## OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

## DISCLAIMER



## FAIR USE POLICY



## CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

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

RESTRICTED

20093

DP/ID/SER.A/1634
15 March 1993
ORIGINAL: ENGLISH

IMPROVED PRODUCTION OF PENICILLIN

DP/CPR/89/021

THE PEOPLE'S REPUBLIC OF CHINA

Technical report: Fifth visit to the Guangzhou pharmaceutical factory and research institute, 7-16 October 1992*

Prepared for the Government of the People's Republic of China
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of F. R. Batchelor, chief technical adviser

Backstopping Officer: Mr. Z. Csizer
Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

* This document has not been edited.

V.93 82812

# TABLE OF CONTENTS

ABSTRACT

The Chief Technical Adviser's main goal was to demonstrate that:

i) a technically complex project can only be implemented successfully by the work of a cohesive team;

ii) without compliance to the GMP, no quality work can be assured, and

iii) quality assurance should start with the preparation of proper records such as SOPs (Standard Operation Procedures), BPRs (Batch Production Records) and TRs (Test Records).

## Introduction

The objective of this visit was to be present with Dr Cole and Mr Bird and liaise with them ensuring proper co-ordination of all aspects of the project. Although fermentation and recovery are separate parts of the process of manufacturing penicillin V one does impinge on the other and it is essential that the groups work efficiently together. Furthermore both are entirely dependant on rapid and reliable measurement of penicillin content by the analytical department. It is thus essential that the staff are properly trained and the need for and benefits of co-operation between the various parts of the operation clearly demonstrated. Hopefully on this occasion these benefits were amply demonstrated by the way Dr Cole and Mr Bird and the CTA worked together to solve a number of problems.

The building which has been the cause of major hold ups in the project is still not completely finished. Considerable progress has however been made and the new Factory Director and the NPD are both to be commended for their efforts. The two most important floors are already partially occupied and the culture maintenance and analytical staff have moved in, also the Braun mini fermenters had been installed. Though there is still much to be done the technical advisers were all able to spend their time usefully. We were also able to work at least an extra hour each day by starting earlier and by the re-organisation of luncheon arrangments.

Language still presents some difficulties but two or three of the staff have made good progress with the English language. So much so that the unavoidable absence of Cai Shi Chaio on one day did not affect work as much as it would have done at the start of the project.

## Fellowship Visit

Four members of the Guangzhou staff Xion Li-Hong, Feng Xing-Hua, Chen Tian-Nu and Kuang Jing-Wen visited the USA. It had been intended they visit amongst other places the America Type Culture Collection to learn about culture presentation and particularly to be trained in the use of the freeze drier. For some reason which neither theynor the backstopping officer in Viennna understand quite why the changes were made. They attempted to call Dr Jong (TA) at ATCC whom they had understood had been involved with the arrangments but were unfortunately unable to make contact with him at his home telephone number.

The CTA and Dr Cole are both disappointed that this training did not go according to schedule. It will mean Dr Cole will have to spend some time teaching the routine use of the freeze drier and related aspects of culture maintenance which it was expected would have been covered during the fellowship studies. In view of the cost of the fellowship every effort must be made to ensure that the training is relevant to the project in hand.

## Modification of Project Objectives

The original objective for the project included not only penicillin V but downstream production of 6APA and semi-synthetic penicillins. Although not formally agreed at a Tripartite Meeting the downstream processing now seems to have been taken out of the project. Not only does this mean that some of the equipment already purchased by UNIDO for the project eg the pH stats is no longer appropriate or useful but it also raises the important question as to the reason for the penicillin V project. Most of the penicillin G and V produced in the world is used for downstream production of semi-synthetic penicillins and cephalosporins. The CTA would like the implications of this change to be discussed at the Tripartite Meeting in December 1992.

## Good Laboratory and Good Manufacturing Practice

Now that the new building is beginning to be occupied, it is possible for the technical adviser to begin writing up standard procedures for the main activities and in all cases this being started for specific procedures in fermentation, recovery and analysis.

Detailed fermentation records are being kept and this is enabling training to be given in the interpretation of the data.

From a general point of view however there is a great need to concentrate on several elementary aspects, including proper cleaning of the laboratories, particularly those used for culture maintenance and on such simple matters of proper labelling of everything including reagents, raw materials and analytical samples through both pilot plant and laboratory.

Local management should prepare standard operating for the safe handling of large volumes of hazardous materials such as concentrated sulphuric acid and also clear instructions as to what to do in cases of spillage. Since there are provisions in the project for 'ad hoc' technical advisers, the CTA suggests one might be considered to give advice on all aspects of the above subject while leaving the existing advisers to concentrate on technical matters of their expertise.

## Analytical Matters

This will of course be covered in detail in Mr Bird's report but the CTA would like to highlight a number of points.

The introduction of HPLC for a more specific method for measuring penicillin V has highlighted what the CTA had predicted, that not all of the material measured by the iodometric assay was penicillin V. While the iodometric assay gives satisfactory results with solid penicillin V and with the more purified process streams, it grossly overestimated the amount of

penicillin in the fermentation broth. For some reason, as yet not understood, the assay indicated up to 6000 units of penicillin in the un-inocculated medium where no penicillin could have been present. The use of more specific penicillinase (B lactamase) to convert the penicillin to penicilloic acid rather than use sodium hydroxide will be investigated in the hope of minimising the problem. Alternatively the hydroxylamine assay may be more useful for this work.

The value or rather necessity of the HPLC has been demonstrated. At the end of the fermentation penicillin V was being overestimated by up to 25% using the iodometric assay. Thus the fermentations were not as good as believed and just as important the recovery was much better than appeared, particularly as it was from rather dilute solutions.

It is very important that the analytical staff understand what the fermentation and recovery groups are trying to do and that they work together as a team. To work efficiently the recovery staff need assay results rapidly in order to make decisions which would improve recovery.

The CTA would also like to stress the new equipment is there to be used and to generate data, constant practice will improve the operator's technique.

It should also be remembered that the HPLC method is not just for penicillin V and p hydroxy penicillin V but can also be used for phenyl acetic acid.

Fermentation

This will be reported on in more detail by Dr. Cole who was returning to Guangzhou fcllowing his attendance at a conference in Beijing.

However, because of following the increase in titre prior to the recovery of penicillin from a fermentation the CTA was able to demonstrate a number of points. It is very important not just to collect data on the fermentation but also to look at the data and analyse what has happened. In order to do this it is desirable to plot out graphically the increase in titre and to record on that graph (and elsewhere) major routine as well as non-routine occurences. The curves obtained enables one to see changes that are not regularly apparent from a column of figures. From a graph one can see not only the change of titre but also the rate of change, and any change in that rate and it is often possible to make at least tentative deductions on cause and effect. It is also of course much easier to see if the assay measurements seem to be consistent. The titre tends to have a consistent trend in one direction - up or down - it does not jump about during a fermentation.

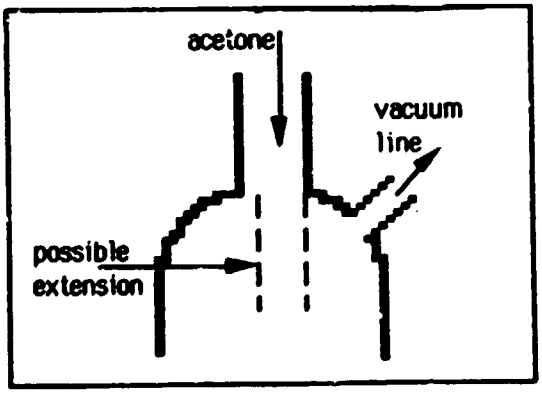
As an example, the graph plotted from the data referred to above, is appended together with indications of where a significant act such as addition of a phenoxyacetic acid side chain was added.

## Penicillin V Recovery

The CTA took part in the recovery of a batch of penicillin V. Unfortunately the batch was atypical due to a misunderstanding on the part of the fermentation staff which may have contributed to the lower than usual titre which does not help recovery. All of the side-chain precusor had been added at one time rather than been fed throughout the fermentation. Nevertheless the CTA was able to prepare an assay schedule and a record sheet for a mass balance during penicillin recovery. With the co-operation of the analytical group and the help of Mr. Bird, results were obtained which enabled the CTA to demonstrate how such data should be used. With the batch in question, it indicated some general areas where penicillin was being lost. The documents were refined and are included in this report. Copies of the modified documents have already been faxed to Guangzhou together with some detailed experimental plans designed to identify more completely where the penicillin is being lost and suggest what can be done to improve the yield.

This particular batch - E65 - gave an overall yield of penicillin V of around 60% even though the broth had a very low titre of only 22,000 units. While it is difficult to measure the large volumes accurately unless they are in suitable calibrated vessels, the data indicated significant losses during filtration - perhaps suggesting some penicillin V was precipitated. One of the experiments was to test the critical nature of pH4.5 which must be measured with an accurate and properly standardised pH meter. There is not one available to the recovery staff and the CTA found that the one used by fermentation could not be set using the buffer available.

Another major loss potential was indicated by the volume of acetone solution after filtration - steps 5-8. It had been assumed that the loss of acetone was due entirely to evaporation under the vacuum used to filter. Loss of activity indicated there might be an additional reason. The CTA examined the receiving vessel (glass lined tank) in detail. It was apparent that acetone running in the top of the vessel could run straight into the vacuum line resulting in loss of acetone containing penicillin V.




**It is essential this entry port for the acetone is modifed to avoid the possibility of acetone being sucked straight over into the vacuum line. A simple extension of the inlet line will achieve this.**

The Guangzhou staff have been asked to complete data sheets of penicillin V recovery runs and fax them to the CTA so that further suggestions can be made from time to time based on the information received.

The drying of the recovered penicillin V needs to be modified. It would be better if it could all be done at once rather than leave half as wet-cake standing overnight. The fact that there were two separate batches also complicates recovery estimates because proper milling and mixing should be carried out before sampling for assay.

The CTA has made arrangements with the Guangzhou staff to arrive a few days prior to the Tripartite Meeting to carry out another recovery run with them.

## Measurement of Success

The Factory Director has introduced the excellent idea of rewarding the staff of the project for success. The CTA fully supports this idea which is already bringing enthusiasm to the project.

The one difficulty however is the measurement of that success. The problem of the assays used for penicillin V before the introduction of HPLC highlights the potential problems.

The use of HPLC for the specific assays of penicillin V indicate that the fermentation staff have not been as successful as thought, though this is no fault of theirs because they had no assays other than the iodometric which has been shown to give high results for fermenation broth, to base their work on. More unfortunately the extraction staff were being judged for failing to recover penicillin V which was not actually in the fermentation but which the iodometric assay seemed to indicate was present. As the penicillin becomes purified the iodometric and HPLC assay move closer together and read the same on pure material.

The CTA and his advisory team would welcome being consulted on the judgment of success of the individual groups.

Finally the CTA would stress the project is also a team effort and it is only by all groups - culture maintenance, fermentation, penicillin recovery and the analytical departments all working together, will the project be really successful.

# MASS BALANCE

## ASSAY SCHEDULE DURING PENICILLIN V RECOVERY BY PRECIPITATION ROUTE

1) FERMENTATION BROTH
   Record total volume of broth.
   Dilute sample 1/10 with water and then filter.
   Assay immediately by Iodometic and HPLC.

2) BROTH ADJUSTED TO pH 4.5
   Record total volume of broth.
   Take sample just before end of filtration.
   Dilute 1/10 with water and filter.
   Assay immediately by Iodometric and HPLC.

3) FIRST (pH 4.5) FILTRATE
   Record total volume of filtrate.
   Dilute sample 1/10 with water.
   Assay immediately by Iodometric and HPLC.

4) MOTHER LIQUOR SECOND (pH 2.5) FILTRATE
   Record total volume of filtrate.
   Take sample for assay 15 mins after start and again near end of filtration.
   Dilute sample 1/5 with water and assay immediately by Iodometric and HPLC.
   A - 15 mins from start B 15 mins from end.

5) ACETONE SOLUTION/SUSPENSION OF pH 2.5 PRECIPITATE
   Record total volume of acetone solution.
   Dilute sample 1/100 with 50% water 50% acetonitrile.
   Assay immediately by HPLC only.

6) ACETONE SOLUTION pH 2.5 PRECIPITATE AFTER FILTRATION STEP
   Record total volume of acetone solution.
   Dilute sample 1/100 with 50% water and 50% acetonitrile.
   Assay immediately by HPLC only.

7) MOTHER LIQUORS AFTER PENICILLIN V PRECIPITATION
   Record total volume.
   Dilute sample 1/10 with water.
   Assay immediately by HPLC only.

8) ACETONE WASH OF PENICILLIN V
   Record total volume of washings.
   Dilute sample 1/5 with water assay immediately by HPLC only.

9) SOLID DRY PENICILLIN V
   Weigh - assay by Iodometric and HPLC.

# MASS BALANCE
# ASSAYS, VOLUMES, AND WEIGHTS DURING PENICILLIN V RECOVERY

| STEP | Volume or Weight | U/ml Iodometric | U/ml HPLC | Kg HPLC | Step Yield HPLC | Overall Yield HPLC |
|---|---|---|---|---|---|---|
| 1) Fermentation Broth | | | | | 100% | 100% |
| 2) Broth adjusted to pH 4.5 | | | | | | |
| 3) First (pH4.5) Filtrate | | | | | | |
| 4a) Mother Liquor pH2.5 Filtrate after 15 mins | | | | | | |
| 4b) Mother Liquor pH 2.5 Filtrate 15 mins from end | | | | | | |
| 5 Acetone solution/ suspension pH2.5 PPT | | X | | | | |
| 6 Acetone solution after filtration | | X | | | | |
| 7 Mother liquor after Pen V Precipitation | | X | | | | |
| 8 Acetone wash of Penicillin V. | | X | | | | |
| 9 Solid/Dry Penicillin V. | | | | | | |

# APPENDIX

## EXPERIMENTS ON WHOLE BROTH TO DETERMINE REASON FOR LOSSES IN EARLY STAGES OF RECOVERY

### EXPERIMENT NO. 1

Take about 2 litres whole broth from $1^{m3}$ fermenter (15-20°C) fresh and well mixed.

Take sample - dilute 1/10 filter and do both iodometric and HPLC assays.

Take 500 mls - adjust to pH 4.5 with 30% $H_2O_4$ (using pH meter) - Hold for 30 minutes (15-20°C). Then take 50 ml sample - dilute 1/10 filter and assay HPLC and Iodometric.

Take 500 mls broth - adjust to pH 4.5 with 30% $H_2SO_4$ (pH meter) - hold 30 minutes (15-20°C) - then filter and wash filter (Buchner) with 50 mls of cold water - measure collected volume of water - Assay 50 ml sample by diluting 1/10 - Correct results for dilution factor.

| RESULTS | | HPLC | IODOMETRIC |
|---|---|---|---|
| HPLC | Iodometric | % Original | % Original |
| | | 100 | 100 |

## FOLLOW UP EXPERIMENT NO.2

### (IF FIRST EXPERIMENT CONFIRMS LOSSES)

**To determine whether exact pH for first precipitation is critical.**

Take 2 litres fresh well mixed broth from $1^{m3}$ at least 110 hours old.

Divide into 6 portions

1. Untreated.
2. Adjusted pH 4.0 (using meter).
3. Adjusted pH 4.25 (using meter).
4. Adjusted pH 4.5 (using meter).
5. Adjusted pH 4.75 (using meter).
6. Adjusted pH 5.0 (using meter).

Filter and dilute sample 1/10 for Iodometric and HPLC assays. There is no need to measure volume of filtrate - we are simply looking at titre to see if any penicillin precipitated or destroyed.

Note sample 1. should be assayed at time 0 - while samples 2 . - 6. should be filtered and assayed at after 30 minutes standing at the experimental pH.

**RESULTS**

| SAMPLE | IODOMETRIC | % | HPLC | % |
|---|---|---|---|---|
| 1. | | 100 | | 100 |
| 2. | | | | |
| 3. | | | | |
| 4. | | | | |
| 5. | | | | |
| 6. | | | | |

## FOLLOW UP EXPERIMENT NO. 3

## To determine loss while holding at pH 2.5 and 15° C.

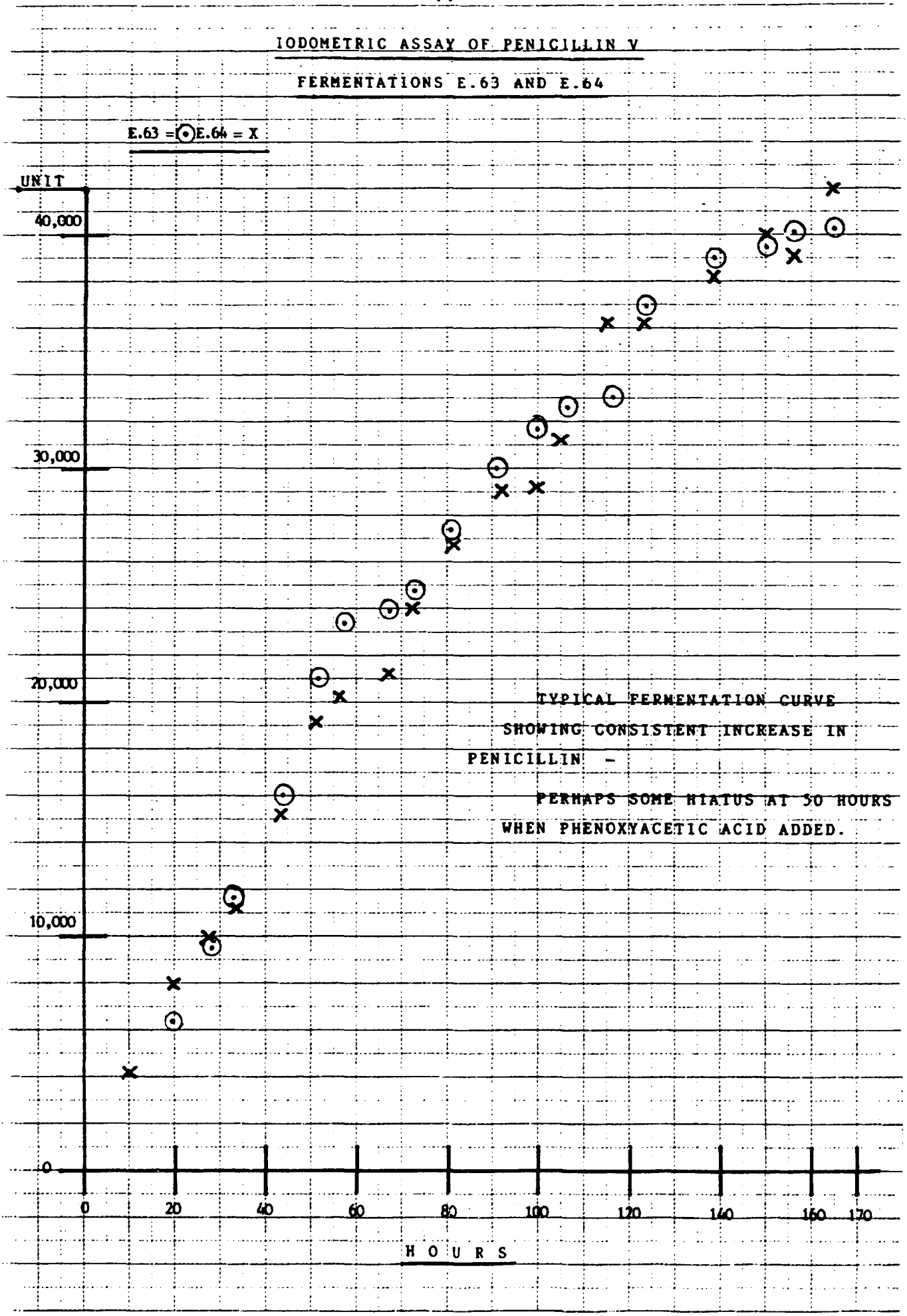
Take 1 litre of pH 4.5 filtrate from pilot-plant process stream - temp (c. 15°C). Divide into two equal portions.

1. 500 mls at pH 4.5 - (15 C). Dilute sample 1/10 assay immediately.

2. 500 mls Acidify to pH 2.5 with 30% $H_2SO_4$ using pH metre - hold at pH 2.5 and 15°C for 2 hours. Adjust pH to pH 6.0 with 2N NaOH with good mixing. Ensure all precipitate is dissolved - make up to 1 litre - dilute sample 1/5 Assay, Iodometric, HPLC.

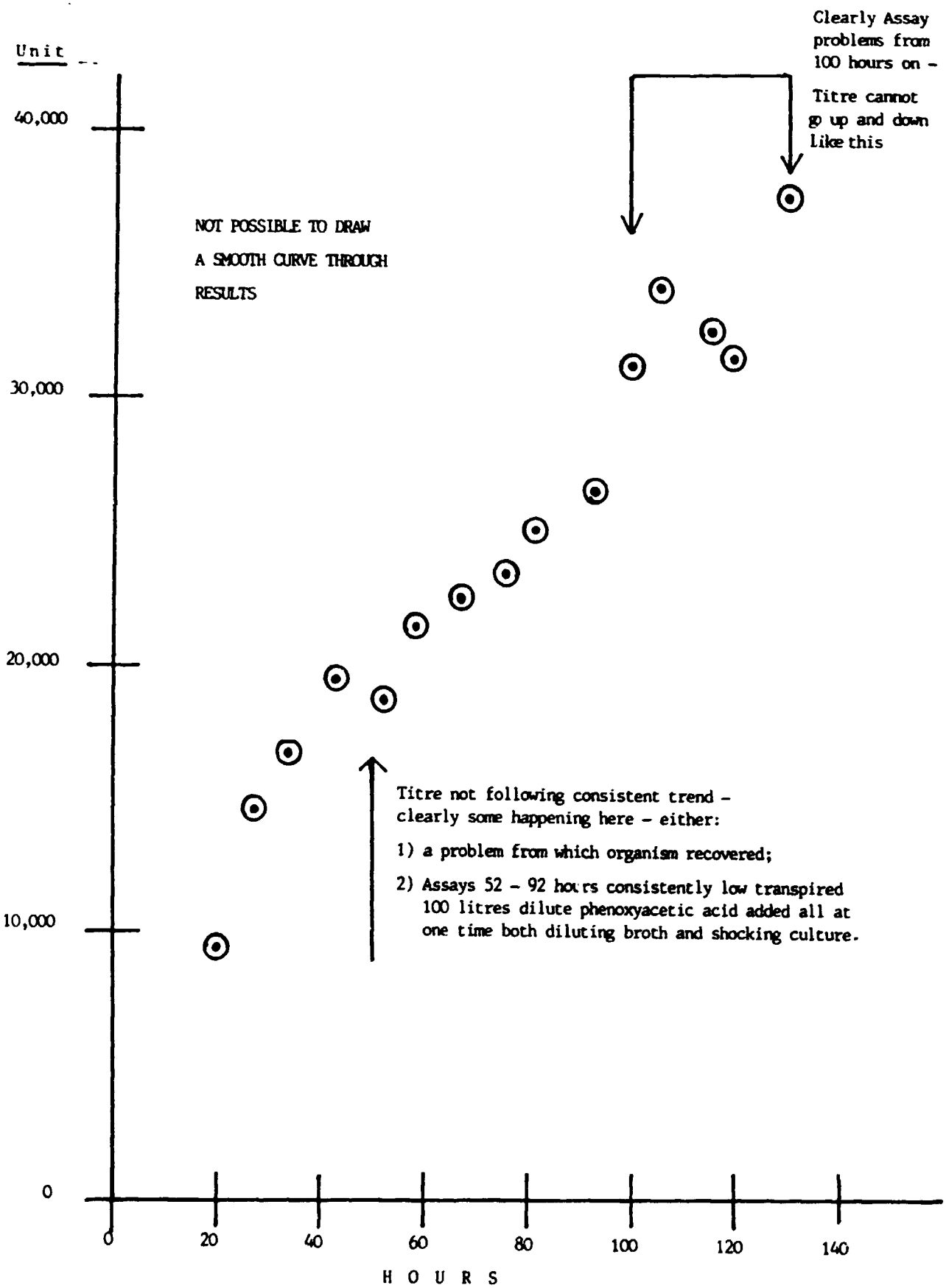
**RESULTS**

| SAMPLE | IODOMETRIC | % | HPLC | % |
|---|---|---|---|---|
| 1. | | 100 | | 100 |
| 2. | | | | |


IODOMETRIC ASSAY OF PENICILLIN V
FERMENTATIONS E.63 AND E.64
E.63 = ⊙ E.64 = X
UNIT
40,000
30,000
20,000
10,000
0
0
20
40
60
80
100
120
140
160
170
HOURS
TYPICAL FERMENTATION CURVE
SHOWING CONSISTENT INCREASE IN
PENICILLIN -
PERHAPS SOME HIATUS AT 50 HOURS
WHEN PHENOXYACETIC ACID ADDED.

# IODOMETRIC ASSAY OF PENICILLIN V

## FERMENTATION E.65.



Backstopping Officer's Technical Comments on the Report of Mr. F.R. Batchelor

The Good Laboratory Practice (GLP) and Good Manufacturing Practice (GMP) have been established to assure product safety and quality. The key message of the new regulations is to validate:

Validate analytical methods; validate sterile, aseptic and non-sterile manufacturing processes; and validate even computer systems used in the pharmaceutical industry.

The simple definition for validation was provided to the industry in 1977 by the U.S. FDA. According to this definition, validation is establishing documented evidence that a system will do what it purports to do.

The most important message of this report is that the introduction of GLP and GMP as well as the performance of process validation have to be conducted prospectively. Prospective process validation involves carrying out the tasks sequentially as the project is being developed.

In the case of a development project, the proper documentation of the design of experiments, the standard operating procedures (SOPs), the batch production records (BPRs) and test records (TRs) should be established at the beginning. Only by having properly kept records can one monitor, analyze and evaluate the program of the project. Records prepared properly are tools that can be used retrospectively for process validation cost effectively, scientifically soundly. Retrospective reviews of records often lead to valuable process development benefits, therefore the importance of record keeping cannot be overemphasized.