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Growing Crystals for X-ray Diffraction Analysis

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Overview

Source: Laboratory of Dr. Jimmy Franco - Merrimack College

X-ray crystallography is a method commonly used to determine the spatial arrangement of atoms in a crystalline solid, which allows for the determination of the three-dimensional shape of a molecule or complex. Determining the three-dimensional structure of a compound is of particular importance, since a compound's structure and function are intimately related. Information about a compound's structure is often used to explain its behavior or reactivity. This is one of the most useful techniques for solving the three-dimensional structure of a compound or complex, and in some cases it may be the only viable method for determining the structure. Growing X-ray quality crystals is the key component of X-ray crystallography. The size and quality of the crystal is often highly dependent on the composition of the compound being examined by X-ray crystallography. Typically compounds containing heavier atoms produce a greater diffraction pattern, thus require smaller crystals. Generally, single crystals with well-defined faces are optimal, and typically for organic compounds, the crystals need to be larger than those containing heavy atoms. Without viable crystals, X-ray crystallography is not feasible. Some molecules are inherently more crystalline than others, thus the difficulty of obtaining X-ray quality crystals can vary between compounds. The growth of X-ray crystals is similar to the process of recrystallization that is commonly used for purifying compounds, but with an emphasis on producing higher quality crystals. Often, higher quality crystals can be obtained by allowing the crystallization process to proceed slowly, which may occur over the course of day or months.

Principles

There are a number of methods for growing X-ray crystals, such as heating and cooling, evaporation, and vapor diffusion, each with its' own advantages and limitations.¹ Described herein is one of the most useful methods for growing X-ray quality crystals, liquid-liquid diffusion.² Successful X-ray crystal growth depends on the proper choice of solvents. The compound must be soluble in one solvent but insoluble in another. Liquid-liquid diffusion involves carefully layering a low-density solvent on top of a higher-density solvent in a thin tube, such as an NMR tube. The rate of diffusion can greatly influence the size and quality of the crystals- rapid diffusion favors smaller crystals, while slow diffusion favors the growth of larger and higher quality crystals. The utilization of thin tubes, such as NMR tubes, slows down the diffusion of the solvents, thus creating an environment that facilitates the growth of higher quality crystals. Commonly used solvents for the lower layer, which the compound is dissolved in, are methylene chloride or chloroform. The compound is dissolved in the less dense solvent, but this can prove problematic as the top solvent can start to evaporate prior to crystal formation. The optimal condition is to have the compounds dissolved in the more dense solvent. The top layer is the anti-solvent or precipitant. Frequently used anti-solvents are hexane, pentane, diethyl ether, or methanol. Once the two solvents have been carefully layered, they are allowed to slowly diffuse into one another. The compound becomes less soluble in the binary solution, facilitating the formation of X-ray crystals.

Procedure

1. Preparation of the Crystal Tube and Filter

1. Place an NMR tube in an Erlenmeyer flask.
2. Prepare a pipette filter.
 1. Construct the filter by placing a piece of lint-free wipe (1 in. by 1 in.) in the pipette, then use a rod to firmly wedge the wipe into the bottleneck portion of the pipette (**Figure 1**).
 2. Make two pipette filters for every crystal tube needed.

2. Adding the Sample to the Crystal Tube

1. Dissolve the compound (tetraphenylporphyrin, 10 mg) in 0.75 mL of solvent (dichloromethane).
2. With a pipette, gently add the mixture to the top of the tube, by passing it through the filter.
 1. The particles are filtered out to avoid the creation of nucleation sites, which can lead to small multiple crystals instead of the desired larger single crystals.
3. Once the sample has been placed in the crystal tube, very slowly and gently, add the anti-solvent (1.5 mL of methanol) to the tube through a new filter pipette. Allow the anti-solvent to slowly layer on the previously added solution (**Figure 2**). Do not use a bulb to push the solvent through the pipette, instead allow the solvent to flow through the filter by itself.
 1. Make sure that solvent of higher density is added to the crystal tube first.
 2. Check that the two solvents are miscible with each other. This is done prior to addition of the solvent.
4. Seal the tube with an NMR cap.

3. Crystal Growth

1. Without causing the two solvents to mix, place the crystal tube(s) in a cabinet where they will not be disturbed.
2. Crystallization time will vary with each compound- typically the crystal tubes should be left undisturbed for a week.
3. After a week, inspect the tubes for crystal growth.
 1. Crystal growth typically occurs at the interface of the two solvents.
 2. Visually inspect the tubes for evidence of crystal growth. Be careful not to facilitate mixing of the solvents, in case the compound requires additional time for crystal formation.
 3. If it appears that crystal growth has occurred, further inspect the tubes using a microscope.

4. Crystal Selection

1. X-ray diffraction crystals should have well defined faces.
2. Crystals that have clustered together should be avoided if possible.
3. Leave the crystals in the crystal tube until ready to harvest the crystal, immediately prior to placing the crystal on the diffractometer.
 1. Keeping the crystals in the tube will ensure that the crystals remain solvated. De-solvation can cause the crystals to crack, and hinder the diffraction of the crystal.

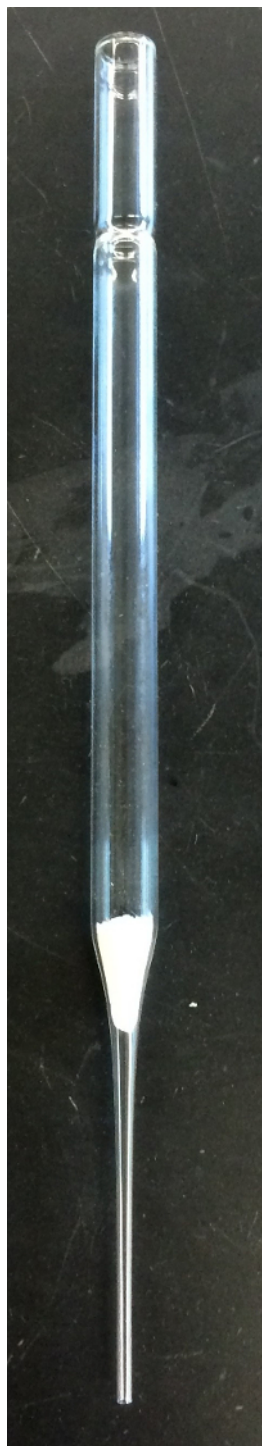


Figure 1. An image of the pipette filter. A small piece of lint-free wipe has been firmly wedged at the bottleneck of the pipette. The solutions are passed through these pipette filters prior to being introduced to the crystal tube.



Figure 2. Once the solution containing targeted compound is placed in the crystal tube, the anti-solvent is slowly layered on top by passing it through a new pipette filter.

Results

The technique of liquid-liquid diffusion was used to create X-ray quality crystals of tetraphenylporphyrin. Using dichloromethane as the solvent and methanol as the anti-solvent, the liquids were allowed to slowly diffuse over the course of a week without being disturbed. Large, well-defined, dark purple-reddish crystals formed at the interface of the two solvents (**Figure 3**). The growth of the crystals can be visually observed. The crystals grew with very well defined faces, which can be seen with a microscope.

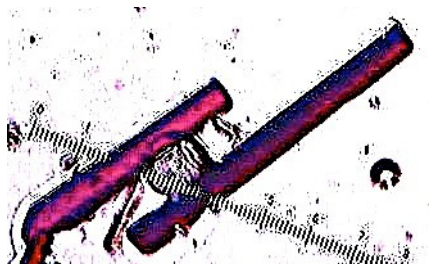


Figure 3. X-ray diffraction quality crystals of TPP. Crystals that are clumped together or that are growing out of one another should be avoided. Large single crystals with well-defined faces typically yield better results.

Applications and Summary

X-ray quality crystals can be grown by liquid-liquid diffusion. The slow diffusion of the binary solvent system allows for the creation of crystals suitable for X-ray diffraction. This method allows the crystal lattice to form slowly, often leading to larger and more well defined crystals. The use of NMR tubes facilitates the slow diffusion of the solvents, allowing for optimal crystal growth. This process can take anywhere from a few days to several months. Often during the crystallization process solvent molecules are incorporated into the crystal lattice. Thus it is important to avoid allowing the crystals to "dry out". Thus, one of the advantages of liquid-liquid diffusion is that the crystals typically grow at the interface of the two solvents, which circumvents this phenomenon.

Liquid-liquid diffusion is one of the most useful techniques for producing X-ray quality crystals, which is the most essential component of X-ray crystallography. Obtaining X-ray quality crystals is typically the limiting factor on conducting X-ray crystallography experiments. X-ray crystallography essentially creates a three-dimensional picture of a molecule's structure, making it the least ambiguous method for determining the complete configuration of a compound. Since structure and function of molecules are intimately related, the ability to decipher a compound's three-dimensional structure is extremely useful for a variety of chemical and pharmaceutical applications. Researchers and pharmaceutical companies use X-ray crystallography to determine the structure of proteins to examine how small molecules interact with enzymes for the purpose of drug discovery and design.³⁻⁵ X-ray crystallography is also one of the most useful methods for evaluating metal complexes. This technique divulges valuable insight on how metals interact with each other and its ligands. The first ever identified quintuple bond between two chromium atoms was identified using X-ray crystallography.⁶ This technique can also be used to explain the luminescent properties of metal complexes.⁷ Crystallography has also been widely used in host-guest chemistry, as this method has been instrumental in revealing valuable information about non-covalent interactions between molecules.⁸

References

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