Standard Operating Procedure

Task: Crystallization of organic and inorganic materials
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Date: 06/08/2021
Revision Date:

Background

- Crystallization is a fundamental chemical purification method of organic and inorganic materials. Crystallization serves two main purposes: generation of high purity material and producing single crystals suitable X-ray diffraction.
- Crystallization can largely be summarized as a series of techniques or tricks to generate a supersaturated solution of a target material that thermodynamically favors transfer from the solution to solid phase. The rate of this transfer is fundamentally important: a rapid phase transfer often leads to a powder or microcrystalline material, fantastic for purification; slower phase transfer is often better for the generation of single crystals due to minimization of the number of nucleation sites and favoring growth of a small number of single crystals of greater size. Disturbing solutions of single crystals can also impact the overall process.
- This SOP describe as number of techniques for crystallization both under air and inert atmosphere. These techniques can be used for both bulk purification and growth of single crystals.
- *Vocabulary:*
    - **Mother liquor** refers to the solution remaining after crystal growth
    - **Seeding** refers to the process of intentionally adding a nucleation site to your crystallization. This can be a too small crystal from before or traditionally, an animal whisker.
    - **Antisolvent** is the solvent that causes crystallization. It is often the layering or diffusing solvent that the compound of interest in not (or very sparingly) soluble in.

Training Requirements:

- Lab safety training
- Glovebox training if applicable
- Chemical hazards training wheels pertinent to target materials

Potential Hazards:

- Hazards associated with specific chemicals being handled

Special PPE Requirements:

- Choose PPE based on specific chemical hazards

Materials Needed:

- Compound of interest. For single crystal growth, start with high purity material
- Solvent

- Appropriate glassware

How to approach a crystallization

- The first thing to know before setting up a crystallization is the basic solubility and stability profile of the material you are working with. The Inorganic Workup/a solvent ladder is a great way to determine this. Alternatively, the solubility of structurally analogous molecules (unified by charge, counterion choice, ligand) are usually similar and can serve as a starting point.
- In *general* most combinations and techniques will yield crystals (if at all) within 24-72 h. Techniques such as layering and vapor diffusion are significantly slower at low temperatures and will be impacted by solvent choice.
- Many successful crystallizations occur from “dilute saturated solutions”, meaning that the material crystallizes from a solution in which it has relatively low solubility, such that saturated solutions have low concentration of analyte.
- If crystals are expected but do not form, take a glass rod and scratch at the sides of the glass. This can induce crystal nucleation. ***DO NOT DO THIS*** if the material is a known shock-induced explosive.
    - The glass can also be pre-scratched to provide sites for nucleation.
- Single Crystals:
    - To grow single crystals, it is best to work with dilute saturated solutions as they are closest to the saturation point. This means that even small changes in temperature (cooling), concentration (slow evaporation, hanging drop), or solubility (vapor diffusion, layering) can readily generate the supersaturated solutions necessary for crystallization. Working with dilute saturated solutions also helps mitigate the likelihood of bulk precipitation and minimizes the amount of material needed in a given crystallization.
    - Scale: a good rule of thumb is to use at least 10 mg in each attempt to ensure there is sufficient material to grow crystals. If the material is highly soluble in the solvent used, more material may be necessary. Thus, choosing solvents in which the material is poorly soluble will lead to saturated solutions at low concentrations (and small amounts of material).
    - It is best that the solutions be left alone as much as possible. Place in locations where they are unlikely to be disturbed. Superstitious chemists will not look at a crystallization until the next day.
    - Growing crystals can be difficult and therefore it can often be worth taking >50 mg of material and attempting multiple different conditions at once
- Bulk crystallization:
    - Scale is limited by glassware, solvent needed, and amount of material.
    - If the yield of crystals is low, a second crop of crystals can often be harvested by collecting the mother liquor and setting up another crystallization either at lower temperature or after concentrating the mother liquor solution.

Techniques:
Hot crystallization

- *Background:* Hot crystallization works by dissolving a material in a given solvent at high temperatures to generate a saturated solution. Upon cooling, the solution becomes supersaturated and induces crystallization. Hot crystallization is a very popular method of bulk purification and is often coupled to hot filtration to remove any insoluble impurities.
- *Glassware:* Erlenmeyer flasks are a standard choice, as they minimize solvent loss and are best suited to bulk purification. NMR tubes are a classic choice when targeting single crystal growth and aim to use <1 mL of solvent.
- *Procedure:*
    1. Choose a solvent. The compound should have low solubility in the chosen solvent at room temperature. A good place to start for commercial chemicas is *Purification of Laboratory Chemicals* handbook or a published procedure for a structurally similar species.
    2. Weigh out material into glassware and record the mass. Add an appropriate amount of solvent and heat the solvent while mixing (stir bar, swirling the flask, occasionally inverting NMR tube). Try not to exceed the boiling point of the solvent.
    3. Once the material has fully dissolved (or is no longer dissolving), filter.
    4. Cover the flask and allow to cool slowly. Once cooled to room temperature, the flask can be further cooled in an ice bath or in a fridge or freezer. Leave at least overnight for best results.
    5. For bulk crystallizations: collect the precipitated crystals via filtration. Wash crystals with a solvent they are not soluble in. Worst case, wash with cold solvent used in the crystallization. Collect the crystals, dry, and record the mass. If the yield is low, a second crop can be collected by concentrating the mother liquor and leaving in a cold place.
- *Tips and tricks:*
    - If targeting single crystals, leave the solution in the oil bath to cool more slowly. This will be more favorable for larger crystal growth
    - The EasyMax can also be used for rigorous temperature control, allowing for known cooling ramps that may assist in generation of single crystals.
    - Diethyl ether, pentane, and dichloromethane are poor choices due to their low boiling points. Alternatives include THF or dioxane, heptane, and dichlorethane.

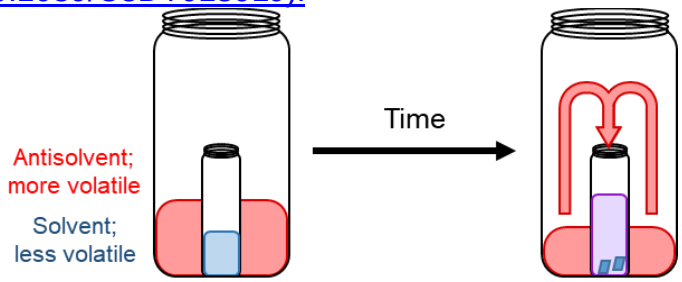
Cold crystallization

- *Background:* Cold crystallization works under the same principles as hot crystallization, but the material must be fully soluble in the solution at the starting temperature (usually room temperature). This is a popular method for both bulk purification and single crystal growth.
- *Glassware:* Round bottom flasks are a standard choice for bulk purification because they can be easily stoppered before cooling in a fridge or freezer. For single crystals, vials and NMR tubes are popular. For crystallizations inside a glovebox freezer, 20 and 4 mL vials are the best fit.
    - Be careful about cooling Teflon-sealed NMR tubes in particular, as this can break the Teflon-glass seal upon contraction.

- *Procedure:*
    1. Choose a solvent. The compound should be completely soluble at room temperature. A good place to start for commercial chemicals is *Purification of Laboratory Chemicals* handbook or a published procedure for a structurally similar species.
    2. Weigh out material into glassware and record the mass. Add an appropriate amount of solvent to fully dissolve the material. Sonication and stirring can be used to speed up this process.
    3. Once the material has fully dissolved (or is no longer dissolving), filter the solution.
    4. Cover the flask and allow to cool. This process can take hours to days depending on the material and solvent.
    5. For bulk crystallizations: collect the precipitated crystals via filtration. Wash crystals with a solvent they are not soluble in. Worst case, wash with cold solvent used in the crystallization. Collect the crystals, dry, and record the mass. If the yield is low, a second crop can be collected by concentrating the mother liquor and leaving in a cold place.
- *Tips and tricks:*
    - Use as little solvent as possible to maximize crystal formation.
    - If targeting single crystals, after filtering the saturated solution, add 5% of solvent. This can help avoid the rapid cooling of placing the vial in a freezer from inducing powder formation.
    - Benzene, DMSO, and water make poor solvent choices due to their high freezing point.

Vapor Diffusion

- *Background:* Vapor diffusion works by slowly diffusing vapors of an antisolvent into a saturated solution of material. This results in a supersaturated solution that can induce crystallization. This method is largely used for growth of single crystals. See “A quantitative study of vapor diffusions for crystallizations: rates and solvent parameter changes” for more info (https://doi.org/10.1039/C8DT01891J).



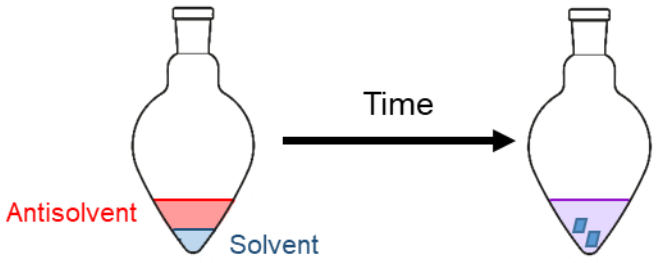
- *Glassware:* Small inner vial and large outer vial. 4 mL vials in 20 mL are popular. Can also use crystallization tubes in either 4 mL or 20 mL vial. For single crystals, usually dissolve ~10 mg of material in <1.5 mL of solvent for the inner vial and ~5 mL of antisolvent in the outer vial. For large scale, could use 20 mL vials in a Mason jar and add sufficient .
- *Procedure:*

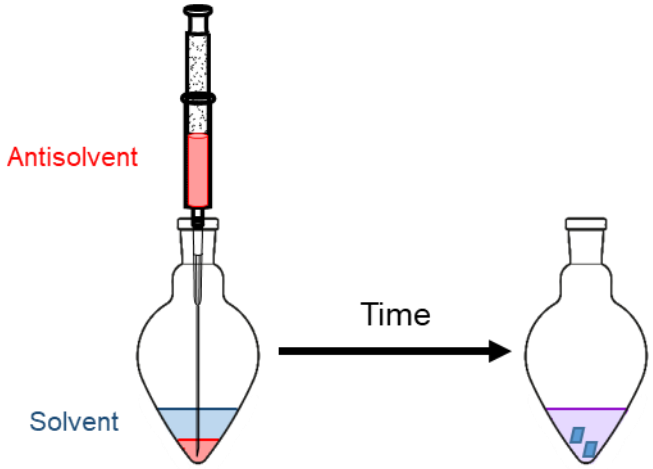
1. Choosing a solvent and antisolvent. The solvent and antisolvent must be miscible, consult a solvent miscibility chart. The antisolvent must also have sufficient vapor pressure to reasonable migrate into the inner vial. Usually high boiling antisolvents (such as toluene) are poor choices due to how slowly they migrate.
2. Weigh out material into a vial and record the mass. Dissolve in a minimal amount of solvent. If you use too much, it minimizes room in the vial for the antisolvent and can result in an overall solvent mixture that does not induce crystallization.
3. Once the material has fully dissolved (or is no longer dissolving), filter the solution into the inner vial. This is important to remove any trace insoluble that can influence nucleation.
4. Place the inner vial into the empty outer vial.
5. Add the chosen antisolvent to the outer vial.
6. Seal the system by placing a cap on the outer vial.

- *Tips and tricks:*
  - Use as little solvent as possible to maximize crystal formation.
  - If targeting single crystals, after filtering the saturated solution, add 5% of solvent. This can help avoid the rapid cooling of placing the vial in a freezer from inducing powder formation.
  - Consult a miscibility chart before starting. For example, pentane and MeCN are immiscible. Alternatives would be Et_2O into MeCN or pentane into either DCM or acetone.
  - If running cold in a fridge or freezer, benzene, DMSO, and water make poor solvent choices due to their high freezing point.
  - If run at RT, if nothing crystallizes out, moving the mixture into a cold location can help nucleation/crystal growth.

Layering and Reverse Layering

- *Background:* Layering operates under the same principles as vapor diffusion, but is often faster and is better suited if high bp solvents are necessary (ex: toluene, DMF, DMSO, etc). In layering, instead of antisolvent vapors mixing with a saturated solution, the antisolvent layer mixes via diffusion with the saturated solution. Layering involves adding antisolvent on top of the saturated solution while reverse layering involves adding antisolvent *below* the saturated solution. Layering is good for both bulk purification and growth of single crystals.



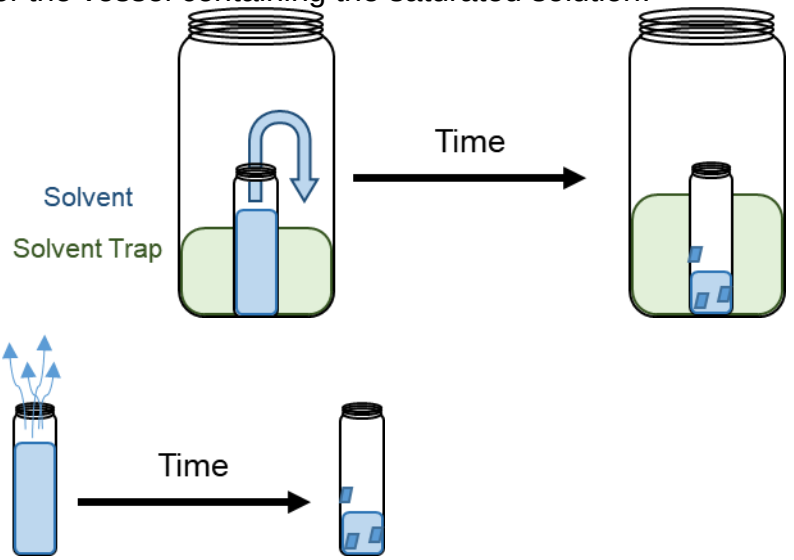


- *Glassware:* Any sealable glassware is good for layering. Note that the larger the surface area of the glassware (ex: Schlenk flask) the more difficult it is to get clean layers. For single crystals, growing in narrower vessels such as NMR tubes or 4 mL vials is common. For reverse layering, pear flasks or any flask with a conical point are ideal for syringing in the antisolvent. NMR tubes may also work. Volume of antisolvent used will change depending on glassware and the relative solubility of the material trying to be crystallized, but in general ratios of 1:1 solvent:antisolvent or greater work well.
- *Procedure:*
    1. Choosing a solvent and antisolvent. The solvent and antisolvent must be miscible, consult a solvent miscibility chart. Note that the density of the antisolvent will help dictate the rate of mixing.
    2. Weigh out material into a vial and record the weight. Dissolve in chosen solvent. If you use too much, it minimizes room in the vial for the antisolvent and can result in an overall solvent mixture that does not induce crystallization.
    3. Once the material has fully dissolved (or is no longer dissolving), filter the solution into the crystallization glassware. This is important to remove any trace insoluble that can influence nucleation.
    4. Add antisolvent:
        - For layering: slowly pipet the antisolvent along the sides of the glass. Avoid dripping antisolvent into the saturated solution. The slower the antisolvent is added, the better layer separation you'll achieve
        - For reverse layering: fill a syringe with antisolvent. Attach a long needle and insert into the conical point of the flask containing the saturated solution. *Slowly* syringe in the antisolvent.
- *Tips and tricks:*
    - Use as little solvent to make your saturated solution as possible to maximize crystal formation.
    - If targeting single crystals, after filtering the saturated solution, add 5% of solvent. This can help avoid precipitate from forming during initial mixing.

- Consult a miscibility table. For example, pentane and MeCN are not miscible. Alternatives would be Et_2O into MeCN or pentane into either DCM or acetone.
- If running cold in a fridge or freezer, benzene, DMSO, and water make poor solvent choices due to their high freezing point.
- If run at RT, if nothing crystallizes out, moving the mixture into a cold location can help nucleation/crystal growth.

Slow Evaporation

- *Background:* Slow evaporation works by slowly concentrating a saturated solution until it achieves super saturation and subsequently crystallizes. Slow evaporation is most commonly used to generate single crystals. It is very important that during this process, you do not allow all the solvent to evaporate, as any crystals that formed could crack and become unusable. The rate of slow evaporation is directly tied to the vapor pressure of the solvent and the opening of the vessel containing the saturated solution.



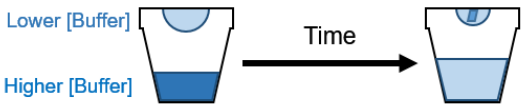
- *Glassware:* Inside a glovebox, a two vial method is preferred and is constructed out of a 4 mL inner vial and a 20 mL outer vial. In the outer vial, a high boiling point solvent “sponge” such as toluene is used to absorb evaporation solvent vapors. On the bench top, 4 or 8 mL vials with plastic caps are preferred. The cap can either be punctured several times with a needle or needles can be placed in the cap.
- *Procedure:*
    1. Choosing a solvent. The boiling point and vapor pressure are very important in determining how fast evaporation will occur. High boiling point solvents are not a great choice (ex: toluene). Low boiling point solvents (DCM, pentane) can sometimes evaporate too fast. Running these solvents cold can help slow the process.
    2. Weigh out material into a vial and record the weight. Dissolve in chosen solvent.

3. Once the material has fully dissolved (or is no longer dissolving), filter the solution into the crystallization glassware. This is important to remove any trace insoluble that can influence nucleation.
4. If inside the glove box, place the inner vial inside the outer vial and add a few mLs of the solvent sponge to the outer vial. Seal the system by capping the outer vial.
5. If outside the box, cap, and puncture/place needles inside the cap.
6. Try and place in a low traffic area, but still visible so that you can avoid all of the solvent evaporating.

- *Tips and tricks:*
  - Try and avoid an excessive amount of solvent (<1.5 mL in a 4 mL vial), as this can slow down the process/leave excess residue of material on the sides of the glassware
  - Pentane, Et_2O, and DCM practically fly at RT, be careful. Benzene is a good nonpolar alternative.
  - If running cold in a fridge or freezer, benzene, DMSO, and water make poor solvent choices due to their high freezing point.

Hanging Drop

- *Background:* Hanging drop crystallizations are most commonly used in protein crystallography and can also be suited to growth of single crystals that are water soluble and stable. Hanging drop crystallizations work by adding a drop of water containing sample on a glass slide and then inverting the slide over a buffered, salty water solution. The surface tension of water stops the drop from dripping into the salty solution and through differences in salt concentrations, water moves from the drop to the buffered solution. One advantage of hanging drop, is that the solubilizing solution can also be buffered, thereby allowing for crystal growth of materials impacted by buffer content or pH.



- *Glassware:* Glass slides, and a 24/96 well plate
- *Procedure:*
  1. Choose an appropriate buffer and pH to generate the species of interest. It is also possible to just use neat water.
  2. Dissolve material in the water or buffer solution. The solution should be near the saturation point. Set aside.
  3. Generate a large volume buffered solution of water for your reservoirs. It is important that the buffer concentration be larger than that used to dissolve the sample, otherwise the drop will grow in size and dilute your sample, not generate a supersaturated solution.
     - Note that the buffer in the reservoir does ***NOT*** need to match the buffer in the hanging drop.
  4. Fill the reservoirs of the crystallization apparatus with buffer solution. Be sure to not overfill; you don't want the hanging drop to make contact with the buffer solution.

5. Place a drop of water in the middle of the glass slide. Then carefully invert the slide and place over one the wells containing buffer. Grease can also be placed on the slide to create a good seal and help avoid evaporation.
6. Set in a location where they will not be disturbed and check every few days. It is fairly easy to monitor the formation of crystals, as they are readily apparent on the slides.

- *Tips and tricks:*
    - If crystals are not forming or are too small, consider trying various buffer identities, buffer concentrations, and pH. These all influence crystal growth
    - Consider saving small crystals as seeds to place in a second round of hanging drop crystallizations.

Additional Tips and Tricks

- If cold layering/vapor diffusion always powder out, one thing to try is to generate your saturated sample and pre-cool that in the fridge or freezer. After that reaches equilibrium and precipitates some amount of the material, filter the solution into your crystallization glassware and continue as normal.
- When identifying whether a crystallization worked, look for clear, distinct faces. Generally, if you can see the faces, it will be large enough for diffraction. If the crystals look circular, these end up usually being amorphous materials.
- In general, if the crystal is in the mother liquor, most crystallographers prefer that you do *NOT* decant the mother liquor off the crystal. They prefer to do this as close to mounting to avoid/minimize the chance of the crystal cracking.
- If your sample is exceptionally air sensitive, the crystals can be placed on slides and coated in paratone oil inside the gloveboxes. Also coordinate with the crystallographer before removing from the inert atmosphere.
- Exceptionally lipophilic crystals can dissolve in paratone oil. Check with a small crystal before coating the entire sample.
- Materials that contain countercations such as Li^+, Na^+, or K^+ often crystallize better in the presence of ethereal donors. A good place to start is Et_2O or THF but the addition of appropriate crown ethers and cryptands may be necessary.
- Materials containing BArF counteranions are prone to be isolated as oils/sticky solids and then “oil out” during the crystallization process. One potential trick is to lyophilize the material out of frozen benzene first to start from a powder.
- Crystals grown cold can occasionally “melt” and redissolve before they can be isolated/mounted on the goniometer. To address this, upon removal from the fridge/freezer, rapidly decant the sample and immediately coat in oil.
- Sometimes whole classes of structurally analogous molecules will refuse to crystallize. It can therefore be worthwhile to substitute counterion choice (BArF↔PF_6↔BF_4, etc), substituents (many people swear that dipp improves crystallinity), additives (ethereal donors, hydrogen bond acceptors/donors), etc.
- If all crystallizations only yield small crystals, save some as seed crystals to serve as nucleation sites for a subsequent crystallization, often resulting in a larger crystal. To seed a crystallization, setup your saturated solution of material and then carefully add the seed crystal(s). It is important the solution is at or very near the saturation point; as otherwise the seed crystal may simply dissolve.

- Occasionally a solved crystal does not line up with the expected compound and it becomes a question of whether the crystal structure accurately reflects the overall material. It can be useful to collect spectroscopic data on the same crystals used for XRD. The solid state crystals can be readily analyzed by solid state IR and rR even when coated with paratone oil. NMR can also be very helpful, though best collected on samples not coated in paratone oil.

Additional Resources

- Inorganic Workup SOP
- Purification of Laboratory Chemicals handbook
- A quantitative study of vapor diffusions for crystallizations: rates and solvent parameter changes. https://doi.org/10.1039/C8DT01891J
- https://hamptonresearch.com/crystal-growth101.php
- http://www.xray.bioc.cam.ac.uk/xray_resources/whitepapers/xtal-in-action/node3.html
- https://www.chemistryviews.org/details/education/2532131/Tips_and_Tricks_for_the_Lab_Growing_Crystals_Part_1.html