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

20284

DP/ID/SER.A/1661
22 July 1993
ORIGINAL: ENGLISH

IMPROVED PRODUCTION OF PENICILLIN

DP/CPR/89/021

THE PEOPLE'S REPUBLIC OF CHINA

Technical report: The last joint mission of the CTA with the instrumental analyst and the fermentation consultant*

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,
A. E. Bird, expert in instrumental analysis and
G. Hanscomb, consultant in fermentation

Backstopping Officer: Z. Csizer, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

* This document has not been edited.

V.93 87688

Table of Contents

Abstract

Most of the items ordered by UNIDO arrived before or during this visit.

Recommendations in the report of the previous visit for installation of air conditioning in the HPLC laboratory, for the use of penicillinase for the iodometric assay and for adequate labelling of analytical samples have not been met. The latter point was complied with during this visit.

The UV detector for the HPLC had been away for repair for at least one month just prior to this visit. It failed again early in the visit. Rapid provision of a detector on loan was arranged.

HPLC assays on five fermentation runs during December 1992 (after the visit of experts Bird and Batchelor) and January 1993 gave titres at harvest in the range 18,000 to 28,500 u/ml. However, some or all of these results are too high because the procedure of diluting samples before filtration for analysis, recommended during expert Bird's visit in October 1992, was not complied with. Few HPLC assays were carried out during this visit because of the problems with the detector.

A file management system was installed in the HPLC computer and staff were trained in use of it for copying and deleting chromatogram and method files.

The fermentation graduate staff were found to be well able to prepare, steriiize and operate the 20 litre fermenters and associated control equipment.

Reasons for poor fermentations of Penicillin V in the 20 litre fermenters were identified and improved operation demonstrated.

Marked differences in the process operated in the 1,000 litre fermenters to that used in the 20 litre fermenters were also determined. The desirability of obtaining a reproducible process in the 20 litre fermenters that is more like the 1,000 litre process, in terms of medium concentration, inoculum level and yield, were stressed.

An inventory of spare parts was made and outstanding items identified.

Desirable new and replacement equipment was also identified.

Problems were encountered with the mechanical seals of both 20 litre vessels and arrangements made for servicing through the B. Braun Agency in Hong Kong.

F. R. Batchelor's Contribution

PROGRESS ON EXTRACTION

Since the December visit only 8 batches have been completed. Batch 7-75 gave a good yield of 68.3% (8.28kg) with a purity of 96% (comparable to the usual purity of crude penicillin) clearly demonstrating the process could work well. Two subsequent batches 7-77 and 7-78 were of good purity but yields were lower 41 and 49% respectively. These yields may in fact be understated considerably because of difficulty in measuring the broth volume (see below) and may be 56 & 64%.

A new numbering system started in February 1993 and only 6 batches FO1 - FO6 have been extracted. Unfortunately the fault in the HPLC equipment recorded in Mr Birds report has meant that only iodometric assays could be used. Since we know the iodometric assay gives falsely high readings on the fermentation broth it has not been possible to calculate the exact yields. The weights of Penicillin V isolated from the runs were 6.08, 6.58, 9.4, 7.1 and 7.56kg respectively and would suggest the recoveries were all in the range of 50 - 65%.

The data had been properly recorded and prepared for me to examine. I would have liked the data to have been forwarded by fax beforehand to enable it to be studied, a commentary on the data need not necessarily be restricted to the individual visit. Furthermore we would have been aware of some of the problems which have occurred with both HPLC and fermentation equipment and would have been able to initiate some corrective action in advance of the visits making them the more useful.

Comments and observations following examination of the data and discussion with Mr Zeng.

1. Deposition of precipitate in the line during filtration.

Mr Zeng reported that he found varying amounts of the pH 2.5 precipitate deposited in the transfer pipe between the tank and the filter press. The problem is that the line is horizontal and of large diameter so that precipitate is deposited when the flow rate through the filter is low. This is likely to occur towards the end of the filtration and particularly when the precipitate is more dense when the yield of penicillin V in the fermentation is higher. Examination of the recovery data show a variable loss at this stage but it was not possible from the records to know whether the higher losses were in fact linked with the presence of precipitate in the line. Corrective action has been taken and modification will be made to the system either to prevent the settling out or to enable it to be recovered and added back to the process stream.

2. Measurement of Volume of Broth.

Mr Zeng reported that in some cases the volume from the fermenter after transferring to the holding tank was sometimes recorded to be as high as 900 litres compared with the more usual 700 (lowest 680). He believed that was due to the presence of a thick layer of foam which was included in the volume and which made it very difficult to estimate the true volume. Unfortunately the accurate measurement of this volume is essential to determine the starting (100%) level of penicillin V for the recovery steps and it must be recorded just as accurately as the concentration of the penicillin V by HPLC. The simplest way would be to break the foam with an antifoam agent such as octanol (There is a problem here of needing an antifoam which does not affect mould growth. Octanol should be readily available for convenience it could be diluted with a little acetone or ethanol if desired. The minimum quantity to break the foam is all that should be used).

If octanol or a suitable substitute (not the antifoam oil used in the fermentation as this will block the filter cloths) then it might be possible to measure the true height of the liquid either by construction a float from a small closed glass vessel on a string the glass bottle with fall freely through the foam but float on the surface of the liquid broth. If the foam is very thick and stable even octanol may not break it making calculation of a materials balance difficult.

Accurate measurement of the liquid volume could show the real recovery of penicillin V to be perhaps 20% or more higher than recorded at present if the observations reported are correct.

3. Dissolution of the crude penicillin V (pH 2.5ppt).

The records showed a substantial variation in the amount of acetone used to dissolve the crude penicillin V precipitated at pH 2.5. The amount varied from 60 - 80 litres. It was said to be based on experience but had no relation to the original titre of the penicillin. Such a 30% variation would mean that the water content of the slurry could sometimes be very high and the ratio of acetone and ethanol during the potassium acetate precipitation of the penicillin V would vary considerably. Both these, and particularly the percentage water, would be expected to affect the solubility of potassium penicillin V and thus the yield.

It is suggested that for the time being and unless there is evidences for a change then 80 litres should always be used.

The data also showed that a substantial amount of penicillin V was left undissolved in the pH 2.5 precipitate after the acetone extraction. For two batches, .75 and .78 where both assay and weights were available the amount

of penicillin V left unextracted was 637gms and 760gms respectively ie over 5% of the total penicillin V. It is suggested more care is taken in mixing the solid with the acetone, it should be gradually slurried with small quantities of the acetone and finally given a good mix with a mechanical stirrer rather than all the acetone being added at once and the slurry being hand stirred. It is also desirable to reserve say 5 litres of the acetone for an additional slurry and wash of the precipitates. These recommendations will be tried on the next run.

4. Potassium Acetate Precipitation.

At present a saturated solution of potassium acetate in ethanol is used and the amount to he added judged by past experience. The exact concentration of the acetate should be known and a 2% excess added after having calculated the amount of penicillin V free acid present from the assay. The number of milligrams of penicillin V free acid is the number of units divided by 1685 (ie 1520 x 388/350). The molecular weight of penicillin V (acid) is 350 penicillin V potassium salt is 388 and potassium acetate is 98. Therefor the total theoretical amount of potassium acetate required is:

$$\frac{\text{kg Pen V acid}}{350} \times 98 = \text{Potassium acetate}$$

The whole calculation is thus:

$$\frac{\text{units penicillin}}{1685} \times \frac{1}{1000} \times \frac{98}{350} \times \frac{103}{100} = \text{potassium acetate}$$

Pen V acid (overage) (gms)

The amounts being used at present were only just adequate and with any improvements in recovery would have become limiting.

5. Drying of Wet Cake.

The vacuum drier has still not been repaired and the only drying equipment available are the wooden cabinets heated by light bulbs, not only are these inadequate and slow, only half the present batch size can be dried at any one time. At the beginning of this visit they had been moved to another building and for two days had no electrical supply. The result was that this batch of penicillin V was left almost 48 hours in the open room with high humidity. It was quite obvious from a visual examination that the very humid atmosphere was affecting it and it was clearly moist and overnight became visibly more yellow denoting some degradation. I cannot re-emphasise too strongly that water/moisture is the biggest problem in the stability of penicillin powder.

If the drying cannot be improved at least an extra acetone wash should be used to dry the solid as much as possible. The same problems applied to the batch extracted while I was present. Particularly dry penicillin was left open on the bench for more than 24 hours. Over the period the humidity was very high more than 90% so water was being absorbed by the penicillin. If the penicillin is properly washed with (ethanol/acetone and then acetone). It may be desirable to use an ethanol/acetone wash before acetone alone in case the amount of potassium acetate left in the mother liquor is not soluble in pure acetone. Residual potassium acetate would increase the impurity level and give a more hygroscopic and less stable product. The acetone it should dry quickly, a few hours in a vacuum oven is preferable though even simple heating should suffice providing there is an air flow provided.

Once dried the product should be bagged immediately. The penicillin should preferably be dried to a moisture content with the EP.USP standard but at least it should be dried to constant weight.

EXTRACTION OF FERMENTATION RUN WHILE CTA PRESENT

22.5.93

Foam Stability

The first obvious point was confirmation of the amount and stability of the foam. 960 litres was reduced to 800 by the addition of 500 ml of octanol. Whether more octanol would have done better could not be tested because no more octanol was available. For the next run however it is hoped to have additional octanol. It will then be possible to set up a test using 6 x 1 litre measuring cylinders adding 0, 0.5, 1.0, 2.0, 5.0, and 10.0 octanol and observing the foam reductions. An additional experiment will be to centrifuge a sample to determine the various volumes.

This problem of measuring the actual volume in the fermenter makes it very difficult to calculate the overall process yield from whole broth the variation in foam stability will make it difficult to obtain reliable data. It is therefore suggested that for the time being the progress of fermentation be measured by the assay of the filtrate and that the efficiency of extraction be measured form the filtered broth recognising that there will be some variable less during filtration.

Recovery of Precipitated Potassium Penicillin V

Acetone evaporates very rapidly and cools the cake. It is therefore wrong to continue to suck air through the cake for a long time. This will only evaporate acetone and allow water from the very humid air to condense on the cold cake. Rather than assist drying it will make the cake wetter and explains why the potassium penicillin V being produced dries slowly into hard plaster like lumps. As soon as all the acetone (in liquid form) is sucked off the

material should be placed on trays in the drying ovens. The acetone will then dry off readily without water condensing. The operation will be quicker giving less degradation, a purer product and a good crystalline form.

Use of Plate and Frame Filters

1) Steel Filter for Removal of Mycelium ie pH 4.5 Filtration

From the data from previous runs and by observation of a current run it was obvious that insufficient wash water has been used to displace the broth in the system and then to wash to mycelium to get the maximum recovery of penicillin. While the pipe-work is of too large a diameter for the volumes in use, it is accepted this cannot be easily corrected. It is possible however to reduce the hold-up volume a little if the number of plates used in the filter are reduced to the minimum required to take the solids from the fermentation. This can only be done by observation and experiment. It is however easy to wash properly, this should be done by allowing the liquid in the holding tank to just empty and then chase with wash water say 50 litres at a time and repeating this till the effluent water from the filter is about one tenth of the colour of the original effluent. This will certainly be more water than is currently being used even with less than half the plates 135 litres was required at a guess perhaps 200 litres may be required on a full scale batch but this should be checked. There was a misconception that washing more slowly would require less water. This is not so, it is not the efficiency of the washing but the need to replace the water in the system including the mycelial waste which is the problem. If one were washing too fast the effluent liquid would become like clear water, not stay dark.

The total filtrate and water wash must be properly mixed before a sample is taken. It was observed that samples were taken and sent for analysis before all the wash was in the tank thus giving a falsely high titre for the total volume which included all the wash water.

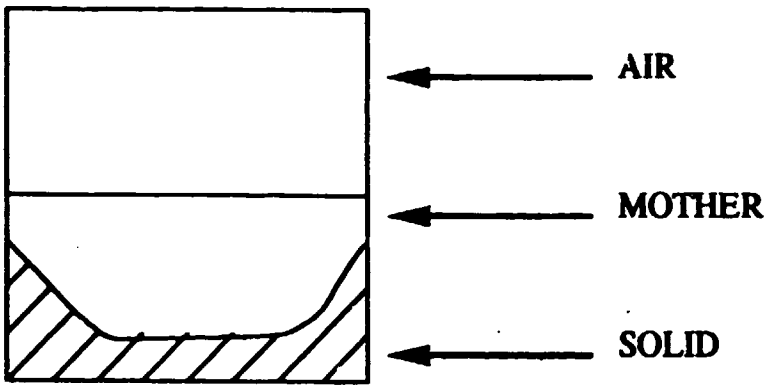
Plastic Plate and Frame Filter Used For Collection of pH 2.5 Precipitate

Not only had the recorded data indicated a potential loss at this stage but observation of the filtration showed that turbid liquor clearly still containing some penicillin V was being sent to waste. Some taps showed higher quantities of solid than others. Furthermore the drip pan under the filter had between .5 and 1 Kg of solid in it which was going to be thrown away. For some inexplicable reason the operator believed the material was not crude penicillin but inert material. Further examination of the filter showed penicillin leaking round the sides of the plates, the top of one section blocked and numerous imperfections in the filter cloth which would allow the passage of precipitate. A further problem is that the filter cloth has shrunk so that the necessary holes for liquid flow do not match with the holes on the filter plates and frames and

allow solid particles to pass because there is quite a small margin of error in fitting the filter cloths.

In view of the problem it has been recommended that a quantity of celite (filter aid) be added to the pH 2.5 solution. 1 - 2 Kg for the fermenter batch should be sufficient, probably just 1 Kg. The effluent liquid should then be recycled back to the holding tank until all of the taps are producing clear liquid. Whether all the frames are necessary can only be determined by experience. To start with all should be used some could then be removed for the next batch if it is clear there is excess space.

The use of the filter aid will also make it easier to extract the precipitate with acetone. The manner of use of the filter was also the cause of washing difficulties. Rather than use full flow all the time and the full air pressure to dry the cake pressure was being switched on and off. This caused the filtrate to fall off the cloth to the bottom, inside the plate then looked like this:



Further pressure then went through the lie of least resistance did not push out the water nor did the water wash the filter cake. The whole operation was thus inefficient and unnecessarily time consuming. The staff have now been shown how to operate the filter to better effect and in the pilot run extracting the penicillin from a seed tank worked very well giving efficient filtration and washing in less time.

Disolution of Precipitated Pen V (free acid) in Acetone

The use of the filter aid will undoubtedly improve the recovery at this stage. The sticky pen V precipitate was simply being hand stirred in acetone and then filtered. The data showed clearly that the residual solid was often 50% penicillin V and after weighed 1Kg ie contained 500 gms penicillin V. This was partly due to the difficulty of dissolution and partly due to the residual cake being saturated with highly concentrated penicillin V in acetone which was difficult to wash efficiently.

After filtration therefore the moist cake of undissolved material including the filter aid should be resuspended in a volume of fresh acetone this filtered off and the cake then washed through with further acetone. The amounts can only be determined by experiment on a full scale batch but I would suggest the origin acetone suspension be 80 litres the resuspension in a further 20 litres and the final wash of the cake a further 5 - 10 litres. After the final acetone wash has been sucked into the holding tank then the acetone should be well mixed before a sample is taken to calculate the penicillin V concentration and the amount of the potassium acetate to be added. It was noted on this visit that a sample was taken before the finals wash was mixed in to give the total thus overestimating the penicillin V present. MATERIALS MUST BE PROPERLY MIXED BEFORE SAMPLING.

Precipitation of Potassium Penicillin V with Potassium Acetate

The above changes will undoubtedly lead to a drier cake and there will be less water in the acetone solution of penicillin V to which the ethanolic potassium acetate will be added. A small amount of water is needed to facilitate the reaction between penicillin V and potassium acetate. If it is found there is a problem in precipitation of the penicillin V ie it does not begin to crystallise as it usually does then a small amount of water (50 - 100 mls) should be added. On no account should additional water be added if crystallisation presents no problems.

FROM: Dr F R Batchelor, Consultant. Fax no: 293 863006

ATTENTION: MR KALERT, Service Dept. Braun Biotech, Gmbh

CC Unido
Re: Unido order 15.1.0768 M

Dear Mr Kalert

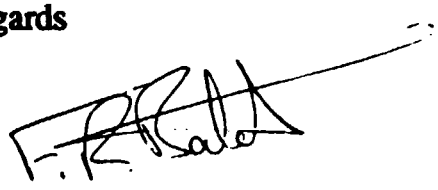
I attach an extract of Mr Hanscomb's report on his visit to Guangzhou. Mr Hanscomb has worked for Beecham for over 30 years during which time he has used several makes of fermenter including Braun and is currently assisting me with the Guangzhou Project

My main point of concern is that your engineer suggested the problem arose because of the medium being used ie it was the customers fault. These fermenters were supplied by you knowing that they were specifically for penicillin fermentations involving solid materials in the medium. I had a number of telephone calls myself with people at Braun to ensure this was known. Despite this the inoculum equipment supplied was suitable only for bacteria.

I would be grateful if you would ascertain the present situation from your people in Hong Kong. I specifically wanted robust equipment knowing the difficulties involved in China and it is very disappointing to find your equipment not living up to its promise. We need these two fermenters operating correctly before Mr Hanscomb makes his next visit.

The equipment had very little use before the problem appeared and I would expect the equipment to be repaired under warranty and some guarantee that the seals will have a normal life.

Regards



Dr F R Batchelor

Chief Technical Adviser, UNIDO

The Spinney, Mynthurst, Leigh, Reigate, Surrey RH2 8RJ

Extract of G. Hanscomb's Report

Preliminary installation difficulties had been previously identified and resolved by a B.Braun engineer. Apart from the DCU display on V1, programming of both systems for sterilisation and operation, by either the DCU or computer, together with logging of fermentation data and data retrieval was fully functional.

Full and detailed instruction manuals were supplied with the equipment and these were supplemented with two further applications manuals, copies of which I had obtained from B.Braun (Biotec) U.K..

During the visit, however, serious problems did develop with regard to the mechanical seals when the upper seal of each vessel appeared to fail for no obvious reason. Service arrangements were eventually made through the B.Braun Agency in Hong Kong. A Chinese engineer from Shanghai, together with a local representative in Guangzhou, Mr. Wun, arrived to investigate the damage to one vessel. I did not consider them to be well prepared since they had no engineering drawings of the seal arrangement, they did not have an adequate tool kit and appeared to have little experience with B.Braun fermenters. These comments were passed to Ms. E. Zheung at the Hong Kong Agency.

After removing the seal no damage could be observed, by either the engineers or myself, and it was considered suit able for replacement. When this was completed and tested it still leaked.

During my final week an engineer, Mr. A. Hung, from the B.Braun Agency in Hong Kong arrived with Mr. Wun. They did have a drawing of the seal and an adequate tool kit. The failed seal was again removed and inspected. This time there was obvious signs of wear to the carbon face of the seal. The seal was taken away for repair in Hong Kong but this was likely to take about two weeks.

It was decided not to attempt repairs to the seal of the other vessel until the first one was completed satisfactorily.

The failure of the seals is a cause for concern since they are expensive to replace and require well trained engineers to fit them. The reasons for failure were not established. It is intended to raise the question of design and robustness with the manufacturers in Germany and to have repairs effected under the warranty agreement.

The fault on the DCU was identified as a faulty electrical connection and corrected.

A. E. Bird's Contribution

INTRODUCTION

This is a report on the fourth visit by the Technical Advisor for instrumental analysis to the Guangzhou Pharmaceutical Factory. The objectives of the visit were to review the analytical work carried out for the Pen V project since the Advisor's third visit in December 1992, to install a file handling programme in the HPLC computer and train GPF staff to use it, to investigate the suitability of an iodometric assay based on the use of potassium iodate and to familiarise GPF staff with some more advanced aspects of HPLC analysis. These objectives were achieved.

In preparation for the visit I spent two days learning about two computer file handling programmes, chose the more suitable one for GPF, obtained a copy on floppy disc and wrote simplified instructions for its use.

My trip lasted 12 days, from Saturday 1st May to Wednesday 12th May, 9 of them in Guangzhou.

I. HPLC

A. General issues

Most of the items ordered by UNIDO after my visit in December 1992 had been delivered before my arrival in Guangzhou, but the most important item, the sample clarification filters for HPLC of fermentation broth samples, was in customs awaiting documentation for duty free import. It was due to be collected from the airport on the day I left, Tuesday 11th May. Fortunately some of the filters that I had sent by post after the December visit were still unused, so it was possible to carry out broth assays.

I spent some time installing the pH meter and magnetic stirrer and demonstrating their use.

The recommendation in the report of my December visit to install a temperature control unit and window blinds in the HPLC laboratory has not been acted on. A small dehumidifier has been installed, but this is of no value during the working day because the windows and door have to be kept open to try to maintain an acceptable temperature. I spoke twice to Mr Liu Guangtao (Director of the Research Institute) about the need for temperature control and my concern that the HPLC will not work satisfactorily in the very high summer temperatures. He assured me that he has told the factory management about the need for a temperature control unit.

The UV detector had broken down and been returned to Waters for repair under warranty during March. It had been returned to GPF only a day or two before my arrival. On the 4th of May the detector failed again, with different symptoms from those of the March failure. The internal diagnostics indicated that the problem might be due to a dirty flow cell but application of the recommended cleaning procedure did not cure the fault. No-one was available in the Waters Guangzhou office who could deal with the problem so I contacted Waters in Hong Kong with a request for urgent assistance. They arranged for an engineer from the Guangzhou office to call on the evening of 5th May. He stayed until midnight but could not repair the detector. He arranged loan of another detector during the repair period, which will probably be about a month due to the need to obtain parts from the US. The loaned detector was collected on 6th May. It was a different model and came without an instruction manual. Although the GPF operator (Miss Cheng Meiling) who collected it had been given basic instruction in how to operate it, considerable time was needed to adjust settings on the detector and in the HPLC computer to obtain satisfactory chromatograms. This had to be done partly by trial and error because of the absence of an instruction manual.

No flow of mobile phase through the column was obtained when the system was first turned on after I arrived. The operator indicated that this had happened when the guard column insert had been changed. I found that the plastic foam protecting pad, which should be removed when the insert is installed, had been left in place. Compression of the foam

produced an impermeable barrier. The instructions that come with the inserts clearly state that the foam should be removed. I wrote this instruction on the lid of the box in the hope that it will be noticed and remembered in future.

The HPLC system is sometimes used by Quality Control Department staff with a different column from that used for the Pen V work. This involves disconnecting and re-connecting columns. Miss Cheng asked if it is possible to connect both columns permanently. I examined the pump and injector instruction manuals and found that this is not possible with the equipment currently available. Insertion of a high pressure switching valve between the outlet from the injector and the inlet to the columns would be needed. I do not recommend UNIDO purchase of this because the requirement arises from use of the system which is not related to the Pen V project and which is infrequent.

B. File management

Two files (a data file and a back up copy) are stored on the system hard disc for every chromatogram run. The file management arrangements provided in the system are inadequate, especially for copying files to a floppy disc and reloading them from a floppy to the hard disc. I provided a file management programme (PC tools) to enable these operations to be carried out efficiently, and some blank floppy discs. Installation of PC tools was carried out satisfactorily and I trained Miss Cheng, Miss Nie Qin (the translator) and Miss Huang Yan Fei from the QC laboratory to use the system. A copy of the instructions that I provided is given as Annex 1. By the end of my visit all old chromatogram files had been copied to a floppy and deleted from the system disc and a separate floppy had been made containing copies of the method files. This is required so that method details can be reloaded if a fault occurs on the system hard disc. Notes that I provided to Miss Cheng on regular copying and deletion of files and updating the methods floppy are given in Annex 2.

C. Pen V assays

The following results had been obtained since my visit in December.

Run No.	Date	Pen V (u/ml)	
		HPLC	I_2 (NaOH)
7-75,140hrs	Dec.	25229	31761
6-77,140hrs	Dec.	25100	35705
7-78,140hrs	Jan.	17903	28054
6-79,116hrs	Jan.	25648	31556
6-79,140hrs	Jan.	28515	34106
6-01,92hrs	Feb.	18124	24397

HPLC results, which are not listed here, were also obtained on samples during extraction of the first three runs and on several shaker flask samples. Four of the latter were as high as 29000 u/ml. However, all these figures, both HPLC and I_2, are probably too high

by 5 - 8 %, that is about 1000 - 2000 u/ml for the HPLC and 1200 - 3000 u/ml for the I_2 results. This is because the instruction to dilute broth samples before filtration for assay, given during my visit in October 1992 and complied with during my December visit, was not followed after the end of that visit. [The reason for the instruction and the origin of the 5 - 8% figure are given in the report on my October visit.] No record was kept of when the analysts reverted to filtering samples before dilution but they think it was soon after the end of my December visit. No sensible reason was given for the change, which was initiated by the staff carrying out the I_2 assay and copied by Miss Cheng for HPLC so that her results could be compared with the I_2 ones. When I discussed this with Mr Liu he was not aware that the change had been made and said he had told the analysts to follow my instructions. He spoke to them about it and subsequently I found that for I_2 assay samples were being filtered both before and after dilution, thus doubling the work load. I told Mr Liu that this is not necessary but, although he accepted that dilution before filtration is the correct procedure, he wanted results using filtration first for comparison with the large amount of data already obtained in this way. HPLC assays will be done using only dilution before filtration.

Few HPLC assays were carried out during my visit because of the problem with the detector. Run 7 - 06 gave results of 16700 and 14500 u /ml after 120 and 164 hrs respectively. The reason for the decrease at the later time is not known. Assay were also done on samples from the various extraction stages of this fermentation.

D. Training

I gave Miss Cheng detailed explanations of the use of internal standard methods and of how to set up the HPLC system so that calculation of the final result is done by the computer rather than by hand. I emphasised the disadvantages of both these procedures as well as their advantages.

Miss Cheng had already used an internal standard method (for cefoperazone, from the Chinese Pharmacopoeia), but did not understand the background to it.

Use of the computer to calculate final results is fairly complex to set up, will save little or no time with the number of samples being run at GPF and leads to the danger that the analyst will accept uncritically the result being provided by the machine. Consequently, while demonstrating how to set up the procedure, I advised against its use.

The effect of changes in the integration parameters and the choice of parameters to use in particular chromatographic situations is one of the most difficult aspects of use of the Waters Baseline 810. Several opportunities arose for me to demonstrate how to make such changes to achieve satisfactory integration. In this context I emphasised the importance of the analyst looking critically at the output from the machine and making changes if it is not acceptable. A copy of notes on this and various other aspects that have been given to Miss Cheng is provided in Annex 3. Most of these points have been made verbally during previous visits but I felt that they needed to be re-emphasised in writing.

By the end of this visit I was confident that Miss Cheng is competent to continue operating the HPLC without further guidance, although she may need to consult other people (eg. Mr Qiu in the QC laboratory who went on the Waters training course, or Waters staff in Guangzhou) if unusual problems occur.

II. IODOMETRIC ASSAY

Non compliance with the recommendation to dilute broth samples before filtration for assay has been described above in section I C. I also found that the recommendation in the report on my December visit to use penicillinase instead of NaOH for hydrolysis of broth and extraction samples was not being complied with. Some assays had been done with both penicillinase and NaOH during December but all subsequent assays were with NaOH. Results with penicillinase were 2000 to 5000 u/ml less than those with NaOH, in line with the difference seen during my December visit and confirming interference by non-penicillin components of the fermentation medium when NaOH is used.

More than half the penicillinase that I had supplied in December was still available (enough for at least 100 assays) and the analysts gave no coherent reason why they had stopped using it. Mr Liu was aware that they had stopped and approved of this. He said that it does not matter whether penicillinase or NaOH is used because both give a result higher than HPLC. I repeated the explanation given in December that penicillinase gives a reliable assay for total penicillins whereas NaOH gives total penicillins plus a variable amount of non-penicillin components, but I was unable to convince him that there is any point in using penicillinase. He also said that using penicillinase takes longer (which is not correct) and was concerned that an adequate supply would not be available. I had told him in December that I would supply more and had taken some with me on this visit.

A fairly recently published alternative version of the iodometric assay uses NaOH hydrolysis and reaction of the penicilloic acid with potassium iodate in acid to generate iodine which is then titrated. I thought it possible that this might not suffer interference from non-penicillin broth components and arranged some tests with the method. However, application to Pen V reference standard gave extremely variable results under 3 different sets of conditions, so I did not pursue work on the method any further.

III. GOOD LABORATORY PRACTICE

No record had been kept of the date of breakdown of the HPLC detector or of the date when the analysts reverted to filtration of broth samples before dilution. I explained that all faults etc. and changes to standard methods should be noted, see Annex 3.

The need for adequate labelling of samples submitted for analysis has been emphasised in the reports on all my visits. At the end of my December visit I wrote notes on this which I gave to Mr Liu and, with his agreement, to the senior staff in the extraction and

fermentation areas. I hoped, naively, that this would have the desired effect.However, the first fermentation sample I saw on this visit had no label at all and the second one had a single number which was not either the fermenter number or the run number! The issue was raised, by Mr Hanscomb with Mr Luo (in charge of fermentation) and subsequent samples were correctly labelled. In the circumstances it is difficult to have any confidence that correct labelling will continue when the Technical Advisors are not on site.

IV. MAJOR FINDINGS

Recommendations made during previous visits about the sample preparation procedure for HPLC and iodometric assay and on the use of penicillinase for the iodometric assay were not being complied with.

HPLC assay results were available for only four fermentation runs since the previous visit in December 1992. Other runs could not be assayed by HPLC because of failure of the detector. Three of the four runs had high apparent titres of Pen V at harvest, above 25000u/ml, but the validity of the assays is uncertain.

All training on HPLC needed from the TA has now been carried out and an adequate supply of accessories is available for the analytical work needed during completion of the project.

RECOMMENDATIONS

GPF management need to install a temperature control unit and window blinds in the HPLC laboratory before the very hot summer weather arrives.

Basic aspects of Good Laboratory Practice such as adequate labelling of samples and record keeping for faults and changes to procedures need to be complied with at all times, not only when Technical Advisors are on site.

Closer supervision of analytical staff is required to ensure that procedures are not changed without authorisation.

The recommendations in Annex 2 for file management of the HPLC system should be complied with.

Fermentation titres and isolation yields are to be based on HPLC assay in which broth samples are diluted before filtration.

ANNEX 1

USE OF PCTOOLS WITH BASELINE 810

WARNINGS

1.Use PC tools **ONLY** to copy or delete chromatogram files (.DAT or . BAK) and method files (.MTH) in the Max Data1, Max Data2 or Max Data3 directories. **DO NOT** delete or make any changes to files in other directories, especially those in Max Program, or to files in drive D. These files are used to operate the system and it will not work if they are deleted or changed.

2. Always remove the floppy disc from the computer as soon as you have finished with it. If you leave the disc in the computer a problem will occur next time the computer is turned on. If this happens you must turn the computer off, remove the floppy disc and turn on again.

To format a floppy disc

All new floppy discs **MUST** be formatted before they can be used. Use PC tools to do this as follows. [NOTE. It is possible to format a disc in MSDOS without using PC tools. I do not recommend this because there is a possibility of formatting disc C (the computer hard disc) by mistake. **YOU MUST NEVER TELL THE COMPUTER TO FORMAT DISC C.** If you do, all the operating programs will be deleted, the system will not work and it will be difficult and expensive to get it going again].

Use double sided high density 1.2 or 1.4 MByte 5.25 inch discs.

Enter pctools as described below.

Insert a blank disc.

Click on **Disc** at top of screen.

Click on **Format data disc**. A message appears, Select drive to format. Click on **A**.

A box appears with options. Click on **1.2M, 80 tracks, double sided. Then** click on **Format**.

The progress of formatting is displayed on screen. When it is complete a box appears asking for a new volume label. Leave this blank.

Click on **Exit**.

The disc is now formatted and ready for use.

To enter PCTOOLS

At the **C:\>** prompt or at the **C:\max\program>** prompt (use Alt F10 to exit from the Baseline main menu if necessary)

Type **cd \pctools** and press enter

This gives the **C: \pctools>** prompt

Type **pcshell**, enter

The pctools screen appears with menus across the top, actions (copy, move etc.) across the bottom, directories and sub- directories down the left hand side and the files in the current directory down the right hand side. The current directory can be changed by positioning the cursor with the mouse over the wanted directory name and

clicking. To use pctools to copy and delete chromatogram files select the Max data 1 directory in this way. The file names of all the chromatograms and methods in Max data 1 are listed on the right hand side of the screen. If there is more than one screen full, the up and down arrows on the right hand side of the list can be used to scroll through the screens. Place the cursor between the arrows and click to move through the list.

To copy files from the hard disc (drive C) to a floppy disc (drive A)

Insert a formatted floppy disc in drive A.
Enter pctools and select the Max data 1 directory as described above.
Select each file that you want to copy by positioning the cursor on it and clicking or select a group of files as described below.
Click on **copy** at the bottom left of the screen.
A message appears asking for the target drive. Click on **A**.
The system now copies all files selected in the previous step to the disc in drive A.
If you want to copy a group of files with a common name or extension, eg. all penVF files or all .BAK files, use the following method to select them. This is much quicker for a large number of files than clicking on each one.
Click on **Options** at the top of the screen
Click on **File select filter**
A box appears asking for the file names and extension.
Type in the common part of the file names and the extension, and put a ? or * in the spaces for the non common part. Eg. To select all PenVF files from PenVF 1 to PenVF 100 type **PenVF ???** or **PenVF*** in the name box and **.DAT** in the extension box. To select all files with a .BAK extension type **???????** or * in the name box and **.BAK** in the extension box.
Click on **Select** or press enter.
To copy all of these files use the copy sequence given above.

Notes

1. The .DAT and .BAK files under any one file name in Max data 1 are identical. Copy only the .DAT files; do not copy both .DAT and .BAK.
2. When the floppy is full a message appears on screen ' Not enough free space...'. When this happens click on **Exit**, remove the floppy and insert a new formatted floppy to continue copying files.
3. To check that files have been copied insert the floppy (if it is not already there), click on **Drv A** (at the top left of screen) and the list of files on the floppy appears on the right hand side of the screen.
4. Label the floppy with a brief description of the files that have been copied on to it, eg. PenV chromatograms.

To delete files from the hard disc (drive C)

Enter pctools and the Max data 1 directory as described above.
Select the files that you want to delete, as described above when selecting files to copy.
Click on **delete** at the bottom left of the screen.

A message headed 'File delete' appears. Click on **Delete** or on **Delete all** (if more than one file has been selected).
The system now deletes all files that were selected in the previous step. If you make a mistake and want to recover a deleted file, click on **Undelete** at the bottom right of the screen and follow the instructions that appear. This will only work if the computer has not been turned off after the deletion operation was carried out.

Notes

1. Take great care when deleting files to make certain that you have copied to a floppy any files that you need to keep permanently.
2. Delete both .DAT and .BAK files.
3. Do not delete .MTH files unless you are certain that the method they describe will not be required again.
4. You can delete files from a floppy in the same way; insert the floppy, click on **DrvA** at the top left of screen to display the list of files on the floppy and select and delete as above.

To copy a file from a floppy (drive A) to the hard disc (drive C)

If you need to work with a chromatogram that you have copied to a floppy and deleted from the hard disc load it back in to Max data 1 as follows.
Insert the floppy.
Enter pctools as described above.
Click on **DrvA** at top left of screen.
Select the file you want to load.
Click on **Copy** at bottom left of screen.
A message appears headed Select target drive. Click on C.
The list of directories in drive C appears on the left hand side with a message, Select where files are to be copied. Click on **Max data 1** in the list of directories.
The file is copied to the hard disc and can now be worked with as usual in Baseline.

To load PC tools on a computer where it is not already installed

Insert the floppy disc containing the PC tools program in to drive A.
At the C:\> prompt type **md C:\pctools** and press enter.
At the C:\> prompt type **copy A:\pctools*.* C:\pctools*.*** and press enter.
The PC tools program loads on to the hard disc in drive C. The file names are shown on screen as they are copied.

ANNEX 2

RECOMMENDATIONS FOR WATERS 486 FILE MANAGEMENT

1. Once a week when the system is in use (or more often if a large number of samples are run) copy the .DAT files to a floppy disc. Then delete the .DAT and .BAK files from the hard disc. Label the floppy and retain it as the permanent record of the chromatograms.

2. When a new method is entered in to the system, or when an existing method is changed, copy the new or changed .MTH file to the Methods floppy. DO NOT delete .MTH files from the hard disc. Keep the Methods floppy with the Baseline system discs so that methods can be re-entered in the computer if a hard disc failure(or any other problem) occurs that removes the programmes from the computer.

3. DO NOT delete files from any directory other than Max Data 1, Max Data 2 or Max Data 3.

4. Always remove the floppy disc from the computer as soon as you have finished with it. If it is left in, a problem will occur next time the computer is turned on.

ANNEX 3

HPLC WORK MISCELLANEOUS NOTES

1. Make a note in the HPLC log book of any faults that occur or changes that are made. Note the date, the nature of the fault or change and the action taken. For example, note when the guard column insert is replaced, when a new column is installed, when any breakdown occurs. The log book must provide a full record of the history of the system.

2. Note any changes from the standard analytical procedure in the note book where the results of the analysis are recorded. For example, if the standard procedure is to dilute before filtration but some samples are analysed by filtering before dilution, this change must be noted.

3. Look critically at chromatograms to make sure the system has integrated them correctly. This is particularly important if you want to measure a peak which is not completely separated from an adjacent peak. Make sure that the integrator has given a figure just for the peak you want and that it has not combined the area of the two peaks. If necessary re-integrate the chromatogram, either by using the re-process procedure described in the operating procedure I wrote for Baseline 810 (October 1992) or by adjusting the parameters in the Peak Integration screen. Refer to the Waters instruction manual for details on the effect of the various parameters.

4. Read and follow the manufacturer's instructions when installing a new column or other replacement item.

5. Remove the foam pad before installing a replacement guard column insert.

6. I do not recommend use of the facility in Baseline 810 for calculation of the final result. It takes longer to enter the data needed to start a set of assays and would not save any time with the number of samples being run on the system. Also it increases the danger that the analyst may uncritically accept the result that the system provides.

Analytical Matters *

Mr Bird visited me in the UK following his return from Guangzhou and shortly before I left for the current visit. He was thus able to brief me in detail and even provide me with a copy of his report.

I agree entirely with the various comments in his report particularly on the matter of labelling in fact further examples of inadequate, and inaccurate labelling have been observed by both Hanscomb and myself. I cannot emphasise too strongly the need to follow correct labelling procedures as an essential step in Good Laboratory Practice. There is also a need to re-emphasise the requirement to record any events during the course of a fermentation, extraction or analysis. This is still not done for interruptions in air supply, agitation or other changes to the fermenters, shaker flasks or extraction procedure. it is of no use having good quality analytical data without the additional backup information. This information is essential before any conclusions can be drawn from the data and any courses of action recommended.

Since Mr Bird's visit the clarification filters have been delivered and the supply of mobile phase filters located. Progress has also been made with the installation of the air conditioning unit for the HPLC room. It is now in situ but not yet working.

Overall I have been very pleased with the job Mr Bird has done and also with effort put in by the Guangzhou analytical staff. They are now very competent in the use of the HPLC and have a good understanding of its use particularly of the computer software package. Both Miss Cheng and Miss Nie are to congratulated. Miss Nie's command of English has been especially valuable to the project especially when Cai Shi Chao has not been available.

* Comments of F.R. Batchelor

G. Hanscomb's Contribution

INTRODUCTION

This report covers the first visit of the Consultant in Fermentation Antibiotics to the Research Institute of the Guangzhou Pharmaceutical Factory. The work centred around the two B. Braun 20litre fermenters and their application to the Penicillin V(Pen V) fermentation.

In preparation for this visit discussions were held with Dr. M. Cole, Expert in Industrial Microbiology, who provided useful background information to the project.

A one day visit was made to the premises of B. Braun (Biotec) U.K.,at Aylesbury, Buckinghamshire, U.K. to discuss the specification and gain familiarisation with the control system as installed at Guangzhou.

A half-day visit was made to the School of Biological Sciences, The University of Surrey, Guildford, Surrey, U.K. to see a practical demonstration and have further discussion on the control system.

I arrived in Guangzhou on Monday 3 May and departed on Friday 28 May.

I. ASSESSMENT OF THE FERMENTATION RESEARCH LABORATORY

The fermentation laboratory, containing the two B. Braun fermenters, I considered to be of good size for the purpose. It was supplied with the necessary services of electricity, steam, compressed air and cooling water. One limitation was the air supply pressure which was not high enough for operation of some control valves. This was compensated for by using nitrogen gas supplied from a cylinder. As long as the nitrogen supply is monitored then no problems occur.

During the visit the electrical power supply failed briefly on regular occasions (about every other day) for 5-10 minutes each time. This appears to be a general problem associated with the supply, but is not helpful for continuous operations and is likely to result in equipment failure and lost fermentations.

Also, on occasions, the compressed air pressure fell to around 1bar, for 1-2 hours, causing reduced air flow through the fermenters.

The assessment was made during the month of May when ambient temperatures reached 30+ degrees C and the humidity level was around 90%. Under these conditions the atmosphere in the laboratory was neither ideal for working in nor for the use of electronic controls and computers. An adequate air-conditioning system would greatly improve this aspect.

The laboratory was regularly cleaned on what seemed a daily basis. This is good practise and should be maintained.

The fermenters are operated by two graduate members of staff. Mr. Zhu had received 2 weeks training at the B. Braun factory in Germany with further on-site instruction ,of one week, by the visiting B. Braun engineer during installation. Mr. Luo had been trained by Mr. Zhu and also the visiting engineer.

Both graduates had become well used to operating the fermenters together with the control systems. Most guidance was needed in problems associated with the Pen V process.

The laboratory was supported by three assistants.

There was a general lack of safety appreciation in comparison to current U.K. standards. This was pointed out to staff whenever appropriate and some useful items of equipment are listed later.

Highly trained and skilled maintenance staff did not appear to be employed on site. Maintenance and replacement of items such as agitator seals and bearings will have to be arranged through B.Braun, or similar, agencies as will most electrical problems. However routine maintenance will be handled by the operating staff.

II REVIEW AND COMMISSIONING OF FERMENTERS

A total of fifteen fermentation cycles had been carried out prior to my arrival and much experience had therefore been gained in most operational aspects.

The digital control unit (DCU) of one vessel (V1) was still giving an intermittent fault whereby the display was not illuminated. This was reported by Dr. Cole in his last report dated November 1992.

One foam detector probe was not working due to a broken insulator.

One dissolved oxygen probe required repair.

These last two items were repaired using spare parts on site.

On arrival one vessel was in operation while the other was idle.

Preliminary installation difficulties had been previously identified and resolved by a B.Braun engineer. Apart from the DCU display on V1, programming of both systems for sterilisation and operation, by either the DCU or computer, together with logging of fermentation data and data retrieval was fully functional.

Full and detailed instruction manuals were supplied with the equipment and these were supplemented with two further applications manuals, copies of which I had obtained from B.Braun (Biotec) U.K.

During the visit, however, serious problems did develop with regard to the mechanical seals when the upper seal of each vessel appeared to fail for no obvious reason. Service arrangements were eventually made through the B.Braun Agency in Hong Kong. A Chinese engineer from Shanghai, together with a local representative in Guangzhou, Mr. Wun, arrived to investigate the damage to one vessel. I did not consider them to be well prepared since they had no engineering drawings of the seal arrangement, they did not have an adequate tool kit and appeared to have little experience with B.Braun fermenters. These comments were passed to Ms. E. Zheung at the Hong Kong Agency.

After removing the seal no damage could be observed, by either the engineers or myself, and it was considered suit able for replacement. When this was completed and tested it still leaked.

During my final week an engineer, Mr. A. Hung, from the B.Braun Agency in Hong Kong arrived with Mr. Wun. They did have a drawing of the seal and an adequate tool kit. The failed seal was again removed and inspected. This time there was obvious signs of wear to the carbon face of the seal. The seal was taken away for repair in Hong Kong but this was likely to take about two weeks.

It was decided not to attempt repairs to the seal of the other vessel until the first one was completed satisfactorily.

The failure of the seals is a cause for concern since they are expensive to replace and require well trained engineers to fit them. The reasons for failure were not established. It is intended to raise the question of design and robustness with the manufacturers in Germany and to have repairs effected under the warranty agreement.

The fault on the DCU was identified as a faulty electrical connection and corrected.

111 SPARE PARTS AND OTHER ITEMS REQUIRED

A full list of the spare parts available on site, that had been ordered originally, was drawn up. This is given in Annex 2.

Outstanding items required include:-

2 pH probes
2 Double mechanical seals
2 Agitator drive belts
1 Dissolved oxygen probe
O-ring seals - various - these require identification and organising into suitable storage boxes
2 Sets of metric size Allen keys

Silicone grease for O-rings

Other auxiliary items required include :-

Air conditioning equipment - see above
2 Bench pH meters (includes one for Microbiology lab.)
2 Variable speed peristaltic pumps, suitable for external control (for additions to fermenters)
1 Water bath (general purpose)
1 Sonication bath (general purpose)
1 Digital thermometer (general purpose)
2 pipetting systems e.g. Pipette-boy (general purpose)
5 each Plastic wash-bottles labelled for water and ethanol - 250ml
2 Magnetic stirrers
Improved securing system for silicone tubing to additions manifold for fermenters
1 Gas cylinder trolley (safety)
Securing chains for gas cylinders (safety)
Goggles (safety)
Aprons - light weight (safety)
Rubber gloves in all sizes (safety)

It was recommended that instruction leaflets be obtained for operation and maintenance of dissolved oxygen and pH probes. These are available free of charge from the Agents (Semtech Instruments Co. in Hong Kong) or the manufacturers (Ingold in Switzerland).

Much of the equipment in the Microbiological Support Laboratory was old, in poor state of repair and needed replacing. In particular :-

Autoclave - this failed often and a larger one is required
Bench centrifuge
-80 deg.C Freezer - not working
4 Fridge-freezers were in use and full up . A cold room would be helpful.
2 Incubators
2 Balances

Two new liquid nitrogen storage vessels had been delivered but were not in use. The canes for holding the ampoules were outstanding and only 5 of the 12 cane holders were present. There was also no separate storage Dewar flask for holding spare liquid nitrogen. To operate this system regular, weekly supplies of liquid nitrogen must be ensured

It was apparent that money for revenue items was not readily available and that low running and maintenance costs should be considered in all items purchased.

Following heavy rain at one weekend the Microbiology Laboratory was flooded with water entering via the outside fire escape and under the door. A suitable barrier is required to prevent this recurring.

IV DETAILED WORK PROGRAMME FOR OPERATING FERMENTERS

A detailed programme for daily, weekly and 3-6 monthly operation of the fermenters was drawn up and a copy provided for the graduate fermentation staff. This is provided in Annex 1.

V PRACTICAL TRAINING AND THE PENICILLIN V FERMENTATION

Practical training was on-going throughout the visit and was generally with reference to the Penicillin V ((Pen. V) fermentation.

A. Penicillin V fermentation

On arrival at the Research Institute one 20litre vessel was being run on a Pen.V fermentation. This, in line with previous experience in the 20litre fermenters, was producing a very low titre of Pen.V, while the morphology of the mycelial pellets was considered abnormal. On examination of the fermentation running conditions I determined that the fermentations in these vessels were being inadequately stirred, therefore limiting the oxygen supply. I explained methods of calculating scale-up/scale-down agitation rates based on maintaining uniform power supply per unit volume of the fermenter.

In practise this required the agitator rate to be increased from 288 r.p.m. to nearly 600 r.p.m. in order to be equivalent to the 1000litre fermenters used in the Pilot Plant. Even at the lower speed being used difficulties had been encountered in controlling foaming. This was likely to be an even greater problem with higher stirring.

I recommended that in the next fermentation in 20litre fermenters the following changes be implemented to try to achieve adequate oxygen supply and to minimise the effects of foaming :-

a) stir at 550 r.p.m. and monitor dissolved oxygen levels

b) reduce the working volume from 20litre to 17.5litre to provide an increase in the head space above the liquid level, also this would allow a proportional reduction in the air flow rate

c) raise the antifoam detector probe by 2.5cm to try to reduce excessive antifoam addition

d) lower the top impeller proportionately to stay within the liquid volume and not create excessive splashing

One fermentation was completed using these recommendations. A Pen.V titre of 13,900 u/ml was attained as measured by High Performance Liquid Chromatography (HPLC). The same sample assayed by the iodometric method gave 20,400 u/ml. During the course of the fermentation foaming had been controlled until the very latter stages, although it was necessary to provide a scheduled feed of oil. The oxygen supply had not become limiting and in fact the dissolved oxygen level had not fallen below 30% of saturation. I noticed that the initial growth rate had appeared to be slow, as indicated by the rate of fall in dissolved oxygen level at the start and the initial accretion rate of Pen.V, compared with that normally achieved in the 1000litre fermentations.

A comparison of fermentation running conditions between the two scales of operation eventually revealed marked differences in the way they are operated. These differences relate to inoculum volumes and inoculum preparation methods as well as to the calculation of medium component concentrations in the final fermentations.

The method of operation at the 1000litre scale would not easily scale down to the 20litre scale unless one of the two vessels were dedicated as an inoculum growth vessel. I do not consider this to be the best use for one vessel. It is necessary to investigate in the 20litre fermenters the use of an increased inoculum volume from that being used of 1% up to a 10% level (a more usual amount for fungal fermentations). To provide this quantity from shaken flasks will require some modification to the shaker table to accept a larger number of 1litre flasks. This was requested to be progressed at the factory.

The use of inoculum taken from the larger (500litre) fermenter would require some suitable transfer equipment to be made up. This may be useful for comparative purposes at a later date.

An increase in medium concentration of around 40% may be necessary to be closer to the situation in the 1000litre fermenter. This will increase biomass production and oxygen demand, therefore requiring a higher agitation rate.

An on-going programme of investigation is therefore required to obtain reproducible results in the 20litre fermenters which are equivalent to those obtainable at the 1000litre scale using the peanut meal/soybean meal medium presently in use. In the longer term the peanut and soybean meals may possibly be replaced by an alternative complex nitrogen source such as corn steep liquor (C.S.L.).

A preliminary experiment was set up in shaken flasks to study replacement of peanut and soybean meals by C.S.L. using a range of concentrations. Results indicated that although titres of Pen.V, as estimated by the iodometric method, reached 20,000 u/ml, the results using HPLC only reached 5,200 u/ml. This suggested the presence of a higher concentration of other natural penicillins produced from other precursors in the C.S.L. However, a full investigation was not possible at the time.

Previous results of Pen.V titres in 1000litre fermentations are generally unreliable due to :-

a) lack of HPLC results

b) failure to dilute samples prior to filtration

c) the presence of air (foam) in the samples making volume measurements unreliable

The use of a foam collapsing agent, such as octanol , was suggested to overcome the sample volume measurement. It was found that in whole broth samples taken at harvest from a 1000litre fermentation that around 20-25% of the volume was air. With allowance made for the air and correct assay procedures followed, for HPLC assay, the result of 32,000 u/ml Pen.V was obtained for fermentation 7-F-08 in the 1000litre fermenter. Since the amount of entrained air is likely to be variable for different samples a set procedure will be required for consistent results.

The problems of foaming during sterilisation of the 20litre fermenters had been overcome by adding 5 - 10ml of polyglycerol ester (PGE) antifoaming agent to the fermenter when the temperature reached 95deg.C (just before rapid boiling). It was found that 5% v/v PGE dispersed well in groundnut oil and it was recommended to use this in the oil feed during the fermentation to provide improved foam control.

Photocopies of three published articles relating to investigations into foaming in fermentations were presented to the fermentation staff for general interest (Annex 3).

ANNEX 1

A. Detailed work programme for B. Braun fermenters

Daily

1. Observe correct operation of set points and recorded parameter data
2. Be aware of symptoms indicating faulty operation e.g. :-
   faulty / noisy agitator
   leakage of gases or liquids from vessel or pipework, leakage from addition systems
   supply services are correct e.g. :- supply pressures, dryness of air
   unusual smells e.g. :- electrical burning or contamination
   signs of foaming in vessel
3. Check adequate supply of additions
4. Record observations of all events both expected and unexpected with time.
5. Take samples for measurements and assays

Weekly or per Fermentation Run

1. Prepare vessels and accessories according to B.Braun operating instructions
2. Calibrate electrodes
3. Check presence of O-rings, rubber diaphragms, all fittings/plugs and inlet filter are present
4. Batch medium and sterilise according to operating manual
5. Operate process according to process instructions. Note. medium sheets, process instruction sheets and running/event sheets should accompany each vessel
6. At harvest, or termination of each run, heat treat where possible to kill off the culture
7. Empty contents and clean as in operating manual
8. Clean and inspect all O-ring seals, where the seal is broken, for splitting of rubber or flattening (deformation)
9. Inspect inlet air filter and replace as required
10. Inspect, clean and treat as necessary electrodes as described in Ingold instructions

3 - 6 Monthly

1. Check all readily accessible O-ring seals and replace as necessary e.g. plugs not removed each run, harvest and sample valves
2. Check operation of all service valves and maintain as appropriate
3. Check diaphragms of control valves and replace as necessary

Notes

a) Maintain minimum stocks of all replaceable spare parts - it is useful to have a card index for this
b) In particular identify and store in suitable storage containers all O-ring seals and other small parts
c) Spare parts required include; mechanical seals, pH electrodes and drive belts
d) Bearings and mechanical seals should only be replaced by B.Braun engineers

ANNEX 2

B.Braun Spare Parts as at 13 May 1993

2 Automatic control valves for heat exchanger
1 Automatic control valve for steam supply
3 Ceramic insulators for antifoam probe
10 Inlet air filters (Pall)
6 x 250 ml pH 7.0 buffer solution
7 x 250 ml pH electrolyte
3 LED for push button switches
1 pkt. PTFE hose
15 Dissolved oxygen electrode repair sets
4 Bottles electrolyte for dissolved oxygen electrodes
1 Pressure gauge for vessel
4 Pneumatic actuators
9 x 250 ml pH 9.0 buffer solution
7 x 250 ml pH 4.0 buffer solution
2 Inspection lamp bulbs
2 Inoculation needles
10 Diaphragm valve membranes - small
2 Pressure controllers - miniature - for airflow
1 Spring for wash/wiper
2 Ball valves - red handle
4 Lock washers
4 Ball bearing races
4 Pump heads for addition systems
2 Spare addition systems with steam seals fitted
15 Diaphragms for diaphragm valves
3 Automatic valves for steam
3 Steam traps
1 Dissolved oxygen electrode cable
1 Foam probe cable
5 Fuses for speed controller
2 PTFE seals for wiper
2 Viewing window glasses (1 for lamp)
1 Pressure gauge - small
2 Cylinder filters
2 Support springs
10 Tops for addition vessels with seals
1 Heating element
10 Tubing clamps for pumps
1 Ball valve - green - 3-way
1 other valve
4 O-ring for agitator flange

2? O-ring for head plate
O-rings - many assorted which require identifying
Quick connectors for exhaust cooler
1 Spare addition manifold
2 Thiocarbamate solution for pH electrode cleaning
3 Pepsin solution for pH electrode cleaning
1 Circulation pump - Grundfos
Rubber diaphragms - many - for addition ports
Silicone rubber tubing for additions systems
Glass fibre for packed filters
2 Synchronous drive motors for addition pumps

In addition electrodes for pH and dissolved oxygen with pressure housing and amplifier were available for the 1000litre fermenter.

ANNEX 3

Publications presented to fermentation staff

1. Micro MFCS Applications Handbook - Version 1.0
2. Improvements - function extension - Biostat with DCU system

   These were obtained from B.Braun (Biotec) U.K. and should provide further flexibility in control aspects in the future

3. "An investigation on the efficiency of antifoaming agents in aerobic fermentation" C.L. Duitschaever, C. Buteau and B.S. Kamel. Process Biochemistry, December 1988, 23 (6) 163 - 165
4. "Efficiency of natural oils as antifoaming agents in bioprocess." F. Vardar-Sukar, J.Chem.Tech. Biotechnol.1988 43 39-47
5. "Foam behaviour in biological media. - 111 Penicillium chrysogenum cultivation foam" B. Konig, K. Kalischewski and K. Shugerl. European J. Appl. Microbiol. Biotechnol. 7, 251 - 258 (1979)

   These photocopies were provided for general interest but with particular relevance to the current process

6. Catalogue of Whatman Labsales Ltd. 1992. St. Leonards Road, Maidstone. Kent. UK.

Fermentation*

1. Mini-Fermenters

Prior to Mr Hanscomb's visit to Guangzhou I was able to brief him in detail. His one month visit was deliberately arranged so that it overlapped with that of Mr Bird and the CTA so the maximum benefit could be obtained. His work is report in detail separately but there are two points I should like to make. Firstly it was very gratifying to find how much had been learned on the visit to Braun clearly both their training and the effort put in by those Guangzhou staff attending had all been worthwhile and this meant Mr Hanscomb was able to concentrate on experimental matters rather than on how to operate the vessels.

Secondly I am concerned about the performance of the Braun fermenters. The mechanical seals should not be giving trouble at this early stage. A letter has been written to Braun drawing their attention to the problems, a copy of the letter is attached to this report.

2. Fermentation (Pilot Plant)

There is no doubt that penicillin V titres above the agreed target are being obtained. It only remains to obtain these more regularly. Some of the problems are outside the control of the local operating staff. The main reasons for the poor yielding fermentation are power cuts and reduced air pressure caused when other demands take priority. Both these can rapidly cause irreversible damage to a fermentation if the vessel is without proper aeration for any length of time. All such events should be recorded so it can be seen how much variability there is in performance when these two factors have not been limiting.

* Comments of F.R. Batchelor

Backstopping Officer's Technical Comments

Mr. G. Hanscomb described a series of scale-down experiments, that is just the reverse of what a research and development programme would suppose to perform. A development project, if properly planned, has the following main phases:

1. Developing of the product prototype;

2. Developing of the laboratory scale processes:
   - developing of the manufacturing processes,
   - optimizing of the processing parameters,
   - demonstrating of the manufacturing consistency;

3. Scale-up at pilot scale:
   - scale-up of the laboratory scale processes,
   - optimizing of the processing parameters,
   - demonstrating of the manufacturing consistency;

4. Scale-up at industrial/commercial scale:
   - scale-up of the pilot scale processes,
   - optimizing of the processing parameters,
   - demonstrating of the manufacturing consistency;

5. Commencing of routine production.

The Guangzhou Pharmaceutical Factory and Research Institute, contrary to the above, has a well functioning industrial scale operations and an obsolete, but still functioning, pilot plant. The Research Institute, however, was without an adequate fermentation research laboratory. Through the subject project DP/CPR/89/021 such a fermentation research laboratory has been established.

The experiences gained through the project have sometimes been discouraging. As in many developing countries, the first problems, that the UNIDO experts/consultants faced, were the lack of proper infrastructure and essential supplies. Failures in the electric power supply, low compressed air pressure, high, 30+ degrees C ambient temperature with 90 % relative humidity that creates failures in the electronic components of the instrumentation are the most typical problems. A temperature control unit and window blinds would also be needed in the HPLC laboratory before the very hot summer weather arrives.

Safety measures are also not kept at the same rigour as in the industrialized countries. Out of the 3 main principles of the safety measures in the biological industry, even the personal safety measures are not kept (the other two are: product safety and safety for the environment).

A major problem with the equipment was also experienced. The Braun fermenters of 20 litres had failures of the mechanical seals and bearings. The failure of the seals is a cause for concern since they are expensive to replace and require well trained engineers/technicians to fit them. The

reasons for failure could not be established. It is intended to raise the questions of design and robustness with the manufacturer in Germany. The unfortunate problem with the reliability of the fermenters also illustrates that the tenders are not the best way to purchase equipment of this level of sophistication.

The scale-down experiments could be characterized the usual difficulties. To optimize the main processing parameters such as energy supply (media composition, heating/cooling), oxygen supply (agitation, dissolved oxygen), size and age of inoculum, foam control, etc., a series of complex problems should be solved. Mr. Hanscomb showed not only that he masters the know how of the commissioning the fermenters, but he also demonstrated, in very elegant model experiments, how to diagnose and solve the problems related to the actual technological issues of Penicillin V fermentation.

Mr. A. E. Bird's fourth mission experienced the usual frustrations. The reason of this was that most of his technical recommendations have not been met. There were certain problems with the HPLC as well (the UV detector had broken down). Since penicillinase gives a reliable assay of total penicillins whereas NaOH gives total penicillins plus a variable amount of non-penicillin component, using penicillinase provide a very practical alternative in case the HPLC would be out of order.

One of the major findings, namely the lack of good record keeping, is not only shows that the requirements of the Good Laboratory Practices (GLP) are not met, but makes difficult to review the results achieved. Furthermore, the lack of reliable record keeping makes any retrospective validation. impossible.

Record keeping should cover without indicating the order of importance at least the following:

- adequate labelling of samples,
- reporting on faults,
- reporting on changes in procedures,
- all experiments with successes and failures,
- quality and specifications of materials used,
- any unexpected phenomenon,
- schedule of equipment utilization,
- maintenance of equipment,
- inventory of spare parts and consumables,
- sources of supplies, etc.

The proper record keeping can ensure the performance of standardized techniques with consistency, therefore the importance of this recommendation cannot be overemphasized, it is actually a must.

Dr. F. R. Batchelor's seventh technical report as adequate as usual. This occasion he did not give his specific recommendations separately, but they are clearly expressed in the bulk of the report. He gave comments on the missions of Messrs. Bird and Hanscomb, with his comments we fully agree. His very high commitment to the project shows his attached copy to Braun Biotech, GmbH, Germany.

His chapters on the Progress on Extraction and the Extraction of Fermentation Run while CTA Present are clear, very concise and well written,

showing of his lifetime experience. What is more important, however, to see from his report, that most of the process improvement suggestions do not need major investment, many times they do not need investment at all. What is needed the know how of industry, the industrial experience that is never the same, the problems to be addressed are never identical, one cannot think and operate in cliches. What is suggested/ recommended should be in line with the characteristics of the specific industrial (sub)sector, the experience of many years, knowing the pros and cons of technologies previously used, as well as the concrete problems of the micro-environment of the given factory, the macroeconomics of the country, since only by knowing all of these, one can give reasonable, practical and optimal recommendations that are pragmatic at the same time, in other words they are implementable.

The reason of this more theoretical approach of our comments is that the Guangzhou Pharmaceutical Factory and Research Institute, in Guangzhou faces a lot of problems that are beyond the scope of the subject project. These are related to the industrial policies and to the technologies. What UNIDO could demonstrate, through the implementation of this project, was its technical competence combined with highest ethical standards and an excellent team work. With this all main objectives of the project could be achieved within the framework of the available resources.