Growing crystals for structure determination through single crystal X-ray diffraction

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There are a lot of online resources about crystallization techniques for single crystal X-ray diffraction (SCXRD) analysis. See, e.g. http://www.cryst.chem.uu.nl/lutz/growing/growing.html and references/links on this website. For that reason, the current document is not aimed to be comprehensive. It is prepared to help get started and cover simple techniques on how to grow single crystals suitable for SCXRD structure determination.

Preferences for crystals

- Crystals size > 50 μm along the smallest dimension
- No visible defects (cracks, inclusions, misoriented fragments)
- Easily separable from one another and from container
- Should be kept inside mother liquor

Crystallization techniques

There are many crystallization techniques. The choice of the technique depends on material properties, availability of special equipment, skills of the researcher and simply individual preferences. In most cases, however, a few simple techniques work very well.

For crystallization of soluble (solubility c_{eq} > 1 mg/mL) small molecular mass (M_w < 500 Da) organic and metalorganic molecular and ionic crystals as well as inorganic salts the following methods are recommended.

- *Solvent evaporation method* can be tried first as the simplest technique that does not require a lot of material.
- If amount of material is significant (> 30 mg) the *temperature lowering method* is another option that provides good control on crystallization process and, as a result, larger and higher quality crystals.
- The next option is *vapor diffusion method*. This delicate technique, which requires time and patience, can provide high quality crystals even if amount of materials is small (a few mg).

These three methods are discussed below. If none of them works, other options to be considered include *solution layering method*, *ripening driven by temperature oscillations*, *crystallization in gels*, *melt crystallization* and *crystallization by sublimation* among others. They are beyond the scope of this document.

Solvent evaporation

Principle

As solvent evaporates, solution concentration increases and supersaturation builds up.

Advantages

- Very simple setup
- Does not require a lot of material

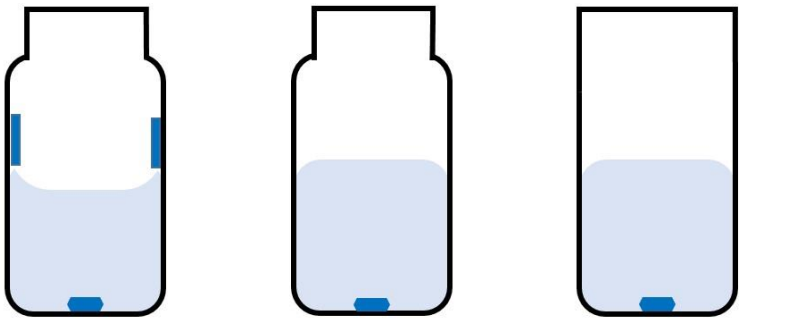
Disadvantages

- Solvent should be volatile
- Hard to control supersaturation, so that often a large amount of tiny crystals forms.

Procedure

- Prepare solution and leave it in an uncapped vial for evaporation. The rate of evaporation can be controlled by partial covering of the vial with parafilm. Very fast evaporation results in massive precipitation of tiny crystals. Very slow evaporation is time consuming.
- Ideally, the experiment should be stopped (vial capped) when some amount of solvent is still present in the vial. First, this should prevent massive precipitation of tiny crystals during evaporation of the last portions of the solvent. Second, the crystals can be easier removed from the vial.
- If crystals appear on walls above the liquid (crystallization at the solution-wall-air interface with the subsequent solution supply by capillary forces) change material of the vial so that the walls will be less wettable by the solution.

Solvent evaporation setup



Good wettability
Crystallization on walls: "creeping"

Bad wettability
Crystallization only inside liquid

Preferable shape of container: no notch; low height to width ratio

Temperature lowering

Principle

Solubility increases (in some cases decreases) as temperature increases. Supersaturation can be created by cooling solution saturated at higher temperature.

Advantages

- Good supersaturation control allows to grow larger and more perfect crystals
- Formation of stable amorphous solute-solvent aggregates is less likely
- Relatively fast method
- Almost any solvent can be used

Disadvantages

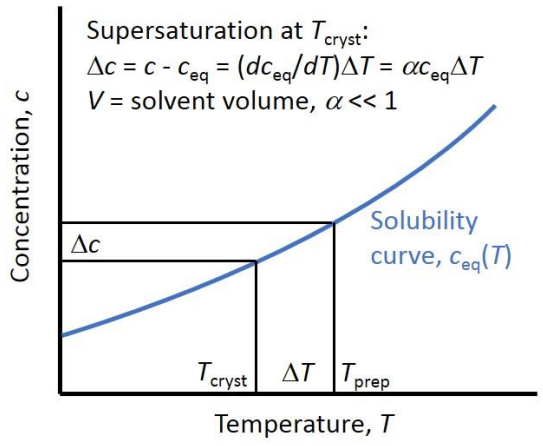
- Requires several steps
- Temperature control is essential
- Hard to use if only a few mg of material is available

Solvent selection

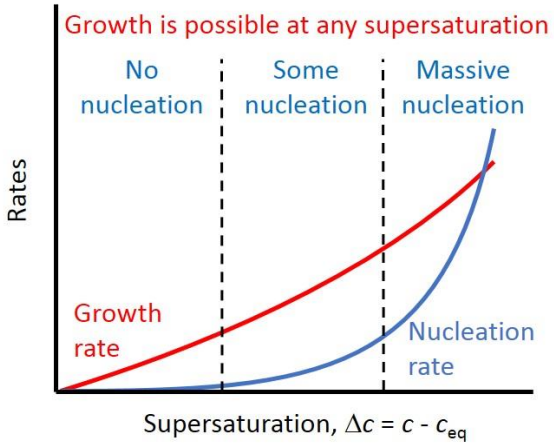
Solubility, c_{eq}, and its temperature dependence, dc_{eq}/dT, are among the main factors determining crystallization. Since c_{eq} and dc_{eq}/dT are correlated, the solubility itself is sufficient to estimate an outcome.

- If c_{eq} is too large then crystallization is hard to control because even small temperature variations can cause massive precipitation/dissolution.
- If c_{eq} is too small it is more likely to form numerous, tiny and imperfect crystals because crystallization of sufficient amount of material would require high driving force for crystallization (relative supersaturation $\ln(c/c_{eq})$).

Solubility can be controlled by mixing "good" and "bad" solvents. However, in the presence of solute even miscible solvents sometimes lead to liquid-liquid phase separation and tiny low quality crystals.



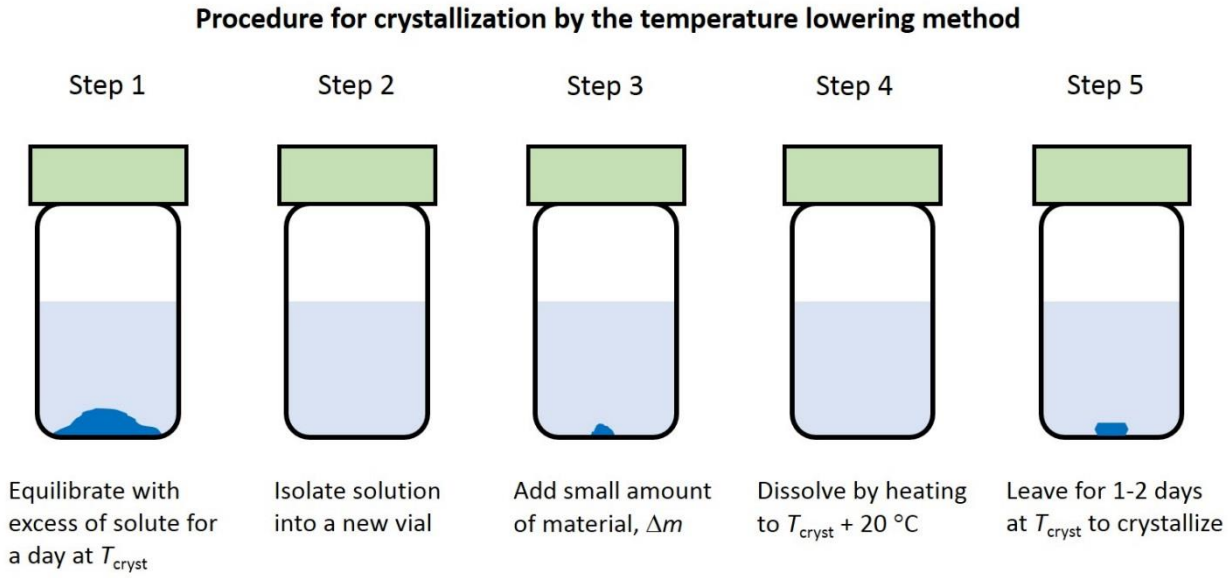
At T_{cryst} solution saturated at T_{prep} liberates mass of crystals $\Delta m = V\Delta c = V\alpha c_{eq}\Delta T$



High Δc – fast nucleation but tiny imperfect crystals
Low Δc – no crystallization

Procedure

1. Prepare solution at a constant temperature (room temperature is fine but more accurate temperature control is preferable) with the excess of material so that some material cannot dissolve and remains on a bottom. Stir solution occasionally and allow enough time (a day or two) to equilibrate solution with undissolved material. The vial should be capped all the time.
2. Carefully transfer solution without undissolved material into a clean vial. Now you have solution saturated at a given temperature.
3. Add a small amount of material into solution. This amount should correspond to amount of material you would like to crystallize (typically 3-20 mg).
4. Cap the vial, heat and stir solution until all material is dissolved. Gentle heating by 10-20 °C (must be below boiling point) can be performed even if an organic solvent is used.
5. Slowly cool the vial to the initial temperature (usually leaving a vial at room temperature suffices, but sometimes placing it into a thermos works better) and wait for 1-2 days to observe crystallization. Keep crystals in solution.



Outcomes

- *After two days crystals do not form.* This means that solution is supersaturated but nucleation does not occur. Seed the solution by opening a vial and adding a tiny piece (<< 1mg) of starting material. Wait for a day. If crystallization does not occur, the procedure was not performed correctly and solution is not supersaturated. Add more material and repeat steps 4-5 of the procedure.
- *A lot of material precipitated but crystals are tiny*. This likely means that supersaturation was too high. Remove some crystals from the vial and repeat steps 4-5 of the procedure.
- *Amount of material is not large but crystals are tiny*. Possible reasons: (1) too many preexisting nucleation centers (undissolved material, crystallites from seeding), (2) homogeneous nucleation because of high supersaturation. In the first case, one can try to repeat crystallization. In the second case, one can try to remove some crystals and repeat crystallization but this will not work if amount of material is already small. If this problem persists consider different solvent or different crystallization technique.

Solvent vapor diffusion

Principle

As vapors of a "bad" solvent (antisolvent) mix with solution prepared with a "good" solvent, solubility of the solute decreases creating supersaturation.

Advantages

- Works for small amount of material
- Crystals typically have better quality

Disadvantages

- Crystallization takes a lot of time
- A lot of limitations on solvents

Solvent selection

- Good solvent and antisolvent should be miscible
- In the presence of solute solvents should not exhibit liquid-liquid separation
- See some solvent combinations in B. Spingler *et al*. *CrystEngComm* **2012**, *14*, 751-757.

Procedure

- Completely dissolve material in a good solvent
- Place vial inside a larger vial containing antisolvent and cap it
- Wait until crystals form (days to weeks)

Vapor diffusion setup