Converting Vapor Diffusion to Microbatch

HAMPTON RESEARCH
Solutions for Crystal Growth

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When the conditions for growing protein crystals by sitting or hanging drop vapor diffusion are known, crystals of comparable or sometimes better quality can usually be produced by microbatch under oil using the following guidelines. Unless stated otherwise, for microbatch, the concentrations of protein and reagent below refer to the concentration in the drop after protein and other reagents have been mixed.

Reagent Concentration

Reagent concentration is typically *lower* in the microbatch drop than in the vapor diffusion reagent well. Set microbatch trials covering a range of reagent concentrations from the concentration used in the reagent well, to half of this reagent concentration. The final reagent concentration in a batch experiment is typically 10 to 20% lower than that in the reservoir of a vapor diffusion experiment.[1,4]

Protein Concentration

Protein concentration is typically, but not always, *lower* in the microbatch drop than the initial protein concentration in the vapor diffusion drop. Set microbatch trials cover a range of protein concentrations from the concentration used to create the drop, to half this protein concentration. In general, the final concentration of protein in a batch experiment is often 10 to 20% lower than the starting protein stock solution used in vapor diffusion.[1,4]

Equilibration

In cases where crystallization takes place rapidly (within minutes, or within 24 hours), or where equilibration is slow (e.g. using low ionic strength reagent formulations such as polyethylene glycol), crystallization may take place in vapor diffusion before the vapor diffusion equilibration between the drop and reagent well is complete. In such instances, the microbatch concentrations of protein and reagent may be close to the concentrations in the vapor diffusion drop immediately after setting the drop, before equilibration takes place. In a vapor diffusion experiment, when crystallization takes place very rapidly, before the vapor diffusion has reached equilibrium (less than 24 hours), significantly lower concentrations (30 to 40%) of protein and reagent should be used for the batch experiment.[1,4]

Spotting a Batch Process in Vapor Diffusion

When crystals form within minutes, or within 24 hours of creating the sample and reagent drop in a vapor diffusion experiment, the process is likely to be batch rather than vapor diffusion.[3] Longer equilibration times (a week or longer) are associated with low ionic strength reagents, such as polyethylene glycols, so the crystallization process in such reagents are quite likely to be batch.[3] Even though shorter equilibration times (complete in as little as 4 to 5 days) are anticipated with higher ionic strength reagents, such as salts, crystals appearing in such reagents in 24 hours or less most likely resemble a batch process instead of vapor diffusion.

Batch Optimization

If a crystallization occurs in 24 hours or less, one should likely think the process batch. Under such a process, subtle changes (+/- 10 to 20%) in reagent concentration, pH, sample concentration, drop ratio, and additives can be explored for crystal optimization. If the crystallization requires more than 24 hours, one can use crystallization time to estimate how far the process is from being batch. Shorter crystallization times, for example 1 to 2 days requiring less manipulation of sample and reagent concentration than longer crystallization times of 4 to 5 days or longer. The longer the time required for crystals to appear, the more likely the concentration of the reagent and the sample would need to be increased to move from a vapor to a batch process for crystallization. The optimal concentrations of buffers and additives are usually held constant when converting to and optimizing a batch experiment.[1,4]

Examples[1,2]

Reverse transcriptase	Vapor Diffusion		Microbatch	
Protein	Drop Initial	5 mg/ml	Drop	10 mg/ml
HEPES	Reagent Well	50 mM	Drop	50 mM
Ammonium sulfate	Reagent Well	1.6 M	Drop	1.3 M

The vapor diffusion protein concentration is that immediately after the drop is created by mixing one-part protein to one-part reagent well, and before any equilibration can occur. In the example of the full-length reverse transcriptase molecule, crystallized with a rapidly equilibrating salt-based reagent, in the form of hexagonal bipyramids, the concentration of the Ammonium sulfate precipitant in the microbatch drop (1.3 M) was lower than the vapor diffusion reservoir (1.6 M). The other concentrations were the same. Note that a more concentrated protein was needed for microbatch compared to the initial protein drop concentration for vapor diffusion.

Carboxypeptidase G2	Vapor Diffusion		Microbatch	
Protein	Drop Initial	3 mg/ml	Drop	3 mg/ml
Sodium cacodylate	Reagent Well	100 mM	Drop	50 mM
Zinc acetate	Reagent Well	0.2 M	Drop	0.2 M
Polyethylene glycol 4,000	Reagent Well	12%	Drop	10%

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The vapor diffusion protein concentration is that immediately after the drop is created by mixing one-part protein to one-part reagent well, and before any equilibration can occur. In the example of carboxypeptidase G2, which was crystallized using a slower equilibrating Polyethylene glycol-based reagent, the protein concentration in the vapor diffusion and microbatch drops were the same, before the vapor diffusion had time to equilibrate with the reagent well. The crystals formed soon after the drops were set, the relatively high polyethylene glycol concentration indicating that little equilibration had occurred before crystallization took place in vapor diffusion.

Glucose Isomerase	Vapor Diffusion		Microbatch	
Protein	Drop Initial	8 mg/ml	Drop	8 mg/ml
MES	Reagent Well	50 mM	Drop	50 mM
Polyethylene glycol 4,000	Reagent Well	18%	Drop	9%

The vapor diffusion protein concentration is that immediately after the drop is created by mixing one-part protein to one-part reagent well, and before any equilibration can occur. In the example of glucose isomerase, crystals appeared within an hour of setting both vapor diffusion and microbatch drops. No equilibration in the hanging drop was needed for crystals to appear. At the moment the vapor diffusion drop was set, the protein, and reagent concentration were identical to that of the microbatch drop.

Trypsin Inhibitor	Vapor Diffusion Drop	Vapor Diffusion Reagent Well	Microbatch
Protein	10 mg/ml	None	12 mg/ml
Sodium acetate	20 mM pH 3.5	20 mM pH 5.0	50 mM pH 5.2
Sodium chloride	150 mM	50 mM	150 mM

The vapor diffusion protein concentration is that immediately after the drop is created by mixing one-part protein to one-part reagent well, and before any equilibration can occur. The example with Trypsin inhibitor illustrates how even in experiments when a protein is crystallized by changing pH using vapor diffusion, it can be possible to grow crystals microbatch without a pH change. Trypsin inhibitor was solubilized in 20 mM Sodium acetate pH 3.5 and equilibrated against 20 mM Sodium acetate pH 5.0. Microbatch crystals were grown in Sodium acetate pH 5.2 and a higher protein concentration.

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References and Readings

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Hampton Research
34 Journey
Aliso Viejo, CA 92656-3317 U.S.A.
Tel: (949) 425-1321 • Fax: (949) 425-1611
Technical Support e-mail: tech@hrmail.com
Website: hamptonresearch.com