Standard Operating Procedure

Task: Recrystallization
Date: May 28, 2014
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Potential Hazards:
- Flammable solvents
- Exposure to toxic vapors
- Contamination of box purifiers with harmful solvents
- Needles

Restrictions:
- No open flames
- For two-solvent recrystallizations solvents must be miscible
- No slow evaporation or uncovered crystallizations containing harmful solvents in box,box freezer, or outside of fume hood

Materials Needed:
- Vary, see procedures below
- If purifying a known compound, check the *Purification of Laboratory Chemicals*

Background:
- Crystallization is a fundamental chemical purification method. Growing crystals can be challenging in some cases, and straightforward in others. It can be frustrating in some cases, and immensely rewarding in others. This SOP outlines different methods of crystallization.
- Theory of crystallization can be summarized in terms of solubility, and the following methods all try to take a homogeneous solution, often near (or even over) saturation of the compound, and alter the system slowly until the solution becomes supersaturated so as to thermodynamically favor transfer of the compound into the solid phase. Solubility of a particular compound will be different in each solvent, and will vary with temperature. Methods to increase concentration, change the solvent polarity, and change the temperature will be discussed.
- The crystallization methods are broken into two categories, single solvent and two solvent methods. Single solvent methods use only one solvent medium, and rely on temperature changes or evaporation to reach supersaturation and induce crystallization. Single solvent methods are ideal for compounds that are soluble in all organic solvents. Two solvent methods use a mixture of solvents to vary the medium polarity over time, leading to a change in solubility of the compound even without a change in temperature or concentration.
- Any method can, in principle, be used for organic, organometallic, or inorganic compounds. A key differentiation is often whether the crystallization will be carried out under air or under an inert gas (nitrogen or argon). Most methods can be adapted to either situation, either in a glovebox or using Schlenk techniques.

Procedures for Single Solvent Recrystallizations:

- *Hot Crystallization (temperature change)*
    - Step 1: Glassware
        - Erlenmeyer flasks work well for recrystallizations as they are designed to minimize solvent loss by evaporation upon heating and are easily secured to be held in a heat bath
    - Step 2: Choose a solvent
        - The compound should have low solubility in the chosen solvent at room temperature, but good solubility in the solvent at elevated temperatures
            - Perform a solubility test
                - Place the tip of a spatulas worth of the compound in separate test tubes
                - Choose a few solvents that might meet the criteria above and label the test tubes with each solvent
                - Add ~0.5 mL of each solvent to the corresponding test tube and make note of any room temperature solubility
                - For samples not soluble at room temperature, use a clamp to hold the test tubes in a heat bath and make note of any solubility at the solvent's boiling point
            - If needed, sonication or heat may be used to induce dissolution
    - Step 3: Dissolve the compound in the minimal amount of hot solvent
        - Heat the solvent before adding
        - Add the solvent to the compound slowly and swirl on hot plate or in heat bath until the solution is clear
    - Step 4: Perform a hot filtration if insoluble impurities are present
    - Step 5: Crystallization
        - Cover and allow the solution to slowly cool to room temperature, then place in an ice bath, fridge, or freezer if necessary
        - If crystals do not form, try scratching the bottom of the glassware (but don't scratch glass that will be pressurized or placed under vacuum)
    - Step 6: Collect the crystals using vacuum filtration
        - Wash with cold recrystallization solvent (or something that does not dissolve the compound, but does dissolve impurities)

- *Cold Crystallization (temperature change)*
    - Step 1: Glassware
        - Scintillation vials, round bottom flasks, or Erlenmeyer flasks work well, choose based on scale and the ability to seal the vessel
        - Scratching the bottom of the glassware can help induce crystal growth (but don't scratch glass that will be pressurized or placed under vacuum)
    - Step 2: Choose a solvent
        - The compound should be soluble in this solvent at room temperature but sparingly soluble at lower temperatures (e.g. -30° C for glovebox freezers)
        - If needed, sonication or heat may be used to induce dissolution
        - If possible, choose a solvent that will either not dissolve impurities at all or will keep impurities completely dissolved at lower temperatures

- Step 3: Completely dissolve the compound in the minimal amount of chosen solvent
- Step 4: Perform a filtration if insoluble particles are present
- Step 5: Crystallization
  - Cool the solution by placing the vessel in a freezer. Check for crystals periodically, and monitor growth until no new crystals are forming (minutes, hours, days, or weeks)
- Step 6: Collect the crystals

Decant the cold supernatant or collect the crystals by filtration, then wash the crystals with small amounts of cold crystallization solvent (or another solvent in which the compound is insoluble)

- *Slow Evaporation (concentration change)*
  - Step 1: Glassware
    - Scintillation vials work well. Choose glassware that based on the desired rate of evaporation (Erlenmeyer will be slower than a crystallization dish), and keep in mind that rates of evaporation can be tuned by piercing septa with needles
    - If only a small amount of sample is available, an old/broken NMR tube with a needle through the cap works well
  - Step 2: Choose a solvent
    - The compound should be somewhat soluble in the chosen solvent at room temperature, but should be insoluble at high concentrations
    - The chosen solvent should be sufficiently volatile to evaporate on a reasonable time scale (more volatile solvents will evaporate faster)
  - Step 3: Completely dissolve the compound in the minimal amount of chosen solvent
  - Step 4: Perform a filtration if insoluble particles are present
  - Step 5: Crystallization
    - Allow solution to evaporate, which will increase the compound concentration towards the supersaturation point
      - The speed of evaporation can be controlled by cooling or warming the sample (slow evaporation tends to work better for single crystals, as crystal grow more slowly)
      - The speed of evaporation can be controlled by covering or uncovering the vessel
        - Slightly unscrew a lid
        - Put a needle (or a few) through a septa/cap
      - In the glovebox, slow evaporation must be performed in a closed system to avoid solvent contamination of others' samples. Place the evaporating solution in a small vessel inside a larger vessel containing a non-volatile solvent such as trimethylsilyl ether, then seal the whole system. The volatile solvent will be drawn into the non-volatile solvent.
  - Step 6: Collect the crystals

        - Decant the supernatant or collect crystals by filtration and wash with a solvent that does not dissolve your crystals (cold solvents occasionally work well for very soluble compounds)

Procedures for Two Solvent Recrystallizations

- *Hot Recrystallization*
    - Step 1: Glassware (*vide supra)*
    - Step 2: Choose two solvents
        - Perform a solubility test if necessary (*vide supra)*
        - Solvent 1: The compound should be soluble in this solvent at room temperature
        - Solvent 2: The compound should be insoluble in this solvent even at the solvent's boiling point, but this solvent must be miscible with solvent 1
    - Step 3: Dissolve the compound in the minimal amount of hot solvent 1 until clear
    - Step 4: Perform a hot filtration if insoluble particles are present (*vide supra)*
    - Step 5: Add solvent 2 dropwise until the solution becomes persistently cloudy
    - Step 6: Crystallization
        - Cover and allow the solution to slowly cool to room temperature, then place in an ice bath, fridge, or freezer if necessary
        - If crystallization is unsuccessful and cloudy suspension remains, gently heat the cloudy mixture on a hot plate until the suspension becomes a transparent solution. Cover and allow the solution to slowly cool as described previously
    - Step 7: Collect the crystals
        - Decant the solvent or collect crystals by filtration. Wash with solvent 2, a mixture of cold solvent 1 and solvent 2, or another solvent that does not dissolve the compound (Caution: Do not wash with room temperature solvent 1!)

- *Cold Recrystallization*
    - Step 1: Glassware (*vide supra)*
    - Step 2: Choose two solvents
        - Perform a solubility test if necessary (*vide supra)*
        - Solvent 1: The compound should be soluble in this solvent at room temperature
        - Solvent 2: The compound should be insoluble in this solvent even at the solvent's boiling point, but this solvent must be miscible with solvent 1
    - Step 3: Dissolve the compound in the minimal amount of solvent 1 at room temperature until clear
    - Step 4: Perform a filtration if insoluble particles are present (*vide supra)*
    - Step 5: Add solvent 2 dropwise until the solution becomes persistently cloudy
    - Step 6: Add solvent 1 dropwise until the solution becomes transparent again.
    - Step 7: Crystallization
        - Cover and allow the solution to slowly cool by placing it in an ice bath, fridge, or freezer.
    - Step 7: Collect the crystals

        - Decant the solvent or collect crystals by filtration and wash with solvent 2, a mixture of cold solvent 1 and solvent 2, or another solvent that does not dissolve the compound (Caution: Do not wash with room temperature solvent 1!)

- *Liquid Diffusion or Layering*
    - Step 1: Glassware
        - Minimal diameter vessels work best, including scintillation vials, old/broken NMR tubes, or any other glass tube
    - Step 2: Choose two solvents
        - Solvent 1: The compound should be soluble in this solvent at room temperature
        - Solvent 2: The compound should be insoluble in this solvent, but this solvent must be miscible with solvent 1
        - Solvent densities and polarities will play a role in the crystallization process, so multiple combinations are often necessary to find the ideal system
            - Larger difference in density → slower diffusion
            - Larger difference in polarity → slower diffusion
    - Step 3: Dissolve the compound in the minimal amount of solvent 1
    - Step 4: Perform a filtration if insoluble particles are present
    - Step 5: Crystallization
        - If solvent 1 is more dense than solvent 2, add the solution of the compound in solvent 1 to the vessel, then carefully add the less dense solvent 2 to form a layer
        - If solvent 1 is less dense than solvent 2, add solvent 2 to the vessel, then add your solution of compound in solvent 1 to form a layer
        - Layering can be challenging. Work slowly, and try not to disturb the surface of the solvent interface. It can be helpful to pipette the solvent along the walls of the flask for steady flow
        - Cover the apparatus, carefully place in appropriate environment, and leave undisturbed
            - Cooling the crystallization can slow down diffusional mixing, and reduce solubility, aiding crystal formation
    - Step 6: Collect the crystals
        - Decant the supernatant or collect crystals by filtration
        - Wash the crystals with solvent 2, a mixture of cold solvent 1 and solvent 2, or another solvent that does not dissolve the compound (Caution: Do not wash with room temperature solvent 1!)

- *Vapor Diffusion*
    - Step 1: Glassware
        - 4 mL scintillation vial inside of a 20 mL scintillation vial
    - Step 2: Choose two solvents
        - Solvent 1: The compound should be soluble in this solvent at room temperature

        - Solvent 2: The compound should be insoluble in this solvent, but this solvent must be miscible with solvent 1
        - Solvent volatility will play a role in the crystallization process, so multiple combinations are often necessary to find the ideal system
            - More volatile solvents → Faster mixing by vapor diffusion into one another
    - Step 3: Dissolve the compound in the minimal amount of solvent 1
    - Step 4: Perform a filtration if insoluble particles are present
    - Step 5: Crystallization
        - Place 4 mL vial inside of 20 mL vial and carefully pipette solution of compound in solvent 1 into the 4 mL vial
        - Pipette ~5 mL of solvent 2 into the 20 mL vial surrounding the 4 mL vial
        - Cap *only* the 20 mL vial, place in appropriate environment, and leave undisturbed so that the two solvents can establish equilibrium
            - Cooling the crystallization can slow down vapor diffusional mixing, and reduce solubility, aiding crystal formation
    - Step 6: Collect the crystals
        - Decant the supernatant or collect crystals by filtration
        - Wash the crystals with solvent 2, a mixture of cold solvent 1 and solvent 2, or another solvent that does not dissolve the compound (Caution: Do not wash with room temperature solvent 1!)