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A comparison of microbatch and vapour diffusion for initial screening of crystallization conditions

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Abstract

Six commercially available proteins were screened using the "sparse matrix" solutions of Jancarik and Kim (with modifications by Hampton Research Inc.). The screens were performed using the most common vapour diffusion method and three variants of the microbatch crystallization method, including a novel evaporation technique. Out of 58 crystallization conditions identified, 43 (74%) were identified by microbatch, while 41 (71%) were identified by vapour diffusion. 26 conditions were found by both methods, and 17 (29%) would have been missed if microbatch had not been used at all. The evaporation technique provided the best microbatch method finding a total of 34 conditions.

1. Introduction

The microbatch method [1] has become well established for the crystallization of biological macromolecules. Small droplets (around 2 μl) of protein and precipitant are dispensed under oil, using a fine multichannel dispensing tip. The method is used for screening [2] where a large experimental space is searched for crystals, and for optimisation [3] where large, well formed crystals are produced for X-ray analysis.

Screens based on the sparse matrix approach introduced by Jancarik and Kim [4] are widely used by workers in the field. However, at least 73% of screening in Europe is done using the vapour diffusion method alone, although microbatch has the advantage of using less protein (0.5 to 1 μl instead of about 4 μl per trial) and of being less labour intensive.

We wished to establish whether microbatch is as effective as vapour diffusion in identifying new crystallization conditions. It is often assumed that because vapour diffusion allows the concentration of the droplet to change slowly, a larger area of the experimental space is searched. Therefore this study also investigated a variation of the microbatch method introduced by D'Arcy [5], where crystallization conditions might be scanned in a similar way to vapour diffusion. In this case the droplets were dispensed under a mixture of silicone oil and paraffin liquid which allowed slow evaporation of water.

Other studies e.g. Ref. [6] have compared vapour diffusion and microbatch crystallization. However, they have not been performed on a systematic basis since their primary aims have been to obtain crystals as quickly as possible rather than to compare the crystallization methods.

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2. Materials and methods

Six proteins readily available from Sigma Chemical were selected to be screened. 48 screening solutions were chosen from Hampton Research's Crystal Screen I. The two solutions not used were the two least successful solutions in a survey conducted by Hampton Research [7].

Four different methods of protein crystallization were compared:

(i) Vapour diffusion hanging drop method;
(ii) Microbatch with the same protein concentration as in (i);
(iii) Microbatch with the same protein concentration as in (i) but allowing evaporation;
(iv) Microbatch with high protein concentration.

The protein screening conditions are shown in Table 1. The protein solution was first filtered with 0.45 μm Millipore filters. All four methods were performed using the same initial protein solution, and within 24 h.

The hanging drop vapour diffusion method used siliconized cover slides and Linbro tissue culture plates, both from Hampton research. 750 μl of screening solution was added to each reservoir well. Glass microcapillary pipettes from Sigma Chemical were used to transfer 4 μl of protein and 4 μl of screening samples to the cover slides. The cover slides were sealed to the reservoir wells using silicone grease.

The three microbatch screens were dispensed into three Nunc HLA plates under a thin layer of n-decane, using the Douglas Instruments IMPAX 1-5 machine. Plates (ii) and (iv) were then topped up with 5 ml of paraffin oil, while a 50% mixture of silicone oil and paraffin oil was used for plate (iii).

The plates were stored at 4°C, and were observed every few days. The numbers and sizes of crystals were recorded.

Salt and PEG crystals were distinguished from protein crystals as follows.

(i) The shapes of lysozyme and thaumatin crystals were compared to the well-known shapes of crystals of these two proteins. This allowed positive identification of the majority of crystals of these proteins.

Table 1
The concentrations of protein stock solutions and dispensing conditions according to method

Protein	Concentration (mg/ml)			
	(i) Vapour diffusion	(ii) Microbatch low concentration	(iii) Microbatch low concentration with evaporation	(ii) Microbatch high concentration
Concanavalin A from Canavalia ensiformis, type VI	20	20	20	30
Bovine haemoglobin	15	15	15	20
Lysozyme from chicken egg white	25	25	25	40
Myoglobin from horse skeletal muscle	15	15	15	20
Thaumatin from Thaumatococcus danielli	30	30	30	50
Trypsin from porcine pancreas +2% w/v benzamidine inhibitor	40	40	40	60
Dispensing parameters				
Volume	4 μl + 4 μl	1 μl + 1 μl	1 μl + 1 μl	1 μl + 1 μl
Reservoir	750 μl			
Oil		paraffin	50% silicone + 50% paraffin	paraffin
Storage temperature	4°	4°	4°	4°
Operator time	2 h	< 10 min	< 10 min	< 10 min
Machine time	–	48 min	48 min	48 min

Table 2
The number of crystallization conditions found by the four methods

Method	Number of crystallisation conditions found	Number of conditions unique to method
Microbatch with low protein concentration (ii)	26	3
Microbatch with low protein concentration and evaporation (iii)	34	2
Microbatch with high protein concentration (iv)	27	3
Total microbatch: all three methods together	43	17
Vapour diffusion	41	15

(ii) Haemoglobin crystals were identified by their red or brown colour.
(iii) The remaining crystals were tested by two methods. Firstly, they were prodded with a sharp metal probe. The protein crystals broke very easily, whereas a distinct "crunch" could be heard and felt with salt crystals. Secondly, a few nl of a mixture of concentrated toluidine blue and azure blue dye was added to the droplet. This was absorbed by protein crystals but not by salt crystals.

All of the crystals were examined by two people independently using these methods. Several salt crystals were identified. Two or three very small crystal forms which could not be positively identified were excluded from the results.

3. Results and discussion

A total of 133 wells produced protein crystals over a period of 10 weeks. These observations are summarized in Table 2 and are shown more completely in the Venn diagram in Fig. 1. Where a condition occurs in more than one microbatch method it is counted once only.

Using all three microbatch variants together proved slightly more successful than the single vapour diffusion method. This might be seen as an unfair comparison, as three microbatch screens are being compared to one vapour diffusion screen, but the total use of protein and operator time is still less to perform the three microbatch screens.

Of the individual microbatch methods, (iii) is the

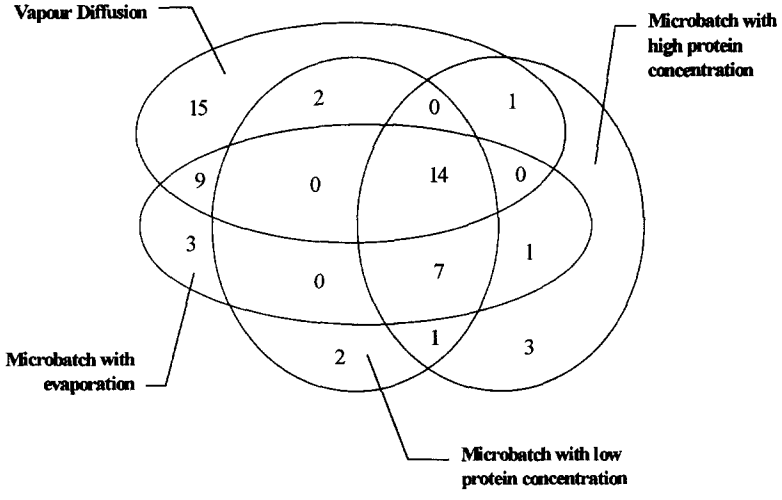

Fig. 1. Venn diagram showing the number of crystallization conditions found by the four methods in combination.

most successful, using low protein concentration and silicone oil/paraffin mixture to allow evaporation. However each microbatch method found between 10 and 12 conditions not found by vapour diffusion.

The total number of wells containing crystals as a function of time is shown in Fig. 2. The line labelled "All VD" represents the cumulated number of crystallization conditions found in the vapour diffusion method and "All MB" represents the cumulated number found with all three microbatch methods. Again, where a condition occurs in more than one microbatch method it is counted once only.

In the first 3 days microbatch and vapour diffusion produced the same number of crystallization conditions. This period gives the highest production rate in the experiment. In the period from 3 days to 4 weeks after dispensing, vapour diffusion method is most successful because the drops have quickly increased in concentration to a suitable nucleation point. The evaporation of water through the oil in the microbatch plates takes longer at 4°C, but after 10 weeks the number of crystallization conditions found by the two methods was similar. Both methods were still producing conditions at an undiminished rate at the end of the experiment.

Crystal quality was assessed by observing crystals and scoring them by appearance. No significant trend was apparent, and no method could be said to give better quality crystals.

Crystal size was found by measuring the two horizontal dimensions visible in the microscope. In general crystals found with the same precipitant solution were larger with vapour diffusion than with microbatch. This may be due to the larger quantity of protein in the vapour diffusion drops, 4 μl compared to 1 μl.

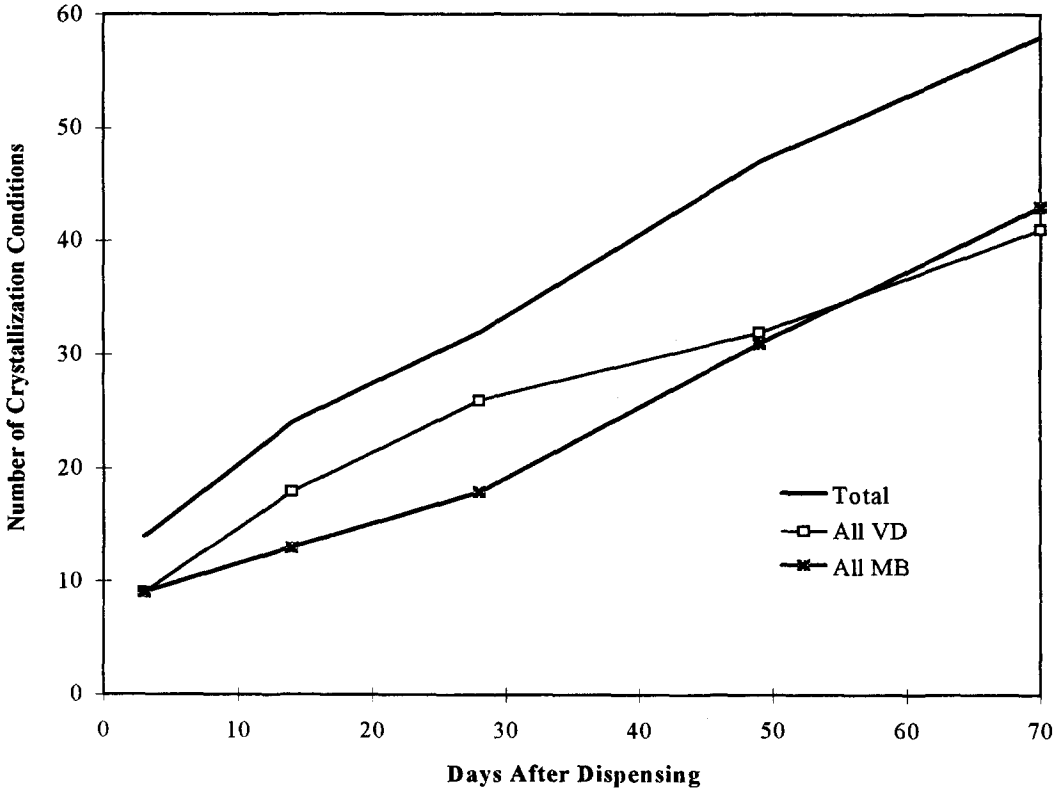

Fig. 2. Graph of number of conditions observed against time. The lowest line (crosses) indicates the number of crystallization conditions for all the microbatch methods combined. Note that around 40% of microbatch conditions were not found by vapour diffusion and vice versa.

4. Conclusions

The best microbatch method used low protein concentration and allowed evaporation with the silicone/paraffin oil mixture. This produced 34 crystallization conditions, which is nearly as successful as the vapour diffusion method. The three microbatch methods combined found more crystallization conditions (43 conditions) than did vapour diffusion. Each of the four methods found a number of crystallization conditions that were unique to that method, indicating that no single screening method will find all the protein's crystallization conditions.

During the first four weeks, vapour diffusion was significantly more successful than all the microbatch methods combined. This might not have been true if the experiments had been performed at room temperature, which would have given a higher evaporation rate, particularly from the trials with the mixture of paraffin and silicone oil. The high protein concentration method was the fastest microbatch method, presumably because the protein concentration was already high enough to produce crystals without further concentration. After 10 weeks the microbatch methods (especially the evaporation method) had caught up with vapour diffusion.

There are four principle factors which determine the priorities of a crystallization project: availability of protein, availability of labour, urgency (based on the time available to get first crystals for optimization), and the need for thoroughness (this increases if the project is very important or if very few crystallization leads are found). The data collected so far suggests the adoption of different screening strategies depending on these factors.

- Limited operator time: microbatch only.
- Limited protein supply: microbatch using as many methods as the protein will allow.
- Limited project time: vapour diffusion and microbatch.
- Need for thoroughness: vapour diffusion and microbatch.

Because the most time consuming step for microbatch is setting up the IMPAX, which takes 5–10 min, it is especially useful when project time is limited.

If protein supply is limited then using multiple microbatch methods gives a thorough search of the experimental space with minimum protein consumption. If the protein supply is so limited that only one microbatch screen can be performed then the evaporation method is the best one to use.

If project time is limited then use vapour diffusion as it will generally reveal crystallization conditions more quickly than microbatch. However it is still a good idea to do the microbatch screens as well, in case vapour diffusion does not produce crystals. In this case start the microbatch screen using high protein concentration as soon as possible to minimise the time spent waiting for first conditions.

References

[1] N.E. Chayen, P.D. Shaw Stewart and D.M. Blow, J. Crystal Growth 122 (1992) 176.
[2] A. Zagari, L. Savino, S. Capasso, F Sica, L. Mazarella, A. Di Luccia and L. Ferrara, Acta Cryst. D 50 (1994) 778.
[3] L. Pearl, B. O'Hara, R. Drew and S. Wilson, EMBO J. 13 (1994) 5810.
[4] J. Jancarik and S.H. Kim, J. Appl. Cryst. 23 (1991) 409.
[5] A. D'Arcy, Hoffman La Roche, private communication.
[6] L.F. Lloyd, P. Brick, Lou Mei-Zhen, N. Chayen and D. Blow, J. Mol. Biol. 217 (1991) 19.
[7] B. Cudney, Hampton Research, private communication.