodur 10, 20, and 30 cm^2 systems in 11 patients and found improved treadmill walking time at 2 and 4 hr postinitiation of the study ($p < 0.05$), but not at 24 hr. After sustained therapy with the 30 cm^2 system for 1 to 2 weeks, no difference in walking time was seen over placebo. Hollenberg and Go[42] studied the short- and long-term effects of Nitrodur on exercise tolerance using a computerized analysis of ST segment changes. Significant improvements were seen at 4 and 7 hr. As a group, the patients demonstrated an improvement of 31% ($p < 0.0001$) for all dose levels. Sublingual GTN, however, induced an even greater improvement than the highest dose of transdermal GTN ($p < 0.003$), and the effects of Nitrodur were attenuated after 2 and 4 weeks.

On the other hand, a study by Thompson[43] using the maximally tolerated dose of GTN in the Nitrodisc system found that, at 2 hr, the mean duration of exercise time increased from 11.3 to 14.4 min ($p < 0.05$), and that at 26 hr, the mean duration of exercise was still elevated to 14.1 min compared to the placebo-treated value of 11.8 min ($p < 0.05$). This represents a 30% increase at 2 hr, and a 25% increase at 26 hr. The consumption of nitrates also decreased by 63% over the time course of the study. Georgopoulos et al.[44] administered Transderm Nitro (5 mg) for 1 to 2 weeks in a placebo-controlled, double-blind crossover study of 13 patients. Although exercise duration was not measured, the exercise-induced depression of ST segment at matched treadmill times was decreased by 50%. The daily frequency of anginal attacks decreased by 67%, and the daily consumption of nitrates decreased by 63%. No tolerance was observed over the 14 days of the study. Finally, Scardi et al.,[45] in a double-blind, randomized, placebo-controlled study, found a statistically significant increase ($p < 0.01$) in total duration of exercise, exercise duration to 1 mm ST segment depression, maximal workload, and total work performed at both 4 and 24 hr after dosing with both the 20 and 40 cm^2 Transderm Nitro systems.

Clearly, the results of these trials are mixed, with several studies showing statistically significant increases in exercise time at 2 and 4 hr after application of the last patch, whereas only two studies demonstrated a significant increase in exercise time after 24 hr. These investigations differ significantly in their criteria for entry and efficacy, as well as in the use of concomitant medications. Study designs vary also: some are crossover, some placebo controlled, some randomized, and some double blind. The patient populations in these studies are also very small, making the statistical interpretations difficult. Finally, there is a question

of tolerance of the exercise protocol in these studies, i.e., there is a "training effect" with repeated exercise testing, especially with those patients who have participated in previous studies. Until more standardized protocols and larger patient populations are used, interpretation of these studies will remain difficult. Currently, a major multicenter clinical trial involving the Transderm Nitro, Nitrodur, and Nitrodisc GTN systems is underway, with particular emphasis on the pharmacological behavior of GTN.

V. DRUG SELECTION

The choice of a compound for transdermal delivery depends upon a number of factors which may be grouped conveniently into three categories: biological, physicochemical, and pharmacokinetic.[46]

A. Biological Criteria

1. The drug must be potent, requiring a parenteral daily dose of milligrams or less. In most cases, this limitation translates into an effective plasma concentration in the nanogram per milliliter range.
2. Drugs subject to an extensive hepatic first-pass effect on oral dosing may benefit by transdermal administration. Dose amount and dosing frequency may be significantly reduced in this way.
3. As for all forms of sustained or prolonged delivery, drugs with short (rather than long) biological half-lives are most appropriate.
4. The drug should not elicit a major cutaneous irritant or allergic response. The definition of "major" in this context is difficult to specify. The clonidine system, for example, has been launched successfully despite a relatively high rate of irritancy provocation.[47]
5. Because transdermal delivery typically provides constant drug input, it is important that the pharmacological effect of the agent be suited to this absorption pattern. The possible induction of tolerance must be carefully monitored, therefore (see above discussion on nitroglycerin).
6. Sensitivity of the drug to cutaneous metabolism[48] within the viable epidermis or to degradation by surface microflora[34] is clearly undesirable. Current understanding of these areas is sketchy, and methods for their evaluation are poorly developed.

B. Physicochemical Criteria

The sequential events that a drug must undergo in order to become systemically available following application in a transdermal device are[3]

1. Transport within the delivery system to the device-skin surface interface
2. Partitioning from the delivery system into the stratum corneum
3. Diffusion through the stratum corneum
4. Partitioning from the stratum corneum into the viable epidermis
5. Diffusion through the viable tissue
6. Uptake by the cutaneous microcirculation and subsequent systemic distribution

It follows that diffusion and partitioning are the key physical processes pertinent to transdermal delivery.

Diffusion—Drug transport is determined primarily by the molecular size and the level of interaction with the medium through which diffusion is taking place (viz. delivery system, stratum corneum, viable epidermis). Most currently used drugs have molecular weights (M) less than 1000 g/mol and the effect of size on diffusion coefficient may be adequately described by a power dependency (e.g., the Stokes-Einstein equation: $D \alpha M^{-1/3}$) or,

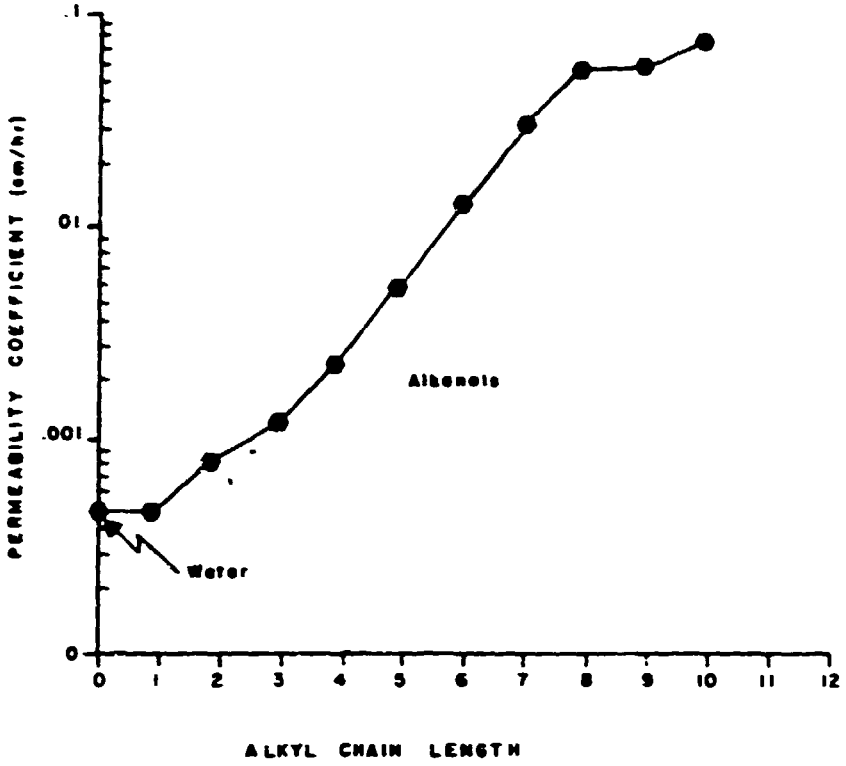

FIGURE 10 Permeability coefficients plotted vs. alkyl chain length for a series of n-alkanols.[54,55]

sometimes, by an exponential function.[49] Generally, these relationships are not particularly powerful and predict that D is much less sensitive to M than, for example, the viscosity of the medium through which the drug is diffusing.

Partitioning—The partitioning criteria for a transdermal candidate are demanding. The molecule must favor the stratum corneum over the device, and the relative affinity of the drug for stratum corneum and viable tissue must be reasonably balanced. Extreme partitioning characteristics are not conducive to successful drug delivery via the skin. Correlations between skin absorption and various oil-water partition coefficients have been reported. Percutaneous penetration through human skin has been related to both heptane-aqueous buffer[50] and octanol-water partition coefficients.[51] Linear free energy relationships have been established between steroid absorption across human skin and (1) benzene solubility[52] and (2) log K (octanol/H_2O).[53] The in vitro penetration of the series of n-alkanols has been compared to the corresponding values of K (ether/H_2O).[54,55] A linear correlation was found up to octanol, but the behavior of subsequent homologs indicated that a change of rate-limiting step was occurring when the penetrant hydrophobicity reached a certain level (see Figure 10). Mechanistically, a plausible explanation for this observation is that stratum corneum to viable epidermis transfer becomes a slower process than stratum corneum permeation for very lipid-soluble drugs. Therefore, an oil-water partition coefficient is a useful qualitative indicator of penetration, the reliability of which is least at the extremes of solute partitioning behavior.

C. Pharmacokinetic Criteria

The dependence of transdermal drug delivery on diffusion and partitioning across stratum corneum and viable tissue implies that the feasibility of the process may be predictable from the physicochemical properties of the drug. Recently, a linear kinetic model has been developed for this purpose and has been validated successfully using in vivo data for nitroglycerin, clonidine, estradiol, scopolamine, and timolol.[56-59]

packing structure of the ordered lipids that Elias[62] has observed in the intercellular channels.* Until a systematic study is conducted on a range of these materials, the exact manner in which they operate is open to conjecture. Hydration has been suggested as the way in which urea elicits its action, and perhaps the most ubiquitous, but infrequently recognized, penetration enhancer is water. In nearly all instances, hydrated skin is more permeable than dry skin. The examples most usually quoted are the corticosteroids which penetrate through occluded skin more extensively than they cross nonoccluded tissue.[1] Perhaps water as a penetration enhancer is one of very few substances which satisfies the six stringent requirements enumerated above.

Thus, it would, in general, be desirable to find an agent whose properties are similar to water, but which demonstrates a more powerful enhancement effect. A commonly cited accelerant is dimethylsulfoxide (DMSO), a colorless liquid with excellent solvent properties. It is miscible with water and many organic liquids, and is reasonably easily incorporated into formulations. The versatility of DMSO is exemplified by its ability to accelerate the penetration of a wide range of compounds including steroids, organic dyes, barbiturates, griseofulvin, phenylbutazone, antibiotics, and quaternary ammonium compounds. Despite considerable investigation, the mode of action of DMSO is not completely understood. It has been suggested that the enhancer replaces integral water within the stratum corneum to form a continuous network through the skin. A recent systematic study, performed in vitro using hairless mouse skin,[63] however, implies that DMSO impairs barrier function by eluting solvent-soluble components from the stratum corneum; in addition, delamination of the horny layer and denaturation of its proteins also seem to contribute in diffusion enhancement.

While the properties of DMSO indicate that it does act as an excellent penetration enhancer, there are problems associated with its use. Concentrations above 60% are required to significantly increase the percutaneous absorption of solutes and, at these high concentrations, DMSO can produce erythema and wheals. Another side effect of DMSO is caused by the metabolite dimethyl sulfide which causes a characteristic foul taste and bad breath. Widespread use of DMSO, therefore, is not common, although it has been used as a solvent for idoxuridine in the treatment of herpes zoster and herpes simplex skin infections.

Other sulfoxides have been examined as penetration enhancers, particularly the alkylmethyl derivatives having the general structure $RSOCH_3$.[64] The optimum chain length appears to be C_{10} to C_{12}, and these compounds have the advantage that their degradation products are less odorous than dimethyl sulfide.[65] They are also active at low concentrations (Figure 12), but the effect appears limited to the enhancement of polar or ionic molecules. It is possible that the activity of these substituted sulfoxides is due to their nonionic surfactant character and that they interact with and alter the structure or conformation of the skin proteins. From a consideration of their molecular structure, they may also be expected to disrupt the structured lipids in the intercellular channels. Phosphine oxides such as the dodecyldimethyl derivative have also been shown to enhance skin penetration.[65]

Chemically related to dimethylsulfoxide are dimethylacetamide (DMAC) and dimethylformamide (DMF). These have been used as penetration enhancers and have been shown to be effective for both griseofulvin and hydrocortisone.[1] It is clear that the dipolar aprotic nature of these solvents is of importance in their mode of action. Although they are not quite as effective as DMSO, they are better tolerated by the skin. However, they have not been subject to widespread evaluation. Other simple solvents, for example, ethanol, have been considered as enhancers. The estradiol delivery system [20] contains a significant amount of ethanol which almost certainly assists the percutaneous absorption of the drug.

Urea is used clinically to enhance skin penetration. Two hydrocortisone preparations

* The latter mechanism has been strongly implicated by recent work from Potts et al. (*J. Invest. Dermatol.*, 86, 478, 1986). It was shown that *cis*-vaccenic acid enhanced percutaneous absorption and caused a concomitant increase in the fluidity of stratum corneum lipids.

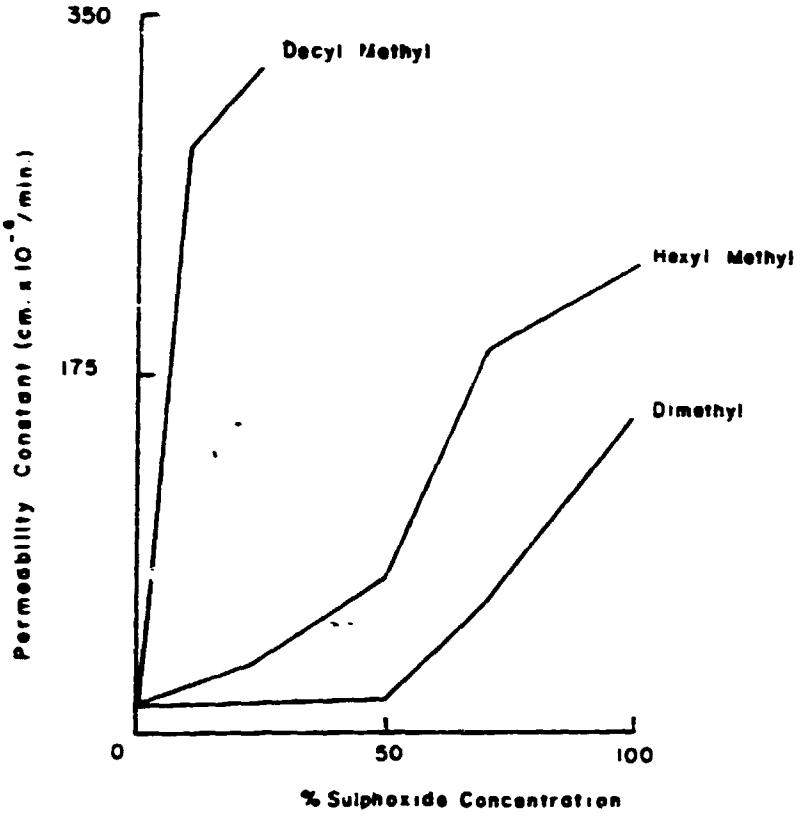

FIGURE 12. Effect of three alkyl sulfoxides as a function of concentration on the in vitro steady-state permeation of salicylic acid across excised human skin.

contain urea to optimize the delivery of drug to the lower regions of the skin. Urea may induce two changes to the barrier function of the skin: (1) it increases the hydration of the stratum corneum, and (2) after prolonged contact, it acts as a keratolytic agent. At low concentrations, the mechanism of action is probably related to the hydration effect. It appears that urea acts rapidly as an accelerant.[66] Using hexyl nicotinate as a model penetrant, the time of onset of erythema was measured in vivo in humans following topical application and was used to assess percutaneous penetration. In oily cream BP, hexyl nicotinate at 0.1% penetrates the skin to give a time of onset of erythema of 14.3 min. Addition of 10% urea decreases this time to 11 min. More extensive data obtained following nicotinate delivery in aqueous cream BP with and without urea are shown in Table 2. However, for use in commercial formulations, urea must be stabilized to prevent degradation. For this reason, its accelerant properties have not been widely exploited.

Pyrrolidone derivatives have been examined as accelerants, and 2-pyrrolidone and *N*-methyl pyrrolidone, in particular, have been shown to be active. They have been examined with a range of solutes including griseofulvin, theophylline, tetracycline, and ibuprofen (see Figure 13).[67-71] At high concentrations, pyrrolidones can be irritating to the skin.

Recently, considerable attention has been directed toward 1-dodecylazacycloheptan-2-one (Azone®), which may be considered to be a "chemical combination" of pyrrolidone and decylmethyl sulfoxide. It has the ring structure associated with the pyrrolidone (albeit a seven-membered ring) and the long alkyl chain with mild polar head group associated with the alkyl sulfoxides. Azone® is a clear colorless liquid which may be incorporated into gel, cream, lotion, or solution formulations. It appears to have low irritancy and is active, at low concentrations, in enhancing the percutaneous absorption of a range of compounds[72-77] including corticosteroids, erythromycin, clindamycin, fusidic acid, griseofulvin, 5-fluorouracil, indomethacin, and hydroquinone. Typical concentrations of Azone® required for

Table 2
PENETRATION ENHANCEMENT BY UREA

Hexyl nicotinate concentration (%)	Erythema onset times (min) (mean ± SE) Urea concentration (%) 0	5	10
0.05	18.5 ± 0.5	17.2 ± 0.4	14.4 ± 0.2
0.1	12.9 ± 0.5	11.9 ± 0.3	10.5 ± 0.2
0.2	11.1 ± 0.4	9.6 ± 0.3	8.1 ± 0.2
0.5	8.7 ± 0.4	8.2 ± 0.4	6.6 ± 0.2
1	7.1 ± 0.3	6.9 ± 0.3	5.1 ± 0.3

Note Times (in minutes) of onset of erythema (mean ± SE, n = 20) induced by hexyl nicotinate delivered from aqueous cream BP as a function of drug and urea concentration in the vehicle

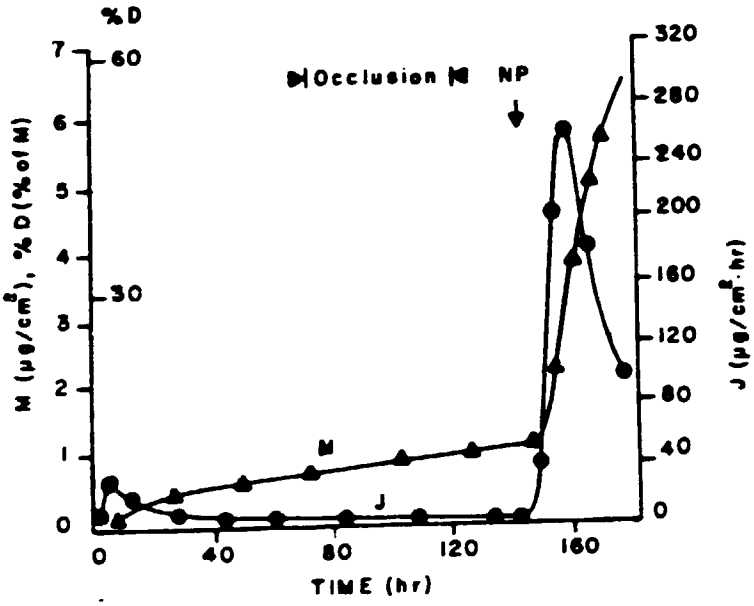

FIGURE 13. In vitro penetration data through dermatomed human skin for ibuprofen applied from an acetone vehicle. The effects of occlusion and the addition of *N*-methyl-2-pyrrolidone (NP) are shown. M is the cumulative amount penetrated; %D is the percent of the applied dose; and J is the derived flux.[67]

optimum effect range between 0.1 and 5%. For this reason, this agent may have particular use in transdermal delivery systems. However, more recent evidence[76,77] suggests that the action of Azone® may depend critically upon the presence of other components in the vehicle (e.g., propylene glycol) and that a degree of synergism may be involved in the promotion effect.

There are other miscellaneous compounds which have been considered as penetration enhancers: propylene glycol has already been mentioned and various amines have been examined. *N,N* diethyl-*m*-toluamide (DEET) has been investigated by Windheuser et al.,[78] and at 5% enhances the penetration of a range of compounds across hairless mouse skin. In a steroid blanching test, it has also been shown effective in vivo in human skin. Since DEET has been used for many years as an insect repellent at concentrations ranging from 10 to 100%, it seems possible that this agent has potential as a penetration enhancer.

One of the major difficulties associated with accelerants is their lack of specificity. They will enhance the absorption of other vehicle components and any impurities will be taken into the circulation. The toxicological implications of this must be considered in the formulation and all the materials must be ultrapure. This is possibly the reason for the lack of accelerants presently in commercial products. However, a fuller understanding of their mechanism of action will perhaps aid the development of compounds with greater specificity. An additional problem, also unresolved at this time, is that the penetration enhancing ability of, and the level of local skin toxicity induced by, the popular promoters are generally highly correlated. In other words, the best promoters of percutaneous absorption cause the most severe irritation. If disruption or destabilization of bilayer structures is a component of their mechanism of action, then this observation is, perhaps, not surprising, and may impose a limitation on the potency of enhancers that can be used practically. However, despite their disadvantages, penetration enhancers will undoubtedly remain a major consideration in the development of transdermal drug delivery systems.

Finally, mention should be made of two alternative methods to achieve penetration enhancement: (1) the use of prodrugs and (2) iontophoresis. Neither of these approaches can claim any existing applications in transdermal drug delivery for systemic effect. The philosophy of the prodrug concept is generally straightforward in its use with respect to the skin: to prepare a labile, more lipophilic precursor of the drug which has improved skin permeability but rapidly hydrolyzes once it has breached the stratum corneum. The area of topical prodrugs has been reviewed recently in some depth.[79] Iontophoresis has been recognized as a means of driving charged materials across lipoidal membranes for a considerable time. There is evidence to indicate that ionic species can be driven across the skin by this technique.[80] However, the problems associated with both formulation of suitable delivery systems and long-term passage of current at a specific skin site remain unresolved. It follows, therefore, that a significant amount of work remains to be performed before the potential use of these alternative approaches is clearly defined.

VII. CONCLUSIONS

In recent years, transdermal drug delivery has elicited a significant response in the pharmaceutical sciences. To a certain degree, the early promise has been realized with the successful and pending introductions into the market of a number of dosage forms. However, initial enthusiasm, which might be characterized (with hindsight) as somewhat excessive, has now been tempered with recognition that the transdermal route of administration has significant limitations and unique formulation requirements. Selection of drug candidates requires a thorough understanding of the kinetics and mechanism of percutaneous absorption, and a careful evaluation of the pharmacodynamic profile is needed for optimal efficacy. These issues can now be tackled more rationally because of developing intelligence in the necessary areas. The notion of skin penetration enhancement remains an enigma with the equation relating promotion to toxicity (local or systemic) undefined. Subsequent advances in transdermal delivery will be much harder to achieve because, in a sense, the most obvious candidates for this mode of input have now been considered. The learning experience, though, has been valuable and has delineated important questions, the answers to which must be known for further progress. There is no doubt that the next efforts will advance our comprehension of the skin's barrier function and of the mysteries of dermal penetration. Transdermal delivery remains, therefore, a challenge that will demand attention for the foreseeable future and will ultimately yield further basic science and commercially applicable rewards.

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76 **Wotton, P. K., Møllgaard, B., Hadgraft, J., and Hoelgaard, A.**, Vehicle effect on topical drug delivery III. Effect of Azone on the cutaneous permeation of metronidazole and propylene glycol. *Int. J. Pharm.*, 24, 19, 1984.

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78. **Windheuser, J. J., Haslam, J. L., Caldwell, L., and Shaffer, R. D.**, The use of *N,N*-dimethyl-*m*-toluamide to enhance dermal and transdermal delivery of drugs. *J. Pharm. Sci.*, 71, 1211, 1982.

79 **Hadgraft, J.**, Prodrugs and skin absorption. in *Design of Prodrugs*. Bundgaard, H., Ed., Elsevier North Holland, Amsterdam, 1985, 271.

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transdermal drug delivery

historical background

advantages and disadvantages

selection of drug candidates

system designs

future developments

CARDIFF

historical background

systemic toxicity

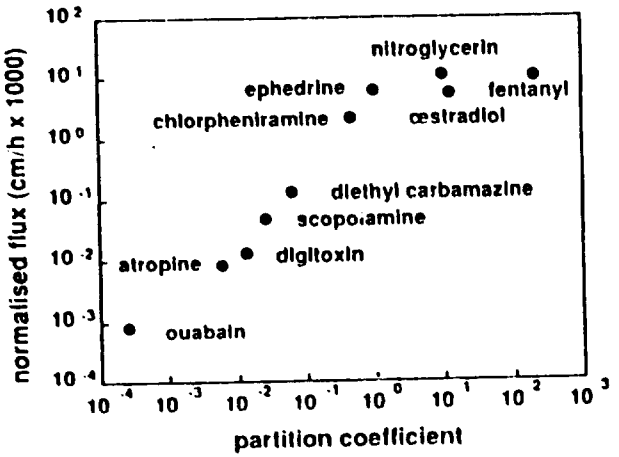
- nicotine. pesticide use in agrochemical workers
- nitroglycerin. headaches in workers in armaments factories.
- hexachlorophene toxicity after topical application to infants.

Therefore drugs placed on skin may penetrate it in sufficient amounts to induce systemic effects

ALZA Corporation (early to mid 70's) developing transdermal patch containing scopolamine for travel sickness. From research papers other drug candidates intimated. Showed importance of physicochemical properties.

CARDIFF

relationship between flux and partition coefficient



from above graph, compounds on market: scopolamine, nitroglycerin, œstradiol, fentanyl.

Others under development include: testosterone & other steroid hormones (combination patch, œstradiol plus progestogen), nicotine, clonidine, bupranolol, timolol, buprenorphine.

Why the interest?

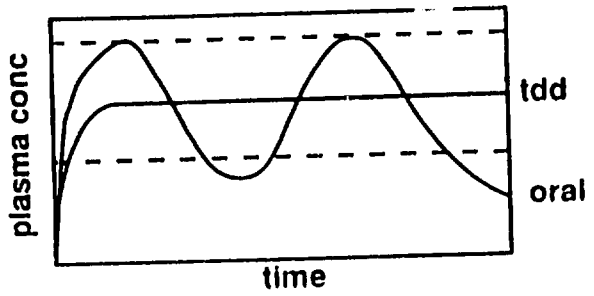
Michaels et al. A.I.Ch.E.J.,21 (1975) 985-996.

CARDIFF

Advantages of transdermal delivery i

*System controls delivery***

constant levels in the plasma



minimization of intra- & inter patient variability.

sustained drug levels in the plasma

dosing frequency reduced (1, 3, 7 days). Improved patient compliance

drug input terminated easily

instantaneous drop in plasma levels

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Advantages of transdermal delivery ii

Avoidance of first pass metabolism

lower daily doses

less variability in plasma levels

eg nitroglycerin subject to extensive first pass effects

peptide delivery using iontophoretic systems. Avoids some of the instability problems of peptides eg hydrolysis in the gi tract, first pass metabolism

Disadvantages of transdermal delivery ii

Limited to potent drugs

System not skin should provide rate control but skin is good barrier and large amounts of drug cannot permeate.

maximum daily dose ~ mg per day.

maximum attainable flux across the skin therefore important.

influenced by physicochemical properties of drug

modified by penetration enhancers.

Since skin barrier not insignificant difficult to design systems with complex release patterns.

Difficult to deliver very lipophilic drugs

build up in stratum corneum, rate of transfer into viable tissue slow, enhancers tend to act on polar drugs.

Disadvantages of transdermal delivery iii

Difficult to deliver ionised drugs

- many drugs are acids or bases, skin surface pH ~5, should not deviate significantly from this. pK of drug important
  - local delivery of cromoglycate
  - peptides, ionization state & maybe molecular size problems, does skin have molecular weight cut off?
  - mask ionization by chemical modification or ion pair formation to increase solubility in skin lipids
  - use iontophoresis

Disadvantages of transdermal delivery iv

Limited phamacokinetic clearance range

- half life of elimination: as with all sustained delivery systems not appropriate for drugs with long biological half life.
- if volume of distribution large, small quantities of drug that penetrate the skin will be dispersed in large volume and plasma concentrations will be small.

Allergic & irritant responses.

- unlike above problems these cannot be predicted and need to be investigated at very early stage of development programme.
  - drug
  - formulation components
  - exacerbation by solvent or enhancers
    - clonidine

Disadvantages of transdermal delivery v

May be metabolized.

- phase I and II enzymes present in viable epidermis.
- some enzymes present in stratum corneum eg cholesterol sulphatase.
- microflora
  - steroid esters
  - nitroglycerin
    - staph. epidermidis.

Tolerance.

- can be induced as result of constant plasma levels
- nitroglycerin (gtn) patches relabelled
- new design strategies for gtn delivery

CARDIFF

selection of drug candidates

daily dose and potency

- a few mg per day

clearance kinetics

- not long half life, small volume of distribution.

tolerance

- any predictable problems?

allergic or irritancy

- if not NCE, any adverse reactions reported?

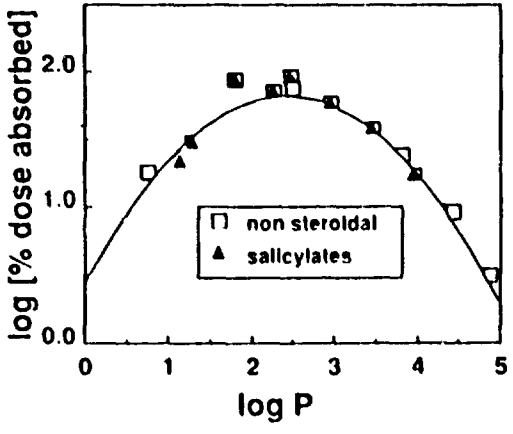
physicochemical properties.

- partition characteristics
- molecular weight
- solubility in water and oils
  - melting point

CARDIFF

partition characteristics

appears to be optimum partition behaviour for transdermal delivery. eg delivery of NSAIDs and salicylates.



usual to use octanol water values from measurement or Hansch data base.

Yano et al., Life Sci. 39 (1986)1043-1050.

CARDIFF

molecular weight

not much known about molecular weight cut off. In general diffusion coefficients are related to molecular volume and to a first approximation the diffusion rate through the stratum corneum can be estimated by assuming it is inversely proportional to the cube root of the MW.

Part of the problem of establishing the relative effects of MW,partition, solubility etc is the difficulty in designing appropriate skin diffusion experiments and being able to deconvolute the data.

CARDIFF

solubility i

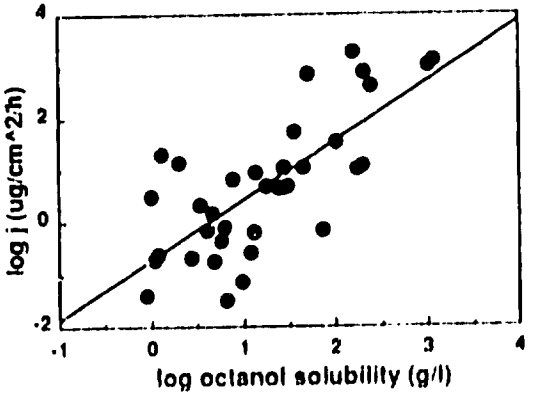
The skin has layers which are both lipophilic and hydrophilic in nature. Therefore transport into the systemic circulation will be favoured by "balanced" solubility in both oils and water.

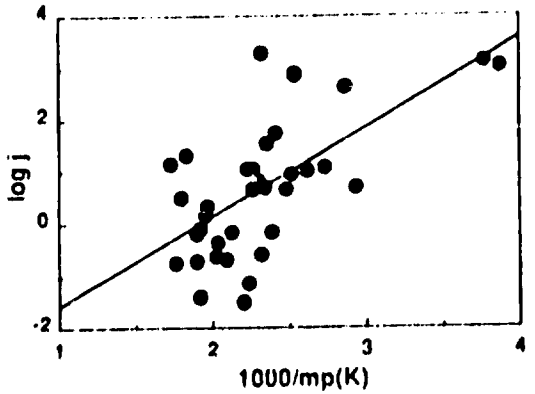
Solubility can be related, through thermodynamic parameters, to melting point. Basically the mp is a refection of the intermolecular forces which have to be broken before a substance can go into solution. In general terms the lower the mp the faster the rate of penetration through the skin.

Nitroglycerin and nicotine are both delivered transdermally, they have low mp and penetrate the skin well.

solubility ii

effects of octanol solubility & mp on drug flux for a range of substances.



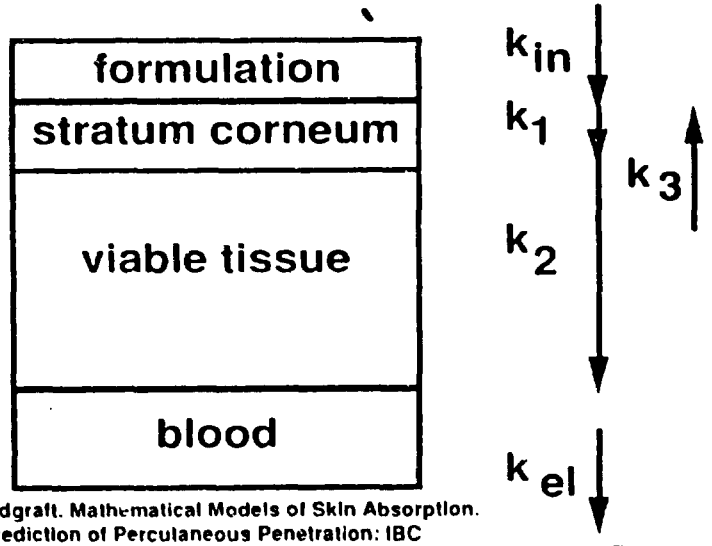


Kasting et al (1987). In *Skin Pharmacokinetics*. B.Shroot, H. Schaeffer, Eds. S. Karger, Basel p138

modelling transdermal delivery i

since the physicochemical properties of a drug are important determinants in the rate at which it crosses the skin, ought to be able to build up a model to act predictively.

$$k = D / l^2$$



J.Hadgraft. Mathematical Models of Skin Absorption. In Prediction of Perculaneous Penetration: IBC Technical Services Ltd., Eds R.C.Scott, R.H.Guy and J.Hadgraft. (1990) 252 - 262

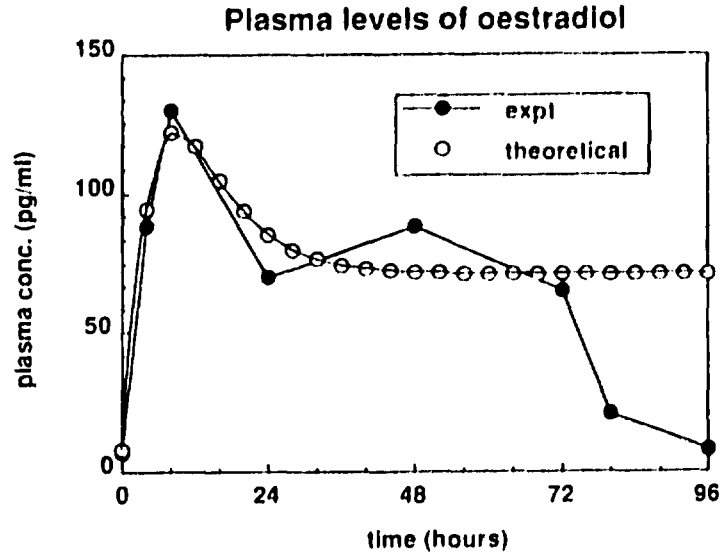
CARDIFF

modelling transdermal delivery ii

modelling achieved using STELLA on the Apple Macintosh.

useful for complex input functions, considering solubility constraints complex elimination kinetics & multiple dosing.

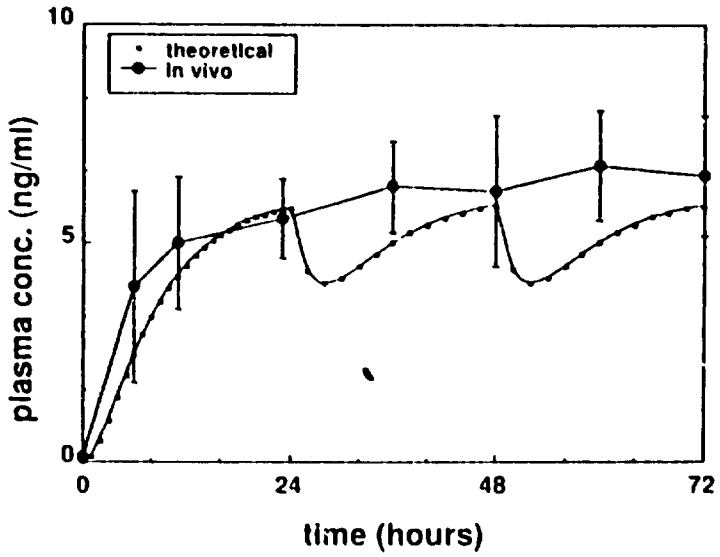
œstradiol



CARDIFF

modelling transdermal delivery iii

bupranolol



R.H. Guy and J. Hadgraft. Int. J. Pharm. (1986) 32, 159-163.

CARDIFF

in vitro skin diffusion studies

determine the maximum flux (J_{max}) of drug across excised *human* skin. This gives estimate of the input of the drug into the systemic circulation, equate this to the known clearance kinetics to give estimate of the plasma levels that can be achieved using the transdermal route.

$$J(max)\ A = Cl\ .c_p$$

provides confirmation of mathematical feasibility studies and confidence in continuation of developmental programme.

V.M. Knepp, R.H. Guy and J. Hadgraft. Transdermal drug delivery : problems and possibilities. CRC Critical Reviews in Therapeutic Drug Carrier Systems. Volume 4, Issue 1 (1987) pp 13-37.

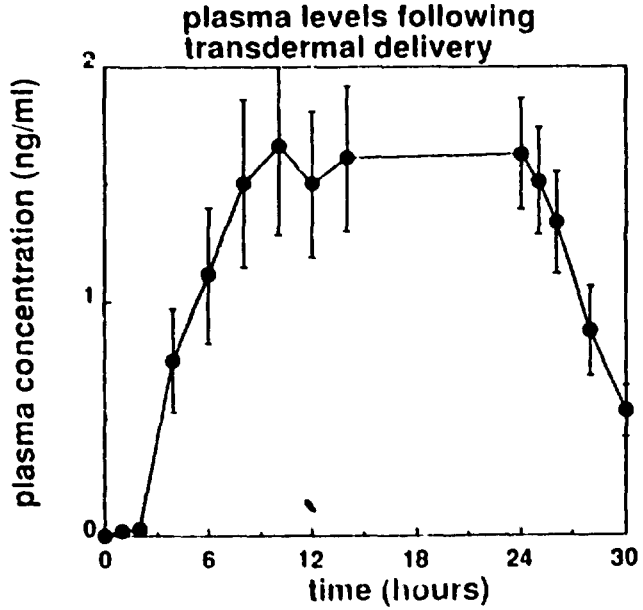
CARDIFF

in vitro - in vivo correlation : Rolipram

concept -> feasibility

mathematical model -> *in vitro* experiment

in vitro data confirm mathematical simulation and suggest that attainable levels are approximately 4 ng/ml.
volunteer study (n=6)



Hadgraft etal., Pharm. Res. (1990) 7, (12), 1307 - 1312

CARDIFF

in vitro - in vivo correlation : pre-term infants

preterm infants difficult to dose

small blood volume, poorly formed gi tract (erratic absorption)

stratum corneum not present at "birth" and therefore very permeable.

ethanol

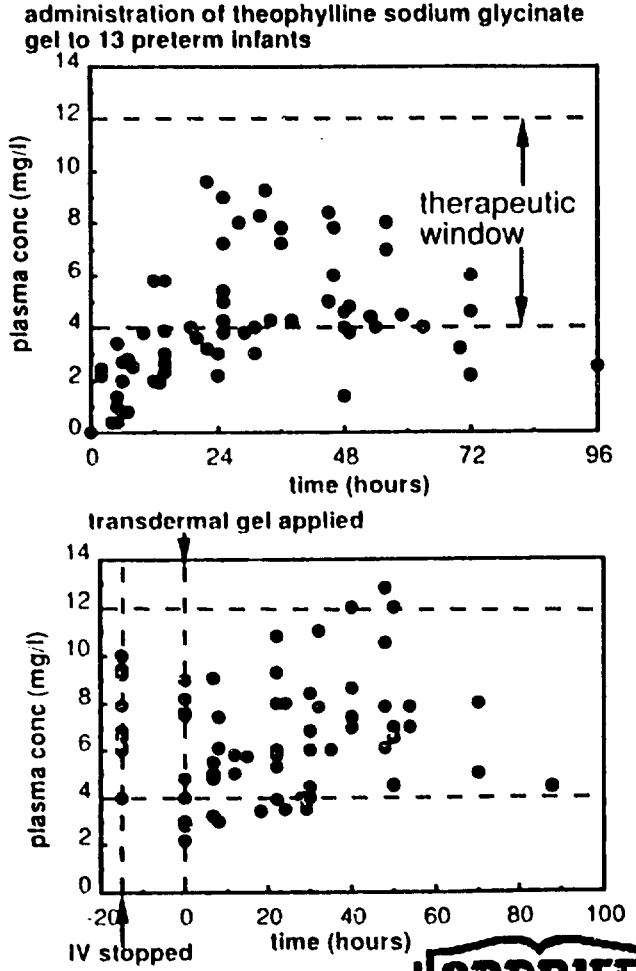
theophylline given to treat breathing difficulties but has narrow therapeutic window.

mathematical feasibility study shows that TDD can be used. Simple gel formulation produced and tested *in vivo.*

Evans et al. J. Pedriatrics 107 (1985) 307-311.

CARDIFF

in vitro - in vivo correlation : pre-term infants ii



CARDIFF

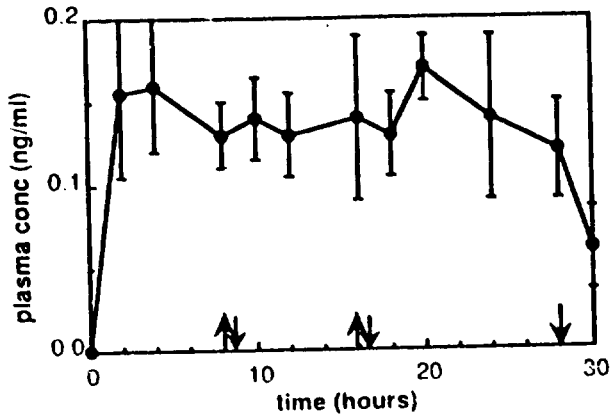
system designs

consider transdermal delivery of gtn

- *simple ointment*
- *Nitrodur ii*
  - matrix type
- *Nitrodisc*
  - microsealed drug delivery system
- *Deponit*
  - matrix, inhomogeneous drug distribution
- *Transderm-Nitro*
  - membrane moderated

CARDIFF

Simple ointment



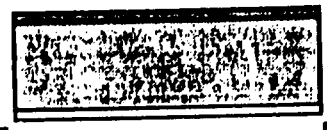
gtn plasma concentrations (mean ± se, n = 12) following application of 400 mg of 2% ointment over a skin area of 10 sq cm. at t=0, t=8 and t=16h.

Chu et al. Angiology 35 (1984) 545-552.

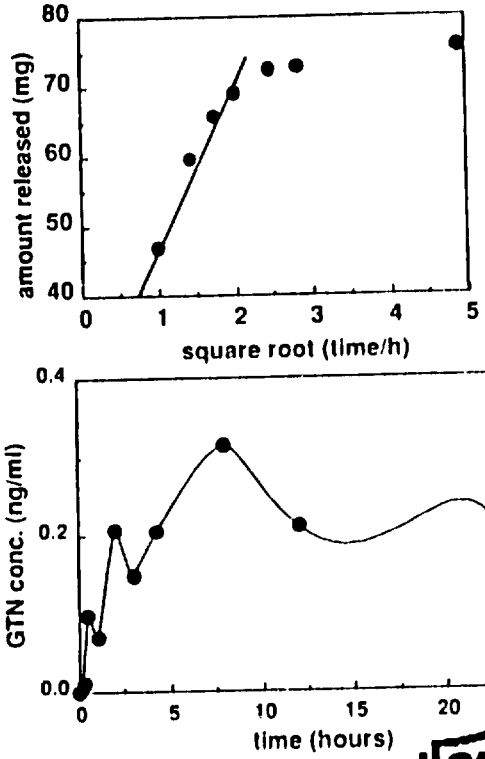
CARDIFF

Nitrodur ii

homogeneous matrix system eg acrylate



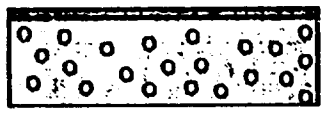
backing membrane
polymeric matrix which may be adhesive
adhesive layer



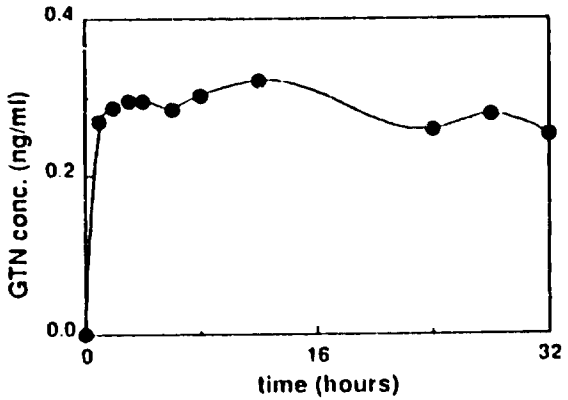
Chien. Amer. Heart J. 108 (1984) 207-216.

CARDIFF

Nitrodisc



backing membrane
polymeric matrix containing "solvent" droplets
adhesive layer



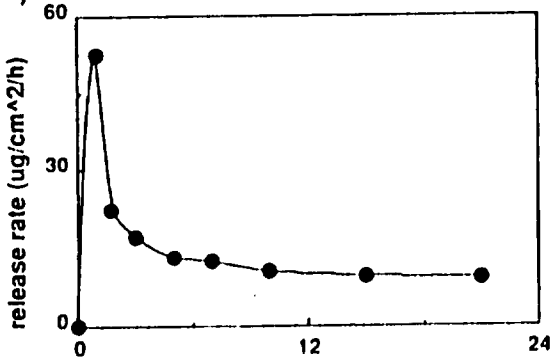
Karim. Drug Dev. Ind. Pharm., 9 (1983) 671-689.

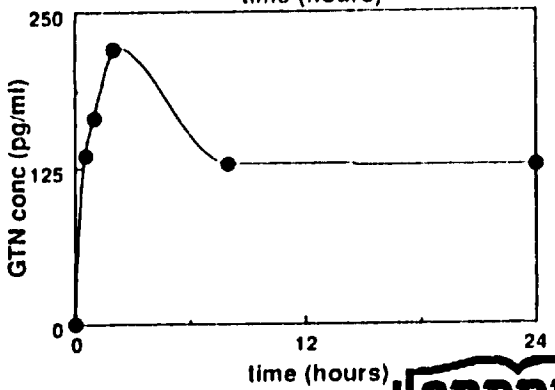
CARDIFF

Deponit

backing membrane
polymeric matrix, containing gtn in solution & on lactose.

matrix built up in several layers so that more lactose (plus gtn) is further from skin surface.



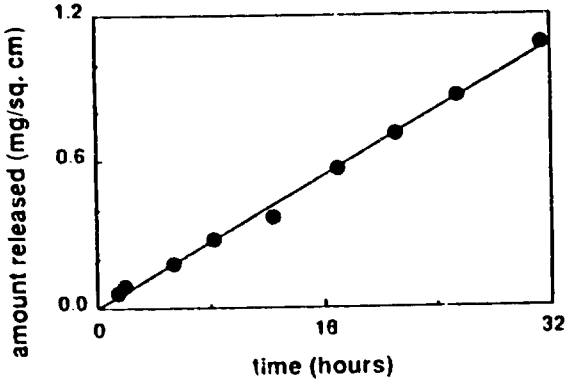


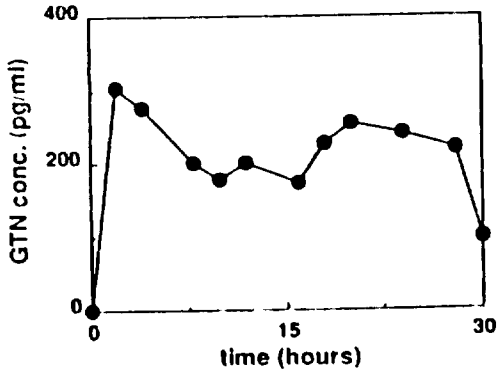
Wolff et al. Pharm. Res. 2 (1985) 23-29.

CARDIFF

Transderm-Nitro

backing membrane
drug reservoir,usually suspension
rate controlling membrane
adhesive, may be annulus round
patch edge

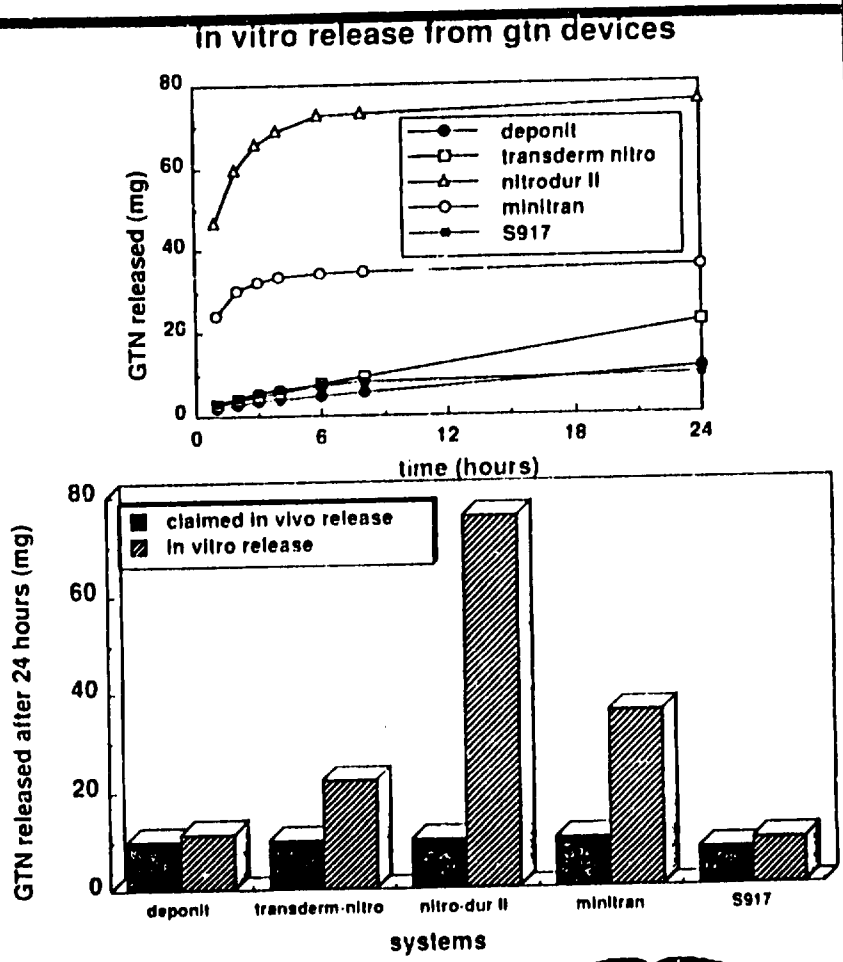




Ciu et al. Angiology 35 (1984) 545-552.

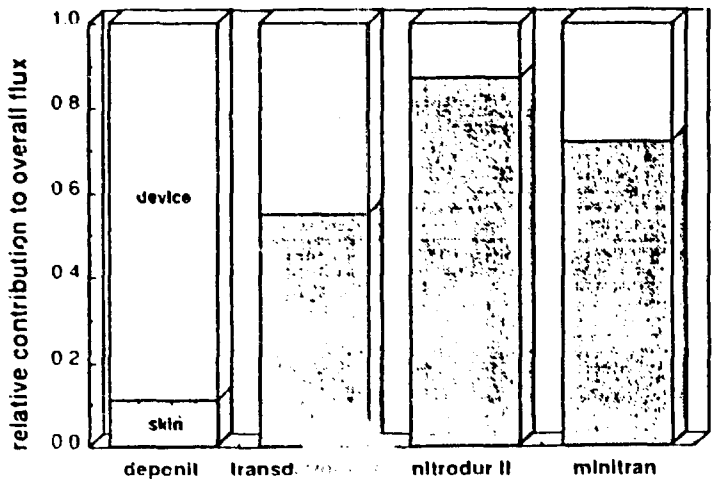
CARDIFF

System vs. skin control?



CARDIFF

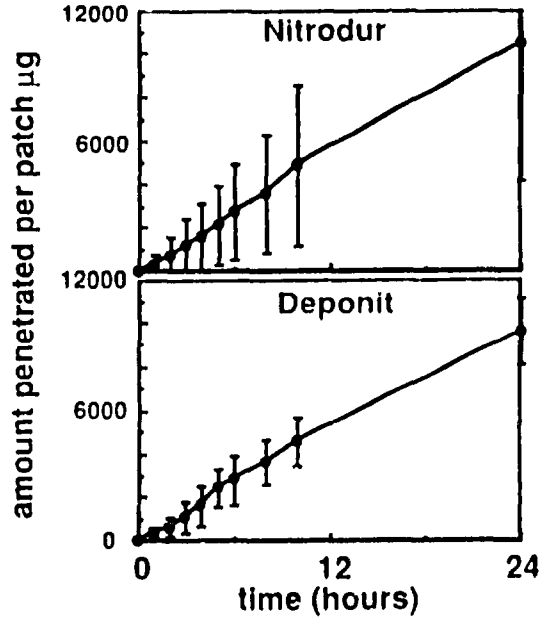
System vs. skin control?



CARDIFF

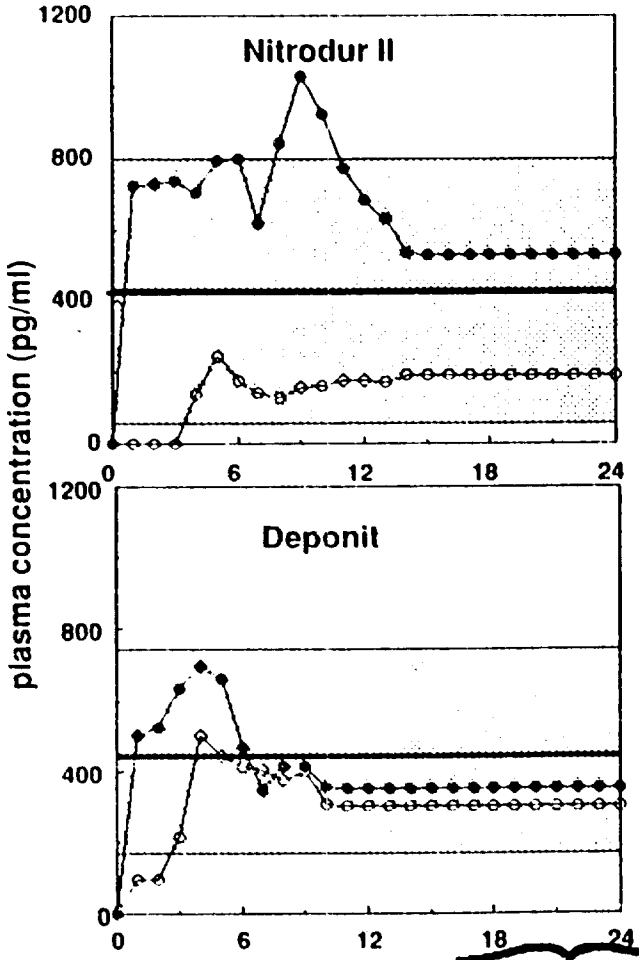
predicting gtn levels from in vitro experiments i

measure *in vitro* flux of gtn through excised human skin (dermatomed to 220μm). Compare Nitrodur II and Deponit. Calculate rates of gtn input and couple with known clearance kinetics using STELLA.



CARDIFF

predicting gtn levels from in vitro experiments ii



CARDIFF

future

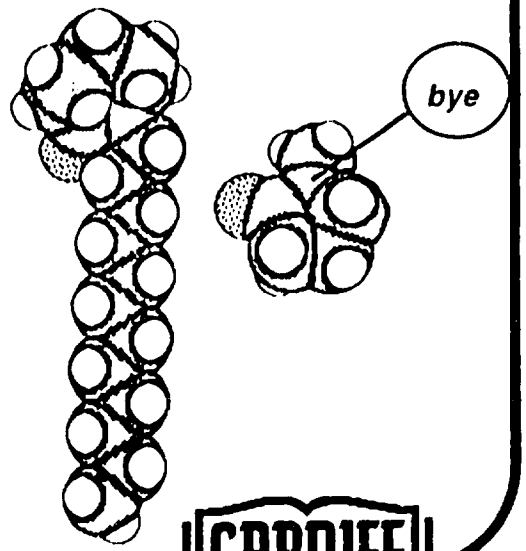
mechanisms of skin penetration / enhancement will be better understood.

will produce greater confidence in predictability.

molecular graphics will be used to predict drug lipid interactions.

modelling diseased states?

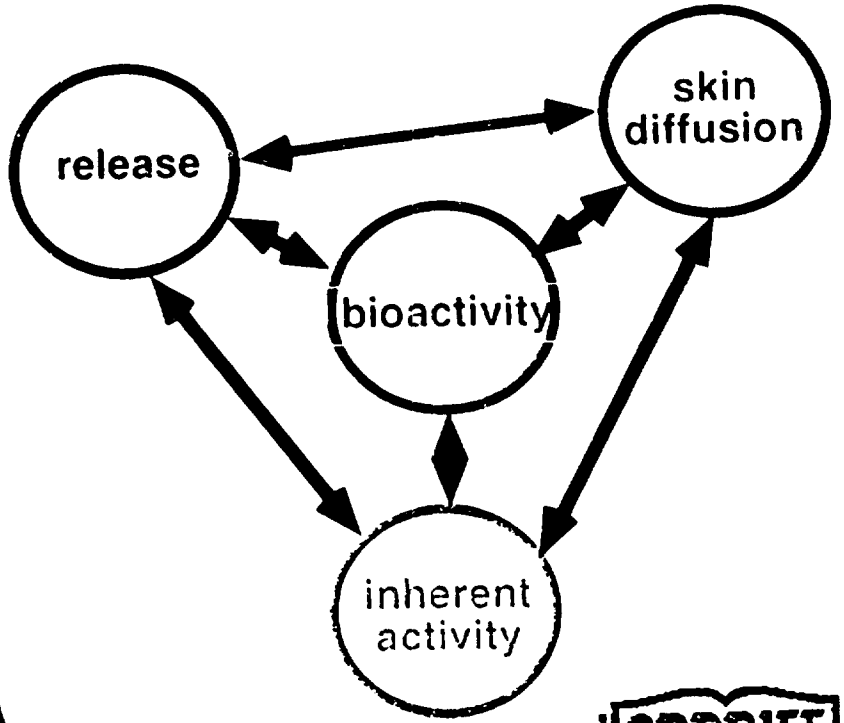
metabolism.



CARDIFF

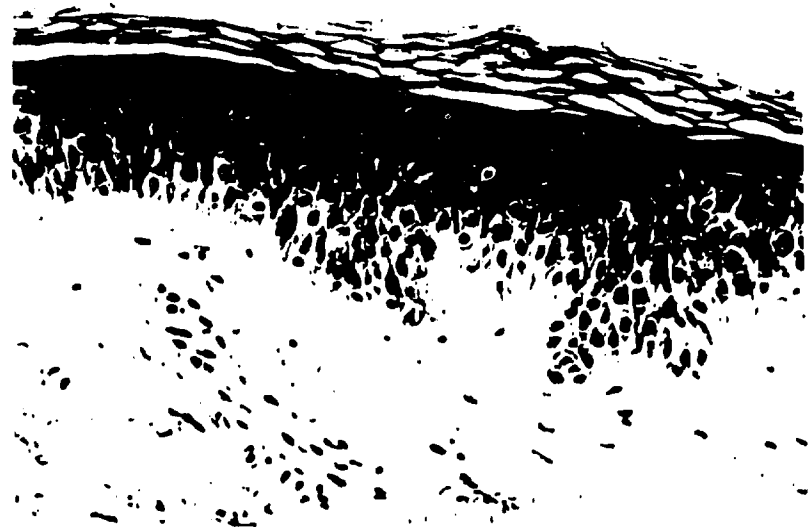
percutaneous absorption

factors affecting topical and transdermal delivery



CARDIFF

route of penetration

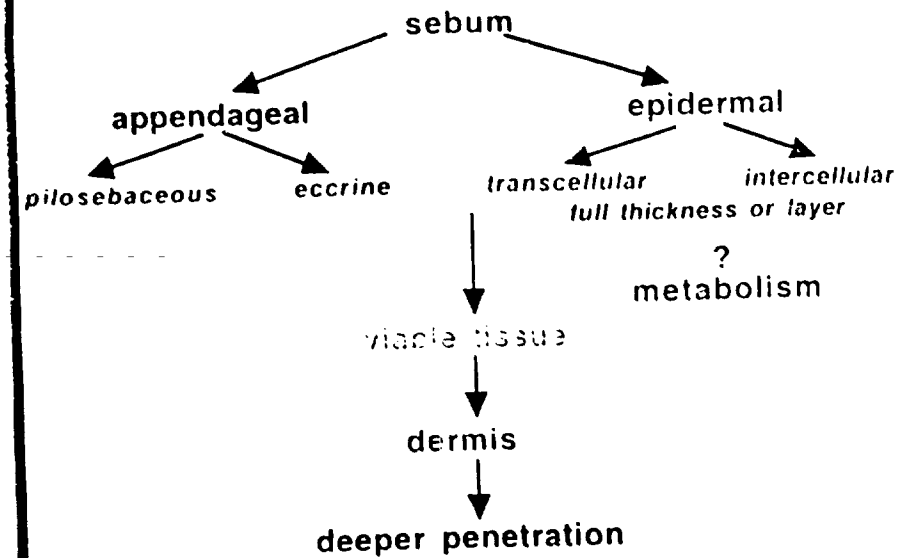


principal barrier: stratum corneum unless drug very lipophilic

how does drug cross stratum corneum?

various routes postulated

route of penetration



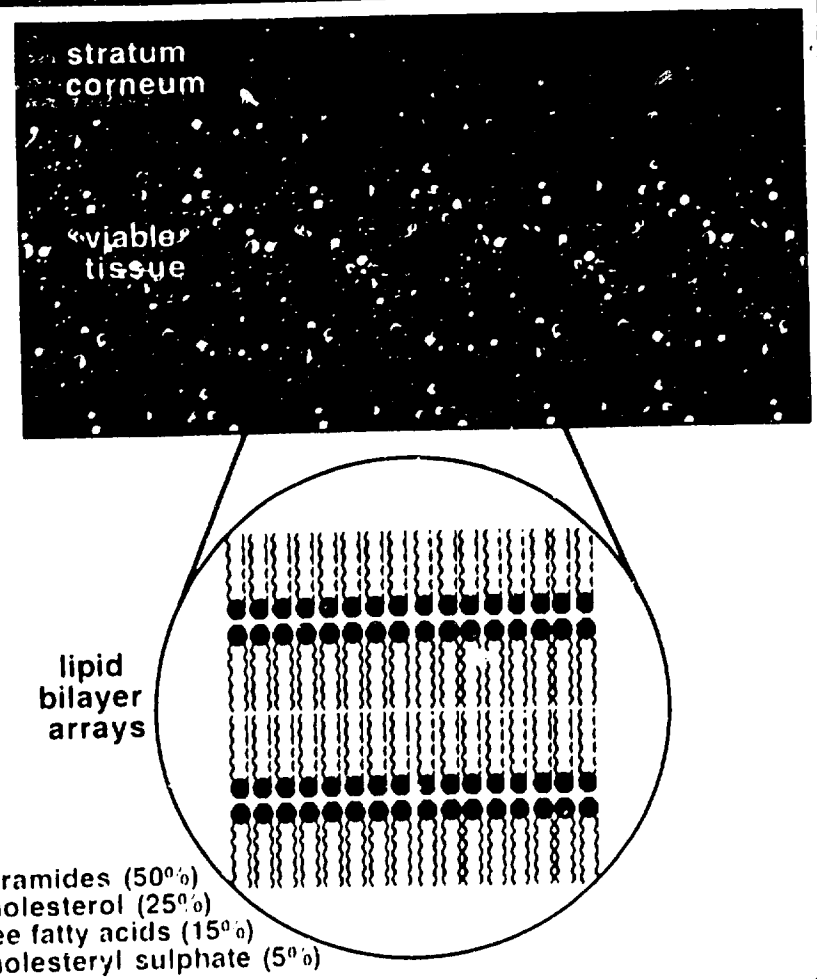
diffusional pathlength >>15μm

visualisation studies

lipids in skin

1st transfer process, partition into skin lipids

schematic representation of skin



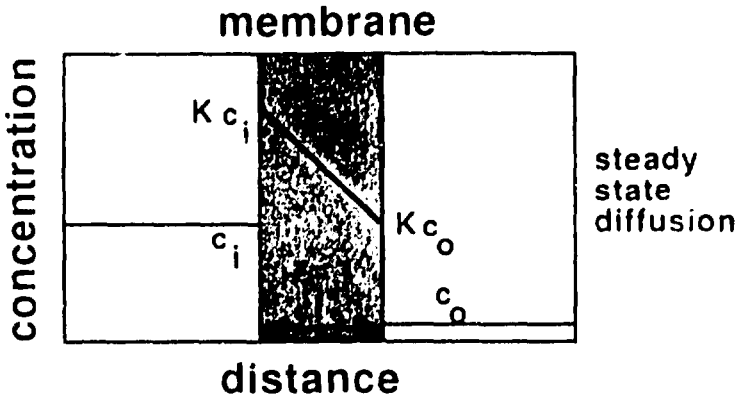
release from formulation

can control penetration eg particle size effects:

comparison of absorption of 0.025% fluocinolone acetonide from white soft paraffin, degree of vasoconstriction (n=10)

preparation	mean	range
coarse particle	0.7	0 - 2
micronized particle	1.4	0 - 2
dissolved in 5% propylene glycol	1.8	1 - 2

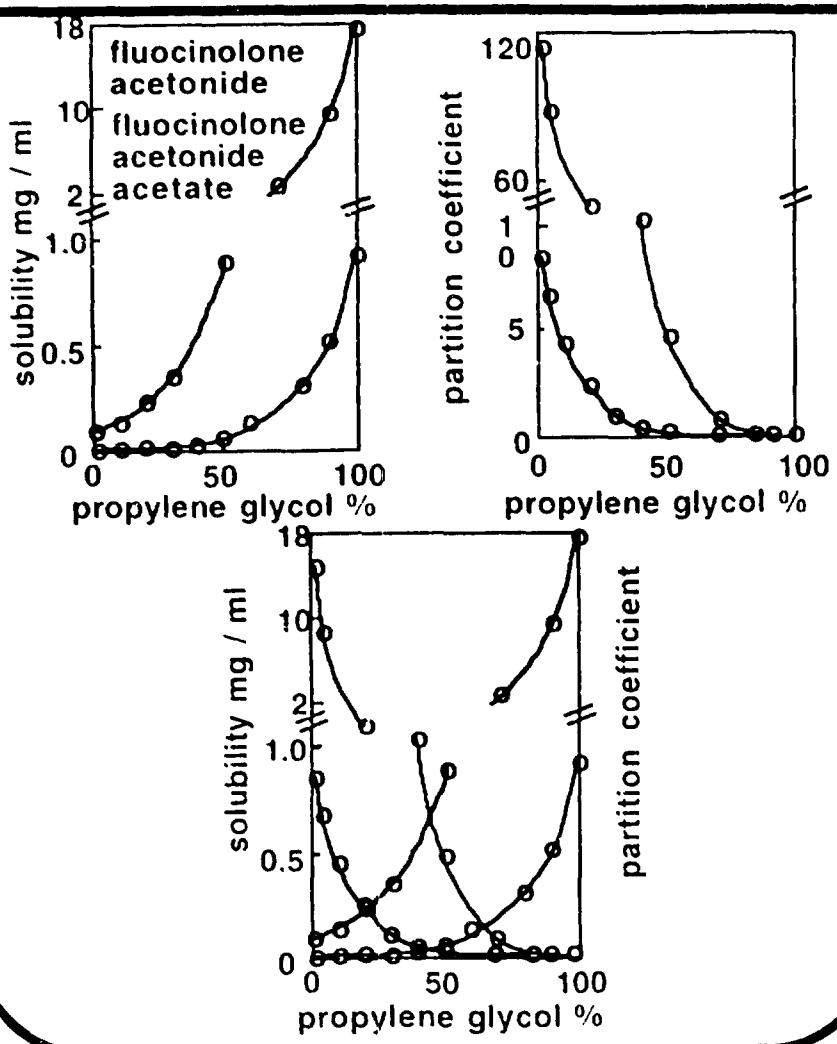
partitioning & solubility



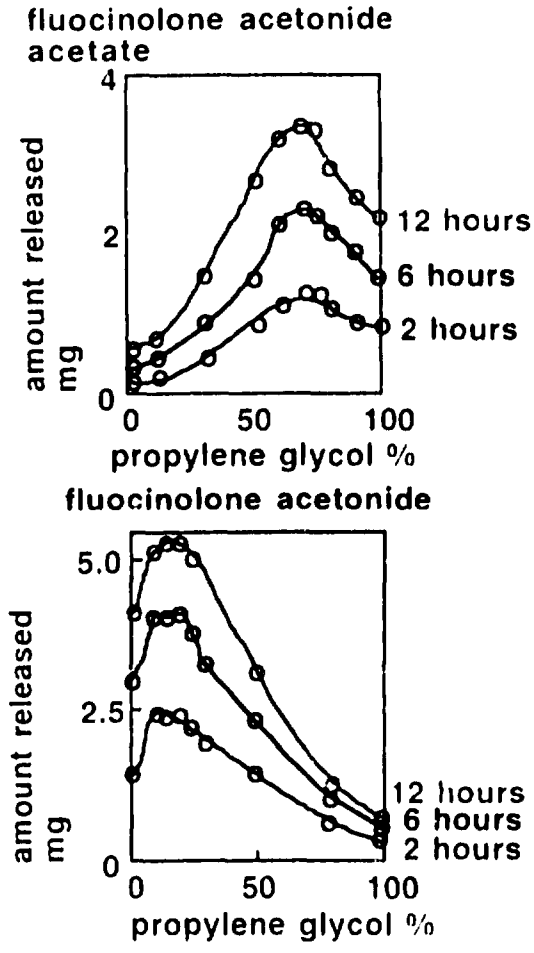
$$J = D\frac{K(c_i - c_o)}{l}$$

permeability coefficient = KD/l

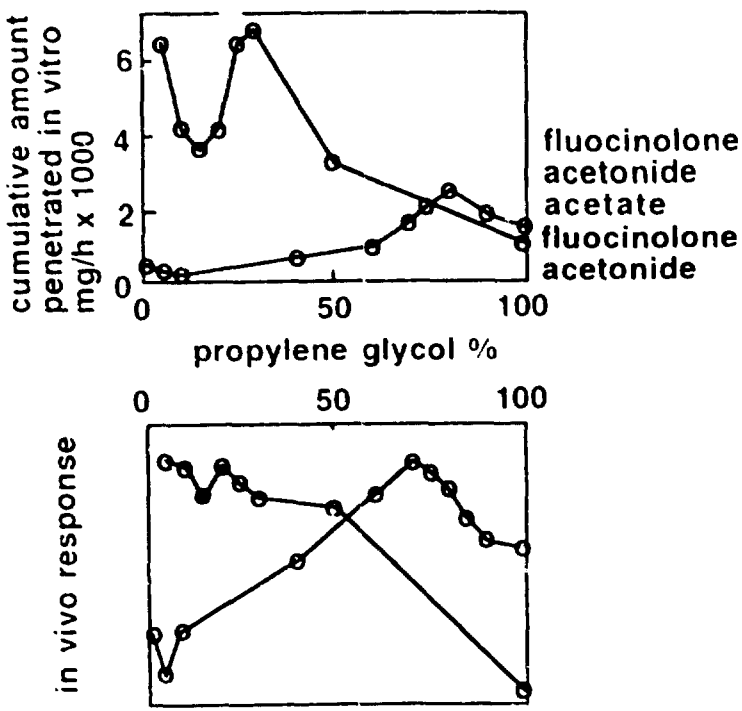
fluocinolone & fluocinonide



fluocinolone & fluocinonide ii



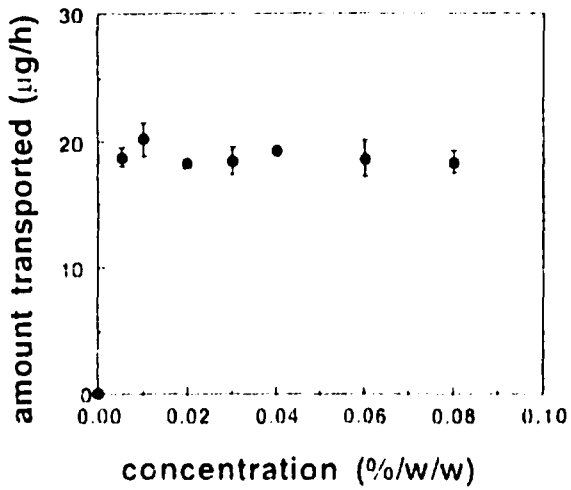
fluocinolone & fluocinonide iii



thermodynamic activity

saturated solutions have thermodynamic activity of 1. Can create different solutions that have different drug concentrations but the same degree of saturation. If the formulation components do not alter the permeability characteristics of the skin, the flux through the skin will be the same.

penetration of HCA through silastic

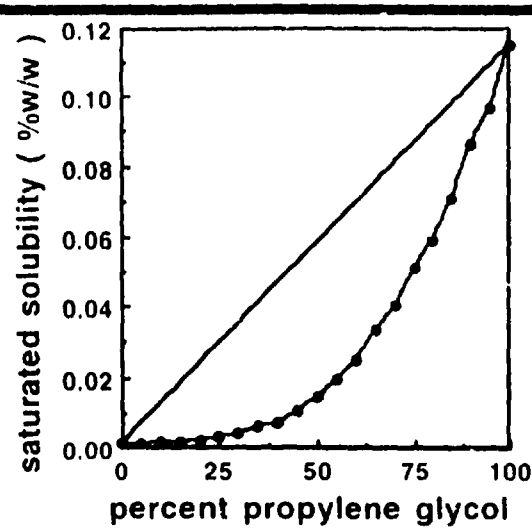


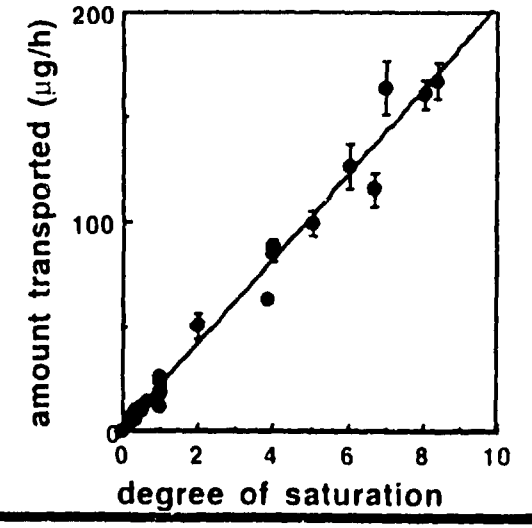
penetration of methyl nicotinate

glycerol %	MN	time of erythema (min)
0	0.04M	4.3
10	0.04M	4.4
40	0.04M	4.7
60	0.04M	4.8
80	0.04M	6.2
100	0.04M	11.5

glycerol %	MN	time of erythema (min)
0	0.07M	4.0
60	0.08M	4.2
80	0.1M	4.3
100	0.2M	4.7

supersaturated systems

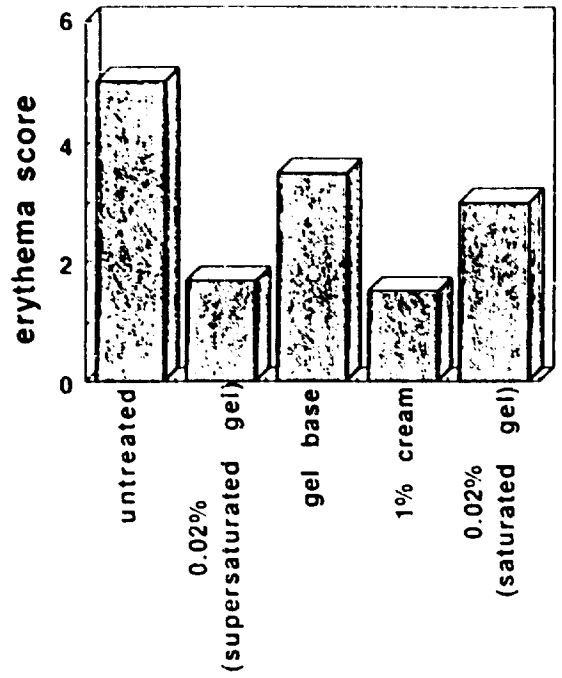




steroid blanching studies

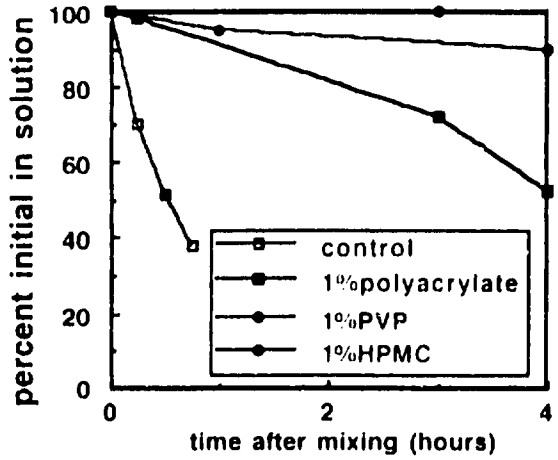
surfactant induced erythema

16 volunteers



stability considerations

need to incorporate polymer additives

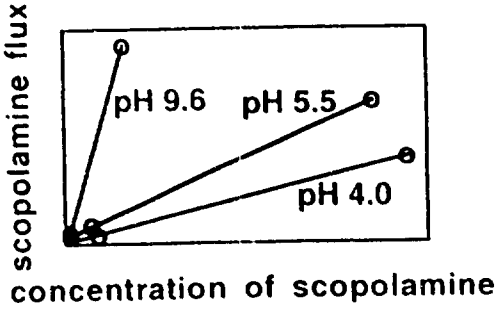


more recent studies show that HPMC can stabilise for at least 72 h.

Formulation design needs twin pack storage to keep components separate.

ionisation

skin surface pH 4.2 - 5.6

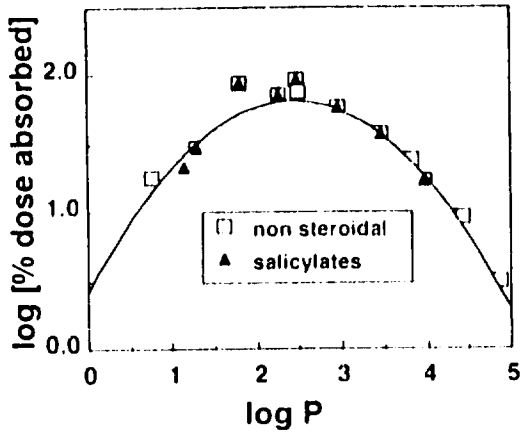


delivery of sodium cromoglycate

prodrugs

partition coefficient

appears to be optimum partition behaviour for transdermal delivery. eg delivery of NSAIDs and salicylates.



usual to use octanol water values from measurement or Hansch data base.

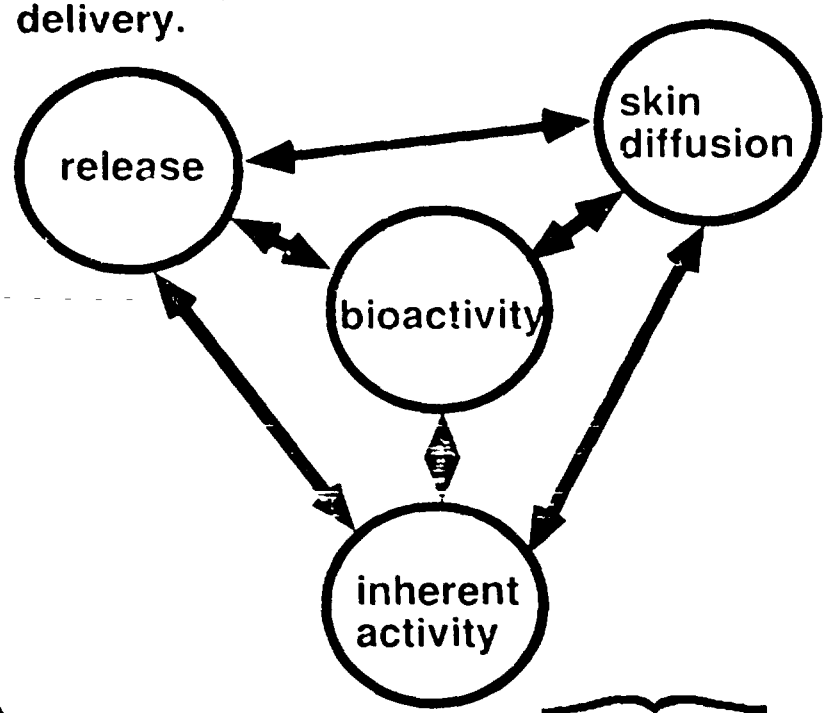
Yano et al., Life Sci. 39 (1986)1043-1050.

Penetration enhancers

Materials that favourably alter the transport properties through the skin.

Chemical & physical enhancement

Uses in topical and transdermal drug delivery.



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Penetration enhancers ii

ideal characteristics

- no inherent pharmacological action
- non toxic
- non allergenic
- specific in action
- immediate action with predictable duration
- chemical & physical compatibility with drug
- odourless, colourless, tasteless, inexpensive

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Penetration enhancers iii

Need to be able to identify what enhancer to choose. Will be a function of the physicochemical properties of the penetrant.

In order to identify this correctly should appreciate the mechanism of action of the different enhancers. This can only be achieved by first understanding the rate controlling steps in percutaneous absorption and the route of penetration.

For most materials transfer through the stratum corneum is the slowest step and therefore the one to study.

Route of penetration via lipid rich intercellular channels, see idealized model of the skin.

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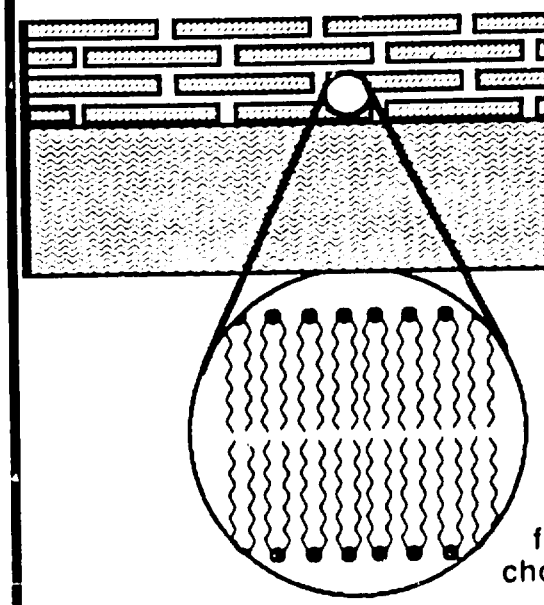
schematic model for skin



enhancers act on lipids:
a. modify disorder, affecting D
b. modify solubility properties.

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Mechanism of action summary

Can alter the following

partitioning into the skin

- intrinsic solubility of the drug in the skin lipids

diffusion through the skin

partitioning from skin lipids to viable tissue.

Most studies have concentrated on the ways in which enhancers interact with the structured skin lipids.

Methods of studying interaction with structured lipids

skin

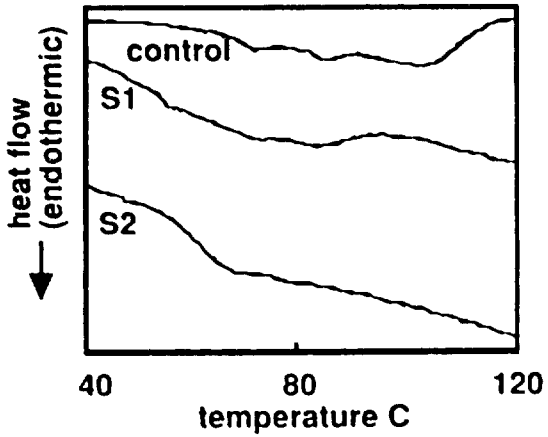
extracted skin lipids

other model structured lipids eg dppc

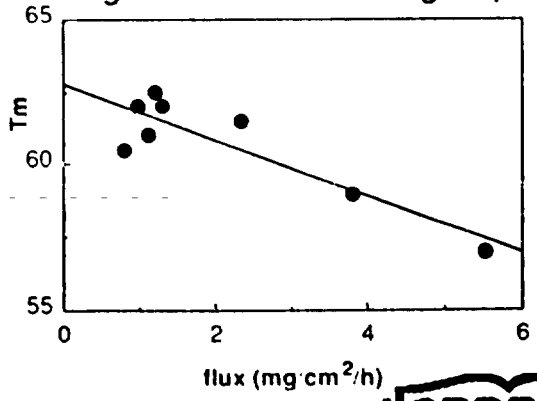
- thermal analysis
- fourier transform infra red
- nuclear magnetic resonance
- electron spin resonance
- x-ray diffraction
- neutron scattering
- fluorescence spectroscopy
- monolayer models
- liposome models

thermal analysis

differential scanning calorimetry



relationship between Tm and flux of salicylic acid, (enhancers: range of oleic acid analogues).



Golden et al J.Pharm. Sci. (1987) 76, 25.

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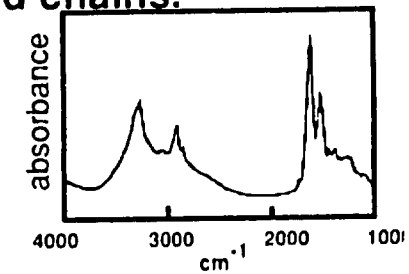
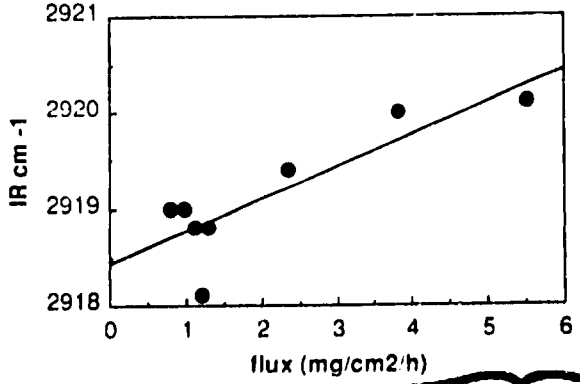
fourier transform infra red i

The CH symmetric (2850 cm-1) and asymmetric (2920cm-1) stretching frequencies reflect the disorder of the alkyl lipid chains.



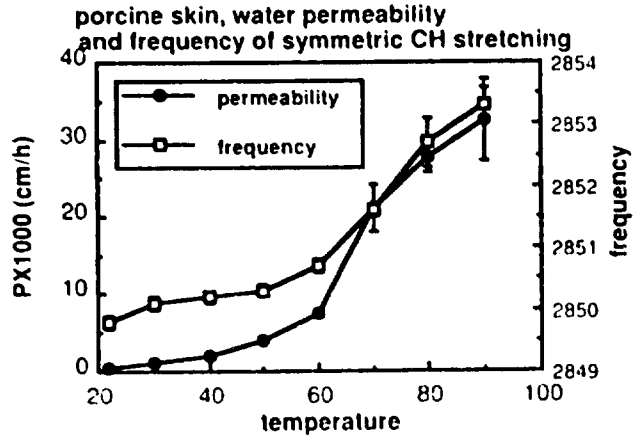
relationship between IR asymmetric CH stretching frequency and flux of salicylic acid (enhancers: range of oleic acid analogues).

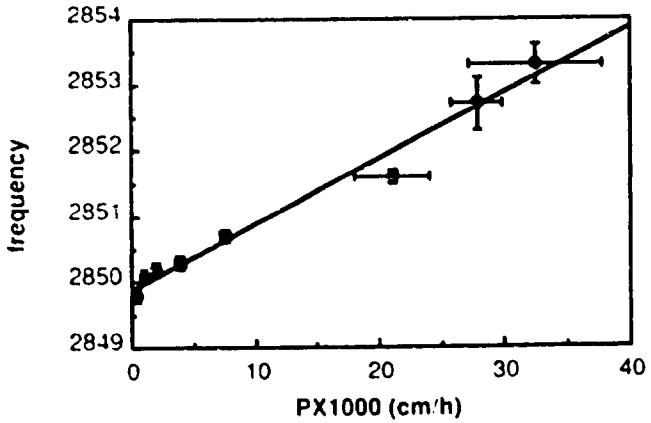


Golden et al J.Pharm. Sci. (1987) 76, 25.

CARDIFF

fourier transform infra red ii





Potts & Francoeur, Proc. Natl. Acad. Sci. 87 (1990)

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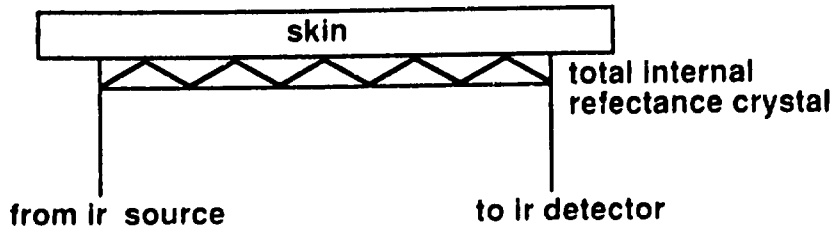
fourier transform infra red, in vivo



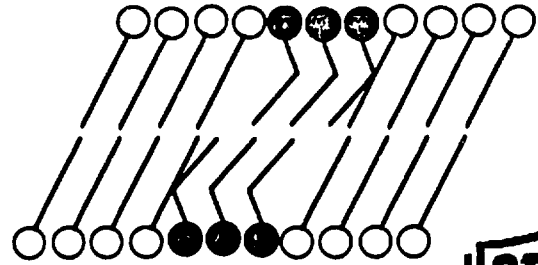
ir only penetrates superficial layers

effects of enhancers on skin lipids

concentration of drug in skin

stripping experiments: depth profile.

oleic acid experiments with perdeuterated material suggests OA pools and increased drug transfer at interfacial defects.



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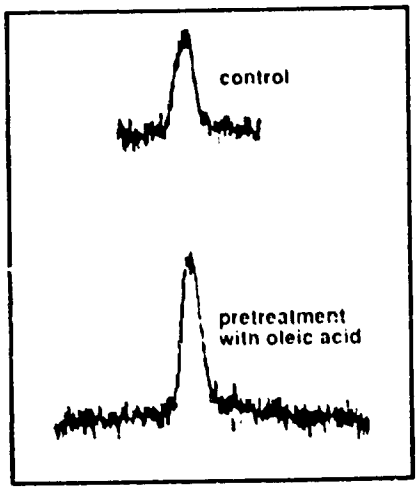
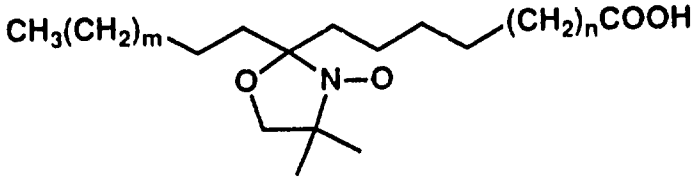
nmr

Can use nmr to monitor in vitro diffusion rates of drugs within the stratum corneum. Need a drug with appropriate "nuclear probe" eg F. Studies conducted on esters of flurbiprofen and influence of oleic acid on diffusion. Width of spectral band gives indication of D.



esr

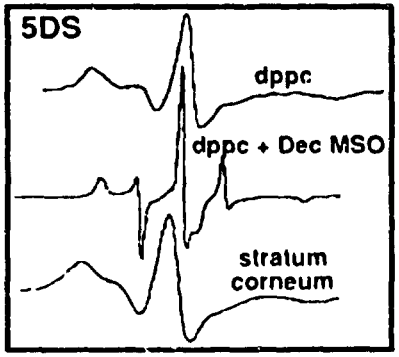
spin probes, (eg doxylstearic acid) can be incorporated into stratum corneum or model structured lipids (dppc liposomes).



the esr spectrum gives information about the molecular environment in which the spin probe is located.

By changing the position of the spin label on the doxyl stearic acid can determine the relative disordering of the acyl chains as a function of the distance from the head group.

comparison of esr spectrum, stratum corneum and dppc liposomes



from spectrum determine order parameter, a measure of the "fluidity" of the microenvironment experienced by the spin probe.

Gay et al. Int J. Pharm. 49 (1989) 39-45

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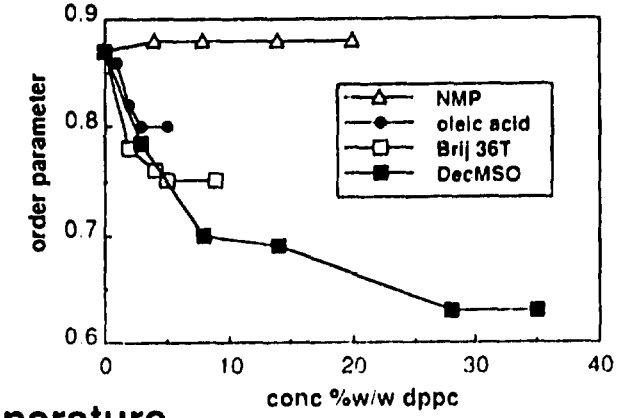
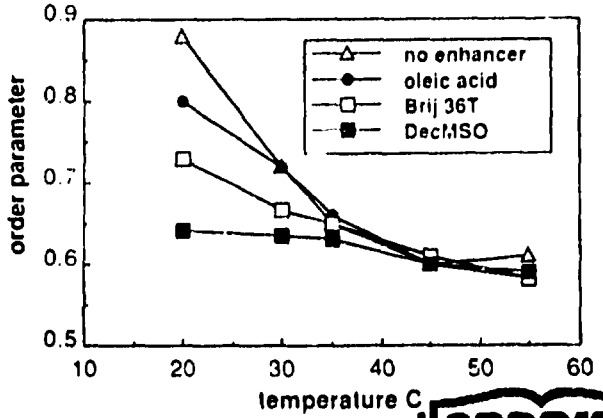
effect of enhancer conc. and temp. on order parameter (dppc)

concentration



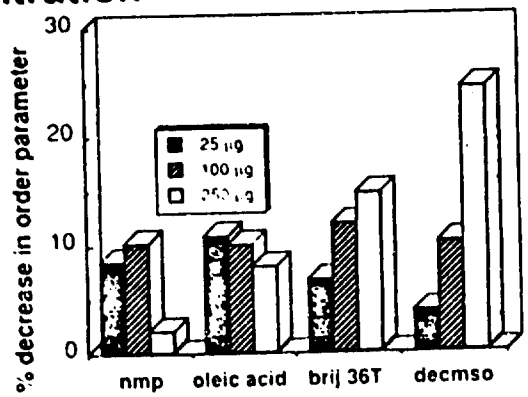
temperature



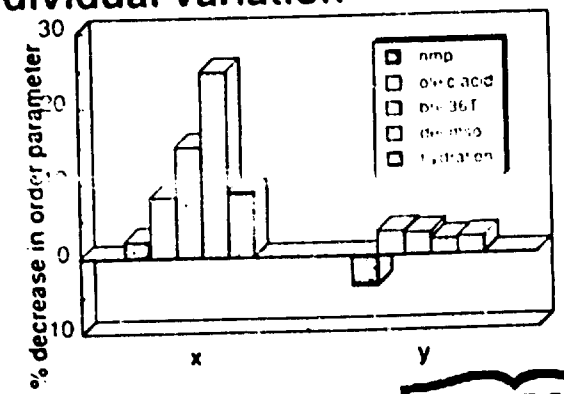
CARDIFF

effect of enhancer conc. on order parameter (stratum corneum)

concentration



inter individual variation



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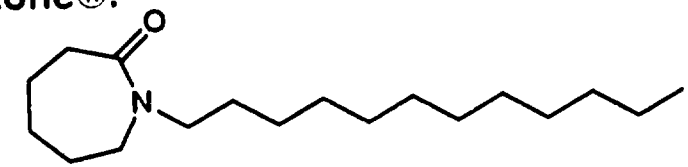
x ray diffraction and neutron scattering

x-ray diffraction on stratum corneum samples provides information about the interlamellar spacing of the lipids. This does not appear to be affected by the presence of enhancers such as Azone®.



will provide information about mechanism of insertion into lipid bilayers

neutron scattering also provides information about interlamellar spacing. It can also give information about location of deuterated materials in the bi (or mono-) layers. Experiments with dppc liposomes suggest pooling of oleic acid and "soup spoon" conformation of Azone

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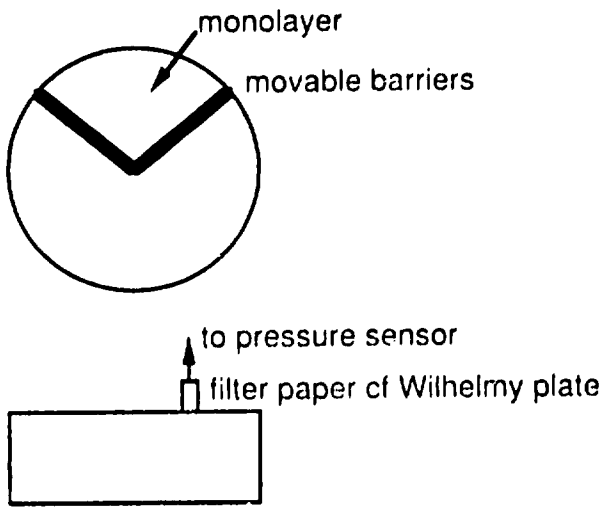
fluorescence spectroscopy

stratum corneum "doped" with fluorescent prcbes cf stearic acid spin probes. The fluorescence life times and frequencies give information about the microviscosity in which the probe is located. Similar experiments to the esr ones have confirmed in skin that there is a gradation in fluidity along the acyl chains. There is a more ordered, rigid structure close to the head groups. This suggests that molecules like Azone are effective because they reduce this ordering and facilitate diffusion of the permeant.

monolayer models i

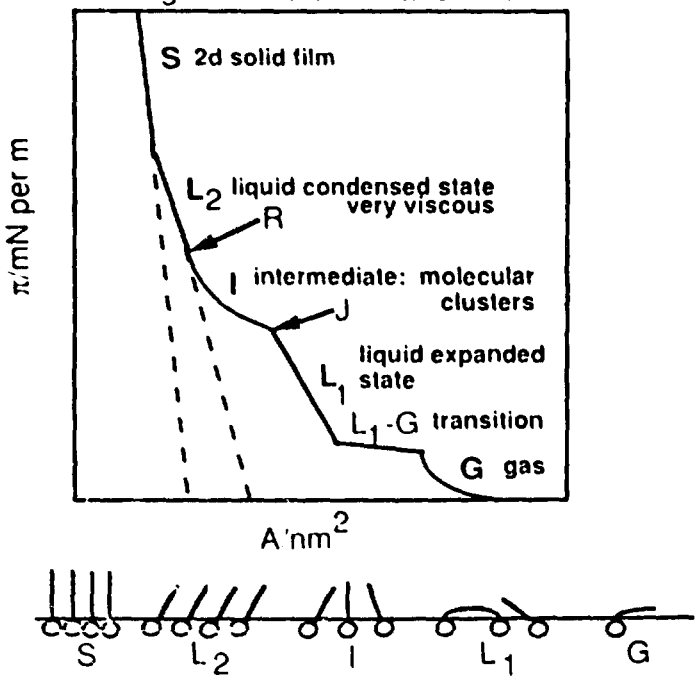
Langmuir trough with dppc monolayer. Determine π A curve and hence calculate area per molecule.

Langmuir Trough

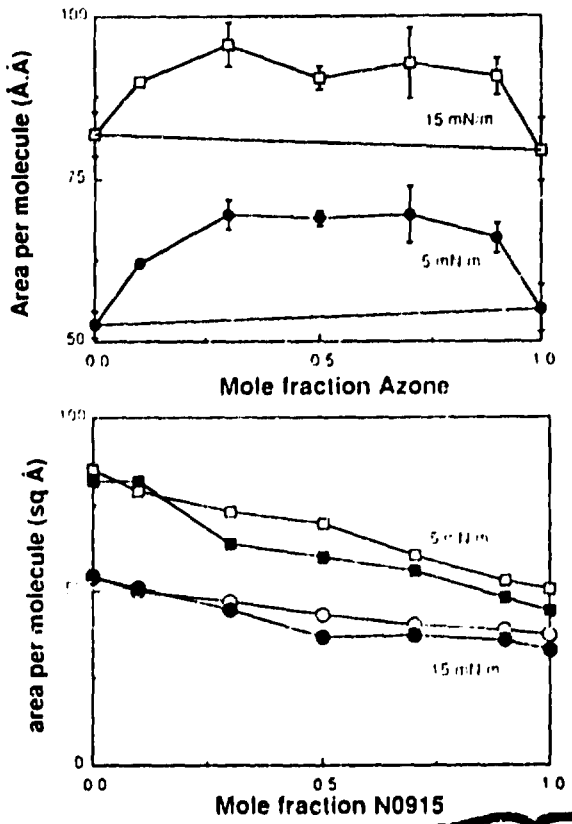


monolayer models ii

schematic diagram for tetradecanoic acid



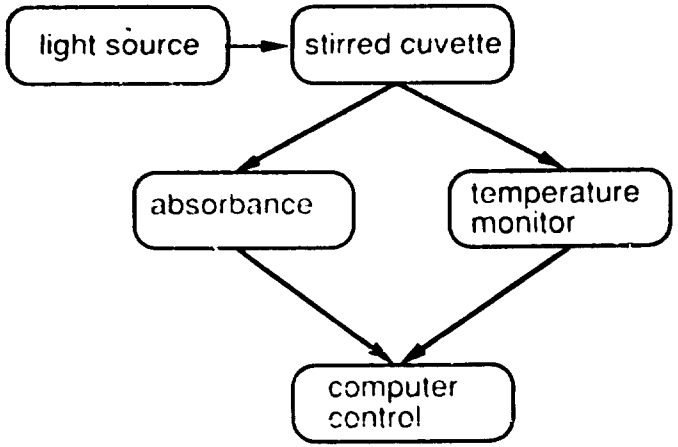
monolayer models iii



D. Lewis and J. Hadgraft, Int. J. Pharm., (1990) 65 211 - 218

membrane fluidity i

as liposomes go through their phase transition temperature, refractive index gradient changes - detected spectrophotometrically as change in scattered light.



J.C. Beastall et al. Int. J. Pharm. (1988) 43, 207-213.

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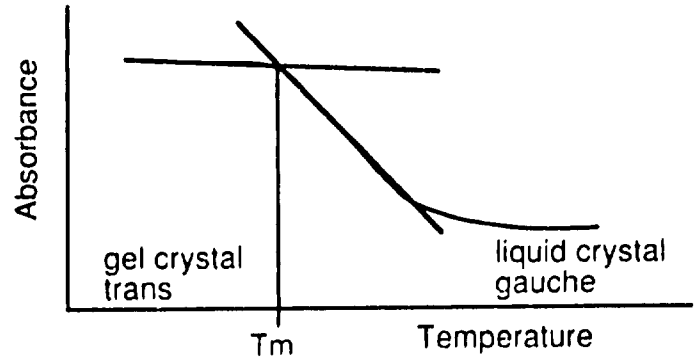
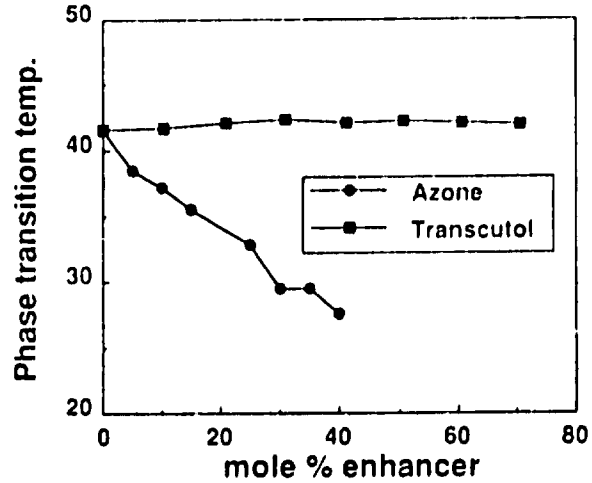
membrane fluidity ii





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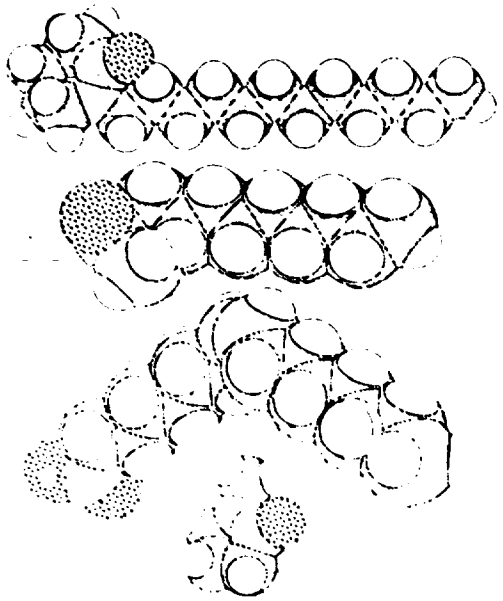
membrane fluidity iii

for compounds that act by fluidising skin lipids the degree of enhancement appears to be related to gradient of line at low enhancer concentration. One "Azone" like material raises phase transition temperature and retards the absorption of compounds through the skin.

examples

dimethyl sulphoxide
decyl methyl sulphoxide
propylene glycol
N methyl pyrrolidone
NN diethyl toluamide
Azone
Silicones
non ionic surfactants eg brij 36T
ionic surfactants
oleic acid
terpenes
Transcutol

molecular graphics & solubility parameters



shapes and polarity important determinants in how enhancers interact with lipids, solubility parameters of the solvent type enhancers will show how they may favourably modify skin lipid properties to enhance drug solubility

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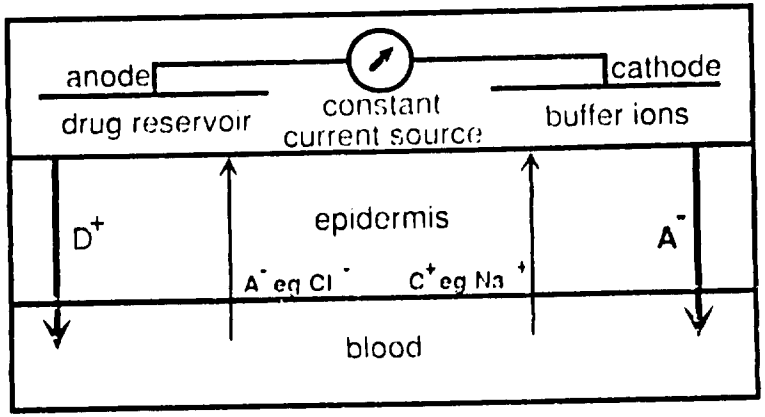
physical enhancement

iontophoresis and phonophoresis

iontophoresis, application of electric current to skin (<1 mA sq. cm, voltage ~10V.

Mechanism



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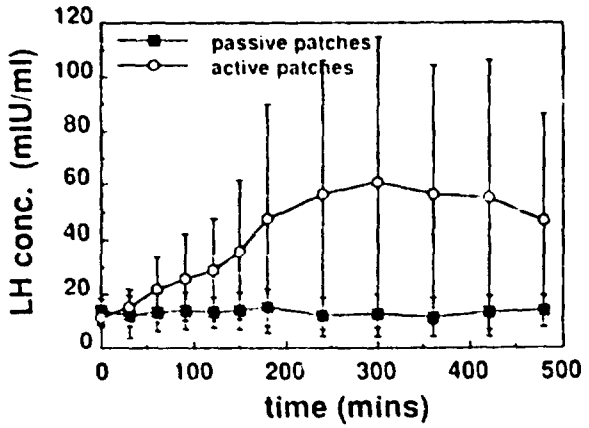
iontophoresis

delivery of leuprolide, nonapeptide (1209D). luteinising hormone releasing hormone (LHRH) analogue. Physicochemical properties suggest very small unenhanced flux across skin. Blood levels of LH determined with & without iontophoresis (70 cm2, 9V, 0.2mA)



Meyer et al., Clin. Pharm. Ther., 44 (1988) 607-612.

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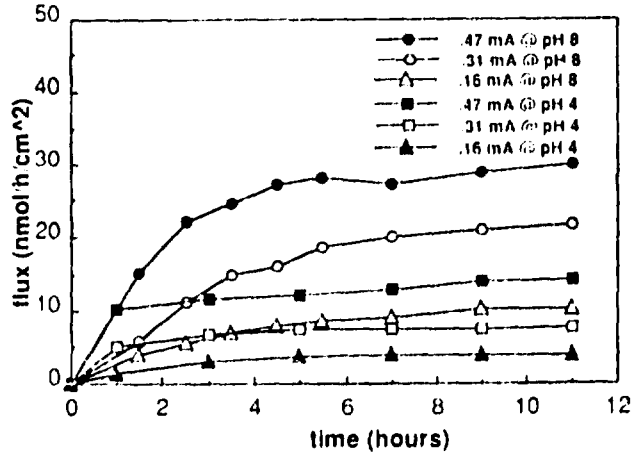
iontophoresis

patient acceptability
- mild tingling
- slight erythema

acceptability to regulatory authorities
- damage to skin

patient control
- effect of current (thyrotropin releasing hormone (TRH)



both ionized &non ionized drug have enhanced penetration - electroosmosis

Burnette et al., In Transdermal drug delivery, Eds Hadgraft & Guy, Marcel Dekker (1989) 247-291.

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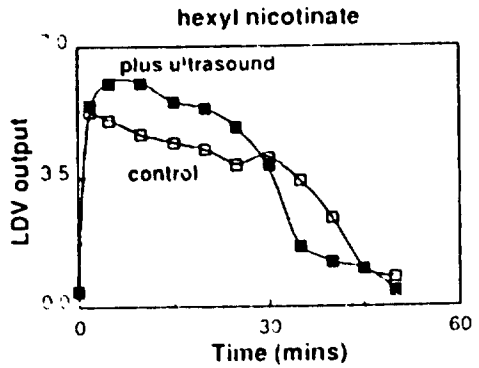
phonophoresis i

application of ultrsound to skin, may act by supplying energy to disorder skin lipids.

steroids

local anaesthetics

esters of nicotinic acid



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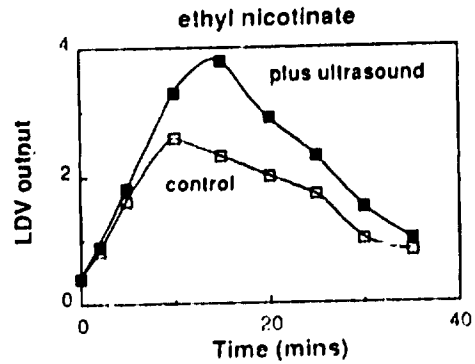

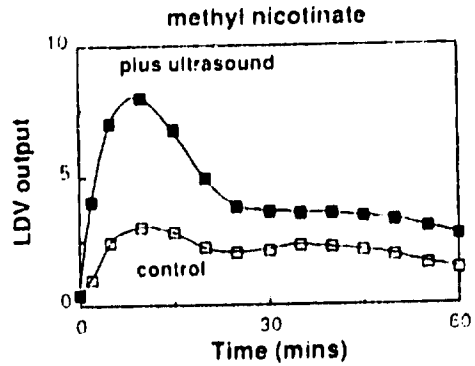

Multiple w/o/w Emulsions as Drug Vehicles

T L WHATELEY

Department of Pharmaceutical Sciences
University of Strathclyde
Glasgow, G1 1XW

CONTENTS

I. Introduction
II. Composition of the Multiple Emulsion and Stability: Influence of the Nature of Oil Phase
III. Methods Available for Stabilizing w/o/w Multiple Emulsions
IV. Mechanisms of Transport of Solutes
V. *In Vivo* Studies

I. INTRODUCTION

Multiple emulsions are emulsions in which globules of the dispersed phase encapsulate smaller droplets, which normally consist of a liquid miscible and, in most cases identical with, the continuous phase.[1] The two major types of multiple emulsions are water-in-oil-in water (w/o/w) in which the internal and external aqueous phases are separated by an oil layer and oil-in-water-in oil (o/w/o) in which water separates the two oil phases. Because of this structure and the presence of the liquid barrier, multiple emulsions are also known as liquid membrane systems. A typical photomicrograph of a w/o/w emulsion is shown in Figure 5-1. The internal droplets that usually contain the therapeutic agent are dispersed in the oil phase (the primary w/o emulsification stage) which in turn is dispersed in the external aqueous phase (the secondary emulsification stage). Drug is released either by internal droplet coalescence with the external aqueous phase or in stable systems by diffusion across the liquid membrane. Obviously if prolonged release is to be

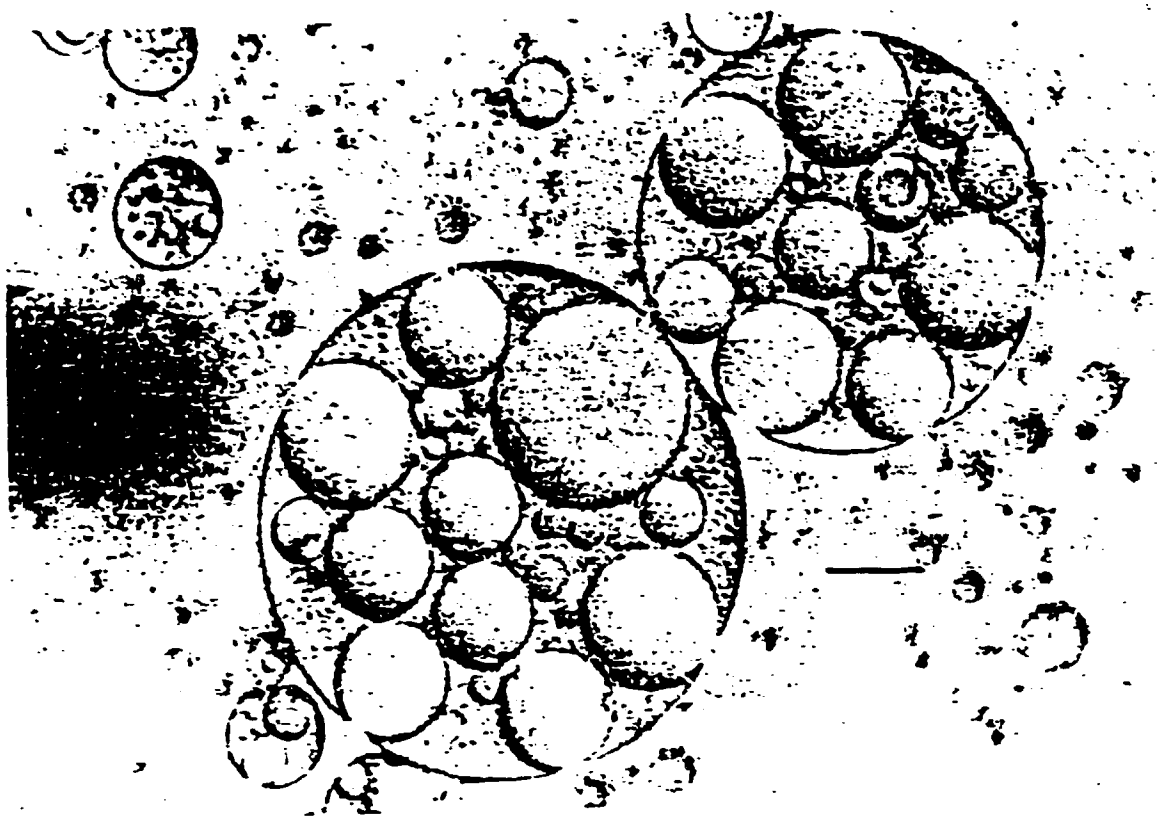

FIGURE 5-1
Photomicrograph of a typical w/o/w multiple emulsion illustrating the polydispersity of both external and internal droplets. Bar marker = 10 μm

achieved stable systems must persist. Such emulsions have the additional advantage of being less viscous than simple water-in-oil emulsions and therefore can be easier to inject.

Multiple emulsion have potential uses as adjuvant vaccines,[2] as prolonged parenteral drug delivery systems,[3-5] as sorbent reservoirs in drug overdosage treatments,[6,7] and immobilization of enzymes.[8] The nonpharmaceutical uses of liquid membranes have included the separation of hydrocarbons,[9] hydrometallurgical recovery of metal ions,[10] and the removal of toxic materials from waste water,[11] all of which depend on the selective transport of solutes across the liquid membrane. This review is concerned with the potential for the use of w/o/w systems for sustaining the action of drugs *in vivo* by prolonging their release from a depot, or for modifying the toxic effects of irritant drugs.

The literature up to 1982 relating to the formulation, mechanisms of breakdown, and factors that control the stability of multiple emulsions was recently reviewed by one of us.[1] Although multiple emulsions, especially w/o/w systems, have potential applications in controlled release systems for drug delivery and for separation procedures as liquid membranes, their use has been limited by their lack of stability.

The production of stable and reproducible systems characterized by the ability of both aqueous and oil droplets to survive successive collisions with neighbors without coalescence and breakdown is important if the potential of multiple emulsions is to be realized.

II. COMPOSITION OF THE MULTIPLE EMULSION AND STABILITY: INFLUENCE OF THE NATURE OF OIL PHASE

A number of factors have been identified as affecting the stability of w/o/w emulsions. These include their method of preparation, the nature of entrapped materials, particularly the effect of electrolytes, their phase volumes, and the concentration and type of emulsifiers.[12] The influence of the oil phase on the stability of the resulting emulsion has until recently received little attention.

Davis and Walker[13] used 6-carboxyfluorescein as an internal marker to measure the yield of multiple emulsion droplets prepared from mineral oils (liquid paraffin and squalene) and vegetable oils (sesame oil, maize oil, and arachis oil). The yield of multiple emulsion droplets was found to be dependent upon the nature of the oil and decreased in the order liquid paraffin > squalene > sesame oil > maize oil > arachis oil. Magdassi et al.[14] observed that optimal stabilities of multiple emulsions were obtained when there is a similarity between the hydrophobic part of the emulsifier and the oil phase. This suggests, not surprisingly, that the stability of multiple emulsions depends to a large extent like ordinary emulsions on the adsorption of the emulsifiers at the oil–water interface and on the properties of the adsorbed layer at that interface. The nature of the oil phase affects the nature of the interfacial film and together these are of crucial importance in determining globule size and stability.

The stability of multiple w/o/w emulsions prepared with isopropylmyristate and a range of pure hydrocarbons has been studied by the present authors.[15] Both the internal aqueous droplets and the multiple oil drops varied in size according to the nature of the oil used in preparing the emulsions and a correlation between the interfacial tension at the oil–water interface and the droplet size was reported. Figure 5-2 shows the change in number of multiple oil drops with time. There is no significant change in number of multiple oil drops prepared with hydrocar-

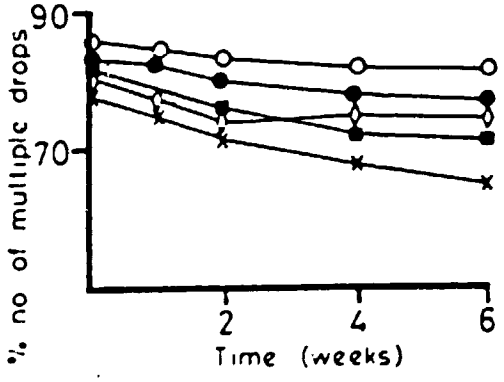

FIGURE 5-2
Stability of multiple w/o/w emulsions prepared with hydrocarbon oils: (○) octane, (●) dodecane, (■) cyclohexane, (◇) hexadecane, (×) toluene. Emulsions were prepared with 2.5% span 80 and 0.2% BSA as primary emulsifiers and 1% polysorbate 80 as secondary emulsifier. From Ref. 15.

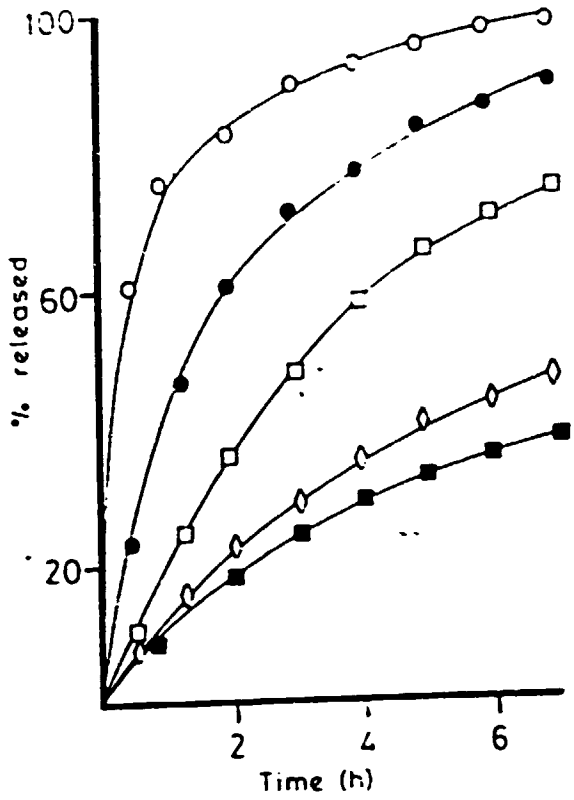

FIGURE 5-3
Release of 5-FU from multiple w/o/w emulsions. The emulsions contained 2.5% Span 80 and 0.2% BSA as primary emulsifiers, with 5-FU (1 mg ml^{-1}) in the internal phase and the following oil phases: (■) octane, (◇) cyclohexane, (□) dodecane, (●) hexadecane, (○) toluene, 1% polysorbate 80 served as the secondary emulsifier From Ref. 15.

bons indicating stability in these systems. *In vitro* release studies of 5-fluorouracil (Figure 5-3) from w/o/w emulsions showed that release was faster for those systems with smaller internal droplets due to increased interfacial area.[15]

III. METHODS AVAILABLE FOR STABILIZING w/o/w MULTIPLE EMULSIONS

In spite of the potential applications of multiple emulsions, there have been very few reports in the literature of attempts to improve their stability. The principal modes of breakdown involve coalescence of internal or external droplets, expulsion of the internal droplets, or osmotic swelling or shrinkage.[16] The methods in the literature can be classified into three: gelation of either internal or external phases, formation of interfacial complex stabilizing films, and the determination of the optimal HLB of surfactant mixtures for the primary and secondary emulsification steps, each of these being combined sensibly with attempts to minimize the osmotic gradient across the liquid membrane.

A. Gelation of Either Internal or External Water Phase

Following an earlier attempt to improve the stability of a w/o/w emulsion by use of an "oil" that solidified at room temperature (e.g., octadecane), Florence and Whitehill[17] investigated the possibility of stabilizing multiple systems by forming a polymeric gel in either the internal or external aqueous phase[15] using a nonionic surfactant—a poloxamer that could be polymerized *in situ*.[18] Irradiation of the system resulted in the bulk polymerization of the stabilizer (the poloxamer), with the consequence that the multiple globules became enmeshed in a network of cross-linked surfactant molecules. Polyacrylamide has also been used to demonstrate this technique, but less toxic systems are required for pharmaceutical use. The main disadvantage of the use of γ irradiation is that the drug has to be incorporated at the primary emulsification step and is therefore exposed to γ irradiation. To overcome this difficulty a series of acryolyl derivatives of poloxamer (Pluronic) surfactants (including Pluronic L44) was found to increase the stability of w/o/w emulsions following cross-linking by irradiation at the interfacial polymerization oil–water interface.[19] The primary w/o emulsion in a typical formulation contained 5% Span 80 in isopropylmyristate and 0.9% of the diacryloyl derivative of Pluronic L44 in the aqueous phase with 4% $(NH_4)_2S_2O_8$ as initiator. Polymerization in the external aqueous phase increases its viscosity, increasing stability by reducing the close approach of particles. However, a polymerized interfacial membrane has been found to be more effective in providing stability.

B. Formation of Interfacial Complex Films

During recent years, work in our laboratory has focused on the formation of stable interfacial films. Surfactants from a series of chemically modified polymerizable, nonionic surfactants can form *in situ* cross-linked membranes after adsorption at the o/w interface.[20] The resulting interfacial membrane was found useful for maintaining the stability of the liquid membrane. This approach, like the previous one, suffers from the disadvantages of possible drug degradation during the polymerization process and/or the presence of residual polymerization initiator in the final product.

Another approach to the formulation of stable interfacial membranes for improving the stability of w/o/w emulsions is by interfacial interaction between a macromolecule such as albumin, or a polyanion such as polyacrylic acid in the internal aqueous phase and a lipophilic nonionic surfactant in the oil phase.[21,22] The formation of an interfacial complex at the primary water–oil interface was found to enhance the stability of the w/o/w emulsion and to cause a delayed release of solutes entrapped in the internal phase of the emulsion. Here, increased stability allows the film to withstand thinning, but unfortunately this stable film acts as an inadequate barrier to solute transfer when it has thinned. A range of PEO–PPO–PEO block copolymers (poloxamers) was found to form a stable interfacial membrane with either polyacrylic acid or albumin.[21,22]

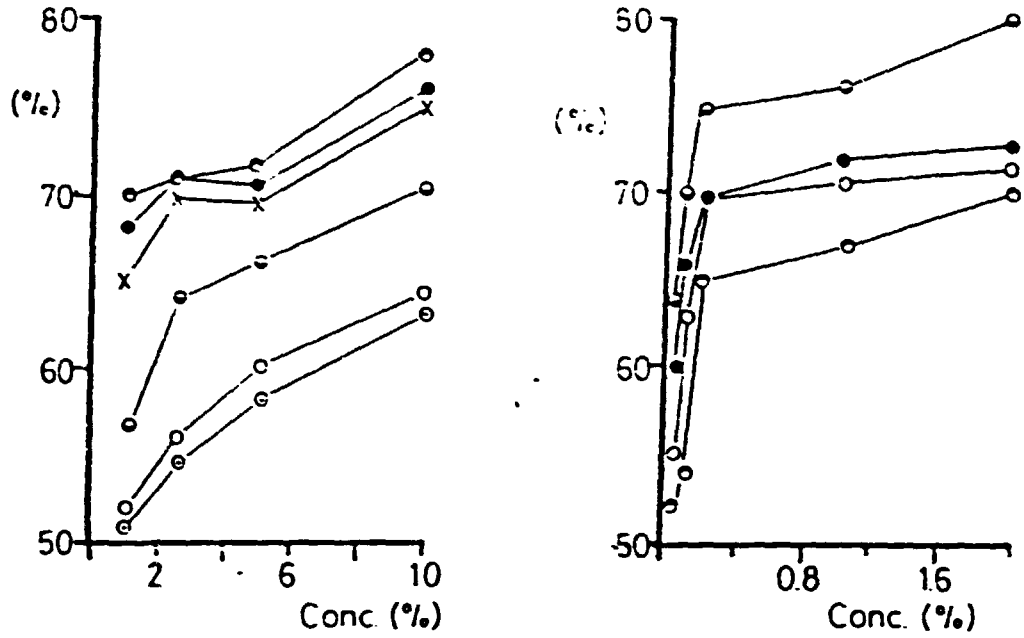

FIGURE 5-4 (left)
The effect of Span 80 concentration on the entrapment of NaCl in w/o/w emulsions of constant BSA concentration in the internal aqueous phase: (⊙) no BSA; (○) 0.05% BSA; (◓) 0.1% BSA; (×) 0.2% BSA; (●) 1% BSA; (◓) 2% BSA. From Ref. 23.

FIGURE 5-5 (right)
The effect of BSA concentration on the NaCl entrapment in w/o/w emulsions: (◓) 1% Span 80; (○) 2.5% Span 80; (●) 5% Span 80; (◒) 10% Span. From Ref. 23.

In a multiple w/o/w emulsion stabilized by interfacial interaction between Span 80 and albumin, the entrapment efficiency of a w/o/w emulsion was studied by incorporating NaCl in the internal phase of the emulsion.[23] Increasing the concentration of Span 80 from 1 to 10% (Figure 5-4) over all the bovine serum albumin (BSA) concentrations increased the amount of NaCl entrapped, which appears to parallel the observed increase in the number of internal aqueous droplets in the emulsions. The effect of BSA on the quantity of NaCl entrapped is not linear (Figure 5-5): there is a region of rapid increase in yield (at all Span 80 concentrations) within the range of 0.05–0.2% BSA. This is followed by only a gradual increase in yield at BSA concentrations above 0.2%. The quantity of NaCl initially entrapped (Figure 5-6) increased with increasing concentration of NaCl, probably due to the formation of a rigid interfacial layer as a result of the salting out effect of NaCl at the inner water–oil interface.

Two competitive effects of NaCl in the internal phase of w/o/w emulsion can be distinguished: because of the "salting out" effect, NaCl has a stabilizing influence on w/o/w emulsions. However, the presence of NaCl in the internal phase of the emulsion could create an osmolarity difference between the two aqueous phases of the emulsion that would induce osmotic flux of water from the external phase to the internal phase of the emulsion. The influx of water into the internal droplets results in the continuous thinning of the oil layer. Successive growth of

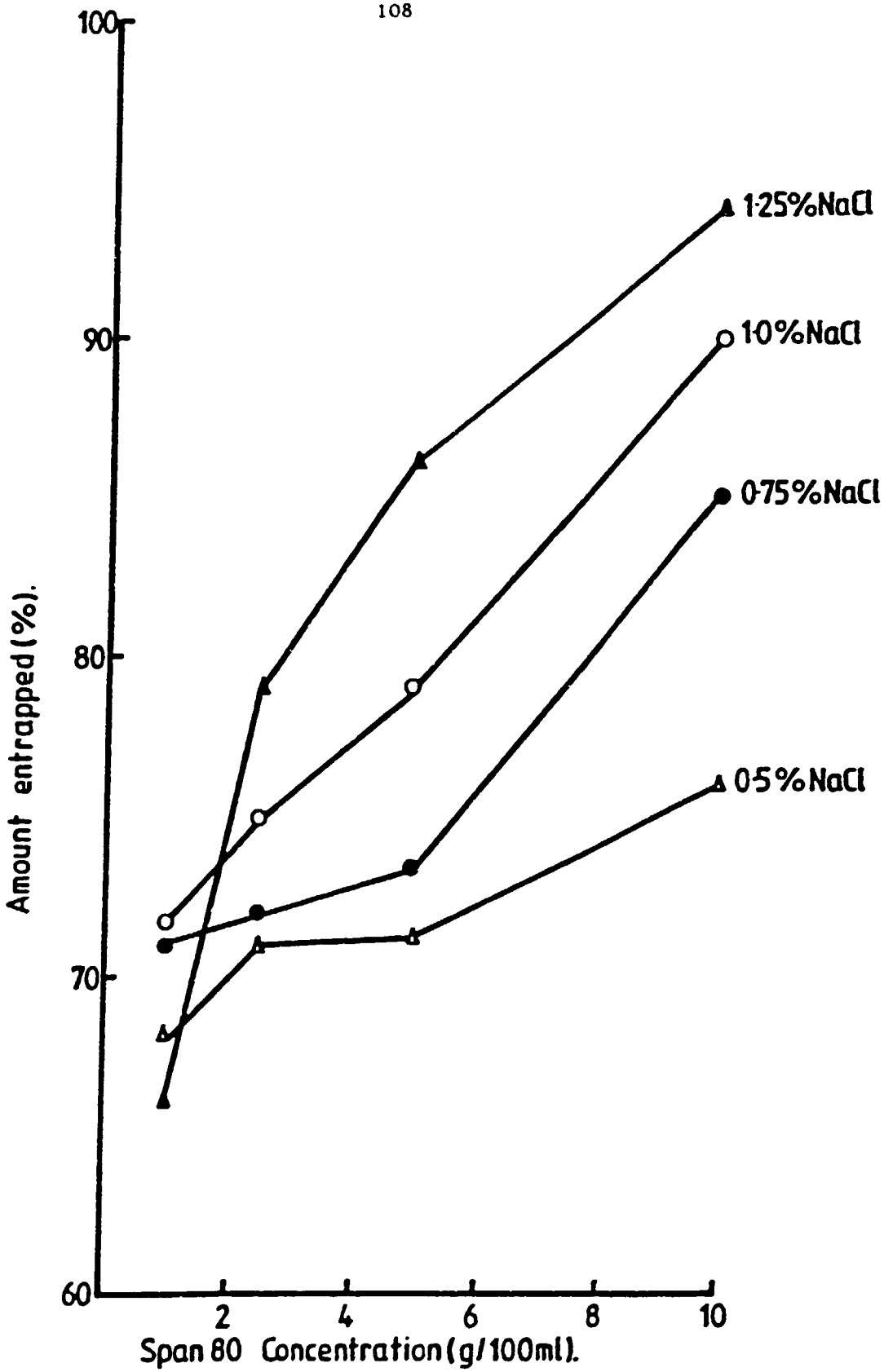

FIGURE 5-6
The effect of NaCls concentration in the inner aqueous phase on the entrapment efficiency of w/o/w emulsions prepared with 0.2% BSA in the internal aqueous phase and varying concentrations of the Span 80 in the isopropyl myristate phase.

FIGURE 5-7
Upper: phase contrast photomicrograph of a swollen w/o/w emulsion containing 1.25% NaCl in internal phase after 4 weeks storage. 0.2% BSA was included in the internal aqueous phase of 1% Tween 80 in the octane (oil) phase. Lower: a similar system after aging for 4 weeks. Bar marker: 10 μm. The very thin external lamellae can be seen.

the internal droplets will depend on the elasticity of the interfacial film and the extent to which it could maintain a low interfacial tension as the interfacial area increases. Unstable systems will break down, thereby releasing the encapsulated salt to the external phase. Multiple oil droplets carrying such thin oil membranes were prepared by interfacial interaction between Span 80 and BSA and were found to be stable for several weeks (Figure 5-7).

C. Hydrophilic–Lipophilic Balance (HLB) Approaches

Multiple emulsions consist of w/o and o/w emulsions and require at least two stabilizing surfactants, a low HLB one to form the primary emulsion and a second higher HLB to achieve the secondary emulsification.[24] Migration of surfactant from one interface to the other on formation of the completed multiple system is one cause of instability. Solubilization of molecules of the primary (lipophilic) surfactant in the external aqueous phase when the concentration of secondary (hydrophilic) surfactant exceeds its critical micelle concentration can also contribute to instability. As the concentration of secondary hydrophilic surfactant increases, more of the primary surfactant may be incorporated into the secondary surfactant micelles, causing the concentration of primary surfactant at the interface to fall, and leading to rupture of the oil layer, which results in the loss of the internal aqueous drops. This has led to studies into the optimal HLB required for both primary and secondary emulsification steps in the formulation of multiple emulsions.

Inversion of multiple w/o/w emulsion to o/w emulsion was studied as a function of the HLB of the external emulsifier, its concentration, and droplet sizes.[24] Inversion was found to occur only when droplet size is reduced below a critical size or if the weighted HLB of the total emulsifiers present in the system approaches the required HLB of the oil phase. Magdassi and co-workers have studied the HLB shift caused by emulsifier migration to the external phase.[25] They observed the optimal HLB for multiple emulsions to be dependent on the concentration of both primary and secondary emulsifiers. A linear correlation was reported between the optimal HLB, the concentration of hydrophilic emulsifier, and the reciprocal concentrations of the lipophilic emulsifier.

When multiple emulsions were prepared with 0.7–1.5 wt% of secondary emulsifier, the yield of preparation did not change significantly (70–80%) at any HLB of the secondary emulsifier. However, at higher concentration of secondary emulsifier (>1.5 wt%), there was only one optimal HLB the yield of the multiple emulsion, decreasing with increase in secondary emulsifier.[50] The difference in behavior of the two multiple emulsions formed from the two concentrations was attributed to the significant difference in the multiple droplet size. Low concentrations of secondary emulsifier produced multiple droplets of high droplet size (low surface area) whereas high secondary emulsifier concentrations produced smaller multiple droplets (large surface area), which have a greater tendency of being expelled from the multiple oil drops.

In a later report, Magdassi et al. investigated the effect of emulsifier type on

the preparation and stability of multiple emulsions.[26] The influence the HLB of secondary emulsifier on the yield of preparation and stability was found to be different for each emulsifier. It is obvious from these studies that in optimizing a multiple emulsion formulation, both the HLB values and surface activities of the surfactant have to be considered.

IV MECHANISMS OF TRANSPORT OF SOLUTES

Mechanisms of transport of solutes from the inner aqueous phase to the external aqueous phase are not well understood. In emulsions where breakdown and coalescence of the internal droplets are minimized, diffusion of unionized materials represents the most important route of release and will be affected by the nature of the solute (its size, lipophilicity or affinity for the particular oil phase, and its dissociation constant). Other factors that can affect the release rate include the pH of the internal phase and the nature of the oil.

To deal with the kinetics of solute release from w/o/w emulsions, the emulsion is usually simplified such that the internal drops are assumed to form one large coalesced subdrop with the oil phase forming a spherical shell of thickness *(L)* around the internal phase. As both the internal drops and the multiple drop are in constant motion, the internal aqueous phase is assumed to be well mixed, transport of the solute from the bulk of internal drops to the w/o interface is considered to be very fast with the middle liquid membrane serving as a barrier against transport. Furthermore, the oil membrane thickness is assumed to be negligible compared to the drop radius, so that it may be considered that the mass transfer area is constant, i.e., uniform flat membrane thickness (Figure 5-8). The equation governing the release from a slab with a nonconstant source has the form[27]

$$M_r = \frac{M_\infty}{V_1 V_2}\left\{V_2 \exp\left[\frac{-ADK(V_1 + V_2)t}{LV_1V_2}\right] + V_1\right\} \qquad (5\text{-}1)$$

where M_r is the amount of drug retained in the internal drops, M_∞ is the initial amount of drug, A is the surface area of the multiple oil drops, K is the partition coefficient of the solute in the oil phase, L is the thickness of the liquid membrane, V_1 is the internal volume of the w/o/w emulsion, and V_2 is the sink solution volume. Since $V_2 \gg V_1$ the equation simplifies to

$$M_r = M_\infty \exp\left(\frac{-ADKt}{LV_1}\right) \qquad (5\text{-}2)$$

or

$$\log M_r = \log M_\infty - \left(\frac{ADKt}{2.303LV_1}\right). \qquad (5\text{-}3)$$

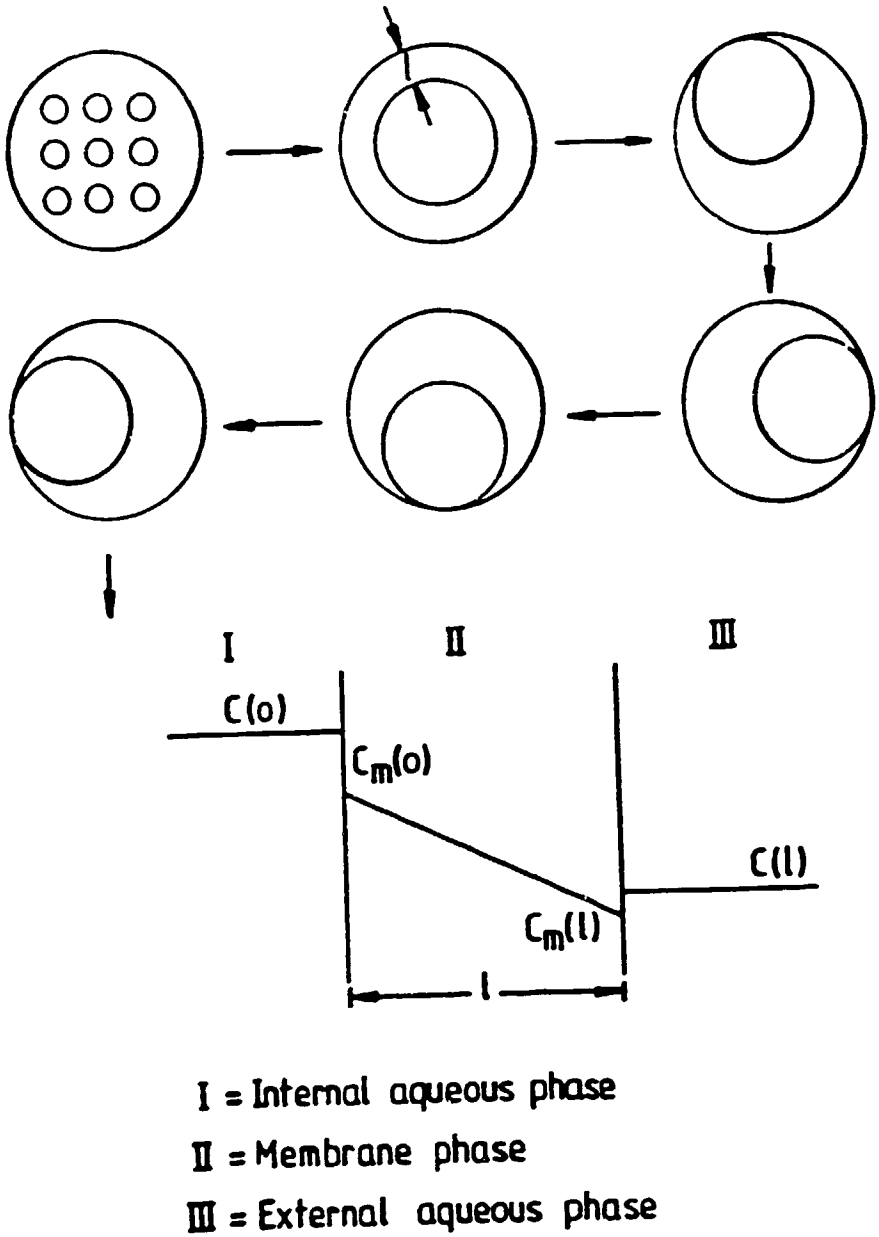

FIGURE 5-8
Uniform planar-sheet model of a w/o/w emulsions, in which internal droplets are assumed to have coalesced into one subdrop with the oil phase forming a spherical shell of thickness, l.

This equation is in the form of a first-order kinetic expression and permits evaluation of release rate constants. It can be seen that the release rate of entrapped solute from w/o/w emulsion will be affected by the diffusion coefficient, the partition coefficient of the solute in the oil phase, and the emulsion droplet size.

A. Micellar Transport

Simple diffusion accounts for the transport of substances between the two aqueous phases of w/o/w emulsions but is of itself insufficient to account for the observed facts particularly with transport of water and electrolytes. Transport in water-loaded inverse micelles and/or swollen micelles of both hydrophilic and lipophilic

surfactants has been suggested,[28] evidence of which was found by Florence and Whitehill.[1] The flux of water through the oil phase will affect the release of ionized species. The permeability of the oil layer of multiple emulsions to water has been studied by using osmotic flux produced by a solute to which the oil membrane is impermeable: this osmotic flux method yields an osmotic permeability coefficient.[29] Water flux through layers of isopropylmyristate and hexadecane has been measured using the microgravimetric method of Schatzberg[30] and Petersen.[31] The total flux (J_t) is the sum of the flux due to micellar transport (J_m) and that contributed by the molecular diffusion (J_s):

$$J_t = J_s + J_m. \tag{5-4}$$

If Fick's law is assumed Equation (5-4) can be written as

$$D_t \frac{dC_t}{dx} = D_s \frac{dC_s}{dx} + D_m \frac{dc_m}{d_x} \tag{5-5}$$

where D_m is the diffusion coefficient of the micelle, C_m is the increase in solubility due to the presence of surfactant, C_s is the solubility of water in the oil phase, and D_s is the molecular diffusion of water in the oil phase. It is assumed that the diffusion layer thickness (dx) is identical for each species. It can be seen that the steady mass loss of water through the oil layer depends on the amount of water carried by the micelles, on their saturation, and the rate of diffusion.

Higuchi[32] has discussed the concept of an effective diffusion coefficient (D_{eff}) that is particularly useful in a system with more than one diffusing species.

$$D_{eff} = \frac{D_s C_s + D_m C_m}{C_t} \tag{5-6}$$

where $C_t = C_s + C_m$. The water permeability P is given by

$$P = \frac{D_{eff} C_t}{L} \tag{5-7}$$

where L is the thickness of the oil layer. In the absence of surfactant the permeability coefficient of 8.29×10^{-9} cm sec^{-1} yields a value of diffusion coefficient for water in hexadecane of 4.10×10^{-5} cm^2 sec^{-1}. In the presence of Span 80, the permeability coefficient increases to 12.7×10^{-9} cm sec^{-1}, illustrating the additional contribution to transport of water of swollen inverse micelles. Addition of hydrophilic surfactants into the oil layer containing Span 80 significantly increased the flux of water (Figure 5-9). The presence of hydrophilic surfactant in combination with lipophilic surfactant in the oil phase would facilitate the formation of swollen mixed micelles,[33] which could solubilize water-soluble compo-

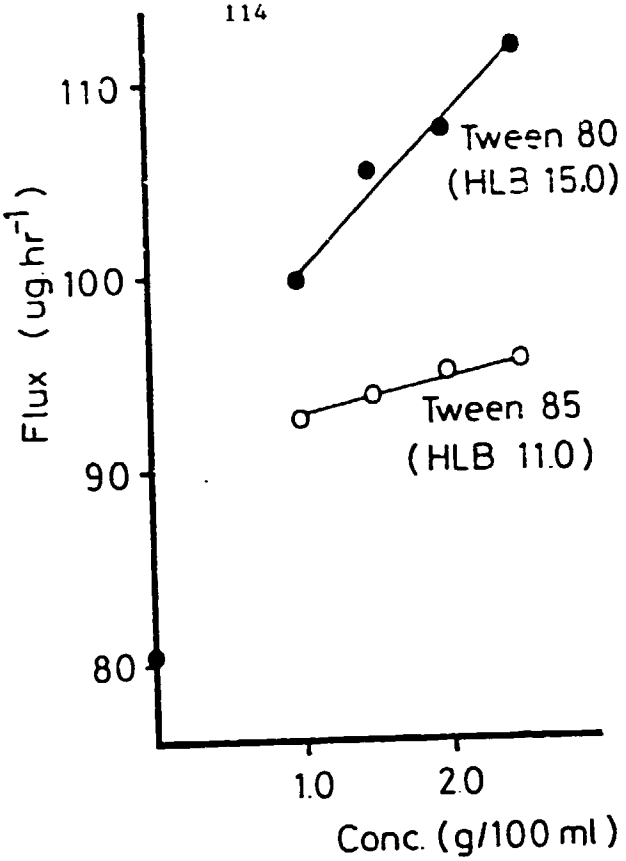

FIGURE 5-9
The influence of Span 80/Tween 80 and Span 80/Tween 85 combinations on the flux of water at 25° C through a thin layer of isopropylmyristate concentration of Span 80 kept constant.

nents. The large micellar units of both lipophilic and hydrophilic surfactants could act as carrier of water and ionized species.

B. Facilitated Transport

This mechanism of solute release involves the incorporation of some material into either the internal aqueous phase or membrane phase, which reacts with the permeating compound to render it soluble in the middle liquid phase. The carrier compounds facilitate the transport of the permeating compound across the membrane. This approach has found widespread use in hydrometallurgical recovery of metal ions.

Much of the published work in the literature deals with liquid membrane formulations for Cu^{2+} recovery. Such formulations usually contain an aqueous solution of sulfuric acid in the internal droplets that is emulsified in hydrocarbon solvent containing a common hydrometallurgical complexing agent such as β-

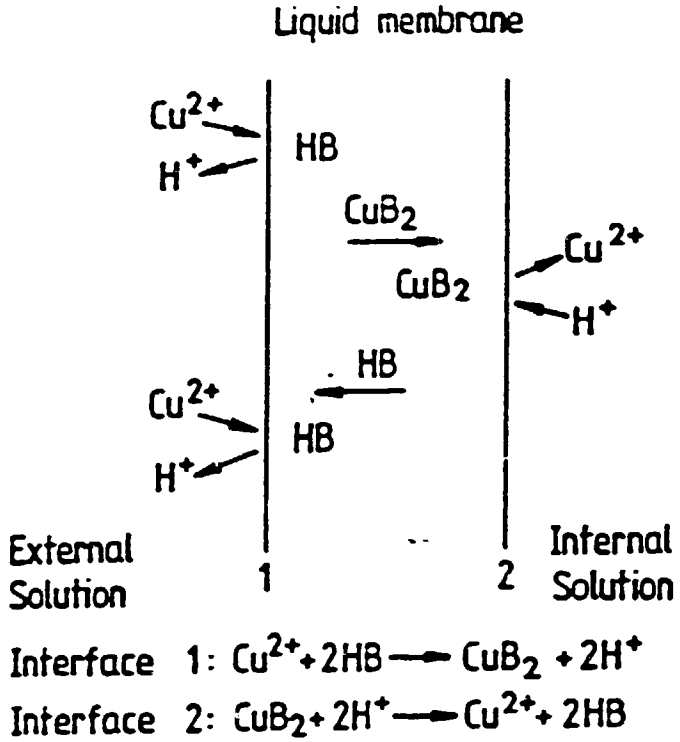

FIGURE 5-10
Simplified diagram of liquid membrane for copper extraction. From Ref. 35.

hydroxyoxime.[34] Other chelating agents for copper extraction have been reported: Kondo and co-workers[35] formulated liquid membranes for extraction of copper using liquid surfactant membranes containing benzoylacetone as a mobile carrier (Figure 5-10). Copper ions in the external aqueous phase diffuse toward interface 1 where the complex between copper ion and benzoylacetone was formed. Diffusion of the complex takes place in the membrane and on reaching the interface 2, a stripping reaction takes place to liberate copper ion into the internal aqueous phase. The rate-controlling step in the extraction procedure was considered to be the chelating complex formation and diffusion process in the aqueous phase close to the interface.[35]

V. *IN VIVO* STUDIES

Oil-in-water emulsion systems have been popular as dosage forms normally for oral administration of oils, as parenteral drug delivery systems, or in the form of cosmetics. Whereas w/o emulsions have high potential as vehicles for lipid-soluble materials and to provide a sustained release dosage system, their high viscosity, which makes them difficult to inject, has limited their use. Discrete depots may be formed and occasionally lesions appear after subcutaneous or intramuscular injection of the emulsions.[36] Multiple (w/o/w) emulsions have low viscosity and initially after injection will form diffuse depots as multiple oil drops: the external aqueous phase would be miscible with the body fluids and so dissipate leaving

globules of water in oil emulsion dispersed in the body fluids. Such systems might be expected to behave like w/o emulsions except that the drug released to the external phase during the secondary emulsification step would provide free drug for rapid release. Because of these apparent advantages w/o/w emulsions have potential to provide controlled release of antigenic material whose immunogenic response is enhanced by the presence of oil adjuvants and of anticancer drugs in cancer chemotherapy.

A. Formulation of Antigenic Material in w/o/w Emulsions

A sustained adjuvant effect due to delayed diffusion through the oil layer was reported when a w/o/w emulsion containing ovalbumin in physiological saline in the internal phase was inoculated subcutaneously into mice.[36] Mice treated with antigen in the multiple emulsion exhibited a slightly better response than those treated with w/o emulsion. Taylor and coworkers[2] studied in humans a comparison of the antibody response and reactions to aqueous influenza vaccine, influenza vaccine containing a mineral oil in simple emulsion, and influenza vaccine containing mineral oil as on multiple emulsion. The antibody response induced by the multiple emulsion preparation was found to be very satisfactory, greater than the response to the simple emulsion vaccine and substantially greater than the aqueous vaccine.

The stimulation by endotoxin encapsulated in a w/o/w emulsion of the nonspecific resistance of mice to bacterial infections was investigated by Hill and coworkers.[37] These workers attempted to encapsulate a bacterial endotoxin in a multiple emulsion in the hope that the administration of the endotoxin to mice would lead to reduced toxicity and a prolonged activity while maintaining the resistance of mice to bacterial infections. They found, however, that the multiple emulsion was as toxic as endotoxin dissolved in saline. This was attributed to the presence of nonencapsulated endotoxin in the external phase. While the protective action of the multiple emulsion was not observed, the multiple emulsion considerably enhanced the nonspecific resistance of mice to bacterial infections.

B. Cancer Chemotherapy

Emulsion systems have been used experimentally in cancer chemotherapy as a means of enhancing the uptake of anticancer agents into the lymphatic system.[38-42] Takahashi and co-workers investigated the possibility of selectively targeting 5-fluorouracil (5-FU) contained in o/w, w/o, and w/o/w emulsion systems to the lymph nodes.[43] The w/o/w and w/o emulsions were eight and three times more effective, respectively, than an aqueous solution of the drug. The same group of workers also found the concentration of bleomycin in the tumor tissue of rats was two to seven times as high after intratumoral injection of w/o and w/o/w emulsion than those produced by local administration of aqueous solution of the drug.[44] In rats bearing subcutaneous tumors AH-66, only trace amounts of bleomycin could be demonstrated in the tumor tissue 30 min after intratumoral

injection of an aqueous solution, whereas considerably higher concentrations of the antitumor agent were found in the tumor up to 3 hr following local injection in the form of an emulsion. In the clinical trials six of eight patients with either squamous cell carcinoma of skin or local recurrence of adenocarcinoma of the breast responded favorably to this treatment.

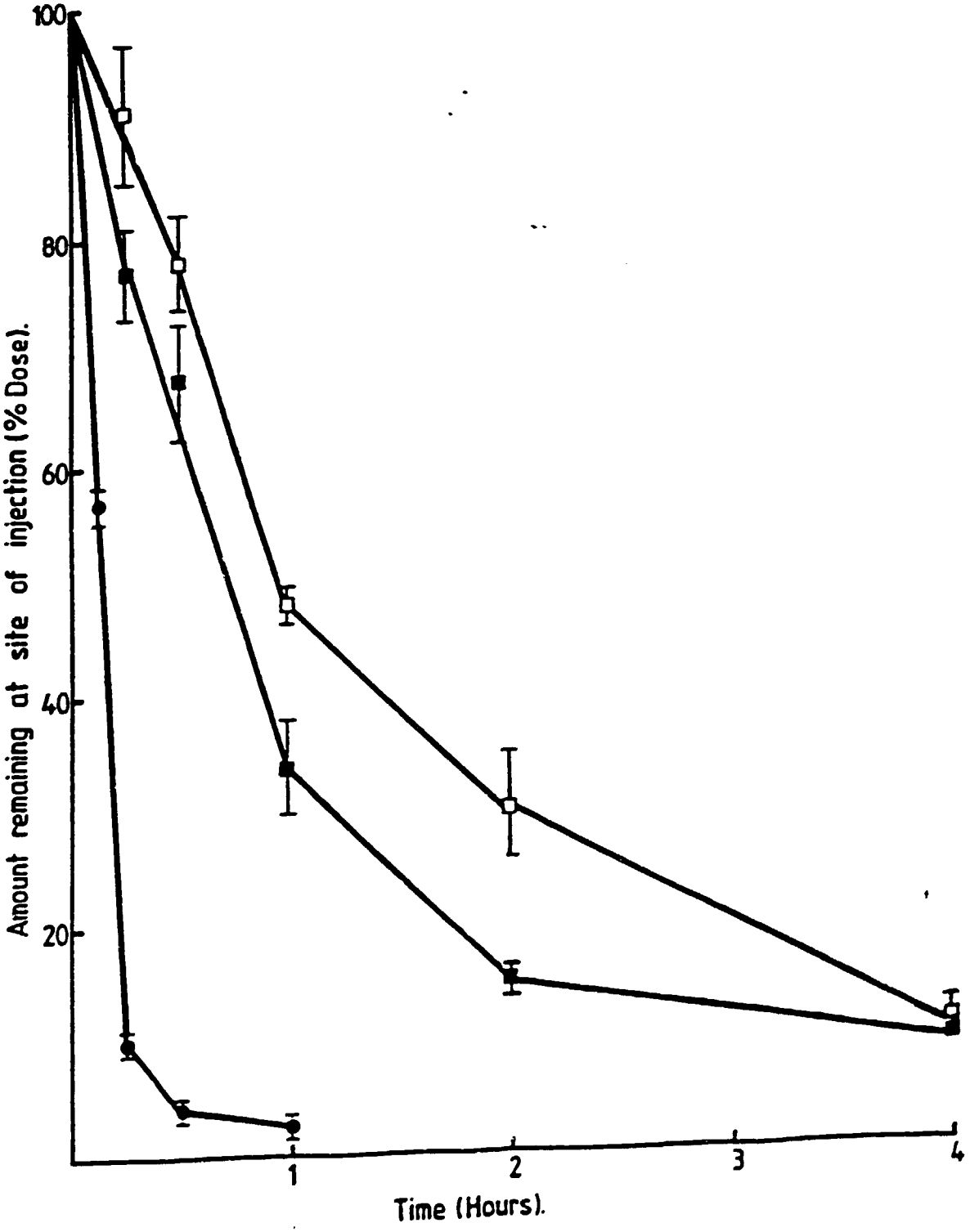

FIGURE 5-11
Disappearance of [5-[^{3}H]FU] radioactivity from the injection site following intramuscular injection of various formulations: (□) w/o emulsion prepared with hexadecane. (■) w/o/w prepared with hexadecane and (●) an aqueous solution of the drug.

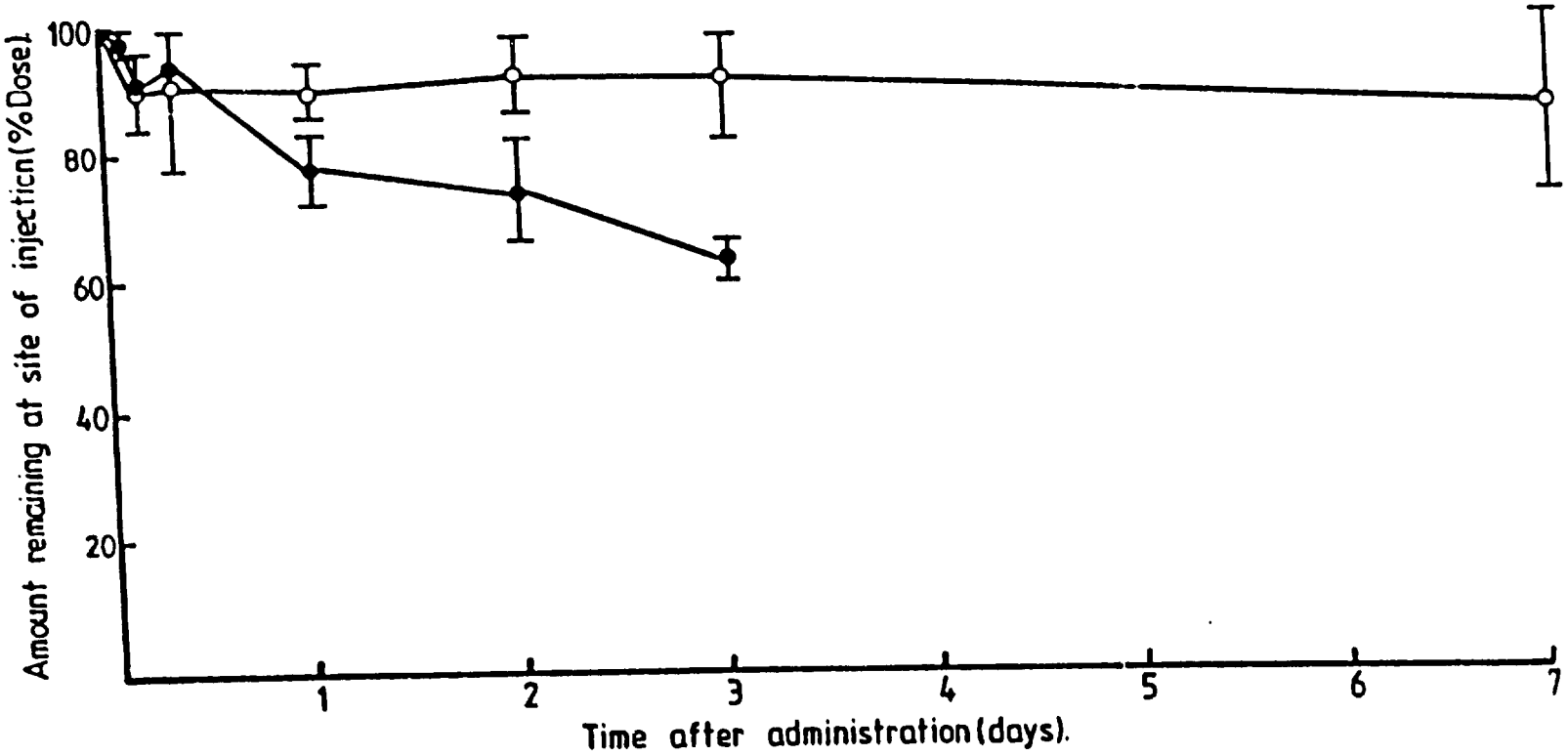

FIGURE 5-12
Effect of increasing Span 80 concentration in the oil phase on the clearance of [^{14}C]hexadecane w/o/w emulsions from injection sites following intramuscular injection; (○) 2.5% and (●) 10%.

Table 5-1
Studies with Drug Incorporation in Multiple Emulsions

Oil	Surfactant	Type	Drug	Test System	Reference
Mineral oil	Arlacel A, Tween 80	w/o/w	Ovalbumin	*In vivo*	36
Palmitic acid	Trioctanoin sodium lauryl sulfate	w/o/w	Insulin	*In vivo*	46
Palmitic acid	Octyl-decyl triglyceride, sodium lauryl sulfate	w/o/w	Insulin	*In vivo*	47
Palmitic acid	Octyl-decyl triglyceride, sodium lauryl sulfate	w/o/w	Insulin	*In vivo*	48
Drakeol 6 VR	Arlacel A, Tween 80	w/o/w	Influenza vaccine	*In vivo*	2
Drakeol	Arlacel A, Tween 80	w/o/w	Endotoxin	*In vivo*	31
Peanut oil	Span 80, Tween 80	o/w/o	Naltrexone Tylmol	*In vitro*	49
Peanut oil	Span 65, Span 80/Tween 80	w/o/w	Naltrexone hydrochloride	*In vitro*	50
Light mineral oil	Span 80, Tween 20	w/o/w	Ephedrine hydrochloride	*In vitro*	51
Sesame oil	Span 80, Pluronic F68	w/o/w	5-[^{3}H]Fluorouracil	*In vivo*	43
Isopropylmyristate	Poloxamers Triton X series	w/o/w	Sulfane Blue	*In vitro*	21
Isopropylmyristate	Poloxamers	w/o/w	Sulfonamides	*In vitro*	52
Isopropylmyristate	Span 80, Tween 80, Triton X-165, Pluronic F87, 88	w/o/w	Methotrexate	*In vitro*	53

Table 5-1 (continued)
Studies with Drug Incorporation in Multiple Emulsions

Oil	Surfactant	Type	Drug	Test System	Reference
Sesame oil Peanut oil	Sorbitan monooleate, Sorbitan sesquioleate	w/o/w	Cytarabine 5-fluorouracil	*In vitro*	54
Octane hexadecane dodecane, cyclohexane toluene Isopropylmyristate	Span 80, Tween 80	w/o/w	5-Fluorouracil	*In vitro*	15

Studies on methotrexate sodium formulated in a multiple w/o/w emulsion showed an enhanced therapeutic activity against L1210 leukemia compared with treatment with the drug in aqueous solution.[5] A single dose of methotrexate sodium formulated as a multiple emulsion tested in mice implanted with the R1 lymphoma was found to be more effective in preventing the death of mice, not only than a single aqueous injection of methotrexate sodium, but also of five daily aqueous injections of the drug at the same dose level.[5]

The present authors have studied the *in vivo* release of 5-fluoro[6-^{3}H]uracil from w/o/w emulsions stabilized by interfacial complexation. Comparative *in vivo* studies of aqueous solution, multiple w/o/w and w/o emulsions showed that formulating the drug in emulsion systems significantly sustained the release from intramuscular injection sites in the rat (Figure 5-11). Two other factors may contribute to the different release profiles observed between w/o/w and w/o emulsions: the higher viscosity of w/o emulsion and the presence of secondary hydrophilic surfactant that could accelerate the dispersion of w/o/w emulsions in the muscles.

Whitehill showed that the intestinal uptake of methotrexate was significantly increased when administered to mice in multiple w/o/w emulsions.[45] The presence of polysorbate 80 in the external phase of the emulsion was thought to account for the enhancement of absorption of methotrexate from the gastrointestinal tract.

C. Insulin Therapy

Another potential area of interest is in insulin and other protein/polypeptide drug therapy. It is known that insulin does not exhibit hypoglycemic activity when administered orally. The possible use of w/o/w emulsions to facilitate gastrointestinal absorption of normally nonabsorbed water-soluble biopolymers has been investigated in rats. Intraduodenal injection of a w/o/w emulsion containing insulin resulted in a significant hypoglycemic activity.[46]

The enteral absorption of w/o/w insulin emulsions in rabbits has been investigated. Multiple w/o/w emulsions containing 100 U/ml of insulin when administered to the jejunum at doses of 100 U/kg produced a significant and consistent increase in plasma insulin followed by a fall in blood glucose.[47] Oral administration of w/o/w emulsion containing insulin produced definite responses in three out of seven rabbits which indicated a possible means of protecting the insulin molecule from proteolytic destruction and for facilitating intestinal absorption of insulin. The same group of workers investigated the potential effectiveness of w/o/w emulsions as oral insulin preparations for short-term treatment of alloxan-diabetic rats.[48] A clear reduction in urinary glucose levels was observed in alloxan-diabetic rats that received insulin formulated in a w/o/w emulsion intrajejunally at a dose of 25–50 U/100 g body weight. Further studies are necessary to elucidate the possible interaction of surfactants, oil, and therapeutic agent. Table 5-1 summarizes some of the multiple emulsion systems that have been studied.

While problems of the long-term stability of multiple emulsions have nearly been solved, it is still unlikely that these systems will be able to produce prolonged release over more than about 6 hr although theoretically, because of the

slow dispersal of some oils from the musculature, (Figure 5-12) a system stabilized by polymers rather than surfactants might prolong the action of water soluble drugs. The traditional surfactant stabilizers, by forming micelles in the oil phase act as carriers that speed up drug loss from the depot.

However the protection these systems afford labile drugs from the environment and the protection afforded the tissues to irritant drugs, might be useful attributes. In addition the lymphotropic properties of these emulsions given orally might also be of value in the formulation of drugs that should be targeted to the lymphatics[55], regardless of any sustained-release potential the emulsions might possess.

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MICROENCAPSULATION AND MICROSPHERES FOR DRUG DELIVERY

T. L. Whateley

Department of Pharmaceutical Sciences

University of Strathclyde

Glasgow, G1 1XW

CONTENTS

Introduction

Applications of Drug Loaded Biodegradable Microspheres

Sustained Release of Polypeptides/Proteins for Depot Injections

Delivery and Targeting by Intra-venous Administration

Targeting to Tumours by Intra-arterial Administration

Delivery of Cytotoxic Drugs to Brain Tumours

Oral Delivery of Vaccines

Preparation of Microspheres of Poly(Lactic-co-Glycolic Acid)

Oil-in-Water Method

Oil-in-Oil Method

w/o/w Multiple Emulsion Method

Multiphase Microspheres

Other Methods

Release of Drugs from PLGA Microspheres

Recent Developments in Microencapsulation

References

INTRODUCTION

Two recent books relating to microencapsulation, microspheres and nanoparticles in pharmaceutical, medical and biomedical applications have been published in 1992:

"Microcapsules and Nanoparticles in Medicine and Pharmacy"

M. Donbrow, CRC Press Inc, 1992

and "Microencapsulation of Drugs"

T. L. Whateley (ed.) Harwood Academic Publishers, UK, 1992.

These two books supplement and bring up to date the books:

"Microencapsulation and Related Processes"

P. B. Deasy, Marcel Dekker, 1984.

and "Biomedical Applications of Microencapsulation"

F. Lim (ed.) CRC Press, Inc., 1984.

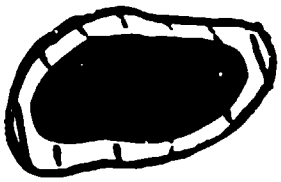
Jalil and Nixon (1992) have recently reviewed the microencapsulation of drugs with biodegradable materials.

Microcapsules and microspheres can be described as small particles (in the 1-500μm size range) for use as carriers of drugs and other therapeutic agents. The term 'microcapsule' has become the term for systems having a definite coating or shell encapsulating the contents in the form of a particle. The term 'microsphere' describes a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles. The distinction between the two terms is illustrated in Figure 1, using mitomycin C, a cytoloxic agent, as an example.

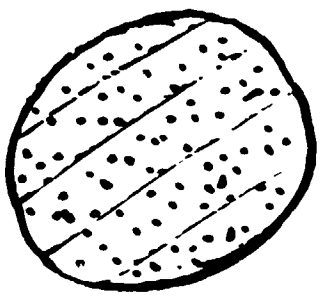
Microcapsules tend to be difficult to prepare in the lower end of the size range indicated above, due to their methods of preparation : hence, they are restricted in the routes of administration to which they are suitable. Microcapsules have wide application for the oral delivery of drugs for the following reasons:-

MICROENCAPSULATED MITOMYCIN C

Drug particles coated with ethyl cellulose



MICROSPHERES CONTAINING CYTOTOXIC (& OTHER DRUGS)



MONOLITHIC

UNIFORM DISTRIBUTION (Molecular dispersion or dispersion of crystals)

FIGURE 1

(a) sustained release is possible; the coating acts as a barrier to drug release. Various mechanisms of release are possible.

(b) taste masking (e.g. for chloroquine, an anti-malarial drug).

(c) protection of drug contents from moisture and/or oxygen.

(d) to allow the combination of incompatible constituents by the protection of one or more component by microencapsulation.

This lecture will concentrate on microspheres, which can be prepared in a wide range of sizes e.g. from nanometres (nanospheres) up to hundreds of micrometres (microspheres). In particular, there is much interest currently in the use of biodegradable polymers for the preparation of microspheres containing a wide range of therapeutic agents. Biodegradable microspheres can, of course, be used for parenteral administration and there is now on the market microsphere preparations for sub-cutaneous administration. Given the wide range of sizes possible, such biodegradable microspheres can be administered intravenously, intra-arterially, sub-cutaneously and intra-muscularly (as well as by the oral route where limited uptake is possible for specific applications).

As discussed under 'Biodegradable Polymers for Drug Delivery' the polymeric system of choice is currently poly(lactic acid) and the co-polymer poly(lactic-co-glycolic acid). The properties, degradation, drug release of these systems has been previously dicsussed.

Some current applications of biodegradable microspheres will be discussed followed by a detailed discussion of methods available for their preparation, with emphasis on the microencapsulation of protein and polypeptide drugs.

Applications of Drug Loaded Biodegradable Microspheres

1. Sustained Release of Polypeptides/Proteins from Sub-Cutaneous Depot Injections

   The development of 'Zoladex', a once-a-month sub-cutaneous implant to deliver the polypeptide, goserelin, was the first drug delivery system based on the biodegradable poly(lactic-co-glycolic acid) (PLGA) polymers. A product, based on microspheres of

PLGA is also on the market, Prostap SR. This product consists of microspheres of mean size 20μm prepared from a polymer of molecular weight 14,000. The method used in their preparation, involving a w/o/w multiple emulsion solvent evaporation process, will be described later in this paper. The injection vehicle for the microspheres contains carboxy methyl cellulose (CMC) in order to increase the viscosity of the medium and to ensure that the microspheres remain in suspension during the administration. A 23 gauge needle can be used for the once-a-month sub-cutaneous injection rather than the larger 16 gauge needle needed for the Zoladex implant, which can necessitate the use of local anaesthetic.

2. Delivery and Targeting by Intra-venous Injection

The delivery and targeting of drugs using microspheres and nanoparticles has been investigated extensively by Davis and Illum (e.g. 1989). By modification of the surface of the microspheres, by, for example, adsorption of non-ionic block co-polymeric surfactants (poloxamers and poloxamines) it has been possible to avoid the normal uptake in the liver by the mononuclear phagocyte system (MPS). Circulating depot release systems should be possible using biodegradable microspheres. The targeting of drugs using microspheres is illustrated in Figure 2 with the use of the example of targeting to the liver.

FIGURE 2 ***TARGETING OF DRUGS TO LIVER***

1st ORDER TARGETING	:	*to organ* *e.g. i/a microspheres* *20-80um* *i/v colloidal particles* *0.1-2μm*
2nd ORDER TARGETING	:	*to tumour* *e.g. i/a microspheres plus angiotensin II*
3rd ORDER TARGETING	:	*selective uptake by tumour cells*

3. Targeting to Tumours by Intra-arterial Administration

The concept underlying regional chemotherapy is an attempt to increase the therapeutic index of the drug by increasing the concentration of drug within the organ harbouring the metastatic deposits with decreased concentration of cytotoxic drug in the systemic vascular compartment.

Microspheres in the size range 20-50μm will be trapped in the capillary bed of the liver following intra-hepatic arterial administration. Second order targeting (i.e. to the tumour(s) rather than to the whole organ) can be achieved by the concurrent administration of angiotensin II (AT II), a vasoconstrictor, which restricts arterial flow to the normal liver but does not affect the capillary blood flow network of the tumour. Delivery of microspheres loaded with mitomycin C (MMC) in this manner has three advantages:

(1) Systemic levels are low, with consequent reduction in side effects.

(2) The sustained release of MMC from the trapped microspheres extends the timescale of exposure of the tumour cells to cytotoxic drug. The embolic effect of the microspheres increases retention of drug at the site of release. This whole process has been termed 'chemoembolisation'.

(3) In the case of a cytotoxic drug such as MMC, which requires bioreduction for activation, the reduced oxygen levels consequent on embolism by the microspheres may enhance the therapeutic efficacy of MMC.

With MMC microencapsulated with ethyl cellulose we have shown that peak plasma levels could be reduced from 812 (±423) ng/ml for free MMC in solution to 80 (± 75) ng/ml for microencapsulated MMC administered as a bolus via the hepatic artery. A Phase I clinical trial showed that the dose of microencapsulated MMC could be increased to 40mg without toxicity. (Goldberg *et al*, 1991; Anderson *et al*, 1991; Whateley *et al*, 1992). However, ethyl cellulose is not biodegradable and we

have been developing methods for the incorporation of MMC into microspheres of the biodegradable and acceptable poly(lactic-co-glycolic acid). Our experience with this system will be used to illustrate the sections of this lecture on the preparation of PLGA microspheres.

4. Delivery of Cytotoxic Drugs to Brain Tumours

The use of cytotoxic drugs to treat malignant brain tumours is limited by drug exclusion from the brain by the blood-brain-barrier. Chemotherapy is limited to a few drugs with high lipid:water partition coefficients, such as the nitrosourea, carmustine (BCNU). Gliomas do not tend to metastasise outwith the CNS and therefore lend themselves to a regional chemotherapy approach where a high drug concentration is generated at the tumour site.

There has been work on the use of BCNU sustained release implants using the surface eroding, biodegradable polymer bis(p-carboxyphenoxy) propane-sebacic acid, a hydrophobic polyanhidride. BCNU is rapidly degraded in aqueous media and is protected within the surface eroding, hydrophobic polyanhydride (Brem, 1990, 1990a; Domb *et al*, 1991).

We are developing PLGA microspheres loaded with the stable, water soluble drug, carboplatin. The biodegradable and acceptable PLGA is suitable for this application with carboplatin and there is the advantage that the hydrophilic cytotoxic drug will not pass the blood-brain-barrier into the systemic circulation.

5. Oral Delivery of Vaccines

There is increasing evidence that small microspheres ($<1\mu m$) can be absorbed from the gastro-intestinal tract to a limited extent, probably via the Peyer's patches of the intestinal walls. Possibly only 1 particle on 10^5 is absorbed, making the mechanism unattractive for drug delivery, in general. However, such an uptake can be adequate to generate an immune response.

Some recent examples of the development of oral vaccines based on microspheres include the following:

Ovalbumin (as a model system); O'Hagen *et al*, 1992

Malaria; Bathurst *et al*, 1992

Staphylococcal Enterotoxin B Toxoid; Gilley *et al*, 1992 and Staas *et al*, 1991.

Preparation of Microspheres of Poly(lactic-co-glycolic acid)

A number of microsphere properties have to be otpimised:

(1) Mean size and size distribution

(2) Surface properties

Re-suspension in an aqueous vehicle for injection without aggregation or sedimentation must be possible: for this a mean size <50μm is normally required together with hydrophilic surface properties.

(3) Drug loading

(4) Drug release rate

(5) Degradation rate of matrix

Other aspects such as sterility, apyrogenicity and residual solvent content (e.g. CH_2Cl_2) clearly have also to be satisfactory.

The discussion of the various methods of preparing drug loaded microspheres of PLGA will be illustrated by our experience in attempts to prepare microspheres in the size range 30-50μm loaded with the cytotoxic agent mitomycin C.

Emulsion-Solvent Evaporation Methods

These are the most commonly used methods : there are several variations of the basic oil-in-water method which will be discussed first :

Oil-in-Water Method

Figure 3 illustrates the basis of this process. The inner, oil phase of the emulsion consists of dichloromethane, CH_2Cl_2, in which the PLGA is dissolved together with

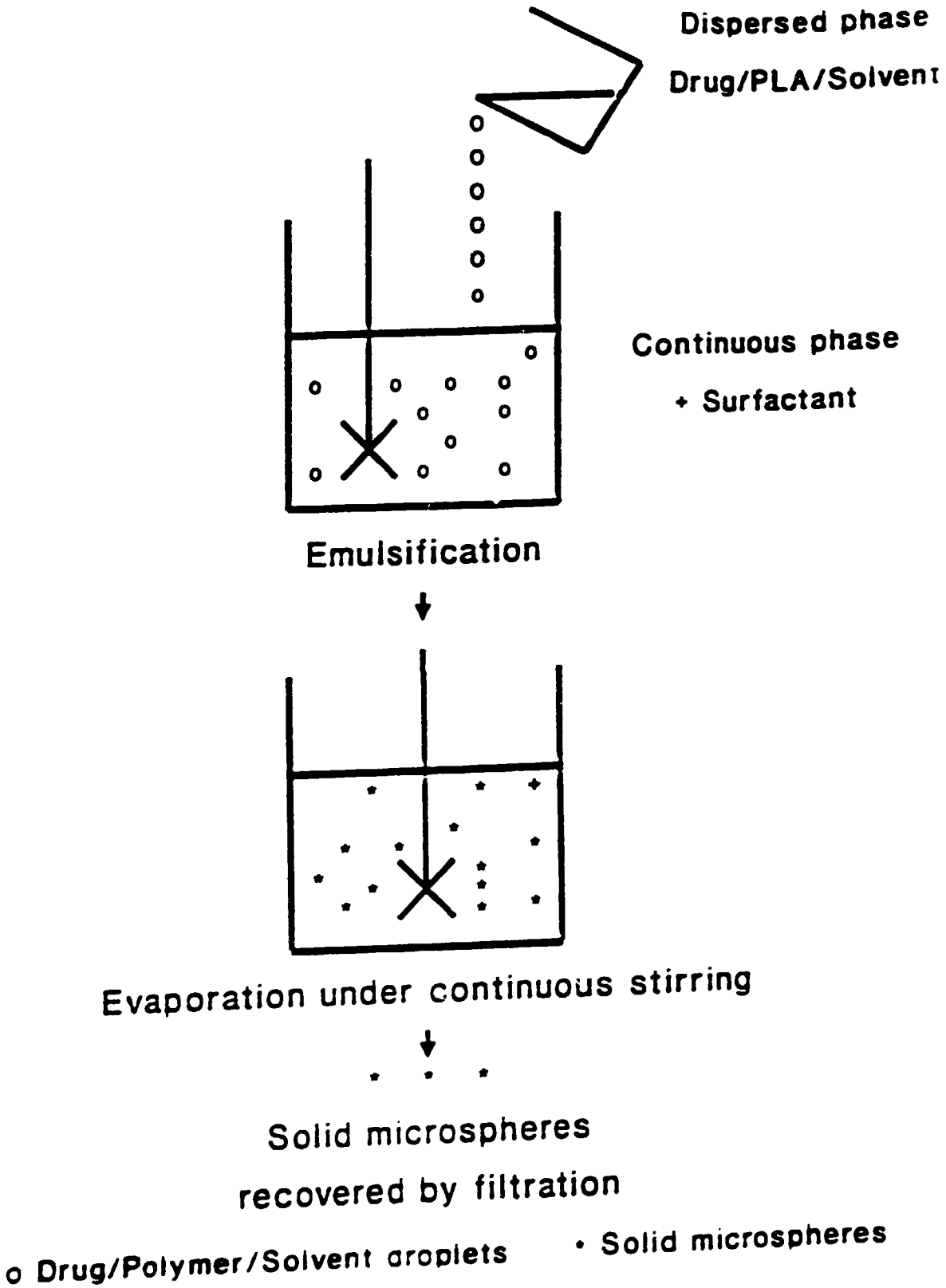

Dispersed phase
Drug/PLA/Solvent
Continuous phase
+ Surfactant
Emulsification
Evaporation under continuous stirring
Solid microspheres
recovered by filtration
o Drug/Polymer/Solvent droplets
• Solid microspheres

FIGURE 3

the drug to be incorporated into the microspheres. The emulsion is formed in water containing, typically, poly(vinyl alcohol) (PVA) as stabiliser for the formed microspheres.

The oil-in-water method has the advantages :

(a) efficient incorporation of lipophilic drugs

(b) wide-range of sizes readily prepared e.g. from large (100μm) to nanoparticle size (<1μm), essentially controlled by stirring rate and conditions.

(c) microspheres have hydrophilic surface properties which allows ready re-suspension without aggregation.

However, this method has the disadvantage that the incorporation of water soluble drugs is very low due to the partitioning of the drug into the large external aqueous phase of the emulsion. The water soluble drug will not be soluble in the inner CH_2Cl_2 phase and will be there as a suspension of drug particles : sonication has been used to improve this suspension process.

Cisplatin has been successfully incorporated into PLGA microspheres (100-200μm diameter) using the oil-in-water method with the external aqueous phase being saturated with cisplatin to reduce partitioning of the drug from the CH_2Cl_2/polymer phase.

We have used a similar approach to prepare PLGA microspheres (30-50μm diameter) containing mitomycin C with loadings of up to 25% for intra-arterial targeting to liver metastases from colorectal tumours. The rate of release of mitomycin C increased rapidly with drug loading e.g. the time for 50% release was 9 hr and 50 hr for drug loadings of 25% and 12% respectively. In these systems the drug is dispersed in the microsphere matrix as discrete crystals and the higher release rate for the higher loaded microspheres can be ascribed to the fact that an interconnecting network of crystals exists at high loadings allowing connected

pathways to form as the crystals dissolve. At lower drug loadings, the crystals in the interior are isolated and are released only on massive degradative breakdown of the microsphere matrix. This mechanism of release is considered in more detail under the discussion of drug release from biodegradable polymers.

This o/w preparative method for water-soluble drugs is sensitive to a number of variables, see Table 1. In addition we have found that batch-to-batch variation in the PLGA polymer makes it difficult to reproduce mitomycin C loaded microspheres.

The incorporation of the poloxamer, Pluronic L101, into the CH_2Cl_2 oil phase at the 10% level was effective in improving the incorporation efficiency for mitomycin C microspheres, possibly by a solubilisation effect. However, residual droplets of the Pluronic L101 surfactant remain in the final product microspheres.

TABLE 1

Variables in the o/w Emulsion-Solvent Evaporation Process for the Incorporation of Water-Soluble Drugs in PLGA Microspheres

Phase volume of organic phase

Phase volume of aqueous phase

Loading of polymer in organic phase

Loading of drug in organic phase

Presence of oil-soluble surfactant in organic phase (e.g. Pluronic L101)

Ultrasonication of organic phase

Stabiliser/surfactant in aqueous phase

Saturation level of drug in aqueous phase

Stirring method and rate

Jalil and Nixon (1990) also studied the variables for the o/w process for both poly(L-lactic acid) and poly(DL-lactic acid) for the water-soluble drug, phenobarbitone and compared the o/w process with an oil-in-oil method, which is now described.

Oil-in-Oil Method

In order to be able to incorporate water soluble drugs efficiently, the use of acetonitrile as the inner 'oil' phase was developed. The external phase is liquid paraffin containing a surfactant (e.g. Span 40) and solvent evaporation takes place at, typically, 55°C over a period of 4 hr.

A serious drawback to the o/o method is the difficulty of obtaining small microspheres (e.g. <50μm). This method had been used by Tsai *et al* (1968) to prepare poly(lactic acid) microspheres containing mitomycin C; however, these microspheres were of size ca 95μm. Microspheres of these sizes are difficult to administer clinically via an in-dwelling hepatic arterial catheter. As this oil-in-oil method is well-suited to the incorporation of water soluble drugs we have attempted to prepare smaller PLA microspheres (e.g. 20-50μm) containing mitomycin C by this method.

Jalil and Nixon (1990) made an extensive and thorough study of the factors influencing the preparation and properties of microspheres of poly(L-lactic acid) and the incorporation of a model, water-soluble drug, phenobarbitone. Both the oil-in-oil and oil-in-water emulsion solvent evaporation processes were investigated. Although high loadings of the drug were obtained using the oil-in-oil method, the microspheres were large.

In their studies on controlling microsphere size in the o/o method, Jalil and Nixon (1990) found that increased stirring rate and increased surfactant concentration (in the external light light paraffin phase of the emulsion) reduced the size of the

microspheres. A range of Spans (sorbitan esters of fatty acids) and Brijs (polyoxyethylene ethers of fatty acids) were investigated (see Figure 4). There was no correlation between the HLB of the emulsifier and microsphere size. The packing of the emulsifier at the interface appeared to affect microsphere size: close packed straight chain saturated fatty acid containing emulsifiers produced smaller microspheres than loose packed emulsifiers containing, for example, three fatty acid chains or a cis-double bond.

Span 40 which was found to give the smallest microspheres, at 2% concentration in light liquid paraffin was used in our studies and a variety of stirring methods and speeds investigated, including the use of a Silverson stirrer. Using the Silverson stirrer at 55°C resulted in microspheres of diameter 60-70μm.

The problem of obtaining small (i.e. <50μm) microspheres from this o/o method was examined by Jalil and Nixon (1990) who found that initially (i.e. 2 mins. after the mixing of the two phases under stirring) small (i.e. << 50μm) droplets of the acetonitrile + polymer phase were observed under the microscope. However, after 8 mins. coalescence had occurred and only large (i.e. < 50μm) droplets were present. We have observed this same phenomenon: it appears that as the solvent (acetonitrile) begins to evaporate out of the droplets, with consequent increase in polymer concentration, coalescence occurs and it seems impossible to obtain conditions of stirring and concentration/nature of surfactant emulsifier to prevent this coalescence during evaporation of the acetonitrile polymer solvent. We are currently investigating this problem further.

A further problem with PLGA microspheres prepared by this oil-in-oil procedure is that they tend to aggregate when re-suspended in aqueous vehicles: this will be due to the hydrophobic nature of the surface from the o/o emulsion procedure with a lack of any hydrophilic stabilising agent (a function served by the poly(vinylalcohol) in the

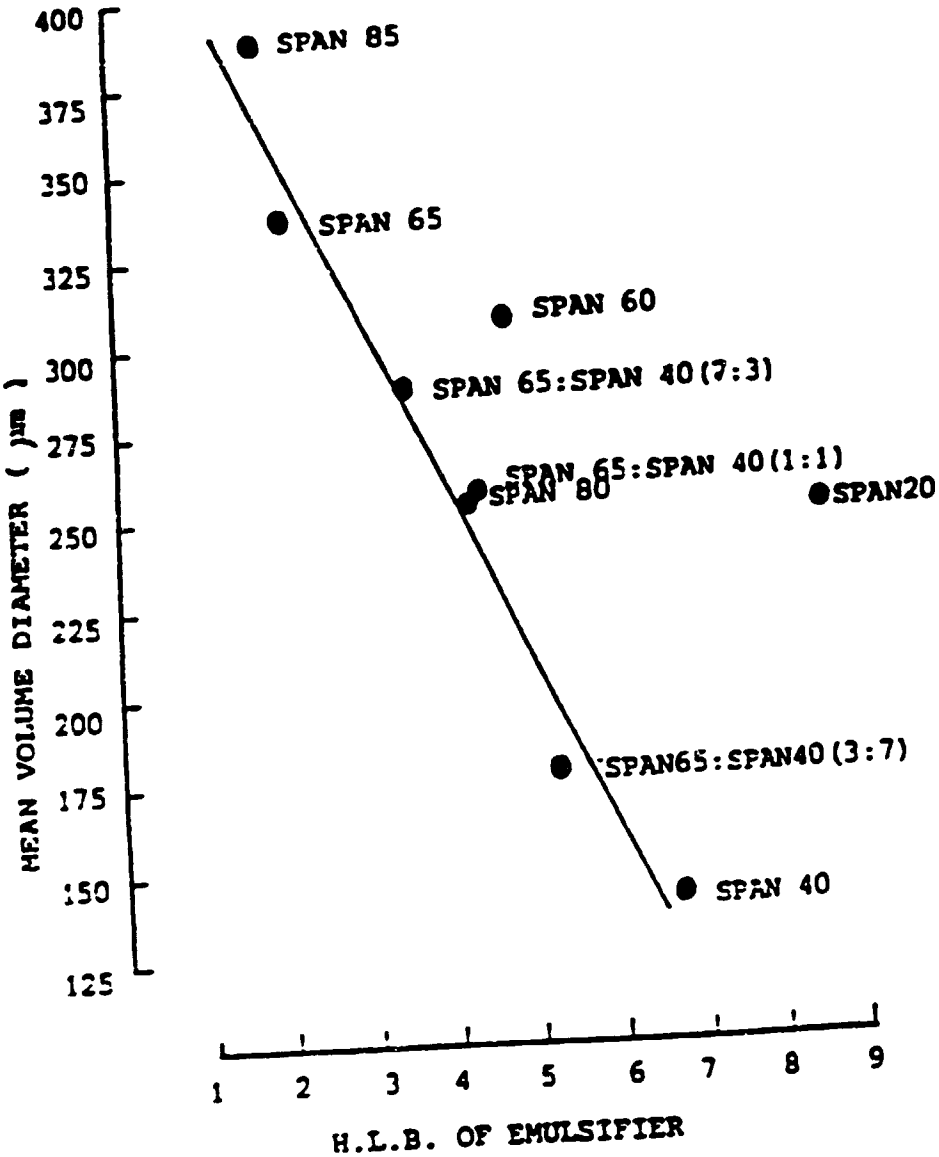

400
375
350
325
300
275
250
225
200
175
150
125
MEAN VOLUME DIAMETER (μm)
SPAN 85
SPAN 65
SPAN 60
SPAN 65:SPAN 40(7:3)
SPAN 65:SPAN 40(1:1)
SPAN 80
SPAN20
SPAN65:SPAN40(3:7)
SPAN 40
1
2
3
4
5
6
7
8
9
H.L.B. OF EMULSIFIER

FIGURE 4

oil-in-water method).

The release rate of phenobarbitone has been reported to increase rapidly with temperature of evaporation i.e. rate of removal of solvent influences the porosity.

Water-in-Oil-in-Water Multiple Emulsion Method

For drugs which are very soluble in water (e.g. the protein and polypeptide drugs) a multiple emulsion method has proved to be very effective. Typically the polypeptide in water (e.g. 0.5ml) is dispersed into PLGA in dichloromethane (e.g. 10ml) to give a water-in-oil emulsion. This w/o emulsion is then dispersed into an aqueous phase (e.g. 200ml) containing PVA and the dichloromethane allowed to evaporate. This process is illustrated in Figure 5. This process overcomes the insolubility of water-soluble drugs in CH_2Cl_2 and has been used commercially for the preparation of microspheres containing LHRH analogues for s/c injection. The possible disadvantages are that denaturation of a protein drug can occur at the H_2O/CH_2Cl_2 interface and that there may be residual water remaining in the microspheres affecting the rate of degradation and stability of the product.

Multiphase Microspheres

The multiple emulsion approach has been taken a step further recently to have a system with 4 phases: a w/o/'o'/o system. The three steps in such a procedure are:

1. An aqueous solution of the drug is dispersed in soybean oil (w/o).
2. This w/o emulsion is dispersed into acetonitrile plus polymer (w/o/'o')
3. This w/o/'o' emulsion is dispersed into liquid paraffin (w/o/'o'/o) and the acetonitrile allowed to evaporate.

The advantage of this procedure is that the drug (e.g. a protein) does not come into contact with a dichloromethane/water interface. Large (200μm+) microspheres are obtained (Iwata and McGinity *et al*, 1992). Figure 6 shows the difference between a multiphase microsphere and a normal monolithic microsphere.

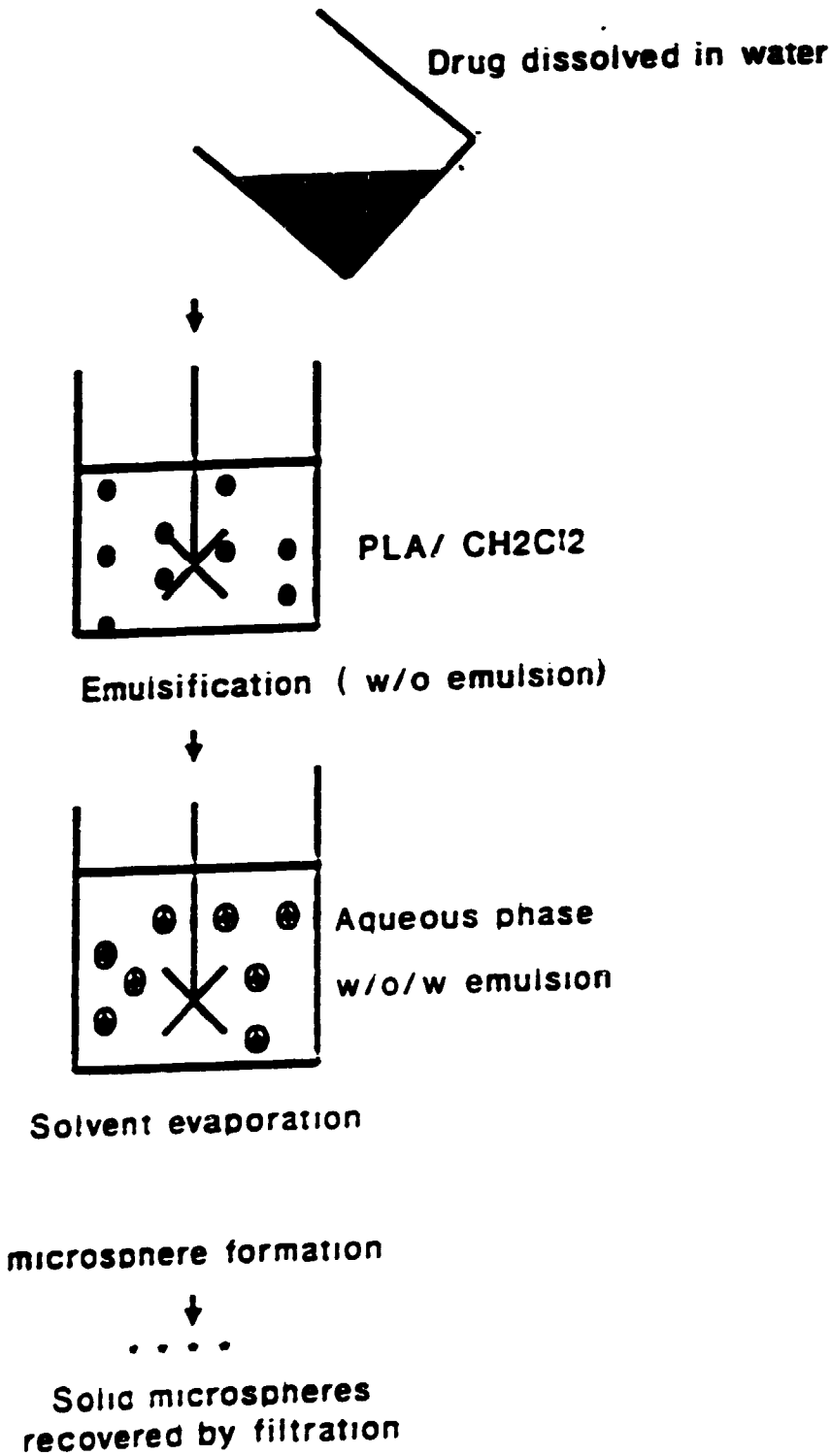
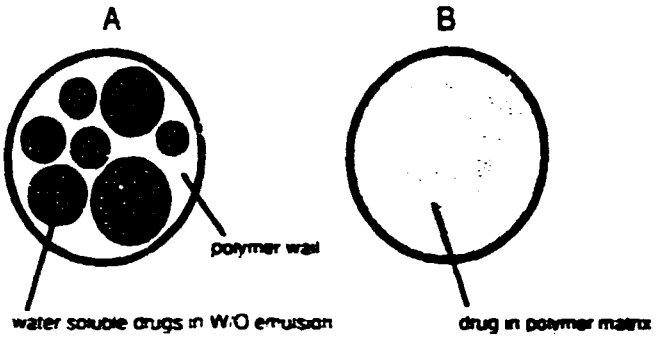

Drug dissolved in water
PLA/ CH2Cl2
Emulsification (w/o emulsion)
Aqueous phase
w/o/w emulsion
Solvent evaporation
microsphere formation
Solid microspheres
recovered by filtration

FIGURE 5



Schematic features of a multi-phase microsphere (A)

FIGURE 6

Coacervation (Phase Separation)

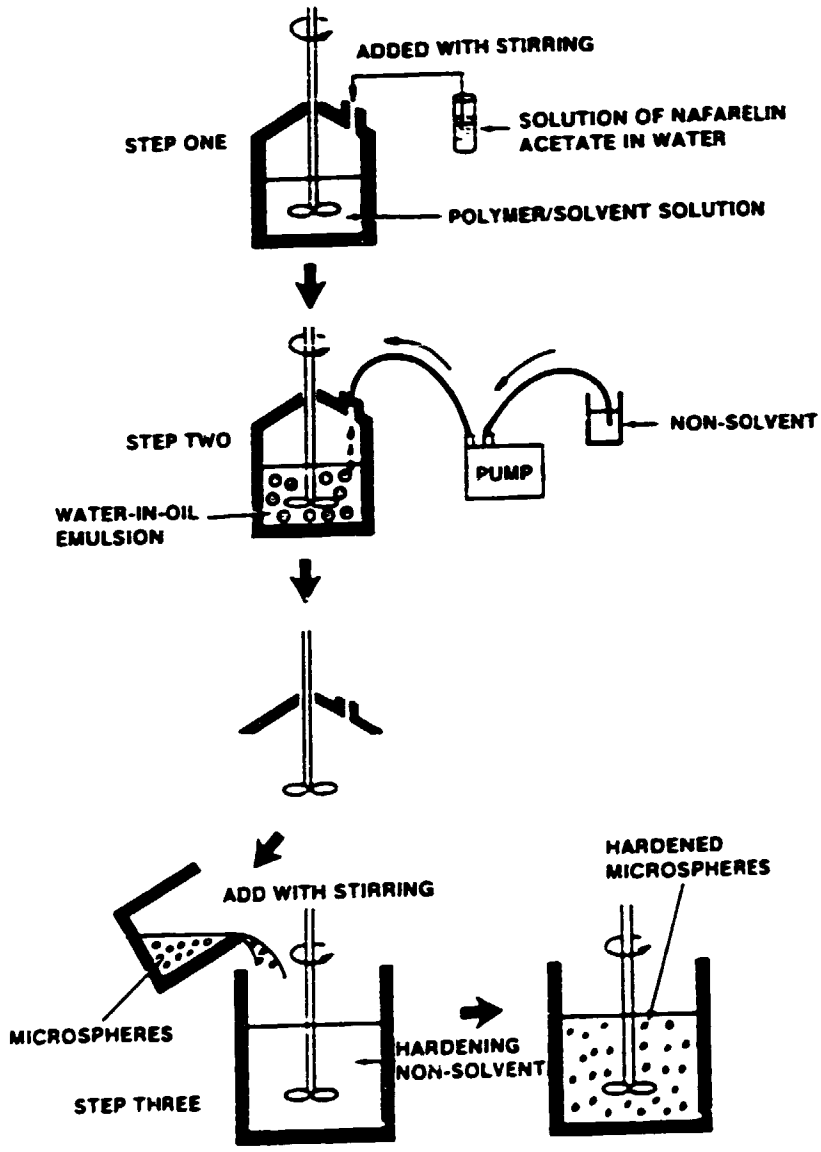
Phase separation of PLGA by non-solvent addition (coacervation) can be brought about by the addition of silicone oil to a solution of PLGA in dichloromethane (see Figure 7). The triangular phase diagram (Figure 8) has been established giving the information for the formation of a stable coacervate droplet phase. Typically, microspheres can be prepared with 3.8% poly(L-lactide) in CH_2Cl_2 plus 10% BSA (relative to polymer mass). At 12°C the addition of 47% ($^{w}/w$) silicone oil (relative to total mass) results in coacervate droplets containing drug particles which can be solidified and hardened in octamethylcyclotetrasiloxane or n-heptane. Encapsulation efficiency was 80% with 95% of microspheres less than 70μm.

This method has advantages for water soluble drugs. However, batch to batch variation of PLGA has caused problems: both the coacervation process and release rate profile are affected by the presence of PLGA oligomers.

An aqueous solution of the drug can be used to form a w/o emulsion in CH_2Cl_2 plus polymer: the non-solvent is then added, precipitating the polymer around the water/drug droplets of the emulsion. This approach has been used to prepare microspheres containing polypeptide drugs for monthly s/c drug delivery.

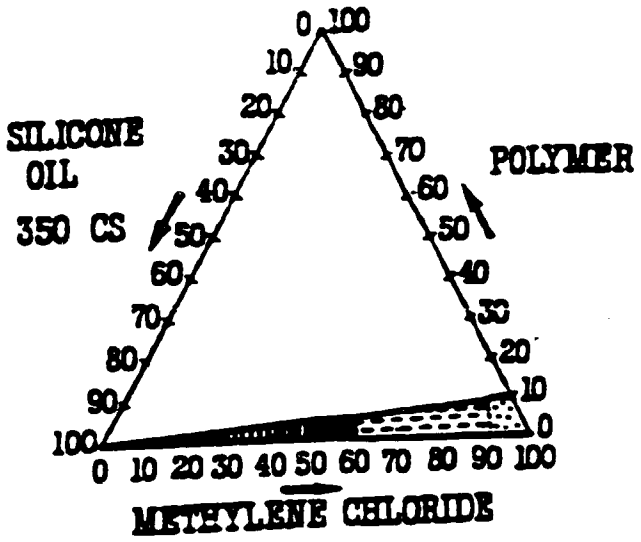
Other Methods of Preparing PLGA Microspheres

A freeze-drying technique for the preparation of microspheres containing the hormone calcitonin has been described. A mixture of calcitonin and poly(glycolide)



Phase Separation Microencapsulation

FIGURE 7


SILICONE
OIL
350 CS
POLYMER
0 10 20 30 40 50 60 70 80 90 100
METHYLENE CHLORIDE

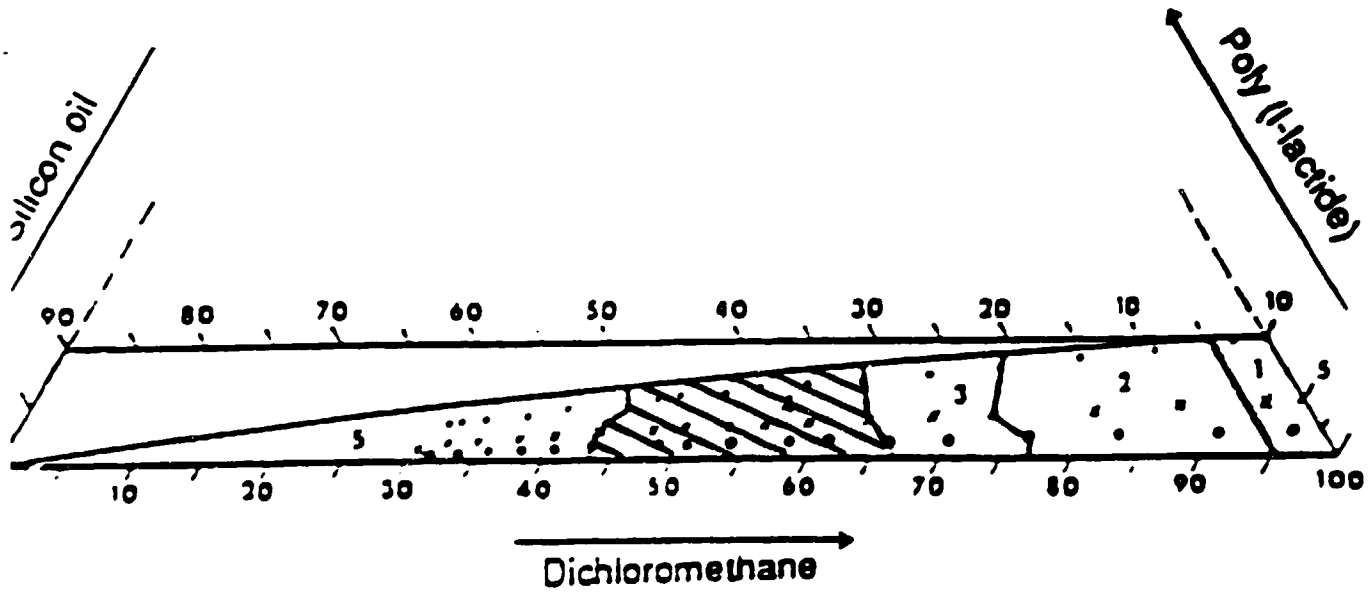

Silicon oil
Poly (l-lactide)
Dichloromethane

FIGURE 8

in hexafluoroacetone is dispersed in carbon tetrachloride: this suspension is freeze-dried and washed with CCl_4. Loadings of up to 7.5% calcitonin were obtained with 90% of microspheres under 5μm in diameter (Lee *et al*, 1990).

A novel method involves spraying a suspension of lyophilised protein particles (1-5μm) suspended in a PLA/CH_2Cl_2 solution through an ultrasonic nozzle into liquid nitrogen covering some ethanol. The liquid nitrogen is allowed to evaporate and the CH_2Cl_2 is taken into the liquid ethanol (see Figure 9). Encapsulation efficiences of 95% are reported with sizes of 50-60μm. Some enzymes have been microencapsulated without loss of activity e.g. ribonuclease and horse radish peroxidase (Khan *et al*, 1992).

An interesting hot-melt method was described by Wichert and Rohdewald (1990) and this is illustrated in Figure 10. An advantage is that contact of active agent with chlorinated solvents is avoided.

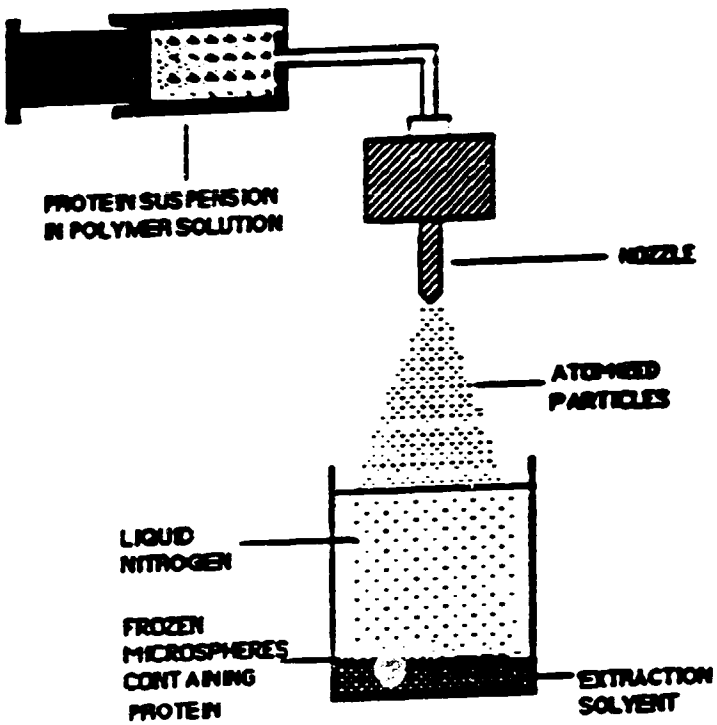
Release of Drugs from PLGA Microspheres

The release of drugs from PLGA materials has been considered under the lecture on Biodegradable Polymers for Drug Delivery. Similar factors and mechanisms clearly apply also to microsphere systems of PLGA.

The additional factor of the effect of particle size on drug release is illustrated in Figure 11, which shows the expected increase in release rate with decreasing size for PLA microspheres containing butamben.

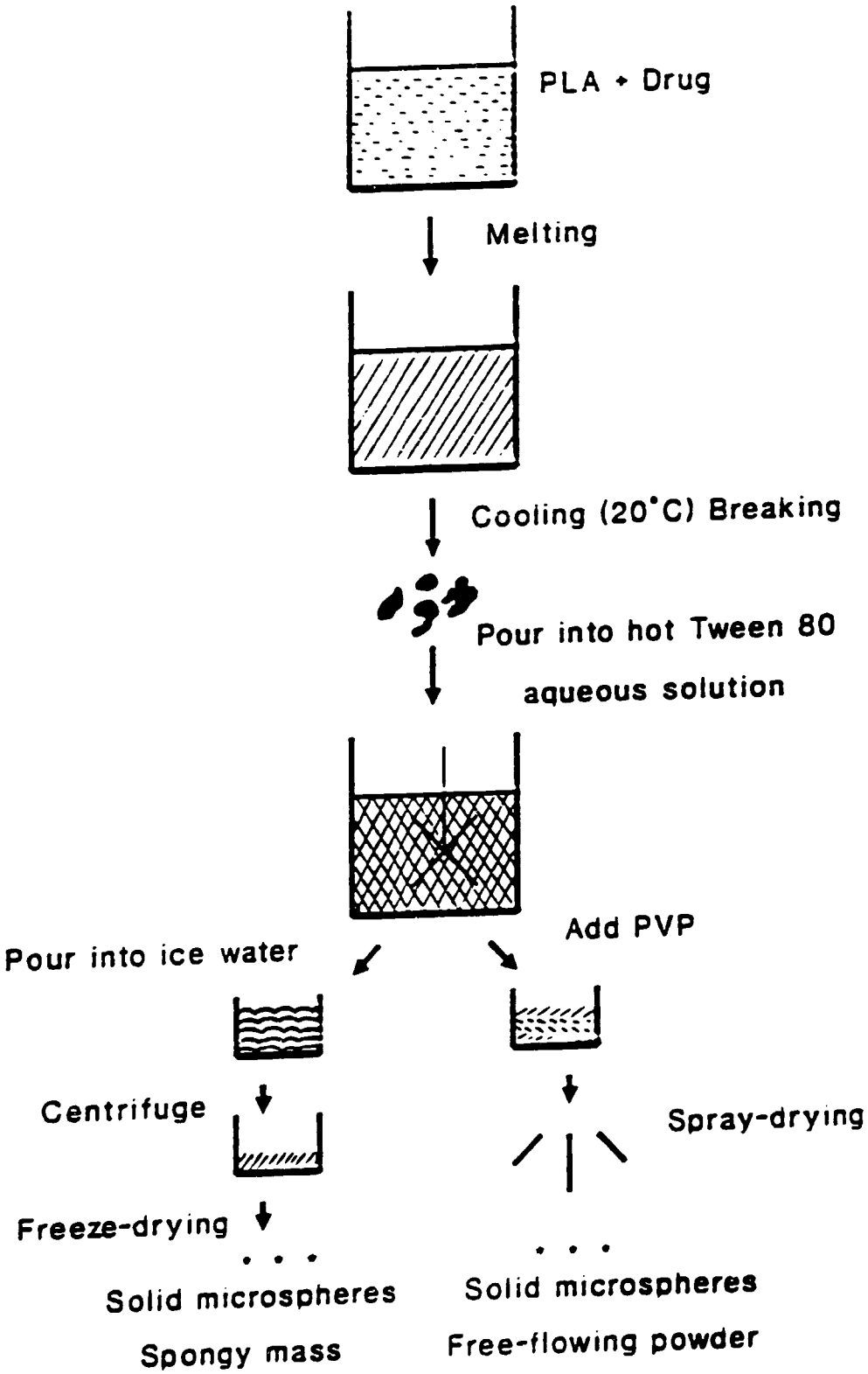
The effect of polymer molecular weight on the release of fluphenazine is clearly shown in Table 2 These results correlate with the increased rate of degradation of the lower molecular weight polymers, as discussed previously. It is interesting to note that the presence of acidic or basic drugs in PLGA matrices can increase the rate of degradation.

The effect of drug loading is also illustrated in Table 2; as discussed under drug



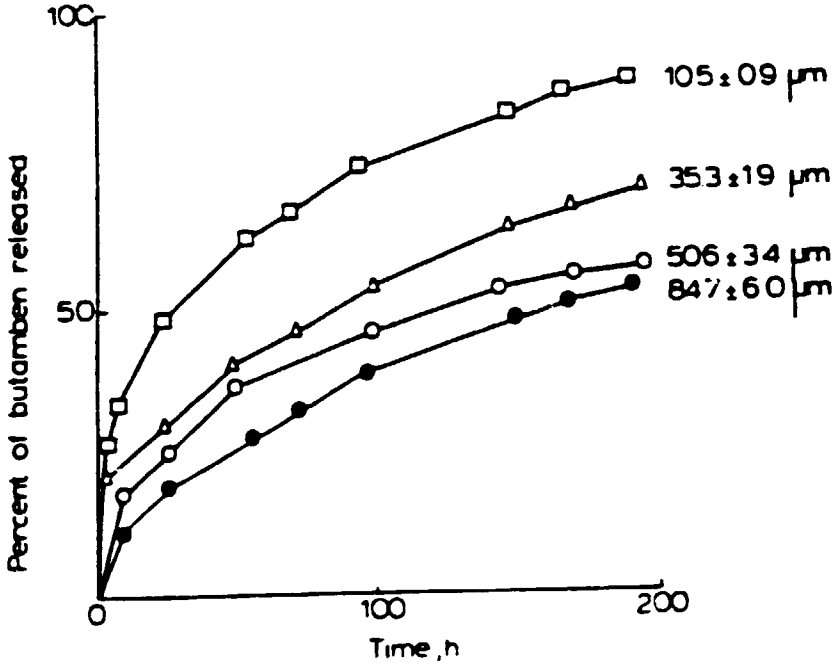
A schematic diagram showing the fabrication of microspheres.

FIGURE 9



Microsphere preparation by melting method (Wichert and Rohdewald 1990).

FIGURE 10



Effect of size on release patterns from polylactic acid microspheres.

FIGURE 11

release from biodegradable polymers, higher drug loading, in general, results in a faster rate of release. The effect of polymer molecular weight is also clearly illustrated by the data (Ramtoola *et al*, 1992).

TABLE 2

FLUPHENAZINE IN POLY(D,L-LACTIDE) MICROSPHERES

POLYMER MOLECULAR WEIGHT	***TIME FOR 50% RELEASE***
2,000	***44 day***
16,000	***164 day***
109,000	***2.3% in 37 day***
FLUPHENAZINE LOADING	
10%	***48 day***
20%	***17 day***
30%	***5 day***

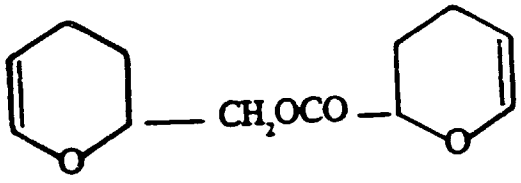
Recent Developments in Microencapsulation

Surface polymerisation of an adsorbed layer on a solid particle has recently been developed as a microencapsulation technique (Graham and Amer, 1992). The microencapsulation of potassium chloride will be taken as an example of the application of the method to provide a sustained release product.

Boron tri-fluoride etherate, as a catalyst in the cationic polymerisation process, is adsorbed onto potassium chloride crystals in heptane. The crystals activated with catalyst are separated and re-dispersed into heptane containing the monomer, for example, 3,4-dihydro-2H-pyranyl-2-methyl-(3,4-dihydro-2H-pyran-2-carboxylate), the structure of which is shown in Figure 12 together with the mechanism of polymerisation. Surface polymerisation of the monomer takes place following the

mechanism in Figure 12 and the potassium chloride crystals are microencapsulated by the polymer.

The release rate from the surface encapsulated potassium chloride was constant over a period of 30 minutes with a time for 50% release of 15 minutes. This technique has also been used to microencapsulate β-estradiol.



3,4-dihydro-2H-pyranyl-2-methyl-(3,4-dihydro-2H-pyran-2-carboxylate).

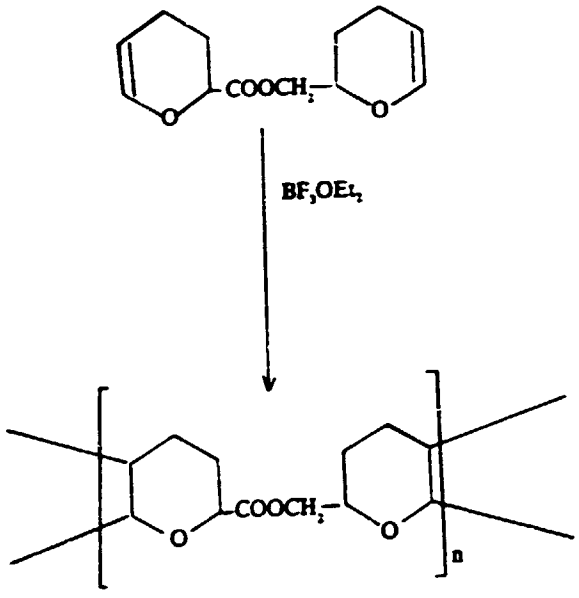


FIGURE 12

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BIODEGRADABLE POLYMERS FOR DRUG DELIVERY

T. L. Whateley

Department of Pharmaceutical Sciences

University of Strathclyde

Glasgow, G1 1XW

CONTENTS

Introduction

Polymeric Implants for Delivery of Protein and Polypeptide Drugs

Biodegradable Polymers

Polyanhydrides

Poly(lactic-co-glycolic acid)

INTRODUCTION

Recent advances in genetic engineering recombinant DNA technologies has led to the development of novel therapeutic protein and peptide drugs. A partial list is shown in Table 1. However, these therapeutic agents are difficult to administer due to their molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance and denaturation. Due to these properties protein and polypeptide drugs are currently generally administered by non-oral routes. Developments in the oral and nasal delivery of proteins and polypeptides will be discussed elsewhere in the Course.

Possible parenteral methods of administration include:

1) Solutions : i/v. s/c or i/m : frequent administration necessary
   : controlled infusion pump.
2) Oil based depot : i/m or s/c : possible, discussed in more detail under 'Multiple Emulsions'
3) Polymeric Implants or Depot of Microspheres : this approach has proved successful for the delivery of polypeptides and will be discussed in detail.

Polymeric Implants for Delivery of Protein and Polypeptide Drugs

Some non-biodegradable polymeric implants systems have been investigated for the sustained release of polypeptides e.g.

Cross-linked poly(vinyl alcohol) hydrogels (Langer & Folkman, 1976).

Ethylene/vinylacetate copolymer (Langer, 1984).

Silicone elastomers (Hsieh *et al*, 1985).

However, the major disadvantage of non-biodegradable systems is that the implant has to be surgically removed once their lifetime has expired. It is also difficult to achieve a constant rate of release (zero-order release).

A number of macromolecular, naturally occurring materials have also been considered, e.g. albumin, gelatin, collagen, etc. In general these can be immunogenic when

TABLE I: A PARTIAL LIST OF PROTEIN DRUGS APPROVED FOR USE OR UNDER INVESTIGATION

PROTEIN	USE/FUNCTION
Human insulin	Treatment of diabetes mellitus
Human growth hormone	To supplement inadequate levels of endogenous growth hormone secretion in children
Alpha interferon	Treatment of hairy cell leukemia and AIDS-related Kaposi's sarcoma
Hepatitis B vaccine	Vaccination against infection caused by hepatitis B virus
Tissue plasminogen activator	Lysis of thrombi obstructing coronary arteries
Erythropoietin	Stimulation of red blood cell production
Murine monoclonal antibody	As immunosuppressant to prevent kidney transplant rejection
Gamma interferon	Treatment of chronic granulomatous disease
Granulocyte colony–stimulating factor	Promotes production of granulocytes; As an adjunct to chemotherapy in correcting neutrophil deficiency
Granulocyte–macrophage colony–stimulating factor	Promotes production of granulocytes and macrophages; Treatment of non–Hodgkin's lymphoma, acute lymphoblastic leukemia and Hodgkin's disease associated with bone marrow transplantation
Interleukin–2	Modulates immune function; Renal call carcinoma
Factor VIII	Treatment of blood clotting factor deficiency in hemophiliacs
Macrophage colony–stimulating factor	Promotes production of macrophages; Treatment of fungal infections; Wound healing
Monoclonal antibodies	Bind to and inactivate endotoxins to prevent septic shock; As cancer-imaging agents

cross-linked.

Biodegradable polymers are clearly the materials of choice for implantation for sustained drug delivery. The polymeric drug carrier needs to be treated as a drug itself in terms of the safety, biocompatibility and lack of toxicity of the polymer and its degradation products. In general, there are two processes of biodgradation - surface erosion and bulk hydrolytic degradation. Polymers where degradation is hydrolytical ly rather than enzymically controlled are preferable in that there will be less patient-to-patient variation.

In general, polypeptide and protein drugs are too large to show significant diffusion through polymer matrices : thus the release of drug can be controlled by the degradation of the matrix. This can allow a more constant rate of release (i.e. closer to zero order release) than with a diffusion controlled release device, where the rate of release tends to decrease with time and fraction released (e.g. Higuchi diffusion control release). Unstable drugs can also be protected from the surrounding environment until released.

Biodegradable Polymers

A range of biodegradable polymers have been considered for the sustained release of protein/polypeptide drugs : e.g.

Poly(alkyl-α-cyanoacrylates)

Poly(ortho-esters)

Poly(amino acids) i.e. polypeptides

Poly(dihydropyrans)

Poly(anhydrides)

and the group of

Aliphatic polyesters :

Poly(lactic acid) or poly(lactide)

Poly(glycolic acid) or poly(glycolide)

Poly(lactic-co-glycolic acid) or poly(lactide-co-glycolide)

Poly(ϵ-caprolactone)

Poly(hydroxy butyrate)

Some structures are shown in Figure 1.

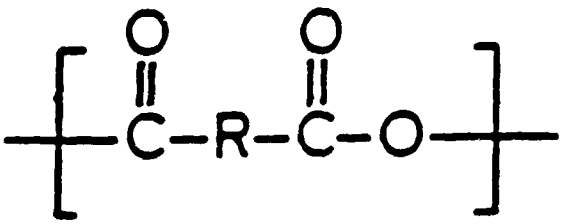
Biodegradable polymers for drug delivery have been reviewed by Leong (1991) and Smith *et al* (1990).

Attention will be focused on the aliphatic polyesters, in particular on the poly(lactic-co-glycolic acid) polymers which are used in current clinical practice for controlled drug delivery, having received regulatory acceptance for long-term parenteral use, in particular for once-a-month sub-cutaneous administration of LHRH agonists for the treatment of prostatic cancer (Zoladex and Prostap SR). They are inert and biocompatible and degrade to the naturally occurring lactic and glycolic acids.

The only other type of biodegradable polymer to be considered in detail is the polyanhydride group as controlled drug delivery systems using these polymers have been taken through to Phase 3 Clinical Trials.

Polyanhydrides

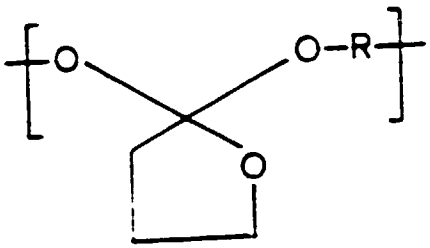
This class of polymer has the general structure shown here in Figure 2.



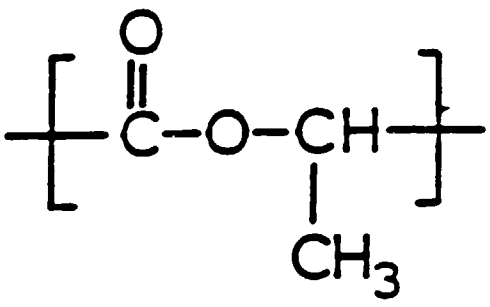
Polyanhydrides

FIGURE 2

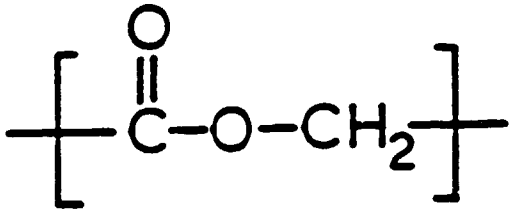
Poly(ortho-esters)



Poly(α-hydroxy acids)



poly(lactic acid)



poly(glycolic acid)

poly(ϵ-caprolactone)

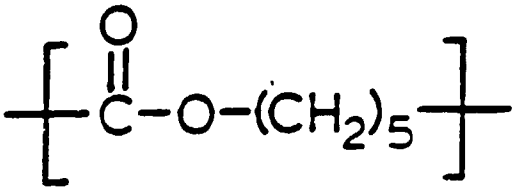


FIGURE 1

A particular polyanhydride has received much attention recently with its application in the controlled delivery of the cytotoxic nitrosourea, BCNU (or carmustine) to treat brain tumours. This work has been pioneered by Brem and his group at John Hopkins Medical Center, Baltimore, USA (Brem, 1990; 1990a) using the biodegradable polymer developed by Langer and co-workers at MIT. (Langer, 1988).

The particular polyandride used is poly(p-carboxyphenoxypropane-co-sebacic acid) PCPP-SA whose structure is shown in Figure 3.

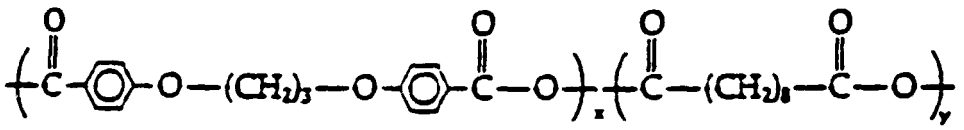
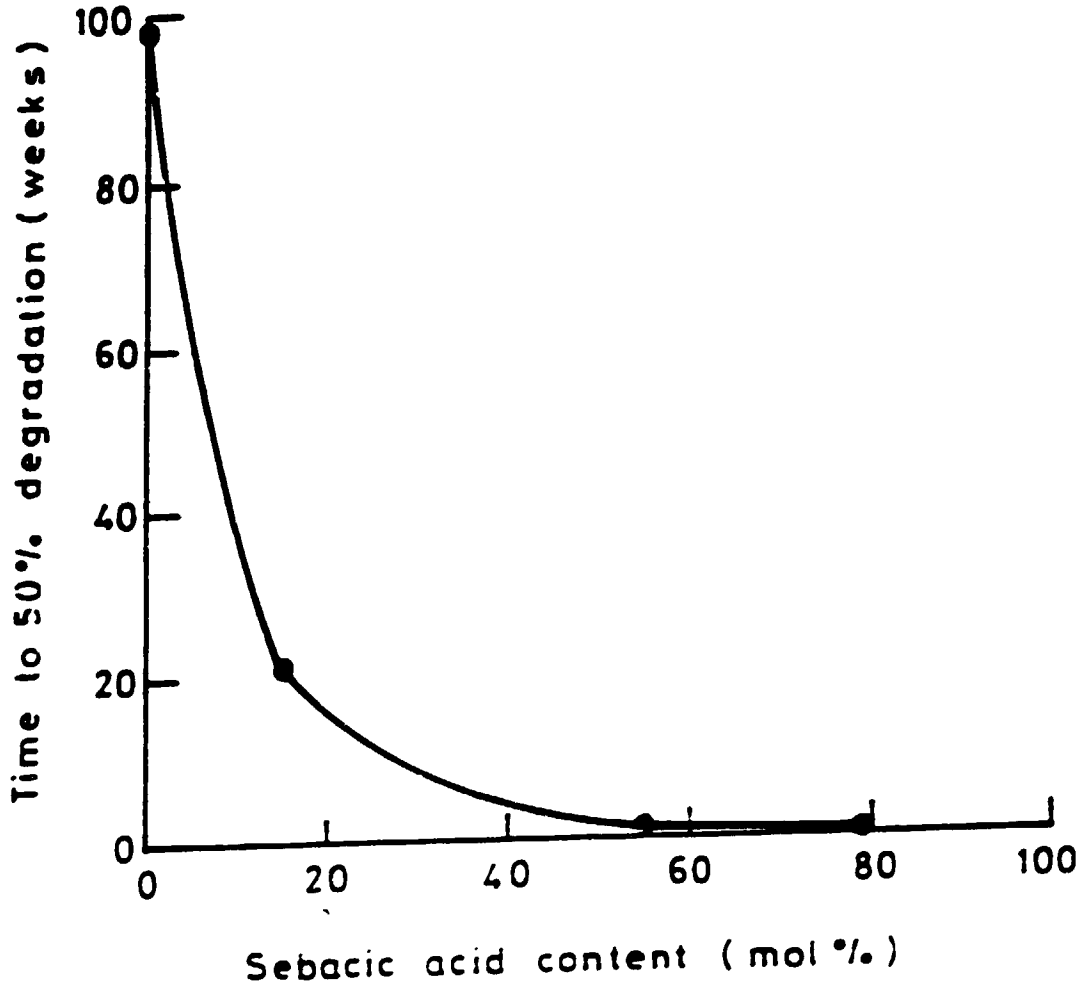


FIGURE 3

This is a hydrophobic, surface eroding polymer and hence can protect the BCNU (which has a half-life of only 12 min. in plasma and aqueous systems) until it is released in the proximity of the brain tumour. Implants are inserted at surgery into the cavity left by the removal of the tumour. Lack of toxicity of the polymer has been shown, as has the efficacy of the drug loaded implant : multi-centre Phase 3 Clinical Trials are being undertaken.

In the Department of Pharmaceutical Sciences at the University of Strathclyde we are following a similar approach but using the non-toxic, acceptable PLGA polymers loaded with carboplatin, which, being water soluble should not re-distribute from the brain, being unable to cross the blood-brain-barrier.

The degradation rate of PCPP-SA co-polymers can be controlled by the sebacic acid content, as shown in Figure 4. Thus, as the release rate is determined by surface



Effect of polymer composition on degradation rate of polyanhydrides.

FIGURE 4

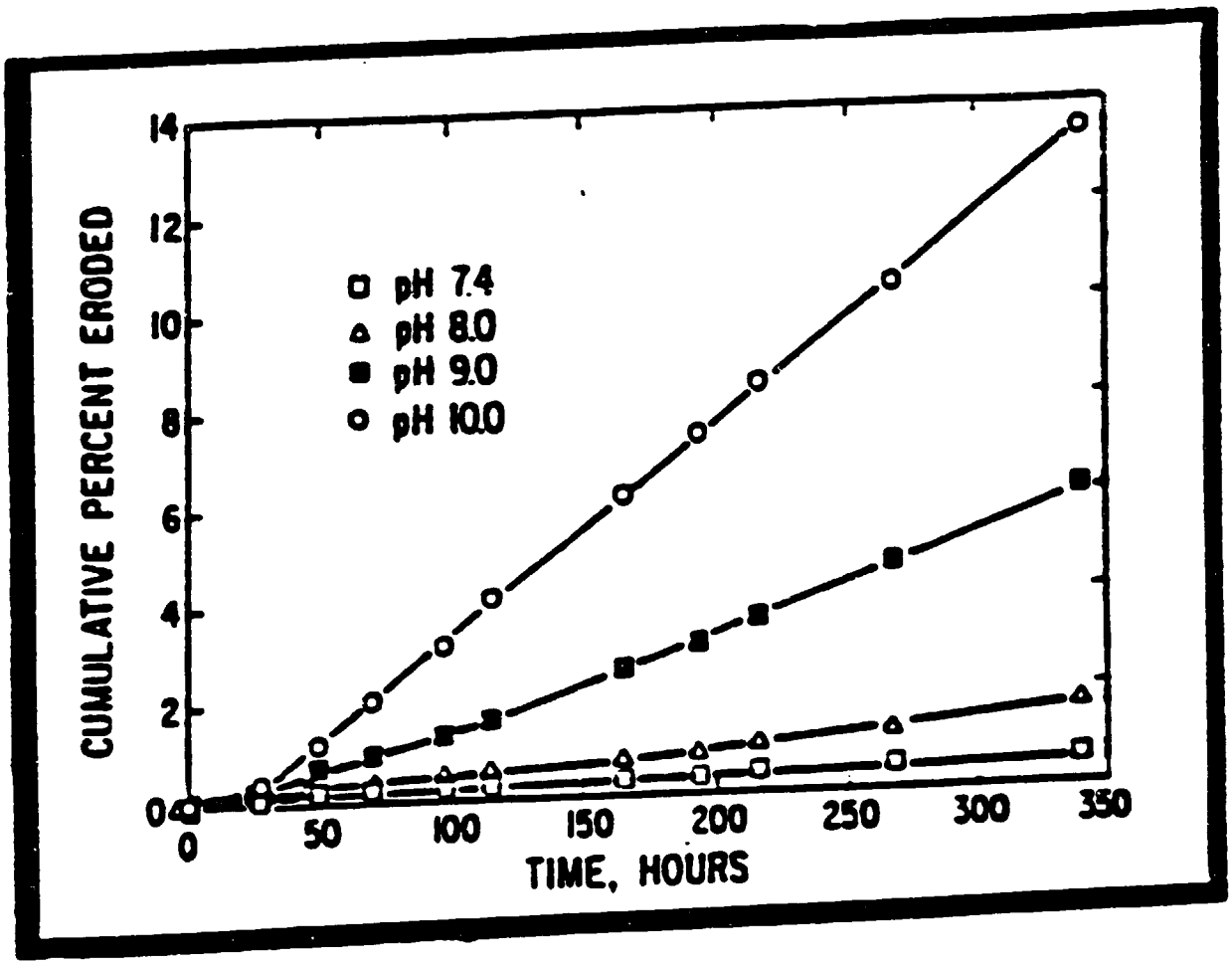
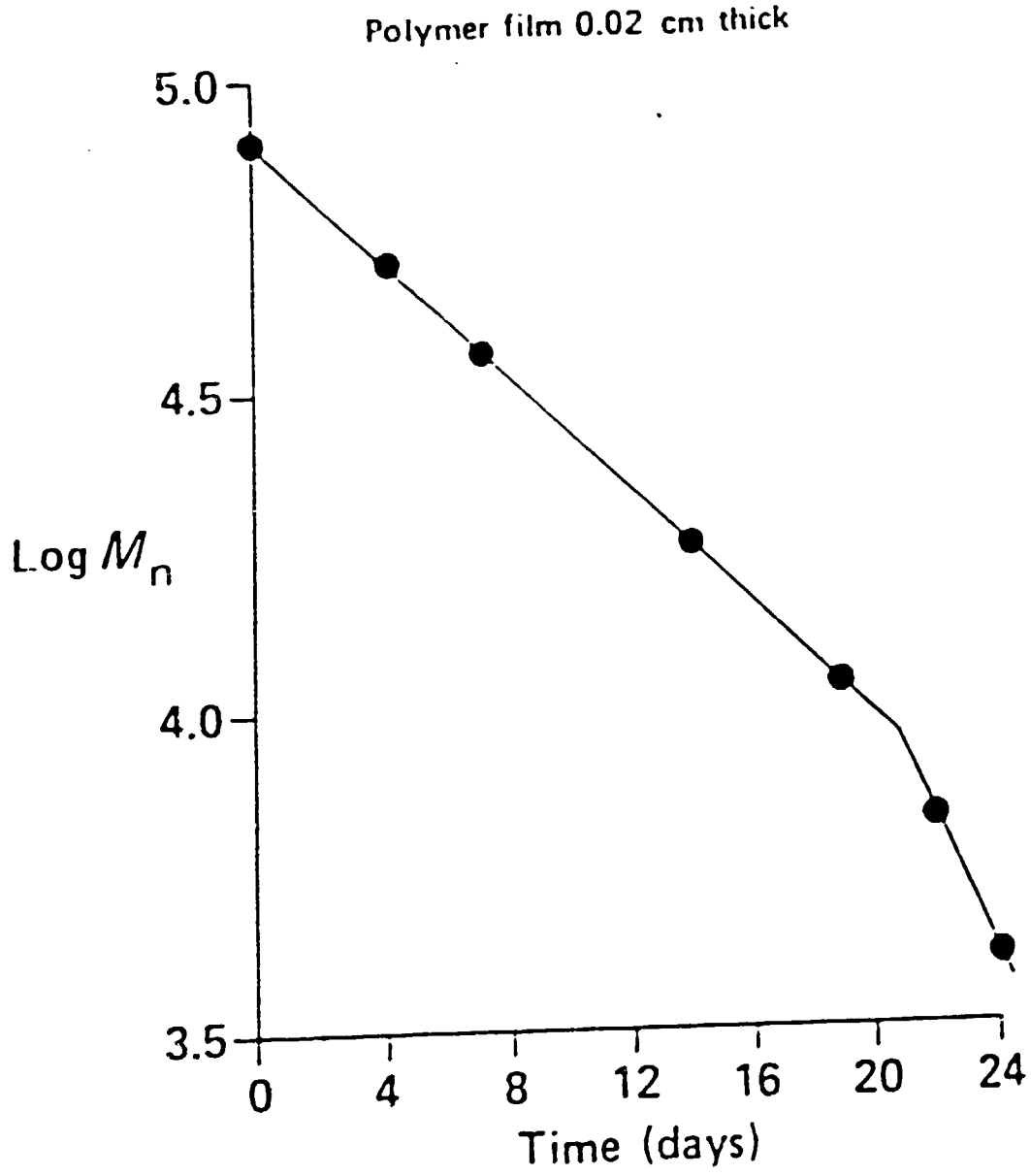

CUMULATIVE PERCENT ERODED
14
12
10
8
6
4
2
0
0
50
100
150
200
250
300
350
TIME, HOURS
pH 7.4
pH 8.0
pH 9.0
pH 10.0

FIGURE 5



In vitro degradation of poly (*d,l*-lactide-co-glycolide) at 37°C in buffer at pH 7.4. Number average molecular weight of degrading polymer as function of time.

FIGURE 9

erosion/degradation, a range of release rates can be readily achieved.

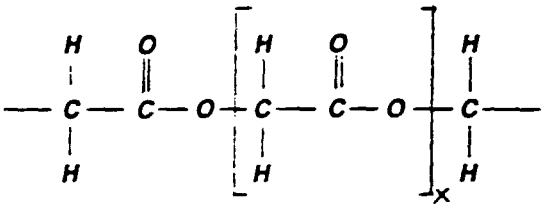
The rate of degradation of PCPP-SA polyanhydrides is sensitive to pH as shown in Figure 5. An increase in pH values causes a faster rate of degradation (Laurencin *et al.* 1990).

Poly(Lactic-co-Glycolic Acid) Polymers

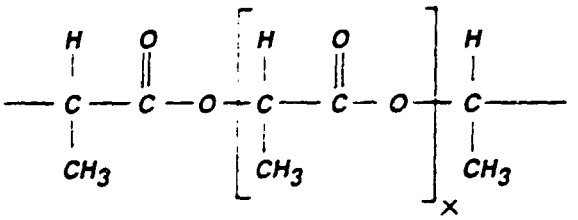
The structure of the monomeric lactic and glycolic acids is shown in Figure 6. It should be noted that lactic acid can exist in both D and L stereo isomeric forms; the L being the form occurring *in-vivo*. Both lactic and glycolic acids occur *in vivo* and are utilised in normal function.

The polymers and co-polymers are available commercially. They can be synthesised via the dimeric lactide and glycolide, whose structures are shown in Figure 7.

The structure of PLGA co-polymers is shown in Figure 8.



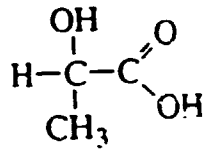
POLY (GLYCOLIC ACID)



POLY (LACTIC ACID)

FIGURE 8

The poly(glycolic acid) polymer is very crystalline and insoluble in solvents other than fluorinated ones. The poly(L-lactic acid) polymer is more crystalline than the poly(D,L-lactic acid) material where the chains cannot pack so well. Crystallinity decreases as glycolic acid is introduced into the poly(lactic acid) systems.

$CH_3CH(OH)COOH$  Lactic acid

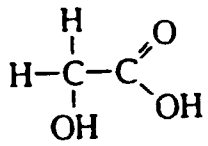
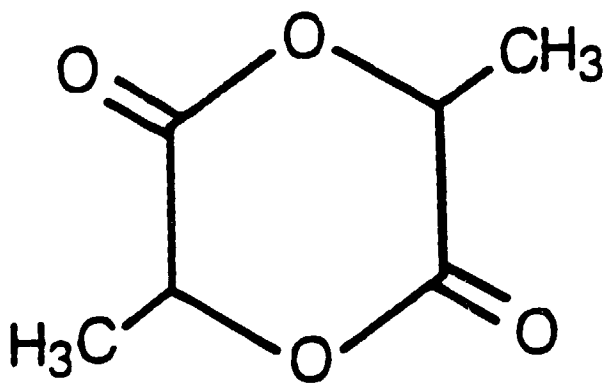
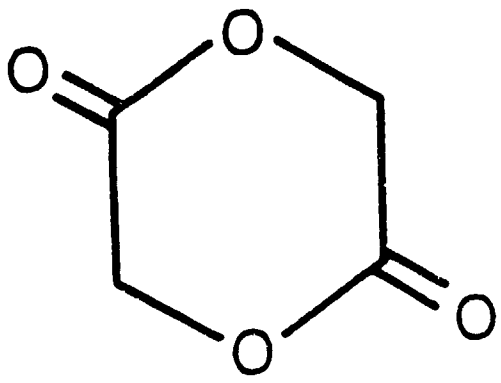
$CH_2OHCOOH$  Glycolic acid

FIGURE 6



Lactide



Glycolide

FIGURE 7

The PLGA co-polymers degrade by a hydrolytic mechanism, which occurs throughout the matrix i.e. it is not an enzymic or surface erosion mechanism. There is an increase in rate of hydrolysis at high and low values of pH typical of acid-base catalysed ester hydrolysis (Makino *et al*, 1985).

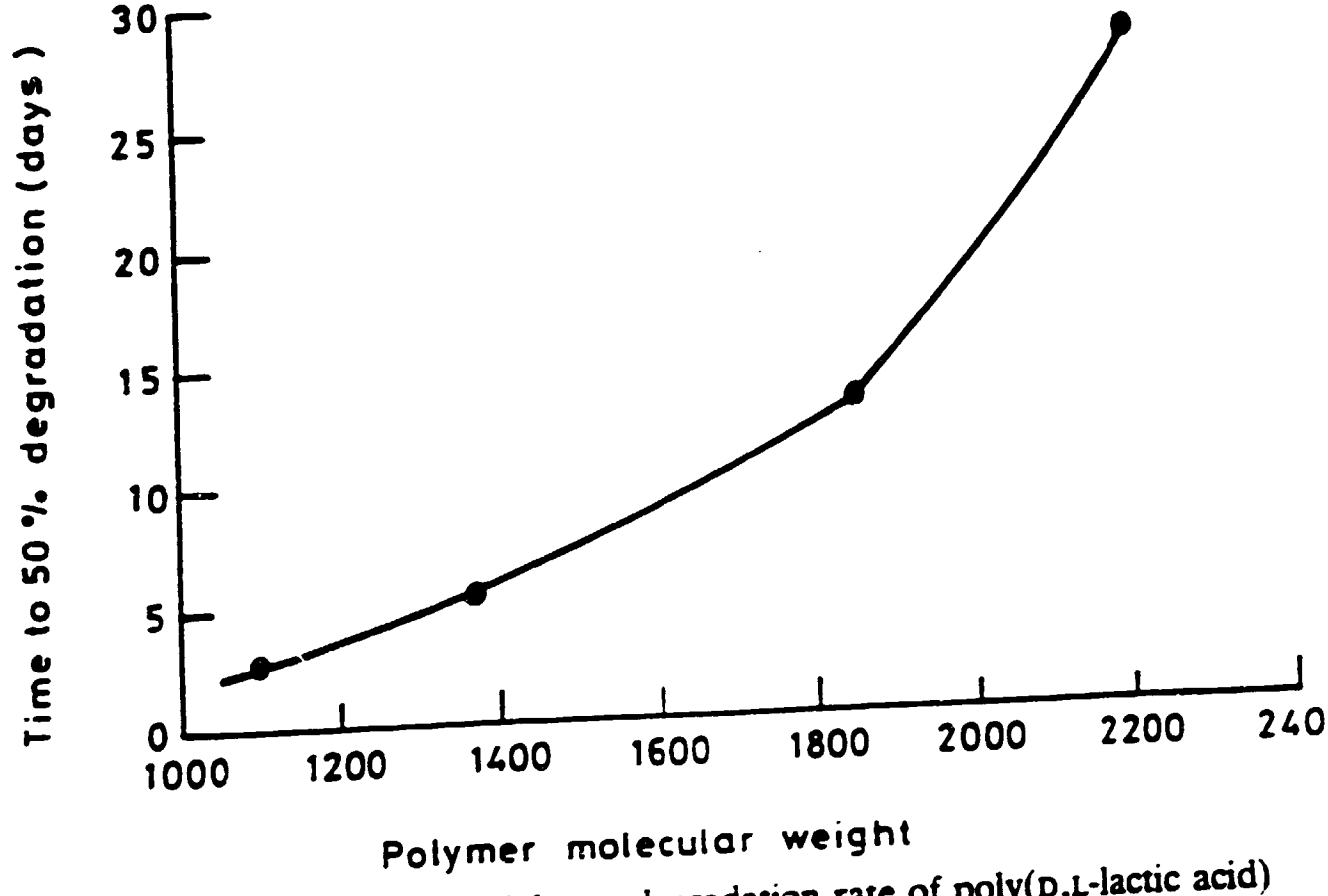
A typical *in vitro* degradation curve at 37°C and pH 7.4, as measured by molecular weight decrease, is shown in Figure 9 (Fildes *et al*, 1991).

There are three major variables which can affect the properties of the co-polymers i.e. molecular weight; ratio of lactic to glycolic acid and γ-irradiation. Each of these parameters has an effect on the degradation rate, which in many cases is the major factor controlling the rate of drug release

Figure 10 illustrates that an increase in molecular weight of PLA decreases the rate of degradation whilst Figure 11 shows that an increasing content of glycolic acid (up to 60-70%) causes an increase in the rate of degradation (Kaetsu *et al*, 1987). It should be noted that the rate of degradation decreases again as the percent of glycolic acid approaches 100%, due to the greater crystallinity in these materials.

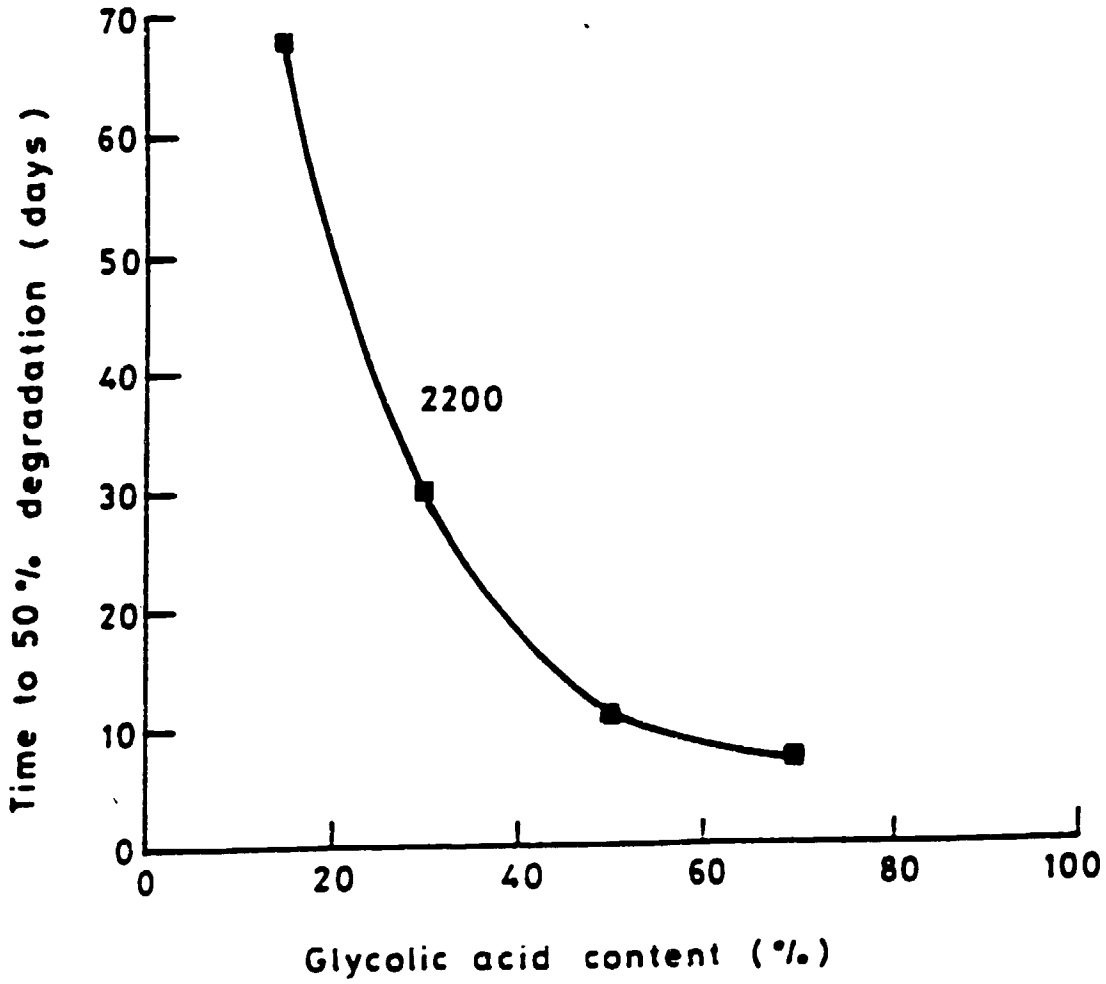
The effect of γ-irradiation (often used for terminal sterilisation of PLGA systems) on the polymer molecular weight (as indicated by the intrinsic viscosity), as illustrated in Figure 12, shows a near-linear decrease in molecular weight with increasing irradiation dose. Thus, γ-irradiation sterilisation has the effect of reducing the molecular weight of the polymer, with consequent effect on the rate of degradation, as previously discussed. These three factors allow control of the rate of degradation of PLGA polymers and consequently on the rate of drug delivery.

Figure 13 shows the process for the preparation of Zoladex type implants for the once-a-month administration of LHRH agonists (Asano *et al*, 1991). The mixture of high and low molecular weight poly(lactic acids), tailored to give the correct degradation and release rate, are melt mixed prior to a further melt mixing with the LHRH agonist. This



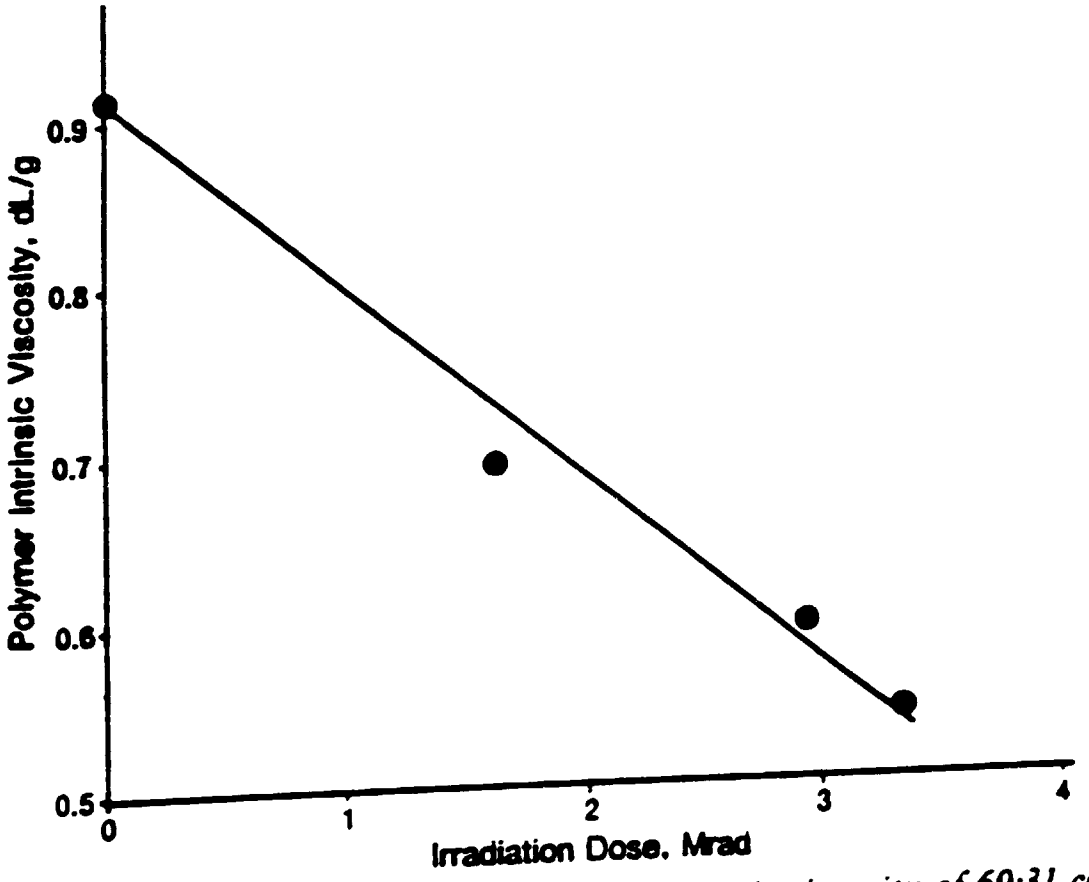
Effect of polymer molecular weight on degradation rate of poly(D,L-lactic acid)

FIGURE 10



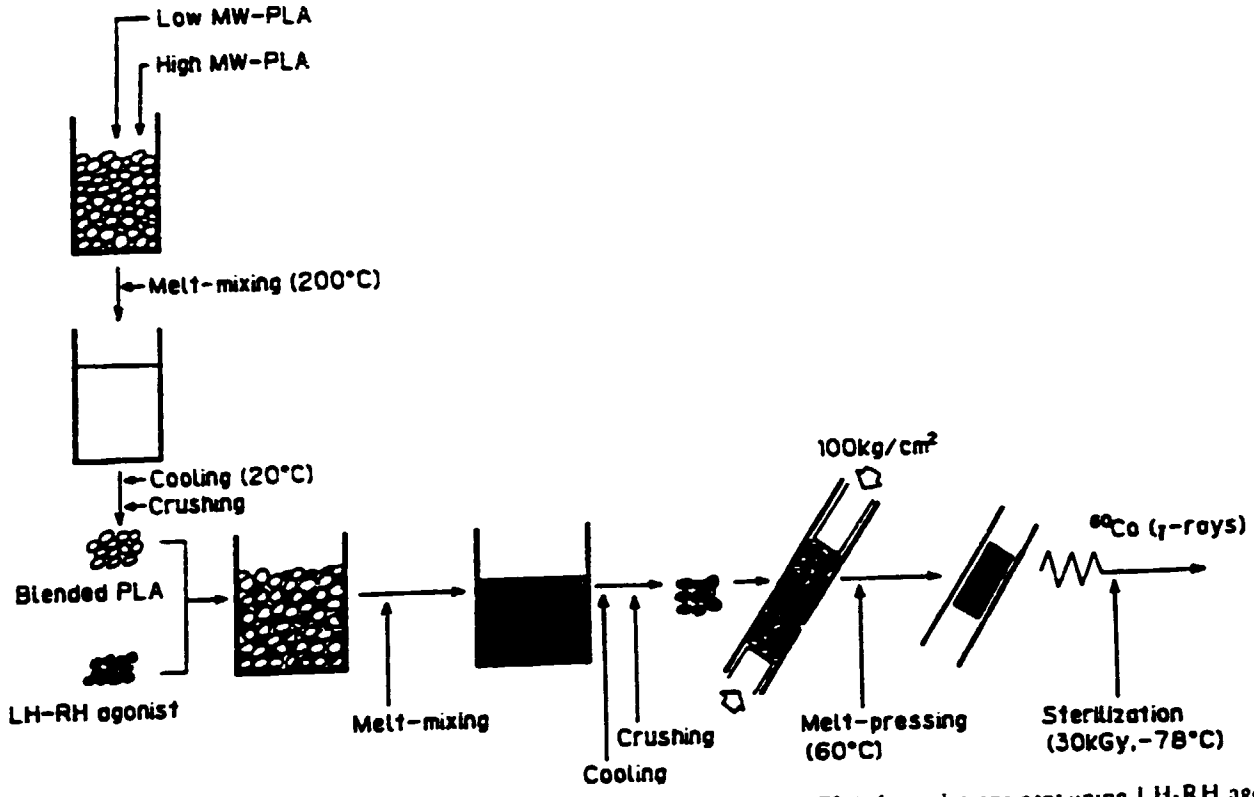
Effect of polymer composition on degradation rate of poly(L-lactic acid/glycolic acid)

FIGURE 11



Effect of gamma-irradiation on intrinsic viscosity of 69:31 co-polymer.

FIGURE 12



Schematic diagram illustrating the preparation of small cylinders of blended PLA formulations containing LH-RH agonist by the melt-pressing technique.

FIGURE 13

mixture is warm-pressed at 60°C and sterilised by γ-irradiation. A special syringe delivery system has been devised for the Zoladex product : however, a 16 gauge needle is needed and this can necessitate the use of a local anaesthetic for the sub-cutaneous injection. Microsphere suspension preparations have been developed, as discussed in the lecture on "Microencapsulation and Microspheres" which can be injected using smaller needles of 21 to 23 gauge. An excellent review of the development of Zoladex is given by Fildes *et al*, (1991).

The general properties of the release of drugs from PLGA systems is well-illustrated in the data in Figures 14a and 14b. These systems are cis-platin loaded microspheres of 75:25 PLGA of molecular weight 58,000 prepared by the oil-in-water emulsion solvent evaporation method with the external phase saturated with the water-soluble cis-platin (Spenlehaner *et al.*, 1988).

Figure 14a shows the release over a 48 hour period for three different drug loadings (18%, lowest curve; 32%, middle curve and 41%, top curve). This data illustrates the general phenomena that a faster rate of release is found with higher drug loadings, over the initial stages of release where degradation of the matrix is not rate-controlling but release is determined by a diffusion-dissolution mechanism. The explanation can be understood by consideration of Figure 15 which shows the distribution of drug crystals (cis-platin and mitomycin C will be insoluble in the polymer and dichloromethane solvent and will be present in the matrix as dispersions of crystals) within the polymer matrix at the 3 different drug loadings. At the highest drug loading, crystals in contact with the surface can dissolve and form channels for water to leach out crystals in the interior. Essentially all the crystals can be part of a network connected to the external aqueous phase.

In the case of medium loading ony a limited number of crystals are in contact with the aqueous phase. Isolated crystals in the interior can only release drug via the diffusion

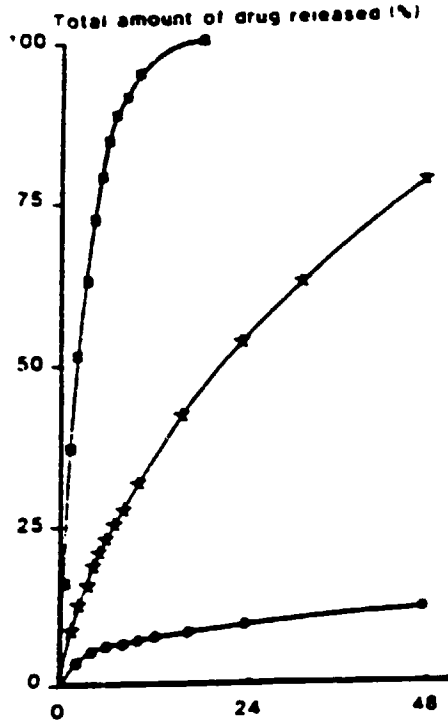


FIGURE 14a

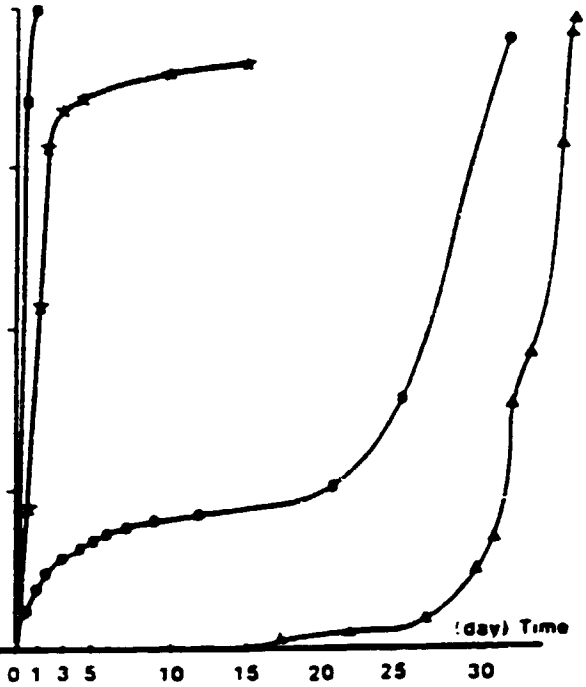


FIGURE 14b

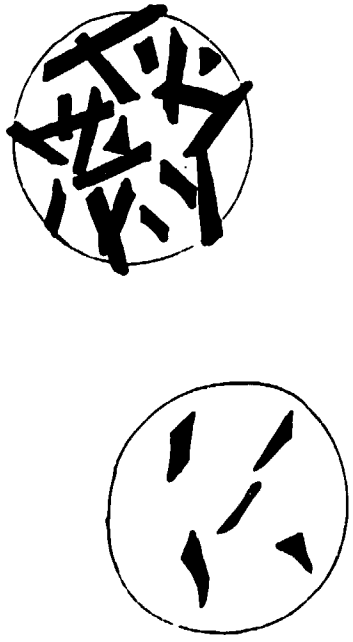


FIGURE 15

of water through the matrix and dissolution, clearly a slower process. At low loading of drug, essentially all of the drug particles in the interior are isolated and as shown in Figure 14a only 10% is released at 48 hour.

Figure 14b, shows data for the same systems of Figure 14a over a time period of more than 30 days (the rightmost curve is for a loading of 9.1%, which showed zero release over the initial 15 day and hence is not included in Figure 14a).

The rapid increase in the rate of release at 20-30 day for the two lower loadings of drug is due to the massive collapse of the polymer matrix due to its hydrolytic degradation. The curve for the drug loading of 18% shows a typical 3 phase release pattern i.e. (1) an initial burst due to material at or close to the surface, (2) a phase of very little release, followed by (3) a rapid release on collapse of the matrix following hydrolytic degradation. The question of the importance of diffusion on degradation controlled drug release has recently been discussed by Shah *et al*, (1992) and Bodmer *et al*, (1992).

The effect of gamma-irradiation on drug release from PLGA matrices is well-illustrated in Figure 16 for PLGA microspheres loaded with 29% cis-platin.

γ-irradiation does not affect the rate of release in the initial few days. It is the massive release on collapse of the matrix which is dramatically affected : γ-irradiation (37.7 kGy) causes the matrix collapse to occur at 10-15 days rather than at 60-70 days in the un-irradiated materials. These results correlate with the data previously presented (Figure ?) where the molecular weight (as indicated by intrinsic viscosity) of PLGA was shown to decrease essentially linearly with γ-irradiation dose.

An injectable 90 day delivery system for testosterone based on PLGA has been reported (Atkins, 1992).

The factors influencing the rate of release of thyrotropin releasing hormone (TRH) from poly(d,l-lactic/glycolic acid) has been investigated in detail recently (Heya *et al*, 1991, 1991a). In particular, the ionic interaction between the basic group of TRH and the

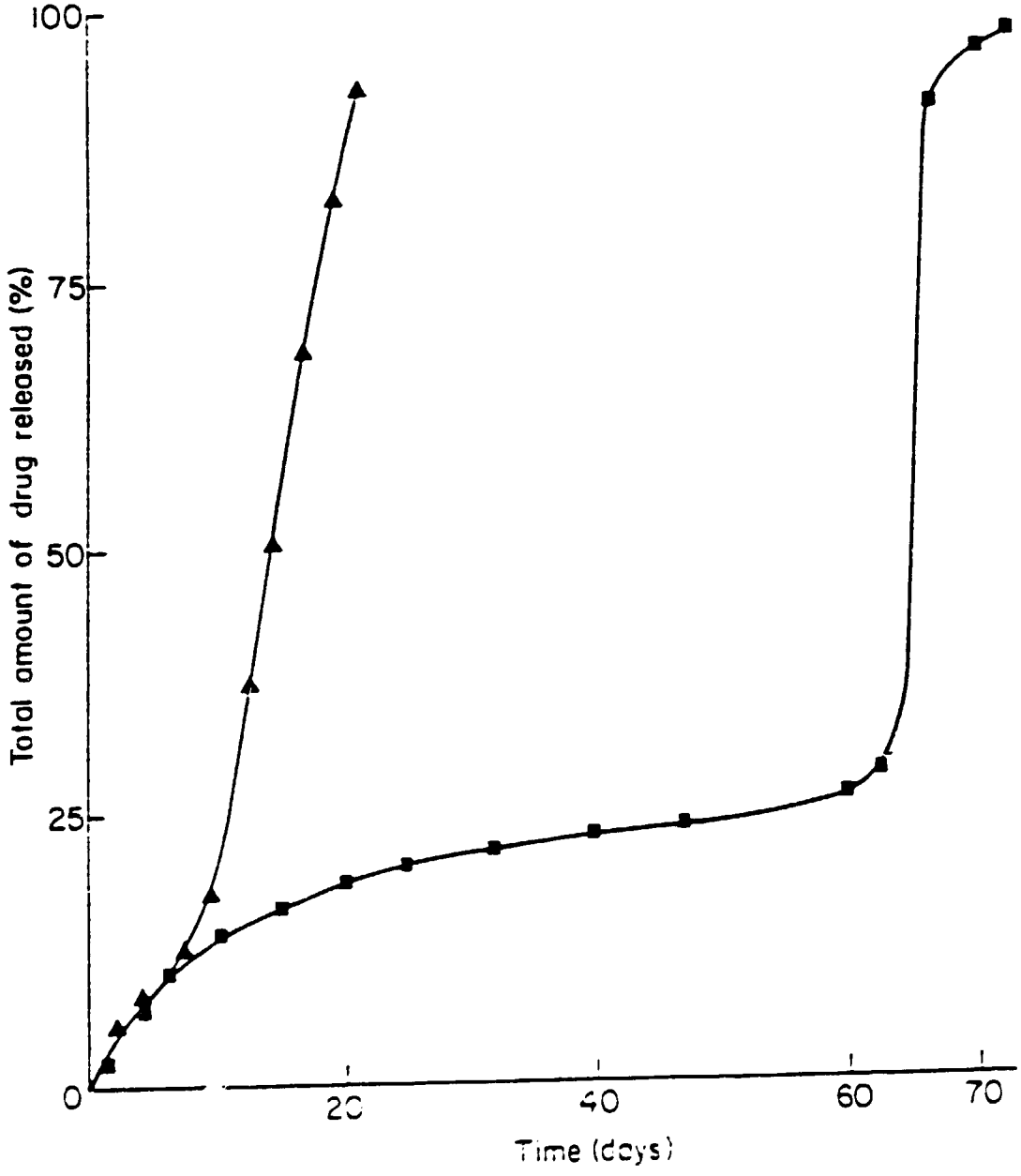

100
75
50
25
0
Total amount of drug released (%)
20
40
60
70
Time (days)

FIGURE 16

carboxylic end group of PLGA affected both the rigidity of the microsphere matrix and the rate of release.

The effect of various additives on the release rate of drugs from PLGA systems has been investigated by a number of groups. The effect of the additon of a low Mw.PLA (2,000) to thin films of a high Mw PLA (120,000) on the release rate of salicylic acid (6.7% loading) is shown in Table 2. Increasing amounts of the low Mw PLA increase the rate of release and the glass transition temperature is reduced (Bodmeier *et al*, 1989).

TABLE 2

High : Low Mw PLA	% Released at 24 hr
100 0	15
75 25	20
50 50	40
25 75	70
0 100	92

The addition of the Pluronic L101 to PLA films eliminated the burst effect for the release of BSA. With 30% L101 there was 15% hydration at 30 days and no loss of L101. The formation of a liquid crystalline phase of H_2O-L101 was postulated (Park *et al*, 1991).

The incorporation of basic amine drugs, can enhance the rate of polymer hydrolysis due to microenvironmental pH effects e.g. thioridazine reduces the polymer molecular weight to one half during the preparation of microspheres. The drug is released rapidly, i.e. 50% in 3 days at pH 7.4 : if the amine group is blocked, [illegible] time is extended. The effect of drug loading on release rate is clearly seen for fluphenazine in poly(D,L-lactide) microspheres. For polymer of molecular weight, 2,000, the T_{50} values for loadings of 10, 20 and 30% were 5, 17 and 48 days respectively. A similar effect was

found for polymer of 16,000 molecular weight (Ramtoola *et al* 1992).

A number of interesting aspects with regard to the degradation of PLGA have been reported.

(a) glycolic acid is released faster than lactic acid.

(b) Degradation is faster in the interior (of implants) due to the lower microenvironmental pH following chain scission but before oligomers in the interior are able to diffuse out (Vert, 1992).

(c) two types of water have been shown by NMR to be present in melt-pressed implants: either dissolved or as water in pores.

(d) free radicals (OH) have been shown to increase the rate of degradation *in vitro* Ali *et al*, 1992).

In general the 50:50 lactide/glycolide co-polymer shows the lowest crystallinity, highest hydrophilicity and maximum rate of degradation. However, samples of 50:50 PLGA of similar molecular weights can show different solubility properties due to the presence of blocks of glycolide (as opposed to a random distribution of glycolide and lactide). Glycolide is highly crystalline and blocks of glycolide result in reduced solubility. Large differences in the 'blockiness' of a sample can be detected by ^{13}C-NMR (Bendix, 1990).

The question of the stability of the drug in the PLGA matrix has obviously to be addressed. For example the cytotoxic drug, etoposide, was found to degrade with a similar mechanism as in aqueous solution, over a period of days in a PLA matrix (Aso *et al*, 1992).

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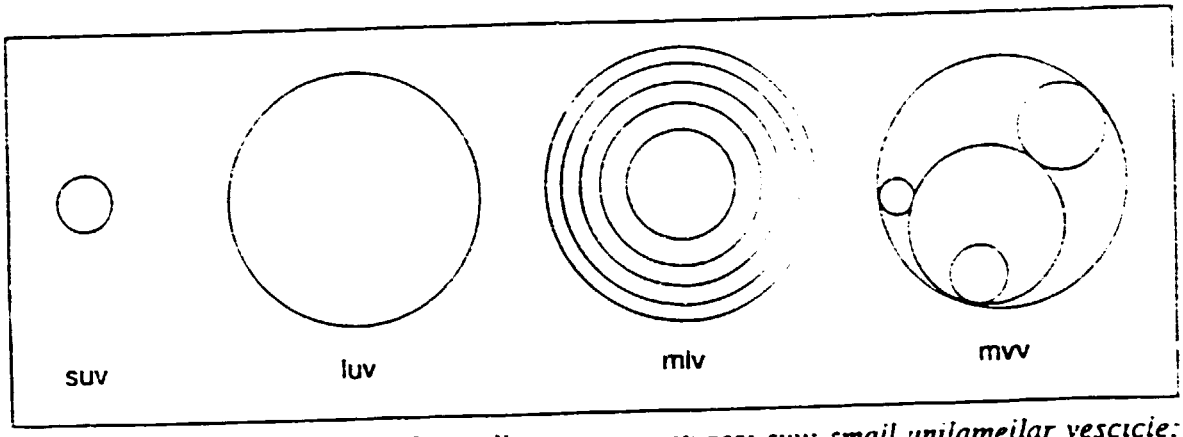
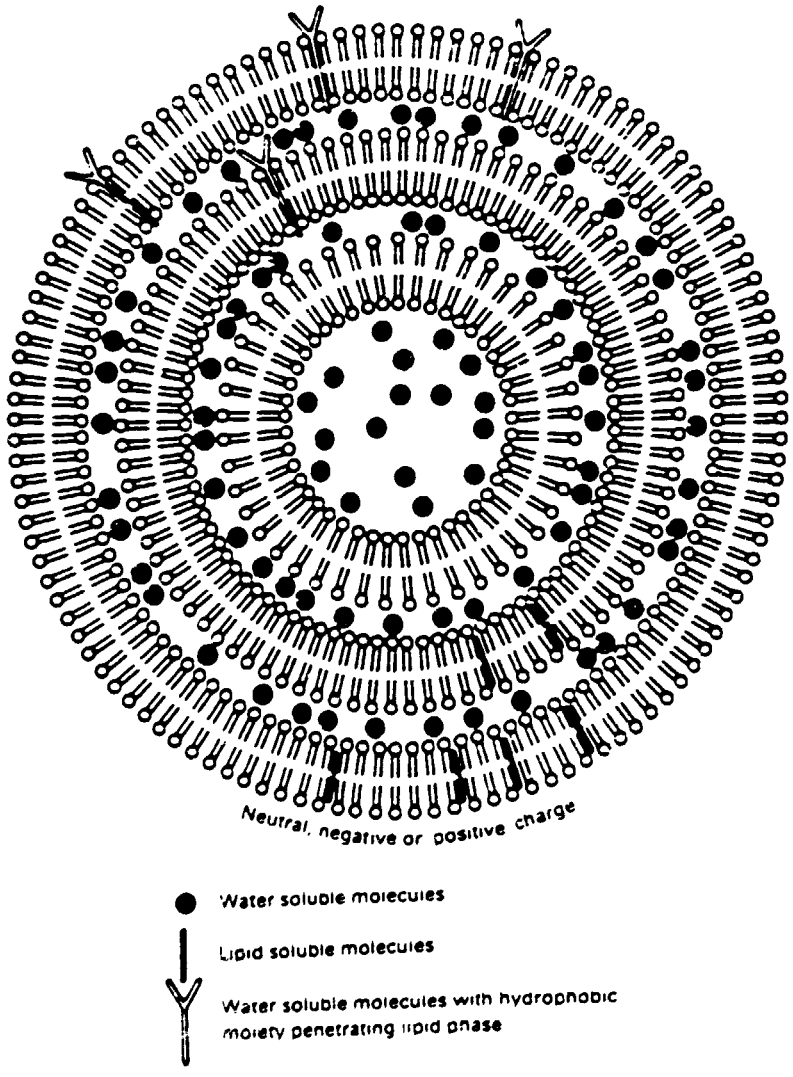
LIPOSOMES AS DRUG DELIVERY DEVICES.

IAN W. KELLAWAY,
WELSH SCHOOL OF PHARMACY,
UNIVERSITY OF WALES,
CARDIFF.

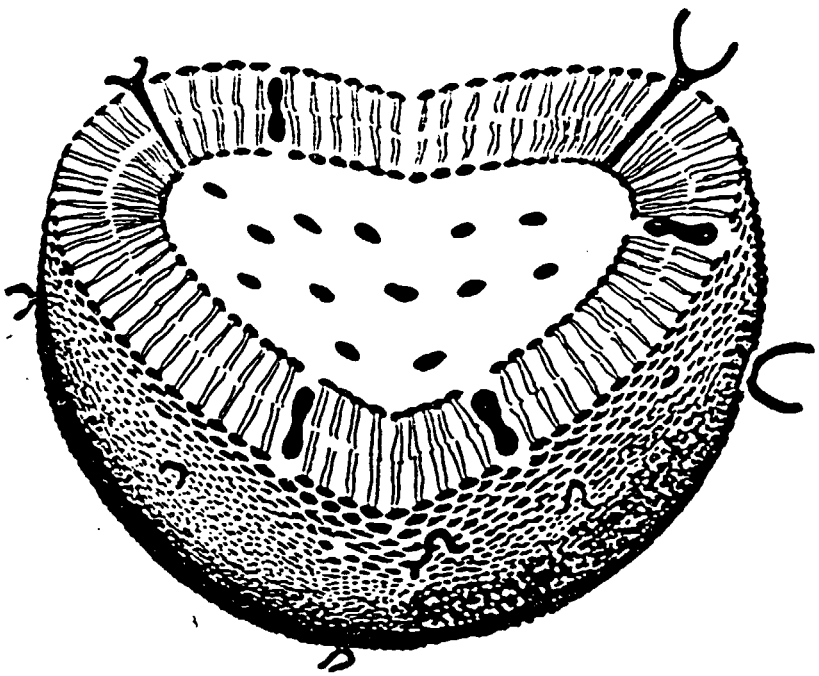


Morphology of the different liposome structures: suv: small unilamellar vesicle; luv: large unilamellar vesicle; mlv: multilamellar vesicle; mvv: multivesicular vesicle.



Schematic diagram of multilamellar liposome.

SCHEMATIC REPRESENTATION OF A UNILAMELLAR VESICLE

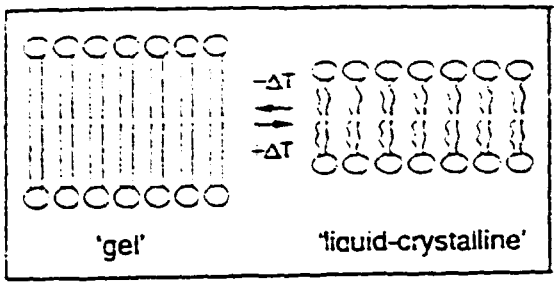


phospholipid

hydrophilic drug

hydrophobic drug

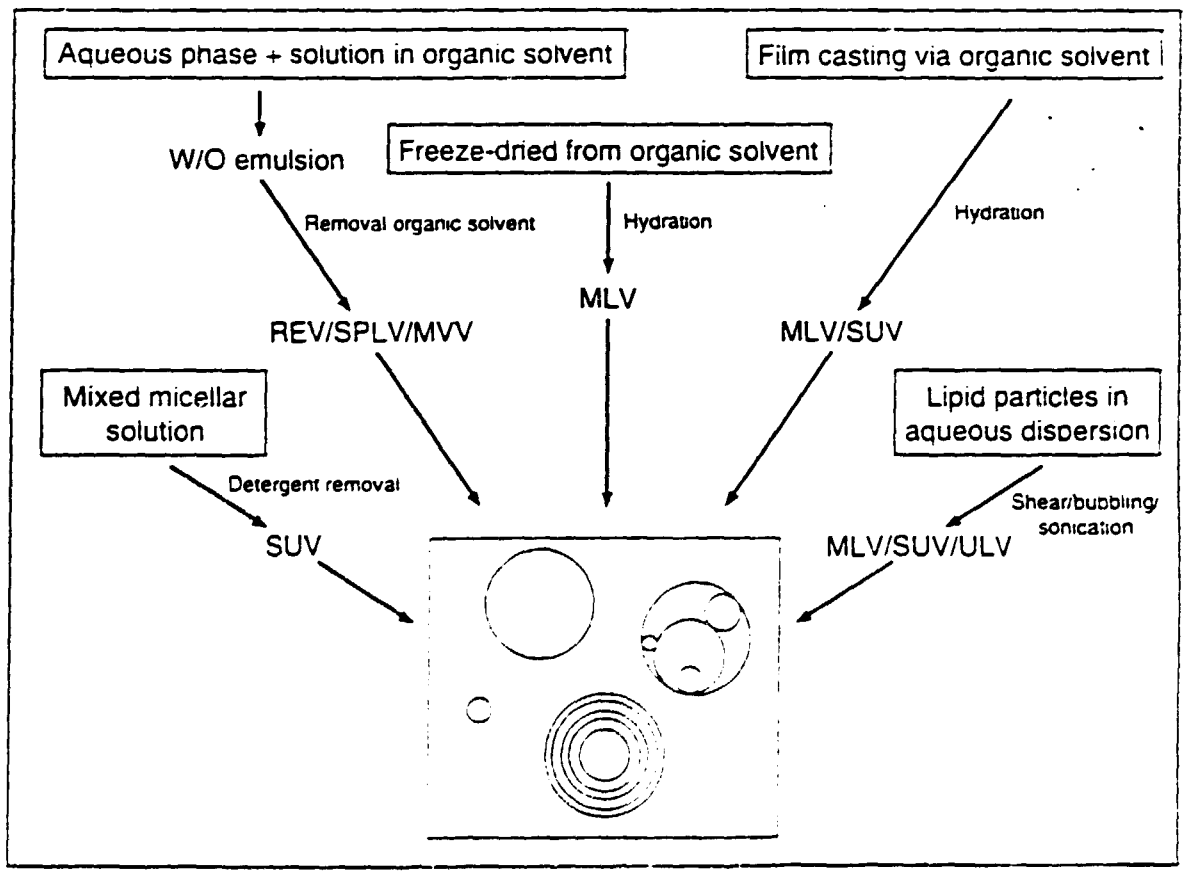
targeting moiety



A schematic view of the 'gel'-'liquid-crystalline' transition of the bilayer.

Transition temperature of some phospholipids regularly selected for liposome preparation.

Phospholipid	T_m (°C)
Egg phosphatidylcholine	-15 to -7
Brain phosphatidylserine	6 to 8
Dipalmitoylphosphatidylcholine	41
Hydrogenated soy-bean phosphatidylcholine (phospholipon 100H®)	51



Schematic diagram of regularly used methods used for liposome preparation. The commonly obtained type of vesicle is indicated. REV — reverse-phase evaporation vesicles; SPLV — stable plurilamellar vesicles; ULV — unilamellar vesicles.

CLINICAL RATIONALE FOR DRUG CARRIERS.

1) GREATER SELECTIVITY THAN ACHIEVABLE BY DRUG DESIGN ALONE.

2) LARGE DRUG PAY-LOAD PROTECTED WITHIN CARRIER PARTICLE FROM DEGRADATION.

3) DECREASED TOXICITY BY CONTROL OF DRUG AND METABOLITE LEVELS IN BLOOD AND ORGAN SITES.

4) CELLULAR UPTAKE BY VARIOUS MECHANISMS ——— ENHANCED CELLULAR DRUG LEVELS.

5) ALTERED PHARMACOKINETICS ———— REDUCED TOXICITY AND ENHANCED THERAPEUTIC EFFECTS.

POTENTIAL LIPOSOMAL - TARGETING BENEFITS.

1) ENDOGENOUS, BIODEGRADABLE, NON-TOXIC CONSTITUENTS.

2) WIDE RANGE OF COMPONENTS

——— LIPOSOMES OF DIFFERENT PHYSICAL AND BIOLOGICAL PROPERTIES.

——— DESIGN FOR SPECIFIC TARGET.

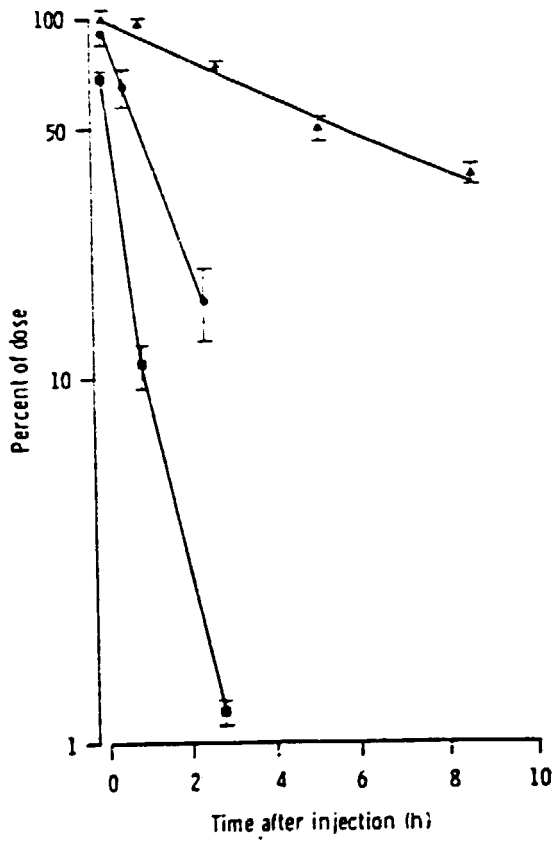
3) NO CHEMICAL REACTIONS AND MILD PREPARATION CONDITIONS

——— OPTIMAL DRUG STABILITY.

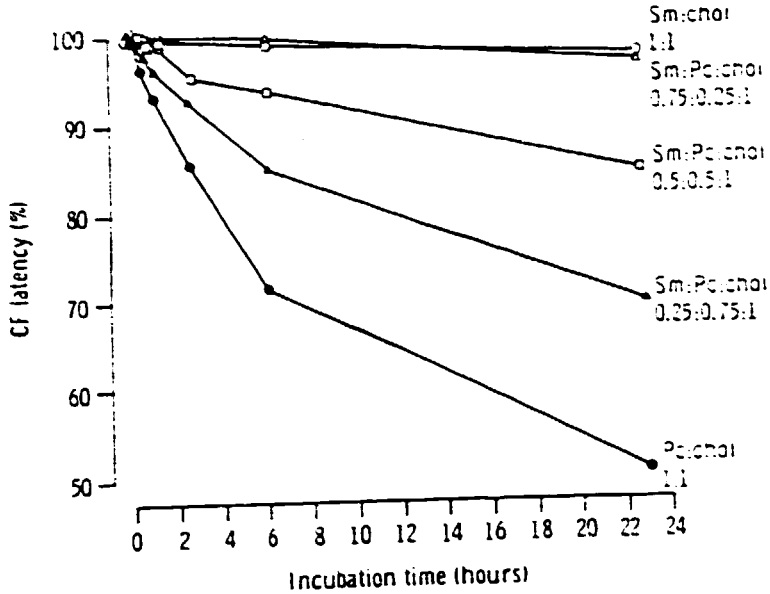
4) IN ADDITION TO CELL ENTRY BY ENDOCYTOSIS ——— FUSION ALBEIT AT LOW EFFICIENCY.

5) READY MODIFICATION OF SURFACE PROPERTIES (MEMBRANE FORMING LIPIDS, ADSORPTION OF MACROMOLECULES; COVALENT ATTACHMENT OF TARGETING ENTITIES).

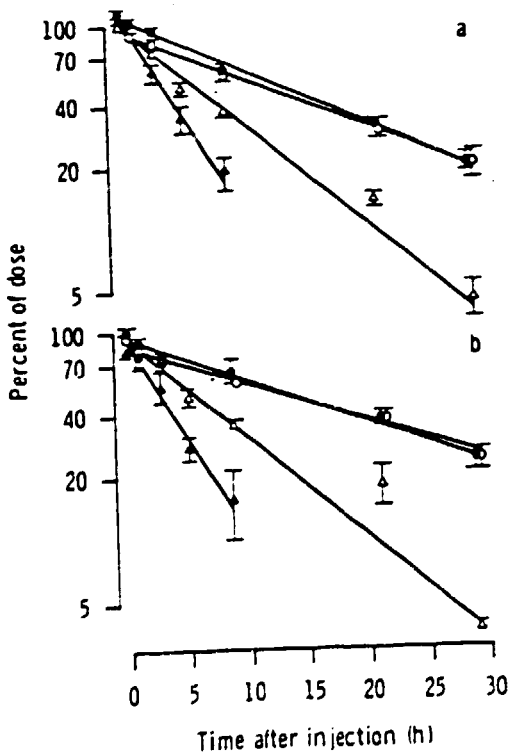
6) POSSIBLE IN VIVO TRIGGERING OF DRUG RELEASE (pH, TEMPERATURE, PHOTOCHEMICAL MODULATION).



Effect of size on the clearance of liposomes from the circulation of mice. Mice were injected intravenously with CF-containing liposomes composed of equimolar cholesterol and DSPC. Vesicles given were reverse phase evaporation filtered through polycarbonate filters of 0.4 (■) and 0.2 (●) μ diameter or small unilamellar (▲), all at a dose of 21 mg phospholipid per kg body weight.

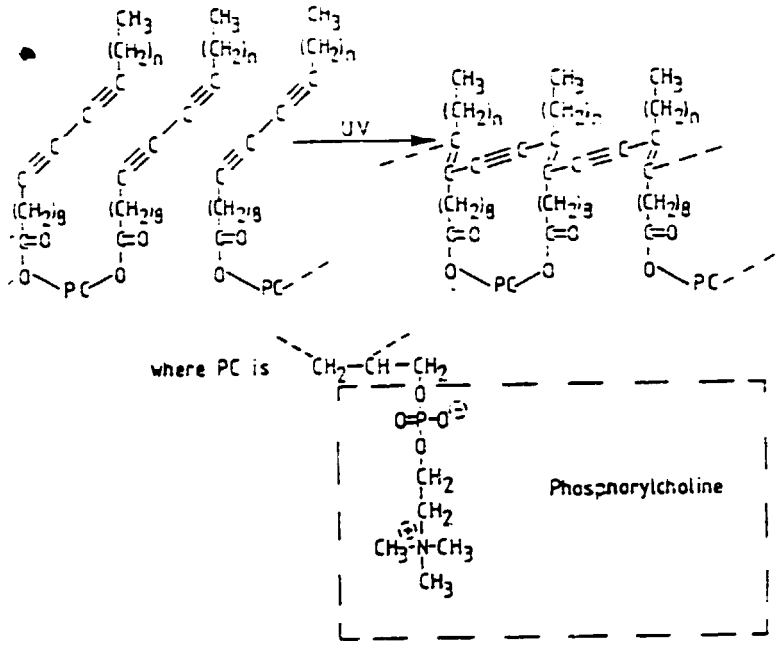


The effect of phospholipid composition of liposomes on their permeability in plasma. Small unilamellar liposomes containing quenched CF and composed of SM (△), 77% SM, 23% PC (○), 47% SM, 53% PC (□), 23% SM, 77% PC (▲), and PC (●) were incubated in the presence of mouse plasma at 37°C. All liposomal preparations contained cholesterol, equimolar to total phospholipid. CF latency values at time intervals are percent of total CF present.



Effect of dose on clearance of small unilamellar liposomes from the circulation of mice. Mice were injected intravenously with liposomes composed of equimolar cholesterol and DSPC and containing CF (a) and ^{14}C-DSPC (b). Amounts of phospholipid given were 0.2 (▲), 0.6 (△), 1.2 (○), and 2.0 (●) mg per mouse.

EXAMPLE OF POLYMERIZED VESICLES.



Effect of ganglioside G_{M1}, cholesterol, or combination of the two on blood, RES ratios for liposomes of varying compositions, 2 h post-injection (mean ± SD, n = 3)

Composition	Size (μm)	Blood, RES	% remaining in vivo
PC	0.17	0.010 ± 0.005	86.0 ± 5.4
PC:CH, 2:1	0.17	0.13 ± 0.08	78.1 ± 0.04
PC:G_{M1}, 1:0.07	0.17	0.17 ± 0.12	79.4 ± 5.9
PC:CH:G_{M1}, 2:1:0.14	0.16	1.7 ± 0.5	75.6 ± 5.7
PC:CH:ASG_{M1}, 2:1:0.14	0.16	0.62 ± 0.44	64.3 ± 1.6
DSPC	0.17	0.015 ± 0.002	91.2 ± 2.60
DSPC:CH, 2:1	0.17	0.007 ± 0.00	101.2 ± 2.4
DSPC:G_{M1}, 1:0.07	0.17	2.0 ± 0.02	76.7 ± 3.1
DSPC:CH:G_{M1}, 2:1:0.14	0.17	3.2 ± 1.0	64.6 ± 3.5
SM	0.17	0.02 ± 0.01	27.1 ± 3.1
SM:CH, 2:1	0.17	0.7 ± 0.2	71.9 ± 4.4
SM:G_{M1}, 1:0.07	0.17	5.7 ± 1.8	12.4 ± 0.7
SM:CH:G_{M1}, 2:1:0.14	0.17	4.6 ± 0.6	72.1 ± 1.5
SM:PC, 4:1	0.17	0.6 ± 0.2	69.0 ± 3.3
SM:PC:CH, 4:1:3	0.17	0.12 ± 0.06	69.9 ± 2.2
SM:PC:CH:SO_4, 4:1:3:0.35	0.17	0.43 ± 0.21	78.4 ± 1.4
SM:PC:G_{M1}, 4:1:0.35	0.16	3.3 ± 0.3	61.8 ± 2.9
SM:PC:CH:G_{M1}, 4:1:3:0.35	0.16	1.5 ± 0.6	88.5 ± 3.0
SM:PC:ASG_{M1}, 4:1:0.35	0.16	0.9 ± 0.5	80.3 ± 2.5

G_{M1} and ASG_{M1} concentrations are expressed as the molar ratio of total phospholipid. The ratio of % injected counts in blood to % injected counts in liver plus spleen is termed blood/RES ratio

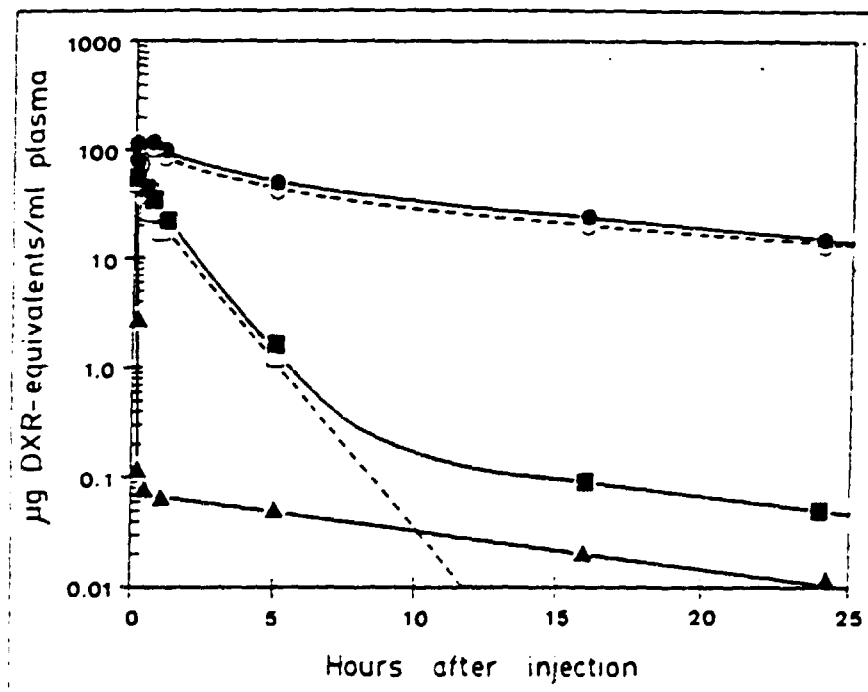
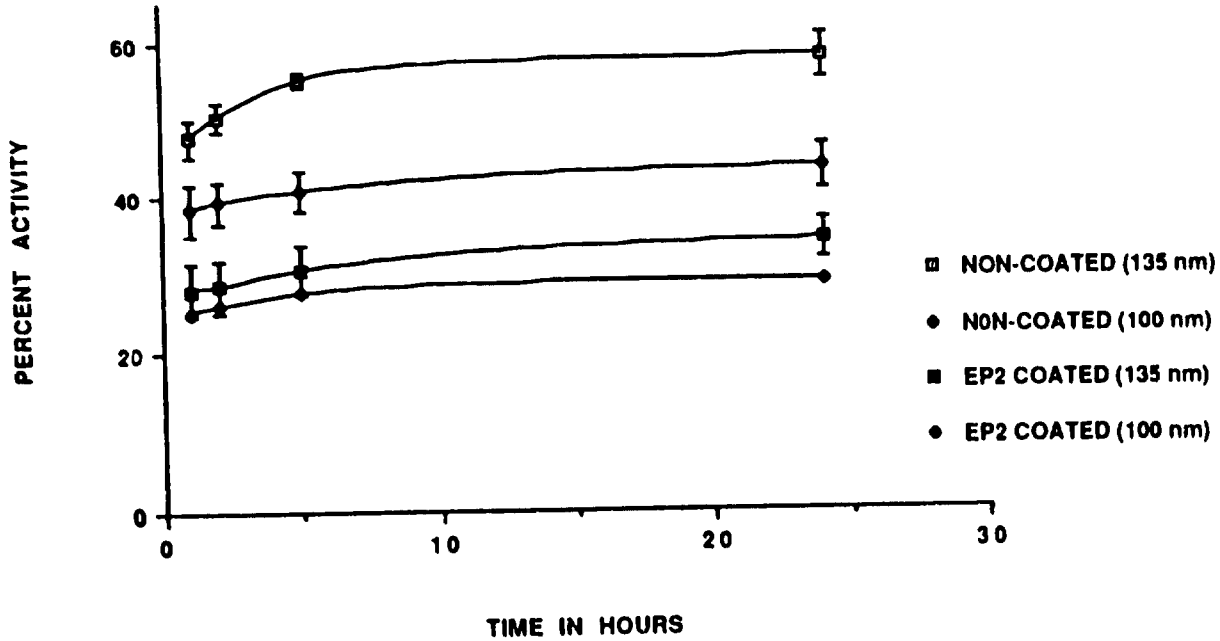


Figure 1. Plasma clearance of free and liposome-encapsulated DXR after IV injection in mice. Symbols represent observed values; lines represent computer-predicted values. Filled symbols and solid lines stand for total DXR concentrations in plasma; open symbols and dashed lines indicate concentrations of liposome-associated DXR in plasma. ▲ = free DXR. ■, □ = DXR encapsulated in PG-PC-Chol; ●, ○ = DXR encapsulated in HPI-HPC-Chol. DXR dose = 10 mg/kg; phospholipid dose = 0.14 mmol/kg for PG-PC-Chol and 0.20 mmol/kg for HPI-HPC-Chol. Sampling times: 5 min, 10 min, 30 min, 1 hr, 5 hr, 16 hr, and 24 hr. Note that concentrations of PG-PC-Chol liposome-associated DXR were undetectable at the 16- and 24-hr time points.

Activity associated with the liver/spleen of 67-Ga labelled DPPC:chol (1:1) liposomes following intravenous adminstration into rabbits. STATIC STUDY



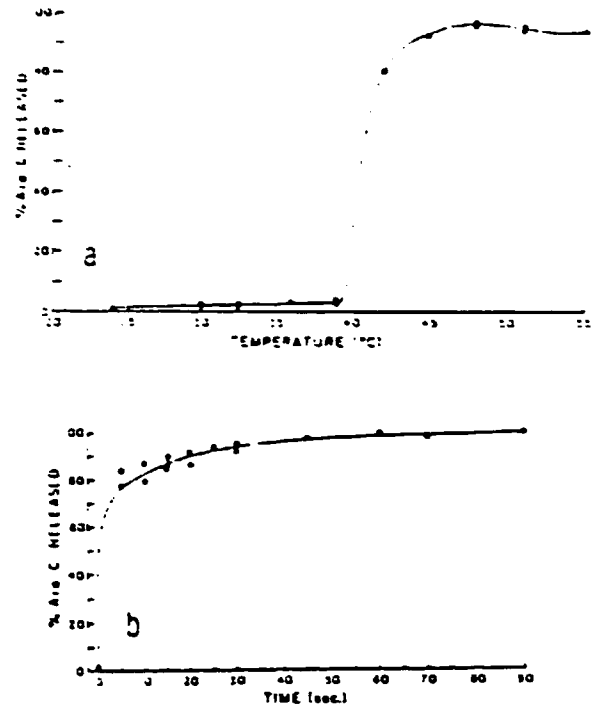
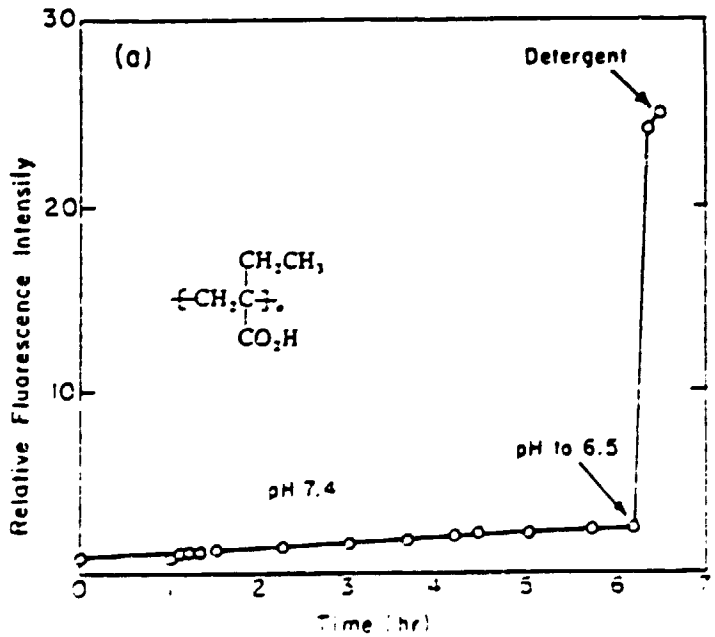


Fig. 6. Temperature-sensitive release of ^{3}H-cytosine arabinoside (ara-C) from DPPC:DSPC (5.5:1) large unilamellar vesicles.
(a) Vesicles were incubated in 100 μl capillaries in 50% mouse serum for 1 minute. Free and encapsulated ara-C were then separated in a Beckman air-driven centrifuge and counted. A dramatic increase in release was obtained at 40°C. The lipid concentration was 3 mg/ml.
(b) Time-course of ara-C release from the same vesicles. The bath was set at 45°C, and the time constant for equilibration of temperature was 1.2 seconds. Six seconds sufficed for near total release, and subsequent experiments with smaller capillary tubes have shown 2 seconds to be adequate.



Efflux of 6-carboxyfluorescein from sonicated phosphatidylcholine vesicles suspended in 50 mM tris-HCl, 100 mM NaCl, 0.01% PEAA, at indicated pH. FIGURE 3a above. Egg yolk phosphatidylcholine.

Table 1. Liposomally entrapped drugs tested for gastrointestinal application

Drug	References
Hydrophilic	
Insulin	Patel and Ryman (1976, 1977a,b), Dapergolas and Gregoriadis (1976, 1977), Patel *et al.* (1978, 1982), Hashimoto and Kawada (1979) Tragi *et al.* (1979), Weingarten *et al.* (1981), Kawada *et al.* (1981), Arrieta-Molero *et al.* (1982), Shenfield and Hill (1982), Dobre *et al.* (1983)
Glucose oxidase	Dapergolas *et al.* (1976)
d-Tubocurarine	Dapergolas and Gregoriadis (1977)
Angiotensin II	Papaioannou *et al.* (1978)
1-β-D-Arabinofuranosylcytosine	Rustum *et al.* (1979)
Gentamycin	Morgan and Williams (1980)
Factor VIII	Hemker *et al.* (1980), Kirby and Gregoriadis (1984)
Factor IX	Ueno *et al.* (1982a)
Heparin	Ueno *et al.* (1982b)
Cysteamine	Jaskierowicz *et al.* (1985)
Lipophilic	
Vitamin K	Nagata *et al.* (1984)
Dolichol	Kimura *et al.* (1989)
Indomethacin	Soehngen *et al.* (1988)

Table I. Liposome-based products in clinical development. Except as noted, all are administered intravenously

Indication	Drug	Phase
Anticancer	Daunorubicin	
	'DaunoXome'	I/II
	Doxorubicin	
	L-Dox	I
	TLC D-99	II
	MTP-PE	II
Bronchodilator[a]	Orciprenaline (metaproterenol)	
	'Metasome'	II
	Salbutamol (albuterol)	II
γ-Imaging agent	^{111}Indium	
	'Vescan'	III
MAI in AIDS patients	Gentamicin	
	TLC G-65	I
Systemic mycoses	Amphotericin B	
	'ABCD'	II
	'ABLC'	II
	'AmBisome'	–[b]

a Inhaled.

b Marketed in Europe.

Abbreviations: MTP-PE = muramyl tripeptide phosphatidylethanolamine; MAI = *Mycobacterium avium intracellulare*; AIDS = acquired immune deficiency syndrome.

DRUG DELIVERY TO THE LUNG

Ian W. Kellaway, Welsh School of Pharmacy, University of Wales College of Cardiff, Cardiff CF1 3XF.

INTRODUCTION

Drug delivery to or via the respiratory tree has been a long-standing pharmaceutical objective. For locally acting agents it is desirable to confine the action of the drug to the lung in order to eliminate unintended side effects which might result following absorption and distribution to other extravascular sites. Oral inhalation is often the preferred route in order that such effects be minimised. The large surface area for absorption provided by the alveolar region, together with reduced extracellular enzyme levels compared with the gastrointestinal tract, ensures that pulmonary administration is a potentially attractive route for the delivery of systemically active agents including the new generation of biotechnology molecules.

RESPIRATORY TRACT MORPHOLOGY AND PHYSIOLOGY

The lung is a specialized tissue with the prime function of gaseous exchange involving oxygen absorption and carbon dioxide and water elimination. Efficient exchange results from a surface area of approximately $70m^2$ and an air-blood barrier of between 0.36 and 2.5 um. It has been estimated that the daily air mass handled by the human lung is approximately 13kg. The lung therefore provides a great potential for toxicity by air-borne toxins and, in addition, some substances that are poorly absorbed in the gut are well retained by and absorbed within the lung.

For convenience, the respiratory tract is often compartmentalised into the following three regions (Table 1):

1. The oropharyngeal (OP) or nasopharyngeal (NP) compartment consists of the mouth or nares and includes the respiratory airways down to the larynx.

2. The tracheobronchial (TB) region begins at the larynx and includes the trachea and the ciliated bronchial airways down to the terminal bronchioles.

3. The pulmonary (P) or functional gaseous exchange region comprises the respiratory bronchioles, alveolar ducts, alveolar sacs, atria and alveoli.

The terms 'upper' and 'lower' respiratory tract are frequently encountered and correspond to the NP, together with the trachea and the P plus bronchial regions respectively.

The NP compartment can entrap larger particles whose inertia causes impaction in the nasal passages or entrapment by nasal hairs. Clearance is believed to occur either by particle solution and distribution into blood or, for less soluble materials, physical clearance by mucociliary transport with subsequent swallowing. The posterior region of the nose is subject to mucociliary clearance whereas particles deposited in the anterior region will only be cleared by such actions as wiping, sneezing or blowing.

A relatively small fraction of all inhaled particles will deposit in the TB region. TB deposition may occur by a variety of mechanisms but principally by inertial impaction, sedimentation and Brownian diffusion - the latter restricted to submicron particles. Mouth breathing of aerosols - the normal route of pulmonary delivery of medicinal agents - by-passes the nasal removal of large particulates, which therefore are deposited in the throat and regions of the TB area. The mucociliary escalator ensures rapid removal (hours) of insoluble deposited particles; soluble particles will dissolve and may enter the blood stream. Mucus is removed by the cilia at a rate which increases as the diameter of the airways increases. This, in conjunction with the tendency for smaller particles towards deeper penetration of the lung, ensures that larger particles are cleared more rapidly. It is for this reason that particle clearance kinetics from this compartment cannot be described by a single rate, although estimates of clearance half-time of 0.5, 2.5 and 5 hr have been quoted for the larger, intermediate and finer airways respectively.

For particles to deposit in the deepest compartment (pulmonary region) successful penetration beyond the NP and TB regions must occur with subsequent retention on the pulmonary surfaces as a result of settling, diffusion and interception processes, the relative contribution being, to a large extent, governed by particle size. The residual volume (approximately 1.2 l of air) ensures that for some particles the time to achieve deposition may be considerably longer than a single breathing cycle and, in some instances, may be minutes rather than seconds. Several mechanisms ensure clearance from this region and include dissolution with absorption, phagocytosis of particles by macrophages with translocation to the ciliated airways, lymphatic uptake of particles and the possibility of direct passage of particles into the bloodstream.

These three compartments were adopted by the ICRP Task Group in their deliberations on the relationship between fraction deposited and particle aerodynamic diameter and subsequently applied to the mouth breathing of monodispersed aerosols (Fig.1). These theoretical profiles are in reasonable agreement with experimentally determined curves (Fig.2).

CELLS AND TISSUES OF THE RESPIRATORY TRACT.

The ultrastructure of the trachea and large bronchi consists of a variety of cells although ciliated and goblet cells predominate. Serous, basal, brush, undifferentiated, Clara and Kulchitsky cells are also present. In the bronchioles, ciliated cells are dominant and Clara cells progressively increase distally along the airways. Goblet cells and serous cells also decrease distally and are absent in terminal bronchioles. Undifferentiated brush, basal and Kulchitsky cells are an uncommon occurrence. The cells and tissues which play an important role in drug deposition and clearance are now described in more detail.

CILIATED MUCOSA

This tissue lines the rear portion of the nose, the larynx and the tracheo-bronchial tree. The cilia are hair-like projections which beat in a coordinated fashion to move the overlying mucus blanket in a direction towards the throat where the mucus is swallowed. The mucus which is primarily an acid glycoprotein, is produced from goblet cells which are

interspersed with the ciliated cells; both cell types are attached to the basement membrane. The mucus is a viscoelastic, tacky fluid which is responsible in conjunction with the cilia for the removal of particulates from the tracheobronchial regions. The efficiency of the 'mucociliary escalator' depends on the quantity and quality of mucus and the number and synchronisation of the cilia. Drugs can alter the viscoelastic properties of mucus and range from mucolytics such as N-acetyl cystine, which reduces the viscosity and elasticity, to mucospicics such as the tetracyclines which enhance both the viscous and elastic nature of the mucus gel. There appears to be a range of mucus elasticity within which cilia can effect transport.

Viral and bacterial infections can lead to changes in both the quantity and quality of the mucus and, in more severe cases, clearance is only possible through the action of coughing or sneezing.

ALVEOLI

The alveoli are polyhedral structures generally less than 300 um in diameter, surrounded by thin walled epithelial cells on all but one side which is open to the atmosphere. The epithelial cells are of several types: Type I are thin cells, overlying a basement membrane some 20 - 40nm thick. A much thicker cell (type II) has a surface covered with microvilli which greatly increase the surface area with the air stream. These cells produce and secrete 'lung surfactant' which is composed of lipid-rich lipoproteins (85 - 90% lipid by weight). The lipid composition is dominated by phosphatidylcholine with dipalmitoyl present in unusually high percentages. This 'cocktail' leads to the generation of low, stable surface tensions, preventing collapse of the lung. Proteins present include serum albumin, together with 10 and 35 kDa non-serum proteins. The latter allow the rapid formation of phospholipid surface films in the alveoli. Respiratory distress syndrome is an example of a disease state related to abnormalities in lung surfactants. Type III, or alveolar brush cells, overlie the alveolar basement membrane and protrude into the airspace with their large microvilli. Other cell types include interstitial cells and macrophages. The alveolar macrophages are mobile, nucleated cells which surround and endocytose small particles. Functions of maintaining sterility by engulfing and killing microorganisms, together

with 'dust collecting' have been ascribed to these cells. Both positive and negative chemotactic responses have been demonstrated by alveolar macrophages. Certain dusts may be cytotoxic to macrophages, for example coal, asbestos and silica. An upper limit of 3 um has been suggested for a phagocytic uptake.

PULMONARY DRUG SELECTIVITY AND PROLONGATION OF THERAPEUTIC EFFECTS

(a) Prodrugs

In addition to improved selectivity of action in the lung relative to other organs, it is possible to obtain prolongation of therapeutic effects and enhancement of pulmonary activity by the design of appropriate prodrugs. Lung accumulation from the blood pool is achieved by many drugs which are both highly lipophilic and strongly basic amines. Such drugs exhibit very slowly effluxable lung pools.

Lung tissue exhibits high nonspecific esterase activity (3) which is species dependent and capable of cleaving carboxylate or carbonate ester linkages. In vivo prodrug conversion to active drug moiety can be controlled by use of different aliphatic or aromatic coupling agents, together with stereochemical modifications.

Terbutaline is an example of a bronchodilator drug for which a number of prodrugs exist (Fig. 3). Terbutaline exhibits little affinity for lung tissue being rapidly absorbed following inhalation with peak plasma concentrations occurring within 0.5h. The di-isobutyryl ester (Ibuterol) results in an increased bioavailability of 1.6 fold over terbutaline following oral administration. However, it is 3 times as effective as terbutaline post-inhalation in inhibiting bronchospasm. Enhanced effects are attributable to more rapid absorption and better tissue penetration. Bambuterol is the bis-N,N-dimethylcarbonate of terbutaline and as such is well absorbed from the gastrointestinal tract and is relatively resistant to hydrolysis leading to a sustained release oral product. However, it is not readily metabolised in the lung which precludes its administration by the pulmonary route.

(b) Polyamine active transport system

The cell types which accumulate polyamines such as the endogenous putrescine, spermidine and spermine, together with compounds such as paraquat, are the Clara cells and the alveolar Type I and Type II cells. The uptake process is saturable and energy dependent. Table 2 illustrates the dependence of both Ki (inhibitory constant) and A (= 1000/Ki) a measure of the affinity of a compound for the polyamine receptor on molecular structure (4). A is seen to decrease with N-methylation and conformational restriction, but to increase for N-(4-aminobutyl)aziridine. Previously, in a study of putrescine inhibition by α,ω -diaminoalkanes, the inhibitory potential increased with chain length, plateauing at 1,7 diaminoheptane (5).

(c) Rate control achievable by employing colloidal drug carriers.

Control of the duration of local drug activity and of the plasma levels of systemically active agents may be achievable by employing a colloidal carrier possessing appropriate drug-release characteristics. Tracheo-bronchial deposition of such carriers may not be desirable as their clearance will occur in a relatively short time period on the mucociliary escalator. Pulmonary deposition will, in contrast, result in extended clearance times which may be dependent upon the composition of the colloid. The mechanism by which clearance is effected will also vary, but will involve alveolar macrophage uptake, with subsequent metabolism or deposition on to the mucus blanket in the ciliated regions or lymphatic uptake. Colloidal carriers, of which liposomes an example, can therefore control both drug delivery rates and availability. Technological problems, however, exist such as the design of delivery devices to ensure deposition in the appropriate regions of the lung without degradation or loss of entrapped drug. Toxicological considerations, foremost amongst which is the processing of the colloid, also require to be addressed.

I. LIPOSOMAL DRUG DELIVERY TO THE LUNGS (see reference 6)

(a) Clearance of liposomes from the human lung

Farr et al. (7) administered ^{99m}Tc-labelled DPPC liposomes to the lungs of healthy volunteers by means of an air jet nebulizer (Hudson). Pulmonary deposition was dependent on breathing pattern and the droplet size distribution of the aerosol. As clearance rates were related to the depth of penetration, it was not possible to show differences between liposomes of different size or type (Table 3). A fraction of the liposome dose which was deposited in the tracheobronchial region was largely cleared by the mucociliary mechanism over 6 - 8 h. However, approximately 60% was retained at 20 h and probably represents the fraction of the dose reaching the alveolar regions. Such clearance kinetics would be appropriate for achieving sustained release of drugs administered as a liposomal dosage form to the lungs.

(b) Pharmacokinetics of liposomal drugs

In 1989 Taylor et al. (8) reported on the influence of liposomal encapsulation on sodium cromoglycate pharmacokinetics in five healthy volunteers. Sodium cromoglycate 20 mg was inhaled from a Hudson air jet nebuliser (172 kPa) as a solution and encapsulated in DPPC/cholesterol (1:1) liposomes. Liposomal drug produced detectable levels in plasma taken 24 and 25 h post inhalation. The free drug produced peak plasma levels more than sevenfold higher than the liposomal drug but was not detectable (i.e. smaller than 0.5 ng ml^{-1}) in 24 h samples (Fig.4). The decline in plasma levels following inhalation of liposomal drug (reflecting the absorption phase) was best described by a biexponential equation with the two absorption rate constants differing by more than an order of magnitude. The authors attributed the rapid absorption phase as due to free or surface adsorbed drug while the slow drug absorption phase was attributed to drug release from the liposomes. The presented data are the first to demonstrate the ability of liposomes to extend the duration of drug plasma levels in man following pulmonary administration.

(c) Therapeutic efficacy

There is a lack of data in man regarding the clinical efficacy of liposomal drugs administered to the airways. Bronchodilator effects and duration of action have been reported for liposomal Beta-2 adrenergic agonists in the guinea pig (9). Airways resistance was measured following intratracheal instillation of free and liposomally entrapped drug (metaproterenol or albuterol). Liposomal metaproterenol was less effective than the same dose of free drug interpreted by the authors as liposomal drug release being too slow to achieve the concentration required for bronchodilator effects (Table 4). Liposomal albuterol in contrast produced an effect which was sustained for a longer duration than the free drug (Fig. 5).

(d) Technological aspects

Nebulization is the simplest method of administering liposomes to the lung, although Taylor et al. (10) have indicated the importance of vesicle size and composition in maintaining liposome integrity (and hence entrapped drug) during the nebulization process.

An alternative approach is to employ a lyophilized liposome preparation in conjunction with a unit-dose dry powder inhaler. The production and stability of lyophilized liposomes to aerosolisation (11) has been reported.

II. DRUG-POLYMER COMPLEXES

The conjugation of cromoglycate (CG) to a dextran carrier was shown to modify its pharmacokinetic profile compared with the free drug after intratracheal administration to rabbits (12). Conjugation prolonged CG residence with a reduction in C_{max}. The half life was increased from 83 $\pm$ 13 min (free drug) to 171 $\pm$ 24 for the conjugate (Table 5).

III. DRUG-CYCLODEXTRIN COMPLEXES

Salbutamol forms complexes with β cyclodextrins and a complex with hydroxypropylbetacyclodextrin (HP-β-CYD) was shown to increase t_{max}, absorption half-life and decrease the C_{max} following intratracheal administration to

rabbit lungs (Table 6) (13). However, complexation did not alter the post peak decline of salbutamol indicating that the absorption was not slowed sufficiently to produce absorption rate limited kinetics. The bioavailability was also reduced to about 80%. The complex dissociated rapidly within the lung. Such an approach of using drug-CYD complexes to prolong pulmonary drug release profiles may be enhanced for drugs exhibiting stronger complexes with CYDs.

DELIVERY OF DRUGS TO THE SYSTEMIC CIRCULATION BY THE PULMONARY ROUTE

The large surface area, thin epithelial membrane provided by Type I cells and a rich blood supply, ensures that many compounds are readily transported from the airways into the systemic circulation. Gaseous anaesthesia and oxygen therapy are examples of efficient clinical utilisation of the pulmonary absorption process. Compounds are absorbed by different processes including active transport and passive diffusion through both aqueous pores and lipophilic regions of the epithelial membranes. Absorption can be both rapid and efficient; for example, sodium cromoglycate is well absorbed from the lung whereas less than 5 per cent is absorbed from the gastrointestinal tract.

Small lipophilic molecules, such as the gaseous anaesthetics, are absorbed by a non-saturable passive diffusion process. Hydrophilic compounds are absorbed more slowly and generally by a paracellular route. Aqueous pores are, by virtue of their size, capable of controlling the rate and extent of hydrophilic compound absorption. Sodium cromoglycate is absorbed by both active and passive (paracellular) mechanisms. The rates of absorption by the paracellular route decreases as the molecular weight of the compound increases.

The efficiency of absorption from the lung is species dependent. For example, insulin is absorbed from the human lung (14) but less efficiently than in the rat (15) or rabbit (16). Human growth hormone (molecular weight 22 kDa) is absorbed from the lungs of hypophysectomised rats with an estimated bio-equivalence of 40 per cent relative to the subcutaneous route and an absolute bioavailability of 10 per cent, sufficient to induce growth (17). A nonapeptide (leuoprolide acetate) has been shown to have an absolute bioavailability following aerosolization to healthy male

volunteers of between 4 and 18% which, when corrected for respirable fraction, corresponds to 35 - 55%.

Protein absorption, however, is postulated to occur through the extremely thin Type I cells by the vesicular process of transcytosis. The passage from lung to blood of proteins in the rat has recently been shown to increase during inflammatory conditions with the observed transport correlating to the severity of the lung injury (19). The pulmonary route therefore warrants further investigation for the systemic delivery of peptides and proteins.

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TABLE 1. The compartmentalisation of the respiratory tract

COMPARTMENT	DEPOSITION	CLEARANCE	PATHOLOGY
NP Nasopharyngeal	Impaction Diffusion Interception Electrostatic	Mucociliary Sneezing Blowing	Inflammation Ulceration Cancer
TB Tracheobronchial	Impaction Sedimentation Diffusion Interception	Mucociliary (hours) Coughing	Broncho- spasm Obstruction Cancer
P Pulmonary	Sedimentation Diffusion Interception	Solubilization Phagocytosis Interstitial (hours to years)	Inflammation Oedema Emphysema Fibrosis Cancer

As reported by the Task Group on Lung Dynamics of the International Commission of Radiological Protection

TABLE 2

POLYAMINE ACCUMULATION

INHIBITORY EFFECTS OF PUTRESCINE DERIVATIVES ON THE UPTAKE OF PUTRESCINE [$H_2N(CH_2)_4NH_2$ 2HCl] INTO RAT LUNG SLICES

	INHIBITOR CONSTANT Ki (uM)	AFFINITY FOR RECEPTOR A $(mM)^{-1}$
N-methylation		
$H_2N(CH_2)_4NHCH_3$ 2HCl	8	125
$H_2N(CH_2)_4N(CH_3)_2$ 2HCl	11.5	87
$(CH_3)_2N(CH_2)_4N(CH_3)_2$ 2HCl	100	10
Conformationally restricted analogues		
$H_2NCH_2CH \overset{Z}{=} CHCH_2NH_2$ 2HCl	40	25
Aziridines		
$H_2N(CH_2)_4N$	7.5	133
 $N(CH_2)_4N$ 	31.5	32

M.C.O'SULLIVAN, B.T.GOLDING, L.L.SMITH, I.WYATT. Biochem. Pharmacol. 41 (1991) 1839 - 1848.

TABLE 3

PULMONARY RETENTION OF NEBULIZED ^{99m}Tc-LABELLED DPPC LIPOSOMES IN VOLUNTEERS (n=4) (REF. 7)

MMAD, mass median aerodynamic diameter; GSD, geometric standard deviation.

Type	Mean size (μm)	Aerosol Analysis		6-h retention ± S.E.M. (%)
		MMAD (μm)	GSD	
MLVs	2.90	3.7	1.5	87.5 ± 2.1
SUVs	0.07	3.2	1.5	76.8 ± 5.1

Inhibition of response to histamine by metaproterenol

TABLE 4

	Free metaproterenol		Liposome metaproterenol	
	27.9 ug	125.0 ug	27.9 ug	125.0 ug
	Resistance responses			
- 15 min	+0.21 ± 0.06	+0.35 ± 0.10	+0.24 ± 0.04	+0.32 ± 0.08
0 time		MPS instillation		
+ 15 min	+0.05 ± 0.02*	+0.03 ± 0.01*	+0.28 ± 0.07	+0.16 ± 0.02*
+ 1 hr	+0.14 ± 0.01	+0.07 ± 0.02*	+0.25 ± 0.06	+0.21 ± 0.07
+ 2 hrs	+0.23 ± 0.05	+0.08 ± 0.05*	+0.29 ± 0.06	+0.26 ± 0.08
+ 3 hrs	+0.30 ± 0.04	+0.18 ± 0.08	+0.33 ± 0.08	+0.27 ± 0.09

The total lung resistance responses to bronchoconstrictor challenge with histamine before and after intratracheal instillation of metaproterenol sulfate. Mean values ± SEM are shown. The asterisk (*) denotes significant reduction in response to histamine compared with baseline controls.

McCALDEN, T.A. and RADHAKRISHNAN, R. Pulmonary Pharmacology 4 (1991) 140 - 145

TABLE 5. Cromoglycate pharmacokinetic parameters (mean ± s.e.m.) after i.v. and i.t. administation of SCG and i.t. administration of a cromoglycate-dextran conjugate (CG-Dx).

	SCG (i.v.)	SCG (i.t.)	CG-Dx (i.t.)
$t_{1/2}$ (min)	7 ± 0.7*	83 ± 13*	171 ± 24*
t_{max} (min)		20 ± 5	75 ± 37
C_{max} (ng/ml)[a]		348 ± 122	184 ± 25
F (%)		36 ± 11	41 ± 6

[a]Normalized for a 4 mg/kg dose
*Significantly different ($p<0.05$) from other treatments (2-way ANOVA and Duncan's multiple range test)

TABLE 6

Summary of the pharmacokinetic parameters (mean ± SE; n = 4) of salbutamol and its complex with HP-β-CYD after i.t. administration to rabbits

	Salbutamol (free)	Salbutamol-HP-β	HP-β-salbutamol
t_{max} (min)	13.6 ± 2.4	23.0 ± 1.2	107.0 ± 4.5
C_{max} (ng/ml)	1035.5 ± 81.5	524.6 ± 43.8	22770 ± 2500
$t_{1/2z}$ (min)	145.3 ± 18.6	168.5 ± 21.4	64.0 ± 2.9
$t_{1/2a}$ (min)	10.6 ± 4.2	32.7 ± 6.1	26.2 ± 7.8
F (%)	109.2 ± 8.1	80.6 ± 8.0	67.8 ± 8.8

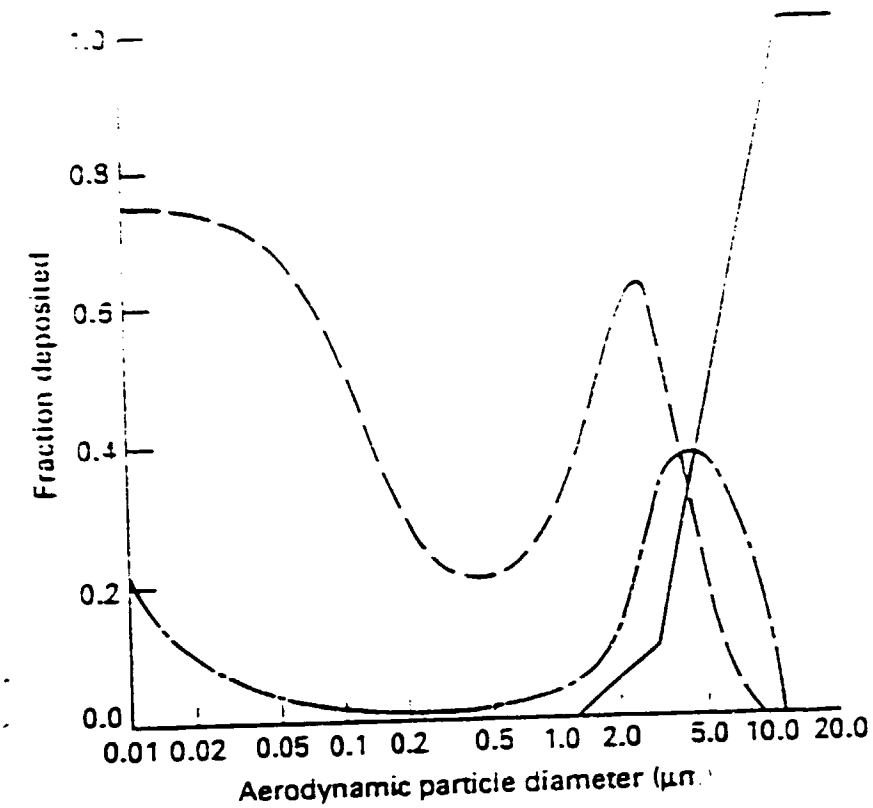


Figure 1 Theoretical deposition of monodisperse aerosols inhaled by healthy human adults in the 'head' (——), tracheobronchial (—— – ——) and pulmonary (– – – –) regions of the respiratory tract during normal mouth breathing. (Adapted from reference 1)

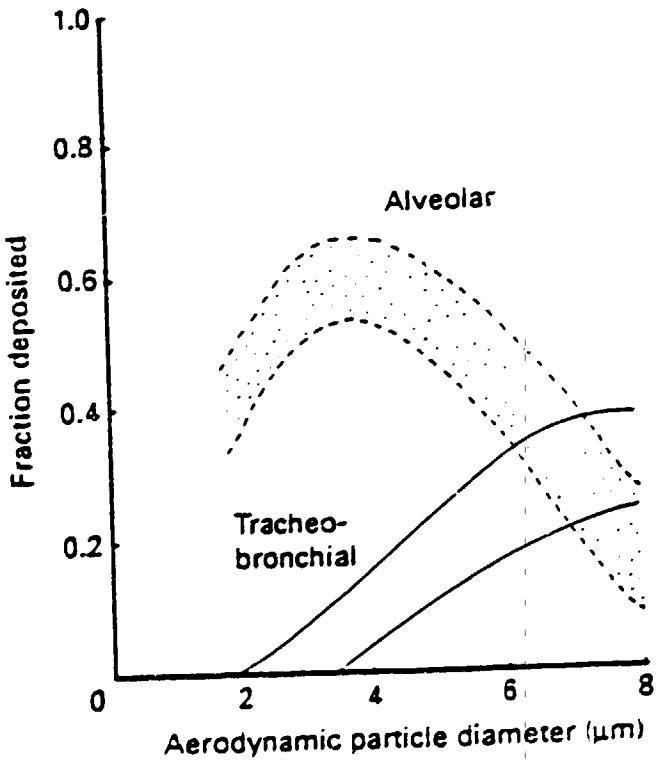
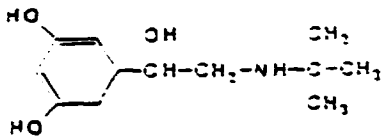


Figure 2 Particle diameter dependence of alveolar and tracheobronchial deposition for mouth breathing. Tidal volume 1 l, breathing frequency 7.5/min, mean flow rate 250 cm^3/s, inspiration/expiration times 4 s each. (Adapted from 2)

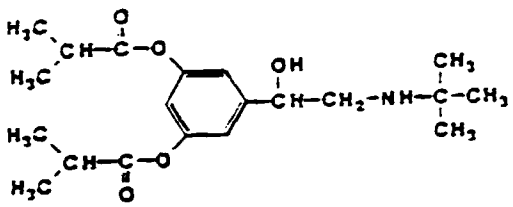
Fig 3.

PULMONARY PRO-DRUG EXAMPLES

TERBUTALINE PRO-DRUGS



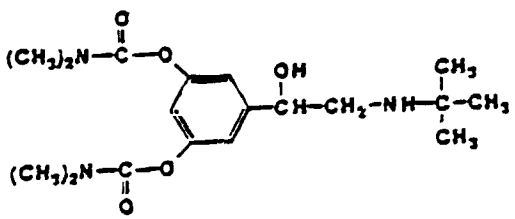
TERBUTALINE



IBUTEROL (DI-ISOBUTYRYL ESTER)

3 times as effective in inhibiting bronchospasm

5 min post inhalation due to better tissue penetration

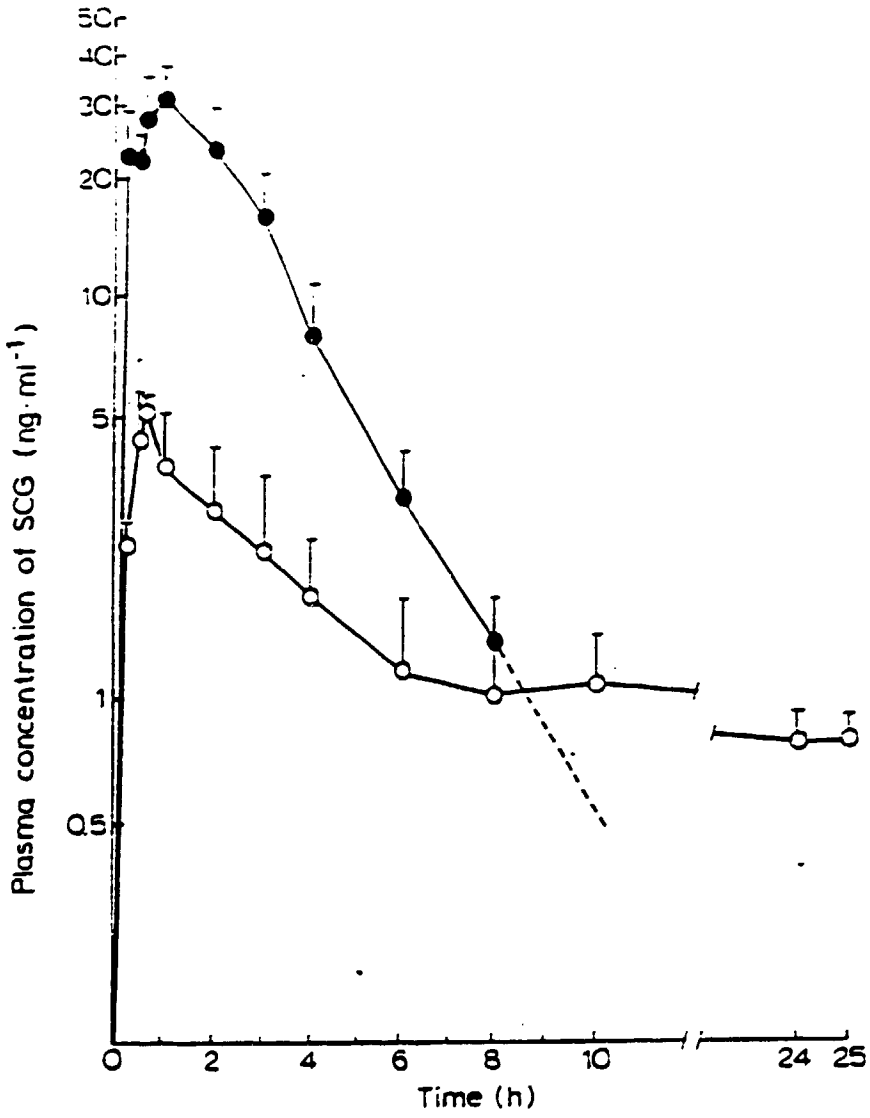


BAMBUTEROL (BIS-N,N-DIMETHYLCARBAMATE)

Not metabolised in the lung therefore

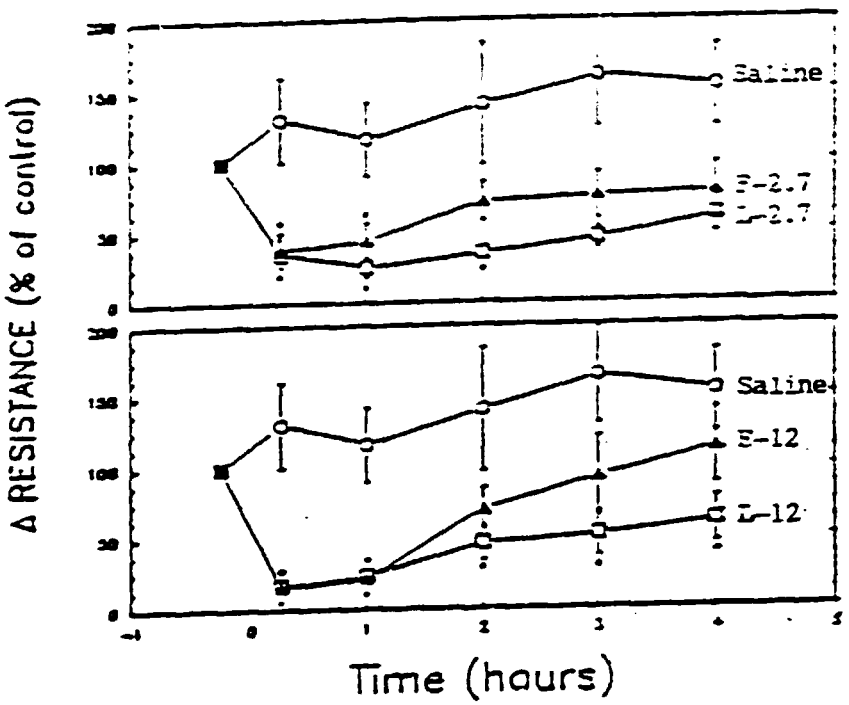
ineffective by inhalation

Fig 4.



Plasma levels following nebulization of 20 mg sodium cromoglycate (SCG) to volunteers. E. point is a mean±S.E. (●). Free SCG (N=5); (○) liposomal SCG (N=4) (Ref *8*)

Fig 5



Figure—Comparison of the effect of free and liposome albuterol on the airways resistance response to histamine.

The mean percentage change of R from baseline ± SEM in response to histamine after intratracheal instillation of free (n = 13) or liposome (n = 9) encapsulated albuterol at 2.7 or 12.0 μg/100 grams body weight. Responses after saline (50 μl/100 g) are included for comparison. The asterisk (*) denotes a significantly smaller response to histamine than in the baseline control or saline experiments.

McCALDEN, T.A. and RADHAKRISHNAN, R.
Pulmonary Pharmacology 4 (1991) 140 - 145

BIOLOGICAL DISPERSION AND THE DESIGN OF SITE-SPECIFIC PROTEIN THERAPEUTIC SYSTEMS

E. Tomlinson

Advanced Drug Delivery Research, Ciba-Geigy Pharmaceuticals, Horsham, West Sussex RH12 4AB, UK

INTRODUCTION

A drug acts when it reaches its pharmacological site of action. However, ideal clinical effectiveness relies additionally on the right amount of drug reaching its site(s) of action at the right rate and frequency, on (often) a drug-free interval (at the receptor site), and on not critically interacting with non-target sites. Few drugs in use today attain such ideality. As more attention is paid to drug/receptor interactions, (often through the use of molecular modelling procedures and/or the use of cloned endogenous proteins which can act as templates for the designed fit of agonist or antagonist drugs), increased effort is being focussed on controlling the biological dispersion of drugs. This activity, which was once reserved almost exclusively for cytotoxic drugs, is an increasingly important aspect of the discovery and drug development process, particularly in the design of therapeutic proteins. Approaches to site-specific delivery include simple low molecular weight prodrugs activated at sites of disease, suicide enzyme substrate inhibitors; polymeric soluble and particulate macromolecular carriers; and unique site-specific therapeutic proteins.

Site-specific drugs and carriers are often trivially referred to as targeting systems or magic bullets. The magic bullet concept is a poor one, since it conjures up the image of an aimed missile have a defined and preordained trajectory. However, in reality, site-selectivity relies on the drug or carrier having a higher affinity for a particular feature of the normal or diseased body. Conceptually, it is more a case of "I don't know where I'm going to, but I'll know when I get there".

THERAPEUTIC PROTEIN SYSTEMS

Many polypeptides and proteins having unique pharmacological properties are expected to be used clinically in the coming decades. These usually have regulatory or homeostatic functions, and include both endogenous polypeptides and proteins, and their (heterologous) derivatives. This latter class of molecules may be produced by inter alia, site-directed mutagenesis, proteolysis, ligated gene fusion, protein aggregation and/or conjugation with (other) biologically active effector functions (Tomlinson, 1989). Proteins can be considered as drugs (for example, drug/antibody complexes).

Targeting of Drugs, Edited by G. Gregoriadis *et al.*
Plenum Press, New York, 1990

Many of the proteins proposed for therapeutic use are glycoproteins whose biological disposition is primarily due to three properties: namely, their chemical and metabolic stability, size and shape, and surface features. The biological half-life of most polypeptides and proteins is short, due generally to a poor chemical stability, and/or rapid liver metabolism and kidney excretion. Also, the instability of paracrine- and autocrine-like mediators is largely due to their degradation by peptidases and proteinases in the vascular endothelium, liver, and kidneys, etc. Further, the terminal amino acids of proteins may serve to control their intracellular metabolic stability (and hence their intracellular residence time). Protein-engineering methods may be used to replace labile amino acids, with, for example, the oxidation-resistant amino acids alanine, serine or threonine, or to produce proteins having differing foldings, potentially leading to proteins which are protected from inactivation.

Pharmacology

For most therapeutic proteins the relation between applied dose and effect is highly critical, particularly as non-linear dose-effect relationships are often found (e.g. with parathyroid hormone, substance P and δ-sleep inducing peptide). As we and others, notably George Poste, have pointed out (Tomlinson, 1989), in selecting proteins for therapeutic use, little rational thought has been applied to their site of action, namely, whether the putative therapeutic protein is to act systematically (i.e. endocrine-like), or whether it is a mimic of an endogenous molecule that is normally produced to act locally (i.e. as an autocrine- or paracrine-like mediator). Endogenous endocrine proteins (e.g. hormones such as insulin), act over long distances from their site of manufacture; they are also stable in blood and, if relevant, their size and surface character enable their (specific) extravasation. However, paracrine-/autocrine-like mediators are produced and released to act locally, and/or have very short chemical half-lives. Such properties ensure that they do not give rise to untoward effects on non-target neighbouring cells. Autocrine- and paracrine-like mediators are often produced at sites of inflammation, tumors and injuries (e.g. transforming growth factors alpha and beta, angiogenenin, fibroblast growth factors, and epidermal growth factor, etc.). As described elsewhere (Tomlinson, 1989), in the site-specific delivery of these mediators, one needs to consider the issues of chronicity in the activation of cells (including their temporal localisation and responsiveness) and, since such agents may be acting as part of a polymediator cascade of events, also the staging sequence through which they act.

CELL RECOGNITION AND PROCESSING

The pathway to a pharmacological site may involve passage into and through various cells. To survive and to maintain their function, both normal and diseased cells take up and process numerous types of materials by a variety of mechanisms. The uptake of hydrophilic macromolecules at the plasma membrane involves invagination and vesiculation of the lipid bilayer to form vesicles. These processes have a putative applicability for cell-selection and cell-access by site-specific therapeutic systems (Tomlinson, 1986). Movement along biological pathways can be either passive or active and include cell fusion, fluid-phase pinocytosis, phagocytosis, and both constitutive and non-constitutive receptor-mediated endocytosis. The binding to specific and/or non-specific regions of cell surfaces can aid other processes which cause cell access of macromolecules - including membrane fusion and simple diffusion. There are two classes of vesicular routings, i.e. those which involve constitutive recycling, and those which occur upon a specific ligand/receptor interaction. For the former class, these processes occur independently of external stimuli, and are for the

purpose of imbibing, cell growth, and intra- and intercellular communication. Recognition and processing can be very sensitive processes, affected dramatically by slight changes in structure. For example, by analogy, an alteration in the gp 120 tryhptophan at position 432 of the HIV envelope can abrogate CD4 binding and thus affect its tropism (Cordonnier et al, 1989).

The capacity and kinetics of cell trafficking events are important considerations in the design of site-specific systems (as are the abundance, specificity, avidity and (cellular) fate of any receptor system). For the use of systems which rely on transport receptors to effect their site location, there is a need to know the dose/uptake relations. For example, studies on hEGF show an edxtraordinary dose-dependency of the pharmacokinetic behavior after iv administration in rats, due not to the saturation of excretion processes such as biliary and urinary excretions, but to the binding saturation of transport receptors (Murakami et al, 1989).

Site-specific delivery with soluble proteins relies on a combination of anatomical and (patho)physiological events, each bringing its own constraints and opportunities. These can involve either anatomically accessible and discrete compartments, as well as normal and dysfunctioning cellular processes of both a passive and an active type.

EXTRAVASATION OF MACROMOLECULES

To be effective, on occasions, macromolecular site-specific systems will need to leave the cardiovasculature in order to reach either extravascular-extracellular, and/or extravascular-intracellular target sites. Extravasation is under strict anatomical and (patho)physiological control. Hence, systems can either be incorporated into phagocytic cells which can extravasate, or pass directly through either interrupted endothelia, or through the cell barrier itself by exploiting fluid-phase and/or receptor-mediated, constitutive and non-constitutive cell transport processes.

Passage through Normal Endothelia via Passive Processes

The structure of the endothelium is complex and varies greatly in different organs and tissues. It is generally comprised of four layers, namely, the plasma membrane/plasma interface (which is formed by the glycocalyx of the cell and the proteins adsorbed onto it); the endothelium (a monolayer of cells which are metabolically very active and effect and monitor the bidirectional exchange of fluid between the plasma and the interstitial fluid); the basal lamina (which supports the endothelium); and the adventitia (a connective tissue which surrounds the lamina and fuses with the surrounding fibro-areolar tissue). Capillaries having a continuous endothelium and an uninterrupted basement membrane are the most widely distributed. Fenestrated capillaries are morphologically distinct from these, and are typified by having a very thin cytoplasm on each side of the nucleus (30-60 nm), and gaps of between 20-100 nm diameter at irregular intervals. Some tissues have sinusoidal endothelial membranes where the membrane is very thin and there is hardly any connective tissue separating the endothelial cells from the parenchymal cells of the underlying organ. These areas are often lined by phagocytic cells. Endothelial cells contain a large number of spherical vesicles of uniform diameter (plasmalemmelar vesicles). These are generally between 60 to 80 nm in diameter. Plasma molecules are selectively transported across the endothelium according primarily to their size, but their charge and their physicochemistry (i.e. hydrophilic/lipophilic balance) are also contributing factors. The capillary wall permeability for soluble macromolecules is well-documented. Soluble materials of less than 30 nm diameter are able to permeate through continuous endothelia (e.g. see Fig. 1). Rippe and Stelin (1989) have

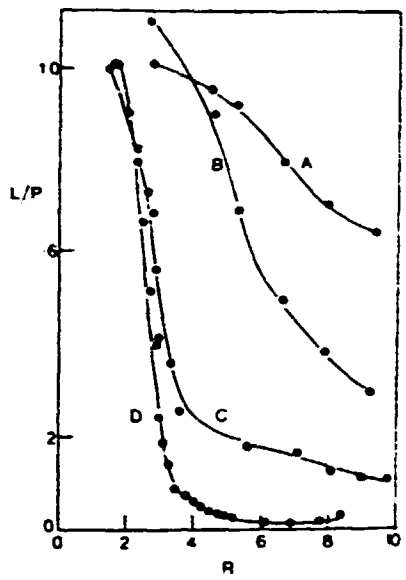


Fig. 1. Relationship between lymph-to-plasma ratios (L/P) for uncharged dextrans and their molecular radius (R, nm) for different organs in the rat; A-D are liver, intestine, leg and lung, respectively (from Tomlinson, 1987). (Reproduced with the permission of the copyright holder.)

recently examined the blood/peritoneal clearances of various endogenous solutes in patients undergoing continuous ambulatory peritoneal dialysis. They demonstrated that solute transport is compatible with a functional blood/peritoneal barrier consisting of a two-pore membrane containing both a large number of paracellular small pores of radius 4.0 to 5.5 nm, and a smaller number of larger pores of radius 20 to 30 nm. In addition, they found that whereas solutes smaller than 2.5 nm in radius permeated across the peritoneal membrane mainly by diffusion across the small pores, solutes larger than 4 nm were calculated to cross exclusively by unidirectional convection across the large pores. Further, molecules larger than 2.5 to 3.0 nm radius (approximately 25 kDa) were simulated to be lost from the peritoneal cavity by non-size-selective lymphatic drainage.

Extravasation of macromolecules occurs by diffusion and convection and transcytosis through the vesicle/plasmalamellar pathways. For macromolecules, the large proportion of extravasation is due to convection. This is related to the relative vascular and (interstitial) extravascular pressure, and is porportional to the rate of fluid movement from the vessel lumen to the interstitium. As pointed out by Jain (1989), this event is proportional to the surface area and the difference between the vascular and the interstitial hydrostatic pressures minus the difference between the vascular and interstitial osmotic pressures. Additionally, transmural arterial pressure has an effect on endothelial (percolation-driven) transport of colloidal particles (Fig. 2) (Chien et al, 1984). Since the osmotic reflection coefficient describes the effectiveness of the transluminal osmotic pressure differences in causing movement of fluid across an endothelium, we can write that the transport of a macromolecule across endothelia is characterised by the three transport parameters of (i) vascular permeability, hydraulic conductivity and reflection coefficient, (ii) surface area, and (iii) both the transvascular concentration and pressure gradients (Jain, 1987).

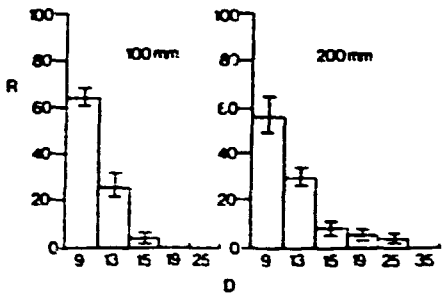


Fig. 2. Effect of transmural pressure (100 mm Hg and 200 mm Hg) on the distribution (R, percent) of Ag and Au colloid particles of different diameter (D, nm) found in the carotid endothelium and subendothelial space (in dogs) (Chien et al, 1984). (Reproduced with the permission of the copyright holder.)

Pathophysiological Opportunities for Extravasation

Inflammation. The hyperpermeability of endothelial barriers at various sites of inflammation is well established. This has been regarded as being of potential use for the selective delivery of anti-inflammatory drugs to inflamed extravascular regions. For example, in areas of inflammation induced by carrageenin, the accumulation of lipid microspheres of approximately 200 nm diameter around endothelial cells of blood vessels, and the penetration of these to the outer layer of blood vessels, has been reported. Inflammation can cause regional changes in the structure, chemical composition and permeability of the endothelium. Permeability changes appear to be due to the effect of histamine and bradykinin which act directly on the capillary venule endothelia vessel wall, with various other mediators (including leukotriene B4 and the complement enzyme C5), effecting a rapid interaction between venular endothelial cells and circulating neutrophils. It is unclear what such hyperpermeability means in terms of the pathogenesis of the underlying disease and the adequate retention of the carrier, particularly when one appreciates that inflamed sites often contain phagocytic cells. Interestingly, intravenously administered radiolabelled 'small' liposome particles can be used to image joints of patients affected by rheumatoid disease (Williams et al, 1986); though it has been found that when the disease is in remission, with no active synovitis, then accumulation of the radiolabel does not occur, suggesting that some accumulation is due to phagocytic activity.

Ischemia/hypertensive vascular lesions. An increased permeability in the endothelia is seen as an important factor in the pathogenesis of hypertensive lesions leading to infiltration and accumulation of plasma material. For example, in experimental malignant hypertension, colloidal iron and carbon particles of between 5 to 50 nm diameter are able to extravasate. Capillaries have also been shown to be permeable at sites of tissue ischaemia, both in the mesenteric artery and myocardium. However, this is not the case with other hypertensive states.

Tumor endothelia. Ultrastructural studies of both animal and human extravascular tumors indicate that a significant fraction of tumor blood vessels have wide interendothelial junctions, a large number of fenestrae and transendothelial channels formed by vesicles, and discontinuous and/or absent basal lamina. In addition, various tissue uptake studies have also demonstrated that the vascular permeability of tumors is significantly higher than where there is a continuous membrane such as in skin and muscle.

Table 1. Average Time for MoAb and Fab to move in the tumour interstitium (from Jain, 1989)

Distance	Average time	
100 μm	about 0.5 h	about 0.2 h
1 mm	" 2-3 days	" 0.5-1 day
1 cm	" 7 months	" 2 months

However, macromolecular drugs such as immunotoxins and drug-antibody complexes, extravasate very poorly and percolate minimally into tumor masses. Jain (1989) has concluded that the poor extravasation of macromolecules could be due to tumors containing regions of high interstitial pressure, coupled with a decreased vascular pressure. This serves to lower fluid extravasation, which in turn leads to low levels of macromolecules extravasating since their transport normally occurs primarily by convention (Jain, 1987). (As tumors grow, their interstitial pressure increases correspondingly.) Also, since large tumors have a relatively lower vascular surface area than small vascularised tumors, then this should lead to a lower transvascular exchange in large tumors compared to small ones (Jain and Baxter, 1988). After extravasation, the flux of a macromolecule in the interstitium occurs via diffusion and convection, the former being proportional to the concentration gradient in the interstitium, and the latter to the interstitial fluid velocity (which, in turn, is proportional to the pressure gradient in the interstitium). In normal tissues, the matrix is composed of collagen, elastin, nidogen, etc. within which is the fluid and a hydrophilic gel made mainly of polysaccharides. In tumors, the large interstitial space and low concentrations of polysaccharides favor the movement of macromolecules in the tumor interstitium (Jain, 1989). But this event does not appear to occur readily, as indicated by a heterogeneous distribution in tumors of administered exogenous macromolecules. It appears that two events serve to inhibit such movement. First, and especially for systems targeted to bind to a surface marker, the binding of a macromolecule to an antigen will lower its apparent diffusion coefficient, and second, because of the large distances and the nature of the network, large molecules such as immunoglobulins can take considerable time to diffuse (Table 1). Related to this is the question of the fate of any low molecular weight drug released from a macromolecular carrier at the tumor surface. Notwithstanding that the concentration gradient for such a drug will permit its diffusion in all directions, Levin and coworkers have computed that the time taken for low molecular weight drugs to diffuse from a well-perfused tumor shell inwards to a poorly-perfused tumor core is long (Levin et al, 1980). For example, even for drugs with high diffusion coefficients (in the region of 10^{-6} to 10^{-5} cm^2 sec^{-1}), to travel 5 mm into the tumor mass would take between 24 and 48 hours. To achieve even this would require high levels of drug to be constantly present at the surface of the tumor mass. It is evident that this could lead to systemic toxicity.

There have been numerous attempts to selectively deliver drug to a tumor target and to maintain it there using various recognition ligands. These include hormones, porphyrins, lectins, sugars and anti-receptor monoclonal antibodies able to recognise some feature on the tumor surface (such as transferrin and low-density lipoprotein receptors). It appears that many of the markers suggested for targeting are not tumor-specific (except perhaps oncofetal markers), but that they are present in an abnormal abundance. In addition, it is known that tumor masses have a clonal heterogeneity - which may preclude the approach of using selective recognition

Table 2. Some Key (Patho)Physiological and Biochemical Issues Affecting the Pharmacokinetic and Pharmacodynamic Behaviour of Site-Specific Macromolecules Acting on Extravascular Tumours

Exclusivity, abundance and trafficking capacity of surface markers
Clonal heterogeneity and biochemical resistance
Intra/extravascular location
Diffusion, convection and binding of drug and/or drug/carrier complex
Multiple sites of drug resistance
Lower hydraulic conductance
A reducing relative endothelial surface
An increasing interstitial pressure
Lower blood pressure
Slow interstitial diffusion
Membrane permeability
Toxic side-effects
Non-linear dose/response relationship
Chronicity of growth and responsiveness

ligands in site-specific drug delivery due to the possibility for the selection of cells which have a biochemical drug resistance.

Thus, access to solid tumors is highly limiting for the use of macromolecular drugs/carriers in cancer, because (a) in humans, the dilation of tumor blood vessels leads to a lowering in hydraulic (transmural) conductance; (b) as tumor masses grow, their relative endothelial surface area (for extravasation) is reduced; (c) interstitial pressure increases, further reducing conductance; (d) the interstitial movement of macromolecules in tumor masses can be in the order of days to move a millimetre; and (e) specific (antigen) binding (by an antibody) serves to delay this movement and to reduce tumor cell penetration. Table 2 defines many of the factors which affect the pharmacokinetics and pharmacodynamics of cytotoxic macromolecular drugs. Clearly, a complex interplay exists between all of these parameters. The overwhelming conclusion that must be drawn from this is that macromolecular drugs are inappropriate agents for directly treating extravascular tumors located in tissues having a continuous endothelium. The same arguments do not apply to accessible tumors such as those in the spleen or the blood. Attempts to improve on the selectivity of anticancer agents has been made by an indirect two-stage approach, in which a bacterial enzyme carboxypeptidase G2 (CPG2) is conjugated to a $F(ab')_2$ fragment (against a subunit of human chorionic gonadotrophin). After localisation (which, following on from the above discussion, must be minimal), a prodrug of a cytotoxic agent able to be selectively cleaved by the enzyme, is introduced (Bagshawe et al, 1988). In mice this has been shown to lead to a reduction in tumor growth.

RETENTION IN THE CENTRAL COMPARTMENT

In many cases, macromolecular drugs and carriers need to persist in the central blood compartment. This may be either because of a need to access a target (cell) within the cardiovascular system, or to remain in the central compartment long enough to be able to remain intact and to extravasate (via either passive or active means). Size, surface charge, chemical stability, and surface physical and physicochemical stability, are the most important features for achieving this persistence. For therapeutic (site-specific) proteins needing to remain in the blood compartment, two prime methods have been adopted. Namely, increasing the apperent size of the protein and/or reducing its (untoward) interactions with blood and tissue components.

Uptake by the Mononuclear Phagocyte System (MPS)

Recognition of macromolecular therapeutic systems by the immune system is mediated through physicochemical interaction. Frequently, opsonization by fibrinogen, fibronectin and other blood components, is a prelude to recognition and thence removal by cells of the formed complex; antigen-antibody interaction and Fc-mediated removal also occurs. Opsonized materials are taken into cells by engulfment after adjerence to, and vesiculation of, phagocytosing cell membranes. Both opsonization and adherence can be diminished if the attractive forces between the interacting therapeutic protein, blood macromolecule and, for example, a cell-surface macromolecule are diminished. Adsorption and adhesion are complex phenomena which are controlled by many factors including hydration, and electrostatic, dispersion and steric forces, and by other short-range interactions (Norde, 1984). Interfacial adsorption is dependent upon a balance between these forces. Colloidal particles will attract each other through van der Waals interactions (short-range), and repel each other through long range, repulsive (e.g. Coulombic) forces. As proteins approach one another there is a net attraction, with a potential energy barrier to interaction at closer proximities, and with strong interaction at very short ranges. Interaction can be avoided by creating a high potential energy barrier. Although in vitro this may be achieved by charge/charge effects, this is likely to be diminished in vivo. Napper and Netschey (1971) have argued that for (particulate) colloids, a high potential energy barrier can be formed by creating a sterically stabilized surface upon introducing a hydrated (i.e. hydrophilic) polymer at the surface of the colloid. The hydration effect is enthalpic in origin; the stabilization effect being manifested by both osmotic effects and chain entanglements, both of which are entropic in origin (Ottewill, 1977). The size of any repulsive barrier should be determined by both the thickness of the polymer layer and its density, as well as by polymer-polymer interactions caused by specific interactions along the polymer chain. It is probable that steric stabilization is akin to the mechanism whereby blood cells and various bacteria and parasites escape detection by the mononuclear phagocyte system (MPS). Surface modifications to proteins can be made to improve their tolerance within the vasculature, due largely to the formation of a surface which makes it energetically unfavorable for other macromolecules to approach.

Chemical Protectants

Many examples of hydrophilic (bio)polymeric protectants have been described for conjugation to therapeutic proteins. Synthetic and biological materials have been used or suggested, and include polyethylene glycols, poloxamers, poloxamines, albumin, immunoglobulin G, carboxymethycellulose, natural xanthans and sorbitans, etc. Conjugations of proteins with hydrophilic polymers have often been reported as being very successful in altering their potency as well as for reducing their immunogenicity and increasing their duration of action. Abuchowski and Davis (1977) first adopted this approach for stabilizing therapeutic proteins by forming protein conjugates with hydrophilic polyethylene glycol chains. Others have increasingly used this approach, and modifications of it, for lengthening the blood half-lives of a number of peptidergic mediators, including lymphokines, and enzymes, such as catalase, asparaginase and urokinase, whilst still maintaining their reactive functionalities. Steric stabilization (or polymer-excluded volume) approaches to avoiding opsonization have also received attention for modifying antibodies (Rihova et al, 1986), and the modification of antibodies with hydrophilic polymers can be additionally utilised to enable the chelation of small inorganics for dianosis purposes (Torchilin et al, 1986).

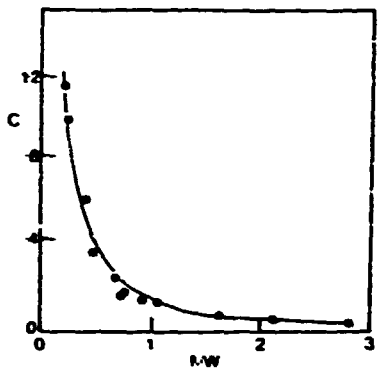


Fig. 3. Effect of size on the systemic clearance (C) of recombinant interleukin 2 (rIL-2) in rats, showing the relationship between C ($ml.min^{-1}$) and effective molecular weight ($MW \times 10^{-3}$) of rIL-2 modified with polyethylene glycol (Katre et al, 1989). (Reproduced with the permission of the copyright holder.)

Covalent attachment of a hydrophilic polymer such as PEG to a small protein will significantly increase its size. This can be modulated by the extent of protein modification and the size of polymer used. Figure 3 shows the experimentally found relationship between the systemic clearance rates of PEG-modified recombinant IL-2 in rats and their effective size and shows that for such systems an abrupt change in clearance occurs at around 70kDa, which of course is predictable from known information on serum proteins and filtration (Katre et al, 1989). IL-2/PEG is reported to have a half-life 10 times that of non-pegylated IL-2, a lower toxicity, whilst still retaining its antitumor activity in patients with advanced cancers.

White et al (1989) have shown that by prolonging the circulating time of SOD and catalase with PEG, conjoint treatment with these increased survival and consistently decreased lung injury and neutrophil recruitment and activation in rats exposed to hyperoxia. In addition, they found that polyethylene-attached antioxidant enzymes decrease pulmonary oxygen toxicity (in rats); indeed, in vitro studies even suggest that PEG itself may be contributing to protection by scavenging hydroxyl radical but not superoxide or hydrogen peroxide.

A decrease in immunogenicity of therapeutic proteins may result from either a reduction in their aggregation, or simply by a masking of any antigenic determinants. It has been found that the primary and secondary IgE antibody responses to protein may be suppressed by chemically conjugating protein with a derivative of polyaspartic acid, i.e. α,β-poly[(2-hydroxyethyl)-DL-aspartamide) - chosen since it had been used as a plasma expander without apparent toxicity (Okada et al, 1985). Although the mechanism of this effect is not fully defined, it has been shown that the suppressive effect of protein modified with polyethylene glycol (Lee et al, 1981) or fatty acid (Segawa, 1981) is due to the induction of suppressor T cells and that conjugation with a copolymer of D-glutamate and D-lysine leads to suppression via tolerance of B cells (Katz et al, 1972).

Conjugation of proteins with hydrophilic polymers can also increase their chemical and physicochemical stability and is known to enhance resistance to proteolysis and heat denaturation (Wileman et al, 1986). Thus, such chemical protection can affect clearance, and, perhaps, immune response.

CHANGING DISPERSION THROUGH STRUCTURAL MODIFICATION

Deletion Mutants

Site-directed mutagenesis can be used to create de novo heterologous proteins which may or may not broadly resemble endogenous material. These approaches are being used not only to improve on the stability and intrinsic specificity of such endogenous proteins, but also (and increasingly) to achieve a selective and often prolonged delivery of the polypeptide/protein to an active site. The efforts to develop further types of plasminogen activators serve as good examples. Recent work has pointed to the altered pharmacokinetic and thrombolytic properties of deletion mutations of human tissue-type plasminogen activator (tPA) in rabbits (Collen et al, 1988). Wild-type tPA is characterised by a rapid clearance by the liver, with an alpha distribution phase half-life of a few minutes. Using a series of deletion mutants (which included removal of fibronectin-like, epidermal growth factor type and glycosylation-site regions), this group have demonstrated that regions within tPA responsible for its liver clearance, its fibrin affinity and its fibrin specificity are not localised in the same structures. They argue that it appears possible to alter specific functions of tPA related to poor pharmacokinetics without decreasing its efficacy. Harber et al (1989) have recently reviewed how the tools of molecular biology and protein engineering may be used to develop 'safer and more effective' plasminogen activators. They describe both domain-deletion and site-directed mutagenesis techniques for the creation of new plasminogen activators, as well as chimeric (or hybrid - see below) molecules. Harber et al (1989) have reviewed the use of domain deletions to produce a shortened form of single-chain urokinase-like plasminogen activator (scuPA), which does not have the NH_2-terminal kringle of scuPA. Whilst it appears that this shortened form of scuPA is not present in vivo; it is surprising to many observers that although it is only 14 aminoacids longer than the low molecular weight two-chain urokinase, low molecular weight scuPA has a similar selectivity for fibrin than is exhibited by scuPA, thus showing that the kringle is not needed for fibrin selectivity. The low molecular weight form also is resistant to plasminogen activator inhibitor I, which should assist in helping to increase its residence in the plasma.

Novel fibrinolytic enzymes have been made which retain the properties of the parent but have modified dispersion patterns. For example, Pohl et al (1989) have patented a fibrinolytically active plasminogen activator of the tissue type, in which both the growth factor domain and the Kringle 1 domain have been deleted, and where various point mutations have been made. These heterologous proteins exhibit different eliminations in vivo (Fig. 4).

In addition, since plasminogen activator inhibitor-1 (PAI-1) (a member of the serpin superfamily), serves to prevent systemic activation of plasminogen, groups have attempted to produce serpin-resistant variants of tPA using site-directed mutagenesis, which could serve to maintain the enzyme in an active form in the plasma (Madison et al, 1989).

Glycosylation

Many endogenous glycoproteins (i.e. serum glycoproteins, lysosomal enzymes and perhaps also sulfated pituitary glycoproteins such as chorionic gonadotropin) interact through their specific carbohydrate residues complexing with (oligosaccharide-specific) recognition systems on the plasma surfaces of target cells. Glycosylation patterns are thus signals used by the body to regulate the dispersion of its own glycoproteins, as implicated for both enzyme and hormone disposition as well as immune surveillance, coagulation, etc.

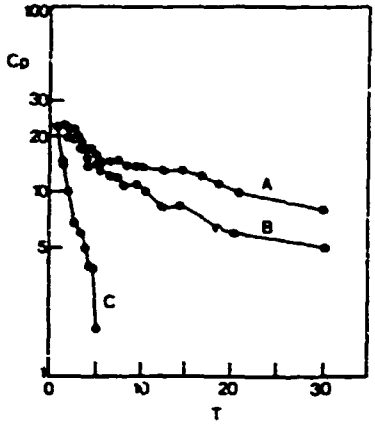


Fig. 4. Elimination of native and modified tissue plasminogen modifier (tPA) from rabbit plasma (Cp is plasma concentration, $mg.ml^{-1}$) with time (T, mins). A and B represent deletion mutants, and C is the native full sized human tPA (Pohl et al, 1988). (Reproduced with the permission of the copyright holder.)

Three biological properties of glycoproteins may be adjusted upon altering their surface distribution of carbohydrates, namely, i.e. circulating blood half-life (and potentially duration of action), immunogenicity, and ability to access (cellular) sites of action. Most glycoproteins contain three or more glycosylation sites, with some proteins having 10 to 30 different sugars at each glycosylation site, with the occupancy of each site varying. Expressed glycoproteins may be engineered in order to affect their selectivity, and activity, for target sites. Again, the extensive work being carried out to modify the glycosylation patterns of tissue plasminogen activator, give that different levels and types of glycosylation result in types of tPA differing in their ability to cleave plasminogen after fibrin stimulation; and/or that a sugar positioned at one of the Kringle rings could result in blockage of fibrin access.

In contrast to possible changes in the amino-acid compositon of a protein, the numerous variations possible in linking simple sugars together affords glycoproteins an almost limitless variability and diversity in structure. Additional modifications, such as removal or addition of peripheral sugars and/or other functional groups such as acetyl, methyl, sulfate and phosphate, are also possible. Oligosaccharides may be N-glycosidically linked (to the peptide at Asn), or O-glycosidically linked (attached to Ser and Thr). The oligosaccharides of the plasma glycoproteins are linked to protein primarily through L-asparagine-N-acetyl-D-glucosamine. Recent studies have examined the function of carbohydrate modifications of the natural hematopoietins (including granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin, and CSF-1). Extensive modifications produced by the addition of an asparagine-linked carbohydrate resulted in rather heterogenous glycosylation patterns, dependent upon expression cell and protein under study. However, it has been demonstrated that the effective half-life of a GM-CSF in the bloodstream of a rat is increased significantly by the addition of N-linked carbohydrate (Donahue et al, 1986). Recent work has shown that by conjugating fragment A of diphtheria toxin to a galactose-containing oligosaccharide such as asialofetuin or asialoorosomucoid, the resultant conjugate targets to hepatocytes, and is up to 3 orders of magnitude more toxic that the native fragment A. If galactosyl residues are to be used clinically to target proteins to hepatocytes it is

important to appreciate that the in vivo uptake of galactosylated neoglycoproteins has been shown to be highly dose-dependent (Vera et al, 1984).

Biotechnological processes. Glycosylation of a therapeutic protein may occur either during expression-cell processing, or post-expression via synthetic conjugation. Biotechnology processes use expression of the protein in a cell system. Glycosylation does not usually occur in prokaryotic cells such as E. Coli. Some glycosylations do occur in eukaryotes, e.g. yeast and mammalian cells, however, the resultant glycosylation patterns can often be different, depending on the cells used. Also, although recombinant bacteria are able to produce large amounts of protein, these are often changed by bacterial proteases; within expression cells it is possible that expressed proteins of low solubility denature and form aggregates. Mammalian cells are particularly suitable both for expressing proteins with complex modifications such as γ-carboxylation of glutamyl residues, and for obtaining homologous (human) glycosylation patterns. Thus it is apparent that biotechnology processes can have a resultant marked effect on the biological disposition and efficacy of therapeutic proteins.

Protein re-modelling. Much recent progress has been made in the re-modelling of expressed glycoproteins intended for therapeutic use in order to affect their selectivity for target cells. Because of the complexity of these reactions, conventional carbohydrate chemistry is of little use and interest is centred on the modifications of proteins with oligosaccharides via enzymatic synthesis. Various chemical approaches to re-modelling proteins post-expression are being developed (e.g. Akiyama et al, 1987). Recently, an important new approach has been described which employs enzymes to elongate and terminate peripheral glycan chains of glycoproteins (Berger et al, 1986, 1987). Mammalian glycoproteins expressed in yeasts are likely to be substituted by mannans, and this group has been able to incorporate sialic acid into endo-β-N-acetylglucosaminidase H-treated oligomannose glycoproteins (Berger et al, 1986). The technology is of potential use in reducing any mannose-receptor uptake of these glycoproteins by cells of the MPS, etc. Although the approach needs optimizing in terms of enzyme activity, it demonstrates the successful incorporation of sialic acid into glycoproteins of the oligomannose type. Berger et al (1987) have also suggested the use of purified galactosyltransferase for the galactosylation of glycoproteins prior to their sialylation or their chemical linkage with oligosaccharides, and argue that this approach appears to be promising for the in vitro re-modelling of glycan chains in heterologous glycoproteins. For example, post-expression protein re-modelling has been used to resurface some of the enzymes indicated in lysosomal storage diseases. In attempts to improve on the affinity for glucocerebrosidase to macrophages, Furbish et al (1981) have reglycosylated the enzyme by simple conversion of the mannose-terminated core structures $Man3GlcNAc_2$ and Man3NAc(Fuc)GlcNAc by sequential treatment with neuraminidase, β-galactosidase and β-N-acetylhexosaminidase. This resulted in a five-fold more efficient increase in uptake by (rat) Kupffer cells over the native enzyme.

Candidate proteins reported amenable for this kind of re-modelling include tissue plasminogen activator, factor VIII, EPO, colony-stimulating factors, α_1-antitrypsin, β- and γ-interferons, interleukins I-III, and even antibodies, etc.

Deglycosylation. Conversely, protein deglycosylation should also affect dispersion (and stability and solubility) of a therapeutic glycoprotein. Indeed, deglycosylation (via chemical means using a mixture of sodium metaperiodate and sodium cyanoborohydride at low pH and temperature), has been shown to virtually eliminate the uptake of the toxin ricin A chain by the liver (Blakey and Thorpe, 1986). Ricin A chain is an oligomannosidic glycoprotein which has a very short half-life due to rapid removal by the

mononuclear phagocyte system, and deglycosylation of the A chain may reduce removal by Kupffer cells. The chemical method described results in 'destruction' of approximately 50% of the mannose and most of the fucose residues, whilst leaving the N-acetylglucosamine and most of the xylose residues alone. Although chemical deglycosylation only marginally delays the clearance of deglycosylated ricin A from the blood compartment (in mice), this is probably due to the production of a smaller macromolecule able to be effectively filtered and excreted through the kidneys. Blakey and Thorpe (1986) assume that linking the deglycosylated ricin A chain to an immunoglobulin would diminish this later effect. Ricin A chain lacking carbohydrate side-chains has also been produced using recombinant technology (Vitetta et al, 1987).

Ligated Gene Fusion Hybrid Delivery Systems

Gene-fusion techniques may be used to produce distinct therapeutic proteins which combine the varied properties of parent proteins. This is exemplified by recent strategies which have been proposed for the targeting of bacterial and/or plant toxins to specific cells using hybrids created by ligating toxin and growth factor genes. Such an approach relies on the deletion of the toxin gene sequence encoding the cell-binding site, which allows the hybrid-fusion protein to display the cell specificity of the growth factor. Recently, a hybrid protein between interferon-γ and tumor necrosis factor-β has been shown to have a greatly increased antiproliferative activity in vitro, compared to either interferon-γ or TNF-β alone, whilst still retaining their antiviral activity and cytotoxic effects. Hybrid protein delivery systems may involve not just adduction of a protein (fragment) to a recognition moiety, but also to a re-ordering of the structure of the efffector portions of therapeutic proteins in order to enhance their pharmacological action.

Harber et al (1989) have pointed to the use of hybrids as novel molecules that combine delivery and effector functions for use in plasminogen activator therapy. For example, high-affinity fibrin selectivity and resistance to plasminogen activator inhibitor I can be introduced into a molecule by construction of a hybrid composed of the A chain (fibrin binding domain) of tissue plasminogen activator, and the low molecular weight form of scuPA (i.e. the catalytic site of urokinase)in a form that is not susceptible to plasminogen activator inhibitor. Such enterprise however is not without its difficulties. As Haber et al (1989) point out, unfortunately the fibrin-binding activity of the recombinant hybrid is less than that of native tPA, with its fibrin selectivity being found in preliminary work to be less than that of single- or of two-chain tPA. Hybrid protein delivery systems may involve not just adduction of a protein (fragment) to a recognition moiety, but also to a re-ordering of the structure of the effector portions of therapeutic proteins in order to enhance their pharmacological action. For example, Murphy (1987) has described a hybrid protein, in sequence, and joined by peptide bonds, of the enzymatically active fragment A of diphtheria toxin, a fragment including the cleavage domain l_1 adjacent to fragment A, a fragment which includes (at least) a portion of the hydrophobic domain of fragment B (though not including the generalised eukaryotic binding site of fragment B), and finally a fragment which includes a portion of a cell-specific polypeptide able to bind the conjugate delivery system to a specific cell feature.

Recent work by Chaudray et al (1989), has produced a single chain antibody-toxin by the fusion of cDNAs encoding anti-Tac antibody variable regions with a fragment of DNA encoding a modified form of Pseudomonas exotoxin (anti-Tac is a monoclonal antibody to the p55 subunit of the human IL-2 receptor). Previous attempts at making such immunotoxins have suffered because the antigen binding site of the antibody is made from two separate

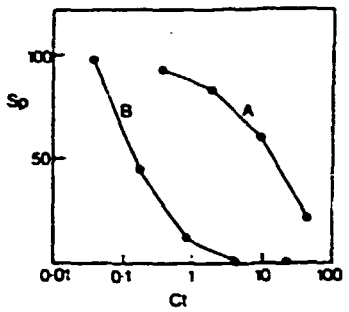


Fig. 5. Comparative in vitro cytotoxicity of hybrid proteins, showing covalently linked immunotoxin (A) and a fusion protein (B). Cytotoxicity was measured as protein synthesis (Sp, percent of control) at various concentrations of toxin (Ct, $ng.ml^{-1}$) (Chaudhary et al, 1989). (Reprinted (with modification) by permission from Nature, Vol. 339, pp. 394-397. Copyright (c) 1989, Macmillan Magazines Ltd.)

polypeptide chains and this new approach has enabled a single chain antibody-toxin protein to be made in E. Coli which contains the two variable domains of the antibody joined by peptide linkage and fused to a modified exotoxin. Figure 5 gives some of their data showing the greater potency of the fusion protein compared to the covalently linked protein, against cells which strongly express the IL-2 receptor. The lower toxicity of the covalent hybrid protein is thought to be due to the linkage being through lysine residues in domain III of the toxin, leading to a reduction in the activity of the toxin. It is not proven that it is a general case that fusion proteins are more active than covalently linked systems, but these authors consider that their approach can be generally used to produce active recombinant immunotoxins with (other) antibodies. In a similar fashion the ricin A chain, which catalytically inhibits the 60S subunit of ribosomes in vitro, linked to an anti-HIV gp120 monoclonal antibody, is considered to be effective in the treatment of acute and chronic HIV infections (Pincus et al, 1989).

Interesting further examples of this approach include the production of phospholipid anchor domain (PAD) fused to a heterologous polypeptide, which could for example be the anchor domain of mDAF (i.e. decay accelerating factor). DAF conjugates or fusions serve to deliver DAF to target cells to inhibit complement activation at the surfaces of such cells and and are claimed to be useful in allograft rejection and autoimmune diseases (Caras, 1989).

Decoy Delivery Systems

Included in this category of novel proteins having both recognition and effector functions, are the recently described immunoadhesins (Capon et al, 1989). Specifically, these are antibody-like molecules, containing the gp120-binding domain of the receptor for human immunodeficiency virus. They have been shown to block HIV-1 infection of T cells and monocytes. Interestingly, such novel hybrids' delivery systems have a long plasma half-life. The fusion of the gp120-binding domain of CD4 to the Fc domain of an immunoglobulin has considerable merit when one appreciates that the formed heterologous protein retains some of the important properties of both of its parent molecules; namely, they bind gp120 and block infection of T cells by lymphotropic HIV-1, and of monocytes by monocytotrophic HIV-1. They are also comparable to antibodies in their long plasma half-life, as

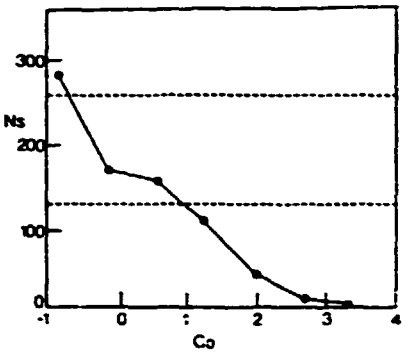


Fig. 6. Neutralisation of HIV with pentameric recombinant CD4-IgM fusion proteins, showing the relationship between number of syncytia (Ns) and concentration of recombinant protein (Co, $ng.ml^{-1}$). The dashed lines indicate the number of syncytia in the absence of any recombinant protein and also the number at 50 percent inhibition (Traunecker et al, 1989). (Reprinted (with modification) by permission from Nature, Vol. 339, pp. 68-70. Copyright (c) 1989, Macmillan Magazines Ltd.)

well as their ability to bind Fc receptors and protein A. The claims of Capon et al (1989) include that the attainment of a high steady-state level of immuno-adhesin makes it likely that effective concentrations of the hybrid will be attained in lymph and lymphatic organs (where HIV may be most active). Similarly, pentameric CD4-IgM chimeras have been produced (Traunecker et al, 1989). These are shown to be 1000 times more active than their dimeric CD4-IgG counterparts in syncytium inhibition assays (Fig. 6). Also effector functions, such as the binding of Fc receptors and the first component of the complement cascade(C1q) are retained (Traunecker et al, 1989), which is contrary to the results found with the CD4-IgG chimaeric delivery decoys which contain the CH1 domain and do not bind C1q. This suggests that it could be the deletion of the CH1 domain which leads to complex aggregation.

Synthetically Linked Hybrid Conjugates

Large changes in the biological disposition of proteins have been reported upon their chemical linkage to other protein (fragments). For example, the toxin gelonin - which has a circulation half-life in mice estimated at 3.5 minutes, when conjugated to immunoglobulin (fragments) has a terminal phase blood half-life in the order of days, with only a slight variation in this time as the conjugated immunoglobulin (fragment) is changed (Scott et al, 1987). The immunotoxin field provides many relationships between protein structure and deposition. Point mutations in the B polypeptide chain of diphtheria toxin that block non-specific binding to non-target cells have been produced. Upon covalently linking this entity to an anti-T-cell monoclonal antibody, it may be demonstrated that because of a change in the non-target tissue distribution of the toxin, it becomes orders of magnitude less toxic than the native toxin to non-target cells (in vitro). The availability of monoclonal antibodies has led many workers to consider these for the targeting of toxic materials to human tumors.

Taetle et al (1988) have recently prepared an immunoconjugate from an antiepidermal growth factor (anti-EGF) receptor antibody and recombinant ricin-A chain. Their data show that this site-specific conjugate gives a dose-dependent killing of cells that express EGF receptors. However, and

Table 3. Some Factors Affecting the Pharmacodynamic Response

Rate and frequency of drug input
Rate of attainment of a critical plasma concentration
Presence of a drug-free interval
Infusion duration
Clearance
Frequency
Route
Dosing interval
Geometry of site of action and proximity of input to it

again typically for these types of anti-proliferative hybrids, the kinetics of cell killing with these conjugates was protracted, suggesting that prolonged exposure may be required for in vivo anti-tumor effects.

A further interesting example is that due to Reiter and Fishelson (1989) who have covalently linked a monoclonal antibody to C3b. This conjugate may facilitate in vitro and in vivo tumor cell destruction by the host complement system. The complement system has the potential to destroy tumor cells but only if directed towards it. Reiter and Fishelson (1989) covalently linked the complement component C3b (which binds to the surface of complement activators) to anti-transferrin receptor antibodies, in the belief that this receptor is expressed to a greater extent on rapidly proliferating cells than on non-proliferating cells. Whilst this approach is imaginative, the previous arguments given above relating to the access and persistence of macromolecular constructs at extravascular solid tumors apply.

RELATION BETWEEN INPUT AND DRUG PHARM^^ODYNAMICS

Although it is largely outside the scope of this contribution, it must also be recognised that there are a number of features of drug administration or input that can be identified which affect the drug's pharmacodynamic properties. These are given in Table 3 and are discussed more fully elsewhere (Tomlinson, 1989, 1990a,b). It is apparent that the constant drug blood levels are often an inappropriate feature for administration of therapeutic protein systems.

FORMULATION

The successful development of macromolecular drugs and drug carriers is a challenge due to their size, complexity, conformational requirements and their often complicated analytical, stability and solubility profiles (Tomlinson, 1987; Pearlman and Nguyen, 1989). Although again outside the scope of this contribution, it is clear (Pearlman and Nguyen, 1989) that one of the greatest challenges for developing such products will be the successful preclinical formulation of such molecules which will require relevant and discriminatory analytical methods.

CONCLUDING REMARKS

This brief contribution has pointed to some of the challenges and developments in the use of proteinaceous therapeutic systems for clinical use. Unfortunately, a belief still persists that macromolecular drugs can be used for successfully treating tumors residing in tissues having a con-

tinuous endothelium. The arguments and examples presented in this paper are intended to show how this has a minimal chance for success.

However, the tremendous advances being made using the tools of modern molecular cell biology are enabling new types of site-specific therapeutic drug to be fashioned. These will be able to take advantage of unique features of accessible lesions in order to effect treatment for a variety of important diseases.

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Theory and practice of site-specific drug delivery

E. Tomlinson

Advanced Drug Delivery Research, Ciba-Geigy Pharmaceuticals, Horsham, West Sussex, U.K.

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Key words: Macromolecular carrier; Drug targeting; Control of gene expression; Genetically-defined delivery system; Membrane transport; Mononuclear phagocyte system; Extravasation; Cell trafficking; Gene therapy; AIDS; Cancer; Parasitic disease

Contents

Summary

I Introduction

II New therapeutic horizons
1. Control of gene expression
2. Pathogenesis of disease
3. Normal physiological processes
4. New classes of therapeutic agents

III Rationale for site-specific drug delivery
1. Properties of peptidergic mediators
2. Drug disposition, metabolism and iatrogenic effects
3. Drug action and timing of drug action
4. Intractable diseases and their location

IV Theory of site-specific drug delivery
1. Ehrlich

Abbreviations: RFLP, restricted fragment-length polymorphism; CNS, central nervous system; HIV, human immunodeficiency virus; MPS, mononuclear phagocyte system; MAF, macrophage-activating factor; MDP, muramyl dipeptide; TDS, targeted delivery system; RES, reticuloendothelial system; CSF, cerebrospinal fluid; AGC, albumin-gold particle complex; LDL, low-density lipoprotein; HPMA, N-(2-hydroxypropyl)methacrylamide; TDS, targeted delivery system.

Correspondence: E. Tomlinson, Advanced Drug Delivery Research, Ciba-Geigy Pharmaceuticals, Wimblehurst Road, Horsham, West Sussex, RH12 4AB, U.K.

2. Concept of a macromolecular carrier
3. DART disease, access, retention and timing
   (a) Access
   (b) Retention
   (c) Timing

V Carrier systems
1. Carriage
2. Site recognition
3. Release

VI Recognition and intracellular trafficking

VII Administration and the entry and penetration of epithelial barriers
1. Nasal and buccal epithelia
2. Intestinal epithelia
3. Rectal epithelia

VIII Administration into discrete anatomical compartments
1. Intra-articular administration
2. Lungs
3. Eye and nasal cavities
4. Oral
5. Intralesional

IX. Interstitial administration

X. Intravascular targets
1. Capillary filtration
2. Mononuclear phagocyte system
3. Diseases of the mononuclear phagocyte system
4. Modulation of mononuclear phagocyte system action
5. Avoiding uptake by the mononuclear phagocyte system
   (a) Saturation
   (b) Modification of carrier surface
   (c) Mimics of natural particles
6. Circulating systems other intravascular targets
7. Normal and abnormal endothelia, and endothelia-related targets

XI. Extravasation
1. Structure and assembly of the endothelial membrane
2. Passage through normal endothelia via passive processes
3. Regional differences in capillary permeation
   (a) Renal endothelia
   (b) Intestinal endothelia
   (c) Post-venule capillaries
   (d) Lung endothelia
   (e) Skeletal muscle, adipose tissue and myocardial endothelia
   (f) Nervous system endothelia
   (g) Liver
4. Receptor-mediated events
5. Pathological opportunities for extravasation
   (a) Inflammation
   (b) Ischaemia hypertensive vascular lesions
   (c) Tumour endothelia

XII Extravascular, extracellular targets

XIII Specific therapeutic uses
1. Carrier-related considerations
2. Disease-related considerations
3. Genetic disorders
(a) Enzyme-storage diseases
(b) Gene therapy
4. Acquired immune deficiency syndrome (AIDS)
5. Control of the immune response
(a) Graft versus host rejection disease
(b) Allergic encephalitis
6. Cancer chemotherapy
(a) Access and retention
(b) Immunotoxins
(c) Regulation of cell function

XIV Clinical use of site-specific systems
1. Clinical utility
2. Pharmaceutical development
3. Safety pharmacology
(a) General considerations
(b) Potential toxicities of particulate systems
(c) Potential toxicities of soluble macromolecular systems
4. Pharmacokinetic/pharmacodynamic assessment
5. Drug regulation

XV Concluding remarks

Acknowledgements

References

Summary

All drugs act on discrete pharmacological receptors; however, their clinical use relies on their pharmacodisposition and pharmacokinetics combining to give an appropriate pharmacological response, coupled with the ability of the body to detoxify itself of any drug that has generally distributed. Although to date it has usually been possible to achieve drugs having these properties, not only are the disease targets becoming more difficult to attain, but the probabilities for success using the various high throughput screening techniques often employed are becoming low. The advent of the control of gene expression has given rise to both a plethora of new molecules and an understanding of normal and pathological processes; this is leading to new approaches to both the design and the clinical use of drugs. For these new classes of drugs, and for many drugs of the conventional type, there is a firm rationale for their site-specific delivery using carrier technology – such that the innate biological pathway of the carrier guide the drug to its pharmacological receptor in a *protective* form. This present contribution examines the rationale for

site-specific drug delivery in terms of disease and drug and carrier properties, and the biological constraints and opportunities for site-specific drug delivery are examined by considering carrier access, retention and ability to control the timing of drug availability at site(s) of action(s).

Although site-specific drug delivery is still rather empirical in its practice, examples are drawn from the literature to show the mistakes and successes in the use of site-specific carriers. These include their employment in macrophage activation, some other forms of cancer chemotherapy, retroviral diseases (including AIDS ARC), gene therapy, enzyme-storage diseases, inflammation, graft versus host rejection disease, and fungal infestations.

In addition, the challenges presented for the successful pharmaceutical development and clinical use of both soluble macromolecular and particulate colloidal carrier systems are discussed.

1. Introduction

Modern medicine is providing relief and comfort for tens of millions of patients. Much of this is due to clinical management of disease using chemotherapeutic agents. It is now estimated that there are over 230 essential drugs in common use throughout the world. In the large majority of cases their use is augmented by the availability of numerous and varied dosage forms.

As we approach the end of this century we are witnessing a change in medicine from being a descriptive science into becoming a mechanistic one. Not only are new and effective methods for diagnosis emerging, but so are a clearer understanding of both the working of the correctly functioning body, as well as the *pathogenesis* of many diseases. These latter advances are aiding the rationale design of many new classes of drugs – including homo- and heterologous peptidergic mediators.

It is quite clear that to turn these new advances into clinical effectiveness not only must the administration of the new classes of chemotherapeutic agent be optimised, but also we must improve upon the ability of drugs to interact with their pharmacological receptor in a way which takes cognisance of the *disease*, its *location* and its *chronicity*. Such a process may be described as *site-specific drug delivery*. Gardner has recently produced a quite excellent summary of the potential and limitations of this process [1]. He identifies a wide variety of chemical and physical approaches which can be considered for site-specific drug delivery. These include those where the drug is selectively directed to a target where it is active (as with carriers), or where the drug is generally distributed and is activated from a non-potent and low-toxicity form into an active form (e.g., as with prodrugs). In contrast, this present contribution attempts to take a global view of the current theory and practice of site-specific drug delivery using macromolecular and particulate carrier systems, with particular attention being paid both to the biological opportunities which are being revealed by the new tools of cell and molecular biology, as well as to the important new insights into physical and biophysical processes being given by the more traditional areas of the natural sciences.

The description will be largely based on a consideration of the *disease*, and the delivery of the drug in terms of *site access, retention and timing* of interaction, coupled to the *duration of effect* of the drug, and the *responsiveness* of the target. These are the key features needed for the design of an effective site-specific system, whether this be produced by either conventional synthetic means of via biotechnology

II. New therapeutic horizons

In many and varied ways there is a revolution under way in clinical medicine brought about by advances in inter alia molecular and cell biology, physiology, pharmacology, biophysics and analytical methods. It is useful to explore some of the impact that the first of these, molecular and cell biology, is having on this new direction, since this will show both why site-specific drug delivery will be of increasing importance in successfully contributing to this revolution, and what the tools and armamentarium of the campaign will be.

II.1. Control of gene expression

The discovery of the genetic code some 30 years ago led to the creation of molecular biology as we know it today. This involved a combining of the reductionist approach to genetics with those efforts designed to elucidate biomolecular structures in order to understand their function. Since the cloning of the first gene in 1973, and the development of other recombinant DNA techniques, there have been many advances in molecular and cell biology which are serving to turn medicine from being a descriptive practice into one which relies far more on developing a mechanistic understanding at the molecular level of the diagnosis and treatment of disease. For example, the geneticist is now largely armed with the molecular biology tools required to collect gene mutations at all of the levels of biological organisation and complexity. Mutagenesis can be directed to the entire genome, to individual genes and to gene clusters, to structural and regulatory segments of genes, and even to single nucleotide positions – such that it is now possible to identify, enrich and amplify genes from the cell of any organism [2]. This level of control has many and varied consequences for the pharmaceutical community in their search for safe and effective medicines, and particularly for the modern medicinal chemist [3].

II.2. Pathogenesis of disease

First, in understanding the pathogenesis of disease, recombinant DNA technology has enabled some of the fundamental molecular mechanisms which regulate the expression of genes at the level of transcription and translation to be elucidated.

An increase in the availability of cloned human DNA sequences and chromosomally restricted fragment-length polymorphisms (RFLPs), has enabled the study

of more than 80 genetic diseases at the DNA level to date. Some of the disorders for which either a partial or a complete genetic analysis has been made include Alzheimer's disease, haemoglobin disorders, muscle disease, lysosomal storage diseases, coeliac disease, chromosomal transformations in Burkitts lymphoma, cystic fibrosis, malaria, AIDS ARC LAS, T-cell leukaemia and HLA-disease associations. (see Subsection XIII.3)

RFLPs may be used also as chromosome markers around the gene or to track the inheritance of unknown genes via linkage analysis, and RFLP hybridisation probes can assist in the study of chromosomal deletions and translocations and the predisposition to disease. Literally thousands of human inherited diseases have now been identified as probably being caused by a single defect in a gene [4,5]. In addition, insights into gene defects using molecular biology tools show us that different types of mutation in the same gene can result in the same clinical manifestation. RFLPs allow the analysis of inherited disease without a need to know precisely the type and location of the defective gene, and this has been useful in determining the aetiology of diseases such as muscular dystrophy and cystic fibrosis; similar approaches are being used to study many polygenetic diseases such as atherosclerosis, psychiatric disorders and rheumatoid arthritis – for which a cluster of genes may predispose an individual to the development of any disease. And all the while new methods are emerging, such as variable number tandem repeat probes which detect genetic heterogeneity (like RFLPs), but by finding repeated DNA sequences. Thus recombinant DNA methodology is offering the promise of both a diagnostic tool and the means to probe the molecular pathology of the disease [6]. When one considers these very recent advances with knowledge of the declared intention of sequencing the entire human genome (see subsection XIII.3), and notwithstanding the tremendous technical and resource issues (and the legal and ethical questions) that shall arise, then clinical medicine must truly be regarded as being on the brink of an *entirely new* and *different* future.

II.3. Normal physiological processes

In addition, the powerful new tools described above are being used to probe normal physiological processes, whether these are occurring intra- or extracellularly. Such studies are being greatly aided by sophisticated and powerful new imaging techniques such as fluorescence-activated cell sorting, confocal fluorescence microscopy and tunnelling microscopy, as well as other techniques which permit a clearer definition of events at the molecular level; other radically new techniques, such as positron emission tomography and in vivo nuclear magnetic resonance (NMR) spectroscopy are enabling the biologist not only to understand the biochemistry of normal and diseased processes, but also even to achieve their visualisation at previously undreamt of degrees of definition.

For example, the successful study of processes occurring in both soft and hard tissues can now be accomplished (including previously difficult areas such as bone metabolism, etc.) Cell processes being elucidated include simple secretory and receptor-mediated events, both of which require signal recognition and feedback

control. These intracellular processes are often mediated by leader sequences which can act in an number of ways, i.e., either through their basicity/acidity properties or via some function of their primary or secondary structure. We shall learn later that to understand the minimum amount of structural information to cause a tropism could be useful in the design of site-specific drugs (Section VI).

II.4 *New classes of therapeutic agents*

The improved control of gene expression in both eukaryotes and prokaryotes is enabling the efficient production of relatively abundant amounts of pure polypeptides and proteins. These can be considered useful as both drugs themselves, or as structures (such as physiological receptors), from which relevant active portions can be identified and copied – perhaps in the design of low-molecular-weight agonist or antagonist drugs [3]. This avenue of drug discovery, involving many modern and powerful techniques such as 2-dimensional NMR, epitope mapping and computer-aided molecular modelling, is producing results in many areas, with those advances seen in understanding and treating brain disorders being the most apparent.

To date only about 100 human proteins have been cloned and expressed, with four broad classes of structure being studied, i.e., hormones, pharmacological and operational receptors, enzymes, and components of the immune system. However, the United States Food and Drug Administration (FDA) was able to announce in April 1987 that not only were there more than a dozen molecules of biotechnic origin now approved for general human use, but that there were more than 150 of this class of molecules currently in clinical trial in humans in the United States. Although we shall return to some of the challenges facing the pharmaceutical community in bringing site-specific systems having a biotechnic component to the clinic and then being generally approved for use in humans (see Section XIV), it is important to realise the power that molecular biology has brought to the production of abundant amounts of relatively pure materials in an increas-

TABLE I

SOME RECENT NOVEL BIOTECHNICS

Product	
Drugs	genes
	endocrine and paracrine-like peptidergic mediators (e.g., Interferon 2α)
	sequence-specific oligonucleotides
	antibody:drug constructs for cancer chemotherapy
Diagnostics	new antibody-related diagnostic testing
	direct HIV antigen diagnostic test
	radioactive Indium 111-labelled monoclonal antibody for renal cell carcinoma
Vaccines	synthetic core viral vaccines
	AIDS vaccine produced by inserting the lymphadenopathy-associated virus surface protein gene into vaccinia virus
Processes	monoclonal antibody based purification process for Natural factor VIII

TABLE II

BIOTECHNIC DRUGS AND VACCINE PRODUCTS AND DISEASE INDICATIONS

Disease symptom	Product
Tumours	immunostimulants
	biological response modifiers
	antibiotics
	cytokines
	lymphokines
Infectious diseases	antibiotics
	interferons
	vaccines
	immunostimulants
Cardiovascular system	plasminogen activators
	antibodies
Bone metabolism	growth factors
	hormones
Inflammation	protease inhibitors
	biological response modifiers

ingly cost- effective manner. (Tables I–III give some of the advances being made by biotechnology in deriving novel classes of drugs and other products).

As we shall discuss later, it is also clear that much of this will come to nought if methods cannot be invented which assure that such molecules can be optimally administered to patients using technologies that ensure that they reach their sites of action in ways which are appropriate for the disease, are cost-effective, do not cause a modulation of the disease, and which are safe and convenient to use.

III. Rationale for site-specific drug delivery

The advent of this new generation of biotechnic drugs and their lower molecular-weight counterparts, and their physical and physicochemical nature; together

TABLE III

SOME BIOTECHNIC PRODUCTS REPORTED AS BEING CONSIDERED BY THE U.S. F.D.A. (AT APRIL 1987)

Product	Company
α-1 antitrypsin	Chiron; Genetech; Transgene
Digitalis antidote	Wellcome
Cholera vaccine	Praxis Biol
Epidermal growth Factor	Chiron/Johnson & Johnson
Factor VIIIc	Chiron/Nordisk
Glucocerebrosidase	Genzyme
Hepatitis B vaccine	SmithKline & French
Interleukin-2	Collaborative Research/Triton Biosciences
Somatropin	Genentech
Superoxide Dismutase	Chiron/Gruenthal/Pharmacia
Tumour Necrosis Factor	Cetus
t-Plasminogen Activator	Genetech; wellcome

TABLE IV

RATIONALE FOR SITE-SPECIFIC DRUG DELIVERY (REFERENCE 9)

Exclusive delivery to specific compartments (and/or diseases)
Access to previously inaccessible sites (e.g., intracellular infections)
Protection of drug and body from unwanted deposition which could have led to untoward reactions and metabolism, etc.
Controlled rate and modality of delivery to pharmacological receptor
Reduction in the amount of active principle employed

with the introduction of some new, though conventional, synthetic drugs; and the intractable nature of many of the remaining disease targets; all these are features of modern medical and pharmaceutical research which are combining to cause a re-appraisal of the clinical utilisation of many drug classes.

Numerous examples of disease can be evidenced for which simply to attain a site of action located in a poorly accessible region would lead to an improvement in drug use; as for example with many intracellular infections, diseases of the central nervous system (CNS), diseases of the immune systems, cancerous states, some cardiovascular-related diseases, haemopoietic diseases and arthritic disease. In addition, it is apparent that many of the intricate dosing regimens and the high doses of drug which are often applied, are frequently necessary because of a poor perfusion of the site of action (e.g., with rheumatoid joints), coupled with an inappropriate pharmacodisposition of the drug. Since the latter may lead to untoward metabolism, often, all of these effects combine to produce deleterious effects.

A further issue of drug action which is often not studied is the *chronicity* of the disease and the responsiveness of the target site. These can combine to demand that drugs must be delivered to their site(s) of action(s) at the appropriate time and at the correct rate and frequency. We shall return to each of these points in turn.

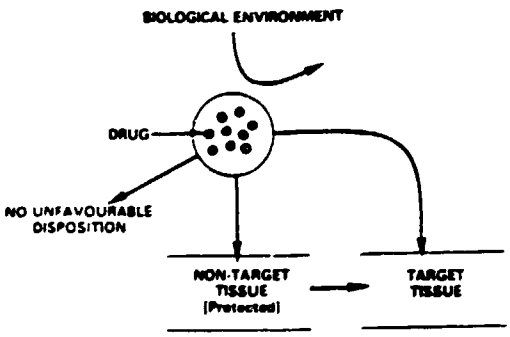

Fig. 1. Aims of site-specific drug delivery (Reproduced with permission, from Ref. 9)

Thus, *site-specific drug delivery* may be defined as *achieving the maximal potential intrinsic activity of drugs by optimising their exclusive availability to their pharmacological receptor(s) in a manner that affords protection both to the drug and to the body alike*.

Table IV gives some of the general rationale for site-specific drug delivery, and these are depicted in diagrammatic form in Fig. 1.

III.1. Properties of peptidergic mediators

For homologous proteinaceous drugs, the dogma has become that because these are akin to the body's own materials their use will result both in effective selectivity towards the target site (i.e., self-targeting) and few untoward effects. However, their use in a clinical setting means that the reality is probably quite different.

Table V gives some of the key issues that need to be considered in the use of peptidergic mediators. It is instructive to examine first some of the physical, physicochemical, and biological properties of peptidergic mediators. First, with respect to the *efficacy* of such peptidergic mediators their local versus general systemic activity needs to be considered. Many endogenous peptides are designed to act within 1–10 nm of their site of production (e.g., as with some of the interferons), and

TABLE V

ISSUES IN THE USE OF POLYPEPTIDE AND PROTEIN DRUGS

Issues
Biotechnology
Processes
Analyticals
Purity
Identity
Administration aspects
Pharmaceutical
Analytical
Biological
Route
Site-specific delivery
Rationale:diseases
Local versus systemic effect
Events required (e.g., lymph-to-plasma ratio)
Route of administration
Transport phase
Passive:active presentation to cells tissues
Pharmacology safety pharmacology regulatory aspects
Processing
Immunotoxicity
Testing: rationale ethics

need a lymph-to-blood ratio of greater than 1.0 (Poste, (1986) NATO ASI abstract.) However, many homologous (and heterologous) peptidergic mediators have a variable and inefficient disposition within the body, brought about largely because of their poor ability to *extravasate* in a selective and efficient manner. Also, polypeptides are degraded in the gastrointestinal tract (by peptidases), are active at extremely low levels, and unstable in biological fluids, and often undergo rapid and efficient extraction from the blood pool by the liver (mainly through the galactose recognition system present on hepatocytes). (Significantly, for materials produced by biotechnology, these latter effects can be affected by the cell-type and down-stream processes used for their production.)

In addition, polypeptide and protein drugs often need to act as part of a polymediator cascade of events. For example (Fig. 2), they may be acting in a cell which is developing in a sequential manner which is being controlled by various (other) mediators, and where all cells have a time-related responsiveness. Thus, chronopharmacological effects (diurnal and circadian rhythms), related to target-cell responsiveness are witnessed with numerous lymphokines and cytokines. Bearing these properties in mind, it is therefore hardly surprising to learn that clinically the dose-response relationships for polypeptides can be rather unusual, with often bell-shaped and even double bell-shaped curves being found [7,8]. All of these properties can combine to require very high doses of peptidergic mediator to be given (relative to natural levels) in order that a suitable biological response be elicited. This could inevitably lead to toxic effects with the chronic use of such mediators.

Thus, the clinical use of paracrine- and endocrine-like peptidergic mediators, when administered using conventional dosing regimens, will often be contra-indicated due to their inability to attain an active site in the appropriate amount and at the correct time, and to the presence of unusual high levels found in either blood or other (non-target) tissues – and which can give rise to immune responses.

Homologous biotechnic drugs may have an element of self-targeting in their intrinsic structure: however, because of their intrinsic properties given above, particularly for locally acting molecules, these macromolecular drugs will often require to be specifically targeted (as well as to have a protection phase in their delivery). Whether this will arise with the use of various synthetic carrier systems or via the de novo synthesis of macromolecular polypeptide therapeutic systems that have all of the functions and attributes of drugs, carrier, protector, etc., is a nice point [9].

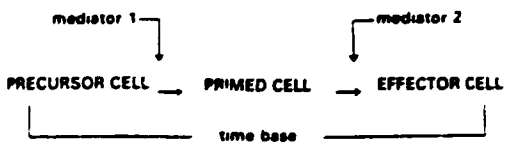

Fig. 2. Chronopharmacology: target cell responsiveness and influence of polymediator cascades

III.2. Drug disposition, metabolism and iatrogenic effects

To date, the design of drug molecules has largely centered around the interaction between a drug and its pharmacological receptor. However, since at (pseudo-steady-state) equilibrium the apparent activity of a drug must be simply the product of its intrinsic activity and its availability to the receptor site, it is surprising that so little attention has been given (in the pharmaceutical industry at least), to understanding the myriad of events which take place from the point of entry into the body, to the drug providing the biochemical stimulus that leads to the pharmacological response. This is even more startling when one appreciates that many of these events can be rate-determining ones, including for example, movement through both epithelial and endothelial barriers. Introduction of drug into the body immediately lays it open to a multitude of temptations and dangers, including degradation, unwanted disposition (either intravascular, e.g., protein binding, or extravascular, e.g., non-target tissues), and the initiation of toxic events (e.g., immune system activation or depression).

In general, after the administration of drugs, their efficacy and safety are maintained by selective interaction with the pharmacological site of action coupled with a reliance on the body's normal detoxication and excretion processes to rid itself of unwanted active principle and its metabolites. This non-discriminating action also removes the principal component from the body, with the result that often a drug's innate pharmacokinetics are manipulated, using perhaps some novel administration dosage form, so as to give a satisfactory level of drug at the pharmacological receptor. These (dose-related) events define the drug's therapeutic index, its dose regimen, and often, its route of administration and dosage form. It is this interplay between a drug's pharmacodynamics, metabolism/excretion and pharmacodisposition which need to combine to lead to an acceptable effective medicine.

Site-specific drug delivery serves to overcome this *void* in our knowledge of events by seeking to exploit the known innate pathways that *carrier systems* have in order to achieve appropriate drug action. This occurs by *protecting* the body and the drug from untoward events, and by achieving exclusive site-specific action of the drug through (variously) *site-selection, access, release of drug and/or adequate retention of carrier and drug*.

III.3. Drug action and timing of drug action

When drugs do reach their site(s) of action(s) they must interact with their pharmacological receptor in an optimal manner. This will involve an adequate retention of drug at the site of action in order for sufficient drug to reach the receptor – given its intrinsic *duration of effect* and the *responsiveness* of the target. In addition to these, in the design of the drug (and/or a site-specific drug delivery system), must be added consideration of whether the drug is acting as either an agonist or an antagonist.

Fast mechanisms of interaction such as responses to drugs involving fast synapses, are in the order of milliseconds: there are also slower mechanisms of action

of greater than 0.1 s (for example, smooth muscle- or gland-cell responses to muscarinic acetyl choline).

III.4. Intractable diseases and their location

The large majority of drugs treat the symptoms of a disease rather than effect a cure. Exceptions to this include the use of antibiotics for fighting various *accessible* infections. However, in contrast to the optimistic outlook for the future presented in Section II, most diseases are still poorly defined in terms of their pathogenesis and their *location*: with the result that the clinical management of many diseases using drugs and various dosage forms has been due to a process of iteration using varied dosage regimens and forms in an increasingly larger patient population in order to find an effective dosing range and format.

The new biosciences are revealing both the rather mundane nature of many of the chemical approaches we have had to date, and the immense complexity and seemingly intractable nature of many of the diseases which will need to be attacked in order to effect sensible treatments and cures. We only have to witness the problem of the acquired immune deficiency diseases brought about by retroviruses, where, for example, with human immunodeficiency virus (HIV) an almost complete genetic analysis is showing us that the regulatory genes of the virus could well be important targets for chemical intervention, but that a further appreciation of the complexity of the location of these targets and the selective nature of any interventionist approach shows the tremendous technical strides that will be needed to arrest the onset of this virus and its associated diseases (see also subsection XIII.4). With diseases located in inaccessible locations, such as those which are poorly perfused (e.g., bone, rheumatoid joints), or extravascular (e.g., some cancers), or intracellular (e.g., some bacterial, fungal and parasitic infections), or in specialised compartments (e.g., within the CNS), it is similarly clear that *selective access is a key feature in the successful use of both existing and future drugs*.

IV. Theory of site-specific drug delivery

IV.1. Ehrlich

Clinical medicine owes much to the seminal views of Ehrlich [10]. He was amongst the first to develop theories of drug action and specificity, and in particular his theory 'Corpora non agunt nisi fixata' ('substances do not act unless they are linked'), the side- chain theory, and his concepts of tropisms of toxins to host cells and to infectious cells – organotropy and parasitotropy (sic) – began an understanding of drug selectivity and disposition that has formed the basis for much of modern chemotherapy research. Ehrlich's efforts were designed to bring chemistry and biology together [10] with the purpose being to 'aim drugs, and to aim in the chemical sense.' His views on toxin and later drug interactions with cells, his prediction of the existence of antibodies and, after their discovery, his discussion on their specific binding, led him to talk of 'magic bullets', and Alexander

Fleming to talk later about Ehrlich's 'wonder weapons'. The impact of the receptor theory of Ehrlich (and of Langley) was limited in their lifetimes, and it was not until the 1950s that this became a major area of research. Ehrlich's efforts were largely designed to produce small molecular-weight drugs, with great accent placed on the pharmacodynamics of drug action, and not, as discussed above in controlling the events which occur prior to this. No wonder so many hundreds and hundreds of thousands of putative drugs have been synthesised and tested and failed either on the basis of their apparent pharmacological action and/or their toxicity (Section III).

IV.2. *Concept of a macromolecular carrier*

Although Gardner has taken a broad view of drug targeting as encompassing simple prodrugs which are generally available systematically and specifically activated at sites of action [1], the modern search for (soluble) *macromolecular drug carriers* which could specifically attain a diseased site through a recognition event, and where drug would be released via specific and/or non-specific processes was first reported on in 1969 [11], some 3 decades after the role of the specificity of drug action was described to the general public in the feature film 'The Magic Bullet' (Warner Brothers, released in the U.K., September 23rd 1940). Ringsdorf's now classical description of a soluble macromolecular carrier has served to remind many that the carrier can be multifunctional [12]. Sinkula has recently examined the development of macromolecular polymer carriers as 'prodrugs' and has pointed out all of the many (putative) attributes that such systems have [13], and these are described in Fig. 3. The characteristics that are desirable in a polymeric carrier of this type were summarised by Sinkula as including (a) the timely release of drug parent molecule from the drug-polymer conjugate at the appropriate site by biochemical or other means, (b) the polymeric backbone must be non-toxic, and non-

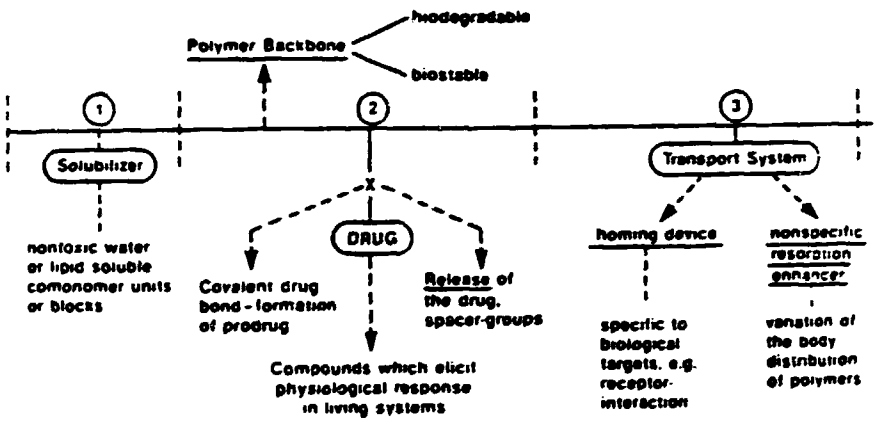

Fig. 3. Idealised description of a soluble macromolecular site-specific delivery system. (Reproduced from Ref. 13, with permission.)

immunogenic, and (c) the backbone must be either biodegradable or easily removed after action.

In addition to this effort, and at first almost separately, there has been the advent of various particulate carriers including liposomes and other capsular and monolithic materials. We shall examine the numerous and varied types of carrier and their properties throughout this contribution.

IV.3 DART disease, access, retention and timing

Recently we have re-examined the concept of carriers in terms of their ability to effect site-specific drug delivery [9,14,15]. It is evident to us that the basis for developing any one modality to fight a disease must be the disease itself. Too often carriers have been produced without great forethought (particularly in the cancer field), and then with almost a hope launched into the body without any cognisance of the pathogenesis of the disease (i.e., its (patho)physiology, biochemistry and chronicity). The presence of human defence systems such as the mononuclear phagocyte system (MPS) has often been ignored, and/or unacceptable claims have often been made on an improved site-specific delivery of drug being attained – though upon examination data show an increase in site access from almost zero to just above zero!

It is clear that apart from disease and the drug, the further essential components of site-specific drug delivery are *access*, *retention* and *timing*, all of which combine to give the specification that must be worked towards in developing a successful modality (Fig. 4).

IV.3(a) Access

Access of a carrier system to a location containing the pharmacological site of action is crucial for their successful use.

The biological opportunities which present themselves for site-specific access using carriers are *anatomical, (patho)physiological and biochemical* [9], and Fig. 5

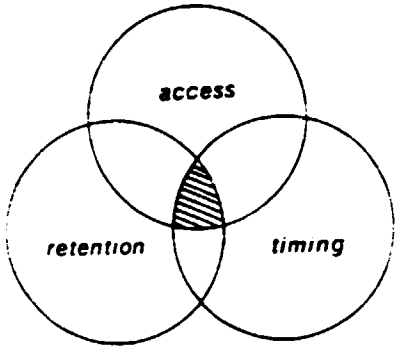

Fig. 4. Prime elements in successful site-specific drug delivery

gives those (known) biological routes which a site-specific system can exploit [9]. Many in vivo diagnostic studies have demonstrated that it is possible to highlight both normal and pathological regions using either simple tracers or more complex constructs such as radiolabelled monoclonal antibodies which interact with organ, tissue, cell and/or disease-specific markers. However, for *drugs* combined with site-specific carriers the delivery processes not only include passage through epi- and endothelial barriers, carriage or transport through the body (including intra- and transcellular transport), and then interaction with the target cells, but they imply protection of the drug and body from one another until the site(s) of action is (are) reached, avoidance of any pharmacological interaction with non-target cells, re-

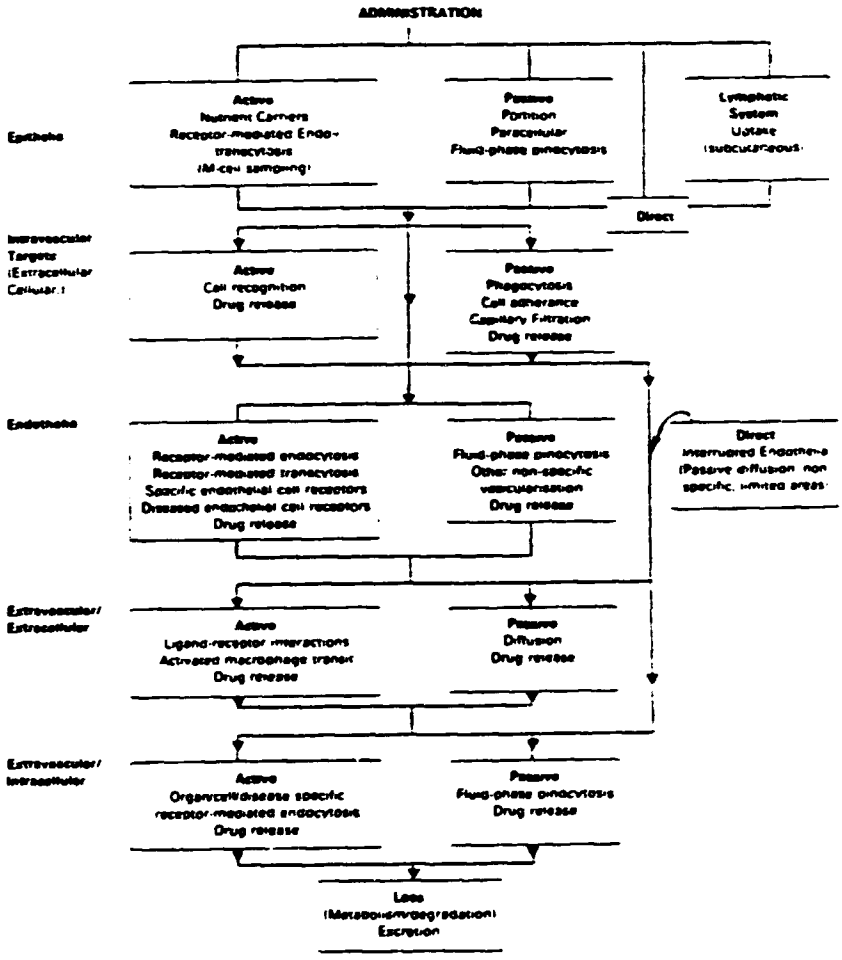

Fig. 5. Anatomical and physiological pathways for site-specific delivery. (Reproduced with permission from Ref. 9.)

lease of therapeutically relevant amounts of drug at the pharmacological receptor at the required modality and frequency, followed by excretion of the carrier and the drug. Drug availability can be due to simple passive events, such as diffusion from a carrier, or active processes including enzyme degradation of carrier and could be argued as being in response to a disease-specific event. (Environmentally sensitive linkages will be discussed later in Section V). For clinical use, carriers should be either biologically degradable or readily excreted, and should interact neither with biochemical nor with immune systems of the body, unless desired.

Access is often affected by various rate-limiting barriers to *diffusion*, although we shall see later that for soluble macromolecular systems *both diffusion and convection* are important (see Section XI). In normal physiological processes diffu-

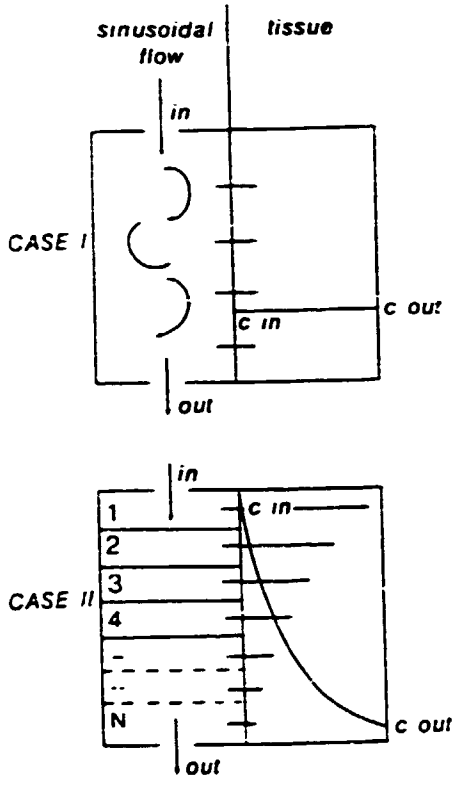

Fig. 6. Effect of stirred and unstirred aqueous diffusion layers on the passive entry of macromolecules into accessible parenchymal cells of the liver (Adapted from Ref. 16, with permission of the author and the copyright holder, the American Pharmaceutical Association.) For case I, the Space of Disse is treated as being a well-stirred compartment resulting in a uniform concentration of macromolecule throughout and hence to its equal uptake along the hepatocyte plasma membrane. In case II, the sinusoidal space is regarded as having a number of sequentially perfused compartments, which results in a concentration gradients being established in the Space of Disse, with a resultant gradient in the rate of uptake by the hepatocytes

sion, and particularly diffusion through unstirred layers, can affect arrival of carrier at a membrane or receptor site. For example, a well-stirred layer does not exist within the Space of Disse of the hepatic sinusoid fluid [16], which, for passive uptake at least, manifests itself as a concentration gradient being formed within the parenchymal cells of the liver (Fig. 6). Resultant concentration gradients have been observed in hepatic cells following uptake of galactose, epidermal growth factor and other (macro)molecules; in some cases these effects appear to reflect axial concentration gradients existing within the hepatic sinusoids [16]. In addition, this effect may be the reason why a *dose-dependency* is seen with, for example, the kinetics of liver uptake of a (radiolabelled) galactosylated neoglycoalbumin (Fig. 7) [17]. A further reason may be the capacity of the receptor system to accomodate ligand (Section VI describes the capacity of constitutive and non-constitutive receptor-mediated processes for site-specific drug delivery).

IV.3(b). Retention

Although it is logical that a carried drug must become available to its pharmacological site of action, we shall see later that some workers have developed systems whereby the drug, although remaining intimately bound to the carrier can still be active (subsection X.4). However, in general, one foresees a release process taking place. Thus, either the drug needs to be released in direct proximity to its pharmacological site of action, or upon release it needs to diffuse to its site of action. The maintenance of adequate levels of drug at the site is important given the *duration of drug effect* and the *responsiveness* of the target site, for if a drug has a long duration of action then persistence of the released drug may or may not

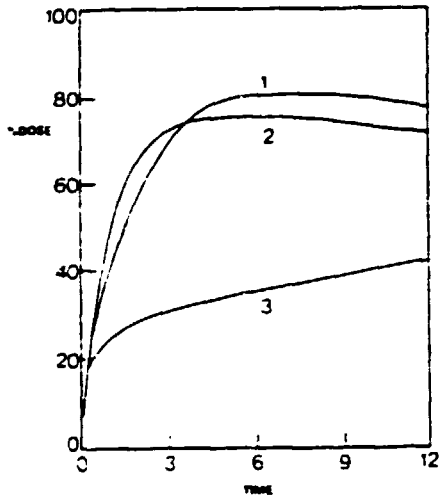

Fig. 7. Dose dependency of the liver kinetics of galactosyl neoglycoprotein, showing percent dose versus time (seconds × E2) where 1–3 indicate doses of macromolecule (mol kg) of 1.2 E-8, 1.2 E-9 and 1.1 E-7 respectively. (Redrawn from Ref. [17].)

be desired depending on whether it is acting as an agonist or as an antagonist

Our own practical studies on drug release from carriers within selected organs have shown us that near-site targeting brings *no* pharmacological benefit if a flux of fluid can remove released drug *immediately* from the vicinity of its release (Lowrie et al., unpublished work, 1986). This concern was first addressed by Stella and Himmelstein [18,19], who used various simulation procedures to show that one cannot simply rely on a carrier system arriving at a particular extra- or intracellular location and releasing its drug for the approach to be successful. If, for example, drug release was non-specific and occured either extra- or intracellularly, and the released drug had a high (non-specific) cell permeability it would rapidly diffuse away from the site of action, resulting in minimal improvement in drug delivery. With a specific intracellular release of drug Stella and Himmelstein were able to predict a measurable improvement in site levels only where the released drug poorly permeated from the site of release [19]. (This is similar to that proposed by Bodor for delivery of 'soft' drugs to the brain, where drugs attain the CNS but because of their conversion to a derivative that cannot leave the compartment readily they tend to accumulate in that compartment [20].)

Stella and Himmelstein have indicated that there are numerous examples in the literature where targeting of biodegradable conjugates which had optimum target site accessibility and selective metabolism failed to give an advantage, and that to have any significant pharmacological effect then a third factor – i.e., retention (of drug) – is important [18].

Paradoxically, effective site access resulting from an efficient extraction of drug

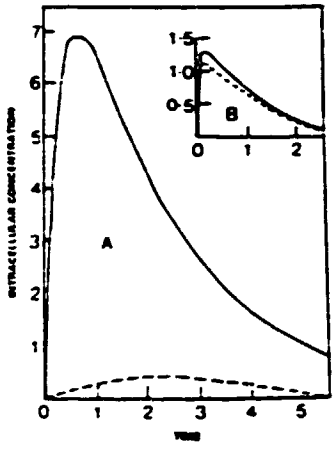

Fig. 5. Simulations giving the importance of *retention* of drugs delivered by site-specific systems. Case A is for a drug conjugate having good access, selective intracellular release of drug, and with the released drug having poor permeability at the site of release. Case B is for a drug conjugate which has good access, selective intracellular release but the released drug has good permeability characteristics. Solid line is for drug released from the conjugate and the broken line is for the parent drug administered without a carrier. The units are arbitrary but the scales for cases A and B are constant. (Modified with permission, from Ref. 19.)

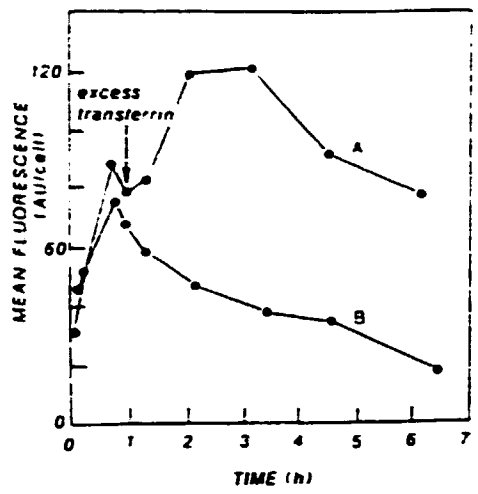

Fig. 9. Cell-asssociated fluorescence after 37°C incubation of K562 cells with fluorescently labelled diferric transferrin, showing the effect of an acid-labile linkage (i.e., *cis*-aconityl) (A), between the transferrin and the label, and an acid-stable linkage (B). The data give that although return of the carrier to the cell surface effectively removes the label from the cell, with an acid-labile linkage the label is released within the cell where it binds to the nuclear membrane. (Reproduced with permission, from Ref. 41.)

carrier from the blood pool (for example) will result in that pool becoming a perfect sink, which could then result in the rapid egress of drug from the targeted site of release. However, and in contrast to the assumptions made by Stella and Himmelstein [18,19] and by others more recently [21], cognisance also needs to be given to the fact that a *carrier* can be retained and act as a local in situ depot delivering drug to the immediate environment at a steady and sustained rate. Thus, if either this case holds, or if the drug released from a carrier into say the cytoplasm or the lysosome has poor diffusion characteristics, or if *it can be retained by some active process at the site of action*, then significant increases in (for example) intracellular target site levels can occur (Fig. 8). The relatively slow process of receptor-mediated trafficking within cells will assume an import in the design of systems that need to be retained at intracellular loci (see also Section VI and Fig. 9).

IV.3(c). Timing

The access and retention of drug (and carrier), and release of drug are related features which rely on various physical and biochemical processes. However, one issue which is little considered is the question of *timing and frequency* of drug arrival at its pharmacological receptor. Urquhart and Nichols have discussed the three prime *pharmacodynamic* factors which have been seen to have a marked influence on drug action [22]. These are: duration of a drug-free interval (at the receptor), attainment of critical thresholds in plasma concentrations and the rate-of-increase in drug plasma concentration. Their findings have been arrived at by observing the

behaviour of conventional drugs using various routes and modes of *administration*: clearly, with site-specific systems these observations will change due to the altered kinetics of disposition of the drug.

We have already given that the time of drug action is important in the design of a site-specific drug, and that target responsiveness (for polypeptides) is also a key feature. There are intriguing interplays between the chronicity of the disease, the availability of drug, and its duration of effect in relation to the design of targeted drugs. It has been recently shown by McIntosh [23] that (for hormone delivery) an awareness of dynamic changes in tissue is vital for optimal drug control, and that hormones (at least) should only be delivered to their pharmacological receptor at times when (their) target cells are most sensitive, since this can affect the nature and the amplitude of the response. McIntosh states that "exploration of the dynamics and mechanisms of drug and hormone action at target tissues has the potential to lead to a new range of useful measurement strategies. When we know to which aspects of a signal target cells are sensitive, and how changes in dynamics of signals bring about alterations in functional response, we can communicate sensitively in the language of biological control" [23]. This means that when we can learn to take drugs to receptor sites (to which they may never before have been), and deliver them to sites at varied frequencies and modalities, then quite remarkable new and novel pharmacological events may be seen.

Chronopharmacology is important in many areas of drug use, including: cancer chemotherapy (see subsection XIII.6.); modalities to combat non-host cells – including parasites and bacterial cells; and in the use of peptidergic mediators and steroids; etc.

V. Carrier systems

We shall shortly examine in greater detail the biological opportunities and constraints for using carriers to affect site-specific drug delivery, though it is appropriate to examine first some of the actual carrier systems that have been suggested, and to study their properties in terms of the design specifications that follow from the considerations given above. (In Section XIV we shall examine some of the regulatory and safety issues relating to the clinical use of synthetic and biotechnic carriers).

Site-specific systems are of two essential types. That is, either particulate car-

TABLE VI

KEY FUNCTIONS OF A SITE-SPECIFIC CARRIER SYSTEM

Carriage
Protection
Site recognition
Site placement and retention
Drug release
Non-modulation of disease/host processes
Excretion/acceptability

riers (capsular, monolithic or cellular), and soluble (bio)(macro)molecular conjugates. Thus the latter type includes both simple prodrugs, larger constructs containing perhaps a biotechnic component (such as an antibody for recognition of a particular biological feature), or even a complete system such as a hybrid fusion protein produced by genetic manipulation (see subsection XIII.4). The functions of carriers are given in Table VI. Colloidal-sized soluble conjugates and particulates are considered to be useful as drug carriers because of their presumed ability to transport a 'high' payload of drug to a site of action, to afford protection to the drug, to give a controllable and sustained release of the drug when required, and to have unique innate biological pathways that can be exploited to achieve the exclusive placement of drug at its site(s) of action(s). It is possible to construct a list of specifications for an idealised site-specific drug delivery system based on the DART principle: these are given in Table VII [9].

Initial studies on developing soluble site-specific carriers appear to be due to Ghose and Cerni for the radioimmunolocalisation of tumours using antibodies [11]. This report was followed closely by those of Ghose and Nigam for direct linking of chlorambucil to antibody [24], Rogers and Kornfeld for using asialofetuin to direct chemically bound proteins to drugs to the liver lysozyme [25], Rowland et al. using *polymers* intervening between drug and antibody [26], Szekerke et al. using glutamyl-containing synthetic polypeptides as carriers for cytotoxic drugs [27], and Trouet et al. proposing that specific intracellular drug action could occur through endocytosis of a drug-carrier conjugate [28]. For particulate carriers, early proposals on their use as carriers are attributable to Sessa and Weissmann [29], and

TABLE VII

IDEALISED CHARACTERISTICS OF A SITE-SPECIFIC DRUG DELIVERY SYSTEM (REFERENCE 9)

Biological factors
Vascular carriage to site of action
Placement at site (via active or passive means)
Epi- and/or endothelial passage
Restricted drug distribution to target site
Drug and host protected from one another
Release controlled by biological processes
Release related to the responsiveness of the target
Drug-related factors
Controlled modality and frequency of release
No premature drug release during transit
Adequate levels of drug carried
Carrier-related factors
Biologically compatible
Biodegradable/excreted
No carrier-induced sequelae
No carrier modulation of the disease
Convenient and cost-effective to prepare and to formulate
System chemically and physically stable in its dosage form

Gregoriadis et al. on liposomes [30], and to Kramer on monolithic microspheres [31]. Other articles and various reviews detailing the attributes of various particulate and soluble conjugate systems have appeared (e.g., Tomlinson. [32]; Poste and Kirsh. [33]; Poznansky and Juliano. [34]; Davis et al., [35]; and Friend and Pangburn. [36]).

V.1. Carriage

Friend and Pangburn [36] have produced an excellent study on the use of various types of carrier. For knowledge of the detail of these the reader is referred to that study, though it is useful here to list briefly some of the macromolecular and cellular or particulate drug carriers that have been proposed as site-specific carriers, and this is given by Table VIII.

V.2. Site recognition

Table VIII shows that a multitude of soluble and particulate carriers have been suggested for effecting site-specific drug delivery. We shall see later that place-

TABLE VIII

CLASSES AND TYPES OF SOLUBLE MACROMOLECULAR AND PARTICULATE CARRIERS

Soluble macromolecular carriers
Proteinaceous carriers
Antibodies
Albumin
Glycoproteins
Lipoproteins
Gelatin
Polypeptides
Lectins
Hormones
Dextrans and other polysaccharides
Deoxyribonucleic acid
Microparticulate carriers
Lipid carriers
Liposomes; low density lipoproteins; chylomicrons; lipid emulsions; waxes
Polymeric carriers
Polyalkylcyanoacrylate; polylactides; polyamides
Proteinaceous monolithic and capsular particles
Synthetic (hybrid) carriers
Triglycerides/block copolymers; niosomes
Cell Carriers
Erythrocytes; erythrocyte ghosts; leucocytes
Carbohydrate particulate carriers
Starch; dextran (cellulose, agarose)
Viruses; retroviruses; viral envelope products

ment of the drug at the site of action can be due to both passive and active processes. With respect to the latter, many attempts have been made to control the biological dispersion of a carrier by linking it to some form of ligand which will be recognised by a particular (normal or abnormal) feature of the body. This ligand may be either simple, for example, a sugar, or more complicated, for example, a fragment of a natural ligand such as interleukin 2 (subsection XIII.5) or an antibody, or even an antibody itself. (Frequently, it is suggested that drugs can be linked directly to antibodies through some simple linker technology.) With antibodies, efforts have largely been centred around the use of murine monoclonals. A priori one would expect that the repeated use of these in humans would lead to immune responses. However, although the head of the US FDA is reported recently as having stated that "for clinical use mouse monoclonals have been shown to be safe ... and that ... in the future one needs to look at treatment reagents (sic) for *effectiveness* rather than safety", this is a contentious point – particularly when related to *use* and any chronic dosing regimen. Because of this, much recent attention has been given to the use of antibody fragments, human antibodies and *chimaeric* antibodies (i.e., where the hypervariable region of the antibody is retained, but the Fc portion is replaced by the human, i.e., non-immunogenic, Fc portion). One can even include here the role of anti-idiotypic antibodies [37] as

TABLE IX

FACTORS AFFECTING THE RELEASE OF DRUGS FROM PARTICULATE CARRIERS (ADAPTED FROM REFERENCE 32)

Drug
Position in the particle
Molecular weight
Physicochemistry
Concentration
Drug:carrier interactions
Diffusion; desorption from surface; ion-exchange
Particle
Type and amount of matrix material
Size and density of the particle
Capsular or monolithic
Extent and nature of any cross-linking, denaturation or polymerisation
Presence of adjuvants
Surface erosion; particle diffusion and leaching
Total disintegration of particle
Environment
Hydrogen ion concentration
Polarity
Ionic strength
Presence of enzymes
Temperature
Microwave
Magnetism
Light

ligands for recognition, as well as the very important and recently discovered use of *antisense antibodies* [38].

Although it is attractive to consider the unique selectivity that antibodies can have for a particular feature within the body, these macromolecules are controlled in their dispersions and their interactions by the same features of extravasation, site-access, and responsiveness of the target that have been described above. Thus, although, for example, it may be possible to raise an antibody to a feature of a cancerous cell, if this is located in a particular extravascular position, its non-accessibility may negate the exquisite nature of the ligand:antigen interaction. Similarly, much attention has also been given to the direct coupling of antibodies and other ligands to particulate carriers in order to adjust their biological dispersion. This field has been reviewed by Toonen and Crommelin for immunoglobulin-liposome combinations [39], who conclude that the relevance of such systems for therapy is currently unclear and may well be restricted to targets within the vascular system.

The potential for the extravasation of various macromolecular carriers will be examined in Section XI.

V.3. Release

Numerous approaches have been taken in developing carrier systems which can release their contained drug either passively or actively due to an internal or an external stimulus. For soluble macromolecular conjugates many chemical approaches have been taken to the coupling of the drug to a backbone of a carrier that will be sensitive to the environment and release drug (see references in Refs.

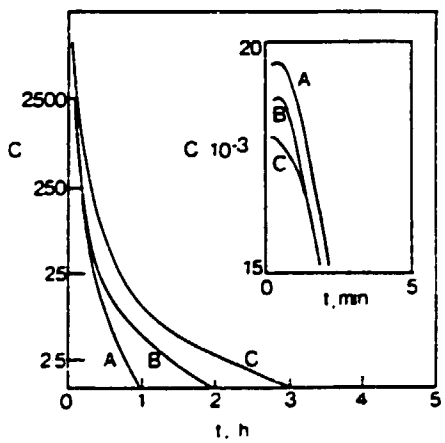

Fig. 10. Appearance of water-soluble drug (C, ng/ml) in phosphate buffer eluent with time (hours) using a dynamic flow through apparatus, showing the influence of time of cross-linking with 1% glutaraldehyde on release. The detail shows release over the first 5 min. The spheres had a mean diameter of 15–20 μm. A, B and C refer to 1, 2 and 6 h cross-linking time, respectively. (Reproduced with permission, from Ref. 45.)

36 and 40). One of the most interesting of linkages developed recently has been a pH-sensitive link which is labile in the endosomes (pH 5.5) of cells rather than the lysosomes (pH 6.5) or the cytoplasm (pH 7.2). Some of our own recent work with a model soluble conjugate, i.e., transferrin linked to fluorescein (Fig. 9), typifies the manner in which the intracellular release (and retention) of a drug can be manipulated from a carrier using this simple piece of chemistry [41]. Clearly with the example given in Fig. 9 the lability of the linkage must be such that drug is released within the endosome prior to the circulating transferrin receptor transferrin:drug complex being trafficked into and out of the cell.

An interesting observation here is that the nature of the linkage can affect the pharmacokinetics of the formed conjugate. Thus, it has been shown that when ricin A chain was conjugated with a monoclonal (Fib-75) IgG2a antibody, using either a disulphide bridge, or a protected –S–S– link, or a sulphide link, in rats a conjugate containing the latter linkage was cleared more slowly from the circulation than was found for the others – which appeared to be correlated to its relative inactivity when compared to the others [42].

There are numerous methods by which a drug may be released from carriers, and particularly for monolithic carriers, factors which will affect this can be identified (Table IX). For example, with proteinaceous carriers which are formed by denaturation of a protein, the extent and type of denaturation greatly affects the release of drug (Figs. 10 and 11). Release from monolithic particles is often complicated by the high initial releases seen (sometimes up to 80% of the incorporated drug within 2 min [43]). Fig. 11 shows that for some drugs microparticles can be

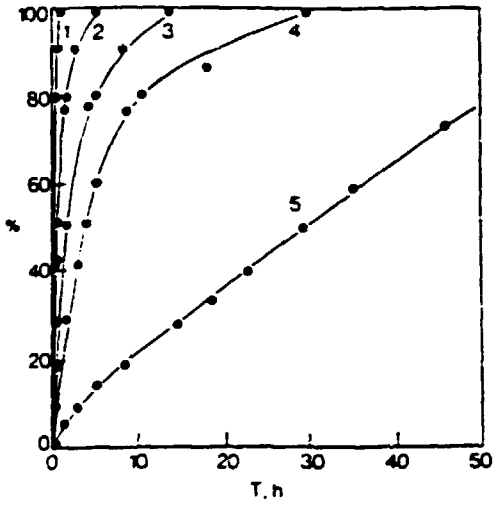

Fig. 11. Release of prednisolone into pH 7.0 phosphate buffer at 37°C from albumin microspheres 1 refers to the solution of micronised drug powder, and 1–4 to release from rabbit serum albumin microspheres (2.4 μm mean diameter) prepared using thermal denaturation at 155°C for 3 h, 6 h, 12 h, and 24 h, respectively. (Error bars have been omitted for clarity.) (Redrawn from Ref. 44.)

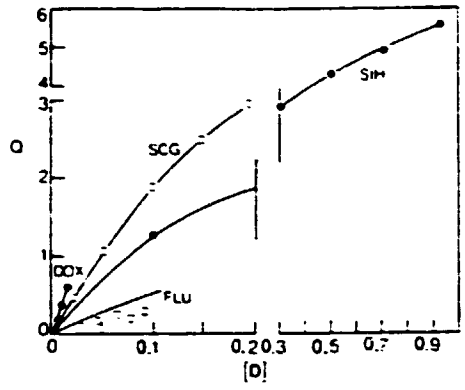

Fig. 12. Incorporation of water-soluble compounds into 10–20 μm diameter human serum albumin microparticles, showing the relationship between the amount (mg) of compound incorporated per 10 mg of formed particle (Q) and the original disperse-phase compound concentration ([D], M). SIH, SCG, DOX and FLU refer to sodium iodohippurate, sodium cromoglycate, doxorubicin and 5-fluorouracil, respectively. Drawn lines are for a theoretical 100% incorporation. (Particles manufactured with 25% w/v protein in the disperse phase of an water-in-oil emulsion.) (Reproduced with permission, from Ref. 45.)

prepared where this initial fast release is absent [44]. Low-molecular-weight water-soluble drugs can be incorporated into monolithic carriers to an extent which appears to be largely determined by the aqueous solubility of the drug [45] (Fig. 12). With lipid-soluble drugs the incorporation is more difficult and often microcrystalline systems appear to be formed [46].

For liposome vesicles drug release can be controlled by adjusting the composition of the constituent lipids. A great deal of inventiveness has resulted in various pH-sensitive (e.g., Ref. 47) and temperature-sensitive (e.g., Ref. 48) carriers. The variability in release which can be obtained at different pHs can be seen in Fig. 13 [47]. Water-soluble drugs may be incorporated into either the aqueous or lipid compartment of liposomes, though the level of drug incorporations are much lower than those found for monolithic systems.

Hashida and Sezaki have introduced a further means of controlling the release of drugs from particulate carriers, i.e., drug conjugation [49]. These workers have demonstrated that the use of a mitomycin C:dextran conjugate (sic) into gelatin microspheres leads to a monophasic release of drug that is independent of carrier size (i.e., surface area), but which is similar to the rate of hydrolytic cleavage of the conjugate.

During the remainder of this article we shall be examining the various roles that these different types of carrier have in achieving access, retention and timing of release. Their more generic properties and some of the concerns that arise with their clinical use are placed towards the end of this article.

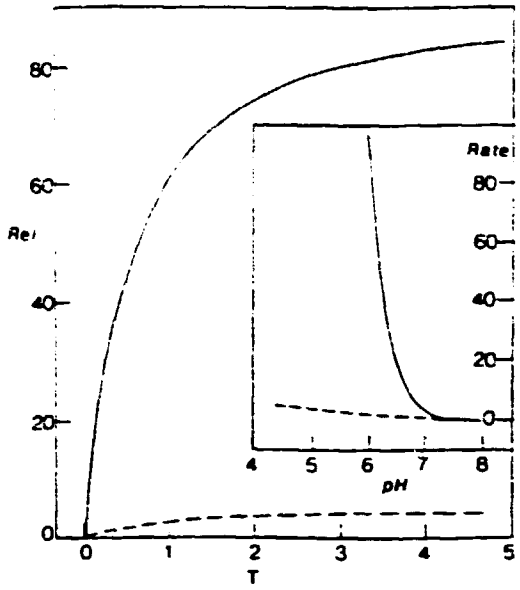

Fig. 13. Calcein release from liposomes of varying composition at pH 6.1. Plot shows release as a percent of maximal fluorescence, i.e., *Rel*, versus time, *T* (mins). the inset gives the initial rate of release from liposomes, given as a percent of the maximum fluorescence per minute, i.e., *Rate*, as a function of pH. Solid and dashed lines refer to oleic acid:phosphatidylethanolamine liposomes and oleic acid:phosphatidylcholine liposomes, respectively. (Redrawn with permission, from Ref. 47.)

VI. Recognition and intracellular trafficking

Frequently, the pathway to a pharmacological site comprises passage into and through various cells. To survive and to maintain their function, both normal and diseased cells can take up and process numerous types of materials by various mechanisms. The uptake of hydrophilic macromolecules at the plasma membrane involves invagination and vesiculation of the lipid bilayer to form vesicles. These

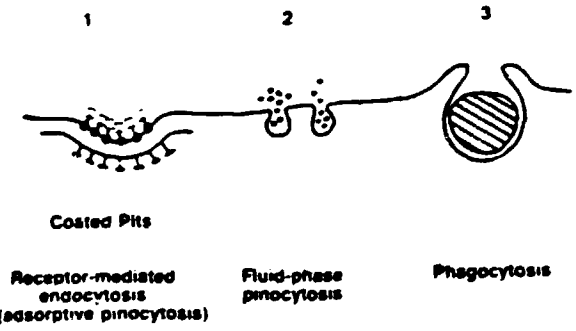

Fig. 14. Endocytic vesicles at cell surface. (Reproduced with permission, from Ref. 9.)

processes have a putative applicability to cell selection and cell access by site-specific systems.

Biological pathways have both a passive and an active nature, and include fluid-phase pinocytosis, phagocytosis, and both constitutive and non-constitutive receptor-mediated endocytosis (reviewed in Ref. 50) (Fig. 14). In addition, the binding to specific and/or non-specific regions of cell surfaces can aid other processes which cause cell access of macromolecules – including membrane fusion and simple diffusion. Hopkins has recently discussed the cellular opportunities (and challenges) for site-specific drug delivery of both soluble macromolecular conjugates and particulate systems [50]. He has identified two classes of vesicular routings, i.e., those which involve constitutive recycling, and those which occur upon a specific ligand/receptor interaction. For the former class, these processes occur independently of external stimuli, and are for the purpose of imbibing, cell growth, and intra- and intercellular communication. Examples of ligands undergoing such events include low-density lipoprotein and transferrin. Recycling processes are mediated though clathrin-coated pits of 80–150 nm diameter, are difficult to saturate, and internalisation of any receptor can occur between 4 and 10 times per hour. For non-constitutive processing, the interaction of a surface receptor with a ligand is the signal for the process to commence. These are very rapid events, and are easily saturable; examples of ligand which are processed in this manner include epidermal growth factor (EGF), platelet-derived growth factor, nerve growth factor and insulin. (To exemplify: for the trafficking of EGF more than 80% of the epidermal growth factor receptors internalise with a half time of 2 min on binding saturable levels of the ligand.) In addition to the well-studied clathrin-coated pits, there is a variety of invagination processes which are capable of forming vesicles and transporting extracellular material into the cell (Table X).

It is instructive to examine the known behaviour of two of the most studied of transport receptor systems, i.e., the galactose receptor in the liver, and the transferrin receptor system. It is known that the removal of terminal sialic acid residues from mammalian serum glycoproteins (which have exposed galactose residues), initiates prompt removal of this galactose-terminated glycoprotein from the circulation. Also, it has been shown that more than 20 plasma proteins are hepatically extracted via the asialoglycoprotein receptor system, and that at least five mammalian hepatic receptors are responsible for the clearance of desialylated glycoproteins (i.e., mannose/*N*-acetylglucosamine, fucose-specific, and 6-phospho-

TABLE X

CELL SURFACE INVAGINATIONS (DRAWN FROM REFERENCE 50)

Type	Property/location
Clathrin-coated pits	Micropinocytic ligand-induced; constitutive, endothelia, fibroblasts
Caveolae (omega shaped)	In fibroblasts, smooth muscles cells, especially in continuous endothelia; fluid-phase transport
Patching and capping	e.g., in leucocytes

TABLE XI

REPORTED DISTRIBUTIONS OF SOME ENDOSOMOTROPIC RECEPTORS (VARIOUS SPECIES)

Cell	Receptor
Hepatocytes	Galactose, low-density lipoprotein; polymeric IgA
Macrophages	Galactose (particles); mannose-fucose; acetylated LDL, α_2-macroglobulin protease complex (AMPC)
Leucocytes	Chemotactic peptide; complement C3b; IgA
Basophils, mast cells	IgE
Cardiac, lung, diaphragm endothelia	Albumin
Fibroblasts	Transferrin; epidermal growth factor; LDL mannose 6-phosphate; transcobalamin II; AMPC, mannose
Mammary acinar	Growth factors
Enterocytes	Maternal IgG; dimeric IgA; transcobalamin-B-12/intrinsic factor
Blood/brain endothelia	Transferrin; insulin

mannosyl receptors). Both the transferrin and the galactose receptor systems have total cycle times of between 15 and 17 min. The actual binding of these ligands takes between 4 and 9 min, internalisation takes between 2 and 5 min, and return to the surface a further 4 to 7 min, depending upon the cell type. Clearly these receptor-mediated processes have a different speed than the passive diffusion events that we are perhaps used to for conventional low-molecular-weight species; hence the capacity and kinetics of cell trafficking events are important considerations in the design of (active) site-specific systems (as are the abundance, specificity, avidity, and (cellular) fate of any receptor system).

Cell processing includes trafficking of ligand and/or receptor to the endosome (Table XI), then into the cytoplasmic region and onwards to discrete intracellular compartments such as the lysosome and the nucleus, and thence through the cell to the opposite membrane (transcytosis), or to the original membrane of cell entry (i.e., diacytosis or retroendocytosis). Ligand/receptor complexes which remain stable in the lower pH of the endosome compartment (pH approx. 5.5) tend to be routed through the cell to new plasma membranes, and it is clear that in some cases the routing through the cell can avoid the lysosome [50]. The molecular signals for this are the subject of intense research. Transcellular transfer or transcytosis is known both for secretory (polymeric) immunoglobulin A in the gut (basolateral to apical surfaces), and the hepatocytes (plasma membrane to bile-duct membrane), and for low-density lipoprotein (in endothelia), and is assumed to occur [50] for transcobalamin/B_{12} complex in enterocytes, growth factors in mammary acinar cells and transferrin and insulin at the blood/brain barrier, though the latter two are much doubted. It is highly encouraging to appreciate that today the molecular basis of numerous cell recognition and subsequent intracellular sorting events are being elucidated using molecular cell biology tools; for example the routing signal sequences for transporting (secreted) proteins to various subcellular compartments including the nucleus and the mitochondria are known (e.g., Ref. 51).

Internalised ligand:receptor complexes may also pass from the peripheral endosome to intracellular locations other than the lysosome, for example to juxtanuclear positions [50]. The various cell routings which exist are mostly specific and exclusive. For example, the long term residents of the plasma membrane are largely excluded from vesicular trafficking (e.g., Thy-1 and histocompatibility antigens), and there are cell-type specific antigens (e.g., Thy-1, H-2, T200) and cell-function specific receptors (e.g., for transferrin, epidermal growth factor, thyrotropin, insulin, asialoglycoprotein and mannose-6-phosphate – see Table XI). These events will need to be determined at the molecular level before they can be exploited for site-specific drug delivery, such that ideally the minimum piece of structural information responsible for any one cell event is determined and then incorporated into the carrier system.

It is perversely encouraging to appreciate that many viruses – which may be regarded as efficient site-specific 'drug' delivery systems themselves – have done exactly this and have learnt to adapt the structure of their various surfaces in order to exploit a particular cell surface antigen in order to affect a particular tropism [52–58] (see Table XII). Viral interaction with cells can be of various types, including cell membrane fusion (with or without a specific cell recognition event), or receptor-mediated cell entry. (See also Section XIIIC on gene therapy using retroviral vectors).

Fluid-phase pinocytosis is a non-specific process and because of its continuous nature and extent may be useful as a general process for transporting macromolecular constructs through epithelia, some endothelia and various blood cells (Sec-

TABLE XII

VIRAL TROPISMS. UTILITIES OF RECEPTORS (SEE REFERENCES 52–58 AND REFERENCES CONTAINED THEREIN)

The existence of a given receptor does not necessarily mean that a cell *lacking* that receptor cannot be infected.

Virus	Receptor (Cell)
Epstein-Barr	Complement CR-2 (on B lymphocytes and epithelial cells)
Rabies	Nicotinic Ach receptor (peripheral nerve cells)
Human immunodeficiency virus	CD4 antigen (on T_4^+ lymphocytes; macrophages, glial and dendritic cells)
Reovirus Type 3	Beta-adrenergic (neurones; murine T cells)
EMC virus, Sendai	Glycophorin A on erythrocytes
Influenza A	Glycophorins on erythrocytes; respiratory epithelial cells, avian intestinal cells
Rhinovirus	80 kDa protein on human cells
Vaccina virus	Epidermal growth factor (on murine L cells)
Lactate dehydrogenase virus (LDV)	Ia antigen [Class II MHC] (Ia^+ murine macrophages)
LDV + non neutralising antibody	Fc receptor
Bengue virus + non-neutralising antibody	Fc receptor
Semliki Forest Virus	Class I^+ MHC (also Class I^+)

tion XI). In contrast, receptor-mediated endocytosis is a much more specific and versatile process for achieving site-specific drug delivery, with molecules from 100 to more than 1 000 000 daltons able to be recognised and processed [50]. In addition, once within a cell, the unique environment can be harnessed to cause drug to be released by either active or passive processes including, lysosomal digestion, pH-controlled events and specific intracellular membrane binding.

Site-specific delivery with soluble and particulate carriers hence relies on a combination of anatomical and (patho) physiological events, each bringing their own constraints and opportunities. These can involve either anatomically accessible and non-accessible compartments, as well as normal and dysfunctioning cellular processes of both a passive and an active type [9]. These biological opportunities and constraints will now be examined, with exemplification of some of the potential therapeutic uses of drug-carrier systems.

VII. Administration and the entry and penetration of epithelial barriers

The utilisation of site-specific systems and any macromolecular 'drug' will largely be contraindicated in non-life threatening or serious conditions if they need to be administered parenterally, unless a convenient and painless method can be developed to achieve effective parenteral administration.

It is thus highly relevant to consider what opportunities there are for the entry and penetration of epithelia in the administration of both soluble macromolecular and particulate carriers. It is ***highly unlikely*** that any such event will be possible with *non-mucosal* barriers, but there is evidence to suggest that both types of system can reach the blood pool via mucosal epithelia [59]. These opportunities are now considered.

VII.1. Nasal and buccal epithelia

Much recent attention has been given to the *nasal* administration of macromolecular polypeptide and protein drugs (see references in Ref. 59). Molecules as large as insulin, interferon and calcitonin have been found to be active after such a route of application. The nasal mucosa comprises two regions – the respiratory region and the olfactory region. The pseudostratified and ciliated region of the former comprises various goblet cells, small mucous glands and epithelial cells, all arranged so as to bring about ciliated movement of materials to the pharynx. These latter cells are absent in the higher olfactory region which has olfactory cells, supporting cells and basal cells.

Harris has concluded that present evidence suggests that nasal administration for polypeptides results in bioavailabilities of between 1 and 20% of applied dose, depending on polypeptide molecular weight and physicochemical properties [60]. Numerous additional factors can also have a role in controlling the entry and penetration of nasal epithelia, including local enzymatic degradation in the nasal cavity, various pathologies and their symptoms (e.g., rhinitis, colds, etc), the formulation and administration systems used, and the deposition and clearance of these systems.

McMartin and Peters have examined the bioavailability of various compounds of differing molecular weight after their nasal administration [61]. Using data given in the literature they showed quite clearly that for potent molecules – where a rapid onset of action is required – the nasal route would appear to be indicated provided the molecule has a molecular weight of less than about 1 kDa [61]. Although for small peptide drugs (sic) this is suggested as a rationale for the development of small *stable* molecules, the corollary of this for larger entities (including soluble biopolymeric carriers) is that administration via this route may only be possible where there is either an added retention [62] at the nasal mucosa or flux enhancers are used. The nasal route may not be a particularly attractive one for the long-term chronic application of drugs and carrier systems either with or without adjuvants, since because all of the dose constantly passes through one small tissue region, then iatrogenic sequelae are more likely. There are already findings that opportunistic pathogens may enter through a formed portal, as well as novel toxicities resulting (such as the lymphomas reported with some interferons administered to the nasal epithelium).

A most interesting possibility for selective delivery does exist with administration to the nasal mucosa, that is for retrograde neuronal transport to take place for the delivery of materials to the central nervous system.

The *buccal mucosa* has often been considered for the entry and penetration of small drugs (e.g., Ref. 63), though it is only recently that it has received attention for the possible route of administration of macromolecules [64]. Results are scarce and it is still too early to state whether this will be a useful route, though it is evident that those soluble macromolecular constructs that have been studied will only pass with difficulty, and that particulate material will not.

VII.2. Intestinal epithelia

The epithelial membrane of the gastrointestinal tract comprises an anatomically continuous barrier of cells which permit the passage of low-molecular-weight material by simple diffusion and various (nutrient) carrier processes. Additionally, low-molecular-weight polar materials are able to diffuse through the tight junctions of epithelial cells (the paracelluiar route), and macromolecules may be absorbed from the lumen by cellular vesicular processes via either fluid-phase pinocytosis or specialised (receptor-mediated) endocytic processes. This may result in the direct transport of material through the epithelial membrane.

The membrane surface of the microvillus is of the typical lipid bilayer containing both transmembrane proteins and surface proteins (Fig. 15). An integral part of the lumenal (or apical) membrane is the glycocalyx, which is a uniform layer of filamentous glycoprotein where its carbohydrate chains are covalently linked to the polypeptide chains in the core of the membrane. The glycocalyx carries a net negative charge and is up to 500 nm in thickness, and has superimposed upon it a secreted layer of mucus which may be up to 5 μm in thickness. Both of these structures are regarded as being rather loosely packed in vivo, and not to represent a significant barrier to the approach of materials to the absorptive surface of cells

LUMEN	Position - stomach emptying - transit		Degradation - bacterial products - lumen enzymes - pH	Binding - food - bacteria
DIFFUSION LAYERS MUCUS GLYCOCALYX	Transport - diffusion limited - convection		Binding - mucus - glycocalyx	Electrostatic repulsion - surface pH and ionic strength Brush-border enzymes
EPITHELIUM Entry and transport	Endocytosis - fluid-phase - receptor-mediated	Carrier transport - dietary - enteroneogenic Fusion M cell uptake	Diffusion - aqueous (paracellular) - lipid (transcellular)	Pathogen mechanisms - toxins - viruses - bacteria
LYMPH	Selectivity - size - charge		Node retention - particles - charge effects	Transport - passive diffusion - immune cell traffic - mechanical effects
BLOOD	Stability - pH - enzymes - immune complexes		Binding - opsonins - cells - transport proteins	MPS uptake
LIVER	Cell entry - passive - active		Metabolism	MPS modulation - activation - depression

Fig. 15. Opportunities and constraints in the entry and penetration of gastrointestinal epithelia by soluble and particulate macromolecular carriers.

[65], although because of its slight net negative charge, it may pose a barrier to the movement of electro-negative materials. Prior to this region is an unstirred aqueous layer through which drugs and carrier systems must pass. This can be up to 100 times the thickness of the mucus layer, is affected by the presence of materials in the gut, and is known to be a rate-determining barrier in the transmembrane movement of hydrophobic drugs (e.g., Ref. 66). It is currently not known what this means for macromolecular drugs and carriers, though the same physicochemical principles should hold.

More importantly, the content of the lumen can be a hindrance to the effective use of drugs and of carriers. One recalls the harsh environment of the stomach and the presence of degrading enzymes to appreciate that such systems need to be either intrinsically stable or stabilised in some form. Various attempts have been made to effect this protection, including reliance on the nature of the matrix material of a particulate carrier (e.g., as shown with liposomes [67]), or development of a protective polymer coating (e.g., an azoaromatic polymer 'film' [68]), or utilisation of various enzyme inhibitors.

Particles can pass through this membrane, but only in specialised cases where either the barrier has been disrupted by chemical means, or where it is incomplete. For example, spheres of 20 nm diameter given orally to suckling mice pass

through the epithelium of the intestinal lumen and become localised in the omentum, the Kupffer cells of the liver (probably via hematogenous translocation), the mesenteric lymph nodes and the thymic cortex [69]; however, with mature epithelia there is little evidence of transport *through* the barrier [70].

Recent studies using nanocapsules composed of polyalkylcyanoacrylate and less than 300 nm in diameter have shown that an improved intestinal absorption of a model compound initially entrapped within the capsules could be explained by either a direct transport of the drug through the intestinal mucosa, or by increasing the availability of the model compound to the absorptive cells [71]. Other work by the same group on the morphology of tissues results in claims that these nanocapsules pass intact through the intestinal barrier by a paracellular route and are to be found in the blood and associated with erythrocytes [72]. These findings are anomalous, and will need much further study before they can be considered as reflecting the true physiological process, or whether, for example, the surgical techniques used in the study cause a novel disruption of membrane structure, etc.

Claims have frequently been made that drugs given orally in liposomes have an enhanced intestinal absorption, and many attempts have been made to increase the gastrointestinal absorption of various classes of drugs, including those which are highly hydrophilic or hydrophobic, polypeptides and proteins (see Ref. 67). As we have discussed above, if one considers the ultrastructure of the gastrointestinal epithelium, it is clear that there are few, if any, opportunities for the simple transfer of such particles across absorptive cells of this barrier, and this has been confirmed by the recent careful in vivo and in situ study of Chiang and Weiner [67]. All of the evidence thus far available suggests that for simple liposomes, an observed increased *drug* absorption is due to either a protection effect or an enhanced availability of drug at the mucosal surface – rather than to a direct transepithelial membrane transfer of the liposome (e.g., Refs. 67, 73 and 74). Also, a recent study with radiolabelled polymethylmethacrylate nanoparticles (mean size, 130 nm) shows that after oral administration to bile-cannulated rats, 10–15% of the dose found in bile and urine is undoubtably due to the uptake not of intact particles, but of low molecular weight polymers arising from a breakdown of the particles [75].

However, it is known that specialised cells that can take up particles exist within the gastrointestinal tract epithelium. Thus, it has been demonstrated that the *M cells* lining the intestine can absorb indigenous bacteria (i.e. *Vibrio cholera*), and transport these through into the Peyer's patches of the gut-associated lymphoid tissue [76]. Mucosal immune responses are initiated within this lymphoidal tissue, and one can postulate that a drug carrier system sampled through the M cell could be used to bring an appropriate immunomodulator to the attention of the mucosal immune system. M cells are specialised absorptive cells that overlay both Peyer's patches and other mucosal lymphoid follicles, and are known to transport macromolecules such as ferritin and horseradish peroxidase [77], viruses [78], and even carbon particles [79], from the lumen of the intestine to submucosal *lymphoid* tissue. Further translocation to the systemic system through lymph fluid and via recruitment of lymphocytes does appear possible. The crucial question is: does this

system provide an opportunity for the uptake of particulate carriers? Present evidence is ambivalent, and indicates that both the capacity and onward translocation of the process can be limiting factors in effecting the entry of particulate carrier systems into the body. Thus, much more work on the utility of M-cell extraction related to specificity, abundance and capacity to handle particulate (and macromolecular) material does appear warranted. For example, although it is shown that living virus particles can enter the general circulation through the intestinal (or indeed the nasal) mucosa, it is not yet proven that their passage through to the circulation is as an intact species or is effected through replication. Hence it must be currently regarded that colloidal particles are not able to traverse the epithelia of the gastro-intestinal tract intact to a significant extent for drug use, except under exceptional and unusual circumstances.

For soluble macromolecules little is yet known on the potential for the entry and penetration of gastrointestinal tract epithelia. For polar polypeptides it has been pointed out that this route will be limited to molecules which can take advantage of either selective transport, or be used in conjunction with adjuvants which adjust the permeability of the epithelium [61].

VII.3. Rectal epithelia

Recent studies have shown that it is possible to develop dosage forms which facilitate the entry and penetration of rectal vault mucosal epithelia of both soluble

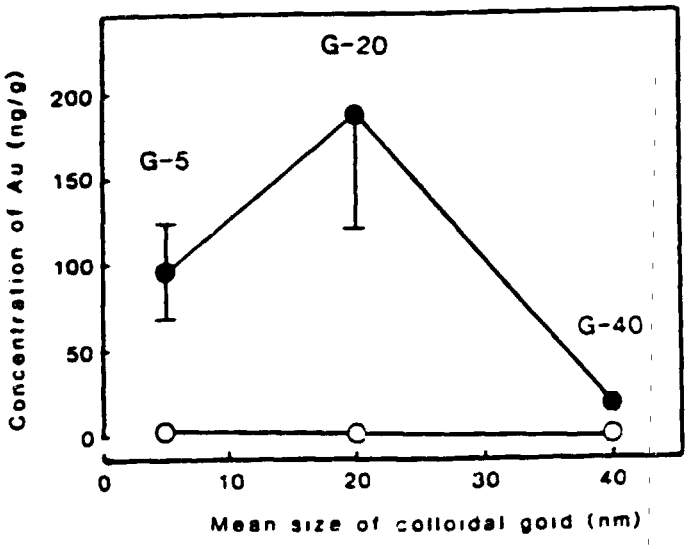

Fig. 16. Effect of mixed micelles of linoleic acid and a non-ionic surfactant on the absorption of colloidal gold particles after administration to the colorectum of the rat. Closed and open datum points refer to appearance of the particles in the regional lymph nodes and the plasma 2 h after application, respectively. (Reproduced with permission, from Ref. 80.)

macromolecules and particulates. For example, a most interesting study has been published by Muranishi and coworkers [80] on the enhanced absorption and lymphatic transport of various colloids after their rectal application using lipid nonionic surfactant mixed micelles to pretreat the mucosal membrane. Not only did these adjuvants appear to prepare the mucosa for the entry (and penetration) of soluble and particulate colloidal materials, but upon microscopic examination they did not show any evidence of severe damage such as disruption and loss of the surface of the mucosal cell membrane (such as is often found with the use of ionic surfactant as adjuvants). This group has been able to show that using, for example, linoleic acid nonionic surfactant mixed micelles as adjuvants, leads to the colorectal epithelial absorption of labelled dextrans (molecular mass up to 70 kDa!) and colloidal gold (up to a diameter of approx. 40 nm) (Fig. 16). These are interesting findings, though the use of adjuvants in the rectal vault could be questioned when considering the presence of enterotoxins, bacteria and opportunistic viruses of similar size to these probe materials. Muranishi et al. found that these particles translocated favourably to the lymphatic system and were retained there, with the higher-molecular-weight materials being observed to have a higher plasma-to-lymph ratio [80].

Interestingly, the use of these types of 'soft' adjuvant mixed micelles is both transient (15 min to return to normalcy), and their effect in promoting absorption of macromolecules differs greatly within the digestive tract: the order of 'sensitivity' being reported as rectum > colon > small intestine > stomach [80].

VIII. Administration into discrete anatomical compartments

Fig. 5 shows that some physiological environments are constrained or bounded by certain anatomical features. Drug/carrier systems may be introduced directly into a discrete anatomical compartment. This could involve inter alia: intraarticular, ocular, bronchial, rectal, oral, nasal, vaginal, anal and intralesional, as well as intramuscular and subcutaneous administration. Usefully, although small particles of less than 5 μm can be taken up by phagocytic tissue histiocytes, this can lead to their retention at these sites of administration. Current approaches being taken appear to be based on the hope that simple drug retention at these sites would enable therapeutic effects to be achieved [81], and since several anatomical compartments exist where direct introduction of *particulates* will lead to their retention due to the physics of the space, then this approach has been used for the treatment of diseases which require a persistent and sustained presentation of the drug at that site.

VIII.1. Intra-articular administration

Particulate carrier systems have ideal characteristics for localising drugs within the joints after direct intra-articular administration. For example, numerous drugs such as the corticosteroids are indicated in rheumatoid arthritis, but because of the low rate of perfusion of these areas, generally dose regimens employing high and

frequent applications of drug are necessary in order to elicit a pharmacological response – which results in iatrogenic effects being common with such drugs. Intra-articular therapy using drug solutions has been examined (e.g., Ref. 82), though because of the rapid clearance of the drug from the joint, multiple dosing has been found to be required – which leads to damage of the articular cartilage.

With intra-articular injection in the treatment of cancer and of chronic inflammation, both drug-bearing liposomes and monolithic particles have been examined, as have simple colloid aggregates of drug and/or radiocolloids. Early studies reported that the anti-inflammatory effect of a steroid, cortisol palmitate, could be sustained for up to 4 days after its intra-articular administration when incorporated into multilamellar liposomes [83]. The persistence of such materials at these sites is of obvious importance, and this has been shown to be related to the size of the particles [84], with larger particles (7 – 15 μm diameter) being retained for longer periods. It is unclear how all particles are retained within joints, though some groups have suggested that this is due to uptake by fixed macrophage cells present within the synovium, with a recent study reporting that particles smaller than or equal to 6 μm in diameter are phagocytosed after intra-articular application [85,86]. The host compatibility of monolithic particulate carriers is most important, with, for example, gelatin and albumin being better tolerated than polylactic acid and polybutylcyanoacrylates [86]. What has been established is that the intraarticular administration of liposome particles containing cortisol palmitate does lead to a successful treatment of experimental arthritis in the knee joints of rabbits, using relatively low doses of steroid [81,87]. Preliminary studies in humans have given similar results. Issues such as cost and suitability of alternative colloidal systems (including stable phospholipid emulsions) have reduced the potential use of these carrier systems.

Interesting further developments have been the use of liposomes of unique composition that can release their drugs at the higher temperatures found at sites of inflammation (e.g., Ref. 88).

VIII.2. Lungs

Ranney has recently reminded us of the importance of the selective delivery of drugs to the lungs, and has pointed out that because of ready access to these organs by both the intravenous and (trans)tracheal routes that diseases located in this region lend themselves to a drug-targeting approach [89]. He has identified three approaches to this: biochemical (that is, by using a specific binding process, etc); biophysical (i.e., capillary filtration, inhalation, etc); and bioadhesion (combination of the previous two). Although these classifications are open to wide interpretation and some debate, it is apparent that a whole host of approaches and systems can be considered.

In this Section we shall examine only the inhalation route as a means of directing drugs to the lung tissue.

The use of particulate carrier systems for the controlled release of drugs in the lungs has often been proposed. The accepted model for respiratory tract disposi-

tion patterns for man (International Commission on Radiological Protection, 1966) predicts a low probability for disposition in the pulmonary region of inhaled particles larger than 5 μm real size. The dynamics of airflow within the lungs, and the physical sizes of the bronchioles, coupled with the ability for particles to be removed from the lungs via the mucociliary escalator, and the difficulty in efficiently administering to the lungs, suggest that only particles having an aerodynamic diameter of less than 2 μm could be of use.

Recent work has shown that instilled microspheres of size larger than 7 μm are strongly retained in the pulmonary region, and that small percents of particles of 3 μm and 7 μm administered by instillation into the lungs of beagle dogs can translocate to the tracheobronchial lymph nodes (though this does not occur above 7 μm) [90]. These data show that approx. 2–3% of the dose clears the lungs after a few days via the mucociliary escalator, thereafter, the 3 μm microspheres clear the lungs but with a biological retention half-time of 850 days, and the larger particles with a half-time in excess of several thousand days. Translocation of particles from the alveoli to the tracheobronchial lymph nodes appears to lead largely to their *extracellular* accumulation, though the dynamics of this are only just beginning to be elucidated [91]. Clearly opportunities for controlled administration and delivery to the lungs exist here. Other studies on particle retention have shown that drug carrier systems can be retained successfully in the respiratory tract [92,93]. Small liposomes of 50 nm are retained for many hours, though as with all particles, those depositing in the tracheal bronchial regions are cleared under normal conditions within 6 h. Mucociliary clearance is impaired in chronic respiratory disease [92], but it has been shown that under such conditions liposomal formulations can be effective (in guinea pigs) in providing a sustained drug input to the lung at the tissue level, leading to both an improvement in drug efficacy and a decrease in drug toxicity [94]. Thus, there is considerable current effort in developing appropriate technologies for administering such particulates by the route of inhalation, and recent patents have indicated that, for example, liposomal sustained-release aerosol delivery systems are technically possible [95].

Whether application of particles in this way can actually improve on the pharmacological action of a drug by altering its access and/or its retention in various regions of the lungs, is still open. It does appear to be an indicated method for the application to various pulmonary pathologies, including perhaps the use of modulators of pulmonary function such as bronchodilators; but for drugs which need to traverse at least the pulmonary epithelium, and perhaps even both the interstitial tissue and the pulmonary endothelium, much further study is required. For example, Ranney [89] points to the example of invasive pulmonary aspergillosis, where amphotericin A given by inhalation – though not within a carrier system – gives no major therapeutic advantage compared to its systemic application.

The use of soluble macromolecular carriers for administration by this route is indicated though little work has been published.

VIII.3. Eyes and nasal cavities

Other compartments for the constrained retention of macromolecular carriers include the eyes and the nasal cavity. For particulates, size is most important for retention. Recent studies have shown that in situ gel-forming systems of nanoparticles can be used to give a prolonged ocular therapeutic effect, combined with ease of application and good tolerance [96]. These systems work by using a latex of approx. 300 nm diameter (e.g., cellulose acetate hydrogen phthalate), onto which is adsorbed drug, and which (in the example cited) has a low enough buffer capacity to gel effectively in the cul-de-sac of the eye. After administration, the latex formulated at a stable pH of 4.4 coagulates within a few seconds when placed into the pH 7.2 environment within the cul-de-sac. The almost instantaneous change from a highly fluid polymeric particle dispersion to a viscous gel ensures retention of the dosage form at the site of action.

The eye contains some highly phagocytosing cells able to take up particulate materials. For example, it has been shown recently that polystyrene latex particles are taken up (presumed to be via phagocytosis) by the rabbit corneal endothelium [97]. However, it is disturbing to note that when carbon microparticles (20–70 nm diameter) are injected intravitreally in monkeys, stimulation of phagocytic cells appears to induce cell proliferation which manifests itself as neovascularisation and retinal detachment [98]. The ocular irritancy which exists with most grains could well be obviated by making them hydrophilic.

Recently, it has been shown that microparticles (diameter larger than 40 μm) have a considerable residence time within the nasal vault when applied to the nasal epithelium [99], which may be of use in treating nasal disorders. (Also, the ability to retain a drug at an absorption site may in fact be more relevant to clinical use than the use of various flux enhancers – refer to subsection VIII.1).

VIII.4. Oral

Many pathoiogical lesions exist *within* the gastrointestinal tract, including such colonic diseases as Crones disease and colon carcinoma. Numerous groups have attempted to develop carrier systems which can release their drug at the diseased site within the gastrointestinal tract. The ability to do this relies on an interplay of various features of gastrointestinal tract physiology, including stomach emptying, gastrointestinal tract transit time of materials, ability to adhere or be retained at selected mucosal regions, protection of the carried drug, and selective release in the desired region. Some of these relationships have been reviewed recently in this Journal [100].

Particulate carriers could be used in the gastrointestinal tract for bringing antigens to the attention of the immune system for purposes of immunisation. One of the most ambitious studies in this respect has been due to Klipstein and co-workers [101], who have been able to demonstrate that oral immunisation could be achieved with heat-labile enterotoxin encapsulated in orally administered and pH-dependent (albumin) microspheres. Here the microspheres act as both a protec-

tant against acid as well as an adjuvant, giving rise to a strong serum and mucosal antitoxin response.

VII.5. Intralesional

The parenteral administration of materials into discrete compartments can also include intra-lesional administration, including to, for example, tumour masses. Indeed, it has been suggested that the most efficacious means of administration of cytotoxic agents and immunostimulants agents could be by localised intra-tumour injection [102]. Retention of both solid particles and even water-in-oil emulsions containing drugs seems possible. For example, water-in-oil emulsions of bleomycin injected directly into carcinomas and adenocarcinomas in humans have longer drug retention times compared to when given as aqueous solutions [49]. However, many questions arise with such an administration route, including the potential for inflammatory responses, and the dangers of inducing metastatic cell loss and local immune responses. Intra-tumour administration does seem to be an extremely limited case for the use of macromolecular and particulate colloidal carriers, with less prosaic questions such as needle size, injection volume, duration of leakage from the injection site, etc., being complicating, though highly important, factors.

IX. Interstitial administration

The administration of soluble macromolecular and particulate carrier systems interstitially has received little attention; however, this route does offer some unique opportunities for selective drug delivery and targeting.

Three prime routes of administration have been considered for colloidal carrier systems, namely into the peritoneal cavity (for delivery of cytostatics to the lymph nodes of the thorax), intramuscularly (as a depot for numerous drugs) and subcutaneously administered for targeting of regional nodes.

Subcutaneous routes of administration for site-specific systems have an interesting appeal in that not only can targets within the lymph system be accessed by both particulate and soluble conjugate carrier systems, but there is also drainage into the general cardiovascular pool (via the thoracic duct); and for particulates, their administration into the interstitium provides an opportunity for either uptake by the lymphatic system or as a local depot of drug. Small molecules of up to 1 kDa, when administered either peritoneally or interstitially, pass directly into the bloodstream, whereas soluble macromolecules and small particulates enter the lymphatic system through clefts in the terminal vessels (Fig. 17) [103]. Leak has shown recently that access of colloids and macromolecules may be related to the presence of high-density anionic sites within the intercellular clefts of mesothelial and lymphatic endothelial cells, such that these clefts can provide channels between endothelial cells which permit movement of macromolecules and colloids [104]. A further suggestion is that particles can also enter the lymphatic system by being transported in pinocytic vesicles through the cells [105]. The dynamics of macromolecule exchange across lymphatic capillaries has been well studied [106]

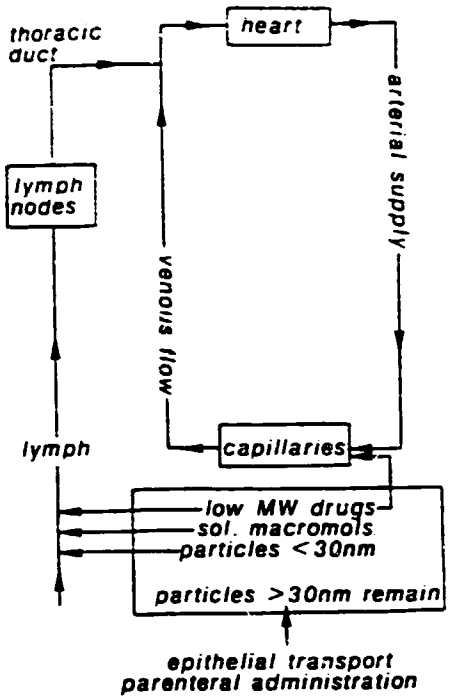

Fig. 17. Lymph and blood system circuitry, showing the fate of low-molecular-weight compounds, soluble macromolecules, particulates > 30 nm in diameter and particulates < 30 nm in diameter, after either parenteral interstitial, or transepithelial administration.

(see also Section XI). In contrast to endothelia lining the cardiovascular system, the terminal lymphatics do not possess as developed a continuous and uninterrupted subendothelial basement membrane. If present, this tends to be variable in extent, and indeed is often absent – the lining endothelium being the only cell membrane present [106,107]. Also, adjacent endothelial cells may have gaps of between 20 nm to more than 100 nm, which are known to be extremely enlargened at sites of both inflammation and extensive tissue movement [108]. Hence in some pathologies, access into the lymphatics is possible with large particles of up to perhaps 1 μm diameter. However, the terminal lymphatics drain into larger vessels (the collecting lymphatics), which have an endothelium possessing an extensive basement membrane and with few endothelial gaps.

Weinstein and coworkers have discussed extensively this route of administration for both diagnostic and therapeutic reasons [109,110], though there have been recently other important studies on the disposition of liposome particles [111] and for other colloidal systems [105] within the lymphatic system.

Clearance of colloidal materials from interstitial sites depends upon a number of factors, including size and surface character of particle (sic), formulation medium, administration point with respect to lymphatic anatomy, the composition and

pH of tissue interstitial fluid, hydrostatic pressure differences, intrinsic smooth muscle action and extrinsic muscle action, as well as disease within the interstitium. These physiological opportunities have long been exploited diagnostically for examining for both lymphatic drainage and malignant lymph nodes. The size dependency for interstitial particle uptake has been determined for both small animals and humans, and in lymphoscintigraphy, a wide variety of inert colloids in the 40 – 100 nm range have been successfully used to outline the lymphatic system (see references in Ref. 105).

Once within the lymphatic system particles may be phagocytosed – though this is a saturable process. Lymph-node status is important in the staging of many malignancies, though as recently pointed out [110] the scope for tumour therapy via the lymphatics is far more limited than it is for diagnostic purposes. For example, in the case of breast cancer, regional nodes are currently considered to be an *indicator* of metastatic spread, rather than a site at which spread can be arrested [110]. Local control may be useful in other malignancies such as Hodgkin's, which disseminate early via the lymphatic chains. Also, subcutaneous or intramuscular administration of cytostatics entrapped in liposome particles can result in an improved access to the lymph nodes and to suppression of tumour metastases within the lymphatics (see references in Ref. 111), and recent work has shown that coating liposomes with a non-specific immunoglobulin can result in these particles becoming accumulated in lymph nodes [112].

Formulation of particles appears to affect their lymphatic uptake. For example, a *lymphotropic* system has been developed recently using either lipid-based particles or particles administered in an oil [49], and both approaches appear to facilitate uptake into the lymphatic system.

Numerous groups have shown that particles injected intraperitoneally into rodents can pass through the lymphatics and enter the cardiovascular circulation intact (e.g., Refs. 111, 113 and 114). Hirano and coworkers have demonstrated that this is dependent upon the size and nature of the materials used to produce the carrier [111]. Thus, for liposomes of approx. 200 nm diameter their composition does affect the absorption rate of these colloids into the lymphatic system (from the point of administration); it is unclear whether this then affects their passage through the lymph capillaries, but there is little doubt that surface character will affect phagocytosis [111]. Notwithstanding that it is technically difficult to determine particle retentions at lymph node retentions, this latter group has pointed to a trend in their findings which shows that surface charge of the liposomal particles affects their rate of absorption into the lymphatic system from the peritoneal cavity, with the apparent half-life for absorption of positive liposomes, (i.e., the least absorbed), being 9.7 h, whilst that for negative liposomes was found to be 5.1 h (assuming first-order processes). The anionic binding sites present on the lymphatic endothelial may be the cause of this retention at the endothelial surface, which manifests itself as a slower absorption. These data suggest that inclusion of a charged lipid into a liposome can reduce its systemic availability, whilst neutral particles can rapidly pass through the lymphatics to the bloodstream. The corollary to this is that charged particles will probably be better retained at the nodes.

Results do show a wide inter-animal variation, which could be due to differences in the lymphatic systems and or differences in their physiologic status, and it may be that these latter effects will limit the number of cases where lymphotropic carriers can be used.

Attention has also been given to the fate of immunoglobulin carriers upon their subcutaneous administration. Monoclonal antibodies pass into the lymphatics and move in the lymph to regional lymph node chains under the impetus of hydrostatic pressure differences, intrinsic smooth muscle action and extrinsic skeletal muscle

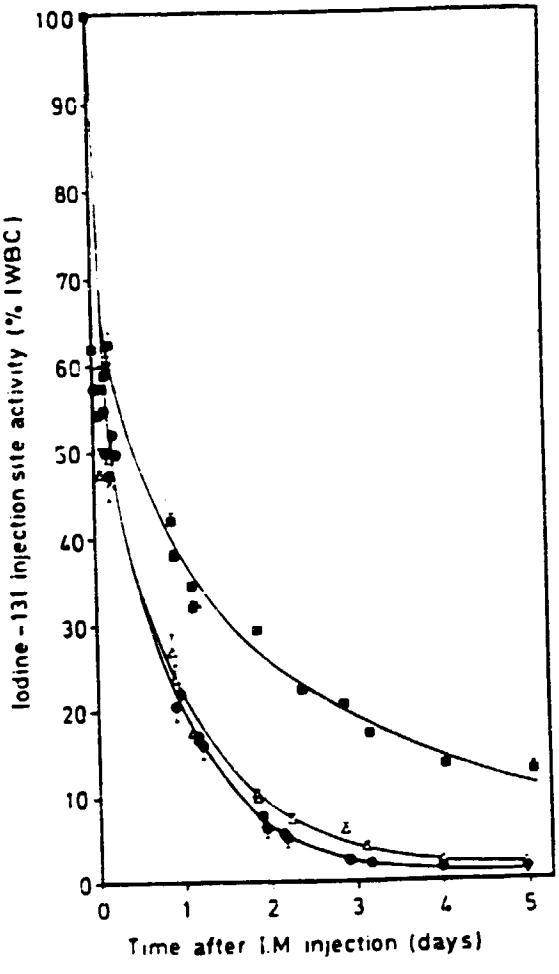

Fig 18 Clearance of iodine-131 activity from the injection site after intra-muscular administration into the rabbit of radioiodinated rose bengal (data are as a percentage of initial whole-body radioactivity counts) Circles, triangles and squares refer to rose bengal administered as an aqueous solution at pH 7.4, incorporated into human serum albumin microspheres cross-linked with 1% glutaraldehyde, and 5% glutaraldehyde, respectively (Reproduced with permission, from Ref. 115)

action. There is no retrograde flow within the system. Weinstein and coworkers [110] have argued that, since the lymphatic system is specialised to extract proteins from the interstitial space and to filter them through the regional lymph nodes, this would be a useful way of selectively delivering monoclonal antibodies to those nodes. They have found that a very efficient delivery to the cells of the regional lymph nodes can occur, with up to 20% of the administered dose binding specifically to cells in the regional lymph nodes, coupled with very little uptake in distant, non-draining nodes, and a pattern of sequential overflow from nodes proximal to the administration point to those further along the system (and thence onward into the general systemic circulation). Used clinically, this approach has produced mildly encouraging results when used against certain types of tumours. Clinical work has taken place using a polyclonal antibody against ferritin, which occurs in many tumours. Injection of these antibodies into a cannulated lymph vessel resulted in accumulation in a region of known lymph node metastases in a patient with Hodgkin's disease [110].

Since particles of 50 μm and above will be retained in the interstitial tissue after subcutaneous and intramuscular administration, they can act as a sustained-release depot. This has been used for the controlled release of a variety of peptidergic mediators, as well as for potentiating and providing a sustained-release formulation for prolonged local anaesthesia. In the latter case, drug release is dependent upon both drug amount in the particle and particle surface area (see references in Ref. 35). An interesting feature here is the found relative biodegradation of different particles given either intraperitoneally or subcutaneously; that is, poly(ethylene carbonate) particles degrade relatively much faster after administration by the former route. In vivo studies have shown that the clearance of model drugs from an intramuscular injection site may be delayed, and the whole-body retention of the compound extended, upon its incorporation into albumin microspheres cross-linked with glutaraldehyde (Fig. 18) [115]. The magnitude of the alteration to the compound's pharmacodisposition was found to be related to the density of the cross-linked particle matrix, with results showing that the greater the density, the slower was the rate of their biodegradation, and hence the slower was the release of the model compound.

X. Intravascular targets

As we have seen above (Sections VI and VII), entry of macromolecular site-specific systems into the cardiovascular blood pool may be via various routes, including: epithelial transport (viz. fluid-phase pinocytosis, paracellular and transcellular passive diffusion, receptor-mediated trans- and endocytosis, nutrient carrier processes, lymphatic translocations), and/or direct parenteral administration. Important therapeutic targets exist within the cardio-vasculature, and it is possible to access many of the cellular and extracellular locations using targeting methods.

X.1. Capillary filtration

Colloidal particles of sizes larger than the narrowest capillary beds will become filtered by these. This phenomenon has been exploited to deliver drugs to various disease states, including cancer, emphysema and thrombus formation, using particles of approx. 10 μm diameter and above. The rationale is that within a diseased organ, at the point of filtration there will be a high concentration of drug for an extended period of time leading to an increased availability of drug for either direct action or absorption through endothelia. This hypothesis is largely *unproven*, but a number of groups have worked on this approach with some degree of pharmacological and clinical success.

All such particles injected intravenously (apart from the portal vein) will become entrapped in the capillaries of the lungs. Indeed this is the basis of the use of radiolabelled human serum albumin particles for the scintigraphic examination of various tumour masses within the lungs. (Interestingly, although the supply to the lungs is arterial in origin, tumour masses are indicated as cold spots, i.e., the particles go to everywhere within the lungs except the tumour mass.)

Martodam and coworkers have exploited this physical approach to entrap particles of 15 μm diameter in the lungs of emphysemic rats after intravenous administration [116]. Further, attachment of a human leucocyte-elastase inhibitory peptide resulted in the alleviation of the symptoms of emphysema [116].

Given the circuitry of the cardiovascular system, to overcome filtration by the lung, particulates need to be administered intraarterially for them to become located into other organs. This approach has been used for both diagnostic and therapeutic purposes. To illustrate the potential that this has, the injection of particles greater than 15 μm into, for example, the mesenteric artery, portal vein or renal artery, leads to their complete entrapment in the gut, liver or kidneys, respectively. Torchilin has utilised this route of administration in using microgranules of Sephadex, to which were bound different thrombolytic enzymes, including fibrinolysin, streptokinase and urokinase [117]. Approx. 10–80 mg of enzyme can be bound to 1 g of this carrier. The immobilised enzymes were shown to be highly effective in the treatment of thromboses and thromboemboli of vessels that were accessible via catheterisation. This was demonstrated by the successful lysis of an experimental thrombus in a dog femoral artery by means of catheterisation of the artery and administration of a dose of immobilised enzyme (fibrinolysin) 100-times lower than that required with normal intravenous injection of the enzyme. Although Sephadex is completely degraded within about 1 h, it does not lend itself readily to use as a carrier, because to link drugs chemically is a somewhat difficult procedure, and long-term release in vivo, (i.e., greater than 15 days), does not appear possible.

Intraarterial delivery to cancerous sites by filtration uses one of two approaches, i.e., either where drug is incorporated into the particles (e.g., Ref. 43), or where the presence of a coadministered particle increases the dwell time within the particular capillary of a solution of drug [118]. Intraarterial administration of a bolus of particles of size greater than 15 μm in rabbits bearing intrahepatic V2 carci-

nomas, results in particles distributing to tumour capillaries, in preference to the surrounding (normal) capillaries, by a factor of four [119], which is regarded as being due to a quantitative difference in capillary networks of the tumour compared to those of the host organ [120]. As Ranney [89] has argued, because the lungs and liver have a double blood supply (e.g., for the lungs: pulmonary and bronchial), such approaches are appropriate in terms of protecting normal tissues in these organs. For example with the lungs, at detection, most pulmonary neoplasms receive a large fraction of their blood supply from bronchial arteries (or chest-wall collaterals), which are accessible to catheterisation. However, it is not yet possible to conclude whether this *gross* organ selectivity of a carrier, and subsequent release of drug can lead to any improvement in the pharmacological efficacy of any one drug.

X.2. *Mononuclear phagocyte system*

An important aspect of the use of carrier systems for achieving site-specific drug delivery is that they should modulate neither the disease nor the body's normal physiological processes, and to ensure efficient drug use and to avoid possible iatrogenic effects, they must not be recognised and taken up by the body's own defence apparatus until their payload has been delivered (Table VII). Foreign colloidal materials that are able to move freely through the cardiovascular system are generally cleared within minutes from the circulation by the mononuclear phagocyte system (MPS). For particulates, clearance has been shown to be related to a large number of factors including size, dose, surface charge, nature of the particle matrix, particle stability, and physiological state of the species (e.g. Refs. 121, 122).

For soluble macromolecules their complexation with antibodies and other endogenous materials needs to be avoided, since various immune complexes can form which are then extracted by the MPS.

The MPS can be regarded as either a constraint to site-specific delivery, as a disease target itself, or indirectly as an opportunity for targeting. The MPS is a connective tissue system of cells distributed throughout the body (Table XIII). The cells are mesenchymal in origin, and include the phagocytically active reticulum cells of the reticular connective tissue, the Kupffer stellate cells lining the wall of the hepatic sinusoids, the Hortega cells (microglia) of the central nervous system, the histiocytes (i.e., tissue macrophages) and the blood macrophages or mono-

TABLE XIII

CELLS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

Kupffer cells	Stellate cells lining the walls of the hepatic sinusoids
Blood-borne macrophages	
Tissue macrophages	Histiocytes
Reticulum cells	Of the reticular connective tissue
Hortega cells	Microglia of the central nervous system

cytes (see Ref. 123 for a more complete description). Cells of the reticuloendothelial system (RES) do include cells of the MPS; however, other RES cells do not phagocytose – including the endothelial cells of the liver which take part in the clearance of foreign particles by endocytic (though non-phagocytic) processes. In addition, Langerhans cells and related cells can be potent accessory cells but are often non-phagocytic and may lack both the Fc and C3 receptors [124]. The main functions of the MPS include the clearance of a large variety of potentially harmful substances from plasma, catabolism of ingested macromolecules, participation in the immune response and the synthesis and secretion of various effector molecules. Materials that are able to be cleared from the blood by the MPS include effete red blood cells, circulating tumour cells, inert colloids, autologous tissue debris, immune complexes, denatured proteins, specific glycoproteins, bacterial endotoxins, steroids and lipoproteins. Macrophages and other phagocytic cells synthesise and excrete monokines, including enzymes such as collagenase, as well as several effectors such as pyrogens, prostaglandins, procoagulants, colony-stimulating factor(s), interferons and tumouricidal factors (reviewed in Ref. 125).

Phagocytosis is a potential mechanism for the uptake of colloidal drug-delivery systems (Section VI), and it is of interest to note that such cells include platelets, which have been shown to take up latex particles (of 87 nm diameter) in their open-channel system, followed by particle location in platelet vacuoles, and with phagocytosis being chronologically similar to that given for polymorphonuclear leukocytes [126]. When foreign particles are introduced intravenously, about 90% are extracted by the Kupffer cells, 5% by the spleen and a few percent enter the bone marrow. Considerable species variation is observed, with the rabbit in particular exhibiting a much greater bone marrow uptake than other observed species [127]. The dominance of the liver in extracting materials from the blood does not reflect the concentration of macrophage cells in the body but rather their accessibility. (The output of blood from the heart after 100% transit through the lungs gives about 28% going through the liver and then through the spleen and intestine, with less than 10% going through the skeleton).

Macrophages are able to bind and to engulf a variety of particles through both a process involving specifically adsorbed opsonins, as well as through non-specific processes [128]. In addition, macrophages present on their surface membrane receptors for the Fc part of the immunoglobulin molecule and for the C3b component of the complement sequence. They also characteristically express Ia antigens. Serum factors which promote phagocytosis are called *opsonins*, which may be divided into immune and non-immune components, though both mediate the onset of phagocytosis through their bi-functional binding to the target and to their receptors on phagocytes. The recognition of foreign particles depends upon opsonin selectivity, and perhaps also their specificity for various cell surface structures [129]. A recognition mechanism in human monocytes which does not require exogenous opsonin but involves the human complement system has been identified [130]. The immune components include immunoglobulin G and complement C3b, though each appear to have a somewhat different subsequent mechanism of clearance by macrophages, and are known to be able to operate independently of one another. The

processes of uptake are complicated: for example, C3b-mediated adherence to macrophages may involve cleavage of this component on the surface, so that it now becomes coated with C3d and is released back into the blood for prolonged circulation. Fibronectin and fibrinogen may also be involved in these processes.

Interaction of particles with cells follows the sequence of opsonisation, followed by adherence to cell surfaces, and then engulfment, with, finally, attempts at their digestion. It is of interest to note that ingestion of non-drug bearing particles can lead to stimulation of the glycolytic and arachidonic-acid pathways and the hexose monophosphate shunt [131], and that both lysosomal enzymes are released within the cell and interleukin-1 is secreted.

The various approaches which have taken in attempting to avoid the uptake of site-specific systems by cells of the mononuclear phagocyte system will be examined in some detail shortly, though it is useful here to explore some of the early approaches which have been taken for interfering with the phagocytic process by hindering the adsorption of opsonins. Thus, it is now demonstrated that various surface manipulations of particles will lead to qualitative and quantitative alterations in opsonisation and macrophage uptake. For example, gentle treatment of a person's own red blood cells with *N*-ethylmaleimide results in splenic extraction after reinjection, but more severe treatment leads to their hepatic uptake [123]. Such procedures cause complement C3b to become opsonised, whereas red blood cells treated with glutaraldehyde are opsonised by IgG, which leads to Fc-mediated adherence. Also, gelatin-coated particles are opsonised by fibronectin. Related to these findings are those which show that when autologous red cells are coated with low concentrations of non-complement-fixing IgG, the main site of clearance is the spleen, whereas coating with complement-fixing IgM causes liver sequestration; which is in contrast to the removal of *soluble* immune complexes by an Fc-mediated, complement-independent process [124].

In humans, the removal of sialic acid from red blood cells exposes terminal galactosyl residues, resulting in their hepatic uptake, whereas the further (and sequential) removal of surface galactose residues results in *N*-acetylglucosamine or mannose being revealed, which leads to the uptake of the erythrocytes by Kupffer cells. A number of additional factors affect the interaction of particles with macrophages apart from the presence of specific surface ligands such as mannose and galactose. For example, it has been shown that with sterylamine incorporated (at 15% c) into the surface of egg phosphatidyl:cholesterol liposomes, the number of larger vesicles (100 nm and 160 nm diameter) associated with rat peritoneal macrophages in vitro was 100-fold less than that found for smaller vesicles of 25 nm [132]. This is of potential use for achieving selective drug delivery, since the total entrapped aqueous volume introduced into the macrophages by large vesicles is an order of magnitude greater than found with small vesicles. Also, increasing the sterylamine content, i.e., increasing the net surface positive charge on the particles, increased their interaction with peritoneal macrophages [132]. In contrast, work with crystalline metal sulphide particles gives that in vitro, negatively charged particles are far better phagocytosed by cultured mammalian cells [133]. This is somewhat peculiar, since macrophages themselves exhibit an overall negative

charge, but is in accord with the reported phagocytosis of other negatively charged particles such as quartz, and certain bacteria [134]. Other factors influencing clearance of blood borne particles in vivo include therapeutic levels of ultrasound – which may be due to either mechanical or biochemical effects [135].

X.3. Diseases of the mononuclear phagocyte system

Many diseases either reside within the cells of the MPS, or may be fought by marshalling the MPS to effect various events (Table XIV) [136]. Apart from having an important role in resistance to many infectious diseases, the cells of the MPS have many dysfunctions. For example, in rheumatoid arthritis, phagocytic cells may overproduce collagenase and secrete prostaglandins, which can be responsible for much of the pathology associated with this disease. Also, numerous facultative parasites are able to survive and proliferate within the cytoplasm of these cells [137], and since the chronic persistence of parasites stimulates the infiltration of more macrophages this results in the formation of severe granulatomous lesions. In addition, neoplasms of histiocytes and monocytes can occur (e.g., including histiocytic medullary reticulosis, monocytic leukaemia, hairy cell leukaemias and certain forms of Hodgkins disease).

Since the cells of the mononuclear phagocyte system readily take up particles by the process of phagocytosis, selective delivery to these cells using colloidal carriers is indicated. Although access is assured, persistence of drug and/or carrier to give a therapeutically significant effect is still an open question. Clinical success has been gained in this field with passive targeting to fungally infected cells of the MPS. Systemic mycoses caused by *Candida albicans*, *Aspergillus* sp. and *Mucor* sp. are mainly intracellular fungal infections which are difficult to treat due to problems of selective drug access. It has been demonstrated both in vitro and in vivo, both in animals and in humans, that there is a marked improvement in the selective tox-

TABLE XIV

SOME MACROPHAGE-ASSOCIATED DISEASES AMENABLE TO TREATMENT WITH PARTICULATE DRUG CARRIERS (SEE REFERENCE 136 FOR A FULLER LIST)

Disease	Drug
Intracellular parasites	
Leishmania	Antimony drugs
Histoplasmosis	Amphotericin B
Leprosy	Sulphones
Neoplasms	
Histiocytosis X	Doxorubicin
Enzyme storage disease	
Gaucher's disease	Glucocerebrosides
Rheumatoid arthritis	Antiinflammatory agents

icity of liposomal polyene antibiotics (e.g., amphotericin B) when administered systemically as a liposomal preparation [138]. In vivo this results in an improved drug therapeutic index due to normal mammalian tissue/cells becoming protected. It has been argued that polyenes transfer effectively from liposomes to fungal cells, but not to mammalian cells [139]. The successful *access* to the diseased cells, plus the almost complete abolishment of nephrotoxic effects, gives that this delivery approach is indicated in the therapy of systemic fungal infections in both cancer and in (other) *immunodebilitated* patients. Further successful uses of liposomal anti-infective drugs have been reported [109,140], as have the use of cytotoxic agents and organic antimonial drugs for the treatment of facultative and obligate intracellular parasites living within the endocytic vacuoles of mononuclear phagocytes (see references in Ref. 34).

Many viruses infect monocytes and macrophages, and this can lead to a plethora of diseases including hepatitis and pneumonia (*Cytomegalovirus*), and stomatitis and encephalitis (*Herpes simplex* I); etc. The use of particulate systems for treating viral diseases has been recently examined [141]. This can be considered in two ways, either by treating a virally infected macrophage, or by providing a vehicle for drugs which are able to stimulate macrophages to effect cytostasis or cytolysis of other (virally infected) cells. Fidler has demonstrated that an important aspect in the treatment of viral infections is the ability to deliver antiviral agents selectively to the sites of virus replication [141]. Several groups of viruses infect cells of the MPS, and hence naturally targeted particles can be used to direct antiviral drugs to infected phagocytic cells, though it is not yet possible to ascertain how this could be made exclusive for the population of infected cells. Viral infections usually manifest themselves by inflammation, including perivascular infiltration by mononuclear phagocytes (see also subsection X.4).

The body rids itself of unwanted substrates by degrading these within the lysosomes of various cells, including cells which are phagocytic. Abnormalities in this process, which are often due to the absence of the cellular machinery for an enzyme to enter the cell and to be correctly routed to the lysosome, can manifest themselves as a number of storage diseases. An opportunity exists here for the selective delivery of various drugs and enzymes into these phagocytosing cells using particulate delivery systems. This is exemplified by the study of Umezawa and coworkers on direct enzyme replacement using liposomes containing beta-galactosidase in (murine) globoid cell leukodystrophy [142]. We shall discuss this in greater detail later (subsection XIII.3), though it is interesting to note here that the types of particle which are indicated for targeting to macrophages are those which are lipid-like, or hydrophobic, i.e., which can be readily opsonised, and include liposomes, monolithic particles having a hydrophobic surface, and even altered erythrocytes [143].

X.4. Modulation of mononuclear phagocyte system action

The uptake of particles by cells of the MPS can be turned into an advantage in the treatment of low burdens of tumour masses. That is, mononuclear phagocytes

TABLE XV

EFFECT OF LIPOSOMAL IMMUNOMODULATORS ON SPONTANEOUS B16 MELANOMA METASTASES IN THE LYMPH NODES AND LUNG OF C57BL-6 MICE AFTER INTRAVENOUS ADMINISTRATION (ADAPTED FROM REFERENCE 141)

Treatment	Surviving mice/total mice on day			
	40	60	90	200
Control (saline)	30/30	14/30	1/30	1/30
MAF (in saline)	14/14	10/14	2/14	1/14
Liposomal MAF	15/15	13/15	10/15	10/15
MDP, 100 μg (in saline buffer)	15/15	9/15	1/15	1/15
Liposomal MDP, 6 μg	15/15	9/15	9/15	9/15

may become activated by effector molecules to acquire the ability to destroy neoplastic cells which may be in *extravascular* locations. It is also considered that such an approach may be able to overcome the tumour cell heterogeneity which hinders the use of other selective delivery modalities (see also subsection XIII.6).

Antigen- and mitogen-stimulated T-lymphocytes release lymphokines which can interact in a highly specific manner with target cells that bear appropriate receptors. Activation can be caused by numerous molecules, and appears to include an initial priming step that can be induced by lymphokines, followed by a second triggering step. It is clear that lymphokines can also act as this second trigger, though other molecules may be more effective [144]. In addition, secretory products of the macrophages themselves regulate macrophage activation by both a positive and a negative feedback control.

Much attention has been given to macrophage activation by bacterial cell wall components and their synthetic analogues. Such molecules (e.g., muramyl dipeptides) and lymphokines (e.g. MIF, MAF, γ interferon and tumour necrosis factor), although active in vitro, both have the potential for being immunogenic, are poorly distributed in vivo to macrophages, and are rapidly excreted and catabolised by the liver. For these reasons, attempts have been made to deliver such molecules to mononuclear phagocytes by exploiting their ability to phagocytose colloids. Lymphokines and muramyl peptides encapsulated within liposomes are highly effective in activating macrophages both in vitro and in vivo (reviewed in Refs. 141 and 145) (see Table XV). Recent work using a lipophilic derivative of muramyldipeptide (i.e., muramyltripeptide-phosphatidylethanolamine, MTP-PE), which, although active, always remains anchored into the lipid bilayer of a liposome, has shown that drugs do not necessarily have to leave their carrier to be effective, so long as they are available to activate the macrophage [141]. Evidence also suggests that once activated, macrophages are able to extravasate and seek out their target cells, though which macrophages do this and why are still unknown. This approach is limited by the number of macrophage cells present which are able to be activated, and is of potential use only where there are low burdens of tumour masses [145,146], although there is evidence that the combined use of various lymphokines and other immunomodulators in liposomes can give rise to a potentiated activation (Table XVI) [141].

function changes with age. For example, Brouwer and Knook have found that the clearance capacity of macrophages shows a moderate age-related decrease, which probably correlates with a decreased endocytic capacity of Kupffer cells [125].

X.5. Avoiding uptake by the mononuclear phagocyte system

Avoidance of the MPS is crucial if the full potential of site-specific drug systems is to be realised. Numerous and varied approaches have been proposed, and these are collected together in Table XVIII.

For particulate systems it appears that modifications to their surfaces based on a knowledge of the thermodynamics of opsonin adsorption form a profitable route to help avoid the MPS [147]. For soluble macromolecular species, the system design specification is that immune complexes are not formed. Additional approaches to avoiding uptake by cells of the MPS include using mimics of natural carriers e.g., low density lipoprotein (e.g., Ref. 148), erythrocytes (e.g., Refs. 143 and 149), and blockade of the reticuloendothelial system [150].

Most of the approaches have limited clinical applicability. For example, the practicalities of producing natural particles such as red blood cells on a large scale appear formidable, although study of why these avoid uptake by the MPS could be invaluable in designing carriers.

X.5(a). Saturation

Attempts have been made to block temporarily the functioning of the MPS by using inert colloids such as carbon, methyl palmitate, latex beads, dextran sulphate, etc. Early studies attempted to alter liposome distribution by prior blockade of the MPS with empty liposomes [121]. Although this goal of thence increasing uptake of drug-bearing liposomes by tumour tissue was not achieved, it was found that the phagocytic function of the MPS was depressed. Other workers have

TABLE XVIII

SOME REPORTED METHODS FOR AVOIDING UPTAKE OF SOLUBLE MACROMOLECULAR AND PARTICULATE COLLOIDAL MATERIALS BY THE MONONUCLEAR PHAGOCYTE SYSTEM

Process	Example
Alteration of particle surface	
Charge	Charged liposomes
Hydrophilic	Albumin nanoparticles
Hydrophilic and sterically stabilised	Poloxamer/poloxamine copolymers adsorbed to hydrophobic surfaces; polyethylene glycols
Mimics of natural particles	Erythrocytes; use of glycophorin
Blockade of MPS	
by saturation	Dextrans; empty mannosylated liposomes
by receptor-block	Anti-Fc receptor antibodies

successfully imaged tumours with labelled neutral liposomes using prior blockade of the MPS with small unilamellar vesicles which had incorporated a o-aminomannose derivative of cholesterol into the lipsome bilayer [150] (this latter method was based on the finding that phagocytosis of aminomannose-modified vesicles proceeds in vitro at a relatively fast rate). It should be noted here that suppression of the MPS often leads to the re-direction of particles to both other blood cells and the lungs [123]. Also, although it is possible to block the MPS reversibly, repeated intravenous injections of liposomes are accompanied by long-term paralysis of the MPS [151], and hence clinically, this approach does not seem viable.

However, it is interesting to examine the mechanism of blockade. Lehnert and Tech have shown that a reduction in phagocytosis after preloading macrophages with particles is due to a loss in surface sites required for particle recognition and binding, and not to limitations in the phagocytic capacity or particle loading of macrophages per se [128].

Recently, a novel approach to blockade has been discussed [50], i.e., the use of pretreatment with monoclonal antibodies to the low affinity Fc receptors of macrophages. An example quoted gives, for the chimpanzee, that an injection of 1 mg per kg antibody prolongs the blood half-life of immune-coated red cells from 50 min to 23.5 h.

X.5(b). Modification of carrier surface

In general, when soluble macromolecular and particulate carriers are introduced into the biological fluid they are immediately adsorbed to by flexible and globular proteins and other endogeneous macromolecules (Fig. 19). As given above, these include opsonins which result in recognition and adherance of the carriers to the cells of the MPS. Materials are then taken into the cells by engulfment upon vesiculation of the phagocytosing cell membrane. Both of the processes of opsoni-

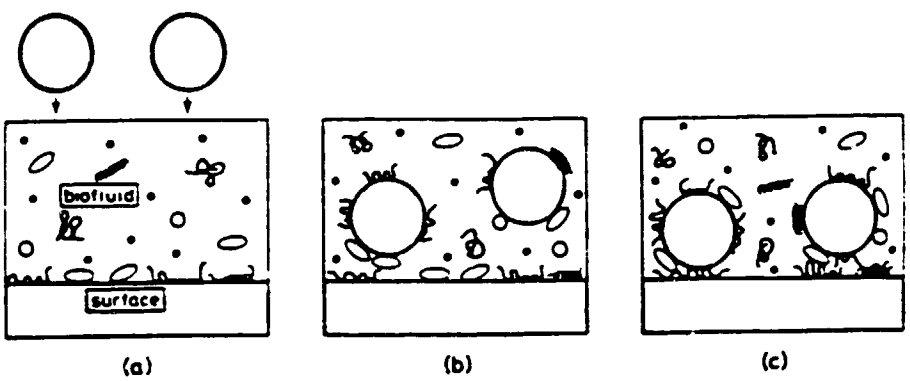

Fig. 19. Fate of microparticles in a biological fluid, where (a), (b) and (c) refer to entry of particles into a biofluid contacting a surface which is covered with a layer of adsorbed material, adsorption of soluble flexible and globular macromolecules from the fluid onto the particles, and deposition of the particles onto a surface. (Reproduced with permission, from Ref. 152.)

sation and adherence can be diminished if the attractive forces between the interacting particles, surfaces and macromolecules are diminished. Adsorption and adhesion effects are complex phenomena, and are controlled by many factors including electrostatic, dispersion and steric forces, by hydration, and by other short-range interactions [152]. Interfacial adsorption is dependent upon a balance between these forces. Colloidal particles will attract each other through Van der Waals interactions (short range), and repel each other through longer range, repulsive (e.g., Coulombic) forces. As particles approach one another there is a net attraction, with a potential energy barrier to interaction at closer proximities, and with strong interaction at very short ranges. For avoidance of opsonisation one wishes to avoid interactions. This can be achieved by creating a high-potential energy barrier.

It has been shown that particles coated with either negative or positive macromolecules can be taken up by different organs at different rates [153] and as described by Napper and Netschey [154], a high-potential energy barrier can be formed by creating a *sterically stabilised* surface by introducing a hydrated (i.e., hydrophilic) polymer at the surface. This results in a surface which is both hydrophilic and stabilised. The hydration effect is enthalpic in origin, and the stabilisation effect is manifested by both osmotic effects and chain entanglements – both of which are entropic in origin [155]. The size of this repulsive barrier should be determined by both the thickness of the polymer layer and its density, as well as by polymer–polymer interactions caused by specific interactions along the polymer chain.

Surface modifications to macromolecules have long been employed to aid their tolerance within the vasculature. It does appear that often this is due to a reduction in the extent of interaction with biological macromolecules [156], largely due to the production of a surface which makes it energetically unfavourable for other (macro)molecules to approach. Synthetic and biological materials used for this masking have been polyethylene glycol [156,157], poloxamers [158–160], poloxamines [158], albumin [161], immunoglobulin G [162] and carboxymethycellulose [163]; other materials which have been suggested include the natural xanthans [147]. Apart from being used for monolithic particles, the approach has been used to reduce the liver uptake of emulsion droplets [164–166].

Steric stabilisation (or polymer-excluded volume) approaches to avoiding opsonisation have recently received much detailed attention, particularly with the use of block co-polymers tightly adsorbed onto particles (where the hydrophobic portion of an A-B-A block co-polymer is adsorbed to the surface of a hydrophobic particle, e.g., using poloxamers of different hydrophilicity/hydrophobicity), and the utilisation of chemical (covalent) grafting in order to attach hydrophilic macromolecules covalently to the surface of particles.

Poloxamers are A-B-A-type block co-polymers, where A is hydrophilic, i.e., poly(oxyethylene), and B is lipophilic, i.e., poly(oxypropylene). Such block copolymers appear to orientate at hydrophobic surfaces with their hydrophilic chains 'perpendicular', which provides a 'steric stabilisation' to particle stability. Davis and Illum have found that the most effective copolymers in causing particles

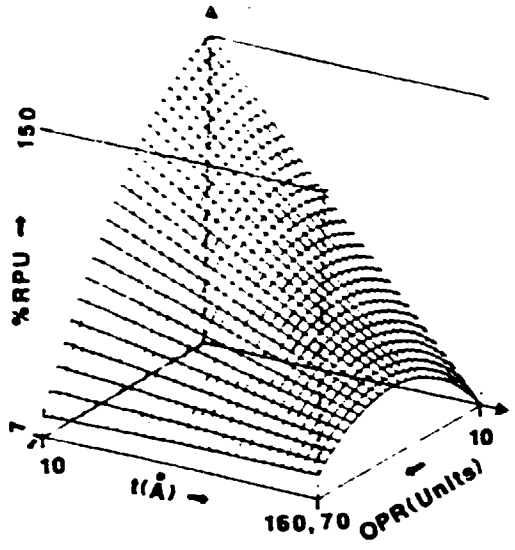

Fig. 20. Relationship between percentage relative phagocytic uptake (RPU) of polystyrene latex particles coated with block copolymers, and the thicknesses of the adsorbed layers of poloxamer block copolymers (*t*, Ångstroms), and the number of oxypropylene units per copolymer (OPR). Data taken from Refs. 147, 158 and 159. The experimental values for RPU have been found to be best described as a function of OPR, oxyethylene units per polymer and the zeta potential of the coated-particles (Patel, H.B. (1987) Unpublished data). (Figure reproduced with permission.)

to avoid clearance by the liver (sic) are those which have both a large hydrophilic block and a large hydrophobic block within the same molecules [147]. These appear to combine to give maximal retention on the particle surface and minimal adsorption of opsonin. It has been shown recently that a decrease in uptake by phagocytic cells of particles coated with poloxamers is directly proportional to an increased thickness of the adsorbed coat (Fig. 20) and their resultant surface charge. It is also apparent that the *density* of the chains and their chemical reactivity will be important in effecting stability to aggregation and opsonisation. It is not known what influence any ligands incorporated for recognition purposes will have on this process.

We have examined the adsorption of a putative opsonin – fibrinogen – onto polystyrene latex particles uncoated and precoated with poloxamers, as well as their aggregation behaviour and their in vivo distribution (Hutchinson et al. (1986) unpublished results, see also Ref. 14). We have found that poloxamer-237-coated particles have an adsorbed layer thickness of 13 nm compared to a value of 18.5 nm found using poloxamer 338. Fig. 21 gives the maximum isothermal adsorption of fibrinogen onto different particles. It is apparent that uncoated particles strongly adsorb fibrinogen in vitro, and this results in particle aggregation. Also, it has been found that protein adsorption is markedly decreased for poloxamer-coated parti-

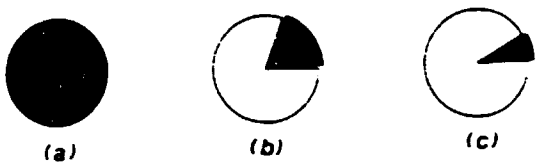

Fig. 21. Relative adsorption of ^{125}I fibrinogen onto 331-nm diameter polystyrene latex particles (a), onto poloxamer-237-coated particles (b), and onto poloxamer-338-coated particles (c), respectively. Shaded area refers to fibrinogen adsorption relative to an uncoated particle. (Hutchinson et al. (1986) unpublished results, and Ref. 14.)

cles in vitro, though less so with poloxamer 237 than with poloxamer 338, and Fig. 22 gives the distribution of polystyrene latex particles (mean diameter, 331 nm) in mice 10 min after an intravenous bolus of approx. 10^{10} particles. Uncoated particles were found primarily in the liver and the lungs (the latter being due to an accumulation of aggregated particles). It is seen that poloxamer-237-coated particles were cleared mainly by the liver, whereas poloxamer-338-coated particles remain mainly in the blood pool. These results are consistent with those of Davis and Illum, who have further found that the effect of adsorbing different poloxamers onto particles is not only to reduce their uptake by the liver, but also to divert these *non-extravasating* particles to other vascular targets, including blood monocytes and bone marrow cells [147].

X.5(c). Mimics of natural particles

It is probable that steric stabilisation is akin to the mechanism whereby blood cells and various bacteria and parasites escape detection by the MPS [130]. Some groups have attempted to exploit the behaviour of natural particles such as erythrocytes [143] and bacteria to increase the circulation times of particles. For example Utsumi and coworkers [149] have demonstrated that incorporation of the sialoglycoprotein of human erythrocytes into liposomes of 0.2–1.0 μm diameter, causes a marked reduction in their clearance by the liver.

X.6. Circulating systems/other intravascular targets

A priori, the avoidance of uptake by the MPS should enable carriers to remain

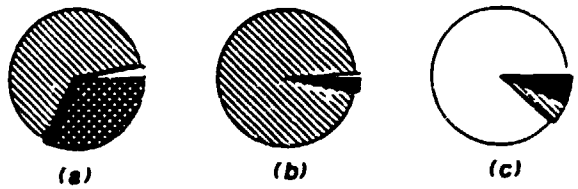

Fig. 22. Distribution of particles described in Fig. 21 after their intravenous administration as a bolus to mice. Open areas, shaded areas, diagonally hatched areas, and squared areas, refer to relative levels of dose found after 10 min in blood, spleen, liver and lung, respectively. The large amount of particles accumulating in the lung with the uncoated particles is due to their aggregation and subsequent filtration by the lung capillaries. (Hutchinson et al. (1986) unpublished results, and Ref. 14.)

for considerably longer periods within the circulation. This can be considered in numerous ways: that is, increasing the statistical probability for a competing process to occur (e.g., extravasation or interaction with an intravascular target cell), and or raising the potential for the development of a long-term circulating drug depot. With the latter, such a system could be useful for the intravascular delivery of both conventional and polypeptide and protein drugs, including antiinfectives, antithrombolytics, fibrinolytics, cytostatics, antiparasitic agents, and enzymes, etc.

The pioneering work of Abuchowski and Davis [156,167] in grafting hydrophilic polyethylene glycol chains onto various polypeptides and macromolecular drugs in order to stabilise them against recognition and uptake by the cells of the MPS not only renders these molecules non-immunogenic, but dramatically increases their blood circulation times – presumably by imparting a high degree of steric stabilisation. This approach has been used for increasing the blood half-times of peptidergic mediators such as interleukin-2, as well as for a number of enzymes, such as catalase, asparaginase and urokinase, whilst still maintaining their reactive functionalities (see, for example, Fig. 23). Similar findings can be found in other studies (e.g., Ref. 161). A decrease in immunogenicity may result from either a reduction in the aggregation of materials or simply by masking any antigenic determinants. It is apparent that this (physico)chemical approach to enhancing the properties of various drugs and carriers in the blood pool will be increasingly applied to both macromolecular and particulate synthetic and polypeptide drug carrier constructs.

In addition to the above, various types of natural particulate systems have been suggested as circulating depots, including erythrocytes containing asparaginase in the treatment of cancer.

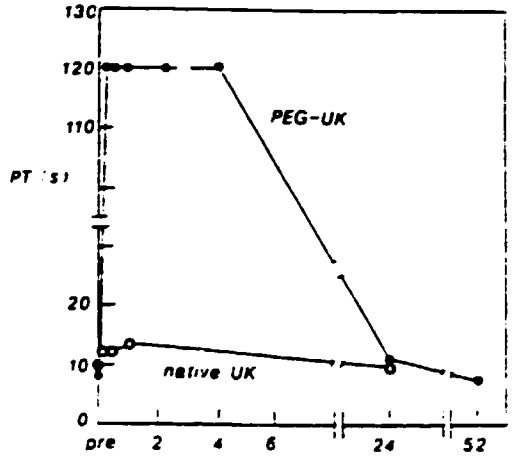

Fig. 23. Influence of chemical grafting of polyethylene glycol (PEG) on the ability of urokinase (UK) to affect the prothrombin time (PT) in vivo in beagles with time. (Reproduced with permission, from Ref. 168.)

Blood cells are now defined in terms of subsets having differentiation markers, with detailed classifications becoming possible. These subsets represent defined intravascular targets which may be recognised by various ligands including antibodies, hormones, and simple sugars, etc. Microparticles linked to such ligands have been successfully targeted to blood cells both in vitro (e.g. Ref. 169) and in vivo [170]. For example, it has been demonstrated that the covalent attachment of anti-rat erythrocyte $F(ab')_2$ antibody to liposomes both enhanced the binding of these particles to rat erythrocytes in vivo, as well as reducing vesicle uptake by the liver [170]. This study also found that the contents of the liposomes were also delivered to the cells to which they were bound. Similarly, a recent attempt to enhance cell elimination has mirrored this finding [171], which indicates that a particulate carrier can be directed to a freely available cell by the exploitation of an antigen antibody interaction. Although such approaches appear attractive, the *internalisation* of particles by various blood subset cells differs (e.g., Ref. 172), and this aspect needs further consideration before any carrier for targeting to intracellular intravascular locations can be developed.

Excitingly, recent work has shown that by constructing transferrin-coated liposomes these can be used in vivo to transport exogeneous DNA to bone marrow erythroblasts in anaemic rabbits (see also subsection XIII.3 on gene therapy), presumably because such cells have many transferrin receptors on their surfaces [173].

X.7. Normal and abnormal endothelia, and endothelia-related targets

A large and varied number of antigens are present on the surface of endothelial cells. Some of these are involved in transporting endogeneous materials into and through the cells of endothelial membrane, but it is interesting to note that many of these antigens are organ-, tissue- and cell-specific markers, all of which could serve a purpose in selective drug delivery.

Although knowledge is currently scant, some information is available. For example, specific cell-surface antigens occur on the endothelium of high endothelial venules in (i) mucosal lymph nodes of Peyer's patches (which recognises a feature of B-lymphocytes), and (ii) peripheral lymph nodes (which recognises a feature of T-lymphocytes) [174,175]. In addition, organ-specific antigens are described on capillary endothelial cells [176]. These latter studies have established that "capillary endothelial cells obtained from different organs express surface-associated antigens that are organ-specific as well as others that are cell-type specific. Brain endothelial cells share Thy-1, BAT, and MBE antigens (with other murine brain cells), while also expressing ME-2 and ACE-derived antigens common to endothelial cells from various sources. Ovary-derived endothelial cells express ovary specificities as well as endothelial antigens; lung capillary endothelium expresses lung-associated antigens as well as general endothelial cell markers". In addition, disease-related markers exist on capillary endothelial cells. For example, recent work has shown the presence of a unique (apical) endothelial marker on endothelia derived from an AIDS-associated Kaposi's sarcoma [177].

Targeting with a ligand able to recognise and attach to such cell markers has

been often mooted, but as yet few studies have been reported. Undoubtedly, many endothelial markers can be imaged using radioimmunolocalisation techniques, but whether this is of significance for chemotherapy is uncertain, particularly because many of these markers are not internalised and any released drug would be removed by normal blood flux. Although most imaging studies have been carried out with soluble conjugates, particulate carriers have been successfully directed to endothelial targets where a pathological condition exists. It is known that there are a number of diseased states of the cardiovascular system where subendothelial structures that are normally thrombogenic come into contact with blood. This is the case in transluminal angioplasty of stenotic vessels, which causes the exposure of intimal and often medial vascular wall structures. The work of Smirnov and coworkers demonstrates that by binding either anti-collagen type-I antibodies or human fibronectin to cholesterol oleate-containing liposomes then in perfused in situ arteries partially denuded to expose underlying basement-membrane structures, such constructs are selectively bound by endothelia-free zones of arterial segments [178]. This finding has been suggested for use in preventing platelet aggregation and thrombus formation on fissures of atherosclerotic caps, as well as for the local delivery of anti-platelet drugs (which, in some cases, need to be present at the moment of plaque rupture). Other targets suggested for local delivery by this method include exposed antigens of activated and spread platelets, newly formed fibrin and other specific antigens present on the plaque surface. The delivery to these lesions of drugs both able to inhibit further platelet aggregation over the plaque surface and to lyse the formed clot, has also been proposed [178]. Previous studies by the same group have shown that in myocardial infarct, the exposure of cardiac myosin (of myocytes) enables anti-myosin antibodies linked to liposomes to accumulate at these sites [117].

XI. Extravasation

Previous Sections have shown that after administration and access to the general circulation there will be many occasions when drugs (within carriers) will need to leave the cardiovasculature in order to reach either extravascular-extracellular or extravascular-intracellular target sites. Such a process of *extravasation* is under strict anatomical and (patho)physiological control. Numerous selective and non-selective opportunities present themselves for the extravasation of site-specific systems (Fig. 5). Hence, systems can either be incorporated into phagocytic cells which can extravasate, or pass directly through either interrupted endothelia or through the cell barrier itself by exploiting fluid-phase and/or receptor-mediated, constitutive and non-constitutive cell transport processes. In the following Sections the constraints and opportunities for extravasation of site-specific delivery systems are examined

XI.1. Structure and assembly of the endothelial membrane

The structure of the endothelium is complex, and varies greatly in different or-

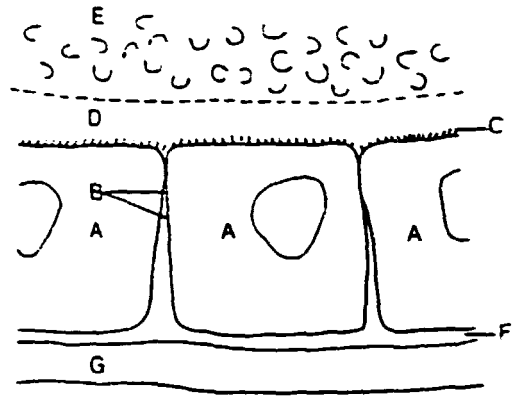

Fig. 24. Blood capillary endothelial membrane. A–H refer to the endothelial cell, the interendothelial gaps, plasmamembrane fibrin layers, aqueous diffusion layers, turbulent blood flow, exoendothelial space, and basement membrane, respectively.

gans and tissues. It is generally comprised of four layers (Fig. 24), namely the plasma membrane – plasma interface (which is formed by the glycocalyx of the cell and the proteins adsorbed onto it), the endothelium (a monolayer of cells which are metabolically very active, and effect and monitor the *bidirectional* exchange of fluid between the plasma and the interstitial fluid); the basal lamina (which support the endothelium); and the adventitia (connective tissues which surround the lamina and fuse with the surrounding fibro-areolar tissue). Table XIX and Fig. 24 give the anatomical features and the distributions of capillary endothelial membranes. There are quite significant differences to be found throughout the body in terms of their structure, assembly and functioning, all of which could be important in the design and use of macromolecular carriers.

Capillaries having a continuous endothelium and an uninterrupted basement

TABLE XIX

ANATOMICAL FEATURES OF ENDOTHELIAL REGIONS

Type characteristics	Tissue
Continuous	
Skeletal, smooth, cardiac muscles; connective tissue; cns; pancreas, gonads; lung	Tight junctions; vesicular trafficking; continuous basement membrane
Discontinuous	
Fenestrated	
Exocrine and endocrine glands; gi tract; renal glomeruli, peritubular capillaries; choroid plexus, intestinal wall; pancreas	Interruptions 20–80 nm; thin membrane 4–6 nm thick; continuous basement membrane
Sinusoidal	
Liver; spleen; red bone marrow; suprarenal and parathyroid glands, carotid and coccygeal bodies	Gaps about 150 nm; basement membrane absent in liver; interrupted in spleen, bone marrow

membrane are the most widely distributed. Fenestrated capillaries are morphologically distinct from these, and are typified by having a very thin cytoplasm on each side of the nucleus (30–60 nm), and gaps of between 20 and 100 nm diameter at irregular intervals. These gaps have a thin membrane (4 nm thick) which is thought to be derived from the basal lamina. From Table XIX it may be seen that some tissues have sinusoidal endothelial membranes where the membrane is very thin, and there is hardly any connective tissue separating the endothelial cells from the parenchymal cells of the underlying organ. These areas are often lined by phagocytic cells. The sinusoids of the liver are lined by highly phagocytic cells (Kupffer cells), and those of the bone marrow by flattened, phagocytic reticuloendothelial cells. In the spleen, the endothelial cells (stave cells) are greatly elongated, and contain a large number of pinocytic vesicles (up to 100 nm in diameter) at both the apical and basolateral surfaces; in addition, the splenic cords, which contain macrophage cells and lymphocytes, are regions of high phagocytic activity. Further, it is now appreciated that within each capillary bed there are variations in endothelial structure. The pioneering works of the Simionescus (e.g., Ref. 179) show that there are differences (at least in the rat) in the microvasculature. Thus, in the venules the endothelium is thinner (170 nm) than the arterioles, and *transendothelial* channels also appear to be more frequent. Also, the endothelial cells of the arterioles are joined by highly organised assemblies of tight junctions and communicating (or gap) junctions, whereas capillary endothelium contains only so-called occluding junctions; also the endothelium of the venules comprises a much

TABLE XX

LYMPH-TO-PLASMA CONCENTRATION RATIOS ([L]/[P]) IN SUBCUTANEOUS TISSUE FOR SOME ENDOGENEOUS MACROMOLECULES (SEE REFERENCE 106 FOR REFERENCES)

Macromolecule	Size (Stokes–Einstein radius) (nm)	[L]/[P]
Human[a]		
Albumin	3.7	0.63
Immunoglobulin G	5.6	0.51
Dog[b]		
Albumin	3.7	0.22–0.38
Immunoglobulin G	5.6	0.17–0.28
Fibrinogen	10.0	0.05–0.16
Transferrin	4.3	0.44
Dextran	2.0	1.00
	2.64	0.50
	3.5	0.23
	3.8	0.15
	5.0	0.06
	8.25	0.04
	13.0	0.05

[a] Interstitial fluid to plasma ratios

[b] Using hindpaw lymph

looser organisation of cell contacts which have many discontinuities (with approx. 30% having gaps of about 6 nm). This differentiation abounds. For example, communicating junctions are absent in capillaries and pericytic venules, and are very small and infrequent in muscle venules.

Endothelial cells contain a large number of spherical vesicles of uniform diameter (*plasmalammelar vesicles*). These are generally between 60 and 80 nm in diameter, and are believed to be responsible for much of the transport of materials across endothelia. It is estimated that there are between 10000 and 15000 vesicles per cell, with about two-thirds 'open' on the plasma membrane and the remainder within the cytoplasm. The opening to the plasma is via a small neck (rather like a vase) of 10–30 nm in diameter, such that their presence can double the available surface of the cell plasma membrane. It is probable that at times these vesicles are fused to form a transendothelial channel. What is known about vesicles is that their transit time across the cell is about 1 s, and they do not preferentially travel towards the lysosome of the endothelial cell [180], but that their aetiology and their ability to transport materials is under direct hormonal and metabolic control.

In addition to these structures, there are many markers on endothelial cells, some of which are part of intracellular processing events (see Section VI).

XI.2. Passage through normal endothelia via passive processes

The endothelial cell surface has a key role in homeostasis [181]. In addition to the morphological aspects presented above, these surfaces appear to have many microdomains of differing charge and/or charge density which appear to relate to varied transendothelial routes. These vary immensely in their composition, with anionic sites being formed by proteoglycans and sialoglycoconjugates, and cationic sites found on fenestrated endothelia by heparan sulphate proteoglycans [181].

It is now recognised that both small molecular-weight solutes and a large number of (endogenous) macromolecules up to 30 nm in diameter are able to pass through this barrier under certain normal and pathological conditions. This has a tremendous importance for site-specific drug delivery.

Plasma molecules are selectively transported across the endothelium according primarily to their size, but their charge and their physicochemistry (i.e., hydrophilic/lipophilic balance) are also contributing factors. The capillary wall permeability for soluble macromolecules is well documented (vide Taylor and Granger [106]); some reported values of macromolecule solute lymph-to-plasma ratios in subcutaneous tissue are given in Table XX. Taylor and Granger show that soluble materials of less than 30 nm diameter are able to permeate through continuous endothelia (e.g., Fig. 25), which is in contrast to that found for liposome particles of 30–80 nm diameter which are not able to pass the (alveolar) endothelia [146].

The events that lead to the passage of macromolecules across endothelia are a mixture of physical processes (hydrostatic pressures, fluxes and dynamic equilibria) and certain cellular events. For the *passive* transfer of materials across the endothelium much experimental work has been done to prove the thesis of Starling that the movement of *fluids* across capillary walls is due to a combination of hy-

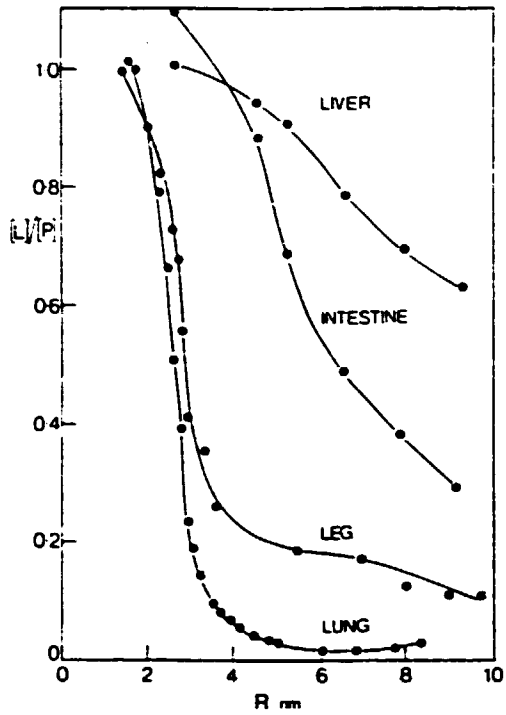

Fig. 25. Relationship between mean lymph-to-plasma ratios ([L]/[P]) for uncharged dextrans and their molecular radius (R, nm), for different organs in the rat. (Adapted and redrawn from Ref. 106 and references contained therein.)

drostatic and colloid osmotic pressure. Clearly, whilst the two prime mechanisms of bulk and diffusional transport are *independent*, transport rates are not, since the capillary walls are acting as semi-permeable membranes – hence the driving force and the concentration gradient are related to one another by osmotic effects. The rate of movement of fluid across the endothelium is often shown experimentally to be directly related to the difference between the hydrostatic and colloid osmotic forces [106], which is consistent with the results of Mayerson and coworkers, who have shown that there is a dependency between the rate of trans-endothelial flux and molecular size for dextrans and proteins [182].

Although the fluxes found for large macromolecules are much slower than those for water and low-molecular-weight solutes, these are still functionally important. The circulation route of plasma proteins is from the capillary blood to the interstitial fluid to the lymphatic capillaries, lymphatic vessels and thence to plasma. Because of the volume of these compartments and the relative rates of flux, approximately half of the total amount of plasma (sic) proteins reside within the interstitial compartment at any one time, and approximately half of the total amount circulate in a 24-h period. Although a full consideration of the various models used to describe flux and flux rates is outside the scope of this present contribution, it

is believed that there are two *paracapillary* circulations, namely a filtration absorption circulation – that includes the total capillary filtrate, the total interstitial fluid together with that part of the interstitial fluid which returns to the capillary blood via the endothelia directly (i.e., the bidirectional flow) and a circulation of that part of the capillary filtrate that is not re-absorbed (and contains the proteins), and which then enters the lymphatic capillaries and is carried to the major lymphatic trunks back into the venous blood [106].

Fig. 25 shows that large materials can pass through the capillary walls of many organs, and that for all organs except lung, the lymph-to-plasma ratios for fractions of uncharged dextrans (even those greater than 4 nm in radius) are significantly greater than zero. This suggests a form of large-pore pathway, which could be related to the existence of the plasmalammelar transendothelial pathway (see page 150). Similar relationships occur for other tracers, including polyvinylpyrrolidone.

Although, in general, it is the hydrodynamic radius which determines the passage of materials through capillary vessel walls, the presence of a *charge* can have a profound effect on this routing, depending upon the anatomical region. For example, Pietra and coworkers have established the apparently paradoxical enhancement of plasma-to-lymph movement of highly charged dextrans through the endothelium of the lungs (though not through either the endothelium of the skin or the renal glomeruli) [183]. Since most endothelia bear a net negative charge, the mechanism of a cation-exchange function of the fixed negative charges has been postulated to explain these findings [184], where, as a result of fixed (anionic) charges, cationic macromolecules are reversibly exchanged with small cations on, inter alia, the plasma membrane surface, the opening of the 'large' pores, and the interstitium – whereas the location and passage of negatively charged macromolecules should be restricted to the narrower channels. This would result in a *higher* equilibration rate for *anionic* macromolecules than for cationic macromolecules, due to the relative capacities of their different transport routes.

Organ-specific variations (in rat) in the passive transport of macromolecules are beginning to be found. Thus, it has been shown that amphipathic soluble macromolecules having a p*I* in the range 4.5–7.2 do not bind significantly to plasma membrane; in addition, coated pits, which are part of the receptor-mediated entry machinery, bind cationic ligands almost exclusively in liver sinusoids, where they are bound to by both polycations and anions; in aortic endothelia, binding sites for oppositely charged (particulate) materials coexist in the same microdomain [185].

It is now regarded that the plasma clearance values for colloidal materials larger than 4 nm are a measure of the volume of fluid transported by endothelial vesicles. Renkin and coworkers have concluded that macromolecules only slightly smaller than pores can be transported through both pores and vesicles [186]. For serum albumin and dextrans (molecular mass, 20 kDa) both processes contribute equally to the extravasation process, whereas for dextrans of molecular mass of only 10 kDa about 10% of the amount entering the lymph does so via vesicle processes. (The volume of *fluid* transported by vesicles is only a small fraction of the lymph flow observed. Most of the water and small molecules in the lymph must therefore reach there by filtration and diffusion through the pores.)

Macromolecules and colloids return from the interstitum to plasma via the lymphatics, and there may also be regional differences in this, though little work has yet been carried out [187]. Blood vessels of most tissues are permeable to lymphocytes, and it is possible that macromolecules may be transported while associated with circulating cells, although no evidence exists for this in vivo.

Passive extravasation is affected by pathological conditions. Damaged capillaries tend to have an increased permeability, and the effects of arrested blood flow and of resultant hypoxia appear to be significant only after the arrest has been total and prolonged – approx. 3 min give a 50-fold rise in filtration. Metabolic changes appear also to result in changes in capillary permeability which are mediated through a reduced oxygen concentration, an increased carbon dioxide concentration, and a local increase in pH due to an accumulation of various metabolites [106].

XI.3. Regional differences in capillary permeation

The mass transfer of material across the capillaries also depends on the blood flow, which is given by the cardiac output. This, combined with unique architectural variations, results in regional differences in the extravasation of both soluble macromolecules and particulates [106]. Some of these differences are highlighted in the subsections XI.3(a)–(g).

XI.3(a). Renal endothelia

The endothelium here is unique, including the presence of a relatively thick basement membrane and an epithelial layer comprised of indigitating 'foot' processes. There are also anionic groups present on the endotheliai surface, and heparan sulphate proteoglycan in the basement membrane. These features combine to show that molecular parameters such as size, shape, flexibility and deformability, and physicochemical parameters such as charge and hydrophobic/hydrophilic balance determine the transport of macromolecules through this vital membrane [188].

XI.3(b). Intestinal endothelia

This is a much studied membrane, and it is now regarded that intestinal capillaries are *more* restrictive to the passive passage of endogeneous macromolecules than are the capillaries of the lung, liver and subcutaneous tissue capillaries [189]; it now appears that fenestrated capillaries of the intestine have similar properties to the continuous capillaries (apart from those at the blood/brain barrier) in their ability to restrict the movement of materials. This may be due to either the fenestrae themselves, or to the basement membrane.

XI.3(c). Post-venule capillaries

The work of Palade and coworkers has shown that in the post-venule region a fraction of the intercellular junctions are open to the extent of about 2 nm, which may have a relevance in the site-specific delivery of drugs to these and other regions [190].

XI.3(d). Lung endothelia

The capillaries in the lung are selective to macromolecule passage in that the in lymph-to-plasma ratio decreases with molecular size. Thus, it has been found that as the molecular *radius* of a macromolecule increases from 3.7 nm to 11.0 nm, the ratio falls from 0.7 to 0.25 [106]; in addition, at a lymph-to-plasma ratio of about 0.4 the rate of decrease in extravasation is much slower as the radius increases – which is similar to most other tissues observed [106].

XI.3(e). Skeletal muscle, adipose tissue and myocardial endothelia

Estimates for these tissues are limited, though those data which are available give a molecular size selectivity. Considerable information is available, though, on the effects of various pharmacological and pathological interventions on the permeability of muscle capillaries to macromolecules. For example, it has been found that histamine and bradykinin increase the permeability of muscle capillaries, though ischemia (up to 2 h) does not appear to have any effect on the permeability of muscle capillaries to plasma proteins. In contrast, thermal injury, diabetes mellitus, increased venous pressure, hypertension and vibration stress do.

XI.3(f). Nervous system endothelia

Access of macromolecules to one of the most desirable of all compartments, the brain, is very difficult. We shall learn later of a possible role for blood/brain endothelia receptor-mediated processes (see subsection XI.4); however, in terms of passive entry into the *central* nervous system it is known that the brain microvasculature represents a further different structural type. Thus, the lining is formed by a continuous, non-fenestrated endothelium with tight junctions and negligible pinocytic vesicle activity. This assembly is greatly affected by pathological conditions including osmotic shock, thermal injury, arterial hypertension, air or fat embolism, hypovolemia and traumatic injury. These can give rise to opportunities for selective drug delivery. For example, during dysfunction of the blood/brain barrier, increased pinocytic activity is an important mechanism for macromolecule entry into the CNS. This is the case in acute hypertension, ischemia, compression injury, and seizures, and is also found after administration of histamine [191].

In addition, certain regions of the brain (e.g., the choroid plexus) have fenestrated capillaries with endothelia connected by gap junctions. It is known that pinocytic transport occurs in these endothelia, and materials such as horseradish peroxidase readily gain access to the cerebrospinal fluid (CSF) in this way. Prior to entering the CSF the macromolecules must pass the choroidal epithelial membrane, and it now appears that it is this latter membrane which is the rate-limiting barrier to the movement of macromolecules from blood to the CSF [192], which gives rise to the postulate that macromolecules can find their way into the CSF via both leaks within the epithelial membrane and vesicular transport [106].

Permeability studies on the endothelia of the *peripheral* nerves show that there is considerable passage of macromolecules across the capillaries supplying both peripheral nerves and ganglia. This is presumed to be due to the fact that some of these are fenestrated.

XI.3(g). Liver

Macromolecules of different sizes are transported at different rates through the liver blood–lymph barrier (Fig. 26), and it has been suggested that the rate-limiting barrier for this is the interstitial matrix [193]. As expected with such a metabolically important organ, the extraction of proteins and their transport into and through lymph is a most complex one. It is believed that the interstitium of the liver is in direct contact with the peritoneal cavity space complex, and this has a marked influence on trans-sinusoidal movement. Water and macromolecules are continuously entering the interstitium and being removed via the lymphatics and the peritoneal cavity; in turn, the peritoneal fluid is absorbed by lymphatics and capillaries within the peritoneal lining [194].

To date, it does not appear that many (if any) site-specific systems have been rationally designed in cognisance of any of the normal physiological processes and anatomical features which have been described above. It is hoped that this position will change as more information becomes available on the selective extravasation of *endogeneous* materials.

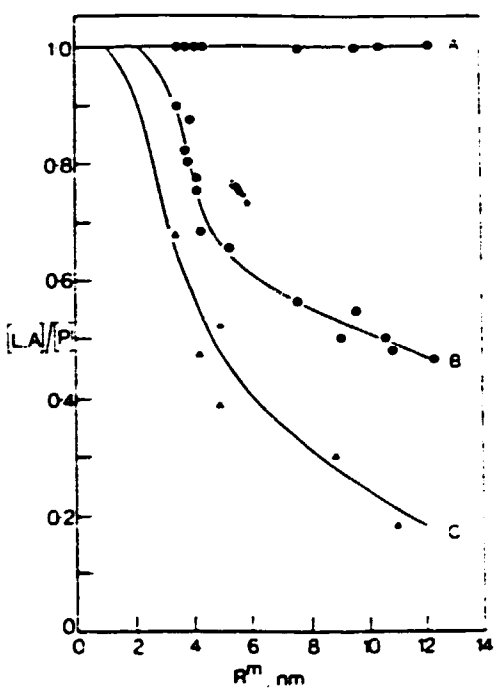

Fig. 26. Relationship between the lymph or ascites-to-plasma ratios ([L.A]/[P]) for endogenous proteins and their effective molecular radius (R^m, nm) in the liver after acute venous hypertension (A), under normal circumstances (B), and during chronic venous hypertension (C). (Adapted and redrawn from Ref. 106 and references contained therein.)

XI.4. Receptor-mediated events

Endothelial cells are able to process both soluble macromolecules and particulate materials via pathways which include active formation of endocytic vesicles. Considerable evidence exists to show that endothelia undergo endocytosis of both the fluid phase and the constitutive and the non-constitutive receptor-mediated types [50]. The size of endocytic vesicles (20–100 nm) is of importance when considering whether particulate carriers could use this route to either enter cells (i.e., endocytose), or even pass through them (i.e., transcytose). Some evidence exists with in vitro systems to suggest that *particulates* of less than 40 nm are able to enter such pathways, in particular when receptor-mediated events are involved. Similar studies are beginning with whole organs. For example, the presence in (mouse) *continuous* endothelia of the lung, heart and diaphragm of receptor-mediated *transcytotic* routes for albumin has recently been shown [195]. It appears that either receptor-mediated endocytosis and/or transcytosis can occur in the endothelium, and further recent work has provided evidence to suggest that low-density lipoprotein can be transported in both ways [196]. The work with albumin is particularly interesting, since it could provide a general route for transcytosis of materials in selected tissues. In detail, workers in the former study have, for example, perfused homologous and heterologous albumin-gold particle complexes (AGC) into continuous endothelial capillaries of mice in situ [195]. Using gold particles of mean diameter 5 nm they have been able to show that for the endothelia examined (i.e., in lung, heart and diaphragm), AGC is first adsorbed onto specific binding sites and then transported in transcytotic vesicles *across* the endothelium by receptor-mediated transcytosis and, to a lesser extent, by fluid-phase processes. Although no binding could be found when the complex was injected in vivo (even with the portal vein and hepatic artery ligated to minimise liver uptake), these workers have suggested that the existence of albumin receptors on such continuous endothelia may provide a specific mechanism for the transport of albumin, and hence, for adducted particles.

Further, after passage through discontinuous endothelia, site-specific delivery systems can enter parenchymal cells and hence leave the cardiovascular system through receptor-mediated events. For example, low-density lipoprotein (LDL), the carrier for cholesterol ester, which is monolithic and has a diameter of about 22 nm, is able to pass through liver sinusoids, enter the Space of Disse and then be processed into the liver hepatocytes after interaction between the apolipoprotein ligands on the surface of LDL and specific receptors on the surface of the hepatocytes. Interestingly, manipulation of the surface of LDL by acetylation causes it to be recognised and be taken up via an endocytic pathway by liver *endothelial* cells rather than the parenchymal cells [197]. Numerous other studies exist which show that not only can particles interact with underlying parenchymal cells in the liver, but also that this can be modified by altering particle surface with ligands specific for the plasma membranes of various liver cells – leading to their redistribution within the liver, as well as to other organs such as the spleen [198]. Such ligands are often simple carbohydrates, and this redistribution reflects the diverse

carbohydrate recognition assemblies present throughout both of these organs.

Low-density lipoprotein itself has been examined as a carrier of drugs and radiolabels because of its unique deposition, and has been studied for the imaging of injured and healing, arterial walls and the adrenal cortex [199], and for targeting to cancerous cells (e.g., Ref. 148).

These data reflect the principle that particles of the correct size may have their surface altered to enable them to be taken up not only by specific organs but by specific cell types within that organ, and that endothelial uptake and passage of particles are also possible.

We have seen (page 154) above that the *passive* entry of macromolecular systems into the central nervous system is unlikely in the normal state; however, the blood/brain surface does possess markers in some abundance, including (in the rat) the receptor for transferrin [200]. Other intriguing findings give rise to optimism that transcytotic pathways for macromolecules exist, for example, transferrin is found within the brain [201], and although horseradish peroxidase stains cerebral endothelia and is not present within the brain, microperoxidase (molecular mass, 2 kDa) transfers in vesicles – though whether this is bound or not is open to some question [202].

Other unique barriers include the blood/nerve barrier. The recent results by Seitz and coworkers show a partial permeability of the normal mouse blood/nerve barrier for both homologous and heterologous immunoglobulin G, that may be due to receptor-mediated events [203], and which appears to be a further opportunity for site-specific drug delivery using macromolecular assemblies.

XI.5. Pathological opportunities for extravasation

XI.5(a). Inflammation

The hyperpermeability of endothelial barriers at various sites of inflammation is well established. This has been regarded as being of potential use for the selective delivery of antiinflammatory drugs to inflamed *extravascular* regions. Numerous studies have been carried out. For example, in areas of inflammation induced by carrageenin, the accumulation of lipid microspheres of approx. 200 nm diameter around endothelial cells of blood vessels, and the penetration of these to the outer layer of blood vessels, has been reported [204,205]; although some groups have shown that in (other) models of inflammation such extravasation does not occur [146]. Interestingly, an enhanced antiinflammatory response has been demonstrated with cortisol palmitate incorporated into liposomes after intravenous administration to rat models of inflammation [206]; and a recent clinical study in 138 patients with rheumatoid arthritis proved with bi-weekly intravenous administration of liposomal steroids, a slight improvement in drug efficacy was achieved, together with a much lower frequency of side-effects when compared to controls [207].

Inflammation can cause regional changes in the structure, chemical composition and permeability of the endothelium. Permeability changes appear to be due to the effect of histamine and bradykinin which act directly on the capillary venule

endothelial vessel wall, with various other mediators (including leukotriene B4 and the complement enzyme C5), effecting a rapid interaction between venular endothelial cells and circulating neutrophils. Arfors and coworkers have postulated that the increased transport of macromolecules is actually due to the production of openings in the endothelium, which vary in size between 80 and 1400 nm and which are associated with an increase in the contraction of the endothelial cells [208]. (They found also that whilst macromolecules of size up to 37 kDa passed via diffusion-related processes, larger molecules were transported by *convection*.)

What this hyperpermeability means in terms of the pathogenesis of the underlying disease and the adequate *retention* of the carrier, is unknown, particularly when one appreciates that inflamed sites often contain phagocytic cells. Interestingly, intravenously administered radiolabelled 'small' liposome particles can be used to image joints of patients affected by rheumatoid disease [209]; though remarkably, it was found that when the disease is in remission, with no active synovitis, then accumulation of the radiolabel did not occur. This group has suggested that some of this accumulation is due to phagocytic activity.

XI.5(b). Ischaemia/hypertensive vascular lesions

An increased permeability in the endothelia is seen as an important factor in the pathogenesis of hypertensive lesions, leading to infiltration and accumulation of plasma material. For example, in experimental malignant hypertension, colloidal iron and carbon particles of between 5 and 50 μm diameter are able to extravasate [210]. Also, as given previously in subsection XI.2, capillaries have been shown to be permeable at sites of tissue ischaemia, both in the mesenteric artery [211] and in the myocardium [212]. However, this is not the case with other hypertensive states. For example, in spontaneously hypertensive rats, the endothelial wall, although exhibiting various severe types of damage, does not have an altered permeability for colloidal carbon. Fig. 26 shows that in acute venous hypertension there is a high degree of permeability for molecules of radii up to at least 14 nm, though in chronic states this is lower than found with normal conditions. Further evidence of the complex relation between endothelial permeability for macromolecules and disease has been presented by Majack and Bhalla [213]. These workers demonstrated that in the spontaneously hypertensive rat, the endothelial wall, whilst exhibiting various severe types of endothelial damage, does not have altered permeabilities for colloidal carbon – reflecting an adaptive and protective function in (chronically) hypertensive species; though what is most interesting is that in mesenteric postcapillary venules (diameter 9–32 μm) permeability alterations occurred, and were mediated through the formation of inter-endothelial gaps (due possibly to an over-production of histamine). These workers have also shown that antigenically stimulated lymphocytes can induce angiogenesis in vivo, and that a major cause of lymphocyte-induced angiogenesis can be attributed to the release of lymphokines acting on capillary endothelial cells. Since organ-specific antigens are present on the capillary endothelium, it follows that lymphokine release may occur locally when lymphocytes sensitized against organ-associated antigens are re-exposed to these antigens during their traffic through the local microvasculature.

This leads to the suggestion that neovascularisation, vasculitis and other vascular manifestations associated with inflammation, could appear localised because of a selective restimulation of lymphoid cells at specific organ sites. This may be exploitable for the selective location of site-specific systems.

XI.5(c). Tumour endothelia

Poste has described as a 'fading prospect' the possibility of an increased permeability of tumour endothelia enabling access of colloidal particles [146]. Although it is true that the permeability of the microcirculation in tumours is often higher than that found for normal tissues, it is highly variable and, as Poste points out, is an unpredictable component of tumour physiology. For inorganic colloids only limited access has been shown, and then when particles are as small as 30 nm in diameter. Knowledge on the vasculature of tumours is scanty. In some models of cancer endothelial cells can be seen which are leaky (e.g., Lewis lung carcinoma), but due to a build-up of necrotic tissue, areas of endothelial damage are often poorly perfused and only limited access is possible. Unfortunately, in the studies performed to date, particles targeted to tumour masses have had to *compete* with the MPS cell pool, and although 'fading', the prospects for accumulation of small drug-bearing particles may be brighter if this competing pool was negated by, for example, using sterically stabilised particles (see subsection X.5). Poste has given a detailed discussion on tumour endothelia and their role as a barrier to colloidal particle-passive extravasation, and shows that in contradistinction to arterial endothelia, changes in venous structures are quite common – malignant tumours, for example, generally invade veins [146].

For active targeting to tumours, irrespective of the serious challenge that tumour cell heterogeneity and extravasation pose, during endothelial proliferation in, for example, tumour angiogenesis, high levels of receptors may be expressed which are normally present at low levels (see subsection XIII.6).

A further approach using particulate carriers in cancer chemotherapy has been the use of particles containing magnetic material. Here, drug-bearing systems can be directed into the tumour mass using two-dimensional magnetic fields. This approach has been used for monolithic particles [214], and for oil-in-water emulsions [215], and is a further means of avoiding the competing MPS cell pool [216]. The method relies on knowledge of the position of the tumour mass, and it is unlikely to have any great applicability in cancer chemotherapy, except perhaps for some lung-associated tumours [89], and in cancerous areas in joints.

XII. Extravascular, extracellular targets

The above discussion demonstrates that numerous pathways can lead site-specific systems to both extravascular-extracellular and -intracellular positions (Fig. 5). Extracellular targets can be located by either passive diffusion or convection, and/or via specific receptor – ligand interactions.

Extracellular matrix components present as the basement membrane have a number of important physiological roles, including cellular translocation and

TABLE XXI

PARTICULATE CARRIER SYSTEMS - UTILISATION

Compartment/method	Targets
Discrete compartments (0.005–100 μm)*	
Eye	Infection
Lung	Allergy
Joints	Arthritis
Gastrointestinal tract	Crone's disease, immunisation
Intralesional	(e.g., intratumour)
Bladder	Infection
Cerebral ventricles	Infection
Interstitial administration (0.005–100 μm)*	
Subcutaneous	Lymph node targeting (e.g., some cancers)
Intraperitoneal	
Intramuscular	Depot for anaesthetics, peptides
Intravascular targets	
Diseased macrophages (0.1–1.0 μm)*	Parasitic, fungal, viral, enzyme storage diseases; autoimmune diseases; gene therapy
Capillary filtration (>10 μm)*	Cancer, emphysema, thrombi, drugs acting on local endothelia
Circulating depot (0.1–1.0 μm)*	Antiinfectives; antileukaemics; antithrombotics; antivirals; release of polypeptides and proteins
Other blood cells (0.1–1.0 μm)*	Cancerous; platelets; gene therapy (bone marrow erythroblasts); immune cells (vaccination/adjuvant); antivirals
Extravascular targets	
Macrophage activation (0.1–1.0 μm)*	Abnormal cells (e.g., cancerous, virally infected)
Continuous endothelia (<0.04 μm)*	Fluid-phase non-adsorptive endocytosis; receptor-mediated endo-/transcytosis
Discontinuous endothelia (<0.15 μm)*	
Basement membranes	Spleen, etc.
Parenchymal cells	Liver
Diseased endothelia (<0.5? μm)*	Hyperpermeable (e.g., inflamed-rheumatoid arthritis, malignant hypertension); proliferating endothelia (invasive carcinoma)
Denuded endothelia	Myocardial infarct, transluminal angioplasty
Basement membrane	Inflammation, wound healing
Ex vivo (> 0.5 μm–50 μm)	
Cells	Cell separations (e.g., for neuroblastoma of bone marrow); cell targeting (e.g., for gene therapy)

* Probable size, size range of particles applicable.

TABLE XXII

PARTICULATE DRUG DELIVERY SYSTEMS (ADAPTED FROM REFERENCE 32)

Matrix material	Diameter (μm)	Intended or suggested use	Actual or suggested active molecule
Chylomicrons	0.1–0.5		Factor VIII
Low-density lipoprotein	0.017–0.025	delivery of neoplastic cells	methotrexate, anticancer agents
High-density lipoprotein	0.007–0.012	delivery to adrenals & ovaries after intravenous injection	Gram-negative lipopolysaccharide
Polyalkylcyanoacrylate	0.2	lysosomotropic after intravenous injection	antimitotics (e.g., daunorubicin actino-mycin-D doxorubicin
		blood glucose regulators	Insulin
	0.213	Intraarticular injection	triamicinolone diacetate
Ferromagnetic polyisobutylcyano-acrylate	0.22	biodegradable TDS with extracorporeal guidance	^{3}H-dactinomycin
poly(methylmethacrylate)	0.1–1.0	vaccine therapy	vaccines
Polyamide	60–120 (mean 72)	oral for saccharose intolerance	L-invertase
Polyacrylamide	0.3, 18, 36	intraperitoneal, intravenous for acute leukaemia	L-asparaginase
	0.25–3	intramuscular subcutaneous for reduction in enzyme antibody effects	L-asparaginase
	0.7	lysosomotropic after intravenous injection to treat enzyme deficiencies (e.g., adult Gaucher's disease)	enzymes
	130–630	oral-controlled delivery	tetracycline hydrochloride: theophylline
Polyacrylidextran		biodegradable TDS for protein delivery	proteins, e.g., L-asparaginase
DL-polylactic acid	25–75	subcutaneous delivery of local anaesthetics	dibucaine: tetracaine
	125	subdermal antimalarial implant	quinazoline analogues
Sulphonic ion-exchange resin	38–297	oral, anthelmintic	levamisole
Carnauba	30–800	chemoembolism TDS for intraarterial delivery of cytostatics	5-fluorouracil: CCNU, methotrexate
Ethylcellulose	225 (mean)	arterial chemoembolism for delivery of cytostatics to kidney and liver	mitomycin-C
Ferromagnetic ethylcellulose	307	extracorporeal guidance to tumours	mitomycin-C
Modified cellulose	40–160	Nondegradable (model) parenteral TDS for delivery to lungs	methotrexate

TABLE XXII

Matrix material	Diameter (μm)	Intended or suggested use	Actual or suggested active molecule
Gelatin	0.301	intraarticular injection	triamcinolone diacetate
	i.e. 1.9	intralymphatic delivery (lymphotropic) as microsphere-in-oil emulsion for delivery of cytostatics	5-fluorouracil
	0.28	delivery to liver and spleen after intravenous injection	bleomycin water-soluble drugs
Gelatin core with dextran conjugate of drug	15	lung, after intravenous injection	mitomycin-C dextran conjugate
Dextran cross-linked functionalised by carboxymethylation	10–30	intra-tumour direct delivery	doxorubicin, mitomycin-C
Self-forming microspheres:			
(i) Polymercaptal	0.8	oral and hemoperfusion for treatment of heavy metal poisoning	mercaptal
(ii) Insulin	0.2	oral and intramuscular delivery for treatment of diabetes	insulin
(iii) Haemoglobin	5–60	oxygen transport function	haemoglobin
Polystyrene	3–25	percorneal retention studies	
Agarose		injection into tumour tissue	mitomycin-C
Ferromagnetic starch	1–50	extracorporeal manoeuvering to tumour tissue after IV injection	ethanolamine and albumin as model compounds
Starch	43	temporary intestinal hypoxis as a radiation protective	non-drug use
Starch (Spherex®)	40	intraarterial administration of cytostatics	5-fluorouracil hepatic BCNU renal actinomycin-D
Albumin	0.1–1	IV immunosuppressives delivery: infestations of the MPS (e.g., histoplasmosis, typhoid)	mercaptopurine-[8-^{14}C]-hydrate
	0.169	Intraarticular injection	triamcinolone diacetate
	0.66	intravenous delivery to liver (MPS)	5-fluorouracil-6-^{3}H
	2–12	general drug carriers	
	10–30	Intraarterial delivery to tumours	doxorubicin, bleomycine, 5-flurouracil methotrexate
	10	intravenous delivery to lungs of anti-allergic compounds	sodium cromoglycate

TABLE XXII

Matrix material	Diameter (μm)	Intended or suggested use	Actual or suggested active molecule
	7, 15	treatment of emphysema (IV)	leukocyte elastase inhibitor
	10–200	intramuscular or subcutaneous depot	norgestrone; progesterone
	all sizes	variously – intraarterial, intravenous	anti-asthmatics; analgesics; bronchodilators; narcotics; mucolytics; antibacterials; antituberculars; hypoglycemics; steroids; antitumour agents; amino acids
	10–40	supplementation of drug therapy using internal radiation via intra-arterial delivery of radiolabelled microspheres	yttrium 90
Ferromagnetic albumin	1–2	delivery to tumours via extracorporeal guidance	doxorubicin
	1–2; 2–4; 3–7	Intravenous delivery to lung; renal artery delivery	doxorubicin
	1	probe for neurological function	myelin basic protein
	7	Immunoglobulin incorporation using staphylococcal protein A	

produced de novo are beginning to be applied in other therapeutic areas (e.g., see subsections XIII.2, XIII.4 and XIII.5).

XIII.2. Disease-related considerations

Diseases often result in a unique biochemical marker being produced. For example, surface antigens may be expressed by virally infected cells, or spike proteins may be shed at the surface of cells infected by enveloped viruses, or tumour-associated markers (particularly oncofetal) on cancerous cells may have a greater abundance than on normal cells. We have already examined the role that the dysfunction and resultant abnormalities of endothelia can have in site-specific drug delivery in the central nervous system, and for targeting to various sites of inflammation. The utilisation of these markers for the in vivo radioimmunolocalisation of lesions has been reviewed by Carroll and Zalutsky [220].

In addition, it is possible to consider in the context of site-specific drug delivery, receptors for simple low-molecular-weight substrates, especially since they often exhibit a disease- dependency. For example, both cardiac adrenergic and muscarinic receptors appear to undergo quantitative shifts in their expression as a result

TABLE XXIII

EXAMPLES OF SUGGESTED SOLUBLE MACROMOLECULAR DRUG DELIVERY SYSTEMS

Material	Use
Proteins	
Antibodies,	
Antibody fragments, e.g.,	
collagen-specific drug toxin conjugates	Injured sites of blood vessel walls
	Tumour cells
Albumin drug-conjugates	Cancer cells (lysosomotropic)
Glycoproteins	Hepatocyte-specific agents (infectious disease especially viruses)
Lipoproteins	Liver, ovaries and gonads targeting in cancer chemotherapy
Lectins	General carrier/recognition ligand
Hormones	
toxin drug-hormone conjugates	Targeting to tumours
Dextrans	
Enzyme-drug-dextran conjugates	Targeting to tumours
	General carrier
Deoxyribonucleic acid	
Drug-conjugate	Lysosomotropic carrier for cancer
Synthetic polymers	
Polylysine	General carrier for targeting to cancer cells
Polypeptide-mustard-conjugates	Lung targeting
	Tumour targeting
Polyglutamic acids	General carrier for tumour targeting
HPMA	Lysosomotropic carrier for cytotoxics
Pyran copolymers	General carrier for cytotoxics
Poly-L-aspartic acid	Hydrolysable targeting carrier for cancer

of such diseases as diabetes and myocardial ischemia; and neurotransmitter receptors in selective regions of the brain have altered expressions (though not binding capacities) in, for example, Parkinson's disease, Alzheimer's dementia and schizophrenia (reviewed in Ref. 220).

Importantly, a disease can lead to the down-regulation of receptors, with an apparent alteration in targeting potential – as is observed with some genetic disorders. For example, four classes of mutations in the structural gene for the receptor for low-density-lipoprotein lead to either a deficiency of receptor at the plasma membrane, or to insertion of a receptor which is either unable to bind LDL, or to carry it into the cell [221]. Alternative receptor-mediated events can be used to circumvent these (and presumably other similar) pathological problems (e.g., Ref. 222).

Specific successes and failures in the use of site-specific drug delivery systems will now be examined.

XIII.3. Genetic disorders

XIII.3(a). Enzyme-storage diseases

As discussed in the introductory Sections, molecular biology is helping to create a mechanistic understanding of disease. Included in this are the lysosomal storage diseases, for which patient molecular biology for the 30 known human diseases is known. Considerable genetic heterogeneity is evident with regard to the biosynthesis of the necessary enzyme molecules. These are glycoproteins, and often post-translational mutations appear to be the cause of the diseases. For example, in mucoliposes types II and III, the mannose 6-phosphate moiety of the enzyme is erroneously expressed. This is the signal necessary for hepatic extraction and translocation via endosomes to the lysosomes where substrates await. The correction of primary defects can be treated with gene products by one of two routes, either of which requires some element of targeting: (a) with enzyme supplementation/replacement: and (b) with replacement of defective genes by normal genes.

Although infusions of enzymes can lead to encouraging alterations in various biochemical responses, no consistent clinical effect can be shown. Such (genetically produced) enzymes degrade at 37°C and/or are proteolytically degraded, and give rise to immune responses through the production of anti-enzyme antibodies (as foreshadowed in subsection III.1). Data on the lysosomal processing of enzymes, lectin – cell surface interactions, and the added understanding of mechanisms and routes of endocytosis, do indicate that receptor-mediated targeting can direct glycoprotein hydrolases to the storage cells of the peripheral organs where they will reach the lysosomes (and perhaps even some secondary lysosomes) after endosomal trafficking [222]. Various carrier systems have been suggested to exploit these phenomena, including microspheres, low-density lipoprotein, fibroblasts, leucocytes, encapsulated cells and soluble conjugates (reviewed in Ref. 34). An example of the use of liposomes is that recently reported on the successful delivery of β-galactosidase to the liver and spleen cells of Twitcher mice (acting as a model for globoid cell leukodystrophy) [142].

These carriers serve either to act as circulating depots for enzymes or are both actively and (mainly) passively taken up by cells to intracellular loci where enzyme substrates have accumulated (e.g., in the lysosomes of Kupffer cells). (The recent book edited by Barranger and O'Brady contains a suggestion on the use of lectin receptors to increase delivery to cells where sphingomyelins accumulate [222]; this appears to be appropriate, and should be a fruitful area of research. Naturally, like many problems requiring targeting, to understand the signals responsible for directing materials to certain intracellular positions may be vital.)

For some enzyme storage diseases alternative approaches of using activators and stabilisers of enzymes are being discussed. Two challenges for site-specific delivery become apparent. Firstly, in some cases, these new classes of effector molecules have to be directed to specific regions of the central nervous system, e.g., neurones: and secondly, the recognition signals required for these secondary mediators are unlikely to be the same as for the glycoproteins, although an approach here could be to turn these activators into neoglycoproteins.

XIII.3(b). Gene therapy

The control of gene expression has raised the possibility for some fundamentally novel types of therapeutic interventions. This is particularly the case where a genetic defect is responsible for the condition. Hence, studies on the molecular basis of hereditary diseases have given hope for new strategies for their treatment, using either gene therapy or by exploiting other regulatory and/or structural cell processes. Gene therapy is a method by which a correctly functioning gene is introduced into the body, where it is then able to express a desired protein. The approach still has many technical, ethical and legal issues to be resolved, though it is being evaluated in many laboratories. Inherited diseases of the haemopoietic system such as thalassemias and combined immune deficiency diseases are likely candidates for treatment in this way [4]. Thus, the second strategy for treating the primary defect leading to lysosomal storage diseases would be to replace defective genes with normal ones. We have earlier alluded to the advances being made in molecular and cell biology that have caused several groups to initiate the construction and sequencing of a physical human genetic 'map' (see subsection III.3). Although the task is a mammoth one (the human genome has approx. 3 billion base pairs, i.e., 3300 centimorgans), the combination of using RFLPs, in situ hybridisation techniques, and new methods such as pulsed-field gradient electrophoresis, has brought the construction of this map into view. It is considered that as these and other techniques of sequencing improve, disorders of Mendelian inheritance (such as cystic fibrosis) will be able to be exactly defined, and that as more knowledge develops, disorders which exhibit familial clustering and which are perceived to result from a multifactorial inheritance, such as coronary artery disease and congenital malformations, will become well defined [223]. Thus, as the genes relating to different diseases become known and then available, the hope is that somatic gene replacement should also become possible.

Formidable problems exist, including the need for strict definition of the gene, such that, for example, no activation of a proto-oncogene could occur, etc. In its simplest case (for example with type-I Gaucher's disease and type-B Niemann-Pick patients), the potential exists for delivery of the correct gene into the stem cells of the bone marrow. For disorders where the central and peripheral nervous system are involved, any similar adventure would require a delivery system of ultimate site selectivity and release. Some groups have suggested the use of (non-toxic) retroviruses for this (see Ref. 222).

The targets for gene therapy (Table XXIV), and the existing strategies for the delivery and expression of genes in vitro and in vivo have been recently reviewed [224]. In principle of course, genetic defects could be cured by either in situ repair of a defective gene or by the addition of a functional gene to the genome. Although possible in prokaryotes, the former approach (i.e., homologous recombination) is not efficient enough for utilisation in humans, and hence most current effort is on approaches intended to insert a functional gene into the *genome*. In vitro techniques (see Ref. 224) may be classed as (a) chemically mediated uptake; (b) physical (e.g., microinjection); (c) fusogenic, (e.g., using DNA-loaded vesicles such as fusogenic liposomes); and (d) viral (i.e., for both DNA and RNA viruses).

TABLE XXIV

BIOCHEMICAL LESIONS AND DISEASE TARGETS FOR GENE THERAPY (ADAPTED WITH PERMISSION, FROM REFERENCE 224)

Abnormal gene	Disease: tissue
β-Globin	Sickle cell: blood
	β-Thalassemia: blood
α-Globin	α-Thalassemia: blood
Pyruvate kinase	PK deficiency: blood
Glucocerebrosidase	Gaucher's disease: blood:bone
Adenosine deaminase	Immunodeficiency: blood:liver:spleen
Hypoxanthine-guanine phosphoribosyl transferase	Lesch-Nyhan syndrome: blood nervous system
Purine nucleoside phosphorylase	Immunodeficiency: blood
Factor VIII	Haemophilia: blood
Factor XI	Christmas disease: blood
Phenylalanine hydroxylase	Phenylketonuria: liver/nervous system
LDL receptor	Familial hypercholesterolaemia: liver

Wilson has recently argued that the use of genetically engineered (i.e., recombinant) retroviruses has a number of significant advantages over other techniques (see Table XXV) [224]. What is currently apparent is that, although it is possible to infect, for example, haematopoietic tissues with retroviral delivery systems in vivo, normal expression of the inserted genes has not yet been satisfactorily achieved in vivo; the reasons for this are unclear.

The tissue tropisms of viruses have caused others to suggest that an approach to the delivery of genes in vivo would be to construct a viral vector using an envelope glycoprotein gene from a strain with the appropriate tissue tropism [225]. (Such a tropism could be fairly non-specific if a tissue-specific promoter of the expression was incorporated into the delivery system.) The remarkable property of such a carrier is that its use could be extended to the transport of any drug including polypeptides. However, for the in vivo use of retroviral carriers, large problems with immunogenicity are to be expected (see also Section XIV).

Liposomes have been used to target genes in vivo. Thus, transient expression of a rat pre-proinsulin 1 gene has been observed in cells of the liver and spleen [226]. However, for sites other than the liver and spleen, this would appear to be a rather unsatisfactory delivery mode unless the cells of the mononuclear phagocyte system can be adequately avoided (see strategies in subsection X.5). It is not clear whether this route for delivery would result in the DNA being available directly or indirectly into the nucleus, although the combination of this approach with a gene conjugated with a (retro)viral vector could assist this process. Delivery of mRNA solely into the cytoplasm could still produce the desired effect, though the mRNA would need to be constantly presented to the cytoplasm, rather than only once as is assumed to be necessary in the delivery of DNA to the nucleus – where it would integrate with the chromosome of the host.

TABLE XXV

RETROVIRAL VECTORS AS GENE DELIVERY SYSTEMS (ADAPTED WITH PERMISSION FROM REFERENCE 224)

Advantages
All target cells may be infected
All target cells can express viral and exogenous genes
Limitless number of cells may be infected
Generally have stable single copy integration at random sites
Structure of integrated viral DNA known
Wide and controllable host range available
Normally not harmful to cells
Flexible genome that can accomodate up to 10 Kb new genetic information
Replication-defective systems can be engineered
Disadvantages
Deletion of viral sequences during replication
Recombination with endogeneous viral sequences to produce packageable, infectious recombinant virus
Activation of cellular oncogenes
Introduction of viral oncogenes
Inactivation of genes

XIII.4. Acquired immune deficiency syndrome (AIDS)

The recognition of the importance of retroviral diseases, including acquired immune deficiency syndrome (AIDS) and other associated manifestations of infection, with human immunodeficiency virus, has led to a rapid proliferation in studies aimed at understanding the pathogenesis of these diseases, so that suitable approaches to either immunisation or drug intervention can be devised. These efforts have led to an understanding of the tropism of the virus, and particularly the involvement of its surface proteins (especially gp 120), with host cells having the CD_4 receptor on their surface. Entry of human immunodeficiency virus is thought to involve a cell recognition and fusion event, followed by rapid integration of the viral DNA into the host genome (see also Table XII and related text). In addition, some aspects of the means by which the various diseases associated with this viral infection arise are known, and interesting new findings such as the role of the core proteins in the pathogenesis are beginning to emerge.

These findings have led Broder to describe numerous putative strategies for intervention (Table XXVI) [227]. In the context of this present contribution it is fascinating to note how many of these have both a biotechnic component and an element of selective delivery to various cells or specific interaction with the causative agent, and is it not exciting to note the almost veracious manner in which targets for intervention such as the regulatory genes of the virus are discussed. Clearly, if the use of vaccines is to be complemented by pharmacological approaches such as those described in Table XXVI, then some form of site-specific delivery will be required. Whether this be by one of the numerous chemical routes [1,40] or via synthetic or bioengineered routes (or both) remains to be seen.

TABLE XXVI

STRATEGIES FOR INTERVENING IN THE PATHOGENESIS OF ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS) (DRAWN FROM REFERENCE 227)

Antibodies for the virus, cell receptor
Drugs to block or inhibit:
fusion
retroviral uncoating
RNase activity
pol gene-mediated 'integrase' activity
Antisense oligodeoxynucleotides (i.e., to inhibit *tat*-III or *trs* protein and so directly block expression of the viral genome)
'Antisense' viruses (i.e., a genetically engineered virus having a piece of mRNA which will bind to the messenger made by the wild-type virus and produce the same effect as above)
Myristylisation-, glycosylation- and protease-inhibitors or modifiers
Interferons

XIII.5. Control of the immune response

XIII.5(a). Graft versus host rejection diseases

We have seen in the above Sections (particularly Section X) that the blood compartment is a sea of opportunities for site-specific drug delivery. This understanding of the cell physiology of numerous blood cell subtypes has recently led workers to propose a unique approach to the treatment of graft versus host rejection disease which can occur after organ transplantation. Thus, a site-specific system has been developed which fights rejection from an understanding that the cytotoxic T cells of the immune system (which are activated at times of rejection) do so with their surface receptor for interleukin-2 now exposed [228]. Using genetic manipulation techniques, this group and others have conjugated that portion of interleukin-2 which interacts with its receptor on an (activated) T cell, with a toxic material – such as the toxic portion of diphtheria toxin. This cytotoxic site-specific system is suggested as being able to enter T cells specifically just as they prepare to reject the graft, and then to destroy them.

One interesting observation here again, is the de novo synthesis of a site-specific drug delivery system which has all of the features of site selection, access, retention and timing that we have argued for throughout. Similar approaches to producing hybrid (or fusion) protein site-specific systems are now emerging (e.g., Refs. 229 and 230). Table XXVII gives other genetically defined delivery systems.

XIII.5(b) Allergic encephalitis

Other workers have attempted a quite unique approach to manipulating the immune response to suppress various autoimmune or allergic events by introducing a carrier *containing* the disease target [231]; that is, in animal models, by introducing into the host liposomes coated with myelin basic protein, a suppression of allergic encephalomyelitis can be demonstrated. This may be due to the *target-carrier system* being a more attractive target to destructive T cells than is their normal target, i.e., endogeneous myelin. (Conversely, this finding may aid in designing carrier systems that can take drugs to discrete subpopulations of lymphocytes.)

TABLE XXVII

EXAMPLES OF GENETICALLY DEFINED SITE-SPECIFIC DELIVERY PRODUCTS

System	Company/Property
Antibody carriers	Cytogen (with specific linker chemistry)
Immunotoxins	Xoma – Xomazyme-Ivm – an anti-T-cell lymphocyte antibody conjugated to the A chain of ricin
Antibody	Centocor – 17-1A antibody indicated in colorectal cancer
Antiplatelet antibody	Centocor – for prevention of clot formation
Antivirus antibodies	Centocor – human antibodies for cytomegalovirus
Antiinfectives antibody	Centocor – for Gram-negative infections
Anticancer-immunological	American Cyanamid (CL-259763)
Glucocerebrosidase	Genzyme
Anticancer-immunological	Lilly (KS1/4-vinblastine)
Anticancer-immunological	Quadra logic technologies
	Eastman Kodak
	Merck
Antitumour protein hybrid	Teijin – antitumour Fab immunoglobulin and a substantial portion of fragment A of a diphtheria toxin
Hybrid protein	Harvard College – various fragments of diphtheria toxin linked together – and to a cell-specific polypeptide ligand – through peptide bonds
Fusion protein	Seragen – portions of interleukin-2 and diphtheria toxin, designed for attacking activated T cells

XIII.6 Cancer chemotherapy

Throughout this review mention has been made of the suggested role for site-specific drug delivery in cancer chemotherapy, albeit often in a negative way. It is certainly true that there is an enormous need to be able to deliver selectively cytotoxic agents which are potent against cancer cells in vitro to tumour masses in vivo, and, as discussed in Section IV, much of the modern impetus for site-specific drug delivery came from this almost emotive requirement. However, the tenets of access, retention and timing still apply here, and if the process of drug targeting in cancer chemotherapy is examined in the light of these, it is seen that many problems exist, particularly with tumours located in extravascular positions (Table XXVIII). We shall now examine the issues of access and retention in cancer chemotherapy, though these should be considered in cognisance of the properties of cell dynamics and kinetics of growth and responsiveness (to drugs) that characterise tumour cells.

XIII.6(a). Access and retention

We have examined previously the ability of macromolecules and particles to extravasate and in particular the potential uniqueness that the endothelia of tumours have (see subsection XI.5(c)).

A seminal study of the problem of access and retention in selective drug delivery to tumour masses is that due to Levin and coworkers, who have used a mixture of semi-empirical mathematical modelling and experimental procedures, to judge

TABLE XXVIII

SOME KEY ISSUES/CONSIDERATIONS IN THE DESIGN OF SITE-SPECIFIC DRUG DELIVERY SYSTEMS FOR USE IN CANCER CHEMOTHERAPY

Access
Exclusivity, abundance and trafficking of surface markers
Clonal heterogeneity and biochemical resistance
Extravascular location
Diffusion, percolation and binding of drug/carrier complex
Multiple sites
Retention of carrier/release and availability of drug
Membrane permeability
Drug diffusion and binding
Toxic side-effects
Dose/response relationship
Timing of availability/responsiveness of target
Polymediator cascades
Chronicity of growth and responsiveness

how to reach and maintain adequate levels of drug at/within the cells of a tumour located in the brain (sic) [232]. These workers made the assumption that the tumour was either distant from capillaries in normal brain, or was poorly perfused. Their other objective was to accomplish this without causing an unacceptable systemic drug toxicity.

In terms of drug delivery, the most suitable tumour to treat would be a small, well-perfused, non-infiltrative metastatic tumour. However, as Levin and coworkers remind us, metastatic tumours (in the brain) are not vascularised until they are approx. 200–400 μm in diameter. Also, most metastatic and primary tumours have diameters greater than 2 cm (approx. $4 \cdot 10^9$ cells), are infiltrative, and are developing hypoxic, and poorly perfused central regions by the time of diagnosis.

We have seen previously that the undamaged blood/brain barrier does contain some possibilities for targeting through receptor-mediated processes, and that brain penetration can be enhanced with damage or dysfunction (see subsections XI.3(f) and XI.4). Often, in clinical practice, the blood/brain barrier has a 'leakiness' that appears to be related to those malignancies found with glial and metastatic tumours, and which may be due to gaps forming between endothelial cells. Although this permits macromolecules to enter the extravascular extracellular fluid of a tumour, this does not seem a priori to be useful in terms of drug action. A tumour mass may be defined as having three regions, i.e., the poorly perfused low-permeability core, the well-perfused high-permeability outer shell, and the less permeable, less well-perfused, outermost shell. Even with highly permeable tumours, Levin and coworkers have demonstrated that for systems that are unable to cross the blood/brain barrier easily, the total exposure of tumour cells in these additional regions will depend upon the *persistence* of a diffusion gradient from permeable tumour to less permeable regions external to the main tumour mass.

clinical trials have now commenced. Much recent attention has been generated with these systems (see Table XVII). For example, attempts have been made to adjust the targeting of the conjugated molecules by adjusting the chemistry of the linkage between the toxin and the antibody so as to alter either the uptake by non-target cells such as the liver (e.g., Ref. 237), or the stability of the conjugate [42]. Also, recombinant DNA procedures have been used for the de novo synthesis of immunotoxin constructs (e.g., Ref. 235) (Table XVII). In addition, the literature contains numerous references to the use of immunoglobulin fragments to overcome non-specific binding events and to improve on extravasation, as well as combinations of monoclonal antibodies to overcome tumour cell clonal heterogeneity. However, it is unlikely that such approaches will ever be considered as general ones, though there is some merit in the current optimism that the use of immunotoxins used in conjunction with other treatment modalities may be a clinically viable approach.

XIII.6(c). Regulation of cell function

We have seen above that tumour cell heterogeneity may be a barrier to the development of effective drug delivery methods, since this could lead to the selection of resistant clones and cell adaption to the presence of drug. Methods have been proposed to circumvent the adaptive mechanisms that tumour cells have. Thus, studies on oncogenes have demonstrated that the genesis of a cell involves the replication and expression of nucleotide sequences peculiar to any one cell; these include both structural genes and some of the various controlling elements. This leads to the possibility that those cell mechanisms which are responsible for either maintenance or expression of the transformed state themselves become the target. Thus,

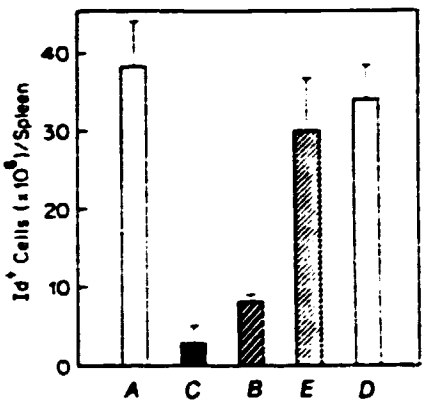

Fig. 28. In vivo treatment of BCL_1 leukaemia in mice using ricin-A chain immunoconjugates. Mice were injected intraperitoneally with $5 \cdot 10^6$ BCL_1 tumour cells. Three weeks later groups of four mice were treated with a single intravenous injection of saline buffer (A), or 0.5 mg of antibody-ricin-A conjugate (B), or an Fab'-ricin-A conjugate (C), or the antibody alone (D), or the unconjugated Fab' fragment (E), respectively. Id^+ cells are those viable cells in the spleen which bear the BCL_1 idiotype, and which are a good indicator of tumour burden. (Reproduced with permission from Ref. 235.)

for neoplasma. such targets include oncogenes. regulatory elements associated with oncogenes. or even the initial transcription or translation products of oncogenes [34]. This leads to strategies being suggested that include the use of selective inhibitors of oncogene-coded proteins that are selective; antibodies that recognise and inhibit oncogene-coded proteins; and synthetic oligonucleotides that can bind to and inactivate the oncogene itself. Similar to the strategies proposed for AIDS (subsection XIII.4) these approaches require a high degree of site selection. Attempts have been made to produce gene-specific oligonucleotides using a number of non-ionic nucleic acid methyl phosphonate molecules [238]. This work demonstrated that oligonucleotides complementary to a specific sequence exhibited on bacterial ribosomal RNA could inhibit protein synthesis in *Escherichia coli* – though not in cells of the rabbit reticulocyte system.

As we have seen above in subsection XIII.3 on gene therapy, and subsection XIII.4 on targeting in AIDS, other advances in nucleotide delivery include the development of retroviral vectors, and the production of 'antisense-RNA' for inactivating mRNA.

XIV. Clinical use of site-specific systems

Site-specific drug delivery serves to improve the therapeutic index by, variously, optimising drug access, and the amplitude and nature of drug interaction with its pharmacological receptor, as well as protecting the drug and the body from any (unwanted and deleterious) disposition which could lead to harmful degradation products and complicated dosage regimens (Table IV). This involves, inter alia, not only the use of novel cell-transport processes, but also novel patterns of drug release, which are used with a clinical appreciation of the *status* of targets (cellular or otherwise) within the patient. Poste (NATO ASI Conference abstract, 1986) has discussed the clinical use of lymphokine and cytokine peptidergic mediators, and shows that the exposure of target cells to prior mediators, the need for polymediator cascade adjustments, unique dose/response relationships, and chronopharmacology (i.e., the influence of diurnal and circadian rhythms on target cell number, anatomic localisation and responsiveness) must be clinically considered. To this list should be added the influence and the status of the disease on the (possible) passive and active processes used to process a site-specific carrier to its site of action.

In addition to questions concerning the clinical effectiveness of site-specific systems, it is probable that regulatory agencies will require information on a specific system *index* for target tissue, its general biological distribution, and system stability and catabolism routes. For site-specific systems which have a biotechnic component (e.g., Table XXVII), there are unique safety and efficacy issues. These are already being addressed by both regulatory bodies and the pharmaceutical/biotechnology industry alike; for example:

> "Biological monoclonal antibody products prepared by hybridoma technology; drugs and biological products produced by recombinant DNA technology ... intended for investigational use in human currently present a potential

for major advances in medical diagnosis or therapy. These new technologies pose unique quality control and safety problems that must be overcome before any such products are licensed and commercially marketed" (Federal Register, Jan 9, 1984, p. 1138)

XIV.1. Clinical utility

The proof of the clinical effectiveness and safety of site-specific systems, particularly those of biotechnic origin, will provide the medical community with a large and interesting scientific challenge.

The most intriguing question of all is: 'how can the efficacy and safety of site-specific systems be elucidated if these are designed as constructs which utilise human-exclusive cellular and extracellular processes?' [9.239]. In part, the answer to this involves the development of specific and unique tests (for example, for antibodies these may include antigen specificity, complement binding, etc.), though repetitive parenteral administration of human-specific systems in heterologous animals will probably give results that are meaningless in terms of either human toxicity or pharmacological profiles.

The determination of the *effect* of a site-specific system product is, of course, crucial. As mentioned above, an immediate point here is the species-specificity of a system, and how, if it is exclusively human-specific, can its *efficacy* (and *safety*) be determined? The challenge for pharmacology and toxicology is very complex, with issues needing to be considered such as up- and down-regulation of receptor-mediated processes being used to affect cell entry [50], biphasic dose/response relationships [7.8], and often involvement of peptidergic mediators as part of a polymediator cascade, and the pre-sensitisation of cells by supplementary mediators to affect target cell sensitisation and responsiveness, etc.

Involvement of clinical research staff in site-specific delivery products and their use in exploiting (patho)physiological states is vital. To underscore this in very simple terms one has to examine some of the clinical indicators of efficacy and safety for biotechnic site-specific systems and conventional molecules. Hence, for example with the latter, study of their pharmacology requires extensive animal and human studies, whilst with a human-specific biotechnic system considerations are largely theoretical with only limited human trials being of use. Similarly with toxicology studies, for synthetic low-molecular-weight entities, extensive animal and human studies are required, whereas animal studies are just not sensible with human-specific (biotechnic) products, unless they are considered to be part of an extensive tier assessment of safety (see subsection XIV.3).

XIV.2. Pharmaceutical development

Many of the issues for the use of macromolecular drug carriers with and without a biotechnic component and/or having a human-specific event, are entirely novel, and relate to identity, purity, effect and use. Most of the key features for the successful pharmaceutical development of site-specific soluble macromolecular and

TABLE XXIX

CONSIDERATIONS IN THE PHARMACEUTICAL DEVELOPMENT AND CLINICAL USE OF BOTH SOLUBLE MACROMOLECULAR AND PARTICULATE BIOTECHNIC AND SYNTHETIC SITE-SPECIFIC SYSTEMS

Specification activity
(i) Pharmaceutical development
A Production
Purity
Evaluation of novel production safety hazards (e.g., sparking with particulates)
Process reproducibility
B Characterisation
Identity
Conformation
Size
Size distribution
Charge, aggregation
Density
Surface configuration, homogeneity of attached moieties:
– polymers
– ligands
– spacers
C In vitro functionality
Drug-loading efficiency
Drug release
Retention of recognition characteristics, etc.
D Stability
Characterisation of the breakdown products:
– in storage formulation
– in biological fluids
Parameters to be assessed on storage:
– chemical stability
– character
– in vitro functionality
– sterility and functionality
– colloidal character (e.g., aggregation, size, charge, etc.)
– surface properties (including conformation and epitopic character)
(ii) Safety pharmacology (non-human)
A General consideration
General safety
Sterility and pyrogenicity
– major organ function tests
– acute and subacute toxicity studies
B Potential novel toxicities (adapted from Refs. 107 and 146)
MPS uptake
Uptake in specialised immune compartments (e.g., skin and gut)
Depression exhaustion of MPS
– bone marrow stem cell kinetics altered
– bacterial and viral infections
– immunological depression
– haemorrhagic and endotoxin shock
– altered drug response

TABLE XXIX

Specification activity
Low level activation of MPS
- Interleukin-1
- Amyloidosis
- Hyperplastic liver foci
- Altered stem cell kinetics
- Altered drug metabolism
- Altered response to drugs
C. Biotechnics
For biotechnics (and specifically monoclonal antibodies), factors affecting safety include:
Hybridoma background:
- murine-murine
- human-human
- interspecies (chimaerics)
Contaminants:
- general safety
- pyrogens
- sterile
- free of hazardous viruses
- free of detectable DNA
Interaction with the host:
- immunogenicity
- cross-reactivity
- hypersensitivity to foreign epitopes
- anti-idiotypic responses to normal cells
- immune complex disease
- potential MPS toxicity
D. Species-specificity
Issues include altered drug disposition/metabolism, and the need for a tier assessment of safety to include knowledge on:
- pathology of lymphoid tissues
- antibody and cell-mediated immunity
- host cell resistance
- phagocytic cell function
- immune/immunotoxicity reaction testing
- antigen specificty
- complement binding

(iii) Metabolism

Issues here include species-specific metabolism (related to novel patterns of drug release at receptor sites), and possible use of novel paracrine- and endocrine-like peptidergic mediators. These could manifest themselves in novel:

- dose responses
- absorption sites/rates
- bioavailabilities (at receptor)
- organ, tissue, cell disposition
- disease-dependent release
- excretion routes rates

TABLE XXIX

Specification activity
(iii) *Efficacy* Major considerations in the clinical development of a site-specific system, relating to effect, utility and efficacy could include: – novel pharmacokinetics and disposition – novel modalities of cell tissue receptor exposure – utilisation of novel cellular transport processes – species-specific drugs and delivery modalities – novel drug interactions – novel drug metabolism – local versus general distribution – biphasic drug action – chronopharmacology – new routes of administration: transmucosal; specific regional uptake in gastro-intestinal tract; novel cell transport processes; – new patterns of drug release: bolus first-order pulsatile; feedback control; disease-related release of drug; (Analytical techniques will need to encompass the identification of very low levels of site-specific systems and their degradation and metabolic products.)
(iv) *Extended non-clinical development* These parallel activities will include: – reproductive toxicology – chronic toxicology – selection of market formulation – definition of marketed specifications(s) – development and implementation of market-related scale-up – confirmation of specification following scale-up

particulate carriers are grouped together in Table XXIX. These challenges for successful development will often relate to the activities of preformulation and formulation groups. Thus, to the physical and physicochemical skills extant in an innovative and resourceful pharmaceutics department, will need to be added (variously) colloid and surface chemistry skills as well as protein chemistry and biochemistry. Unique issues, such as considerations of the effects of formulation additives and processes on the integrity and safety of these novel products will require examination; for example, what would be the effect of a specific buffer on the epitopic character of a proteinaceous carrier system?

The *identity* of materials produced by recombinant DNA technology present a particular problem. Thus, although it may be currently impossible to elucidate the structure of any natural protein, the primary structure of the product can be found using a combination of techniques, such as two-dimensional NMR, fast atom bombardment mass spectrometry, immunochemistry, protein content analysis, spectral analysis and electrophoresis, etc. Further, the analysis of dosage forms and body fluids for the content of biotechnic molecules and site-specific systems, requires advanced analytical techniques having high degrees of sensitivity and selectivity because of the low doses used, and the presumably high degree of target organ

extraction of the carrier on first pass. Because of the unique method(s) of their production, in which viral vectors and other quite exotic cell processing and expression tools and purification steps are used, a number of novel concerns arise with respect to the *purity* of *biotechnic* products. These include microbial contamination (viral, bacterial and mycoplasmal), nucleic acid contamination (i.e., possible untoward oncogenic sequences), and processing contamination (e.g., column leachable material, reagents, etc.) (see Table XXIX).

XIV.3. Safety pharmacology

XIV.3(a). General considerations

To systems produced by biotechnological means, apart from general safety concerns with biotechnics (including those relating to their production, e.g., hybridoma background, microbial contamination, nucleic acid and processing material contaminants; interaction with the host, e.g., immunogenicity, cross-reactivity – e.g., hypersensitivity to foreign epitopes, anti-idiotypic responses to normal cells, immune complex disease and MPS toxicity), will be added the influence of formulation variables on the effectiveness, safety and in vitro stability of the site-specific system (Table XXIX).

It is likely that site-specific drug delivery systems will be considered first for the delivery of drugs of known action and toxicity, although in the longer term their potential is more likely to be reached with new entities – particularly hybrid systems. For the former case, it is probable that carrier usage will require the following general information to be provided; that is, where the carrier system is taken up (localised) within the body; what the dose/response relationship for the system is; what its therapeutic index is; and what the metabolism of the carrier and the drug presented in this form is.

Various (potential) iatrogenic sequelae can be envisaged with the use of carriers with existing drugs. For example, an altered drug distribution may lead to autotoxicity, with MPS uptake giving rise to, say, toxic events or distribution to specialised immune compartments in, for example, the skin and the intestines. Hence, it is likely that the use of approved drugs in such carrier systems will require full-scale toxicity testing.

The evaluation of novel toxicities will be a priority in determining the clinical and commercial feasibility of site-specific systems, including those which have a biotechnic component and/or utilise human-specific and/or disease-specific processes to effect site access or retention, etc. For homologous biotechnics, although in animals there will be an induction of the immune response, repetitive parenteral administration will give effects that will be largely unrelated to either toxicity or pharmacological profiles in humans. Thus, long-term studies in animals will be meaningless. For heterologous molecules, additional specific and unique tests will be needed. For both types, safety pharmacology testing will require new approaches, including the use of transgenic species and various tier assessments, where a knowledge of toxic behaviour is built up in a composite manner using an assembly of various highly specific tests (conducted largely in vitro), including pathology

of lymphoid organs, antibody- and cell-mediated immunity, studies on host resistance, phagocytic cell function and the ability to induce hypersensitivity (Table XXIX).

XIV.3(b). Potential toxicities of particulate systems

The potential toxic effects that could arise due to the use of carriers are due to both the nature of the matrix material itself, and the effect(s) of colloidal particulate material. Table XXIX includes some of the potential problems which could arise with particulates; though it should be appreciated that many of these concerns have arisen with the development of liposomes which (at the time) tended to accumulate readily in the liver and the spleen [114,139,146,151,206,240], though some data are available on the effects of human albumin microspheres on the lungs after their capillary filtration [241].

The major concern is with potential immune responses and alteration (by either depression or activation) of the functioning of the MPS. Also, as for any novel administration or delivery mode, the potential toxic effects that new patterns of tissue exposure bring will need to be determined. To exemplify this it has recently been reported that in the mouse, liposomes administered intravenously are capable of transiently decreasing plasma fibronectin levels, which was correlated with an appearance of fibronectin in the liver – and may be associated with fibronectin acting as an opsonin (see subsection X.5(b)) [242]. As Norde has pointed out, such interactions could lead to changes in the secondary and tertiary structure of materials, which may have unforeseen biological consequences [152].

XIV.3(c). Potential toxicities of soluble macromolecular systems

The potential toxicities of some synthetic as well as biological macromolecular carriers have been studied. Clearly, synthetic polymers are chosen to be carriers because of either their degradability and/or their ability to become rapidly excreted from the body. Although little work has been reported on drug carriers per se, there is a body of literature relating to compatibility of polymer material used as protheses, etc. For soluble macromolecules, it has been found that numerous haematological and immune responses can take place, including antibody responses to various soluble copolymers [243]. The latter study found for copolymers of various peptides and substituted phenols with either *N*-(2-hydroxypropyl)methacrylamides (HPMA) and poly(*N*-(2-hydroxyethyl)aspartamide), that there was a stimulation of an in vitro and an in vivo anti-haptenic response depending upon the molecular weight of the conjugate and the level of hapten content in the carrier. However, these polymers did not stimulate B cells, and neither activated mitogenic responses nor stimulated lymphocytes (mouse or human) to polyclonal antibody response.

HPMA has been examined for its possible effect on the (porcine) complement system in vitro [244]. The complement system is an important part of the host defences, and its activation could at times be harmful. Thus, it has been found that HPMA homopolymers and copolymers terminated with carboxylic acids, amines, aromatic groups or puromycin, have no significant effect on the complement sys-

tem in vitro. Although, again, these types of study are only glimpses of what would be required if soluble macromolecular carriers were to be developed for general human use.

For biological macromolecular carriers, numerous concerns have been expressed about the use of murine monoclonals in terms of hypersensitivity and allergic reactions, etc. (Table XXIX). Perhaps a useful indication of the general safety of such systems is to be found in the data from an important recent study on immunotoxins, where it was shown that in rats a melanoma antigen-specific *murine* monoclonal antibody conjugated with ricin A chain was well tolerated at doses of 1 mg per kg per day after intravenous administration; though at higher doses numerous effects in haematological and serum chemistry were observed [245]; also, most of the observed changes were considered by these workers to be probably due to the toxicity of the ricin A chain (rather than due to the antibody portion of the drug carrier).

XIV.4. Pharmacokinetic/pharmacodynamic assessment

What role and challenges present themselves here for pharmacokinetics, in terms of simple data analysis as well as for understanding dose/response relationships and even the (clinical) management of the system [246]? If a system was largely extracted by a target organ, then the application of classical systemic pharmacokinetics would be inappropriate (see subsection IV.3), and it is clear that it will be the *pharmacodynamic* assessment of site-specific drug delivery systems which will be needed both to prove their efficacy and for their clinical management.

Few, if any, data exist to indicate whether current practices and models are appropriate for site-specific systems, though it is apparent that novel approaches will be required. It is encouraging that some useful new theories are beginning to emerge on the interchange between drug carrier pharmacokinetics and pharmacodynamics, and which may eventually have some use in deciding what molecules are candidates for drug targeting. For example, recent attempts have been made to describe the pharmacokinetics and efficacy of site-specific drug delivery systems both theoretically [21,247] and practically [49,198,248,249]. Much more study in this area is indicated.

XIV.5. Drug regulation

From the above, it is apparent that submission of site-specific systems will include data on their efficacy, which will address the issues of a specific index for target tissue(s), general biodistribution (including kinetics), in vitro and in vivo stability, and catabolism routes and rates, as well as general-excretion rates. For site-specific systems that contain a biotechnic component it is evident that currently existing regulations will be used; that there will be no further issue of formal guidelines (at least by FDA); that there will be case-by-case consideration; and that (in the U.S.A.) a full NDA will be required. To indicate the complexity of a submission document, it is instructive to examine some of the data that will probably

TABLE XXX

SOME POSSIBLE DATA REQUIRED FOR THE SUBMISSION OF A GENETICALLY DEFINED SITE-SPECIFIC DELIVERY SYSTEM TO REGULATORY AGENCIES

Gene synthesis
Confirmation of cloning and expression with sequence insertion
Host strain, and storage of same
Fermentation composition
Purification
Physicochemical characterisation
Sequence, origin of other genes
Restriction enzymes used
Vector construction
Nature of host cell
Master cell bank
Production:purity
Pyrogenicity:viral contamination
Identity
Efficacy:utility
Safety

be required for submission of a site-specific product produced from a synthetic gene (Table XXX). Some of the regulatory issues pertaining to the use of these systems have been addressed by various groups (e.g., Refs. 250–252).

XV. Concluding remarks

This contribution has pointed to many of the advances being made in the field of site-specific drug delivery. Undoubtedly, these and other approaches to selective drug delivery and targeting offer many opportunities in the re-examination of existing drugs in terms of improving on their potency and adjusting their toxicity. However, and in conclusion, it is in the *design and discovery* of new drugs or new modalities that the processes of site-specific delivery will have most long-term impact for many areas of disease. Thus, bearing in mind the tenets of access, retention and timing, to be able to cause effective moieties to attain a site of action (perhaps for the first time), and/or to direct them there in both a selective manner and at a rate and frequency that is either unique or optimal for the ultimate interaction with the pharmacological receptor (be it pulsatile, zero- or first-order, etc.), and hence to reach the maximal potential intrinsic activity that a drug has, must bring forth a plethora of unique opportunities. In this respect, we have recently concluded that site-specific drug delivery should not be regarded only as an add-on benefit for drug use, but also as an intimate and integrated part of the intellectual and experimental exercise of designing therapeutic systems for combating disease [9].

As more becomes known about (patho)physiology and the local biochemistry of disease, and molecular biology tools extend our present ability to manipulate and control gene expression, and protein engineering tools enable the de novo synthe-

sis of therapeutic systems that have defined functions of transport, protection, spatial orientation and temporal release, then a revolution in our thinking and, ultimately, the management of disease, will be embarked upon. However, since it is central to the design of site-specific systems that they effect *exclusive delivery of drug to the pharmacological site of action at a frequency and modality appropriate to the disease*, then site-specific approaches must begin from a clear understanding of the molecular basis of both the pathological condition and the normal physiological processes which surround it.

We have seen that a major priority in determining the commercial success of site-specific delivery systems will be the understanding and evaluation of their (potential) novel toxicities. Unquestionably much of this will relate to two features: the development of sensitive and specific *analytical* procedures, and the ability to examine for the efficacy and safety of *species-specific* product. With the latter, take, for example, the cell processing in an animal of human immunoglobulin A (IgA). Thus, it is known that asialoglycoproteins are taken up in the rat by a receptor system different from polymeric IgA (i.e., secretory component); however, although these two pathways are separate – with only a very low level of missorting found in the rat, when human IgA is presented as a ligand to the rat it is processed by the hepatocytes along elements of both pathways, and it is probable that receptor switching occurs intracellularly [253].

We have seen that with macromolecular multifunctional carriers, two types of system can be considered as drug carriers, i.e., particulates and soluble (bio)(macro)molecules. In this present contribution the use of colloidal and non-colloidal particles for site-specific drug delivery have been described in terms of the biological opportunities and constraints for their use, with particular attention being paid to the issues of site-attainment and the optimal pharmacodynamics of the drug. It is seen with particulates that because of their general inability to leave the general circulation, they will probably only have a use that is restricted to either biochemical or cellular targets within the vasculature, to discrete anatomical compartments, to extravascular targets at highly specialised areas, or where the pathological state permits it. Many more opportunities exist with soluble constructs because of their ability to extravasate (Section XI).

Gardner has addressed the putative limitations of both soluble macromolecular and particulate site-specific drug delivery systems (Table XXXI) [1]. Although these have been arrived at largely from the viewpoint of their use in cancer chemotherapy, it is hoped that the reader will have been encouraged by the present review to look beyond these to appreciate that quite unique opportunities do exist within the body for selective drug delivery using a variety of approaches (Tables XXI–XXIV). New ground is being broken all the time (subsections XIII.1–6). Witness for example the use of liposomes to deliver genetic information [254,255], or the incorporation of fusogenic viral sequences into liposomes to effect fusion with biological membranes [256], or the use of magnetic microspheres in vitro to sort diseased cells from normal ones (e.g., Ref. 257). Also this paper has only briefly discussed the role that particulate carriers can have as adjuvants in immunisation (e.g., Refs. 101 and 258.)

TABLE XXXI

SUGGESTED LIMITATIONS OF MACROMOLECULAR SITE-SPECIFIC SYSTEMS (ADAPTED FROM REFERENCE 1, THOUGH SEE CONTRASTING COMMENTS IN TEXT, SECTION XV)

Particulate systems
Preparative methods
Stability of carriers on storage
Loading
Parenteral administration
Poor ability to cross endothelia
Recognition and removal by the MPS
Limited access to non-phagocytic cells
Toxic effects (immunogenicity)
Poor recognition ability
Degradability
Soluble macromolecular systems
Probable parenteral administration
Drug loading
Poor drug accessibility to target site
Competitive uptake by MPS
Loss of recognition ability in vivo and upon chemical linking of drug
Poor efficiency of drug delivery to intracellular sites
Antigenicity of macromolecule and drug/carrier complex
Inapplicable for targeting to normal tissue

Although there has been a great deal of enthusiasm evident with the area of site-specific drug delivery, a great amount of investigative science is still required, and it is just not possible to link any (putative) drug to a carrier for there to be a desired effect. It is apparent that a full consideration of the opportunities and constraints for targeting have to be made; this point is exemplified by two recent publications on antiviral chemotherapy, i.e., contrast approaches taken in Ref. 259 with those in Ref. 260. In addition, many of the rather elaborate site-specific systems being proposed are just too sophisticated, and simple adjustments to either the structure of a drug and/or its manner of administration could suffice. These competing approaches must be considered for they could often be more cost-effective and simpler to use. See, for example, the use of lipid emulsions in reducing the systemic human toxicity of amphotericin B [261], as opposed to the use of various liposomal preparations that have been suggested previously [138].

In conclusion, the biological sciences are telling us what disease is, and are providing us with new materials which can be used directly as drugs or as templates from which new entities can be designed. If we wish to carry the analogy of using the body's own 'drugs' or analogues of them to a logical conclusion, then we must also learn to deliver them in a similar way to the body, i.e., often exclusively and when required. Site-specific drug delivery will help to meet these challenges.

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Impact of the New Biologies on the Medical and Pharmaceutical Sciences

E. TOMLINSON

This article points to the impact that the new biologies are having on the medical and pharmaceutical sciences. It describes and exemplifies advances being made in understanding the pathogenesis of disease; the new medical procedures being adopted to treat disease, including the use of living cells and gene therapies; the availability of new classes of drugs through targeted drug design; the novel synthesis and production of drugs; and the advent of anticodon drugs and recombinant therapeutic proteins.

The New Biologies and Medical Innovation

A new paradigm in human health care is emerging; that is, disease is being understood and treated at an increasingly higher level of genetic order and function, with the prevention and early diagnosis of disease becoming of increasing importance (Fig. 1). This revolution in medicine is beginning to result in dramatic benefits for the well-being of society. Many advances have been brought about by the availability of the tools of modern molecular and cell biology to pharmaceutical scientists involved in the development of modern medicines.

The discovery of the genetic code almost four decades ago began the movement towards the development of the new science superdiscipline called molecular cell biology. This necessitated the combining of the reductionist approach to genetics that began in the 1930s with those efforts designed to elucidate biomolecular structures in order to understand their function. Through this, the biological sciences have been transformed into a basic and quantitative science that attempts to describe biological events in physical and chemical terms[1]. The molecular biologist is armed with an array of powerful yet simple tools that may be used to probe and regulate cell function, and now has the ability to collect gene mutations at all levels of biological organization and complexity. Mutagenesis (i.e. a method for introducing defined mutations at specific sites in a gene) can be directed to the entire genome, to individual genes and gene clusters, to structural and regulatory segments of genes, and even to single nucleotide positions; it is now possible to identify, enrich, and amplify complete genes from the cell of any organism (Winther 1985). This has led to a more fundamental study of the structure and workings of both individual cells and tissues. Much is known about the structure and function of the cells of the body, and, of particular interest to pharmaceutical scientists, how they can select, transport and utilize various endogenous small and large molecules. One of the most important applications of modern molecular biology has been the definition of the normal cell cycle, i.e. how and why cells divide and then return to their resting state. Modern cell biology is being strongly supported by the use of sophisticated imaging techniques such as fluorescence-activated cell sorting, confocal laser fluorescence microscopy, and scanning tunnelling microscopy (Table 1).

To be able to harness and to manipulate the genetic machinery of any cell so as to control gene expression (i.e. the production of a gene-encoded protein) has many varied consequences for the pharmaceutical community in its search for safe and effective methods for both the diagnosis and the treatment of disease. These include: the evaluation of gene families and the markers of genetic originality, new and important insights into both the molecular specificity of biological action, as well as the pathogenesis of many diseases; the rapid and unambiguous diagnosis of disease (e.g. using gene probes); the production of new classes of low molecular weight drugs; the availability of ultrapure amounts of therapeutic polypeptides and proteins produced by recombinant DNA technologies (i.e. using DNA produced by joining pieces of DNA from different sources), the use of genetic material as drugs; and the recent emergence of the use of living cells for treating disease. Undoubtably, a critical scientific turning point in this progress has been the extraordinary ability to move from manipulating the structure and functioning of single cell organisms to doing so for whole vertebrates, including humans.

Understanding the Pathogenesis of Disease

Genetic analysis and the sequencing of the human genome

Recombinant DNA technology has enabled definitive studies to occur on some of the fundamental molecular mechanisms that regulate the expression of genes, as well as on dysfunction of these mechanisms. For example, an increase in the availability of cloned human DNA sequences and of other molecular biology tools known as chromosomally restricted fragment-length polymorphisms (RFLPs)[2] has enabled the study of more than 100 genetic diseases. RFLPs allow the analysis of inherited disease without a need to know the precise location and type of the defective gene. This has been used recently to determine the aetiology of diseases such as muscular dystrophy and cystic fibrosis. Some of the lesions for which a partial or complete genetic analysis has been made are given in Table 2. RFLPs may be used to mark the position of a sequence in a given

[1] Presumably this would have pleased Lord Rutherford who is credited with the dismissive that . . . "there is only Physics, the rest is stamp collecting"

[2] The genome, i.e. the full complement of chromosomes and extrachromosomal DNA, contains throughout its length unique variations in DNA nucleotide sequences. RFLP technology identifies these patterns and is, for example, able to determine differences in them between a parent and an offspring.

suppression of insulin-dependent diabetes mellitus (IDDM), an autoimmune disease in which the body's immune system destroys the insulin-producing islet cells of the pancreas. Speculation that this autoimmune disease results from an aberration in at least one gene that controls the ability of the body to recognize self from non-self (i.e. a major histocompatibility gene, MHC), has led researchers to introduce an MHC gene into diabetic mice, a step that resulted in those mice no longer displaying symptoms of the disease. Transgenic animals will be regarded more and more as part of the approaches required by the (bio)pharmaceutical industry in its search for safer and more effective medicines. Because they permit the efficient study of specific human processes, their use should also lead to a reduction in the overall number of live animals required to ensure the safety and efficacy of modern medicines.

Discovery, Design and Production of Drugs

The dramatic increase in the knowledge of the molecular origins of many diseases, combined with the availability of novel cell and animal test systems (as given above), has provided pharmaceutical scientists with the potential to address disease targets that were hitherto impossible to study satisfactorily. This widened horizon is beginning to impact strongly on the means by which today's modern (bio)pharmaceutical industry discovers, designs and even manufactures its products.

Targeted drug design

The pharmaceutical industry has adopted and pioneered many varied and erudite approaches in its search for, and development of, new and improved medicines. Now is really a very interesting time for an industry that until recently has operated from a largely chemical viewpoint, and which has needed to rapidly adopt many approaches that are biological in their conceptual origin. For example, for the discovery of drugs, the armamentarium of approaches available to the pharmaceutical scientist has greatly expanded in the past decade. From an empirical approach based on molecular roulette and the screening of tens of thousands of synthetic molecules, through rational drug design based on interactions with pharmacological receptors, the field has become greatly aided by the application of molecular biology techniques combined with a molecular computer graphic examination of cloned cell-surface pharmacological receptors and enzymes. That is, once a target gene has been identified, cloned, and sequenced, a tertiary structure for the protein coded by that gene may be arrived at from both the deduced amino-acid sequence and X-ray crystallography and NMR studies on the protein (the X-ray crystallographer may even be using crystals of protein grown on the NASA space shuttle!). The protein is then represented graphically, and powerful computer programs used to fit small molecules to the protein structure that can act as agonists or antagonists of some unique function of that protein. A limitation in using X-ray crystallography data is that this results in a three-dimensional representation of only one of the many conformations that the complex protein molecule can have, and hence many groups are studying how the preferred (solution) conformations of a protein may be modelled dynamically.

This approach is now beginning to produce results in many areas, with advances in understanding brain disorders and their treatment being most prominent. An important example is the search for improved therapies for hypercholesterolaemia (which may lead to atherosclerosis—the most prevalent form of cardiovascular disease). Here, study is based on the identification and cloning of the enzymes involved in intracellular cholesterol biosynthesis, with potential drug candidates being arrived at from their deduced ability to act as agonists or antagonists of the actions of such enzymes.

Again, the availability of the complete sequence of the human genome and its expressed coding regions should have a profound impact on efforts in targeted drug design. Whilst, hopefully, this should result in therapies having an unparalled specificity and effectiveness, targeted drug design is a new approach which attempts to combine a number of difficult applied sciences, and the complexity of the steps required for it to be effective should not be minimized (Fig. 2 (Blundell et al 1990)). In particular, targeted drug design demands a closeness with biochemistry and an understanding of how the target protein is implicated in the overall pathogenesis of the target disease.

Novel means for synthesizing drugs

Applied molecular biology provides the pharmaceutical scientist with the ability to accomplish syntheses of desired drugs that are either difficult or impossible to make using conventional synthetic chemical routes. One recent approach which may have a broad applicability is that exemplified by the production of 6-deoxyerythromycin, a derivative of erythromycin that had been predicted to have better physicochemical characteristics for oral delivery than other erythromycins. Thus, Weber et al (1991) used a targeted gene integration method to disrupt a gene (*eryF*) in a region of the chromosome of *Saccharopolyspora erythrae* that is essential for that organism's production of erythromycin. *EryF* apparently codes for the cytochrome P450, 6-deoxyerythronolide B (DEB) hydroxylase, an enzyme that converts DEB to erythronolide B (EB). Enzymes normally acting on EB appear to process the alternative substrate DEB to form the biologically active 6-deoxyerythromycin, a possible new drug that indeed has desirable physicochemical and biological properties. This so-called method of targeted gene disruption uses plasmids (i.e. circular double-stranded DNA) to carry small pieces of chromosomal DNA that are directed into an homologous site on the chromosome.

Screening for candidate drugs using transformed cells

Applied molecular biology can also be employed to produce unique cells that possess human surface and intracellular receptors that can specifically interact with both small and large molecular weight molecules, and thus can be used for the rapid in-vitro screening of drug candidates. This may be accomplished by incorporating cloned cDNA (i.e. DNA that is complementary to messenger RNA) into a host cell that upon receptor interaction with a candidate drug undergoes a measurable biochemical change, indicating a specific binding interaction between the receptor and the putative drug

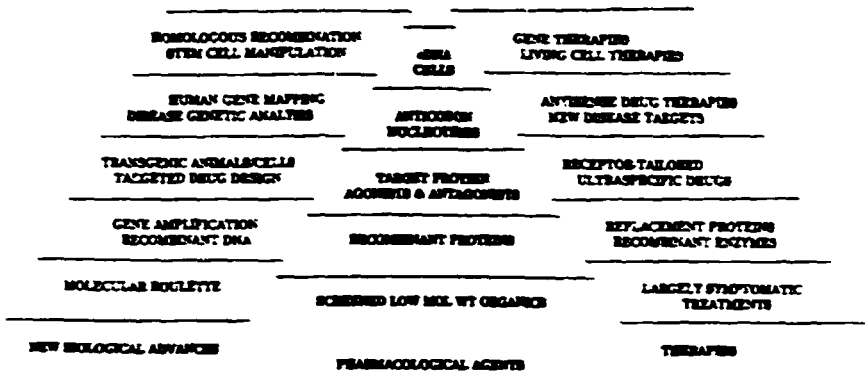

FIG. 1 The new paradigm in human health care: study, diagnosis and treatment of disease is occurring at an increasingly higher level of genetic assembly and function.

Table 1 Some molecular cell biology tools

Recombinant DNA techniques
Site-directed mutagenesis
Ligated gene fusions
Hybridoma technology
For production of antibodies
Carbohydrate engineering
Novel instrumental techniques
Polymerase chain reaction
Immunoblotting
Confocal laser microscopy
Fluorescence activated cell sorting
Scanning tunnelling microscopy

Table 2 Some diseases analysed at the genetic level

Haemoglobin diseases
Muscle disease
Lysosomal storage
Coeliac disease
Chromosomal transformations in Burkitt's lymphoma
Cystic fibrosis
Malaria
AIDS
T-cell leukaemia
HLA-disease associations

chromosome, to track the inheritance of unknown genes (via analysis of family linkages), and to study chromosomal deletions and translocations. In addition, RFLP techniques are being used to study pathologies such as rheumatoid arthritis, atherosclerosis, and some psychiatric disorders, for which a cluster of genes may predispose an individual to the development of the disease. Thousands of human inherited diseases have now been identified as probably being caused by a single defect in a gene. Interestingly, insights into gene defects have shown that different types of mutation can lead to the same clinical manifestation, which has considerable importance for the discovery of effective chemotherapeutic agents.

Newer and improved methods to define genes and their function are constantly emerging, due in great measure to the concerted international effort now focused on the sequencing of the entire human genome. This is a colossal project, and has the potential to make an awesome impact on the medical and pharmaceutical sciences, attempting, as it does, to provide a road map for scientists in their study of normal physiological events and their aberrations. For example, in the US a seventh genome centre has been created recently in Philadelphia in order to concentrate on the mapping of chromosome 22, with the goal to locate and to identify all of the 1000 to 2000 genes that probably reside on that chromosome (abnormalities of which are associated with childhood leukaemia, and some other forms of cancer).

Use of transgenic species

It has long been possible to introduce human genes into the embryonic stem cells of animals in order to produce transgenic species[1] that express a particular gene, including genes that result in a (genetically defined) human pathological lesion in the animal. The increasing availability of novel genetically engineered (small) transgenic animals that can act as models of disease, and also be used both for the testing of drug efficacy and toxicity, as well as producers of therapeutic proteins and even human organs for transplantation (see later), is astounding. In particular, they facilitate the study of disease targets. For example, severe combined immune deficient (SCID) mice, repopulated with complete human immune systems, enable the human immune system (and impact of infections and drugs on it) to be examined. Consider the implications that result from the fact that genes which encode for proteins such as growth hormone and plasminogen activator inhibitor-1 have been stably introduced into mice in order to examine the effects that these proteins have on the physiology of the mouse. Research into transgenic species often produces unexpected results that may have important implications for the discovery and development of improved medicines. Thus, for example, it was reported recently that adult transgenic mice carrying a mutated bovine growth hormone gene are smaller than normal, leading to speculation that the study of this mutant growth hormone could be useful in the development of low molecular weight drugs designed to suppress abnormal growth. Also, there has been much recent work on the role of specific immune regulatory genes in either the development

[1] Produced by artificially incorporating foreign (i.e. human) DNA into a fertilized animal egg which then follows a normal gestation

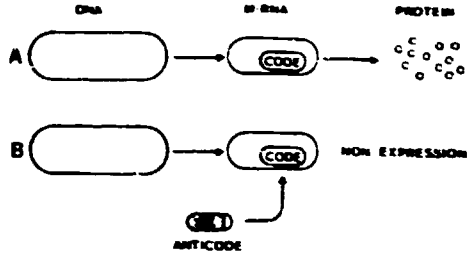

FIG 3 The proposed use of anticodon (antisense) nucleotide drugs, illustrating (A) the transcription of chromosomal DNA and the translation of the resultant messenger RNA into a corresponding encoded (disease-causing) protein, and (B) the specific binding of an anticodon oligonucleotide to the specific code of the messenger RNA—so inhibiting the production of a (harmful) protein

them assemble and secrete normal functioning or disease-fighting proteins?

Table 4 gives some of the pathologies that have been described as being amenable to treatment with antisense drugs. To employ the exquisite selectivity of the antisense oligonucleotide to fight disease is a compelling one, but as found also for many of the new classes of protein drugs (see later), the ability of these novel drugs to reach their intracellular targets may require new methods of administration or a selective intracellular delivery—using perhaps carriers that protect and transport the molecule through the body until the target region is encountered. For chronic therapies, delivery approaches would presumably also need to ensure that the antisense drug was continually present at the cellular site where mRNA was being processed to produce its encoded protein. Methods proposed to achieve this include both linking an antisense molecule to a peptide that can lead the molecule into the cell, and the use of lipid carriers that fuse with the cell plasma membrane and cause the antisense drug to be transported into the cytoplasm of the cell. Clearly, in order to realise the potential of these novel drugs one of the most important challenges for pharmaceutical science is to produce methods that achieve their selective delivery and targeting.

Genetically engineered viruses as pharmacological agents

Many studies since the 1950s have proposed the use of viruses to treat human tumours, but have had little, if any, success. Recently Martuza et al (1991) have reasoned that a mutant virus, based on herpes simplex virus can be genetically engineered so that not only is it deficient in the enzyme thymidine kinase, and can replicate in dividing cells such as those that are found in a tumour, but it is severely impaired in its ability to replicate in nondividing cells in the human nervous system. Such properties appear to make this type of engineered virus suitable for the treatment of malignant gliomas, such as glioblastoma—the most prevalent of brain tumours, and which are almost invariably fatal. Their studies show that after intraneoplastic administration of such a recombinant virus to athymic mice that possessed intracranial gliomas, survival of these mice was prolonged relative to controls. Further genetic alterations in this recombinant virus could lead to an increase in its specificity as well as to a decrease in some of its known side effects. If such an experimental treatment could be shown to have real clinical application, then the development of quality-assured product will surely become a difficult, though necessary task, for the (bio)pharmaceutical community.

Table 4 Some potential applications of antisense (anticodon) drugs*

Target	Therapeutic target
Infectious disease	
Candida albicans	Skin and systemic fungal infections
Herpes simplex virus	Topical and systemic herpes simplex I and II infections
Host disease	
Intracellular adhesion molecules	Ocular, skin and systemic inflammations
5-Lipoxygenase	Asthma
Phospholipase A$_2$	Rheumatoid arthritis
Raf oncogene	Cancer

*Adapted from information provided by ISIS Pharmaceuticals, Carlsbad, California, USA

Therapeutic Proteins

The body contains a plethora of proteins (including enzymes, hormones and receptors) that control and regulate biological function. Some humans can lack a specific protein, leading to the onset of severe diseases, e.g. diabetes or haemophilia. The pharmaceutical industry has long provided patients with replacement proteins (such as insulin and factor VIII) obtained from human or animal sources. Now the application of modern molecular biology techniques to produce these endogeneous proteins has revolutionized the way in which we consider and use proteins as therapeutic agents. A relatively new technique, known as PCR (polymerase chain reaction), has become one of the most important tools in this process. PCR acts rather like a molecular photocopier to provide relatively vast amounts of genetic material by amplifying individual DNA sequences from a single copy in a single cell. The availability of large amounts of genetic material, coupled with the ability to purify, cut and rejoin DNA at any desired site has led to the development and clinical use of homologous as well as heterologous therapeutic proteins (Griffiths 1991), that is, proteins that are either virtually exact copies of naturally occurring ones, such as insulin, or those that are composed of various polypeptide structures derived from different protein sources, e.g. the anticancer immunotoxin protein drugs (see later).

Homologous therapeutic proteins

In the early 1980s, insulin became the first commercially available therapeutic protein to be produced using recombinant DNA technology. Since then numerous homologous proteins that have been produced using biotechnological methods have reached the clinic and the market, including erythropoietin, growth hormones, tissue plasminogen activator, recombinant vaccines (e.g. hepatitis vaccine) and interferons (Table 5). These products are having considerable impact on the prevention and treatment of a wide variety of diverse pathologies, including bone disease, wounds, arthritis, immune deficiencies, hepatitis and cancer. The availability of the vast majority of therapeutic proteins is largely due to the proactivity of the biotechnology pharma-

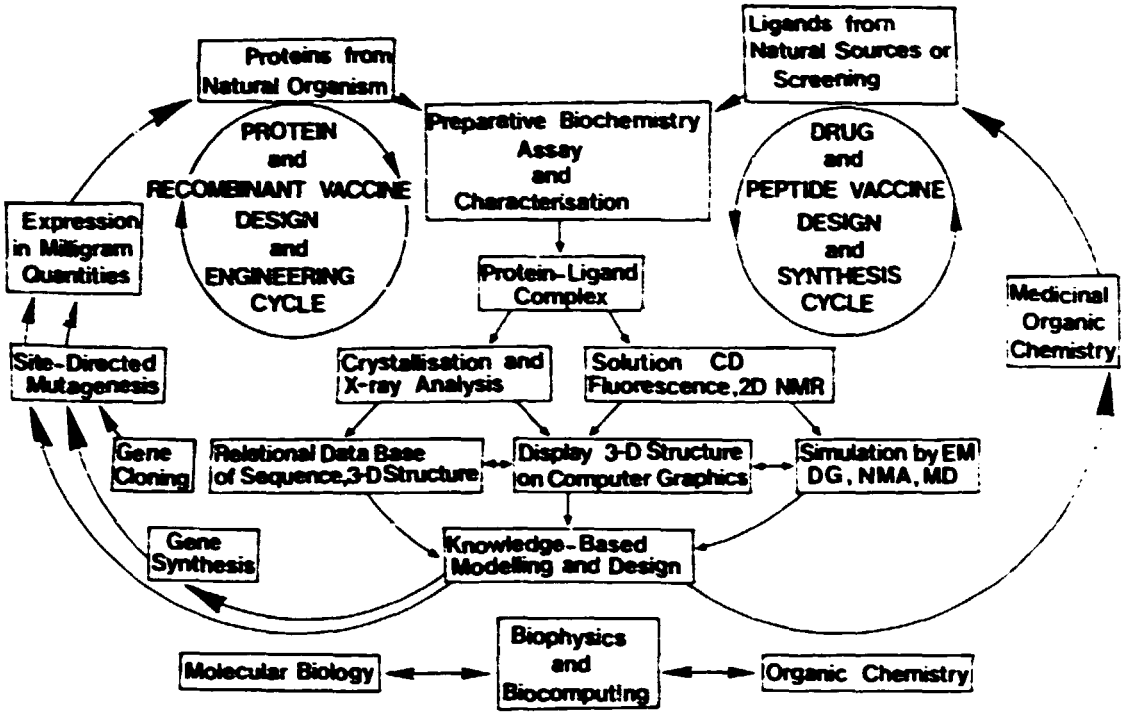

FIG. 2. Scheme illustrating the complexity of targeted drug design (Reproduced with permission of the copyright holder (Biundell et al (1990))

Methods for introducing cDNA into cells are varied, ranging from physical injection to the use of exotic genetically engineered (replication-defective) viruses (Table 3). Importantly, while the primary structure of a pharmacological receptor may be obtained by isolating clones using DNA probes that code for small peptide receptor fragments that have been purified and sequenced, molecular biology can also be used to determine the primary structure of receptors for which no amino acid structure is available. This is because it has been found that most known receptors are members of gene superfamilies, such that many receptor proteins share certain similar (i.e. conserved) amino acid sequences.

While this approach to the discovery of specific drug receptor interactions is a very new one, if it can be developed into a reliable and broadly applicable technique, then it could become one of the most important methods for the screening for new drugs.

Table 3. Methods of introducing genetic material into living cells.

Electroporation
Chemical methods (e.g. calcium co-precipitation)
Physical methods
Microinjection
Microparticle bombardment
Plasmid DNA
Cationic lipid mediated
Direct injection
Homologous recombination
Replication-defective retroviruses

Antisense (anticodon) drugs

Understanding the molecular basis of disease is now enabling completely new classes of pharmacological agents to be produced. Witness for example the advent of antisense (or anticodon) drugs that are designed to bind to messenger RNA molecules that code for aberrant proteins, and to thereby inhibit the production of that protein. The human genome is comprised of more than 100 000 genes, each of which usually codes for the production of a particular protein via the eventual formation of messenger RNA (an oligonucleotide whose specific sequences code for a unique protein structure). Antisense (i.e. anticodon) drugs are sequences of oligonucleotides that are precisely complementary to a given sequence of oligonucleotide and which are designed to bind to messenger RNA (mRNA) 'sense' targets that code for a disease-causing protein, so inhibiting a cell's ability to produce that protein (a case of: never mind the message—kill the messenger, Fig. 3). Thus, rather than require a low molecular weight drug to inhibit the action of a particular disease-causing protein, antisense drugs are designed to act directly on the genetic machinery that produces that protein in the first place, potentially producing benefits that are more pronounced than with conventional drugs. This is an example of what is described above as a new paradigm in the treatment of disease (Fig. 1), that is, disease targets are being addressed at an increasingly higher heirarchial level of genetic assembly. Indeed, as we shall see later, why stop at mRNA, why not intervene at the DNA level, or even control the genetic machinery of living cells to make

cological behaviours and to develop appropriate administration systems that can accommodate these

Autoimmune therapy

The irresistible march towards developing new therapies often produces novel and unexpected ways for treating disease. Witness recent developments in the therapy of autoimmune diseases, such as multiple sclerosis and rheumatoid arthritis, pathologies which appear to arise when the body's immune system reacts against its own tissues. Whereas many strategies for treating these diseases focus on the administration of mainly immunosuppressive polypeptides and even antibodies that block these reactions, recent work has seemingly confirmed an early promise that autoimmune attacks can be suppressed by simple oral administration of the protein antigens that appear to trigger the autoimmune reaction in the first place. Numerous theories arise on how 'antigen feeding' can have such potentially dramatic benefits. One is that it stimulates the local immune system in the gut, leading to an activation of a particular class of immune cells (CD8 cells) that are able to suppress the activities of other immune cells, potentially including those that respond to the normally physiologically present antigen in the first place. Other theories consider that administering antigen orally leads to the specific inactivation of the immune T cells that respond to the antigen. Whatever the mechanism of action, animal studies have been promising enough for at least two human clinical trials to be underway using the oral administration of antigen, i.e. administration of bovine myelin for the treatment of multiple sclerosis, and oral collagen for treatment of rheumatoid arthritis. A further trial that has been approved is for the oral administration of S-antigen in patients with uveitis.

Site-specific therapeutic proteins

As more is known about how the body regulates itself, and how and why endogeneous and applied exogeneous molecules interact with one another, then new classes of therapeutic protein will continue to emerge that will have high affinities for specific targets in the body, be protected from the sometimes harsh environment of the body, and be active only at desired sites of pharmacological action. Examples of these types of heterologous therapeutic proteins follow.

Deletion mutants The ability to control gene expression through site-directed mutagenesis permits the production of non-natural or heterologous proteins that, whilst they may partially resemble natural proteins, have altered pharmacological properties due to different pharmacokinetics, biological dispersions, and chemical stabilities. For example, natural tissue plasminogen activator (t-PA) has a short initial half-life in blood due to its rapid clearance by the cells of the liver. It is now known that certain regions in the structure of t-PA (including fibronectin-like and epidermal-growth-factor-like groups) are responsible for this clearance. These regions can be readily deleted from t-PA using site-directed mutagenesis without affecting either the fibrin affinity or the specificity of the molecule. Thus, this simple molecular biological tool can serve to improve the biological stability and the pharmacokinetics of the activator without decreasing its specificity of action (Fig. 4). Many other candidate protein drugs having improved biological dispersions and physicochemical stabilities are being developed in this manner.

Site-specific hybrid proteins The knowledge that small fragments of proteins have unique affinities for certain body structures is leading to the development of heterologous protein drugs that are comprised of combinations of various polypeptide structures from different proteins, such that they possess both pharmacologically active effector portions as well as portions that strongly bind to target sites, be they either on an enzyme, hormone or intracellular surface. This class of heterologous protein may be described as site-specific hybrid protein delivery systems (Tomlinson 1987). They can be produced either by synthetically linking protein fragments, or by use of a technology that employs "gene cassettes" to produce fused genes from which the complete hybrid protein may be directly produced. Table 6 gives some examples of site-specific proteins that have been developed or proposed for therapeutic use. Fig. 5 exemplifies the way in which this new class of site-specific protein drug can act, and illustrates how a novel hybrid protein that contains both a specific polypeptide structure that has a high affinity for the T-cell surface receptor for interleukin-2 (IL-2) and that portion of diphtheria toxin that can cause cell death, interacts with its target T cell. This molecular delivery system has been proposed for the treatment of graft vs host rejection disease, a disease that can occur after organ transplantation. When cytotoxic T cells of the immune system become active and try to reject host cells, they do so with their surface receptor for IL-2 present on their surface, thus providing a specific affinity target for the IL-2 toxin to combine with and then be drawn into the cell by the IL-2-receptor—where the toxin causes the death of that specific subset of T cells.

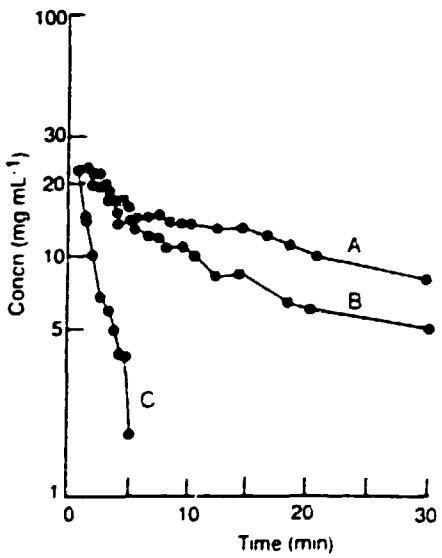

FIG. 4. Elimination of native and modified tissue plasminogen activator (tPA) from rabbit plasma with time. A and B represent deletion mutants, and C is the native full size human tPA. Reproduced with the permission of the copyright holder (Pohl et al 1988).

Table 5 Some recombinant drugs and vaccines either in development or commercially available

Disease symptom	Protein class example
Cancer	Immunostimulants (e.g. Interleukin-2), biological response modifiers (e.g. muramyl peptides), immunotoxins, antibodies, cytokines (tumour necrosis factor), lymphokines, colony-stimulating factors, genetically engineered viruses
Infectious disease	Antibiotics, interferons, vaccines, immunostimulants
Cardiovascular system	Erythropoiesis stimulation (e.g. erythropoietin), plasminogen activators (e.g. tissue plasminogen activator), fibrinolytics (e.g. urokinase), antibodies, haemophilia (Factor VIII), anticoagulants (e.g. hirudin)
Blood volume replacement	Albumin
Bone metabolism	Human calcitonin, growth factors, antibodies, cytokines
Wound healing	Growth factors (e.g. insulin-like growth factors, epidermal growth factor)
Inflammation	Protease inhibitors, biological response modifiers
Hormone augmentation	Glucose regulation (e.g. insulin), dwarfism (human growth hormone)
Immune regulation	Macrophage-inhibitory factor, macrophage-activating factor, therapeutic proteins
Enzyme-storage disease	Glucocerebrosidase (Gaucher's disease)
Immune deficiencies	Engineered vaccines (e.g. based on glycoproteins 120 and 160 derived from HIV envelope)
Lung disorders	DNase, lung surfactant

ceutical industry that not only had the foresight to invest in the biotechnological processes needed to produce such molecules, but also applied its skills in an entrepreneurial manner to work closely with government agencies to ensure that such unique products were qualified in terms of their efficacy, quality and safety. This required whole new methods of preclinical and clinical testing, production, quality assurance, and safety pharmacology procedures. Examples of novelty in the production of these new classes of therapeutic agents include the use of transgenic animals to produce therapeutic proteins, and even the spraying onto tobacco fields of recombinant viruses that contain genes for therapeutic proteins, in order to cause the tobacco plants to produce proteins (an appealing and satisfying use indeed for a plant whose present cultivation must be the antithesis of health care!). Interestingly, large transgenic animals may become the most appropriate way of producing some therapeutic proteins, particularly if they can be expressed in the milk of say bovine herds (Smith 1990); recently, it has been reported that transgenic pigs are able to produce human haemoglobin in relatively large amounts.

Clinical use of therapeutic proteins

Given the rate at which new molecules are being produced and characterized, and since many of these have several biological functions, enormous financial and human resources are required to bring appropriate therapeutic proteins to the clinic and the market. A major priority in determining the commercial success of such proteins is both in understanding and evaluating their novel potencies and (potential) toxicities, and also in achieving their selective delivery to their site of action. The former relate to two aspects: the availability of sensitive and specific analytical techniques, and to the ability to examine for efficacy and toxicity in appropriate cell and animal models. For the latter, unfortunately, many of the current general methods for using therapeutic proteins in the clinic have perhaps too easily been derived from practices developed for conventional low molecular weight drugs. It is apparent (Tomlinson 1990a) that those (homologous) therapeutic proteins that will be successful clinically, will be both those that act mainly on the enzymes and the cells of the blood compartment, as well as those that are able to access tissue parenchyma cells. To understand how to administer protein drugs correctly and to ensure they reach their site of action in an appropriate and convenient fashion, is a further challenge for medical and pharmaceutical science. It is a difficult task. For example, although for a few proteins there is little relation between dose applied and effect, for most it is highly critical, particularly as non-linear dose-effect relationships are often described (indeed bell-shaped, and even double bell-shaped dose-response relationships have been found clinically (Talmadge 1986), e.g. for parathyroid hormone, substance P, and δ-sleep-inducing peptide). Although the intrinsic interaction between proteins and their targets could be responsible for observed nonlinear dose-effect relationships, such behaviour could also be due to a nonlinearity in the way they disperse in the body, caused by inter alia: a poor ability to enter into (saturable) receptor-mediated transport processes; chemical instability; incorrect sequence of administration with other agents; or incorrect time of administration due to inappropriate temporal location and responsiveness of the target (cells). It is already known for example that the sequential use of protein drugs can be used to modulate their intracellular dispersion, as shown in-vitro by Aggarawal et al (1985) for γ-interferon (which appears to influence the cytotoxic action of both tumour necrosis factor α and β by causing TNF receptors to become concentrated (i.e. upregulated) on the surface of TNF-resistant cells). Consider also the cells of the immune system as targets for clinical intervention, and the differing roles of growth factors and immunomodulators. Haemopoiesis leads both to growth and differentiation of numerous varied cells that are acted upon by growth factors, and to resting cells that may be activated by immunomodulators. Many of the molecules that act on both of these two types of cells produce numerous similar biological effects (e.g. tumour necrosis factor and Interleukin-1, and α- and β-interferons), with the resultant effect differing only in magnitude. Since dysregulation of biological control would lead to a plethora of harmful events, it is most important that both the (physico)chemical structure and the method of administration of therapeutic proteins are tailored so as to ensure their required physiological localizations. It will be of increasing importance for pharmaceutical scientists involved in dosage form design to understand these complex pharma-

Table 7. Polymer-protein adducts under clinical development

Polymer:protein	Proposed use
Polyethylene glycols	
Superoxide dismutase	Kidney transplantation, burns, reperfusion damage (phase II clinical trial)
L-Asparaginase	Malignant haematological disorders (phase III clinical trial)
Adenosine deaminase	Severe combined immunodeficiencies (phase I II clinical trial)
Urokinase	Anticoagulant, fibrinolytic
Dextran	
Purine nucleosides	Inhibitors of adenosine deaminase
Carboxypeptidase G_2	Enzyme replacement therapy
L-Asparaginase	Cancer (lower antigen reactivity and increased circulatory persistence)
N-(2-Hydroxypropyl)methacrylamide	
Antibodies	Cancer-specific treatments

Table 8. Reglycosylated therapeutic proteins in or suggested for clinical use

In clinical trial
Glucocerebrosidase (for Gaucher's disease)
Ceramidedetrihexosidase (for Fabry's disease)
Suggested for clinical use
Human growth hormone
Erythropoietin
Factor VIII
Colony-stimulating factors
α- and β-Interferons
Interleukins 1-3
Antibodies
Insulin
Growth-hormone releasing factor

late and produce effective dosage forms for such systems whilst retaining their unique conformations.

Preclinical and Clinical Development of Therapeutic Proteins

Regulatory approval for the clinical use of therapeutics developed using molecular genetics requires information on their identity, purity, effect, and utility—including safety pharmacology (Tomlinson 1990b). Over the past decade these requirements have placed challenging demands upon the (bio)pharmaceutical industry, often necessitating the adoption of completely new approaches. For example, the analysis of the content of such molecules in dosage forms and body fluids has required the development of advanced analytical procedures, such as capillary zone electrophoresis, that provide high degrees of sensitivity and selectivity. Additionally, because of the novel methods employed for the production of recombinant therapeutic proteins, including perhaps the use of viral vectors (i.e. a viral vehicle by which DNA is introduced into a host cell), and other cell processing and gene expression tools, as well as novel purification steps, a number of unique quality assurance procedures are now required with respect to, amongst others, microbial contamination (viral, bacterial and mycoplasmal), nucleic acid contamination (i.e. presence of possible untoward oncogenic sequences of DNA), and processing contaminants (separation column leachable materials). Similarly, safety pharmacology testing has sometimes required the use of transgenic species and the development of various tier assessments where a knowledge of potential toxicity is built up in a composite fashion through using an assembly of various highly specific cell and cell-free tests). The testing of these products needs to be based on a knowledge of their physiological and pharmacological actions: it is, for instance, rather inappropriate to test for a human-specific protein over the long term in an animal that does not have the (human) specific ability to process that protein.

It is apparent from this how important it has become to achieve a merging of various disciplines to assure the clinical effectiveness and safety of many of these new medicines.

The Selective Delivery of Modern Drugs

This article shows that many modern drugs are being designed from a knowledge of both the molecular biology of the body and the pathogenesis of a disease. In considering modern pharmacological agents such as antisense (anticodon) drugs, DNA (gene therapy), double-stranded RNA, and protein drugs (e.g. hormones), as well as their low molecular weight analogues, it becomes apparent that often their delivery to a site of action needs to mimic a physiological pattern of delivery (e.g. the body produces and delivers its own human growth hormone in a pulsatile manner). Recent approaches to achieving the optimal arrival of drug at a site of action include the use of controlled-release polymeric dosage forms, such as oral dosage forms that release their drug in particular regions of the gastrointestinal tract (for example, the large colon appears to be most appropriate for the selective uptake of small proteins), novel routes of delivery (e.g. nasal and inhalation), macromolecular drug carriers (e.g. antibodies, liposomes), and, importantly, the development of drug molecules that have a specific action-site affinity or bioactivation. Selective delivery requires a consideration of both the changes occurring with time in the status of the disease and the functioning of the body, and the (related) chronopharmacology of the drug (Table 9).

Many of the new classes of drug described here will need to be administered parenterally until innovative approaches are developed that provide for both a selective delivery of drug (given its pharmacology), as well as being convenient for patient and physician alike. For Pharmacy, which has often, but not always, been at the forefront of developing means for achieving selective drug delivery and targeting, these requirements present it with a very intriguing challenge for what in the past was largely the domain of that area of its science termed Pharmaceutics.

Table 6. Site-specific hybrid therapeutic proteins.

Structural portions responsible for:		
Site affinity (recognition)	Pharmacological effective region	Suggested therapeutic use
Ligated gene fusion products		
Interleukin-2	Diphtheria toxin	Graft vs host rejection disease
Growth factor	Toxin (e.g. ricin A)	Leukaemia
CD_4	*Pseudomonas exotoxin*	HIV infection
Chemical linkage products		
Insulin	Diphtheria toxin	Insulinomas
Anti-endothelia immunoglobulin	Glucose oxidase	Endothelial wall repair
Anti-fibrin antibody	Tissue plasminogen activator	Fibrinolytic

Similar approaches are under consideration for the treatment of HIV infections by routing drugs into affected cells using protein carriers that are structurally similar to surface regions of HIV and so are able to use the same pathway into the infected cell that the virus has. Hybrid proteins are thus specialized site-specific delivery systems that have been designed from the application of cell and molecular biology to treat a unique pathology. The patent literature contains hundreds of examples of these new forms of protein drugs; we can expect to see a good number of these become tested in humans in the coming years.

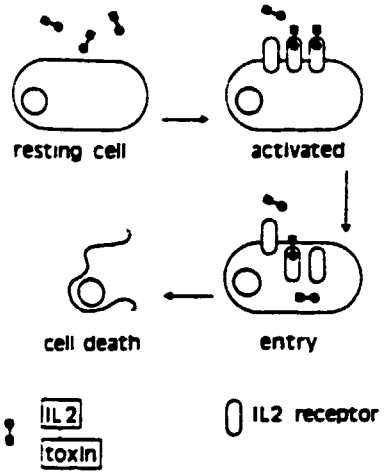

FIG. 5. Scheme depicting the interaction of an Interleukin-2-diphtheria toxin hybrid protein delivery system with activated T cells, as proposed for treatment of graft vs host rejection disease, showing binding of and specific cell entry of the hybrid when the T cell becomes activated, leading to death of a specific subset of T cells. (Adapted from Tomlinson 1991).

Understanding the pathogenesis of disease
- Genetic analysis
- Use of transgenic species
- Mapping of the human genome

Discovery, design, delivery and production of new classes of drugs
- Targeted drug design
- Novel screen for candidate drugs
- Unique methods of production
- Antisense (anticodon) drugs
- Genetically engineered viruses
- Therapeutic proteins
  - homologous proteins
  - deletion mutants
  - site-specific hybrids
  - polymer protein adducts
  - (re)glycosylated proteins
- Autoimmune therapies

Living cell therapies
- Autolymphocyte therapies
- Tumour infiltrating lymphocytes

Gene therapies
- Single gene defects
- Cell-based protein delivery systems

Chemical protectants and (re)glycosylations. Numerous other pharmaceutical approaches may be taken to alter the dispersion of proteins in order both to enhance their pharmacological activity and to reduce their potential for side effects. For therapeutic proteins needing to remain in the blood compartment, two main approaches have been adopted: i.e. increasing the apparent size of the protein so as to reduce its glomerular filtration, and reducing its (untoward) interactions with blood and tissue components by the concealment of active groups. A successful means for accomplishing both of these events is to chemically adduct a hydrophilic polymer to the protein. Such a surface modification makes it less likely for another soluble macromolecule (such as a neutralizing antibody) to approach. Table 7 lists some of the hydrophilic polymer protectants that have been described for conjugation to proteins; other polymers suggested include poloxamers, and albumin. Conjugations of proteins with hydrophilic polymers have often been reported as being very successful also in altering the potency of protein drugs as well as in reducing their immunogenicity (i.e. their capacity for producing an antibody response).

A further method for altering the dispersion of proteins is to adjust the nature of the various types of carbohydrate structure (so-called glycosylation patterns) that may exist on the surface of a protein. This is because glycosylation patterns are signals used by the body to regulate the dispersion of its own glycoproteins (as evidenced for both enzyme and hormone disposition, as well as coagulation and immune-surveillance events). Such (re)glycosylations (and even deglycosylations) appear to be useful to (re)direct proteins to specific cells by causing the altered protein to be taken up, or otherwise, by a cell's carbohydrate recognition and processing machinery. In the future it is considered that this chemical approach will be generally performed on a recombinant protein after it has been produced by biotechnology, and will include the extension or addition of novel carbohydrate structures, or even the total removal of carbohydrate groups. Table 8 lists some of the therapeutic proteins that have been suggested as being appropriate candidates for specific (re)glycosylations. It will be a particular challenge for pharmaceutical science to analyse, formu-

Table 11 Some current disease targets for gene therapy

Novel cell-based protein delivery systems

Condition	Cell type	Delivered produced protein
Wound healing	Epidermal cells	Wound-healing growth factors (e.g. epidermal growth factor)
Parkinson's disease	Fibroblasts	Tyrosine hydroxylase
Alzheimer's disease	Neuronal cells	Nerve growth factor
Cancer	Tumour-infiltrating lymphocytes (TILs)	Tumour necrosis factor
Improved vascular grafts	Endothelia lining surface of synthetic graft	Plasminogen activators

Single-gene defects

Condition	Incidence	Abnormal gene	Target cells
Haemophilia A	1:10000 males	Factor VIII	Endothelia, fibroblasts, hepatocytes
Haemophilia B	1:30000 males	Factor IX	Endothelia, fibroblasts, hepatocytes
Severe combined immunodeficiency	Rare	Adenosine deaminase	Lymphocytes, bone marrow
Other immunodeficiencies	Rare	Purine nucleotide phosphorylase	Bone marrow
Urea cycle disorders	Rare	Ornithine transcarbamylase	Bone marrow
	Rare	Argininosuccinate synthetase	Bone marrow
Haemoglobinopathies (thalassemias)	1:600 in some ethnic groups	β-Globin	Blood cells
Inherited emphysema	1:3500	α_1-Antitrypsin	Lung cells
Lysosomal storage diseases	1:1500	e.g. Glucocerebrosidase	Blood, nervous system
Cystic fibrosis	1:2500 Caucasians	Transmembrane regulatory protein	Lung cells
Lesch-Nyhan syndrome	Rare	Hypoxanthine-guanine phosphoribosyltransferase	Blood, nervous system
Duchennes' muscular dystrophy	1:10000 males	Dystrophin	Muscle cells

Cell-based protein delivery systems

Gene therapies are not limited to repairing the effects of aberrant or absent genes; indeed the approach may be extended to add novel properties to cells to enhance their ability to fight disease. The most advanced efforts in human gene therapy are currently directed towards creating living cells that are genetically augmented with a "therapeutic gene" and then implanted in the patient where they act as molecular pharmaceutical factories and pharmacies, producing and delivering their gene product to a defined anatomical region. The use of certain genetically augmented living cells has been sanctioned for human clinical trial in the US for the delivery of the enzyme adenosine deaminase from lymphocytes that have been taken from a severely immunocompromised patient, genetically augmented with the adenosine deaminase gene, and then reintroduced into the blood compartment where they coexist with normal lymphocytes. (Interestingly, an alternative treatment for the so-called bubble boy syndrome[5] is to use adenosine deaminase that has been chemically adducted with polyethylene glycol so as to lengthen its circulating half-life in blood (see earlier and Table 7).) Clinical gene therapy trials are also underway for the delivery of tumour necrosis factor (TNF) from a subset of lymphocytes that have been augmented with the TNF gene and are putatively able to infiltrate extravascular tumour masses. Other cell-based protein delivery systems based on living cells under active development include those for Gaucher's disease (glucocerebrosidase), disorders of the blood-clotting system (factors VIIa, VIII, IX), blood-contact products such as stents circulatory assist devices and improved vascular grafts, and other single-gene defect disorders such as cystic fibrosis (Tables 10, 11). Gene therapies may also combine protein delivery with other properties of cells, as exemplified by a recently proposed novel approach to wound healing in which a "biological bandage" is produced that consists of a donor skin graft that has been genetically augmented with genes that code for wound-healing growth factors, such as epidermal growth factor (Morgan et al 1987). This living cell system is suggested to have the combined attributes of the occlusive properties of skin grafts and an ability of the cells to produce and deliver therapeutic proteins for a sustained period at the site of action. Allied to this proposal is the recent finding that the cellular protein gelsolin, which is involved in the cell processing of actin, affects cell motility, leading to the notion that living skin cells could be engineered to produce appropriate amounts of gelsolin so leading to an enhanced chemotactic responsiveness of the cells and hence to an increase in the onset of wound closure (Cunningham et al 1991).

Much attention has been focused on using implantable skin cells for the production of small proteins that will then be able to be delivered systemically from a living cell skin patch. For example, St Louis & Verma (1988) have shown that skin fibroblasts, which are both readily accessible and sturdy, are able to be genetically augmented with the gene for factor IX and then easily implanted back into the dermis where they become highly vascularized and produce and deliver enhanced levels of factor IX (a small protein) into the blood pool. Genetically augmented fibroblasts have also been proposed for implantation into the brain to correct neural disorders; for example to contain the gene for tyrosine hydroxylase (an enzyme involved in the intracellular metabolism of dopamine, and whose absence is thought to be implicated in the pathogenesis of Parkinson's disease). Also, neuronal growth-factor genes placed in implanted fibroblasts have been suggested for treating the degenerative disorder Alzheimer's disease.

Only 5 years ago the status of gene therapy was described as being "So near yet so far away". Since then, careful

[5] The "bubble boy" had an adenosine deaminase deficiency that left him severely immune-compromised which necessitated his continual existence within a protective chamber

Table 12 Some novel products arising through the application of the new biologies

Class	Product
Drugs	Genes Sequence-specific oligonucleotides (antisense) Recombinant proteins (e.g. erythropoietin, insulin)
Living Cells	Autolymphocyte therapy Genetically augmented tumour infiltrating lymphocytes for the delivery of tumour necrosis factor
Vaccines	Synthetic core viral vaccines Anti-HIV vaccine produced by insertion of the lympadenopathy-associated virus surface protein into vaccinia virus
Diagnostics	Direct HIV antigen diagnostic test Radioactive indium ^{111}In-labelled antibody for renal cell carcinoma
Processes	Monoclonal antibody-based purification for natural factor VIII

application of molecular and cell biology techniques, combined with an understanding of the conditions that are most amenable to gene therapies, has led to a true realization of the approach. Indeed, in the US up to a dozen companies have been formed to commercialize various gene therapies. Current areas of scientific endeavour include the development of novel methods for introducing pure RNA or DNA into cells by simple injection that results in the transient expression of protein for disorders requiring but short-term therapy (Wolf et al 1990). Such an approach has been proposed both for treating muscle disease, and because the method may perhaps be used for the intracellular expression of genes encoding antigens, for providing immunity. Further, the direct application of DNA has been suggested for the treatment of: muscular dystrophy, cystic fibrosis (by aerosolization to the lung of a transmembrane regulatory gene implicated in the pathogenesis of cystic fibrosis), Parkinson's disease (by direct injection into the brain of the tyrosine hydroxylase gene), and hypercholesterolaemia (by delivery of the high-density lipoprotein receptor to liver with cationic orosomucoid complexes). To date, direct injection of genes into muscle, liver, brain, lung, artery, intestine, peritoneum, skin, mammary, and heart tissues, and resulting in the (enhanced) expression of a variety of proteins, has been reported. An even more remarkable method for delivering genes is the use of high-energy bombardment of living cells with DNA coated onto microparticles such as gold and even the noble gases (Table 3).

However, a serious potential problem with gene therapy is the lack of site-specific integration of genes that occurs using either retroviral or injection techniques. Thus, recent academic and industrial attention has focused on using a site-specific homologous recombination between DNA sequences residing in the chromosome and newly introduced DNA sequences (Capecchi 1989). This technique, whilst relatively inefficient, is claimed to be able to turn single genes in a chromosome off or on. One reported application has been to disrupt the expression of the major histocompatibility complex (MHC) gene so as to create universal donor cells that could be used for transplantation to humans without the need for tissue matching. Among early clinical trials being planned with this approach is the use of retinal epithelial cells lacking MHC protein that can be grafted onto the retina to correct macular degeneration. Gene therapy has a long way to go before it can be considered as a reliable and effective form of medical treatment, but it remains a compelling approach, and represents a further example of the paradigm that I have pointed to in this article, in this case where the genetic machinery of a cell is controlled, not only to make a (protein) drug, but also to secrete or deliver it in a controlled and sustained fashion into a defined body compartment. Advanced drug delivery indeed!

Concluding Remarks

This article attempts to describe some of the remarkable advances being made in human health care that have largely resulted from the impact of the new biologies on medical and pharmaceutical science. Importantly, I have pointed to the fact that the ways in which diseases are being studied and treated are implicated, in or derived from, an increasingly higher order of genetic structure, function or regulation (Fig. 1). This view is an optimistic one, for but a very few of the many hundreds of recent innovations have been described. Many others could have been chosen, including the importance of engineered humanized antibodies in the diagnosis and treatment of disease, the relevance of the many studies on the molecular basis of angiogenesis that are pointing to new ways to treat cancers and wounds and, the discovery and analysis of the cell adhesion molecules, and the implications this has for the treatment of immune diseases. While some of these will inevitably fail, the unsurpassed power and effectiveness of the tools that modern molecular and cell biology provide will ensure that others will arise to take their place and become successful. However, to translate these advances into safe and effective medicines requires the application of a host of additional and complementary disciplines, skills and technologies that have only been briefly mentioned here.

And as for future trends in medical and pharmaceutical innovation? It has been argued that molecular biology heralds the beginning of the fifth Kondratiev wave[6] that will lead to an unprecedented era in medical innovation, and we have seen in this article some of the ways in which molecular and cell biology indeed are impacting on the study and treatment of disease (Tables 12, 13). But with no sign of the fourth wave (i.e. the electronic age) abating, it may well be that a merging of the technologies of molecular and cell

[6] Economic theory includes the belief that waves of innovation (as proposed by the Russian Nikolai Kondratiev) have occurred more or less regularly over the past 250 years in roughly 50 year cycles. The first few years witness a build up of new technological potential, followed by a period during which many new and far-reaching innovations occur, followed by a long period of commercialization. The fifth wave is predicated at least on biotechnology and also on recent advances in physics and mathematics (Foster 1986).

Table 9 Methods for adjusting the biological dispersion of therapeutic proteins (modified from Martuza et al (1991))

Approach	Examples
Site-directed mutagenesis	α_1-Antitrypsin, subtilisin (substitution of methionine amino acids to improve physicochemical stability)
(Re)glycosylation	Glucocerebrosidase (improved disposition into target cells through a carbohydrate recognition pathway)
Hybrid site-specific proteins	
Linked synthetically	Immunoglobulin and toxin fragments
Fused-gene products	Growth factor and toxin fragments
Protectants	
Polyethylene glycols	Interleukins, superoxide dismutases
Dextran	L-Asparaginase
Administration	
Frequency and rate	Growth hormone effectiveness altered
Route	Insulin and site of action
Staging	Combinations of interferons and tumour necrosis factor have different responses dependent upon the staging of their respective administration

Living Cell Therapies

Human cells have been used for therapeutic purposes since the first blood transfusion. Now it is possible to isolate and purify particular cells and to then alter them with proteins and even genes so as to cause permanent and potentially therapeutically beneficial alterations in their function. Cell therapies include the use of autolymphocyte therapy (ALT), lymphokine-activated killer cells, genetically and non-genetically augmented tumour-infiltrating lymphocytes, marrow cells propagated for transplantation, and foetal cell transplants (for example, the use of foetal mesencephalon that is rich in dopamine-synthesizing neurons for treating Parkinson's disease). Most of these cell therapies are controversial, giving rise to significant scientific and ethical questions (particularly when foetal tissue is involved). For example, ALT is an approach for treating tumours by enhancing the patient's immune system through use of the patient's own lymphocytes. The procedure involves removing lymphocytes from a patient, activating these using specific monoclonal antibodies to secrete large quantities of cytokines, then using the patient's own cytokines produced in this manner to activate further samples of lymphocytes that are then reinfused into the patient. Presently, ALT is being tested in patients for the treatment of metastatic kidney cancer. An important question here is how to regulate and to control the use of cell-mediated therapies. ALT for example is not regulated by the Food and Drug Administration as it is not a drug. When it is realized that large SCID animals are being considered to produce human immune system cells for the purposes of immune therapy, and that the possibility even exists for developing transgenic animals that have organs that would be histocompatible with humans, so leading to the availability of universally compatible human organs for human transplantation, then this need for government regulation becomes even more compelling. The living-cell industry is a new one, and it behoves it to adopt a co-ordinated and professional approach to working closely with appropriate regulatory agencies to ensure that the promise that cell therapies bring is attained in a safe and speedy fashion.

Gene Therapies

A recurring realization of this analysis of the impact of the new biologies on human health care is that clinical intervention is focused more and more on treatments that involve knowledge of the molecular basis of disease. Now even the prospect of replacing defective genes is in sight. To deliver correctly functioning DNA into a human in order to treat a genetic disease has long been a goal of medical science; now gene therapies are being considered for the treatment of not only single gene defects but also as a means of creating cell-based protein production and delivery systems (Table 10).

Treatment of single gene defects

One infant in every hundred is born with a serious genetic defect; such aberrations account for almost 10% of admissions to paediatric hospitals in North America. More than 4000 inherited single-gene disorders are known, including sickle cell anaemia, cystic fibrosis, Duchenne muscular dystrophy, haemophilia, Tay-Sachs disease and Huntington's disease. Most lack an effective therapy. The availability of agents that faithfully transmit genetic information into chromosomal DNA opens up the prospect of gene therapies for the treatment of a number of these inherited diseases as well as for other serious and debilitating illnesses (Table 11). Gene therapy can involve either the replacement or correction of an aberrant gene or the addition of a gene to chromosomal DNA in a cell in-vitro or in-vivo (Tomlinson & Livingstone 1989). An important technique for delivering human genes into cells is the use of replication-disabled recombinant retroviral[4] particles that are able to efficiently introduce and express new genes in many different types of mammalian cells. Their use generally leads to the stable integration of new genes into chromosomal DNA and a faithful transfer of genes without rearrangements. To date, more than 50 different genes as large as 4·5 kilobases (i.e. nucleotide bases) have been effectively transferred into mammalian cells using retroviral technologies.

Table 10 Potential applications of gene therapy.

Inherited diseases
Acquired diseases
Infectious
Cancer
Cardiovascular
Autoimmune
Novel therapies
Organ cell transplants
Improved circulatory assist devices, stents, improved biohybrid vascular grafts

[4] Derived from RNA-containing retroviruses

Table 13 Impact of the new biologies on medical and pharmaceutical science

Understanding the pathogenesis of disease
Genetic analysis
Use of transgenic species
Mapping of the human genome
Discovery, design, delivery and production of new classes of drugs
Targeted drug design
Novel screen for candidate drugs
Unique methods of production
Antisense (anticodon) drugs
Genetically engineered viruses
Therapeutic proteins
homologous proteins
deletion mutants
site-specific hybrids
polymer protein adducts
(re)glycosylated proteins
Autoimmune therapies
Living cell therapies
Autolymphocyte therapies
Tumour infiltrating lymphocytes
Gene therapies
Single gene defects
Cell-based protein delivery systems

biology with those of electronics will occur (after all the home of most of the (US) biotechnology industry is but an entrepreneur's stone throw away from Silicon Valley). In fact, today one sees trends towards such bioelectronics: witness the reports of the use of integrated microchip circuitry for the treatment of hearing loss, as well as for helping some paralysed people to walk a few steps, and now dramatically, the announcements on the development of a microchip that could be implanted into a blind person and be linked to conducting nerve cells so that light-stimulated signals are sent to the brain to enable images to be recognized. As Sam Goldwyn would have said: the future ain't what it used to be.

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TABLE OF CONTENTS

I. Transport and Diffusion in Membranes: Concepts and Simulation 1
Dr. Russell Paterson

II. Regulatory Considerations 17
Mr. Robert F. Weir

III. Oral delivery of Peptides and Proteins: Problems and Perspectives 48
Prof. Clive G. Wilson

IV. Laboratory Sessions

A. Automatic Multi-Cell, Multi-Lambda Membrane Permeability System 62
Christopher R. Moran

B. Measurement of the Diffusion Coefficient of Proxyphylline in a Fully Hydrated Poly(ethylene oxide) hydrogel 71

Release of Proxyphylline from a Dispersion in a Poly(ethylene oxide) Xerogel in the Form of a) A tablet and b) A sphere 75

Marion E. McNeill

C. Theory of the Diaphragm Cell 81
Sam McFadzean

D. Preparation of Liposomes 84
Alex Mullen

D. Particle Size Determination of Liposomes 93
Alex Mullen and Moire Owens

E. Particle Size Determination of Aerosols and Microspheres 97
Tony L. Whateley

F. Microencapsulation and Preparation of Microspheres 100
Isabel Crossan

INSTRUCTIONAL STAFF

Course Organisers:

Professor Neil B. Graham	Department of Pure & Applied Chemistry University of Strathclyde, Glasgow
Dr. Tony L. Whateley	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow

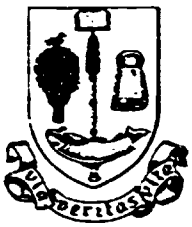
Lecturers:

Professor Jonathan Hadgraft	The Welsh School of Pharmacy University of Wales College of Cardiff, Cardiff, Wales
Professor Ian W. Kellaway	Welsh School of Pharmacy University of Wales College of Cardiff, Cardiff, Wales
Dr. Russell Paterson	Department of Chemistry University of Glasgow, Glasgow
Professor Eric Tomlinson	GeneMedicine Inc, Houston, Texas
Mr. Robert F. Weir	Controlled Therapeutics (Scotland) Ltd East Kilbride, Glasgow
Professor Clive G. Wilson	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow

LABORATORY DEMONSTRATIONS

INSTRUCTIONAL STAFF

Christopher R. Moran	Department of Pure & Applied Chemistry University of Strathclyde, Glasgow
Marion McNeill	Department of Pure & Applied Chemistry University of Strathclyde, Glasgow
Sam McFadzean	Department of Chemistry University of Glasgow, Glasgow
Alex Mullen	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow
Moira Owens	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow
Tony L. Whateley	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow
Isabel Crossan	Department of Pharmaceutical Sciences University of Strathclyde, Glasgow



TRANSPORT AND DIFFUSION IN MEMBRANES CONCEPTS, CHARACTERISATION AND SIMULATION.

by

Russell Paterson

Director Colloid and Membrane Research Laboratories,
Department of Chemistry
University of Glasgow
Glasgow G128QQ
Scotland UK

OUTLINE OF THEORY OF TRANSPORT PROCESSES:

Diffusion processes are only one class of spontaneous processes by which chemical systems may reach equilibrium or, if certain restrictions are imposed, a steady state. Classical thermodynamics requires that there will be a net increase in the entropy of the system and surroundings during any spontaneous process. Irreversible thermodynamics shows us how this rate of production of entropy can be expressed as the sum of products of flows and thermodynamic driving forces within the system. It provides the most general of all possible system dynamic descriptions, its scope extends to all non-equilibrium processes in all energy domains (for example, electrical, chemical, thermal) and is fully capable of describing energy transduction between them.

In this theory, the local rate of production of entropy, σ and consequently hte rate of dissipation of free energy, $\Phi = T\sigma$ to specific irreversible processes occurring within the system. For any local volume, this dissipation function, Φ, is defined by the sum of products of the flows in the system and their conjugate forces, eqn.(1).

$$\Phi = f'_q\left(-\frac{dT}{T}\right) + f_v(-dP) + \sum f_i(-d\mu_i^c) + i(-dV) + \sum f_r A_r \geq 0 \qquad (1)$$

These are, respectively the flows of; heat, f'_q; volume, f_v; molecules f_i; electric current, i; and the rates of chemical reactions, f_r, within each element. The conjugate forces (termed efforts in bond graph theory) are defined by the local differences in temperature, T; hydrostatic pressure, P; chemical potential, μ_i^c; and electrical potential, V across the element and by the affinities, A_r, of the chemical reactions in the element.

In this way the thermodynamics of irreversible processes defines precisely the fundamental scheme of flows and forces for all possible processes in all familiar energy domains.

Experiments performed exclusively in each domain have produced simple, linear constitutive relationships between flows and forces:

$$f_q = \left(\frac{\theta_q A}{l}\right)(-\Delta T)$$

$$f_v = \left(\frac{\theta_p A}{l}\right)(-\Delta P)$$

$$f_i = \left(\frac{D_i A}{l}\right)(-\Delta C_i)$$

$$i = \left(\frac{\kappa A}{l}\right)(-\Delta V)$$

where $(\kappa A/l) = R$, the electrical resistance of the material.

These are the well-known transport equations of Fourier, Darcy, Fick and Ohm, respectively. All take the general form of eqn.(2), in which flow is defined by an effort (or force) e, divided by a resistance, R.

$$f = \frac{e}{R} \qquad (2)$$

In non-equilibrium thermodynamics there is provision for coupling phenomena: That is a force or effort applied to one species will affect the flow of another. A simple example is found in electrokinetics in which a voltage applied to a membrane or capillary will not only cause ions to flow and generate an electrical current (as in Ohm's law) but also produce a flow of water. This is the phenomenon of electro-osmosis. In this case water flows when there is now force (effort) applied to it (such as an osmotic or pressure difference). The water is pulled along, through the membrane by the moving ions: coupling. Ion pumps in biological membranes use the affinity of a chemical reaction (usually ATP hydrolysis) to drive ionic flows, even against their concentration gradients.

These effects can be described precisely by irreversible thermodynamics, but this application is out with the scope of this lecture. We will consider here only diffusion systems an d exclude coupling phenomena. It is worth noting however that such effects exist and will occur in apparently simple diffusion systems and that they may be exploited at some later stage in the formulation of advanced drug release and other bio-medical applications.

Most drug delivery systems are diffusion controlled by a membrane, pessary or other device. It is necessary therefore to be able to assess the capability of new membrane materials by measuring their diffusion coefficients, D_i and permeabilities, $P = (DA\alpha)/l$, to drug molecules. (α is the distribution coefficient for the permeant between membrane and solution, $\alpha = \overline{C}/C$. This term appears because Fick's law, as given above, relates the diffusional flow to the concentration difference in the membrane from one surface to the other, while the practical permeability, as measured in the laboratory, relates the diffusional flow to the concentration difference of permeant between the two solutions on either side of the membrane and so the α factor is required.

To solve the diffusion equations for practical systems and so obtain solutions to particular diffusion problems with defined initial states and set boundary conditions is a major mathematical task involving considerable creativity. A number of books deal with this

subject and give mathematical solutions for large number of common problems, such as diffusion across a membrane or diffusion in spheres or cylinders or other simple geometric forms. Three standard texts, for reference, are listed in the bibliography. (Solutions to heat conduction problems are exact analogues to those of diffusion and historically heat transport problems were solved first, hence the relevance of the book by Carlslaw and Jaeger.)

The complexity of the mathematical solutions, even for simple diffusional problems involving simple shapes, constant diffusion coefficients and elementary boundary conditions, involve complex mathematics and yield complex solutions. As a result it is common to use approximate solutions applicable only in the initial initial or final stages of the diffusion process. A series of such solutions are given in Professor Graham's paper, in Volume I. It is quite clear therefore that diffusion processes commonly devised to obtain controlled release or even the study of diffusion across multilayer membranes (both artificial and natural, as across skin layers) cannot be predicted mathematically. It is necessary to consider computer simulation techniques for the design and prediction of membrane processes.

In this paper the applicability of Network Thermodynamics is explored. The systems which will be described were developed in the author's own laboratory as part of an on-going research programme. The original source references [1,2,3,4,5] give the basic concepts of the method which are introduced in more succinct form here.

Simulation of Transport Processes

The equations of Fourier, Darcy, Fick and Ohm all take the form of eqn.(2), in which the flow is defined by the size of the applied effort, e, dmultiplied by a reciprocal resistance, R. The term resistance is used by analogy with Ohm's law and as a reminder of the purely dissipative role of such processes. (The reciprocal of the resistance is of course the permeability and the equations could equally well have been expressed in terms of permeabilities.) The representation of the diffusion or transport system can be made in a very compact way using the bond graph notation of Paynter, [2]. In bond graph terms eqn.(2) is the constitutive relationship of a generalised resistor, relating effort to flow. The products of efforts and flows, as defined by the dissipation function, have the dimensions of power. In the notation of Paynter [2], power flow is represented by a power bond, pointing in the direction of the half arrow, fig.1a and power dissipated, by fig.1b, symbolising a resistor with constitutive relationship, eqn.(2).

If the power entering a resistive element e_1f_1 and leaving it is e_3f_3, the power dissipated is e_2f_2, given by their difference, eqn.(3).

$$e_2f_2 = e_1f_1 - e_3f_3 \qquad (3)$$

Since a resistor is a series element, flows are conserved, eqn.(4)

$$f_1 = f_2 = f_3 \qquad (4)$$

To represent eqns.(3) and (4), bond graph notation employs a 'through' or 1-junction, fig.1d, in which all power bonds shared a common flow.

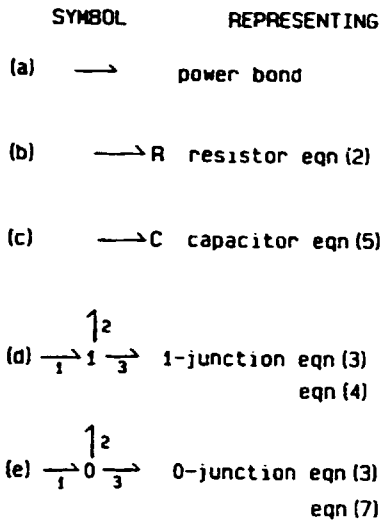


Figure 1: Basic bond graph symbols and their constitutive relationships.

Of particular interest to chemical diffusion studies is the capacitative role of an elemental volume in the non steady state. The relationship between effort and flow is now one of a capacitor, described by eqns.(5) and (6), in which C is the capacitance, relating the charge, q (defined as the time integral of the flow) to the effort.

$$e = e_o + \mathrm{C}^{-1} q \qquad (5)$$

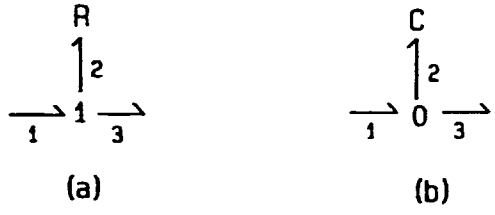
where

$$q = \int_{t=0}^{t} f dt \qquad (6)$$

In a chemical system the 'charge', q, is simply the number of moles of the permeant species in the local volume element and, since the effort is defined as the chemical potential, the capacitance is not a simple constant. The local effort at any time, t, is determined by its initial value, e_o and the subsequent history of accumulation or depletion of permeant in the element, eqn.(5). For a chemical capacitor therefore, the difference between the power entering and leaving, eqn.(3), is that stored (reversibly) without dissipation, fig.1c. The junction conditions are now those of a parallel element at constant effort, combining eqn.(3) and (7) and represented by an O-junction, or constant effort junction, fig.1e.

$$e_1 = e_2 = e_3 \qquad (7)$$

The capacitative role of an element is defined by eqns.(3), (5) and (7) and represented by fig.2b.



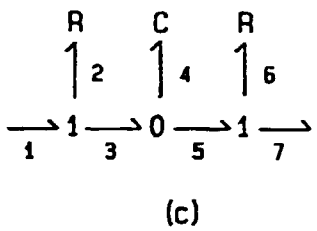


Figure 2: Resistor and Capacitor elements separately, and combined as a lumped diffusion bond graphs, a, b,and c, respectively.

In electrical circuits the role of capacitor and resistor are separate, but in chemical systems they are combined in each element of the medium. To model an element however, it is usual to separate them formally and mathematically. It is common (although not mandatory) to split the elemental resistance into two parts (often equal) as in fig.2c, where power enters the element by bond 1 and leaves by bond 7. In its passage power is dissipated in R_2 and R_6 and stored in C_4. Such a bond

graph, which, on its own, incorporates all the relevant functions of a diffusion element is termed a 'lump'. A 1-lump model of a diffusion element, is therefore characterised by a single capacitor, C_4 and two resistances R_2 and R_6, whose sum equals the total membrane resistance.

The precision of quantitative modelling is greatly improved if the system is subdivided into a number of lumps, each characterised by its own resistance, capacitance and local effort. The greater the degrees of sub-division, the more closely the bond graph will approach a true continuum of states.

Bond graph notation includes a number of additional functional elements [3], but the only one to be used here is the source of constant effort, SE, as shown in fig.3.

[The bond graph model of a simple membrane was demonstrated in the lecture by a hydraulic analog. In place of resistors there were capillaries (which resist flow) and in place of capacitors, simply vertical tubes at regular interval along the capillary path. In non-steady state conditions the flows entering and leaving the vertical (capacitor) tubes would not be equal and so the level in the tube would either rise or fall changing the pressure and so the effort or driving force on the liquid which drives the liquid at that point in the system. The source of effort, SE, and the terminal capacitor, C, are, in this hydraulic analog merely a constant head device and a collecting volume or receiver (a beaker), respectively. Because the laws of transport are analogous for heat, water flow, diffusion and electrical current, as noted, then such a hydraulic model is an exact analog of our diffusion bond graph. The bond graph can however be used to develop sets of differential equations, (such as eqns.(8),(9),(10),(11)) which may then be solved by standard computational methods, by computer.]

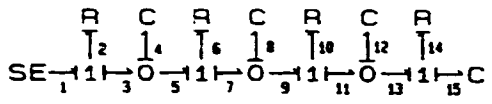


Figure 3: A three lump bond graph of membrane through which diffusion occurs between a constant source, SE, and a limited collecting volume represented by the terminal capacitor, C.

(A source of constant effort is a capacitor with (near) infinite capacity, leaving its effort (effectively) unaltered, in the time scale of the experiment, eqn.(5). It imposes 'infinite bath' conditions. Limited volumes of homogeneous (well-stirred) solution or gas are modelled in the bond graph as capacitors. Unstirred layers of solution at surfaces or layered membrane structures, merely provide additional lumps in the model, each with its own characteristic resistance and capacitance, fig. 2c. A 3-lump bond graph model for diffusion in a membrane exposed to a constant source, **SE**, on one side and a limited collecting volume, **C**, on the other, is shown in fig.3. In a 3-lump model only three internal efforts and charges are defined within the membrane phase. For an **n**-lump model, **n** such efforts are defined, one in each contiguous lump, to define a 'staircase' of efforts, which will approximate ever more closely to a smooth profile as the number of lumps in the model is increased.

CHARACTERISTIC EQUATIONS OF STATE

For all one-dimensional diffusion schemes, simple bond graphs of the type shown in fig..3 are required. With the bonds numbered as shown, the model may be expanded to as many lumps as required. Using the constitutive relationships for each element and beginning with the flows, $\dot{q}$, into each capacitor in turn, it is always possible to characterise a bond

graph with **n capacitors** in terms of **n** first order differential equations [3]. For the three lump, four capacitor, SE-C bond graph, shown in fig. (3) the four characteristic 'state-space' equations are,

$$\dot{q}_4 = e_1 R_2^{-1} - (C_4^{-1}R_2^{-1} + C_4^{-1}R_6^{-1})q_4 + (C_8^{-1}R_6^{-1})q_8 \quad (8)$$

$$\dot{q}_8 = (C_4^{-1}R_6^{-1})q_4 - (C_8^{-1}R_6^{-1} + C_8^{-1}R_{10}^{-1})q_8 + (C_{12}^{-1}R_{10}^{-1})q_{12} \quad (9)$$

$$\dot{q}_{12} = (C_8^{-1}R_{10}^{-1})q_8 - (C_{12}^{-1}R_{10}^{-1} + C_{12}^{-1}R_{14}^{-1})q_{12} + (C_{15}^{-1}R_{14}^{-1})q_{15} \quad (10)$$

$$\dot{q}_{15} = (C_{12}^{-1}R_{14}^{-1})q_{12} - (C_{15}^{-1}R_{14}^{-1})q_{15} \quad (11)$$

Eqn.(9), which defines the flow $\dot{q}_8$, into the middle capacitor, C_8, is typical any additional lumps which may be added to the bond graph to increase its precision. Such 'state-space' equations contain only three terms and involve only the central capacitor, and those on either side of it. A system in which no efforts are defined will decay from its initial non-equilibrium state to equilibrium (free response). Typically the source of constant effort is replaced by a limited volume of diffusant in which case a capacitor, C, will replace the effort source on bond #1, fig. 3. The state space equations will be unaltered except that the effort, e_1, eqn.(9), will be replaced by the constitutive relationship of the new capacitor, q_1/C_1 and an additional first order equation in $\dot{q}_1$, analogous to eqn.(11), is required. The same bond graph and state-space equations apply to single phase diffusion in, for example, sheets rods of membrane or columns of liquid in free diffusion [7].

The state-space equations, once defined, are easily integrated numerically from any chosen initial state, defined by the source effort (if defined) and the charges (moles) in each capacitor. Numerical integrations were performed using standard methods.

FICKIAN DIFFUSION MODELS

To illustrate bond graph procedures, we may consider Fickian systems initially. Although the treatment is in no way restricted to such systems, they are convenient for these demonstrations, not least because rigorous mathematical solutions available in the literature [8][9] against which bond-graph simulations may be readily compared. In particular, since the amount of computation increases greatly with increasing reticulation, it is of primary interest to determine the minimum lumped description consistent with quantitative modelling.

It is usual to define the permeability, P, of a membrane by eqn.(12),

$$f = \overline{P}\Delta c \quad (12)$$

where Δc is the concentration difference across the membrane. For a Fickian system

$$\overline{P} = \frac{\overline{D}A\alpha}{l} \quad (13)$$

where $\overline{D}$ is the diffusion coefficient, A the area, and l, the thickness of the membrane. The distribution coefficient, α defines the equilibrium ratio of the concentrations of diffusant in membrane to contact solution (or gas) phase, $\overline{c}/c$. A comparison of eqns.(2) and (12) shows the (fickian) bond graph resistance to be the reciprocal of the permeability, $\overline{P}$, eqn.(14).

$$R = \frac{1}{\overline{P}} = \frac{l}{\overline{D}A\alpha} \quad (14)$$

Eqn.(14) may be applied to each slice or lump in the bond graph model, and since all efforts are referred to the external phase (solution or gas) this definition allows that the membrane itself may be made up of different layers of differing thicknesses, each with its own diffusion and distribution coefficients. In this way multilayer membranes may be modelled. Using the Fickian model we may show in a similar fashion that the capacitance of a lump (or collecting volume capacitor) is simply αV, eqn.(15), [7][10].

$$C = V\alpha \tag{15}$$

since

$$\dot{q} = \frac{d\bar{n}}{dt} = V\frac{d\bar{c}}{dt} = V\alpha\left(\frac{dc}{dt}\right)$$

where $\bar{n}$ and $\bar{c}$ are moles and concentrations, respectively in the membrane phase.

On the Fickian model chemical potential is no longer taken as effort, it is replaced by concentration. The constitutive equations for capacitors and resistors remain unaltered (eqns.(2),(5) (6)) and state-space equations (such as eqns.(8)-(11) for a three-lumped model membrane (fig.3)) remain, with resistances and capacitances defined as above, eqns.(2) and (5). Bond graph techniques may be used in a Fickian model, but since the product of effort and flow no longer defines power the treatment must be considered pseudo-thermodynamic. Fully thermodynamic treatments are necessary if flows are coupled [5][11].

Simple Diffusion:

Figure 4 shows the effect of varying the size of the collect ing volume upon the quantity of gas, q(t), which will diffuse through a thin sheet of membrane into a collecting volume (terminal capacitor) when it is exposed to a constant pressure of that gas on its outer side. This example is taken from [10]. The upper curve represents the infinite volume case and with a ten lump or larger reticulation the bond-graph predictions agree to better than 0.1% with the mathematical solution.

Limited collecting volumes present no additional complexity, and merely involve a changing in the value of the final collecting volume capacitance, fig..3. Bond graph simulations for an experimental volumes of 1.5 ml and 0.1 ml are shown in fig.4 (middle and lower curves, respectively).

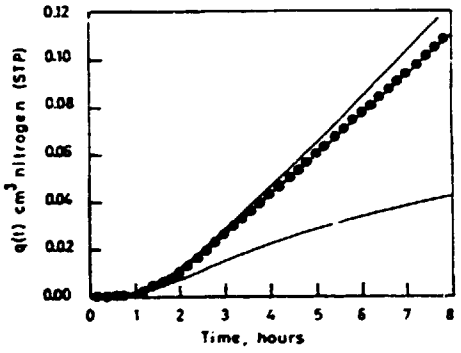


Figure 4: Simulation of the quantity of nitrogen, q(t), diffused from a constant pressure source (53.37mm Hg) through a planar membrane into a collecting volumes of 1000cm^3 (upper curve), 1.4980cm^3 (middle) and 0.1000cm^3 (lower curve). Filled circles mark experimental data [12] for collecting volume 1.498cm^3. Membrane: (ethylene-propylene co-polymer film), area; 45.6cm^2; thickness, 0.1013cm, N_2 solubility 7.118×10^{-4}Barrers, D,3.09×10^{-7}cm^{2}s^{-1}.

APPLICATIONS

The dotted curve superimposed on the former shows that this prediction is in excellent agreement with experimental data [12]. The ease of simulation of infinite band limited volume conditions is to be contrasted with the extreme complexity of dealing with the same problem mathematically [8][9]. The bond graph model provides not only output and flux data, but also predicts experimentally inaccessible concentration profiles in the membrane phase as a function of time, fig.5

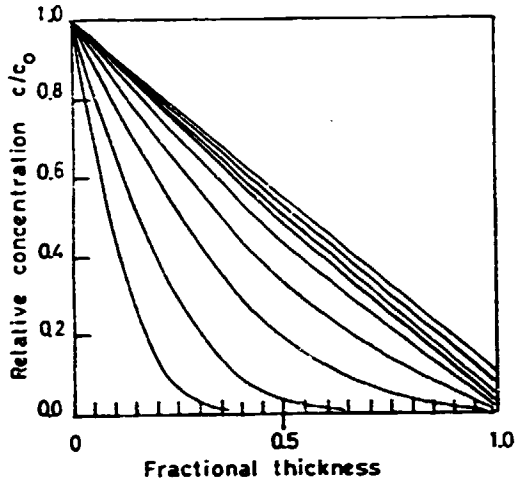


Figure 5: Concentration profiles within the membrane, obtained for the experimental system (collecting volume 1.498 cm^3), using a 20-lump bond graph. Concentrations have been plotted at the centre of each lump and joined to give smooth concentration profiles.

These local membrane concentrations are easily obtained since during the computer simulation, the charge (moles of diffusant) in each lump volume is obtained at each integration step. From this simple bond graph, all the classical diffusion and heat transport properties of slabs, (infinite) cylinders, spheres etc may be simulated. Gas diffusion was chosen here solely because suitable data were available for comparison, the treatment is equally viable for membrane/solution systems and with little modification to thermal diffusion problems.

For systems with a free response the source of effort (SE) is repiaced by a capacitor (C) as noted in the theory above. Figure 6 illustrates the release of diffusant from a membrane dipped into a well stirred solution, and fig.7 the decay of the same membrane from a linear, steady state concentration profile with the membrane clamped between solutions of equal volume.

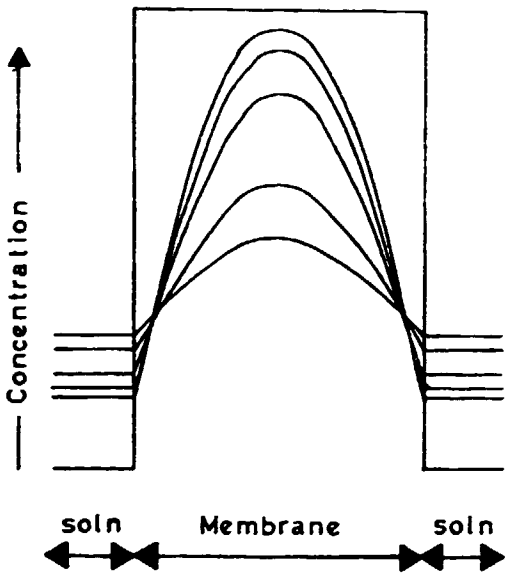


Figure 6: Concentration profiles obtained when a membrane is dipped into solvent. Time span ten minutes, edge effects ignored. Membrane: area 1.5810cm^2, thickness 0.0632 cm, D $1x10^{-4}cm^2s^{-1}$, alpha 10.0 and solution volume 2mL. Concentrations are shown on the common (solution) reference,in the membrane phase $\overline{C} = C\alpha$.

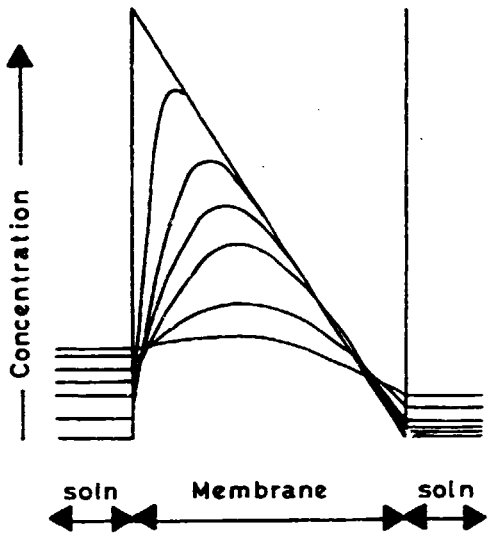


Figure 7: Decay from an initial linear concentration profile steady state. The membrane (as fig.6) now clamped between two solutions (each 1ml).

Diffusion problems in which the membrane or diffusion medium is made up of layers, or, when diffusion polarisation occurs at the interface between two phase (for example solution and membrane) are solved by adding further lumps to the model each with its own volume and diffusion (transport) parameters.

The systems so far simulated have well known mathematical solutions and were used to check the quantitative capabilities of the bond graph models and to set degrees of reticulation (number of lumps) and the step times ofr integration to obtain quatitative simulations. As systems simulated get much more complicated only simulation techniques can be used.

For example for non-steady state diffusion through a multilayer membrane, fig.7a.

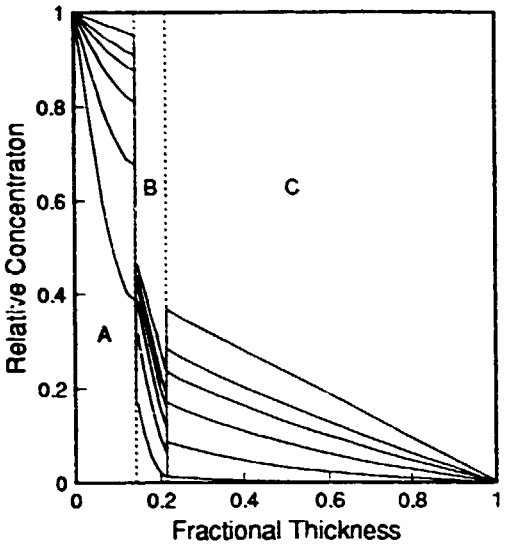


Figure 7a: Non steady state diffusion through a multilayer membrane/solution system, in a time lag experiment. Simulation of a bilayer membrane: active layer, B, 5um, support layer C, 55um, and unstirred solution layer, A, 10um. The distribution coefficients and diffusion coefficients of A,B and C were 1, $2x10^{-5}$; 0.5, $2x10^{-4}$ and 0.8, $1.15x10^{-5}$, respectively. A sixty lumped model was used.

Non-linear Systems:

Since the state-space equations are integrated numerically, the resistance, capacitance, and source terms may be redefined after each incremental integration step-time. This allows precise prediction of non-linear processes in which diffusion coefficients are known to change either as functions of time or as determined by their concentration dependence, progressively altering resistance coefficients during the process, eqn.(14). Such factors as swelling, which alter the lump volume, affect both resistance and capacitance values eqns.(14)(15). C[illegible] ug release devices have been d[illegible] polymers which swell. Such devices depend upon the fact that as drug diffuses out of the polymer device and concentration profiles flatten out, the expected steady reduction in efflux, is compensated by the increase in the diffusion coefficient in the swelling layers.

As an example of the implementation of network thermodynamics to problems of this nature a computer simulation of caffeine infusion from a tea leaf is presented. This particular problem was chosen because a near complete set of experimental data were available [13]. The analogy between this phenomena and drug release from a swelling hydrogel polymer however is **exact.**

A tea leaf is sufficiently thin so that diffusion from the edges of the leaf can be ignored. The problem then becomes that of diffusion from the faces of a swelling plane sheet. Also for added convenience diffusion into an infinite medium only was considered, this ensures that distribution coefficients, α may be ignored eqns(14),(15). The leaf considered had an initial thick ness, $2l$ (.08 mm) and an area, A ($1mm_2$). The overall volume of the leaf was considered to increase linearly with time finally doubling its volume relative to the dry membrane after 60 seconds. These experimentally determined properties do not provide sufficient information for computer modelling. The Network model requires not just the total swelling but also water penetration profiles as functions of time and position in the leaf. In addition the local diffusion coefficients must be known as functions of the degree of swelling. Since for the test system these detailed relationships had not been measured, experimentally reasonable assumptions were made.

Two methods for predicting the water profile were used. The first assumed that it remained linear through the leaf, penetrating from a fully swollen surface at time zero. The position of the water front was then calculated to give an overall swelling rate which agreed with experiment.

Volumes were considered to swell isotropically. The water profiles calculated on this basis are shown at 2 second intervals in fig.8 in which distance is expressed as the fractional thickness relative to the fully swollen leaf.

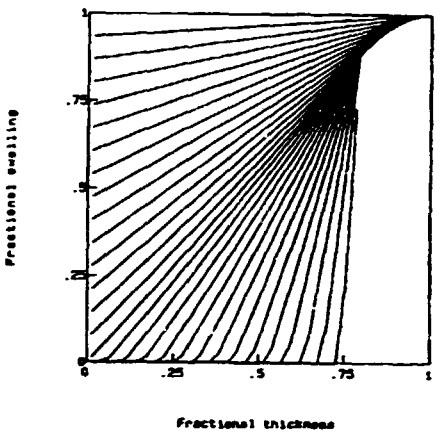


Figure 8: Successive swelling profiles, at 2s intervals, calculated from penetrating front algorithm. Distances measured from the centre of the leaf, expressed as fraction of the fully swollen length.

The dry membrane at time zero has a fractional thickness of 0.8. The second algorithm used, perhaps less realistic, considered the profile to invade the leaf sharply such that volumes were either fully swollen or completely dry. These profiles are unremarkable and are not presented here.

It was assumed that the diffusion coefficient changed due to tortuosity factors alone. There are many relationships available which predict the effects of tortuousity. Prager's derivation, [14] has been used here. This provides the relationship between the degree of swelling at each location and the local diffusion coefficient. The specific tortuousity

correction is not critical in this simulation. In fact, the implementation of Prager's relationship, in this particular model, results in an almost linear function between the diffusion coefficient and fractional swelling. The experimental diffusion coefficient for the fully swollen leaf, $\overline{D}_o$ was $5.0 \times 10^{-7} cm^2 s^{-1}$. Thereafter the diffusion coefficient is predicted by Prager's factor to decrease to $\approx 4 \times 10^{-7} cm^2 s^{-1}$ at .75 fractional swelling, $\approx 3 \times 10^{-7} cm^2 s^{-1}$ at 0.5, and $\approx 1.6 \times 10^{-7} cm^2 s^{-1}$ at .25. In the dry leaf membrane the diffusion coefficient was taken as zero. For the penetrating front example this rapidly changing diffusion coefficient is of particular importance.

Using the network methods outlined above the concentration profiles of diffusant (caffeine) were predicted at 2s intervals during the swelling - diffusion process, fig.9.

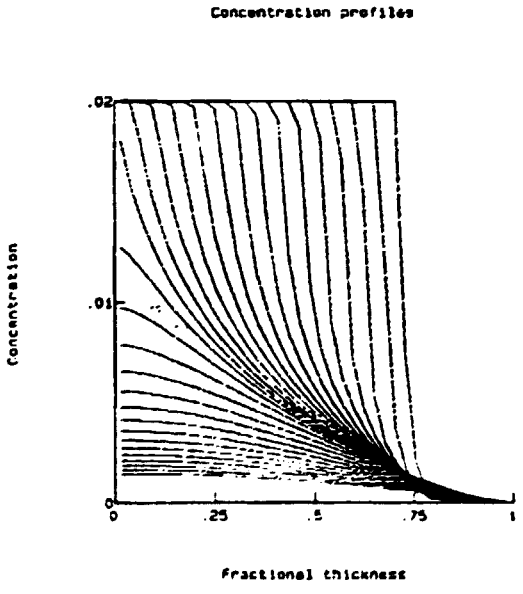


Figure 9: Successive concentration profiles for caffeine within the swelling leaf, at 2s intervals, corresponding to fig.8.

In this example the total amount of caffeine in the tea leaf at time zero was 1.6×10^{-5}mmol ($.02 mmol ml^{-1}$). Concentrations have been plotted relative to the fully swollen thickness and at the same time as for the water profiles shown in fig.8. In this way each concentration profile may be compared to the corresponding swelling profile in the leaf. Figure 10 gives the simulated release of caffeine using the penetrating front algorithm for water uptake (curve 1), and the sharp front algorithm, (curve 2).

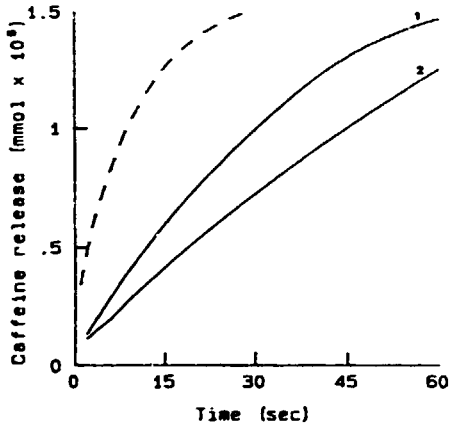


Figure 10: A comparison of caffeine release predicted using the penetrating front and sharp front algorithms for solvent invasion, curves 1 and 2, respectively. Dashed line, comparative release from a fully swollen leaf at equal caffeine loading.

These may be compared with the mathematical solution for release from a fully swollen leaf [9], shown as a dashed line on this figure.

Hydrogel constant release devices operate on the same principle and it is clear that system dynamic modelling of this type will be of considerable use in their design.

Morphine release from a swelling hydrogel has also been simulated. The hydrogel, initially dry, was in the form of a slab, thickness 0.28cm, area $2.75 cm^2$ and loaded with

0.23mmolcm^{-3} morphine. On immersion in water, it swelled to twice its dry volume, with a half-time of two hours. The swelling profile was the same as fig.8 for the tea leaf except that the profiles were recorded at 8 minute intervals rather than 2 second intervals. Morphine concentration profiles in the swelling hydrogel are shown in fig.11 at 8 minute intervals up to 4 hours.

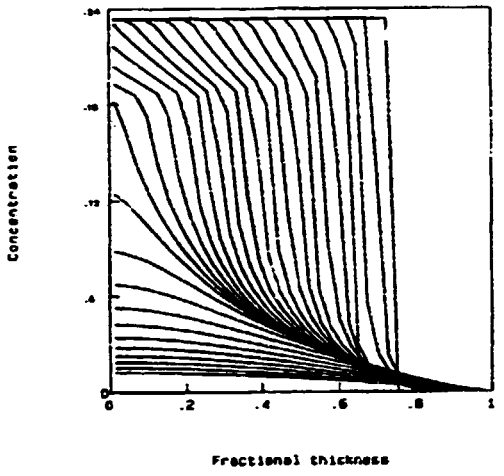


Figure 11: Successive concentration profiles for morphine within the swelling, hydrogel at 8 minute intervals.

The near constancy of the concentration gradient at the outer face, indicates a steady release of drug and this is confirmed by the predicted release into the environment, fig.12

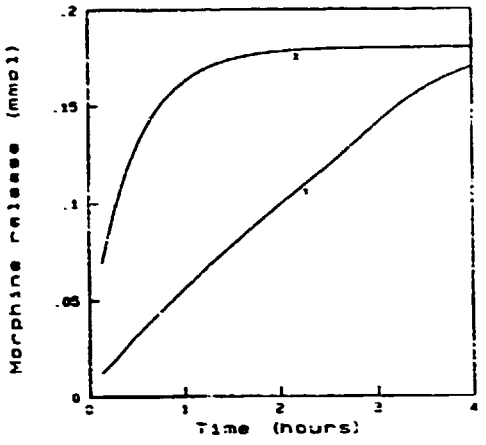


Figure 12: A comparison of morphine release predicted using the penetrating front algorithm for solvent invasion and that from a fully swollen hydrogel, curves 1 and 2, respectively.

The lower curve, being linear, indicates a constant release. The upper curve, for comparison, is the expected release from the fully swollen hydrogel, similarly loaded.

A further illustration of simulations involving variable diffusion coefficients is found in ion exchange. The ion fluxes of exchanging ions (a and b) are coupled through the electrical potential and a common diffusion coefficient Dab is obtained, (using Nernst-Planck theory) which is strongly concentration dependent, eqn.(16).

$$\overline{D}_{ab} = \left\{ \frac{\overline{D}_a \overline{D}_b (Z_a^2 \overline{c}_a + Z_b^2 \overline{c}_b)}{\overline{D}_a Z_a^2 \overline{c}_a + \overline{D}_b Z_b^2 \overline{c}_b} \right\} \qquad (16)$$

Figure 13 illustrates the evolution of a membrane, initially in the hydrogen form and bathed on both side with hydrochloric acid when the left hand side solution is changed to NaCl at time zero.

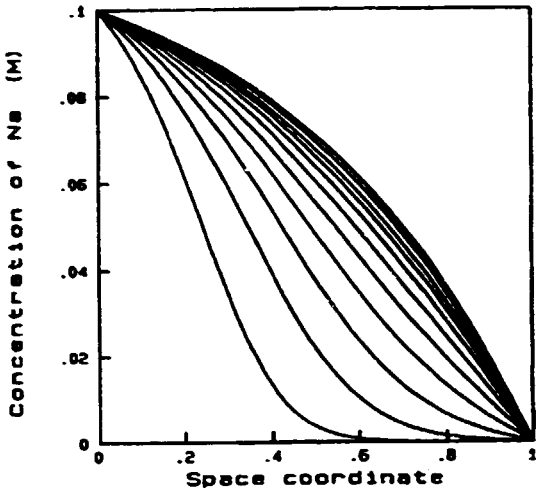


Figure 13: Simulation of approach to steady state diffusion for Na^+/H^+ exchange across a cation exchange membrane, as ref [15]. Sodium concentration profiles at 5,10,15,20,25,30,35,40,45,55,75 and 110s, thereafter invariant as steady state is reached.

The system is that described by Helfferich and Ocker [15] with a membrane thickness 0.1cm, modelled by a 30-lump bond graph. Twelve successive sodium concentration profiles are shown, the final two (at 55s and 75s) effectively superimpose as a steady state is approached.

Variable Input Systems:

The source of effort (SE, fig..3) need not be constant in the bond graph simulation, it need only be known as a function of time. In the thermal bond graph the heat gains and losses through such barriers as walls or insulating layers as a function of varying external temperature would provide a useful application.

In membrane studies the author has applied this principle to determine the system behaviour of a membrane exposed to regular square concentration (or pressure) waves [16]. As the source concentrations oscillates, concentration waves travel through the membrane. The emergent wave is much reduced in amplitude and out of phase with the signal input wave.

From the phase shift, the diffusion coefficient of the permeant may be obtained. Bond graph and mathematical simulations are compared with measured concentration waves, fig.14.

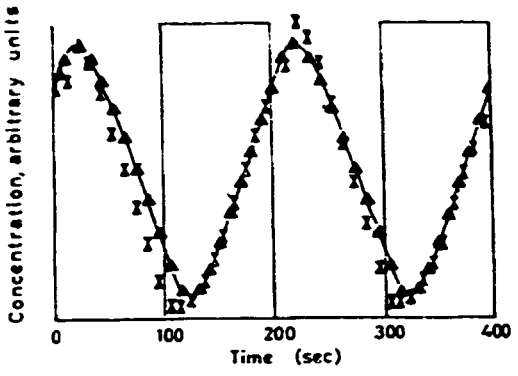


Figure 14: Phase shift between square concentration wave input at the surface of a membrane and the emergeant wave detected at the opposite side in a small collecting volume: ----- mathematical solution - Δ bond graph ; X experimental - taken from [16]. System: dialysis membrane: permeant KCl : detector, conductivity probe.

Square concentration waves were generated using a spray technique. The same technique conveniently allows the determination of diffusion coefficients and permeabilities by the time-lag method [17]. In both cases bond-graph simulations were used to optimise the design of the experimental detection equipment.

The oscillator experiment can now be performed under automatic control using fully computerised equipment recently built in the author's laboratory.

The latest version uses a multi-time lag system. The external solution is stepped from an initial condition of equilibrium (when it is equal to that in the collecting volume) to a higher level and back again at regular intervals in what turns out to be repetitive time-lag experiment, fig.14a.

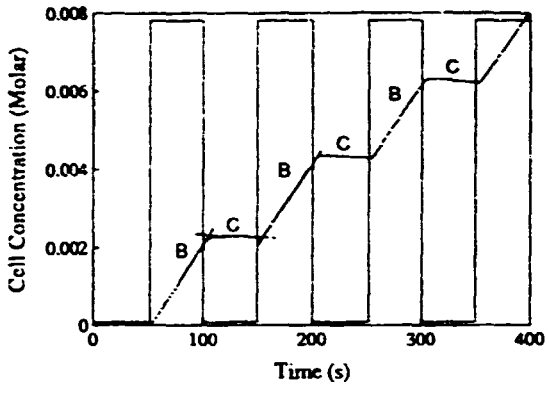


Figure 14a: Experimentally observed emergeant concentration waves from a stepped oscillator experiemtn with an anotec membrane 0.02nm pore size bilayer membrane, with oscillations between 0.1KCl and water alternately, switch period 100s, collecting volume 2.44cm³ and exposed membrane area, 0.771 cm², [19].

Intersections between linear portions A and B give repeated estimates of time lags $\tau = l^2/6D$ and the slope of A - slope of B provide repeated estimates of the permeability. The method has enormous advantages over conventional method in that a large number of precise estimates of diffusion coefficient and permeability, P, eqn.(13), are obtained in a single experiment.

Coupled Systems:

Coupled systems are strictly outwith the scope of this paper, but such systems have been simulated in the author's laboratory and include both chemical coupling between molecular fluxes and electrochemically coupled systems of importance in electrically driven membrane processes such as electro-dialysis, in batteries, or in applications such as chlor-alkali production. (Here we distinguish the free diffusion of two ions in a process of ion exchange (treated above) from multi-ionic electrodiffusion since such systems require a full coupling analysis and cannot be described in terms of a simple diffusion model). In fig.15 concentration profiles for chloride and hydroxide are shown in a simulation of a cation exchange membrane set between two concentrated solutions, one of sodium chloride, the other sodium hydroxide. In this simulation a current densities of 3000 Am^2, was used, as in the chlor-alkali cell and as seen in fig.15, concentration profiles are non-linear, being strongly influenced by the electric fields. Network thermodynamics is particularly useful (possibly uniquely so) in coupling and energy transduction modelling. This is due to its unified approach to all energy domains, based upon flows and efforts, defined by the dissipation function eqn.(1).

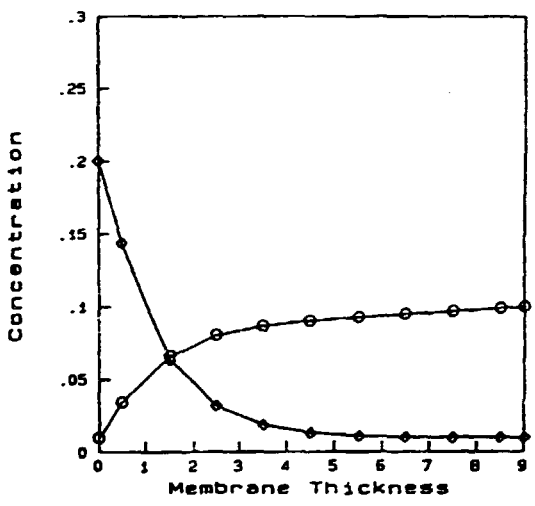


Figure 15: Illustration of membrane profiles for chloride * , and hydroxide o , in a cation exchange membrane in which the current density is 3000Am² modelling the behaviour expected in Nafion membranes in use in chlor-alkali cells.

Two-dimensional diffusion:

The extension of bond graph models into the second dimension is relatively simple. The bond graph, fig.16 is an extended 2D lattice with directional resistances R_x and R_y space equations are obtained as before and the progress of diffusion in two dimensions may be predicted.

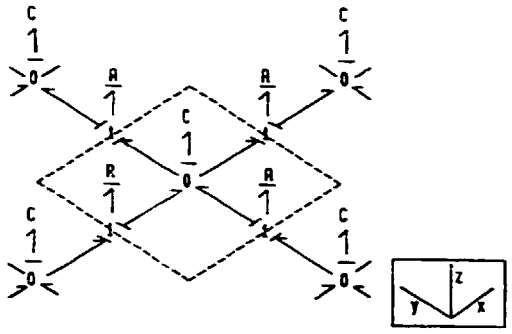


Figure 16: Bond graph for two dimensional diffusion [6].

Concentration profiles become 3D objects and may be displayed as in fig.17.

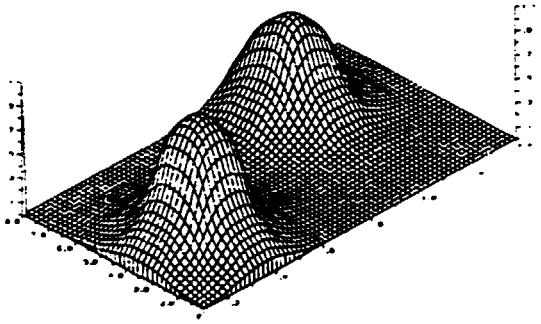


Figure 17: Simulation of free diffusion in a plane.

This is a concentration profile of a diffusion system in which two high concentration spots set asymmetrically interdiffuse on a rectangular plane. To test the quantitative aspects of the model, several systems were simulated for which analytical models were available. These include effusion from a cube through four contiguous faces and from a limited cylinder (disc or pill). The edge effects caused by additional diffusion paths around the edge of a clamped disc were analysed by Barrie, Barrer and Rogers [18]. Figure 18 shows a bond graph simulation of the concentration profiles for such a membrane in the steady state.

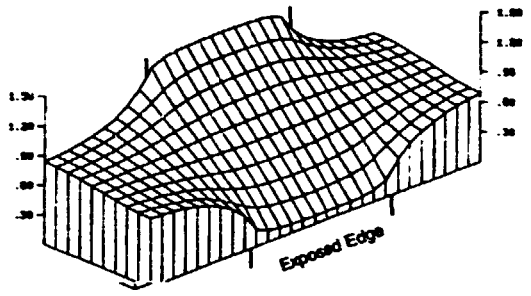


Figure 18: Steady state concentration profiles across a planar disc with edge effects.

The local fluxes are, as always, determined in these bond graph simulations. Quantitative agreement with the theoretical model was obtained, Table 1. Clearly all the additions extensions to the linear bond graph discussed above for one dimensional cases can be transferred to two- and even three-dimensional systems.

Table 1: edge effects on steady state fluxes through a planar membrane : a comparison of the analytical solution [18] and bond graph simulations

$\frac{l}{a}$	$100\frac{(b-a)}{a}$	$100\frac{(J-J_o)}{J_o}$	
		Analytical*	Network**
0.2	18	7	6
0.4	18	13	14
0.6	18	19	19
0.8	15	21	21

The membrane disc : thickness, l; radius, b; exposed radius, a.
The steady state flux, J, with edge diffusion effect, may be compared with J_o; the corresponding flux with no edge effect ($b-a$).
* Percent increase in flux predicted by the analytical method of Barrer, Barrie and Rogers [18].
** Corresponding results obtained using a 10x10 bond graph.

Network thermodynamics and the bond graph theory combine to give a universally applicable system dynamics for membrane systems. It is clear that such a theoretical tool

could (and should) have a major role in the design an modelling of new membrane processes and the optimisation of existing ones.

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THE DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS

REGULATORY CONSIDERATIONS

by Robert F Weir

1. Introduction and History

In the development of pharmaceutical production and marketing, government intrusion is a relatively modern phenomenon.

The exception is the United States. In 1937 Samuel Massengil distributed an elixir of sulfanilanide. Included in the formulation as a solvent was diethylene glycol. 107 people died as a consequence, including many children. This prompted the passing of the 1938 Food Drug and Cosmetic Act which necessitated the approval of any new medicinal product by the FDA before marketing.

Elsewhere in the world the trigger for government controls was the thalidomide tragedy. In 1957 the German company Chemie Grunenthal marketed thalidomide as Contergan in Germany. The product was licensed to various overseas corporations, such as Distillers in the UK and Horner and Merrel in Canada. One of the attractions of Contergan was its claim to be free from toxic effects. However, in 1959 the first reports of CNS interactions appeared and in 1960 phocolmenia (sealed limbs) was reported in Germany. Further reports of teratogenicity (that is, causing congenital malformations in children) led to the withdrawal of the product from the German and British markets in 1961 and to a similar fate in Canada in 1962.

Thalidomide was still awaiting approval in the United States at this time. The appointed assessor had had concerns about reports of peripheral neuritis and for that reason had delayed approval of the product.

The thalidomide tragedy generated world-wide effects. Thus, in 1962 the Food Drug & Cosmetic Act in the United States was strengthened, demanding (among other things) that any proposed clinical trial be approved through the submission of an Investigational New Drug Application (IND), that adverse events be notified to the FDA and that standards of Good Manufacturing Practice (GMP) be implemented.

In the United Kingdom in 1963 the Committee on Safety of Drugs (The Dunlop Committee) was formed. Submission of pre-marketing data to this body was voluntary, but the industry acted in a responsible way. Only in 1971, following the passing of the Medicines Act, did statutory controls come into force, necessitating a major review of the safety implications of a proposed new compound prior to marketing.

Holland, Germany and other European countries introduced similar regulations for pre-marketing approval. Today every country exerts some measure of control. As this paper will illustrate, there are significant moves towards a harmonisation of standards on a world-wide basis.

For completeness it should be stated that the United Kingdom had introduced controls for a limited range of products as early as 1925. The Therapeutic Substances Act was inspired by the problems of quality control connected with biological products, or products requiring biological testing. The Act, which

applied to such products as vaccines and sera, antigens and insulin, included controls on the release of each batch by an independent state laboratory and the right to inspect manufacturing facilities.

2. Intra-Company Government Initiatives

2.1 Operating Standards

Increasingly on an international basis governments are setting standards for research development and production. These standards have been embodied in codes or guidelines (depending on the particular country) known as:

Good Manufacturing Practice (GMP)
Good Laboratory Practice (GLP)
Good Clinical Practice (GCP)

We will discuss later the actions which governments may take in order to ensure compliance with the standards. All scientific and technical aspects of company operations are embraced and may be summarised as follows:

a) **People** A company must employ individuals of adequate academic experience according to the tasks in question. These individuals must be exposed to appropriate industrial experience and trained accordingly. Records of their training must be maintained.

b) **Facilities** Buildings must be adequate in terms of quality and space. They must provide the environment appropriate to the operation; this means control of the microbiological surroundings and of the particulate content of the air.

The housing and use of animals in experimental programmes is similarly standardised in terms of environment, hygiene, segregation and humane systems.

Production facilities must be designed to avoid cross-contamination. In particular cases, such as the manufacture of penicillin containing products, significant physical separation is demanded by certain government authorities.

Equipment and instrumentation must be adequate for the stated tasks. Documented procedures for cleaning, calibration and routine maintenance must be in place, and there must be evidence that staff have been trained to a sufficient level of competence to handle the equipment and instruments.

c) **Documentation** The adequacy of operating systems is proved by the quality of documentation. Similarly, records must be generated and maintained for all relevant activities.

d) **Operations** Unit operations in manufacturing and in laboratories must be validated in order to prove their adequacy and correctness. The actual manufacturing processes must comply with the information submitted to the government as part of the original pre-marketing approval.

Clinical operations, whether within the company or in hospitals, must similarly be controlled in order to confirm compliance with the original approved protocol.

2.2 The Qualified Person

For the purpose of maintaining the quality of pharmaceutical products throughout the European community, an EC directive was established creating the role of Qualified Person. This demands that within each pharmaceutical company one individual is responsible for the decision to release a supply of sales goods to the marketplace.

This role was first conceived in France in the form of the "Pharmacien Responsible". With the creation of the Single European Market on 31 December 1992, and consequent steps towards the free movement of goods, the Qualified Person becomes an extremely critical function for the healthcare industry in Europe.

National governments are charged with approving individuals as Qualified Persons. For this purpose, academic attainment, industrial experience and targeted training are defined. In the United Kingdom an individual meeting these standards and wishing to become a Qualified Person must undergo assessment by one of the professional bodies charged with the maintenance of standards.

3. Government Involvement in Manufacturing Authorisation

Prior to the approval for marketing of a new pharmaceutical product, systems exist for the authorisation of the actual manufacturing facility. However, there is great country to country variation in the associated procedures, and harmonisation is far from complete.

Just as government demands that standards of Good Practice (GMP, GLP and GCP) be maintained, so many governments demand the right to inspect facilities. This generally takes place prior to marketing authorisation and may be repeated from time to time. Compliance may lead to the provision of a specific licence. Failure of compliance leads to government actions in order to generate improvements and will normally lead to a delay in the grant of marketing authorisation. Failures of compliance have necessitated the withdrawal of products from the market from time to time.

In the United Kingdom a specific Manufacturer's Licence is issued, granting permission for the manufacture of certain products. This is linked with the marketing authorisation for the products themselves. Inspection systems similarly exist in continental Europe. There is a measure of cross-acceptance of National Inspection Reports within and beyond Europe, frequently through the medium of the Pharmaceutical Inspection Convention (PIC). The US Inspection systems are considered to be the most intensive and far reaching, frequently being undertaken prior to the grant of clinical trial permission (that is, pre IND inspection) as well as before marketing authorisation.

4. Government Involvement in the Authorisation of Human Studies

Studies on human beings are generally categorised into four groups:

Phase 1 studies, involving kinetic examinations on healthy human volunteers.
Phase 2 studies, for dose ranging investigations.
Phase 3 studies, involving major clinical investigations.
Phase 4 studies, post-marketing clinical investigations.

Phase 1 studies are universally free from government intrusion, but generally require some form of local hospital approval, such as by an Ethical Review Committee. Government controls of clinical studies are far from universal, and even in Europe where relatively sophisticated systems of market authorisation exist, it may not be necessary to seek approval to commence studies on patients.

The original (but not commonly used) system for approval in the United Kingdom involves an application for a Clinical Trial Certificate (CTC). In order to accelerate the commencement of clinical studies, the UK government introduced a system rather similar to that of the US Investigational New Drug (IND) arrangements. The UK system is known as the Clinical Trial Exemption Procedure (CTX).

Both the CTX and IND systems involve negative vetting. This means that clinical studies may commence assuming no objections are raised by the authority within a certain number of days (35 in the case of the UK).

An application for an IND or CTX approval must be made according to the stated government requirements. The degree of novelty of the active constituent

influences the extent and nature of data required. Also critical are the intended route of administration, the dosage, frequency of administration and the duration of the proposed clinical study. The use of a novel excipient or drug delivery system would also require documentation.

The UK government authority (the Medicines Control Agency) may determine that a CTX application is inappropriate. In such an example there is no right of appeal and the corporation must either submit a modified CTX or revert to a full CTC application.

Where the proposed product is based on biotechnology or involves a novel drug delivery system, it is preferable not to submit an application without pre-discussion at the government agency. Failure to do so will almost certainly lead to delays. Informal discussions or a pre-planned seminar for government agency reviewers should serve to develop a mutual understanding of the critical issues involved. This should also serve to accelerate the review and approval process.

5. Government Involvement in Marketing Authorisations

The three major marketing targets for a multinational corporation are undoubtedly United States, Japan and Europe. Although the European community does not have a single marketing authorisation system yet it moves relentlessly, albeit rather slowly, towards that goal.

The data requirements for the three blocks are rather similar but not identical. The US may demand more toxicity and clinical data, with an emphasis on bio statistics. The Japanese authorities generally demand toxicity and clinical information oriented to their own population.

The standard format for EC applications is as follows:

There are five parts listed I - V.

Part I is a summary of the dossier comprising:

I(a) **Administrative Data.** This includes information on the name of the product, its active ingredient, the dosage form, the name and address of the company and the official signatures.

I(b) **Summary of Product Characteristics.** This includes qualitative and quantitative particulars of the composition, covering both active ingredient and excipients. The dosage form and route of administration are stated together with information on the pharmacology and clinical indications, contra indications,

precautions and special warnings. The dosage should be defined in this section, together with relevant information about storage limitations.

I(c) **Contains the Expert Reports.** Under EC legislation it is necessary to file 3 Expert Reports covering chemical and pharmaceutical information, toxicological and pharmacological documentation and clinical documentation. These reports, prepared by an internal or an external Expert, are expected to review the relevant sections of the main dossier in a critical way. Guidance notes exist for those embarking on the writing of Expert Reports.

Part II comprises chemical, pharmaceutical and biological documentation.

II(a) Covers the composition of the product as well as the containers and closures.

Important within this section is the development pharmaceutics information. This would describe the problems encountered during development and the means by which they were overcome. Information on incompatibilities and interactions should be included as well as data on variations between clinical trial formulations and final compositions.

The incorporation of a unique delivery system would be discussed in detail in this section.

II(b) Describes the method of manufacture and the dosage form. This would include information on batch size and unique equipment required for successful manufacture. The individual stages in processing would be described and the validation of these would be discussed in detail.

II(c) Concerns the control of starting materials. For new active ingredients much detail would be expected here about the structure, description, development chemistry, controls during synthesis, impurities, batch analysis and specifications.

II(d) Describes the controls on intermediate products. This may be important where the manufacturing technique generates a controlled release formulation and in-process controls become critical.

II(e) Describes the controls on the finished product. These would cover physical tests such as, dissolution, microbiological procedures and chemical control tests. The results of batch analyses on preferably five consecutive full-scale production batches should be incorporated.

II(f) Describes the stability of the product. Justification for the design and protocol of the studies may be required, particularly where these vary from normal standards. The objective is to prove that the product remains adequately pure and potent during its shelf-life. Stability data may be required on a new active ingredient as well as the finished product.

Part III provides pharmaco toxicological documentation divided as follows:

III(a) - Single Dose Toxicity.

III(b) - Repeated Dose Toxicity.

III(c) - Reproduction Studies.

III(d) - Mutagenic Potential

III(e) - Carcinogenic Potential

III(f) - Pharmacodynamics

III(g) - Pharmacokinetics

III(h) - Local Tolerance/Toxicity

Part IV provides the clinical documentation divided as follows:

IV(a) - Clinical Pharmacology

IV(b) - Clinical Experience

IV(q) - Other Clinical Information

Part V provides for special particulars.

V(a) Relates to the dosage form and would provide information on packaging together with a draft of the label and the package insert.

V(b) Refers to samples which are required for applications in some, but not all, EC territories. The size of the sample, when required, also varies.

V(c) Relates to manufacturing authorisation. A copy of the manufacturing

authorisation should be provided where it already exists for the product in any EC territory.

V(d) Concerns marketing authorisation. Copies of any existing marketing authorisations from countries within or outside the EC should be provided.

Single State or Multi-State Authorisation in the EC?

Corporations operating within the EC now have a choice. They may elect to file individual market authorisation requests in each member state. Alternatively, they may choose (and are increasingly choosing) to follow the so-called CPMP or multi-state route. They may actually elect to initiate both procedures and cancel one or the other at a later time period.

The single state procedure clearly involves the submission of an appropriate dossier to each regulatory agency. Since there is limited standardisation between the member countries this is a cumbersome and costly process for the corporation. The CPMP multi-state procedure is almost certainly the precursor of a single EC market authorisation process and is becoming the route of choice for most of the pharmaceutical companies. The procedure operates as follows:

The first step is achieved when market authorisation takes place in one member state. Thereafter, the multi-state system would begin. The rapporteur, that is the acting agent in the outgoing member state (that is the country which has already granted marketing authorisation) is a key individual and should be consulted prior

to the initiation of the relevant documentation. The rapporteur obtains an assessment report about the product within his own state and sends this to the authorities in the other concerned member states.

The corporation files its application in all of the concerned member states, with a copy to the Secretariat of The Committee for Proprietary Medicinal Products (CPMP). When all of the concerned member states have received the valid application, the 120 day approving period begins. During this time each concerned member state must notify their reasoned objections to the granting of an authorisation. If no objections are raised the state is obliged to grant marketing authorisation. All reasoned objections are sent to the rapporteur and the company involved. Following consultations with the rapporteur, the applicant may appeal against the objections. After submitting the appeal documentation a minimum of 30 working days are required before the CPMP Meeting can review the information. Prior to the meeting, and through the mediation of the rapporteur, the company may elect for a personal hearing, including the presence of Experts to address the CPMP Meeting.

Certain categories of products may not be processed according to the multi-state system. However, this procedure is gaining in popularity because the approval times are generally much less than those recorded for single-state applications.

6. The EC Concertation Procedure for High Technology and Biotechnology Products

A significant advance took place in July 1987 with the establishment of the EC Directive for certain high technology medicinal products. This has become known as the "High-Tech Directive". This concertation procedure has certain objectives, including the following:

1. To allow an early exchange of views among assessors in European Regulatory Agencies about the content of complex submissions.

2. To accelerate the EC approval process.

3. To present a 10 year protection against a second applicant from the date of granting the first marketing authorisation.

The High-Tech Directive divides the relevant products into List A and List B products:

List A products are developed by means of the following:

> Biotechnological processes; recombinant DNA technology; controlled expression of genes coding for biologically active proteins in prokaryotes and eukaryotes, including transformed mammalian cells; and hybridoma and monoclonal antibody methods. Companies wishing to obtain EC approval for products in these categories **must** follow the High-Tech Directive route.

List B products are:

> Other high technology medicinal products that, in the opinion of the competent authority concerned, constitute a significant innovation or area of significant therapeutic interest. These include other biotechnological processes; medicinal products administered by means of a new delivery system; medicinal products containing a new substance or for an entirely new indication; new medicinal products based on radio-isotopes and medicinal products manufactured using processes that demonstrate a significant technical advance. The use of the High-Tech Directive is optional for products based on List B.

The obligation falls to the company to prove that the High-Tech route is appropriate for the product in question. The timetable for the consultation procedure is 180 days. However, the clock may be stopped when the obligation turns to the applicant to provide supplemental information. A period of 4 - 6 weeks is normally required to enable the rapporteur Member State to produce an initial assessment report. As for the general CPMP Multi-State Application (see Chapter 5), a rapporteur is selected and becomes a key individual.

An Appeals procedure exists, similar to that for products in the multi-state CPMP system.

Experience with the High-Tech concertation procedure is limited so far. A large proportion of applications have been from smaller companies aiming for

specialised markets. The benefits from the processing of difficult biotech applications by this route remain to be determined. However, the system appears to be an important step in the evolution of the EC market approval arrangements.

7. Inter - Government Initiatives

The previous chapters have indicated some of the steps which are taking place towards a common EC registration system. More details of these may be gained by studying the EC publications (see Chapter 9).

Outside of the EC, the Product Evaluation Report (PER) scheme was founded in 1979 within the European Free Trade Association (EFTA). This scheme aims to simplify the registration process, and thereby facilitate intercompany trading in pharmaceutical products. The initial member countries were Austria, Finland, Norway, Sweden and Switzerland. Germany, Italy, the Netherlands and United Kingdom have joined. After a lengthy period of collaboration with Sweden, Australia joined the scheme in 1990, as did Canada. It is likely that Hungary has now been accepted into the PER.

The scheme which is controlled from EFTA headquarters in Geneva operates through the exchange of Evaluation Reports on pharmaceutical products with the objective of speeding the individual state registration process.

Various consultation documents describe further steps towards simplification of the government role in marketing authorisation throughout the EC. These include the establishment of the European Medicines Evaluation Agency (EMEA). This body, which is intended to be responsible for both human and veterinary pharmaceutical products, would not replace national authorities but function as a co-ordination point for scientific and technical assessment of quality, safety and efficacy. It would also co-ordinate the reporting of adverse events

(pharmacovigilence), a subject of increasing concern to regulatory authorities. In the long term the EMEA could be expected to grow and become the registration authority for the EC.

8. Other Regulatory Considerations

8.1. Medical Devices

In most countries a distinction is drawn between a pharmaceutical product and a medical device. In the United States a simplified form of processing exists for recognised devices which are considered to be 'like and similar' to those previously marketed. Marketing authorisation is achieved through the use of the 510K documentation system.

Within the EC attempts are being made to standardise the approach to the authorisation of devices. It is possible that certain novel drug delivery systems may be categorised as devices.

The Active Implantable Medical Device Directive was published in 1990. A medical device is defined as an instrument, apparatus, appliance, material or any other article whether used alone or in combination, together with any accessories or software for its proper functioning intended by the manufacturer to be used for human beings in the diagnosis prevention monitoring treatment or alleviation of disease or injury, investigation, replacement or modification of the anatomy or of a physiological process and the control of conception, and which does not achieve its principal intended action by pharmacological, chemical, immunological or metabolic means, but which may be assisted in its function by such means.

An active medical device is defined as any medical device relying for its functioning on a source of electrical energy, or any source of power other than that directly generated by the human body or gravity.

An active implantable medical device is defined as any active medical device which is intended to be totally or partially introduced surgically or medically, into the human body or by medical intervention into a natural orifice and which is intended to remain part of the procedure.

In total, a series of four directives should cover the complete field of medical devices and will lead to a standardisation in EC authorisation.

8.2 Drug Master Files

It is important to bear in mind that, in making a request for market authorisation, a company is obligated to submit "all relevant information". On occasions this may be difficult because part of the "relevant information" has not been made available to the company. For example, the basic details of a drug delivery system which the company has licensed-in may be covered by strict confidentiality arrangements, preventing the company submitting sufficient details to the government agency.

In these circumstances, the difficulty is overcome by the use of the Drug Master File system (DMF), which enables the out-licensing company to provide, in confidence, an information package to the government agency with a cross-reference to any marketing authorisation request.

The EC system was revised in 1992 and the concept of a European Drug Master File (EDMF) was initiated. Confidential information covering a delivery system, a unique active constituent, or an excipient is provided in EDMF format in parallel with the submission of the marketing authorisation request.

Under US regulations two types of Drug Master Files exist. The so-called Type 2 DMF fulfils the same role as the EDMF and operates in essentially the same pattern. The close association between the EDMF and the marketing authorisation request within the EC is not necessarily so strict under US rules. Additionally, the FDA recognises a Type 1 DMF which describes the facility prepared and proposed for pharmaceutical manufacturing. This latter submission should stimulate a visit by the FDA Inspector, leading to a decision about the acceptability of the facility. Deficiencies will be listed on document 483 and classified according to severity. Correction of these deficiencies may be necessary before the approval of a New Drug Application (NDA) can take place.

8.3. Abridged Applications

In most western countries the opportunity exists to recognise the case for approval of certain products on the basis of a limited supply of information. This is known as an Abridged or Abbreviated Application (an ANDA according to the US procedures).

In order to achieve classification as an Abridged Application, the proposed new product must comply with one of several classifications. Thus, the active constituents should have been approved already in comparable dosages, the

proposed therapeutic indication may be different or the combination of known and approved active constituents is different. As justification for such applications already published data may be submitted. The abridged application system recognises the ethical considerations of avoiding unnecessary repeat animal and human studies. It is conceivable that, in certain circumstances, it may be justified to seek marketing authorisation under these rules for a product based on a unique drug delivery system. However, the definitions, as outlined in the previous paragraph, must be taken into account.

9. Further Reading

9.1 Mann, R.D. (1989). The historical development of medicines regulations. International medicines regulations: Proceedings of Centre for Medicines Research Workshop. Ciba Foundation, London 20/21 September 1988. Edited by Walker, S.R. and Griffin, J.P. Kluwer Academic, London.

9.2 Morris, J.M. (1990) Development pharmaceutics and process validation, Drug Development and Industrial Pharmacy, 16(11) 1749-1759.

9.3 Matthews, B. R. (1990) The drug master file, Pharmaceutical Manufacturing International, 115-118, ISSN 0951-9696, Sterling Publications International Ltd, London.

9.4 Poggiolini, D & Donawa, M. E. (1990), EEC pharmaceutical regulations: the multistate procedure and the CPMP. Pharm. Technol. 2, 104, 106, 108 and 110.

9.5 Cartwright, A. C and Matthews, R. B. (1991) Pharmaceutical Product Licensing - requirements for Europe. Ellis Horwood Ltd, Chichester, England.

9.6 **Rules governing medicinal products in the European community** - These have been published in the form of a 5 volume set.

Volume I "The Rules Governing Medicinal Products for Human

Use in the EC". Published in English, Spanish, Danish, German, French, Italian, Dutch and Portugese.

Volume II "Notice to Applicants for Marketing Authorisation for Medicinal Products for Human Use in the Member States of the EC". Published in English, Spanish, German, French and Italian.

Volume III "Guidelines on the Quality, Safety and Efficacy for Medicinal Products for Human Use". Published in English, Spanish, German, French and Italian.

Volume IV "Guide to Good Manufacturing Practice for Medicinal Products". Published in English, Spanish, Danish, German, Greek, French, Italian, Dutch and Portugese.

Volume V "The Rules Governing Medicinal Products for Veterinary Use in the EC". Published in English, Spanish, Danish, German, Greek, French, Italian, Dutch, Portugese.

An addendum to Volume IV has been published containing guidelines on such topics as Analytical Validation, European Drug Master Files, Production and Quality Control of certain products using biotechnological processes and human monoclonal antibodies, Good Clinical Practice and Clinical Testing.

These publications may be obtained from:

UNIPUB

4611 F Assembly Drive

Lanham

MD 20706-4391 USA

Tel: (800)274 4888

Kinokuniya Company Ltd

17-7 Shinjuka 3-Chome

Shinuku-ku

Tokyo 160-91

Tel. (03) 3540131

Guidelines on the production and quality control of medicinal products derived by recombinant DNA technology

Commission of the European Communities notes to applicants for marketing authorizations*

Committee for Proprietary Medicinal Products *Ad Hoc* Working Party on Biotechnology/Pharmacy

1 Introduction

Developments in molecular genetics and nucleic acid chemistry now enable the genes coding for natural, biologically active proteins to be identified, analysed in fine detail, transferred between organisms, and expressed under controlled conditions so as to obtain synthesis of the polypeptide for which they code.

A common strategy in the development of recombinant DNA (rDNA) derived products is the insertion of naturally occurring or synthetic nucleotide sequences into a vector which is introduced into a suitable host organism so as to ensure the efficient expression of the desired gene product. Many vectors in use at present are bacterial plasmids and much gene cloning has been carried out in *Escherichia coli* and other prokaryotes. However, other vector–host cell systems involving eukaryotes, including yeasts and cell lines of mammalian or insect origin, have been developed and are, in some cases, in use for production. The factors affecting the expression of foreign genes introduced into a new host using a suitable vector are complex and the efficient, controlled expression of stable, cloned DNA sequences is an important aspect of current research.

The ability to synthesize and manipulate nucleic acids allows the construction of genes coding for modified products possessing enhanced biological activity and/or diminished undesirable characteristics, or entirely novel products. In addition, large quantities of useful medicinal products which were previously difficult to prepare from natural sources can now be manufactured using such rDNA technology.

A flexible approach to the control of these products should be adopted so that requirements can be modified in the light of experience of production and use, and with the further development of new technologies. Implementation of these requirements for an individual product must reflect its intended clinical use.

This document is not intended to apply to the control of genetically modified live organisms designed to be used directly in man, for example as live vaccines.

2 Points to consider in manufacture

This document is intended to facilitate the collection and submission of data to support applications for marketing authorization within the EEC for medicinal products derived by rDNA technology and intended for use in man.

Extensive 'scale-up' may be required as laboratory developments progress to full scale commercial production, and this may have considerable consequences for the quality of the product and thus implications for control testing. Although comprehensive characterization of the final product is essential, considerable emphasis must also be placed on 'in-process' control, a concept which has been highly effective in the quality control of bacterial and viral vaccines prepared by conventional methods.

Requirements relating to establishments in which biological products are manufactured (e.g. Revised Requirements for Biological Substances No. 1; WHO TRS 323) will apply to products derived by recombinant DNA methodology as will several of the general requirements for the quality control of biological products. Thus, appropriate attention needs to be given to the quality of all reagents used in production, including components of fermentation media; specifications for these are to be included in documentation and they must comply with any relevant EEC requirements. Tests for potency, abnormal toxicity, pyrogenicity and sterility etc., which apply to products made by conventional methods, will also apply to products made by recombinant DNA technology. It is undesirable to use in production agents which are known to provoke sensitivity reactions in certain individuals, such as, for example, penicillin or other β-lactam antibiotics.

Certain factors may compromise the safety and efficacy of rDNA-derived products; these should be given special attention and are outlined below:

(i) Products from naturally occurring genes expressed in foreign hosts may deviate structurally, biologically or immunologically from their natural counterparts. Such alterations can arise either at the genetic or post-translational level or during production or purification. Other products may be entirely novel in structure, produced by manipulation of naturally occurring genes or by chemical synthesis of new ones. These products may have enhanced biological features and/or diminished undesirable effects compared with their naturally-occurring counterparts. It should be recognized, however, that they may also have unexpected and undesirable biological properties.

(ii) The choice of manufacturing procedure may influence the nature and range of potential contaminants. Thus rDNA-derived products may contain potentially hazardous contaminants not normally present in their equivalents prepared by conventional methods and which the purification processes must be shown capable of removing. Examples of these are endotoxins in products expressed in bacterial cells and DNA of oncogenic potential in products expressed in transformed mammalian cells.

(iii) Unintended variability in the culture during production may lead to changes which favour the expression of other genes in the host/vector system or which cause alterations in the polypeptide product. Such variation might result in decreased yield of the product and/or quantitative and qualitative differences in the impurities present in the product. Consequently, procedures to ensure consistency of production conditions as well as the final product are imperative.

Whilst the requirements set out below should be considered to be generally applicable, individual products may present particular quality control problems. Thus, the production and control of

*This document is the final draft generated by the Ad Hoc Working Party on Biotechnology/Pharmacy and approved (in June 1987) by the European Committe for Proprietary Medicinal Products. Address for correspondence: Pharmaceutical Division, DGIII/B/6, Commission of the European Communities, 200 Rue de la Loi, B-1049 Brussels, Belgium.

each product must be given careful individual consideration taking fully into account any special features.

3 Strategy for cloning and expression

3.1 Expression vector and host cell. A description of the host cell and expression vector used in production should be given. This should include details of the origin and identification of the gene which is being cloned and the construction, genetics and structure of the expression vector. The method by which the vector is introduced into the host cell and the state of the vector within the host should be described. The association of the vector and host cell may be permanent, allowing continuous expression of the product: or self-limiting, for example where the vector is an acceptable cytopathogenic virus.

3.2 Sequence of the cloned gene. Full details of the nucleotide sequence of the gene insert and of flanking control regions of the expression vector should be provided. All relevant expressed sequences should be clearly identified. The DNA sequence of the cloned gene should normally be confirmed at the seed lot stage and at least once after a full scale fermentation. In certain systems, for example where multiple copies of the gene are inserted into the genome of a continuous cell line, it may be inappropriate to sequence the cloned gene at the production level. Under these circumstances Southern blot analysis of total cellular DNA or sequence analysis of the mRNA may be helpful and particular attention should be paid to the characterization of the final product.

3.3 Expression. The strategy by which the expression of the relevant gene is promoted and controlled during production should be described in detail.

4 Control of seed lot

It is essential that production is based on a well defined seed lot system involving a master seed and manufacturer's working seed bank. During the establishment of the seed no other cell lines should be handled simultaneously in the same laboratory suite or by the same persons. The origin, form, storage, use and details of life expectancy at the anticipated rate of use must be described in full for all seed materials. Attention should be paid to the stability of the host–vector expression system in the seed stock under conditions of storage and recovery. Any known instability should be reported. New seed lots should be fully characterized.

A critical part of quality control will involve the full characterization of seed material. Where higher eukaryotic cells are used for production, distinguishing markers of the cell, such as specific isoenzyme and immunological features or karyology, will be useful in establishing the identity of the seed. Details of the tumourigenicity of continuous cell lines should also be obtained and reported. Likewise, where microbial cultures are used, specific phenotypic features which form a basis for identification should be described.

Evidence that the seed lot is free from potentially oncogenic, where appropriate, or infective adventitious agents (viral, bacterial, fungal or mycoplasmal) must be provided. Special attention should be given to viruses which can commonly contaminate the animal species from which the cell line has been derived. For instance, cell lines of murine origin should be checked for contamination according to Appendix I of the *Notes to Applicants for Marketing Authorizations on Requirements for the Production and Quality Control of Monoclonal Antibodies of Murine Origin Intended for Use in Man* (*Trends in Biotechnology*, in press). Certain cell lines contain endogenous viruses, e.g. retroviruses, which may not readily be eliminated. The expression of these organisms, under a variety of conditions known to cause their induction, should be tested for and reported. Furthermore, the purification process should be shown to be capable of removing and/or inactivating any such virus which may inevitably be present in the seed as an endogenous agent or part of the expression vector.

5 Production

Ideally not more than one cell line should be cultivated simultaneously in the same production area. If other cell lines are cultivated in parallel, records must be kept of the cell lines handled and evidence presented for the absence of cross-contamination between them.

5.1 Production at finite passage. Details of the fermentation or culture used to manufacture the product should be provided. For each production run, the presence, extent and nature of any microbial contamination in the culture vessels immediately prior to all harvesting must be thoroughly examined. Detailed information to confirm the adequate sensitivity of the methods used to detect contamination should be provided and acceptable limits of contamination set.

Maximum permitted passage levels for production should be defined and should be based on information concerning the stability of the host cell/vector system upon serial sub-cultivation up to and beyond the level of production. Data on consistency of growth of the culture and on the maintenance of yield of the product should be presented. Criteria for the rejection of culture lots should be established.

Monitoring of the host cell/vector characteristics at the end of production cycles should also be undertaken. For example detailed information on plasmid copy number and degree of retention of the expression vector within the host cell, as well as restriction mapping of the vector containing the gene insert, may be of value.

5.2 Continuous culture production. This approach should only be undertaken when special consideration has been given to the control of production based on continuous culture. Where it is undertaken monitoring of the production system is necessary throughout the life of the culture. The required frequency and type of monitoring will depend upon several factors including the nature of the expression system and product. Information should be obtained on the molecular integrity of the gene being expressed and of the phenotypic and genotypic characteristics of the host cell after long term cultivation. Evidence should be provided that the yield does not vary beyond defined limits and that the nature and quality of the product does not change with respect to specified parameters. The acceptance of harvests for further processing should be clearly linked to the schedule of monitoring applied. The period of continuous cultivation should be specified and this should be based on information concerning the stability of the system and consistency of the product up to and beyond this limit. In cases of long term continuous cultivation, the cell line and product should be completely re-evalu-

Glossary

Master Seed Bank – A homogeneou suspension of the original cells alreac transformed by the expression vect containing the desired gene, aliquoted in individual containers for storage (e.g. in liquid nitrogen refrigerator). In some case it may be necessary to establish separa master seed banks for the expressic vector and the host cells.

Manufacturer's Working Seed Bank – homogeneous suspension of the see material derived from the master see bank(s) by a finite passage level, aliquote into individual containers for storage (e. in a liquid nitrogen refrigerator).

In both seed banks, all containers a treated identically during storage, and onc removed from storage, the containers ar not returned to the seed stock.

Production at Finite Passage – Th cultivation method is defined by a limite number of passages or population dou lings which must not be exceeded d production.

Continuous Culture Production – The number of passages or population doublings are not restricted from the beginning of production. Criteria for the termination of production have to be defined by the manufacturer.

Bulk Harvest – This is a homogeneous pool of individual harvests or lysates which is processed in a single manufacturing run.

Bulk final processed product – This is the finished product, after completion of the manufacturing process, obtained from a bulk harvest. It is maintained in a single container and used in the preparation of the final dosage form. The generation of this final batch has to be clearly defined and unambiguously recorded by the manufacturer.

Final dosage form – The finished product is formulated and filled into final, sealed containers which hold the product in its final dosage form. The containers of a filling lot are processed together and are uniform in their contents and biological potency.

ated, as set out in sections 3.1, 3.2, 7.1 and 7.2, at intervals based on information concerning the stability of the system and the character of the product.

A clear definition of a 'batch' of product for further processing should be provided. Regular tests for microbial contamination should be performed in relation to the strategy for harvesting. Criteria for rejection of harvests and premature termination of the culture should be defined.

6 Purification of the product

6.1 Methods. Methods used to purify the product should be described in detail. Procedures which make use of affinity chromatography, for example employing monoclonal antibodies, should be accompanied by appropriate measures to ensure that these substances, or any additional potential contaminants arising from their use, do not compromise the quality and safety of the final product. Attention is drawn to the *Notes for Applicants for Marketing Authorizations on Requirements for the Production and Quality Control of Monoclonal antibodies of Murine Origin Intended for Use in Man* (*Trends in Biotechnology*, in press).

6.2 Validation of the Purification Procedure. The capacity of the purification procedure to remove unwanted host cell derived proteins, nucleic acids, carbohydrates, viruses and other impurities should be investigated thoroughly, as should the reproducibility of the purification process as regards its ability to remove specific contaminants and the consistent composition of the purified product with respect to any impurities which may be present. Pilot scale studies using, for example, a carefully selected group of viruses which exhibit a range of physico-chemical features relevant to their behaviour on purification, or radioactively labelled DNA intentionally mixed with the crude preparation (spiking) should be undertaken. A reduction factor for such contaminants at each stage of purification, and overall, should be established by using, if necessary, concentrations of DNA and viruses in excess of that expected during normal production.

7 Final processed product

7.1 Characterization of the Purified Active Substance. Rigorous characterization of the active substance by chemical and biological methods will be essential. Routine detailed characterization of the final product may be required if the nature of the expression system makes it impossible to characterize the gene at the production level. Particular attention should be given to using a wide range of analytical techniques exploiting different physico-chemical properties of the molecule; for instance, size, charge, isoelectric point, amino acid composition and hydrophobicity. It may be desirable to include suitable tests to establish that the product has the desired conformational structure and state of aggregation. Examples of techniques suitable for such purposes are: polyacrylamide gel electrophoresis; isoelectric focusing; size exclusion, reversed phase, ion exchange, hydrophobic interaction or affinity chromatography; peptide mapping; amino acid analysis; light scattering; UV spectroscopy; circular dichroism, and other spectroscopic techniques. Additional characterization of the product using for example, electron microscopy or relevant immunochemical techniques may provide valuable information. Biological and immunological characterization should include as wide a range of techniques as possible appropriate to the anticipated biological activity, use, system of administration of the product and duration of treatment. The determination of the specific activity of highly purified material will be of particular value (units of activity/weight of product).

Sufficient sequence information to characterize the gene product adequately should be obtained. The degree of sequence verification required will depend on the extent of other characterization tests. For some purposes partial sequence determination and peptide mapping may suffice, for others full sequence determination may be necessary. Attention should be paid to the possible presence of N-terminal methionine, signal or leader sequences and other possible N- and C-terminal modifications (for instance acetylation, amidation or partial degradation by exopeptidases). Other post-translational modifications, such as glycosylation should be indicated. Special consideration should be given to the possibility that such modifications are likely to differ from those found in a natural counterpart and may influence the biological and pharmacological properties of the product.

7.2 Purity. Data should be provided on contaminants whose presence is anticipated in the final processed product. The level of contamination considered as acceptable, and criteria for acceptance or rejection of a production batch should be given. It is important that the techniques used to demonstrate purity be assessed using as wide a range of methods as possible, including physico-chemical and immunological techniques. Particular emphasis should be placed on tests for viral and nucleic acid contamination and for other unwanted materials of host origin, as well as on materials which may have been added during the production or purification processes.

8 Routine batch control of bulk final processed product

A comprehensive analysis of the initial batches of a product should be undertaken to establish consistency with regard to identity, purity and potency. Thereafter, a more limited series of tests may be appropriate as outlined below.

8.1 Consistency. An acceptable number, for example five, of successive batches of the bulk processed product should be characterized as fully as possible to determine consistency of composition. The studies should include biological, chemical and immunological methods to characterize and assay the active substance and methods to detect and identify impurities. Any differences which occur between batches should be noted. The data obtained from these consistency studies should be used as the basis for product specification.

8.2 Identity. A selection of the tests used to characterize the purified active substance (see 6.1) should be used to confirm the product identity for each batch. The methods employed should include tests for the anticipated biological activity as well as physico-chemical and immunological methods. Depending on the extent of other identification tests, sequence verification of a number of amino acids at the N- and C-terminus or other methods such as peptide mapping should be performed.

8.3 Purity. The degree of purity and also the degree of consistency of the production process. In general, a very high degree of purity can be achieved for

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desirable and attainable will depend on several factors; these include the nature and intended use of the product, the method of its production and purification most products by modern manufacturing procedures.

The purity of each batch should be established and be within specified limits. The analysis should include sensitive and reliable assays for DNA of host cell origin applied to each batch of product prepared from continuous lines of transformed mammalian cells. Strict upper limits should be set for DNA in the product. It is recommended that DNA analyses are also performed on each batch of product obtained from other eukaryotic cells, and limits set for DNA content, until further information on safety is obtained. DNA of prokaryotic expression systems (e.g. of vector or plasmid origin) should be tested for wherever appropriate to considerations of the quality and safety of the product. For products to be administered for an extended period of time, or in high doses, the residual cellular proteins should also be determined by an assay with appropriate sensitivity (e.g. ppm) and strict upper limits set.

8.4 Potency. The potency of each batch of the product should be established (e.g. units of biological activity per ml) using, wherever possible, an appropriate national or international reference preparation calibrated in units of biological activity (see Section 9).

In addition, information on specific activity (units of biological activity per unit weight of product) will be of considerable value and should be reported. A highly purified reference preparation is required to standardize measurements of specific activity (see Section 9).

It is recommended that correlations between potency measurements, involving biological tests, and the results of physico-chemical methods of assay are made and the information reported. If possible, batches should be calibrated using accurate physico-chemical tests, and the biological assays used to confirm – within stated limits – that the product is biologically potent.

9 Specification and reference materials

The studies described in Section 7 will contribute to a definitive specification for the product when considered together with the information obtained from the examination of successive batches, as indicated under Routine batch Control (Section 8).

A suitable batch of the product, preferably one which has been clinically evaluated, should be fully characterized in terms of its chemical composition, purity, potency and biological activity, including where possible full amino acid sequencing, and retained for use as a chemical and biological reference material.

When appropriate the biological activity of the product and its physical characteristics, including the amino acid sequence, should be compared with that of a highly purified preparation of the naturally occurring molecule.

10 Finished product

The product in final containers should be shown to comply with the requirements of the EEC directives where appropriate. In circumstances where this is not possible the omission of tests should be justified by the manufacturer.

11 Pre-clinical safety tests

The requirements for a particular product need to be carefully evaluated in each case. In general, classical toxicological studies in animals may be of only limited relevance. However, some safety testing will usually be required for most products. Reference should be made to separate *Notes to Applicants on the Preclinical Biological Testing of Medicinal Products Derived by Biotechnology.*

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Oral delivery of peptides and proteins: Problems and perspectives

C G Wilson, Department of Pharmaceutical Sciences, Royal College, University of Strathclyde.

The synthesis of analogues of biologically active peptides using solid or solution phase protein synthesis or recombinant DNA technologies has awoken much interest in these drugs as therapeutic agents of the future. The major problem in realising the potential of these compounds is the difficulty in getting sufficient drug at the required site of action. The drugs often show remarkable specificity and potency when directly applied to the tissue but the problems of polarity, high molecular weight and enzymic degradation limit the oral bioavailability. The delivery by the parenteral route is problematic since this route requires requires more nursing care and dose preparation than for oral dosing. The search for mechanisms to increase the fraction of dose absorbed following oral delivery of peptides and proteins is therefore receiving more attention in pharmaceutical laboratories around the world.

Why peptides?

The appreciation that physiological and pathophysiological processes are modulated by the release of peptide and proteins identified a clear therapeutic target. Banting and Best were able to reverse diabetes in the 1920's by subcutaneous bovine insulin and ever since this time, the advantage of a non-injectable insulin has been obvious but not commercially realisable. This molecule represents a considerable challenge in drug delivery but the partial success with the nasal/buccal delivery of smaller molecules such as DDAVP and oxytocin has continued to arouse interest.

Why oral delivery?

Although a wide variety of routes of administration and delivery systems exist, absorption of the therapeutic agent from the gastrointestinal tract is the most desirable approach. Oral delivery is non invasive, relatively free from complications arising from the need for sterile techniques that occur with parenteral formulations and is easily managed, thus increasing patient compliance. However, the oral route presents a large obstacle for peptide and protein drugs

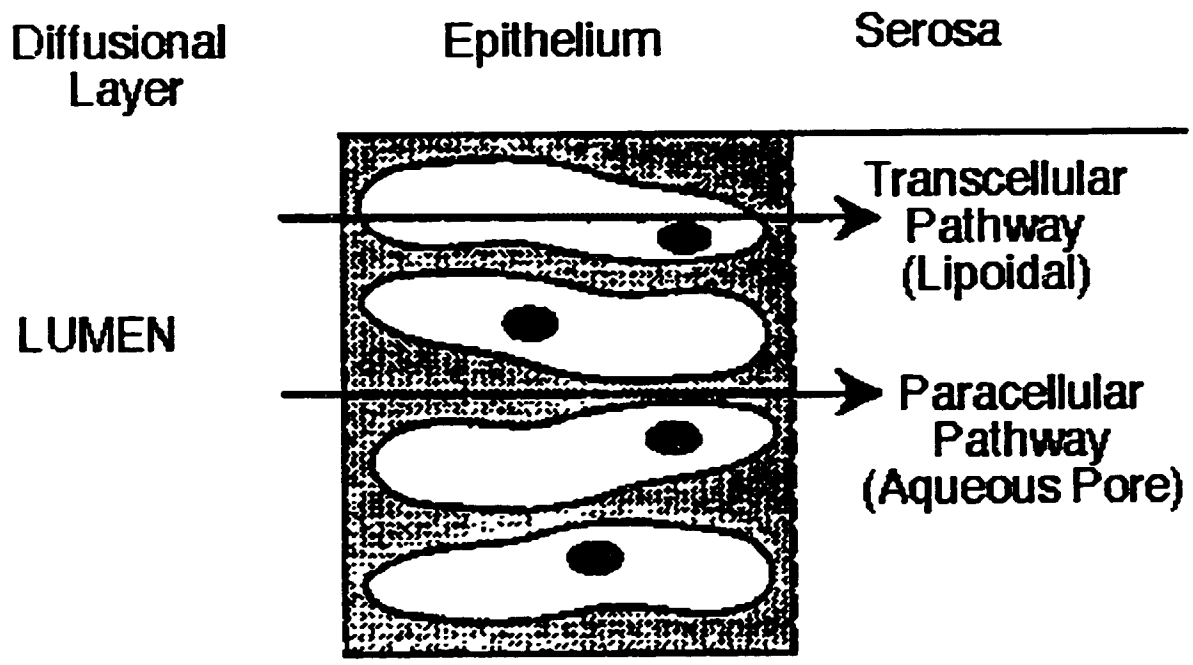

Figure 1. Transcellular and Paracellular pathways

due to the enzymatic and cellular barriers in the gastrointestinal tract .

It is generally believed that the intestinal mucosa is impermeable to macromolecules such as peptides. This is true in terms of nutrition, but is clearly not the case immunologically . Ingested antigens, toxins, and antibodies may pass the mucosal barrier in quantities which may be important in the pathogenesis of local and systemic immunological disease states.

The barriers to peptide absorption:

1. Absorption

The epithelial barrier may be breached through two mechanisms (Figure 1) ; first the *transcellular* movement through the lipid membrane by a diffusional or by an active transport process. Diffusional processes require a partition from the external aqueous phase into the lipid bilayer of the cell membrane and thus lipophilicity and a concentration or electrochemical gradient is prerequisite for solute movement. Active transport against a concentration gradient occurs for amino-acids and some dipeptides but flux decreases markedly with increase in molecular weight.

The second mechanism is via the *paracellular* route, that is to say around the gaps between adjacent cells. The size of the paracellular junctions varies along the gastrointestinal tract; the cells being drawn together by by fusion of membranes at intracellular junctions or through microfilamentous connections between adjacent cells. Experiments with probe molecules shows that the calibre of these tight junctions is variable down the gastrointestinal tract and is relatively low in the stomach and large bowel and most leaky in the small intestine. Generally the apparent pore radius is 7.5 - 8.0 Å in the jejunum. decreasing to 3-3.5 Å in the jejunum and 2 to 2.5 Å in the colon (1). Compounds with molecular sizes greater than these dimensions will therefore be precluded from absorption by the route.

Physicochemical considerations.

Three physicochemical parameters which influence the absorption of peptides must be considered: charge, size and lipophilicity.

Charge

The twenty standard amino acids which make up most peptides have at least an acidic and a basic group attributable to their ionisable carboxylic acid and amino ends. Additionally, five of the twenty standard amino acids have charged side chains. The pKa values of the -COOH groups lie in a small range around 2.2; above pH 3.5 these groups are almost entirely in their carboxylate forms. The NH2 groups all have pKa values near 9.4 and are almost entirely in the ammonium ion form below pH 8.0. The basic amino acids lysine, arginine, and histidine are all positively charged at physiologic pH, while aspartic and glutamic acid are negatively charged above pH 3. In the physiological pH range, both the carboxylic acid and the amino groups of peptides and amino acids are completely ionised resulting in a zwitterionic molecule. Therefore, peptides and proteins will tend to be more hydrophilic than many other biologically active molecules. This feature would most likely preclude absorption of peptides and proteins by transcellular diffusion unless the charges were neutralised through ion pairing. Another factor is the negative charge at physiological pH associated with intracellular gaps which will tend to attract positively charged or neutral compounds and favour the paracellular absorption relative to anionically-charged compounds.

Size

The potential therapeutic agents in this class tend to be very large with molecular weights of 700 - 30,000 daltons. The extent of absorption tends to be very low above a molecular weight above 1,000 daltons, since aqueous channels will exclude high molecular weight compounds from entry.

Lipophilicity

The lipophilicity of peptides and proteins is generally very low, with most compounds having a partition coefficient between 0.02 and 0.08. The ability to traverse the transcellular pathway is therefore extremely limited.

2. Degradation

Perhaps even more important than the permeability barrier in limiting the absorption of intact peptides and proteins from the gastrointestinal tract is the enzymatic barrier. This barrier is well designed to digest proteins to a number of

amino acids and small quantities of peptides consisting of two to six amino acid residues prior to their appearance in the portal circulation. Hydrolysis of peptides and proteins occurs at three sites: in the lumen, at the brush border, and intracellularly.

Proteases in the brush border and the cytosol of the enterocyte are potentially the most important deterrent to the absorption of small, biologically active peptides across the intestinal mucosa. Typically, proteases are anchored in the apical membrane with the active site in an extracellular environment. In addition to the membrane-bound proteases, luminal proteases including trypsin, chymotrypsin, and other pancreatic proteases may be adsorbed onto the brush border of the enterocyte thereby assisting in proteolysis of oligopeptides and proteins. Brush border proteases as a group tend to cleave tripeptides and tetrapeptides although they are also capable of readily hydrolysing peptides in the range of two to ten amino acid units. Specifically, about 60% of the cellular proteolytic activity against tripeptides and 90% of the activity against tetrapeptides can be found in the brush border. In contrast, the general cytosolic proteases attack smaller di- and tripeptides with very little activity against tetrapeptides. The most active area proteolytically is the ileum, which is due to the high activity of brush border aminopeptidase N and diaminopeptidase

Circumventing the barriers

a). Modify the Backbone

Modifications to the amino acid backbone have been extensively used for example cyclization, alkylation of specific amino acid residues, and amide bond surrogates. For example, substitution of D-Ala for L-Gly in the N- , terminal amino acid in methionine enkephalin and the amidation of its C-terminal methionine produces a more stable analogue which has a half-life of hydrolysis that is 15 times that of methionine enkephalin. Modification of the amino acid in the primary structure of the peptide may also improve its lipophilicity. This may be useful in minimising contact of the peptide with the proteolytic enzymes at the site of absorption, since diffusion across the absorptive membrane will be facilitated. The compound 1-deamino-8-D-arginine vasopressin , DDAVP, permeates the intestinal wall of the rat 35 times better than the aminated vasopressin (2). Substitutions with unnatural amino acids such as Oic (3aS,7aS-

octahydroindol-2- yl-carbonyl), Tic (1,2,3,4-tetrahydroisoquinolin-3-yl-carbo- nyl), and Thi (3-2-thienylalanyl) have been used to improve the stability and receptor binding of peptides and proteins.enzymes at the site of absorption, since diffusion across the absorptive membrane will be facilitated (3).

(b) Administer Protease Inhibitor

Coadministration of protease inhibitors has been used as a method of increasing the absorption of peptides and proteins. As early as 1959, investigators were looking into the effects of proteolytic inhibitors on its intestinal absorption. Danforth and Moore (4), using ligated sections of normal and depancreatised rat jejunum, measured the extent of the absorption of insulin both with and without protease inhibitors. They found that when insulin was introduced into the intestine along with diisopropyl-fluorophosphate (a serine protease inhibitor), there was a marked and consistent blood glucose depression whereas insulin alone (up to 800 USP units/kg) had no effect when administered via the intestinal lumen.

Saffran et al. (5) looked at the effects of aprotinin, a potent inhibitor of trypsin, on the absorption of the peptide hormones lysine, vasopressin (LVP) and (1-deamino, 4-valine)-8-D-arginine-vasopressin (DDAVP) after intragastric administration. The results showed that inclusion of the protease inhibitor enhanced the oral activity of both vasopressin and the synthetic analogue, increasing both the intensity and duration of effect.

(c) The use of absorption enhancers

Certain chemicals and drugs can increase (and decrease) mucosal permeability. Drugs such as oxyphenisatin, dihydroxy bile salts, ricinoleic acid and long- chain fatty acids have all been shown to increase the permeability of the colon and the use of long-chain fatty acids to increase the absorption of cefmetazole has been studied recently (6). Penetration enhancers are of four major types: *chelators* such as EDTA, citric acid, salicylates, N-acyl derivatives of collagen, and enamines; *surfactants* such as sodium lauryl sulphate, polyoxyethylene-9-lauryl ether, and polyoxyethylene-20-cetyl ether; *bile salts* such as sodium deoxycholate; and *fatty acids* such as oleic acid and monoolein . Whether these agents act on the cell (transcellular route) or the junctions (paracellular route) is not clear. The incorporation of permeation enhancers into a dosage form may be

essential in the systemic delivery of macromolecules. These chemicals would need to be co-released at the site of drug absorption. The risk involved, however, is increased absorption of potentially toxic or immunogenic substances from the GIT. The ideal absorption enhancer should be highly permi-selective.

(d) Site specific delivery

The colonic route of drug delivery can be used for systemic administration of drugs. Protecting peptide and protein drugs from hydrolysis in the duodenum and jejunum and subsequently releasing these agents in the ileum or colon may result in greater systemic bioavailability. There is also evidence to suggest that the proteolytic activity of the colonic mucosa may be much less than that observed in the small intestine

It would be predicted that absorption of most drugs would be slower from the colon by comparison with that in the small intestine, since the colonic mucosa has a much reduced surface area being flat with fewer folds and the absence of villi. Furthermore, the lumen of the colon is wider, movement more sluggish and the volume of dissolution fluid available is low. However, transit through the colon is considerably slower than that through the small intestine and thus the mean residence time of a drug in the colon can compensate possibly leading to greater amounts of drug being absorbed than might be expected.

Factors affecting residence in the colon are dependent on gastric filling and emptying, on rates of passage in the small intestine and on colonic metabolism (7,8). The rate can vary greatly from individual to individual and even in the same person (9). Transit can be relatively short (8-10 h) or as long as 78 h. The residence time in the various regions of the colon is a further factor which is expected to be important in influencing drug delivery. In most individuals the ascending colon is sufficiently liquid to allow diffusion of dissolved drug across the lumen to the epithelium, whereas further along the colon as more water and electrolytes are absorbed, the milieu becomes dryer, hindering diffusion (Figure 2). Residence time in the colon has been calculated to be 31.9 ± 17.8 h. for American males and 38.8 ± 18.5 h. for females (10) with 18-19% of the time spent in the ascending colon giving a calculated transit time of between 5.8 and 7 hours.

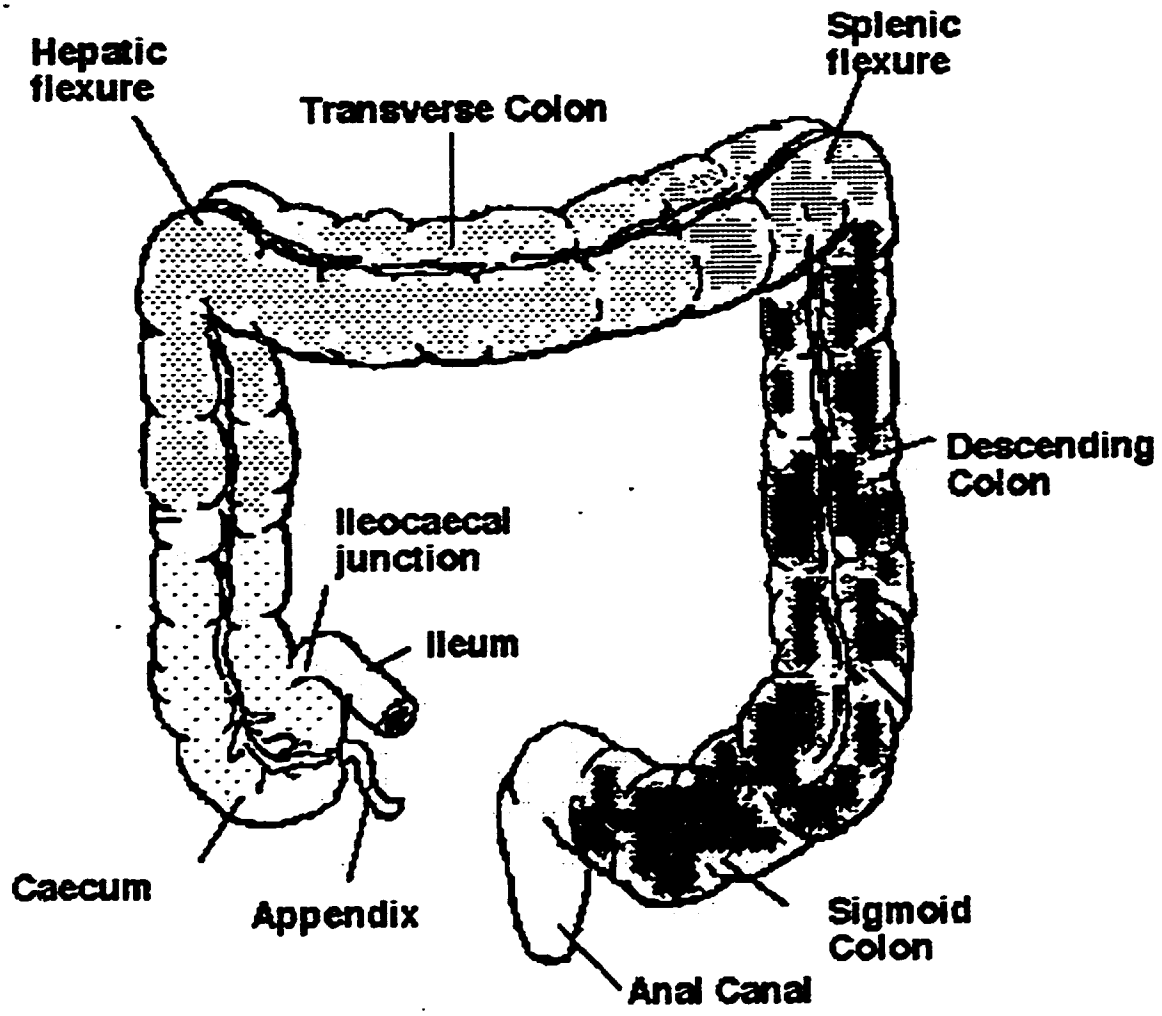

Figure 2. The Colon

Shading illustrates gradual consolidation of contents

Scintigraphic studies conducted within our laboratories on the behaviour of sustained release dosage forms have shown that the ascending and transverse limbs of the colon are important and under-rated sites of drug absorption (11,12, 13). Scintigraphic data also suggests that to optimise the delivery of drugs to the proximal colon a multiparticulate preparation should be considered which remains intact for approximately 5 hours after administration to the fasted patient. This would allow time for gastric emptying and transit through the small intestine. The drug preparation should then disperse allowing release of the material over the following 10 to 12 hours in the ascending and transverse colon. Residence times indicate a figure of 12.9±3.7 hours (range 9.7 - 23.2 hours) for the proximal colon and a further 12 hours for the descending colon (14). Additionally, the right colon exhibits an adaptation for capacitance (15) by comparison with the left colon where motility patterns are strongly propulsive (16). Extending the release profile over longer time periods would not give an added advantage, due to the progressively slower diffusion of drugs through consolidating luminal contents and to the variability of excretion patterns.

The use of enteric coating in formulations of drugs which are unstable in gastric acid or for preparations which cause nausea or irritation when they disintegrate in the stomach is well established. More recently, polymer coatings have found application in the construction of formulations whose delayed release presents the drug to the distal regions of the gut, rather than the upper small intestine. The application of such a principle, which minimises systemic absorption from the small intestine, is obvious in the treatment of diseases affecting the colon. The success of such therapy is dependent on two physiological principles; namely that the pH of the small intestine will be sufficiently high to ionise the carboxylic acid moieties of the polymer (17) and secondly that small intestinal transit is relatively unaffected by eating, nature of the diet and normal everyday activity to ensure that sufficient reproducibility in man. Numerous studies employing gamma scintigraphy have demonstrated that small intestine transit is generally a remarkably robust parameter in gastroenterology and influences of diet, daily habits, posture and even pharmacologically active materials are confined to the stomach and the distal ileum and colon. Davis and others (18) have summarised the data on many investigations completed by the Nottingham group which have shown small intestinal transit to be around four hours with little variation due to

size of meal. Thus the use of a gastroresistant formulation with an appropriate thickness of polymer to deliver drug to the terminal ileum and colon appears to be a workable premise.

Dew and co-workers had shown using X-contrast agents that positioned release could be achieved for capsules and tablets using Eudragit S polymer at a coating thickness of 120 μ. (19). Using an alternative polymer system, our own group has demonstrated caecal release of capsule contents in normals treated to simulate a diarrhoeal state similar to that noted in diarrhoea predominant irritable bowel syndrome (20).

Research on site specific delivery- new methodology

A recent development involving neutron activation techniques, allowing controlled-release dosage forms to be radiolabelled under conditions where conventional methods with short-lived gamma-emitting radioisotopes are impractical, has significantly increased the potential for in vivo analysis of delivery systems under physiological conditions. Such techniques allow the incorporation of a stable isotope, such as barium-138, samarium-152 or erbium-170, into the formulation prior to manufacture in the same manner as any other excipient. Subsequent neutron activation of the intact dosage form thus permits the manufacture under industrial scale conditions at a time not determined by proposed study dates and without the risk of contamination to personnel and equipment. This technique also has the advantage that it is applicable for radiolabelling enteric coated capsules without compromising the integrity of the dosage form (21).

A major problem with the utilisation of neutron activation is the high cost of the enriched erbium needed for tablet preparation. Recent studies in our laboratories (22,23) have investigated the possibility of using non-enriched substrates. Samarium contains a natural abundance of 26.7 % ^{152}Sm, and when irradiated produces ^{153}Sm, a gamma emitter with a half-life of 46.7 hours. Although peptide delivery systems has not yet been investigated, we have utilised a preparation consisting of samarium oxide -loaded drug polymer microspheres (Eudragit RS/ sulphasalazine) . Samples were irradiated for 90 minutes at a neutron flux of 1012/n/cm^2/s. Drug release profiles indicate a satisfactory labelling efficiency with

minimum radiolysis of drug. This system was used to investigate models of bowel disease in man (24).

Pulsatile systems based on a swelling hydrogel have been discussed earlier in the course by Professor Graham. This system has been commercialised (Scherer DDS Limited, Clydebank, Scotland) and was recently used by Davis and colleagues to investigate the absorption of an angiotensin-converting enzyme inhibitor, captopril [(1-(D-3-mercapto-2-methyl-1-oxopropyl)-L-proline (S,S-isomer)] in 8 volunteers (25). The unit was loaded with 25 mg captopril and 5 mg ^{111}In-labelled diethylenetriaminepentaacetic acid (1 MBq) and sealed by means of a hydrogel plug. In six subjects, the drug was delivered to the colon and in two subjects, the terminal ileum. Absorption was extremely variable and it appears that the drug is poorly absorbed in the distal gut, perhaps by metabolism by the gut flora.

This study may reveal a final problem in the delivery of drugs to the colon; the colonic microflora. The human alimentary canal is highly populated with bacteria and other microflora. The density is greatest in the colon/rectum but there is a surprising diversity of organisms in the buccal cavity. In the gastrointestinal tract between these two sites, the GIT is very sparsely populated with microorganisms. The gut bacteria are capable of catalysing a wide range of metabolic events. Many colon- specific drug delivery systems for conventional drugs rely on enzymes unique to gut microflora to release active agents in the colon. However, only two or three enzyme systems have been exploited in this area: azoreductases and glycosidases (including glucuronidase). A large number of polysaccharides are actively hydrolysed by gut microflora leading to the possibility of using naturally occurring biopolymers as drug carriers. In addition, etheral sulphate prodrugs or carboxylated prodrugs may be metabolised in the colon to the parent drug leading to local delivery in the colon (26). Perhaps the utilisation of a delivery system based on a fermented prodrug approach will be the method of choice of the future, provided that the released peptide survives the bacterial endopeptidases.

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UNIDO WORKSHOP

THE DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS

22nd - 27th NOVEMBER 1992

PRACTICAL LABORATORY SESSION

Automatic Multi-Cell, Multi-Lambda Membrane Permeability System

Membrane Diffusion

When a system is at equilibrium, the chemical potential, u_i, of the system components is the same in all phases. If equilibrium does not exist then there will be flow from the phases of higher chemical potential to those where it is lower until the equilibrium has been re-established.

Diffusion is the process by which concentration differences are reduced by the flow of matter. Although the driving force for diffusion is the difference in the chemical potential of the components it is more usual to consider diffusion in terms of concentration gradients. Fick's first law states that the flow of matter is directly proportional to the concentration gradient, Equation (1).

$$J = \frac{-D.dc}{dx} \qquad (1)$$

where J = The Flux of a Component Across a Plane of Unit Area

D = The Diffusion Coefficient

$\frac{dc}{dx}$ = The Concentration Gradient

Fick's second law states that the rate of change of concentration in a volume element within the diffusional field is proportional to the rate of change in

the concentration gradient at that point in the field. Equation (2).

$$\frac{dc}{dt} = \frac{D.d^2c}{dx^2} \qquad (2)$$

When a permeable membrane is introduced between two phases which have differences in chemical potential, flow between the two phases must occur through the membrane phase. Simple diffusion through a membrane is governed by Fick's laws. When a substance enters a homogeneous membrane from an external phase it dissolves in the membrane material to form a thermodynamically stable mixture. The concentration of the substance within the membrane is well defined and determined by the activity of the substance in the external phase. This equilibrium solubility of a substance within a membrane can be expressed as the Partition Coefficient, K.

$$K = \frac{\text{Concentration of solute in membrane, } C_m}{\text{Concentration of solute in external solution, } C_0} \qquad (3)$$

The dissolved molecules are able to share in the molecular motion within the membrane phase and so undergo translation within it. When a particular solute has an affinity with the membrane phase, the concentration of that solute can increase over that in the external phase giving a K value of greater than 1. A K value of unity would indicate that the solute has an equal affinity to either phase and a K value of less than 1 would indicate a preference of the solute for the external phase or solution.

The diffusion of a solute through a membrane from one solution to another can be shown schematically by Figure 1.

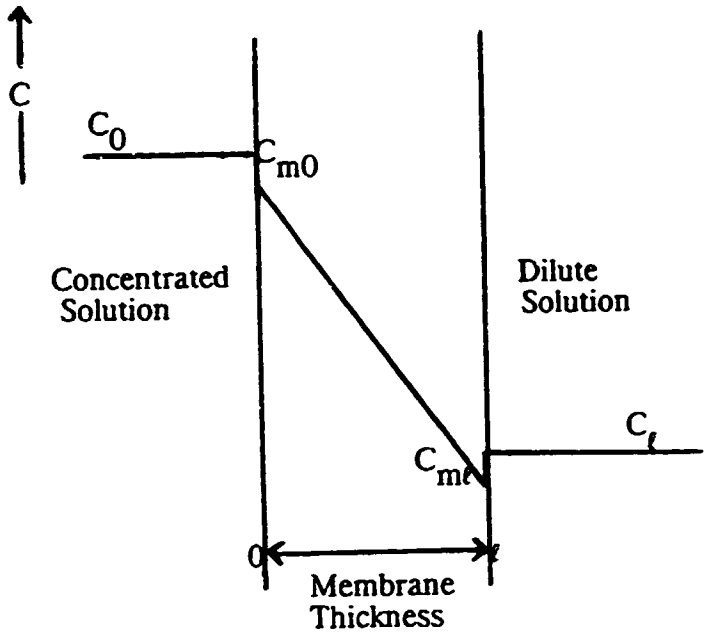

Figure 1. The diffusion of a solute through a membrane.

The flux through the membrane from Fick's first law is

$$J = \frac{D.(C_{m0} - C_{m\ell})}{\ell} \qquad (4)$$

where C_{m0} = The Concentration at the Upstream Membrane Surface.

$C_{m\ell}$ = The Concentration at the Downstream Membrane Surface.

ℓ = The Membrane Thickness.

By using Equation (3), for the partition coefficient, K, of the solute between the two phases, the following relationships can be obtained

$$C_{m0} = K.C_0 \quad \text{upstream surface} \qquad (5)$$

$$C_{m\ell} = K.C_\ell \quad \text{downstream surface} \qquad (6)$$

The flux at steady state can be expressed as

$$J = \frac{D.K.(C_0 - C_\ell)}{\ell} \qquad (7)$$

$$= \frac{D.K.\Delta C}{\ell} \tag{8}$$

where C_0 = The Upstream Bulk Concentration.

C_ℓ = The Downstream Bulk Concentration.

ΔC = The Concentration Difference between the two solutions.

The values of D and K are not often easily measured and, commonly, values of the Permeability Coefficient, P, are used where

$$P = D.K \tag{9}$$

Thus, the mass transfer of a solute across a membrane at steady state conditions can be expressed by Equation (10).

$$\frac{dMt}{dt} = \frac{A.D.K.\Delta C}{\ell} \tag{10}$$

where Mt = The Mass of Solute Transferred.

A = The Membrane Cross-Sectional Area.

From Equation (9)

$$\frac{dMt}{dt} = \frac{A.P.\Delta C}{\ell} \tag{11}$$

and by rearranging

$$P = \frac{(dMt/dt).\ell}{A.\Delta C} \tag{12}$$

Thus, we have an expression for the permeability of a membrane made up of readily measurable experimental parameters.

Figure 2. shows a typical mass transfer profile for the diffusion of a solute through a membrane. The time taken for the establishment of a constant rate of diffusion, or steady-state, is known as the Time Lag, τ. The time lag, τ, the membrane thickness, ℓ, and the diffusion coefficient, D, can be related by the following equation

$$\tau = \frac{\ell^2}{6.D} \qquad (13)$$

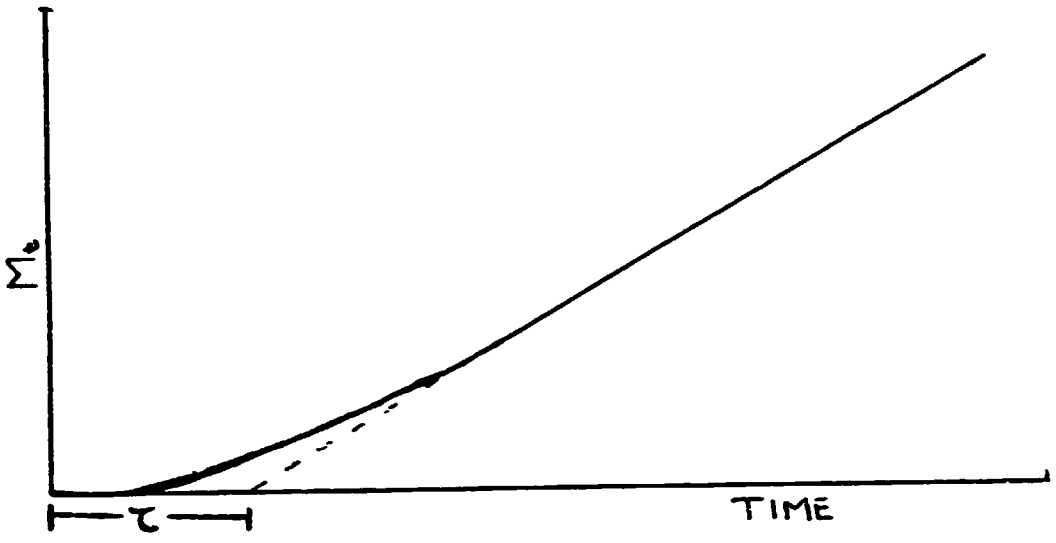

Figure 2. Typical mass transfer profile for the diffusion of a solute through a membrane.

By extrapolating the steady-state gradient back onto the time axis, a value for τ can be obtained. Using Equation (13), a value for the diffusion coefficient, D, can be calculated. The substitution of the values obtained for P and D into Equation (9) will yield a value for the partition coefficient, K.

The Automatic Multi-Cell, Multi-Lambda Membrane Permeability System

An automated 6 cell, multiwavelength diffusion system has been established in conjunction with Dr. R. Paterson's group at The University of Glasgow. The system consists of a series of up to 6 membrane diffusion cells coupled to a UV/Visible spectrophotometer and a computer which permits automatic logging of the data and calculation of the membrane permeabilities. The system provides a powerful and flexible tool for the analyses of up to 6 different sets of data at up to 6 different wavelengths (i.e. 6 different species can be analysed at one time).

The diffusion cell is of the Stagnant Point Flow design. The solution is introduced down a central flow pipe to impinge on the membrane surface, breaking up any stagnant layers before mixing back down the cell and leaving at the rear. The cell has a thermostatic jacket for temperature control. The cell is assembled with the membrane clamped between the two cell halves using a bull-clip. The exposed membrane area is 2.27cm^2 and the half-cell volume, 8.0ml.

Permeability experiments are carried out by firstly circulating distilled water or aqueous buffer solution around the downstream or sink side of the membranes. The downstream loops are passed through flow-cells in the UV spectrophotometer (Cecil, CE 5501 Double Beam UV/Visible Spectrophotometer). The spectrophotometer is fitted with a six-cell unit and an RS 232 interface which provides the link to an IBM PC. Solutions of the solutes under examination are introduced on the upstream or reservoir side of the membranes and the diffusion of solute down the concentration gradient for each cell is monitored by the UV spectrophotometer. The data is automatically collected by the computer, processed and displayed. The mass transfer profiles obtained for each diffusion experiment can be used to calculate Permeability Coefficients, Diffusion Coefficients, Partition Coefficients and Lag Times.

The rate of mass transfer of a solute is detemined from the gradient of the steady-state portion of the mass transfer profile and the permeability can then be calculated using Equation (12).

$$P = \frac{(dMt/dt).\ell}{A.\Delta C} \qquad (12)$$

For experimental purposes, the concentration gradient or difference, ΔC, is assumed to be equivalent to the concentration of the reservoir solution as the concentration of the sink is always negligible relative to the reservoir

concentration i.e. infinite sink conditions were assumed.

The gradient of the steady-state portion of the mass transfer profile can be used to calculate the time lag, τ, and consequently, values for the diffusion coefficient, D, and the partition coefficient, K, can be obtained. The sophisticated computer program enables this information to be calculated quickly and easily for up to six cells and six different solutes.

The automatic diffusion cell system is not only suitable for the investigation of our own and similar novel membrane materials but may be used for the evaluation of existing commercial membranes. The system is capable of carrying out continuous analyses over periods lasting from only a few minutes, up to several weeks.

Chris Moran
University of Strathclyde
November 1992

EXPERIMENTAL

The following experimental data can be entered into the computer and processed for the calculation of Permeability Coefficient, Diffusion Coefficient, Lag Time and Partition Coefficient. The operation of the automatic diffusion cell system will also be demonstrated.

DATA SHEET

Name of Operator	
Date	
Experimental Reference	UNIDO WORKSHOP
Solute	Guaiacol Glyceryl Ether
Molecular Weight of Solute	198.20
Solute Reservoir Concentration (mg/ml)	10.00
Solute Sink Volume (ml)	50.00
System Temperature ($^{\circ}$C)	37
System pH	5.0
System Flow Rate (ml/min)	20.00
Analysis Wavelength (nm)	273.4
Membrane Reference	PUUII/10%AMG/0.45μmHV
Swelling of Membrane (pph)	20.00
Membrane Thickness (cm)	0.015
Membrane Area (cm^2)	2.27

Experimental Data

POINT	TIME(s)	ABSORBANCE
1.	0.0	0.000
2.	313.0	0.003
3.	701.0	0.006
4.	1090.0	0.008
5.	1477.0	0.014
6.	1864.0	0.023
7.	2251.0	0.034
8.	2637.0	0.046
9.	3023.0	0.061
10.	3409.0	0.075
11.	3794.0	0.089
12.	4181.0	0.104
13.	4567.0	0.118
14.	4952.0	0.133
15.	5339.0	0.148
16.	5724.0	0.163
17.	6112.0	0.179
18.	6497.0	0.196
19.	6885.0	0.212
20.	7271.0	0.227
21.	7657.0	0.243
22.	8042.0	0.258
23.	8429.0	0.275

Dr. Marion E. McNeill

1. Measurement of the diffusion coefficient of proxyphylline in a fully hydrated poly(ethylene oxide) hydrogel.

Theory

The diffusion coefficient, D, of a water-soluble compound in hydrated hydrogels is a measure of permeability through water-swollen matrices based on Fick's Laws of Diffusion. Crank related the fractional release M_t/M_∞ of a solute from an infinite slab of thickness, ℓ, to the square root of time, $t^{0.5}$.

$$\frac{M_t}{M_\infty} = 4\left(\frac{Dt}{\pi \ell^2}\right)^{0.5} \quad \text{for} \quad \frac{M_t}{M_\infty} \leq 0.6$$

When $M_t/M_\infty = 0.5$, i.e. the half life time, $t_{1/2}$, this equation simplifies to

$$D = 0.0492\ell^2/t_{1/2} \ cm^2s^{-1}$$

These equations assume release of solute from the slab into an aqueous sink is uni-dimensional from the top and bottom surfaces and release from the edge is negligible.

The value of D depends on the molecular weight of the compound, any interaction between the solute and the polymer, the degree of hydration of the polymer, and the temperature.

Method

Hydrogel : PEO3800/1HT Composition, PEG $\overline{M}_n$ 3800, 1 mol 1,2,6 hexane triol/mol PEG and the stoichiometric equivalence of dicyclohexyl methane-4,4'diisocyanate.

Proxyphylline (Sigma), hydroxypropyl theophylline, MW238

Xerogel slabs a) thin 1.02 ± 0.02mm x 30mm x 42mm
b) thick 2.85 ± 0.05mm x 30mm x 41 ± 1mm

Samples a) were swollen in 6 mg/ml proxyphylline in water at 37°C to equilibrium.
Samples b) were swollen in 2 mg/ml proxyphylline in water at 37°C to equilibrium.

Caleva Tablet Dissolution Apparatus Model 88T conforming to US Pharmacopoeia XXI linked to Cecil UV Spectrophotometer 550 containing flow cells. Solution pumped by a Watson Marlow Peristaltic Pump 503U.

λ max for proxyphylline : 273nm
Volume of sink : 800 ml double distilled water
Temperature of sink : 37°C

a) Circulate distilled water through flow cell.
Set paddle speed to 60 revs/min.
UV spectrophotometer : Autozero at 273nm
Read out scale 1A, Chart speed 60s/cm.
Scan Mode : Time course, Enter time constant, e.g. 2.

Remove swollen slab from swelling solution. Blot surface dry with tissue. Drop into cell of Caleva. Press scan. When absorbance is changing slowly, stop scan. Remove outlet from Caleva cell. Once the tubing has emptied into the cell remove inlet. The following morning, measure the absorbance to determine M_∞. Calculate M_t from the A_t measurements for a range of times, t. Plot M_t/M_∞ vs $t^{0.5}$.

For proxyphylline 1A = 25.9 μg/ml at 273nm

Results

t (mins)	$t^{0.5}$ $(mins)^{0.5}$	A_t	M_t (mg)	M_t/M_∞ (%)

When M_t/M_∞ = 50% $t_{1/2}$ =
Xerogel thickness ℓ_d = 1.02mm

Swelling and expansion of the xerogel is isotropic and depends on the polymer composition and the temperature of the water.

for PEO3800/1HT at 37°C the linear swelling factor is 1.54.

Therefore swollen hydrogel thickness, ℓ = 1.02 x 1.54
= 1.57 mm
= 0.157 cm

$D = 0.0492\ell^2/t^1_{/2}$

= $\quad x10^{-6}cm^2s^{-1}$

b) The effect of thickness on the half life time could be observed by repeating experiment a) with a slab 3 times thicker. The half life should then be 9 times longer than $t_{1/2}$ for a). In order to fit the second experiment which illustrates the effect of an initially dry polymer on the release profile of proxyphylline into the 2 hour laboratory session, experiment 1b) will be run on a sequential TIME PLOT program along with 2a) and 2b).

t (mins)	$t^{0.5}$ $(mins)^{0.5}$	A_t	M_t (mg)	M_t/M_∞ (%)

When $M_t/M_\infty = 50\%$ $t^1/_2$ =
Xerogel thickness ℓ_d = 2.85 ± 0.05
Swollen thickness ℓ = 2.85 x 1.54
= 4.39mm
= 0.439cm

$$D = 0.0492\ \ell_2/t_{1/2}$$

= x10^{-6}cm^{2}s^{-1}

2. Release of proxyphylline from a dispersion in a poly(ethylene oxide) xerogel in the form of a) a tablet and b) a sphere.

Theory

Drugs can be incorporated into hydrogels by swelling the polymer with a solution of the drug then evaporating the solvent leaving the drug substance trapped in the matrix as a uniform dispersion. When the xerogel containing the drug is reswollen in an aqueous fluid the drug dissolves in the penetrating water and diffuses along the aqueous pathways and across the device boundary to the surrounding fluid. The main effects compared to the fully swollen systems investigated in the first pair of experiments are prolongation of the period of release which can be seen by an increase in the half life time, and also a flattening of the release profile. The release kinetics can be described by the following equation.

$$\frac{M_t}{M_\infty} = kt^n$$

When n = 0.5 the diffusion is Fickian. This was illustrated in Experiments 1a) and 1b). When n = 1 the release is zero order i.e. constant. The release profile for Experiments 2a) and 2b) will lie between n = 0.5 and n = 1 and is termed anomalous. It should be possible to calculate the value of n from the data generated by the following experiments.

Method

a) Tablet shaped xerogel : 10mm diameter 3.05 ± 0.5mm thick.
Polymer composition : PEO4050/1.2HT

This composition has a higher $\bar{M}_n$ than the PEG3800 used in the swollen releases but this is compensated by a small increase in the proportion of crosslinker so that the 2 compositions have practically the same equilibrium water uptake of 186pph (parts per hundred parts dry polymer) at 37°C.

Weight of tablets : 280 ± 4mg
Swelling solution concentration : 30mg/ml
Swollen weight of tablets : 814 ± 11mg
Dried weight of tablets : 300 ± 3 mg

b) Spheres : 6mm diameter
Polymer composition : PEO4050/1.2HT
Weight of spheres : 141mg
Swelling solution concentration : 60mg/ml
Swollen weight of spheres : 433 mg
Dried weight of spheres : 143 mg

For both 2a) and 2b) and also 1b).

Volume of sink : 800ml
Temperature : 37°C
Paddle speed : 60 revs/min.

Circulate distilled water from 3 Caleva cells through 3 flow cells in the Cecil UV. Go to 273nm. Accessories program, Accessories, Autozero, Delay 10 minutes, Enter, Delay, Number of Cycles 25, Start (blue button). Collect data and final absorbance values from all 4 experiments, next day in order to calculate M_∞.

Results

Tablet design

t (mins)	$t^{0.5}$ $(mins)^{0.5}$	A_t	M_t (mg)	$\frac{100M_t}{M_\infty}$	t-t′ (mins)	ln(t-t′)	$\frac{100M_t}{M_\infty} - \frac{100M_{t'}}{M_\infty}$	$\ln\left[\frac{100M_t}{M_\infty} - \frac{100M_{t'}}{M_\infty}\right]$

When $M_t/M_\infty = 50\%$ $t^1/_2$ =

Estimated end of burst effect t′ =

Sphere

t (mins)	$t^{0.5}$ $(mins)^{0.5}$	A_t	M_t (mg)	$\frac{100M_t}{M_\infty}$	t-t′ (mins)	ln(t-t′)	$\frac{100M_t}{M_\infty} - \frac{100M_{t'}}{M_\infty}$	$\ln\left[\frac{100M_t}{M_\infty} - \frac{100M_{t'}}{M_\infty}\right]$

When $M_t/M_\infty = 50\%$ $t^1/_2$ =

Plot M_t/M_∞ vs time t for both the tablet and the sphere

Is there an initial burst from surface proxyphylline? If yes, decide on the transition time, t′, (possibly 10 minutes) when the release profile becomes anomalous.

Estimated end of burst effect t′ =

In order to calculate the exponent, n, which fits the release profile from t′ until t for 60% complete the tables for the tablet and the sphere, then plot

$$\ln\left[\frac{100M_t}{M_\infty} - \frac{100M_{t'}}{M_\infty}\right] \quad \text{vs} \quad \ln(t-t')$$

$$\ln\left[\frac{100M_t}{M_\infty} - \frac{100M_{t'}}{M_\infty}\right] = k + n \ln (t-t')$$

Determine the values of k and n from a regression program, or from the plot. n is the gradient and k the intercept. $k = \ln k_1$

Then $$\frac{100M_t}{M_\infty} = \frac{100M_{t'}}{M_\infty} + k_1(t-t')^n$$

For the tablet $$\frac{100M_t}{M_\infty} = \quad + \quad (t- \quad)$$

For the sphere $$\frac{100M_t}{M_\infty} = \quad + \quad (t- \quad)$$

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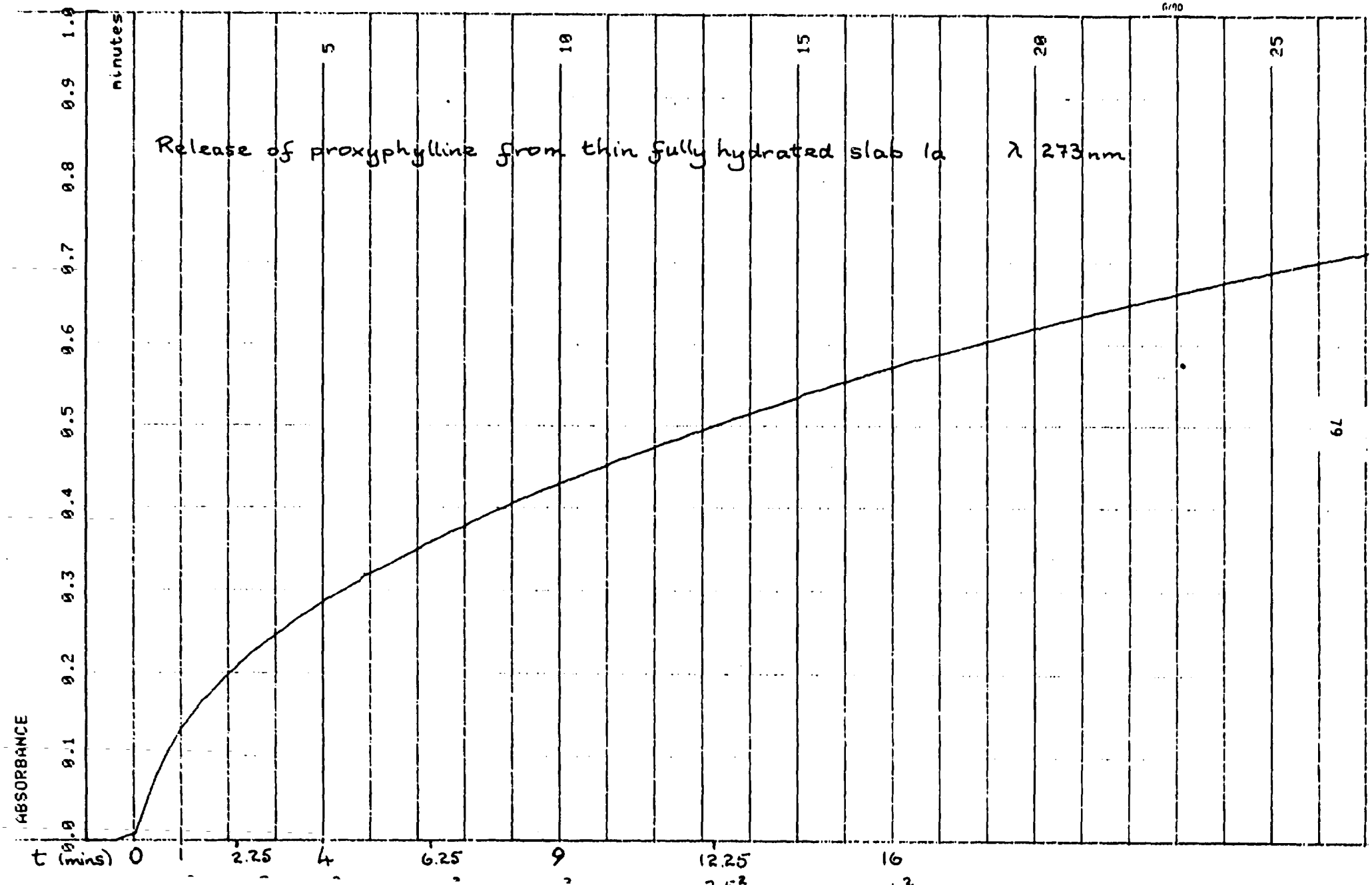


CECIL INSTRUMENTS CE5501 W DOUBLE BEAM SPECTROPHOTOMETER
CELL PROGRAM

Wavelength = 273.0 nm Bandwidth = 2.0 nm Cycle Delay = 10 minutes
Abs values

Sphere Tablet Thick slab

	CELL 1	CELL 2	CELL 3	TIME
CYCLE 1	0.000A	0.000A	0.000A	0h 0.0m
CYCLE 2	0.065A	0.064A	0.175A	0h10.0m
CYCLE 3	0.107A	0.109A	0.259A	0h20.0m
CYCLE 4	0.143A	0.147A	0.329A	0h30.0m
CYCLE 5	0.174A	0.180A	0.378A	0h40.0m
CYCLE 6	0.201A	0.209A	0.413A	0h50.0m
CYCLE 7	0.227A	0.239A	0.452A	1h 0.0m
CYCLE 8	0.250A	0.266A	0.488A	1h10.0m
CYCLE 9	0.272A	0.290A	0.520A	1h20.0m
CYCLE 10	0.292A	0.312A	0.550A	1h30.0m
CYCLE 11	0.312A	0.334A	0.579A	1h40.0m
CYCLE 12	0.331A	0.355A	0.603A	1h50.0m
CYCLE 13	0.349A	0.374A	0.627A	2h 0.0m
CYCLE 14	0.366A	0.393A	0.650A	2h10.0m
CYCLE 15	0.383A	0.411A	0.671A	2h20.0m
CYCLE 16	0.399A	0.428A	0.692A	2h30.0m
CYCLE 17	0.413A	0.446A	0.710A	2h40.0m
CYCLE 18	0.430A	0.462A	0.728A	2h50.0m
CYCLE 19	0.444A	0.477A	0.746A	3h 0.0m
CYCLE 20	0.458A	0.493A	0.761A	3h10.0m
CYCLE 21	0.471A	0.507A	0.776A	3h20.0m
CYCLE 22	0.483A	0.522A	0.789A	3h30.0m
CYCLE 23	0.497A	0.535A	0.805A	3h40.0m
CYCLE 24	0.508A	0.548A	0.817A	3h50.0m
CYCLE 25	0.520A	0.562A	0.829A	4h 0.0m

CECIL INSTRUMENTS CE5501 W DOUBLE BEAM SPECTROPHOTOMETER
CELL PROGRAM

Wavelength = 273.0 nm Bandwidth = 2.0 nm Cycle Delay = 0 minutes
Abs values

Sample 1 : readout: 1.041A λ: 273.0nm Thin slab t 17h $\sim A_\infty$

	CELL 1	CELL 2	CELL 3		TIME
CYCLE 1	0.831A	0.857A	1.054A	$\sim A_\infty$	t 16h0h 0.0m

Theory of the Diaphragm Cell

by: Sam McFadzean

In this example the permeant molecule is an ion. Two electrodes sensitive to this ion (iodide) are placed, one in the Top and the other in the Bottom half cell. The difference in electrical potential between these two electrodes is measured as a function of time during the diffusion experiment.

Theory requires that (in the steady state) the permeability of the membrane may be estimated by the equation:

$$\ln\left(\frac{\theta + r}{\theta - 1}\right) = \mathbf{P}\beta t$$

the symbols are defined in the theoretical development given below:

Proof Consider the Diaphragm Cell - in which the diaphragm is a test membrane

Nomenclature : C_B, C_T Concentrations of diffusant (bottom & top) (mol cm^{-3})

$\Delta C = C_B - C_A$

$\theta = C_B/C_A$

V_B, V_T Half cell volumes (cm^3)

A Area of diaphragm membrane (cm^2)

l Thickness " " (cm)

J Flux density (mol $cm^{-2}s^{-1}$) defined by Fick's Law

f Flux ($mols^{-1}$) = JA

D Diffusion coefficient of permeant (cm^2s^{-1})

Experimental Conditions :

1. The half-cell solutions are stirred continuously and are regarded as homogeneous.
2. We consider only the (pseudo) steady state in which there is a linear gradient across the

diaphragm.

3. Fick's Law applies in the membrane so that

$$J = D\frac{\Delta\overline{C}}{l} = \frac{D\alpha}{l}(C_B - C_T) \tag{1}$$

where $\alpha = \Delta\overline{C}/\Delta C$ is the distribution coefficent for permeant between the membrane and solution phases.

In the steady state the loss of diffusant from the bottom half cell equals the gain in the top, so that defining the flux, f, from bottom to top

$$f = -\frac{dC_B}{dt}.V_B = +\frac{dC_T}{dt}.V_T \tag{2}$$

$$\Delta C = C_B - C_T \tag{3}$$

$$d\Delta C = dC_B - dC_T \tag{4}$$

$$\frac{d\Delta C}{dt} = \frac{dC_B}{dt} - \frac{dC_T}{dt} = -f\left(\frac{1}{V_B} + \frac{1}{V_T}\right) \tag{5}$$

from equation (1)

$$f = \left(\frac{DA\alpha}{l}\right)\Delta C = \mathbf{P}\Delta C \tag{6}$$

from (5) & (6)

$$\frac{d\Delta C}{dt} = -\mathbf{P}\left[\left(\frac{1}{V_B} + \frac{1}{V_T}\right)\right]\Delta C$$

$(1/V_B + 1/V_T)$ is a constant - defined as β, the cell constant.

$$\int_{\Delta C=0}^{\Delta C_t} \frac{d\Delta C}{\Delta C} = -\mathbf{P}\beta \int_0^t dt \tag{7}$$

since dx/x = dlnx, we obtain, on integration,

$$\ln \Delta C_{t=0} - \ln \Delta C = \mathrm{P}\beta t \tag{8}$$

at time zero $\Delta C_{t=0} = C_B^o$ in this experiment

$$\ln C_B^o - \ln \Delta C = \mathrm{P}\beta t \tag{9}$$

From the emf measured in this experiment we obtain the concentration ratio $C_B/C_T = \theta$

$$E = \frac{k}{n} \ln \theta \quad : \qquad \theta = \exp\left(\frac{nE}{k}\right) \tag{10}$$

where $k = RT/\mathrm{F}$

equation (9) deals with concentration differences $\Delta C = C_B - C_T$

We can obtain the final equation - if we remember that the system is sealed, so by conservation of diffusant (we neglect the very small amount of diffusant in the membrane).

$$C_B V_B + C_T V_T = C_B^o V_B \tag{11}$$

Let the volume ratio of the half cells be $V_T/V_B = r$. Rewriting eqn(11) in terms of Θ and r and rearranging gives:

$$C_T = \frac{C_B^o}{\theta + r} \tag{12}$$

and

$$C_B = \theta C_T = \frac{C_B^o \theta}{\theta + r} \tag{13}$$

From equations (3)(9)(12)(13), the working equation is obtained:

$$\ln\left(\frac{\theta + r}{\theta - 1}\right) = \mathrm{P}\beta t$$

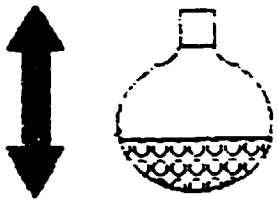
UNIDO COURSE

DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS

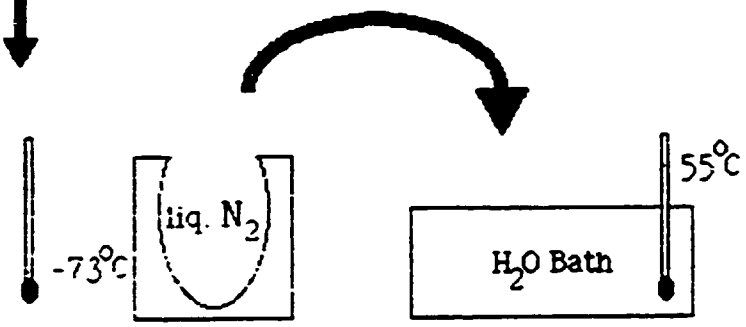
PRACTICAL

PREPARATION of LIPOSOMES

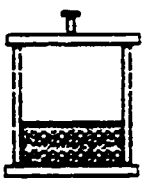
by: Alex Mullen
November 1992



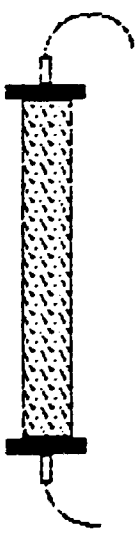
The lyophilised product is rehydrated using an aqueous solution of your drug material. Rehydration is performed under constant agitation for two hours at the required temperature(usually above T_c).



The hydrated lipids are subjected to five freeze-thav cycles in liquid nitrogen.



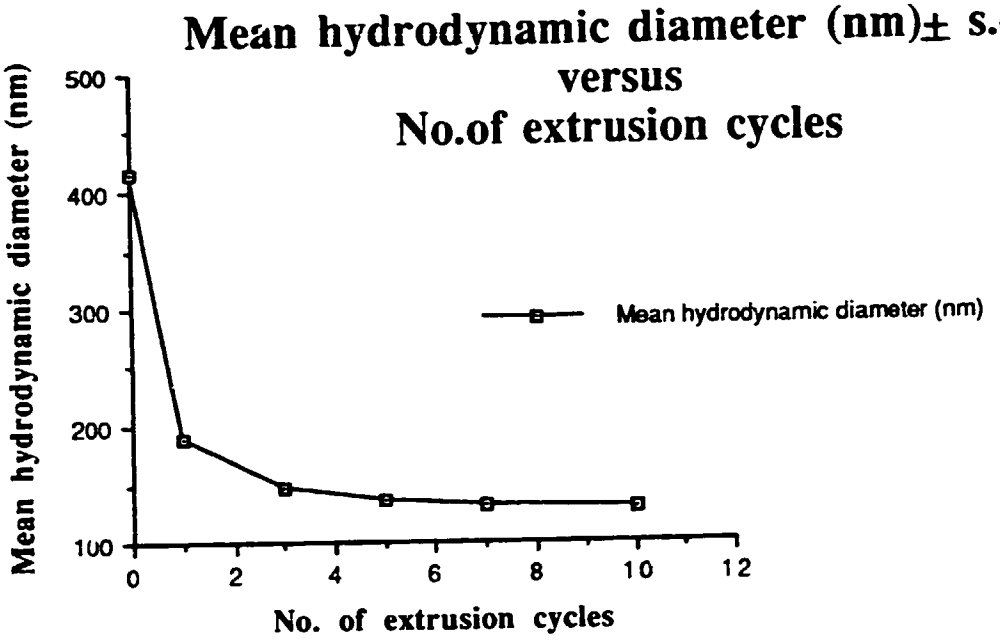
Once hydrated and freeze-thawed the vesicles are reduced by extruding them ten times through two stacked polycarbonate membranes of an appropriate pore diameter.



The extruded vesicles are then separated from the free drug solution by gel chromatography. The liposomes that are obtained can then be characterised.

SIZE REDUCTION USING AN EXTRUDER

The following data was obtained using liposomes prepared from L-α-lecithin, DPC and CHOL in a molar ratio of 7:3:1 respectively. The liposomes were size reduced by passage through two stacked 150nm polycarbonate filters



MALVERN ANALYSIS SUMMARY REPORT

Original sample after hydration

No.	Time	Intensity	Size nm	Poly	Zeta(mV)	Mobility (Ucm/secV)
1	17:51:24	27.1	[illegible]	0.411		
2	[illegible]	[illegible]	[illegible]	[illegible]		
3	[illegible]	[illegible]	[illegible]	0.297		
4	[illegible]	[illegible]	[illegible]	[illegible]		
5	17:54:02	27.5	401.[illegible]	0.285		

	Size nm	Poly
Mean	414.3	0.300
Standard Deviation	15.[illegible]	0.057

MALVERN ANALYSIS SUMMARY REPORT

Original sample after hydration
1 extrusion cycle

No.	Time	Intensity	Size(nm)	Poly	Zeta(mV)	Mobility (Ucm/secV)
1	17:57:45	173.3	187.5	0.003		
2	17:58:24	169.4	176.[illegible]	0.055		
3	17:59:04	167.1	185.3	0.130		
4	17:59:43	164.0	184.1	0.105		
5	18:00:23	155.0	185.[illegible]	0.100		

	Size(nm)	Poly
Mean	183.3	0.080
Standard Deviation	5.7	0.045

MALVERN ANALYSIS SUMMARY REPORT

Original sample after hydration
3 extrusion cycle

No.	Time	Intensity	Size nm	Poly	Zeta(mV)	Mobility (Ucm/secV)
1	[illegible]:37	[illegible]	[illegible]	[illegible]		
2	[illegible]	[illegible]	[illegible]	[illegible]		
3	[illegible]	201.4	[illegible]	[illegible]		
4	[illegible]	[illegible]	[illegible]	[illegible]		
5	[illegible]	195.4	[illegible]	[illegible]		

	Size nm	Poly
Mean	[illegible]	[illegible]
Standard Deviation	[illegible]	[illegible]

MALVERN ANALYSIS SUMMARY REPORT

Original sample after hydration
5 extrusion cycles

No.	Time	Intensity	Size(nm)	Poly	Zeta(mV)	Mobility (Ucm/secV
1	18:09:13	156.5	142.0	0.085		
2	18:09:52	154.4	[illegible]	[illegible]		
3	18:10:32	153.8	133.2	0.[illegible]		
4	18:11:11	152.[illegible]	[illegible]	[illegible]		
5	18:11:51	153.2	131.[illegible]	0.098		

Mean	135.7	0.084
Standard Deviation	[illegible].7	0.032

MALVERN ANALYSIS SUMMARY REPORT

Original sample after hydration
7 extrusion cycles

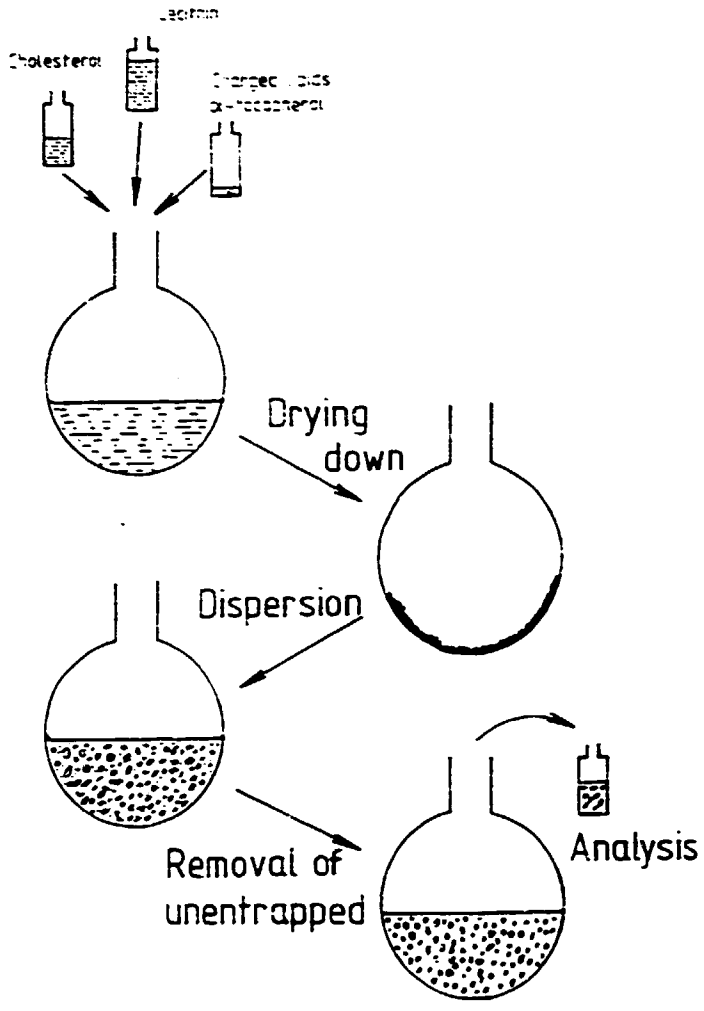
No.	Time	Intensity	Size(nm)	Poly	Zeta(mV)	Mobility (Ucm/secV
1	18:15:55	161.4	133.3	0.121		
2	18:1[illegible]	155.8	132.[illegible]	0.100		
3	18:16:14	157.8	[illegible]	0.013		
4	18:18:53	154.2	125.4	0.100		
5	18:19:33	156.9	126.1	0.082		

Mean	130.4	0.085
Standard Deviation	2.8	0.035

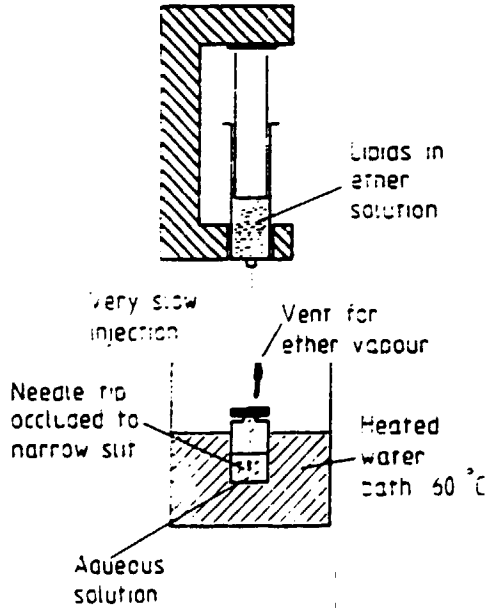
MALVERN ANALYSIS SUMMARY REPORT
Original sample after hydration
10 Extrusion cycles

No.	Time	Intensity	Size(nm)	Poly	Zeta(mV)	Mobility (Ucm/secV
1	[illegible]	31.7	[illegible]	[illegible]		
2	[illegible]	34.[illegible]	[illegible]	[illegible]		
3	[illegible]	[illegible]	[illegible]	[illegible]		
4	[illegible]	[illegible]	[illegible]	[illegible]		
5	[illegible]	[illegible]	[illegible]	[illegible]		

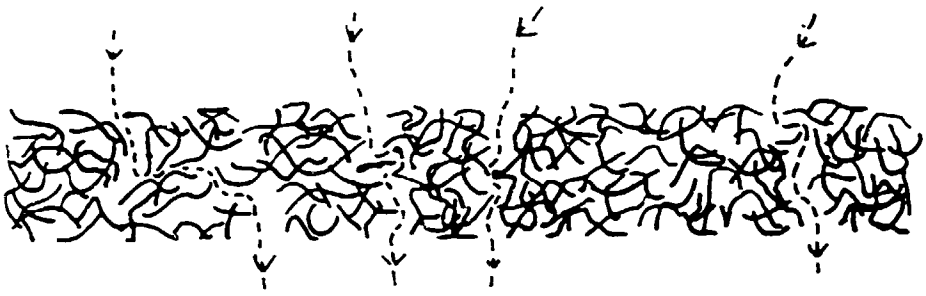
Mean	[illegible]	[illegible]
Standard Deviation	[illegible]	[illegible]



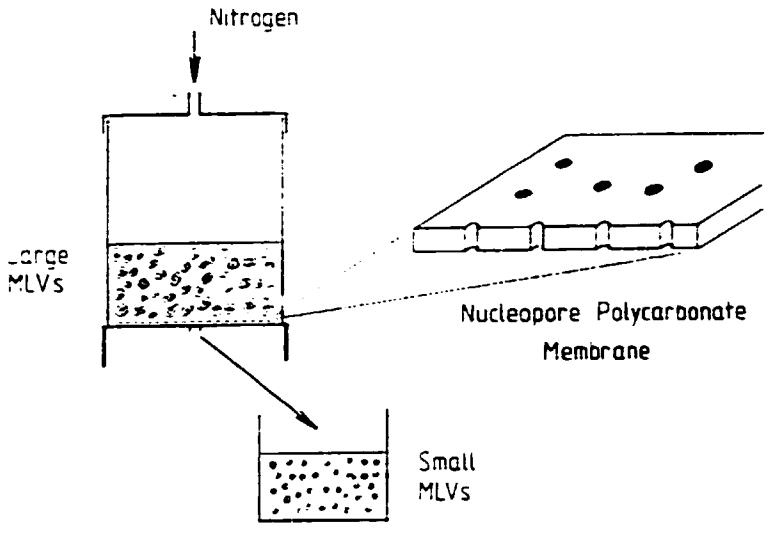
Stages common to all methods of preparation of liposomes.



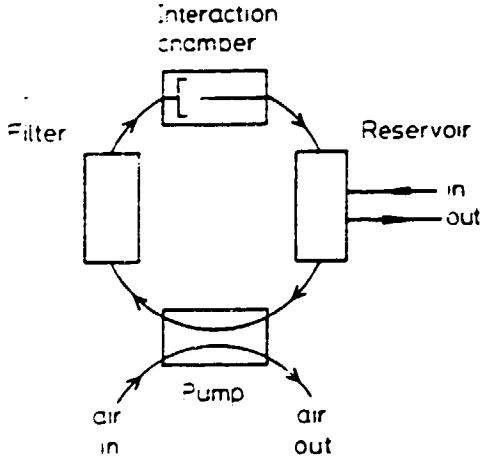
Preparation of LUVs by ether injection. In this method an ether solution of lipids is introduced into an aqueous phase held at a temperature at which the ether vaporizes rapidly. The phospholipids left behind disperse in water forming large bilayer sheets which rapidly seal to give closed LUVs.



Tortuous path membrane. Compare the difference in size and shape of the pores of this membrane with that of the nucleation track membrane



Liposome sizing by extrusion through Nuclepore membranes. MLVs are reduced in size by extrusion through the pores of a Nuclepore polycarbonate membrane. The pores are uniform in diameter, and after several passes through the membrane the liposomes obtained are relatively homogeneous in size, with a diameter equal to or slightly larger than that of the membrane pores.



Diagrammatic representation of the use of a micro-fluidizer to prepare liposomes. A suspension of MLVs is introduced, via the reservoir, and pumped at high pressure through the interaction chamber, where the suspension is separated into two streams which collide with each other at high velocity. Large vesicles are broken up into smaller ones and may be collected after a single pass, or recycled several times to produce further reductions in size.

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Max-Planck-Inst fuer Biophys. Chemie, Goettingen, Germany
Martin-Luther-Universitaet, Halle/Saale, Germany
Rheinische Friedrich-Wilhelm-Universitaet, Bonn, Germany
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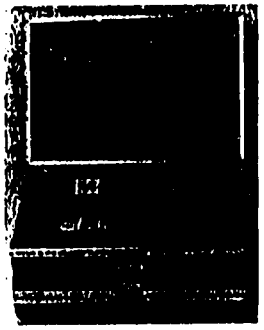
Preparation of Unilamellar Liposomes

Use the last page of this brochure to receive the LiposoFast free for trial



LiposoFast™ - Basic

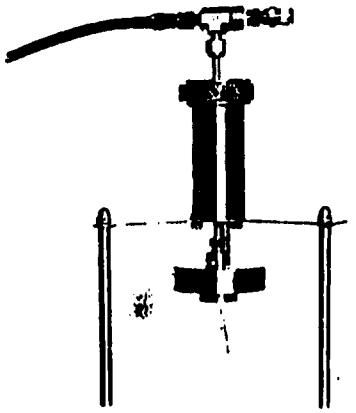
- Preparation of small-volume Unilamellar Large Liposomes by extrusion through polycarbonate membranes

Price: US$480.00 or DM790.00 or FF2,640.00 or CDN$550.00

- Hand operated and easy to use design for volumes between 0.1-10ml. Preparation time: 5min.

According to the following reference:

- MacDonald RC/MacDonald RI/Menco B Taleshita K/Subbarao NK/Hu L
- Small-volume extrusion apparatus for preparation of large, unilamellar vesicles
- Biochimica et Biophysica Acta/1061/1991/297-303



LiposoFast™ - Large

- Applies the same operation principle as the LiposoFast - Basic.
- Stainless Steel and corrosion resistence design. Can be immersed in a temperature controlled water bath.
- Capacity : 50ml.
- Simple and easy to use design.

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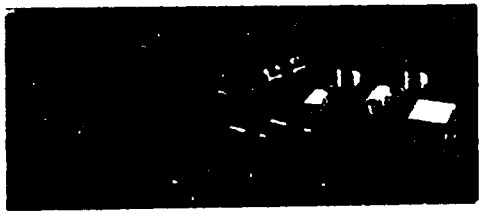
Instructions for LiposoFast™ - Basic



1- Clean the device with ethanol prior to use.

2- Insert the first half of the filter supporting system into the housing, leaving a few mm from the top edge.

3- Lay one or two filter(s) on the "O" ring embedded in the filter support system.

4- Put the second half of the filter support system into the housing so that the "O" rings can be seen through the inspection hole.

5- Tighten the end caps by hand firmly to avoid twisting of the support system parts and to keep the dead volume as low as possible. The filter is pressed firmly between the "O" rings.

6- Hydrate and disperse the dry lipid in buffer to obtain multilamellar vesicles. Repeated freeze-thawing (e.g. 10 times) will improve the encapsulation efficiency.

7- Screw and press the syringe simultaneously to attach the syringe containing the prepared multilamellar liposomes to the luer in the filter support system. Usually 0.25-0.5 ml are being used. Repeat the test for higher quantities. By proper handling the total dead volume is only a few microlitres and reproducible.

8- Pass the liposome emulsion through the filter(s) back and forth (usually 15-29 passes are sufficient).

9- Remove the liposomes from the opposite syringe to ensure that there are no unfiltered vesicles left.

References

LIFOCODE: [illegible]
AUTHORS: [illegible]
TITLE: [illegible]
SOURCE: [illegible]

LIFOCODE: 9118
AUTHORS: subbarao nk/macdonald rc/sakeshita k/macdonald rc
TITLE: characteristics of spectrin-induced leakage of extruded phosphatidylserine vesicles
SOURCE: biochimica et biophysica acta/1063/91/137-154

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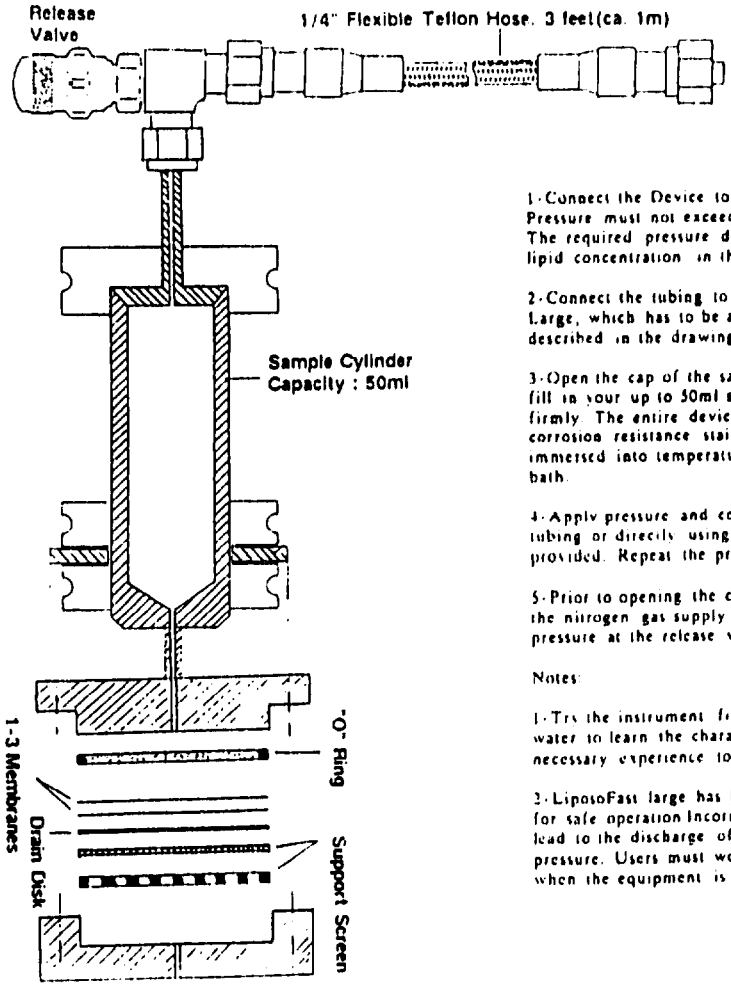
Instructions for LiposoFast™ Large



1- Connect the Device to the Nitrogen gas. Pressure must not exceed 800PSI (5.5 MPa) The required pressure depends mainly on the lipid concentration in the MLV emulsion.

2- Connect the tubing to your LiposoFast-Large, which has to be assembled as described in the drawing.

3- Open the cap of the sample cylinders and fill in your up to 50ml sample. Close the cap firmly. The entire device is made of corrosion resistance stainless steel and can be immersed into temperature controlled water bath.

4- Apply pressure and collect the sample via tubing or directly using the erlenmayer flask provided. Repeat the process 10-20 times.

5- Prior to opening the cap, close the valve of the nitrogen gas supply and release the pressure at the release valve using wrench.

Notes:

1- Try the instrument first with distilled water to learn the characteristics and get the necessary experience to handle the steps.

2- LiposoFast large has been manufactured for safe operation. Incorrect operation may lead to the discharge of fluids under pressure. Users must wear eye protection when the equipment is being used.

UNIDO COURSE

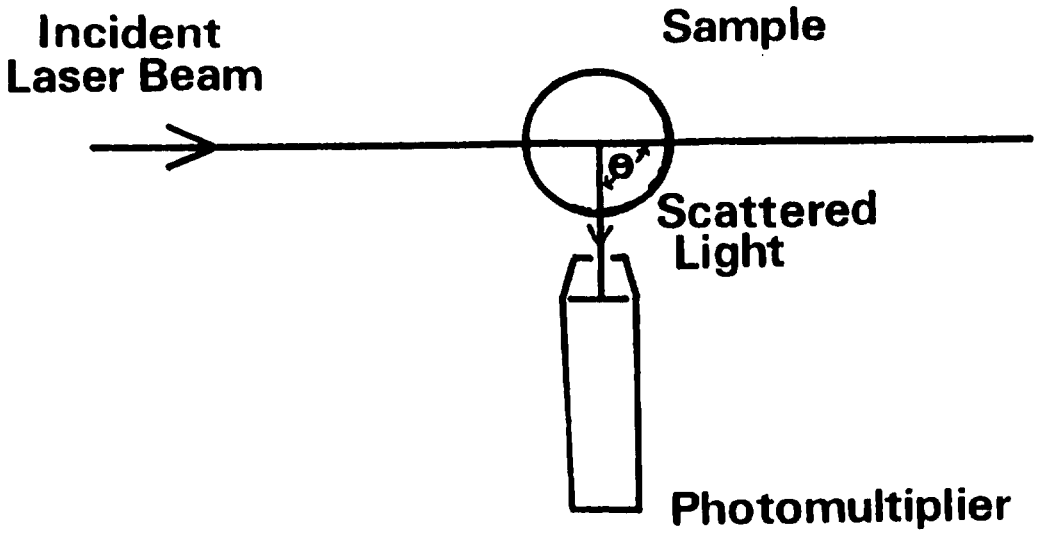
DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS

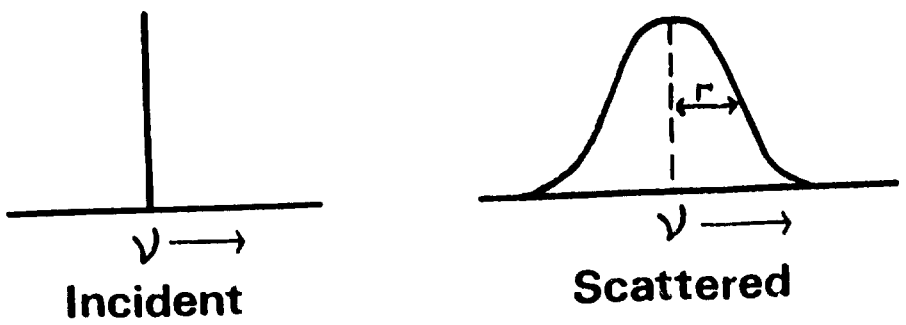
PRACTICAL

PARTICLE SIZE DETERMINATION OF LIPOSOMES

by: Alex Mullen and Moira Owens
November 1992

FIGURE 1





$$\Gamma = 2DK^2$$

$$R = \frac{kT}{6\pi\eta D}$$

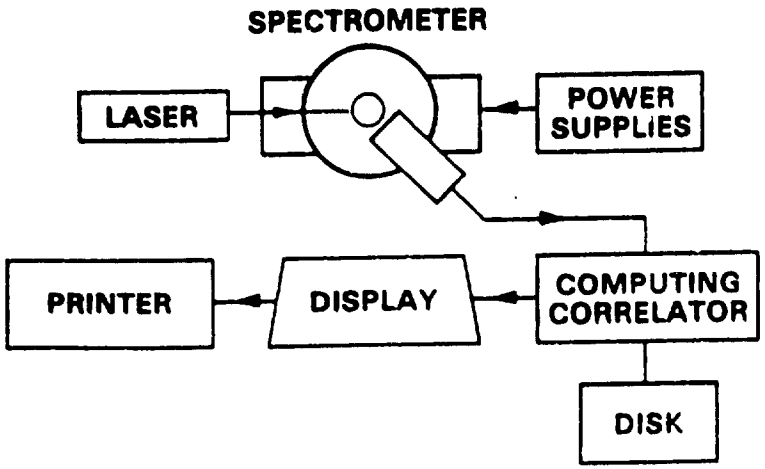
$$\left(K = \frac{4\pi n}{\lambda}\sin\frac{\theta}{2}\right)$$

Basic's

Consider a suspension of particles dispersed in a liquid. Basic physics tells us that at all temperatures other than absolute zero the particles are randomly moving relative to each other under the effects of thermal motion. The rate and the way the particles move is a direct function of their size. larger particles moving slower than smaller ones. The requirement is therefore to measure this rate of particle movement and hence determine the size and other information relating to the particle.

When particles in suspension are illuminated with a laser they will scatter light and because the particles are constantly in motion, the scattered light will fluctuate as the phases of scattering contributions from various particles change relative to each other. Observation of these fluctuations with a photon detector indicate that the changes take place with a time characteristic related to the size of the particles under study and yield a correlation function, $g(\tau)$, which may be used to compute the characteristic movement rate (diffusion coefficient) of the particle and hence particle size from the equations. Additional measurements give information on shape, structure and dynamic properties of the particle

Operational Principle



Particle Size

$$g(\tau) = 1 + \exp(-2DK^2\tau)$$

where D = Diffusion Coefficient. $K = \frac{4\pi}{\lambda} \sin(\frac{\Theta}{2})$

λ = Laser Wavelength. Θ = Measurement Angle

Particle Size may be computed from

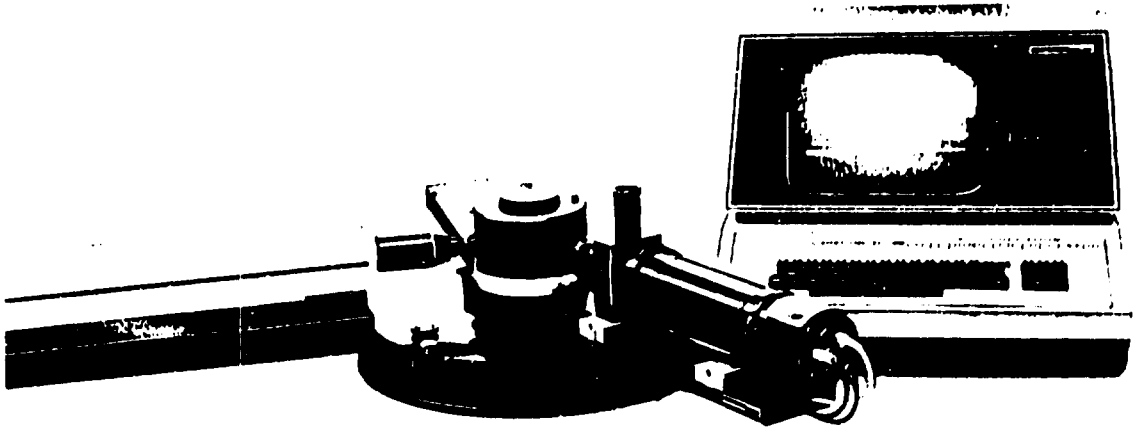
$$\text{Particle Size} = \frac{kT}{3\pi nD}$$

where k = Boltzmanns Constant, D = Diffusion Coefficient, n = Solvent Viscosity, T = Temperature

MALVERN SYSTEM 4600

A complete Research Laser Light Scattering System for Sub-Micron Particle Sizing and Molecular Analysis using **Photo Correlation or Intensity Spectroscopy**

APPLICATIONS
Particle Sizing
Particle Shape
Determination of Molecular Weight
Colloids
Micelles
Proteins
Viruses
Gels
Polymers
Latexes
Micro Emulsions
Clays
Inks
Pigments
Blood
Antigens



Particle Sizing 5 Angstroms - 3 microns.
Diffusion Coefficient 10^{-6} - 10^{-3}
Molecular Weight 10^{4} - 10^{17}

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DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS

PRACTICAL

PARTICLE SIZE DETERMINATION OF AEROSOLS

PARTICLE SIZE DETERMINATION OF MICROSPHERES

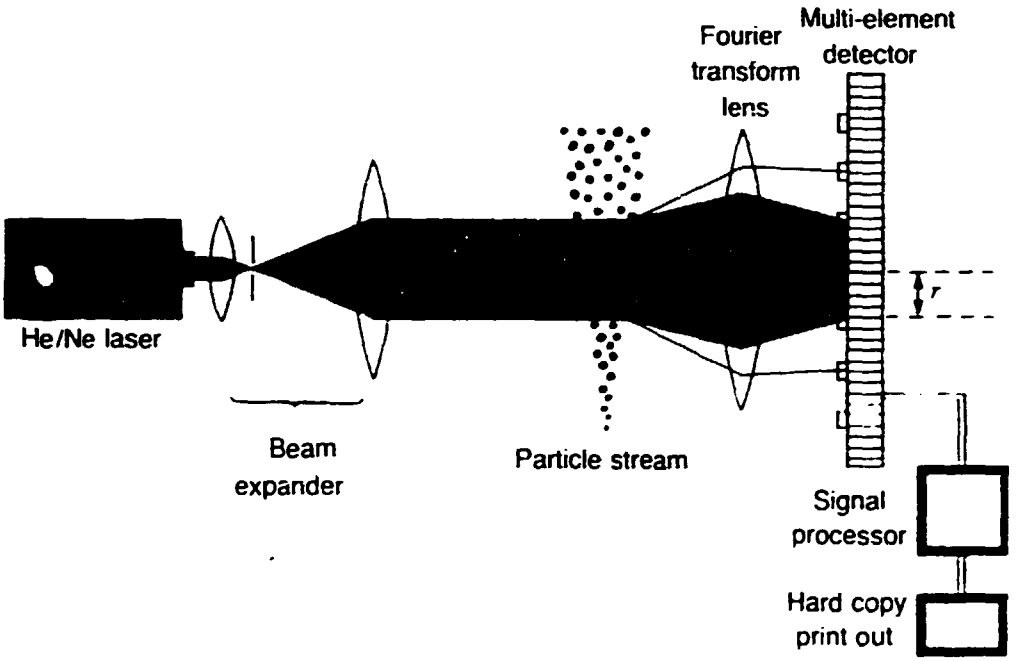
by: Dr. Tony L. Whateley
November 1992

PARTICLE SIZE DETERMINATION OF AEROSOLS AND MICROSPHERES

For particles and droplets in the size range 2-1000 um the same method can be used:

MALVERN MODEL 2600 LASER DIFFRACTION PARTICLE SIZER

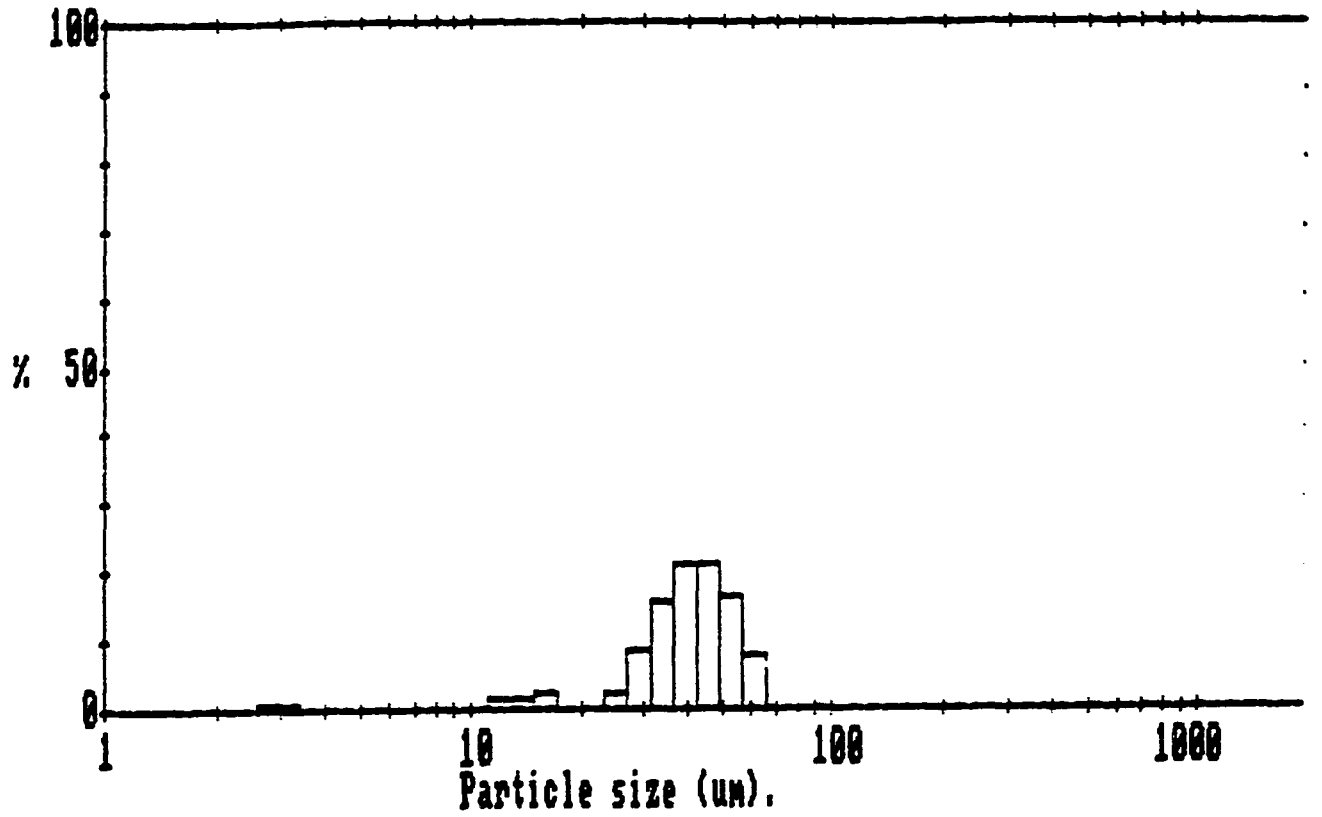
A diagram of the insrument is shown below.



Schematic diagram of the Malvern particle and droplet sizer

Once the sizer has been set up the following sequence of commands can be used to size both aerosols and suspensions of microspheres.

ALI	Align
MEA BAC	Measure background
INS SAM	Inspect Sample to adjust conc. of a suspension
MEA SAM	Measure sample (inject aerosol into beam)
CAL RES	Calculate results
SAVE DAT,1	
DIS RES	Display results
PLO HIS	Plot histogram
PRI	Print



Malvern Instruments MASTER Particle Sizer M3.1 Date 01-01-80 Time 02-55

Size microns	x under	Size band microns		x	Result source=Sample / Record No. = 1 / Focal length = 63 mm. / Experiment type pil / Volume distribution / Beam length = 14.3 mm. / Obscuration =0.1400 / Volume Conc. = 0.0103 x / Log. Diff. =4.58 / Model indp
118.4	100.0				
54.9	89.9	118.4	54.9	10.1	
33.7	26.5	54.9	33.7	63.4	
23.7	8.7	33.7	23.7	17.8	
17.7	8.3	23.7	17.7	0.4	
13.6	4.2	17.7	13.6	4.1	
10.5	2.5	13.6	10.5	1.8	
8.2	2.5	10.5	8.2	0.0	
6.4	2.5	8.2	6.4	0.0	D(v,0.5) = 40.4 µm
5.0	2.5	6.4	5.0	0.0	D(v,0.9) = 54.9 µm
3.9	2.4	5.0	3.9	0.0	D(v,0.1) = 26.0 µm
3.0	1.5	3.9	3.0	0.9	D(4,3) = 36.4 µm
2.4	0.0	3.0	2.4	1.5	D(3,2) = 23.6 µm
1.9	-0.0	2.4	1.9	0.0	Span = 0.7
1.5	-0.0	1.9	1.5	0.0	Spec. surf. area
1.2	-0.0	1.5	1.2	0.0	0.11 sq.m./cc.

Sample details:-ned ca2498v micronised ipa tween 80 probe sonicated 22/5/91

UNIDO COURSE

DEVELOPMENT OF ADVANCED PHARMACEUTICAL FORMULATIONS

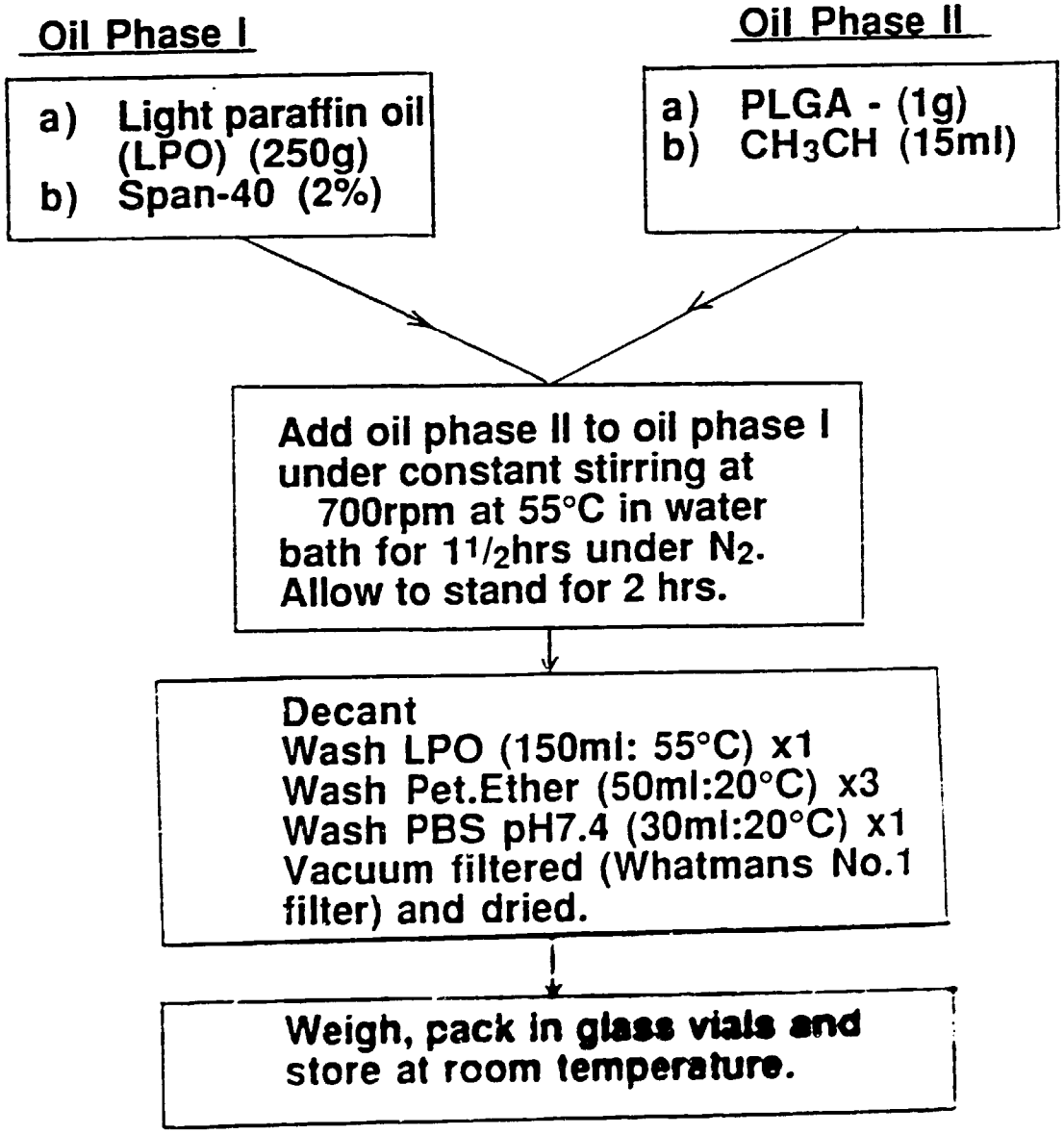
PRACTICAL

MICROENCAPSULATION AND PREPARATION OF MICROSPHERES

by: Isabel Crossan
November 1992

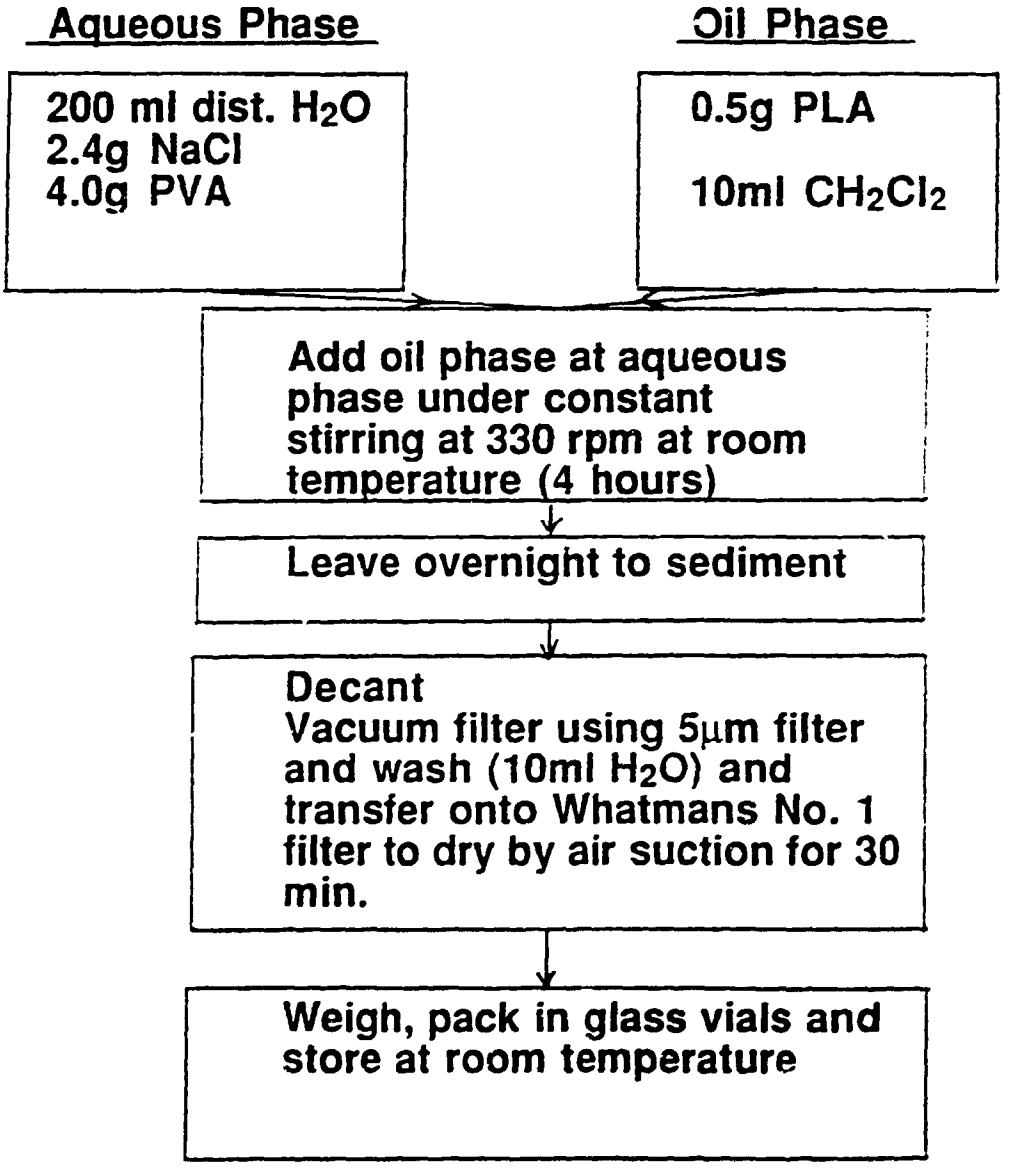
METHOD I. Oil-in-Oil

The oil-in-oil emulsion method (using acetonitrile + SPAN-40 in light liquid paraffin, stirring with Silverson at 690 rpm for 2 hours at 55°C under N2) yielded microspheres of adequate loading for the water soluble MMC but was not suitable for the preparation of small microspheres (<50μm).

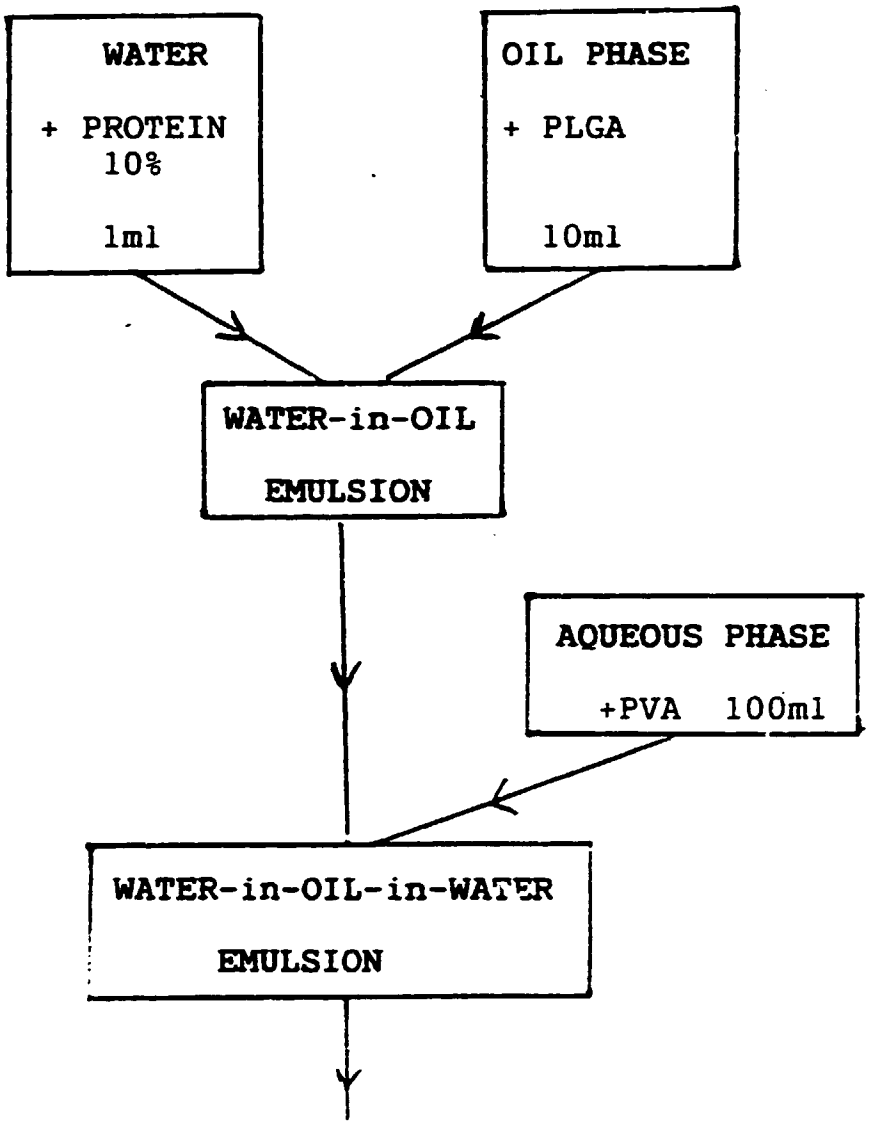


METHOD II. Oil in Water

The oil-in-water emulsion method not previously investigated for MMC (using dichloromethane, polyvinyl alcohol, and sodium chloride in water saturated with MMC stirred at room temperature with an overhead paddle at 330 rpm for 4 hrs) yielded microspheres of suitable size (40-50μm) and surface properties (*i.e.* hydrophilic) and of adequate loading of MMC. Solutions were decanted, filtered and dried under vacuum. Release rate studies were performed using U.S.P. Method II and HPLC assay.



METHOD III WATER-IN-OIL-IN-WATER



OIL IN OIL METHOD

ADVANTAGES : *good for water soluble drugs*

PROBLEMS : *difficult to prepare small microspheres*

: *aggregate in aqueous solutions due to hydrophobic surface*

OIL IN WATER METHOD

ADVANTAGES : *good for lipophilic drugs*
wide range of sizes readily prepared
easily re-suspended (hydrophilic surface)

PROBLEMS : *poor drug loading for water soluble drugs*

MICROENCAPSULATION WITH ETHYLCELLULOSE

SUSPENSION OF DRUG IN CYCLOHEXANE

AT 80^{O} PLUS

ETHYLCELLULOSE

cool slowly

↓

DRUG PARTICLES COATED WITH ETHYLCELLULOSE

↓

isolate, wash