Crystal Growth Techniques

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Solutions for Crystal Growth

Crystal Growth 101

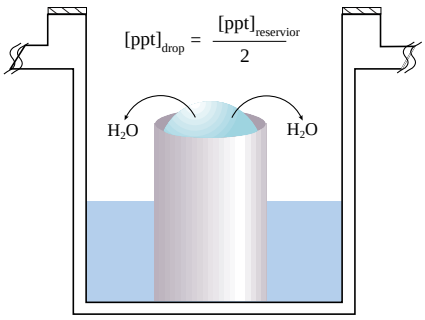
There are several techniques for setting up crystallization experiments (often termed "trials") including sitting drop vapor diffusion, hanging drop vapor diffusion, sandwich drop, batch, microbatch, under oil, microdialysis, and free interface diffusion. Here we offer an overview of these crystallization techniques.

Sitting & Hanging Drop Crystallization

Sitting and hanging drop methodologies are very popular because they are easy to perform, require a small amount of sample, and allow only a large amount of flexibility during screening and optimization.

Using the sitting drop technique (figure 1) one places a small (1 to 40 microliters) droplet of the sample mixed with crystallization reagent on a platform in vapor equilibration with the reagent. The initial reagent concentration in the droplet is less than that in the reservoir. Over time the reservoir will pull water from the droplet in a vapor phase such that an equilibrium will exist between the drop and the reservoir. During this equilibration process the sample is also concentrated, increasing the relative supersaturation of the sample in the drop.

figure 1



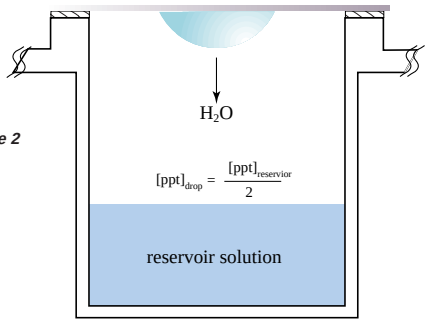
The advantages of the sitting drop technique include speed and simplicity. The disadvantages are that crystals can sometimes adhere to the sitting drop surface making removal difficult. This disadvantage can turn into an advantage where occasionally the surface of the sitting drop can assist in nucleation. The sitting drop is an excellent method for screening and optimization. During production, if sticking is a problem, sitting drops can be performed in the sandwich box set up.

Sitting drop crystallization may be performed using Micro-Bridges® or Glass Sitting Drop Rods™ with VDX or Linbro plates. Both plates can be sealed with clear sealing tape or plain cover slides for easy viewing and access. Sitting drop crystallization may also be preformed using the Cryschem Plate™. The Cryschem Plate is a specially designed plate with a post already in the center of the reservoir.

Using the hanging drop technique (figure 2) one places a small (1 to 40 microliters) droplet of the sample mixed with crystallization reagent on a siliconized glass cover slide inverted over the reservoir in vapor equilibration with the reagent. The initial reagent concentration in the droplet is less than that in the reservoir. Over time the reservoir will pull water from the droplet in a vapor phase such that an equilibrium will exist between the drop and the reservoir. During this equilibration process the sample is also concentrated, increasing the relative supersaturation of the sample in the drop.

The advantages of the hanging drop technique include the ability to view the drop through glass without the optical interference from plastic, flexibility, reduced chance of crystals sticking to the hardware, and easy access to the drop. The disadvantage is that a little extra time is required for set ups.

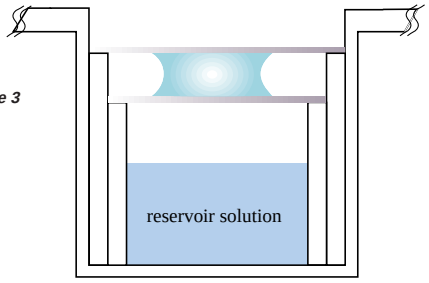
figure 2



Sandwich Drop Crystallization

The Q Plate is specially designed to allow for hanging drop, sitting drop, and sandwich drop vapor diffusion. Here we will address only the sandwich drop feature (figure 3). For sandwich drop the sample solution mixed with the precipitant is placed in the middle of a lower 18 mm siliconized glass cover slide followed by one setting a larger 22 mm siliconized glass cover slide in position along an upper edge which allows for a small amount of space between the cover slides but is close enough such that the drop is sandwiched between the glass cover slides. The advantages to the techniques are an excellent optical pathway for microscopic examination and an alternate equilibration method. The disadvantages include tedious set up and the plate's large footprint.

figure 3



Free Interface Diffusion

Free interface diffusion crystallization is less frequently used than sitting or hanging drop vapor diffusion but it is one of the methods used by NASA in microgravity crystallization experiments. Using this method one actually places the sample in liquid contact with the precipitant. When doing so one attempts to create a clearly defined interface between the sample and the precipitant. Over time the sample and precipitant diffuse into one another and crystallization may occur at the interface, or on the side of high sample/low precipitant or low sample/high precipitant. The technique allows one to screen a gradient of sample precipitant concentration combinations. The technique can readily be performed in small capillaries (figure 4).

Batch

Batch crystallization is a method where the sample is mixed with the precipitant and appropriate additives creating a homogeneous crystallization medium requiring no equilibration with a reservoir. The technique is popular with small molecule crystallographers. The advantages to the technique are speed and simplicity but the disadvantage is that only a narrow space of precipitant/sample concentration can be

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sampled in a single experiment. A batch experiment can be readily performed in a capillary, small container, or plate with a small reservoir such as the Macro-Store Plate™ (HR3-116). One must be very close to the conditions which promote crystal growth in order for this technique to be successful.

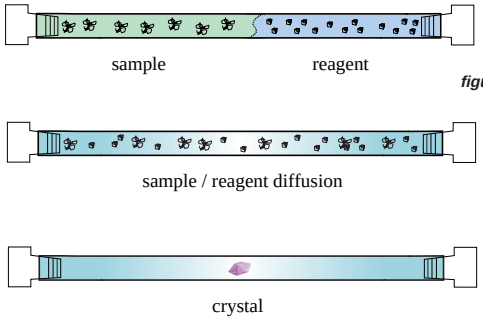

figure 4

MicroBatch Under Oil

In this technique a small drop of the sample combined with the crystallization reagent is pipetted under a layer of oil. For a true MicroBatch, the drop is placed under Paraffin Oil (figure 5) which allows little to no evaporation nor concentration in the drop. A Modified MicroBatch can be performed when the drop is placed under a mixture of Paraffin Oil and Silicon Oil, or straight Silicon Oil (figure 6). Such oils allow water vapor to permeate from the drop and allow sample and reagent concentration. Unless the drop is equilibrated with a reservoir, water will leave the drop until that only solids remain.

The benefits of Crystallization Under Oil include the use of very small sample and reagent volumes with less concern for unwanted evaporation, the minimization of surface interaction with the sample, the ability to precisely control sample and reagent concentrations during the experiment, and the minimization of condensation during temperature fluctuations.

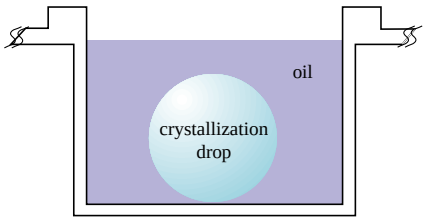

figure 5

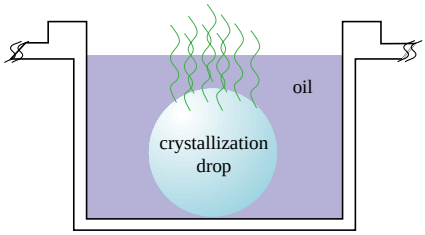

figure 6

Microdialysis Crystallization

Dialysis crystallization involves placing the sample in a Dialysis Button which is sealed with a dialysis membrane. Water and some precipitants are then allowed to exchange while retaining the sample in the cell. The Dialysis Button is placed into a suitable container holding the precipitant or crystallization media (figure 7). For example, one might dialyze a sample requiring a high ionic strength for solubility against a solution of low ionic strength. The technique allows for salting in and salting out, as well as pH crystallization techniques

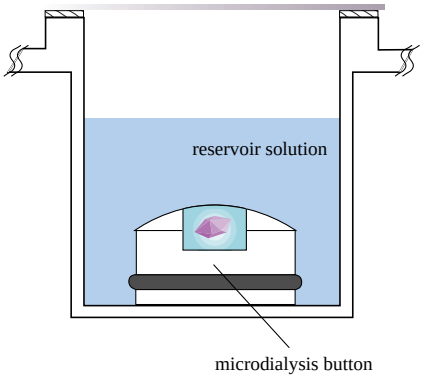

figure 7

Technical Support

Inquiries regarding the crystal growth techniques, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

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