X-Ray Crystallography Laboratory
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Getting Crystals Your Crystallographer Will Treasure

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Table of Contents

What is a crystal structure?

The definition of a crystal Structure.

The determination of the connectivity of the atoms in a compound and the way the molecule (or molecules) pack to form a solid crystalline material.

What information do we get?

A crystal structure provides positive identification of a single crystal taken from a pure batch of material. This provides absolute proof (*provided it was done properly*) that the compound or complex is the stated material. It provides the exact connectivity of the atoms and the bond distances and angles between these atoms in the solid state which results in the complete identification of the compound. It also provides Inter and Intra molecular interactions which may provide insight into the chemistry and properties of the compound.

Why have crystal structures become so popular?

Rarely incorrect and now faster to achieve results! With the advance in technology for x-ray crystal structure determination and the increased speed of computers, single crystal studies are rapidly becoming more routine. The ease of new programs makes the routine structures quick and easy for even non-specialized scientist to be able to perform this analysis. The positive identification of the compound leaves no interpretation of the data leading to incorrect assignments of the structure. It also can answer basic questions regarding bonding within the molecule which can explain the chemistry and properties that exist.

What do we need to bring to the Laboratory?

A single crystal is required in the determination of an x-ray Structure. A single crystal consists of atoms which possess long-range three-dimensional order. Typically appear as regular polyhedral shapes with well defined boundaries. Examples include: Table salt, sugar, gems, quartz and metals.

We can not perform analysis on non-crystalline materials. These amorphous materials contain only short-range order, or random ordered atoms. Example: Glass.

Twined crystals are usually thought of as single crystals that are grown such that they contain a boundary between them. Twinned crystals are for the experienced crystallographer and should be avoided if possible.

Crystal size

Ideal size of a crystal is one which occupies the entire x-ray beam, here at MSU the beam is 0.5 mm generally. This means that the ideal crystal would be a sphere 0.45 mm in diameter. Although this is the ideal size, one can perform x-ray determination on smaller or larger (by cutting) crystals. The capabilities of this depend on the x-ray source, the arrangement of the atoms in the lattice and what atoms are there as well as the diffraction power of the crystals. Unfortunately, the diffraction power of crystals is still relatively unknown until you try the crystals in the diffractometer.

Unfortunately, the shapes of crystals depend on both the internal symmetry of the material and on the relative growth rate of each of the faces. In general, the faces of the crystal that grow most rapidly are those to which the crystallizing particles are bound most securely. These rapidly growing faces are usually the smaller, less well-developed faces. Thus, the larger faces are usually associated with directions in the crystal where there are only weak intermolecular interactions.

Where to start?

Concept of crystal growing

Properties of the compound

Solubility is the single largest and most used property needed to grow a single crystal. Generally, one knows a fair amount of this from the synthesis and other aspects of working with the compound. Stability and reactivity need to be considered. One does not want to cause a reaction with the compound of interest in the solvent system that you are trying to grow the crystals.

Simple recrystallization is usually the first step in growing a good crystal. It is very important that the sample be pure and can be a solid! If you get oils, this could mean that the sample is not pure since contaminants often lower the melting points of solids and can cause them to be oils. So, first thing to do is look at the recrystallization you performed to make the solid the first time. Did you get a solid and are these crystals well defined and good enough for x-ray study?

If these are not good enough crystals, then the method you chose depends greatly on the physical and chemical properties of the sample. Solution methods require solubility of the solute in various solvent systems. Thermal, chemical, and melting properties can also play a major role in choosing a method for crystal growing.

Patience is the major thing you need to remember.

How much material is needed?

The simple rule is that you only need one single crystal. The concentration of the solutions tends to be near what you would expect in order to run an NMR experiment. The most important issue is that the compound is insoluble in the final resultant mixture of solvents that is attained in the vessel of choice. Note: New instruments can do crystals 0.1 x 0.1x 0.1 mm or smaller.

If the crystal for x-ray diffraction is to be 0.3 x 0.3 x0.3 mm, volume = 0.027 mm^3

Typical unit cell is 12 x 12 x12 Å; volume = 1728 $Å^3$

Å = 10^{-10} meters = 10^{-8} cm = 100 pm (picometers)

Therefore, in a typical crystals 1.6 x 10^{16}-unit cells

1.3 x 10^{17} molecules for 8 molecules per cell.

MW= 206.2 then only 2.49 x 10^{-7} moles in the cell. 5.1 x 10^{-5} g, 0.051 mg

Unfortunately, more than one crystal grows in the vessel, so more material is needed.

Typically use a concentration that you would use in an NMR experiment.

What do I grow the crystals in and where?

Clean glassware is very important. The use of new glassware sometimes results in problems due to the lack of nucleation sites (see *crystal growth*), but this can also be helpful. Some crystallographers suggest that the new glassware contains a "variety of dusty contaminants" from the manufacturing process. This has not been observed here in my experience. Most solution methods growing the crystals in vials that can fit inside one another are usually a good idea.

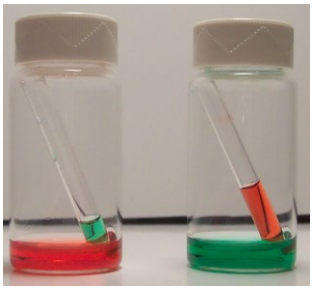
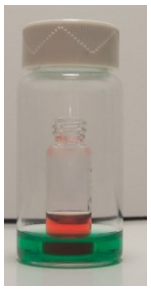
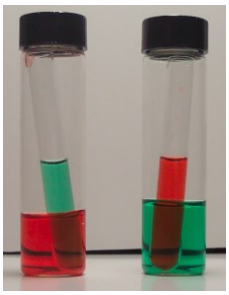
Consider the location of the set-up. You want the setup to be located out of the way, avoid vibrations and disturbances. Set it up so you can see if there are crystals growing without having to move the apparatus. Note if you grow them near a heater or cooler, in the sun or not. All these can change the way crystals are formed.

Keep the container covered so that no dust or dirt can enter and cause crystallization.

The use of vials that fit inside each other allows for the three most common experiments to be tried. The center vial, where the solute of interest is dissolved consists of either a glass tube or small flat bottom vial. Round tube has the advantage that they keep the material concentrated longer. These work well for complexes that tend to be round or ball shaped. These are usually tried first here at MSU. Flat vials work better for more flat materials, and sometimes for compounds that form needles.

The outer vial is such that you can tighten the cap or have it loose (slow evaporation) but dirt and dust does not get in the system. The cap should be resistant to solvents.

Unfortunately, the choice of vial does not follow the above general guidelines. So, if you have trouble with one system, try the other, exceptions have been noted here, In my experience.

Solvent Choice

Consider your solvents carefully. Like dissolves like.

Remember if the compound is polar, then polar solvent with the compound is layered with non-polar solvents.

Avoid solvents in which your compound forms supersaturated solutions since these solutions tend to give crystals which are too small in size (micro crystals).

For compounds soluble in non-polar solvents, evaporation may be the best or layering with polar solvent, this is harder to accomplish.

Hydrogen bonding is very important in the crystallization process. Hydrogen bonding provides energy to the lattice and generally better packing, but not always. Consider whether a hydrogen bonding solvent might help or hinder the crystallization. Amides generally do better with hydrogen bonding solvents.

It is amazing that some solvents tend to direct crystal growth better than other solvents. Benzene is such a solvent. We have had lots of luck using some benzene in the solvent mixture to generate x-ray quality crystals. The aromatic rings fill holes that may form in the lattices, but most of the time, we do not see the benzene co-crystallized with the compound. For organic complexes ethyl acetate works well.

Avoid highly volatile solvents, CH_2Cl_2 and diethyl ether. Unfortunately, these often work very well. They also tend to lead to creation of crystals by slow evaporation.

Avoid long alkyl chains in the solvent, these cause disorder in the lattice if solvent is trapped in the lattice, since there are many conformations allowed and therefore all atoms are not in the same place throughout the lattice.

Table 1. **Table of typical solvents used for growing single crystals of organic compounds.**

Water – alanine, organic acids
Ethanol (CH_3CH_2OH) – Soluble hot, insoluble cold
Methanol (CH_3OH) – Soluble hot, insoluble cold
Iso-propanol
Dichloromethane (CH_2Cl_2) {DCM}
Acetonitrile (CH_3CN)
Isopropyl Acetate ($CH_3C(O)O(C(CH_3)_2)$)
Toluene ($C_6H_5CH_3$)
1,2 - Dichoroethane ($ClCH_2CH_2Cl$) (replacing DCM)
Ethyl Acetate ($CH_3CO_2C_2H_5$)
Acetone, ($CH_3(CO)CH_3$)
Benzene (C_6H_6)
Tetrahydrofuran, THF (C_4H_8O)
Methylformate
Hexane(s)
Heptane
Pentane
Diethyl Ether
Petroleum Ether
Cyclohexane
Cyclopentane
dioxane

Rare solvents to try.

1,1,1-trichloroethane (instead of DCM)
Methylcyclohexane (instead of Cyclopentane)
Methoxybenzene
Pyridine
Dilute HCl. Or phosphate buffered PH, acetic acid, trifluoroacetic acid, formic acid
DMF Often to soluble, no material crystallizes out; used when compound insoluble in all Other choices (inorganic clusters)
DMSO Often to soluble, no material crystallizes out; used when compound insoluble in all other choices (inorganic Clusters)

To help get organics and peptides to dissolve in water, use of solubilizing agents, alcohols, acetonitrile, Dichloromethane, DMSO can be used.

A list of 107 solvents given by: B. Spingler, S. Schnidrig, T. Todorova and F. Wild Cryst. *Eng. Comm.*, **2012**, *14*, 751-757.

Crystal Growth

Producing good quality crystals of a suitable size is the first and most important step in determining any crystal structure. Crystallization is the process of arranging atoms or molecules that are in a fluid or solution state into an *ordered* solid state. This process occurs in two steps, nucleation and growth. Nucleation may occur at a seed crystal, but in the absence of seed crystals usually occurs at some particle of dust or at some imperfection in the surrounding vessel. Crystals grow by the *ordered* deposition of material from the fluid or solution state to the surface of the crystal. More information on crystal growth: *Crystal Growth of Organic Materials*, edited by Myerson, Green, and Meenan, ACS Proceedings Series, 1996.

The main focus for growing crystals is to create an environment that changes slowly over time. This change should produce an environment in which the compound becomes supersaturated and eventually grows a solid, crystal material. This change in environment is most generally accomplished (with small molecules) by addition of a second solvent in which the compound of interest does not dissolve.

Changing the nucleation process is the largest thing one can do, one avoids dust or glass fragments (from pipette) to be the nucleation site. If using new glass and getting lots of small crystals, scratch the glass to create only a few sites so the crystal might grow larger.

If a sample only yields small crystals, the method should generally be altered so as to slow down the growth step. Slowing the crystal growth sometimes requires changing the method used to grow the crystals. Or lowering the temperature at which the crystals are grown.

Physical disturbance of the crystal growing vessel can result in smaller crystals being formed. Choose a location to grow the crystals where there are no vibrations from elevators, doors, rotovaps, vacuum pumps etc… You should set the crystals where you can view them without having to move them, or if you do, wait one week before checking on the crystals.

Patience! Some methods work in a few hours, and other methods require weeks or even months for success.

CRYSTALLIZATION METHODS

The techniques chosen will largely depend on the chemical properties of the compound of interest: Is the compound air sensitive, moisture sensitive? Is it hygroscopic? Can it form hydrogen bonds, does it react with certain solvents etc.?

VAPOR DIFFUSION

This is by far the best crystallization method to use. Very good when only milligram quantities are available. Requires volatile solvents but done properly one generates a less desirable solvent system which then allows for slow crystal growth.

Vapor diffusion is carried out by dissolving a small amount of the sample in a small vial, then placing this inner vial inside a larger vial that contains a small volume of a solvent system in which the sample is insoluble. The outer vial is then sealed. **DO NOT DISTURB THE**

VESSEL. Vapor from the solvent of the outer vial then diffuses into the solution in the inner vial, causing the compound to grow crystals. The vertical surfaces of the inner vial should not touch the outer vial to keep the outer solution from rising by capillary action and filling the inner vial.

Sometimes this is combined with slow cooling or placed in a fridge to slow the diffusion of the solvents, giving more time for the crystals to grow.

NOTE: If a paper says the diffused Hexanes into dichloromethane, in reality they did a controlled evaporation.

Potential Solvent Choices for Vapor Diffusion.	
Solvent	Anti-Solvent
Water	Dioxane
Methylene Chloride	Diethyl Ether (Hexanes-controlled evaporation)
Methylene Chloride	Cyclopentane
Acetonitrile	Pentane or Ether
Ethyl Acetate	Hexane or pentane or Ether
Isopropyl Acetate	Ether
Ethanol	Cyclohexane
methanol	Hexane
Methylformate	Cyclopentane (Hexanes-evaporates)

SOLVENT LAYERING

This is a simple concept. You layer one solvent over top of a second solvent. The two solvents should be miscible in one another. One solvent your compound is insoluble, the other it is soluble. Dissolve some of your compound in the soluble solvent and then layer the two *very carefully*. You must have solvents that can be layered, enough of a difference in properties that an interface develops between the two solvents as you set it up. **DO NOT DISTURB THE VESSEL**. Sometimes a third solvent is used to create a buffer to slow the diffusion rate, which controls the rate of crystallization. Use benzene or toluene at the interface! Rate of crystal growth depends on concentration level and solubility of the compound in the resulting mixed solvent system.

Sometimes this is combined with slow cooling or placed in a fridge to slow the mixing of the solvents, giving more time for the crystals to grow.

SLOW EVAPORATION

Evaporation is by far one of the easiest methods for crystallizing organic and organometallic small molecule compounds. The choice of solvent is important because it can greatly influence the mechanism of crystal growth, when the crystal begins to form and because the solvent may be incorporated into the crystalline lattice. The rate of crystal growth can be slowed either by reducing the rate of evaporation of the solvent, less open area or by cooling the solution. Keep the solution clean by covering it, simple thing to use is a Kimwipe, but some slow the process by putting a rubber septum in then inserting a needle.

If this method provides an oil, this could be not because the compound is impure, but the compound is too soluble in the solvent chosen for evaporation.

This method does not generally provide the best crystal, since the crystallization proceeds only when there is only a small amount of solvent left, causing the crystals to grow upon each other. Also the crystals tend to adhere to the glass walls, which can make it more difficult to retrieve the crystals without damaging the crystals.

Use of mixed solvents can be done to help the crystallization, i.e. dichloromethane solution with some heptanes will evaporate causing an increase in the heptanes concentration and then possibly crystallization.

SLOW COOLING

This is the standard recrystallization method. This can work very well; follow the rule soluble hot, insoluble cold. Remember here we want to have the crystals form very slowly. We do not mind if material is still left in the solution, we want the nice formed solid, not good yield. Slow reduction of temperature works the best.

To generate reduced temperature slowly, isolation of the material from environmental conditions can help, although generally though you most often put these crystals in the fridge or freezer. To reduce the time for the vial and solvent system to cool, one can place the crystallization vial into

another container. Some people use a Styrofoam box, others a Dewar with foam lid. We find that a jar with cotton (or absorbent material from shipping) in the bottom works well since you can still see if crystals are growing without disturbing the crystals. Sometimes this is hard with cotton and some students use a plastic petri dish as isolation. This works better than the glass which conducts the cold quicker.

Another route to using slow cooling is to work with saturated solutions at high temperature, filter away hair and dust particles. To get the slow cooling, one can transfer to an oil or water bath that is near that temperature then allow both to cool. I prefer to have the oil bath used to warm it up and dissolve the material at the high temperature and then shut off the heat and let the oil bath and crystallization vessel cool slowly to room temperature. Since we are cooling the oil and the vessel it is a very slow process and this can generate some very nice single crystals. **CAUTION:** *The oil will be hot and can burn and the compound understudy should be known to be stable and not give off toxic fumes when heated.*

USE OF NMR TUBE

Often crystals have been received by allowing the solvent to evaporate slowly from the NMR tube. The cap fit tight enough to keep dirt out but allows evaporation of the solvent and crystals form.

SUBLIMATION

This is probably the best method for getting x-ray quality crystals. Unfortunately, this cannot be performed for very many compounds and must be performed very slowly and with a small amount of material to get good results. Need to be careful not to have new crystals forming on already formed single crystals.

CHIRAL COMPOUNDS

Chiral compound tends to be more difficult to crystallize than racemic compounds. Nature prefers a center of inversion. Try to make derivatives which possess phenyl rings. If absolute configuration is needed, try and have heavy atoms.

S-Alpha-Methylbenzlamine is good to use with carboxylic acids, can be generated from alcohol or aldehydes. Cheap and usually easily crystallized. Provides one known center, and then can determine other centers.

Improve heavy atom and crystallization.
Have heavy atom present such as Bromide or Iodide (Si, Cl S also work). For alcohols and amines, you can make a derivative using *p*-Bromobenzoate. This usually increases the ability to form good crystals as well as determination of chirality. Include aromatic components in derivative when possible.

THERMAL GRADIENT

Thermal gradient methods can produce very high-quality crystals. Such methods include slow cooling of sealed, saturated solutions, refluxing of saturated solutions, and gradient (zonal) heating. Gradient heating is used primarily for crystallizing solid solutions or mixtures. Small crystals may sometimes be grown larger by zonally refluxing a supersaturated solution. Larger crystals may be grown either by decreasing the thermal gradient or by cyclic heating and cooling of the sample.

Thermal gradient heating sometimes works indirectly. If you set you crystallization apparatus by the cooling vent, one side of the apparatus is cooler than the other and this changes the crystallization properties and can cause crystal formation.

Another way to generate temperature change, both warm and cold, is the use of a programmable incubator. This allows you to be able to lower the temperature, get a couple of nucleation sites, and then warm slightly to get back into the crystal growth phase of crystallization. Annealing is also possible and sometimes provides good crystals. (Annealing= ramp between cooling and warming cycles)

See WWW site for more details: Dr. Paul D. Boyle, http://xray.chem.uwo.ca/crystal_growing/GrowXtal.html

COUNTERIONS OR IONIZATION

Probably the best thing one can do to promote crystallization of an anion or cation is to change the counter Ion. Counter ions which are generally the same size usually pack well.

The counter ions most likely to cause difficulties are Et_4N^+, Bu_4N^+, BF_4^-, and PF_6^-. Some alternative counter ions that are usually ordered are triflate, BPh_4^-, Me_4N^+, $(Ph_4P)_2N^+$, and Ph_4As^+.

If the compound is neutral and does not crystallize or is liquid, consider creating an ion. Deprotonation or protonation can be performed to generate a salt which then may crystallize. Good to confirm the identity of the material.

CO-CRYSTALS AND CLATHRATE

Some have had success with growing compounds in the presence of other compounds, or co-crystallization. This incorporation of another molecule typically occurs with the solvent of crystallization.

The use of triphenylphosphine oxide (TPPO) has been seen to be a useful co-crystallant for some years in inorganic chemistry and has been reported to be useful for organic molecules which are proton donors. (See *J. Amer. Chem. Soc.* **1988**, *110*, 639- 640).

A final group of co-crystals can be thought of as being formed by incorporating the compound of interest or guest molecule into the small vacant regions in the lattice around large, rigid host molecules. This lattice of host/guest molecules is called a clathrate. Structures of porphyrin-based clathrates are very common.

REACTANT DIFFUSION

This is performed when the compound is very insoluble and difficult to work with after it is formed. Perform the final reaction on a small scale compared to the surface area of the two reactants. Layer one reactant on the top of the other reactant and allow diffusion to control the reaction rate and crystal formation.

The other unique way might be to use a U-tube. If you put the glass frit in the bottom of the U-tube, then put one reactant on one side and the other reactant on the other, the two would diffuse through the frit and possibly form crystals as they react. Type of frit can control the diffusion rate.

MACRO METHODS

Protein crystallographers use different techniques to grow their crystals. Some people have used these techniques to grow single crystals of small molecules. Current limits on this technique are the molecule should have a high solubility in water or alcohols or mixture. Advances are being made by companies to help crystal growing of small molecules using these techniques, even with small amounts of organic solvents in the solution.

See Hampton Research, http://www.hamptonresearch.com/

This technique is to saturate the solution with smaller cation or anion than your compound (complex), this then will slow crystallization done, but the larger compound comes out of solution leaving the solution saturated with small anions (cations) that you are not interested in. This is essentially how protein people grow their crystals, the salts and buffer occupy the solvent leaving the protein no choice but to fall out of solution, either as crystal or as another solid form.

See for example: *Principles of Protein X-ray Crystallography*, by Jan Drenth, 2nd ed., Springer-verlag, New York, 1994. ISBN 0-387-98587-5.

SEEDING THE SOLUTION

This is a useful method when one of the other methods provides crystals that maybe of reasonable quality, but they are too small to give proper diffraction. Collect some of the crystals, with the mother liquor (it is best if the seed crystals do not dry out), then deposit these (CAREFULLY) into a fresh or newly created saturated solution.

Seeding a solution with similar crystallized material can also work. Sometimes you have a similar compound that gives good crystals and you can use one of these crystals as a seed.

ODD METHODS

There are many odd methods that have been known to work. Some of these methods have proven to be the only way to get single crystals of the material.
The use of siliconized glassware as crystallization vessel can help when large number of micro-crystals is formed. This can reduce the number of nucleation sites as well as help when the crystals received like to adhere to the glass walls of the container and therefore do not allow collection of the crystals. Sometimes this will reduce the number of nucleation sites. I have personally had success with these types of vials. (Aldrich sells some and there are procedures you can find for doing this:: https://doi.org/10.1002/0471142735.ima03ks21 or contributed by Brian Seed in Current Protocols in Immunology (1997) A.3K.1-A.3K.2 Copyright © 1997 by John Wiley & Sons, Inc)

Melting the compound and letting it recrystallize! This can be tried in a melting point tube, once the sample is melted, turn off the heat and leave the tube in place to allow for slow cooling.

Some people have reported that they have been successful by crystallizing the compound in the presence of a boiling chip, glass chip and even in some rare cases in the presence of some crystalline **mineral powders**.

Key Factors to Good Crystals

- Solvent- Choosing the right solvent or solvent system is very important.
- Nucleation- generating only enough nucleation sites that you get a few large crystals and not lots of small ones.
- Mechanics-the physical method that takes place to get the crystal, diffusion, evaporation, gas-solid change. The location of the apparatus that is growing the crystal.
- Time-the longer it takes to grow the crystals generally the better. Unfortunately, this does not always apply.
- **Patience, Patience**

Crystal Selection and evaluation

Evaluation starts at the microscope. Are the crystals regularly shaped and have well defined edges and no obvious dislocations? Are they big enough to consider usable on the instrument? Sometime this is a matter of practice to see what size will or will not be suitable with a particular instrumental setup.

All the crystals should appear to be the same, if there are a few very nice ones and most are poor (or shape differs) then one of three things exists.

1) If the compound is chiral, then the very nice crystals will probably be those of the trace amount of racemic compound present or is an impurity.
2) Could be that there is more than one compound in the bulk material. This could be caused by decomposition during crystal growth or synthesis
3) Could be that the compound has two or more different packing arrangements that are similar in energy for the solvent system/crystallization used. Polymorphs

Do they look crystalline and single under cross polarized light? As you rotate the polarizer, the crystals should turn light to dark (all the crystal) at some point. One can often see cracks, dislocations, and even twinned crystals clearly under the polarized light. If there appears to be a rainbow effect of colors, then they may not be single. Do not give up yet. If the crystals appear to be large and no pieces on them, or cracks, then typically we try the crystals. Occasionally these types of crystals work, although not very often.

Mount and evaluate the crystal on the diffractometer is the only way to know for sure whether the crystal will diffract or not. This requires about 20 - 30 minutes. Once you know what to look for in evaluation, experience, this can even take less time.

Crystal Mounting General

Crystal mountings must be rigid to hold the sample in the same orientation and must minimize the amount of extraneous material that is in the incident and diffracted beam paths. The sample support is usually made from an amorphous material such as glass that is held in a metal pin and clamped on a goniometer head. Solid glass fibers may be used; however, fibers pulled from glass tubing are actually small capillary tubes and are more rigid than solid glass fibers. These narrow tubes also place less non-crystalline material in the X-ray beam path than solid fibers.

Air stable crystals run at room temperature are glued (epoxy, using super glue (acetone based and dissolves many organics), Duco/amyl acetate, etc.) to the end of a glass fiber. The sample should be mounted with its smallest surface on the end of the glass fiber to minimize absorption effects and to minimize background scattering from the sample mount. Avoid mounting the crystal along the side of glass fiber, making the crystals appear as a flag on a flagpole. Keep the crystal at the tip to the fiber. This can be helped by hanging the crystal upside down while the glue dries. Slightly air or moisture sensitive crystals can be done at room temperature by coating the crystal in epoxy or placing them in capillaries. Use of low temperature data collection is preferred to these options.

A low temperature data collection procedure gives a better data and is the norm in x-ray analysis.

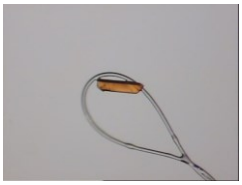
Mildly air unstable compounds can be coated with epoxy or an inert viscous material such as Paratone N™ or Krytox™ oil. These mountings are usually carried out in an inert atmosphere such as a dish filled with argon (nitrogen) gas. The crystal is further kept from reacting during data collection by cooling the sample in a chilled, inert (nitrogen) gas stream. The cold stream also holds the crystal in place by freezing the crystal in place on the fiber or loop. See mounting at MSU.

Very reactive compounds must be mounted in a glove bag or glove box and sealed in capillary tubes. Crystals of these compounds are usually wedged in capillary tubes or are held in place by a small amount of grease. Capillary tubes containing unstable compounds must be sealed by melting the ends of the glass tube.

Capillaries introduce two kinds of problems. The curvature of the capillary distorts the image of the crystal when centering the sample on the diffractometer. Also, the glass itself significantly increases both the background scattering and the absorption of the incident beam of X rays. It is crucial that the capillaries be made from thin glass similar to that found in commercially available capillaries. Thick glass capillaries absorb X rays so much that very little scattered radiation will leave the capillary.

Nylon Loops have begun to be used by small molecule crystallographers, although macromolecular crystallographers have used them for years, because of the ease of mount very small crystals in the loops and the low background they provide. With the advance in detectors, the smaller crystals are now more routinely studied. The loops provide stability in the low temperature stream. One must be careful that the loop size is not such that the crystal bends in the low temperature stream and causing the crystals to move within the x-ray beam. Experience has shown that loops made from 0.2-micron nylon and are 0.1 – 0.3 mm in diameter are suitable for many small crystals.

Recent uses of Lithio Micrographs are being used. These are excellent in many applications but are more fragile than nylon loops. Sold by Mitegen (www.mitegen.com) in various sizes and configurations are thought by some crystallographers to be better than standard loops. Although the background appears lower, they have not held up to routine use in my labs over the last year. They are excellent for very small crystals. **THEY ARE A MUST FOR THE SYNCHROTRON DATA COLLECTION OF SMALL MOLECULES.**

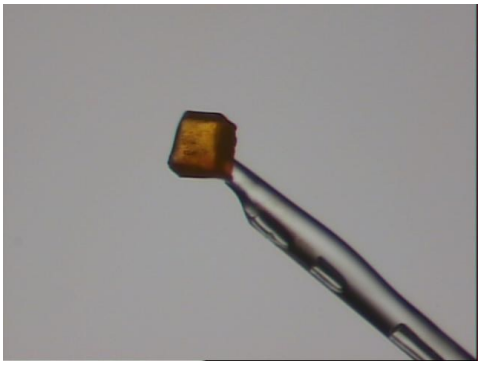


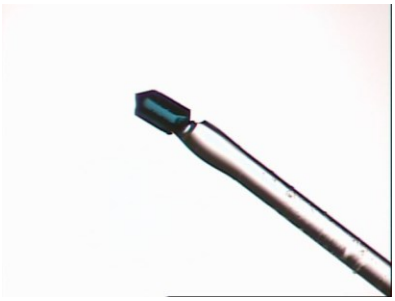
Crystal Mounting at Michigan State University Small Molecule X-ray Laboratory

We mount most crystals with fine glass/quartz fibers which are attached to the copper mounting pin held on by a magnetic base. We routinely used epoxy as the means to attach the glass fibers to the copper pins. After selecting our crystal in a small amount of paratone oil, we then holding the mounting pin by hand, push the crystal with the fiber out of the oil. This will remove excess oil from the crystal and leave a small amount that will allow you to pick up the crystal off the slide. Sometimes you may need to flip the crystal to remove the oil from the other side of the crystal. With practice you can then get the crystal to adhere to the end of the fiber or in a nylon loop. Some rare cases you may need to use a heavier grease to get the crystal to remain on the fiber, apiezon-T grease works. The pin is then carefully placed on the goniometer in the low temperature stream on the diffractometer.

A method for mounting air sensitive crystals in this lab is as follows. For low temperature and/or sensitive compounds, the crystals can be handled indefinitely at room temperature and briefly in room air by using the method of Hakon Hope (ACS Symposium Series No. 357, *Experimental Organometallic Chemistry: A Practicum in Synthesis and Characterization*, Chapter 10, Handling of Reactive Compounds for X-ray Analysis, pp. 257-262, 1987) using Exxon Paratone-N oil. Here the crystals (either dry or along with their mother liquor) are placed on a microscope slide and covered with the Paratone-N oil. The selected crystal is maneuvered into the oil and the mother liquor and/or other crystal fragments are stripped off by pushing the crystal around in the oil or cut with a scalpel. Using a clean glass fiber (already attached to a long-tapered copper pin) pick up the crystal and remove it from the oil. In so doing, the crystal and the tip of the fiber are covered with oil. Excess oil can be removed by wicking, do not remove to much or you can cause decomposition. When the crystal is placed in the cold gas stream, the oil becomes very rigid and provides both the glue and the protective coating for the crystal.

The glass fibers are obtained by drawing thick-walled capillary tubes using the very localized and hot flame from a torch.





Limitations to Crystallography

- **Requires single crystals**. This by far is the greatest limitation to x-ray diffraction analysis. No crystal, no information.
- Crystal quality governs the quality of results obtained.
- Only one crystal of the bulk material. Remember that we are looking at one small crystal in the entire bulk of the material.
- Chirality determination is dependent on how good the crystal and data, as well as contents of the asymmetric cell. Easily determined if one chiral center is known. Newer instruments can sometimes generate data sufficient with only C, and O atoms present.

Sample holding, vials: ½ Dram VWR # SC66011-020

Tube, Culture, Rimless Pk 72 6 x 50 mm VWR # 6820-068

Second solvent/ environment/ dirt reducing vial.

Vial S, Scint, VWR Tinfoil VWR SC 66022-106A

Be sure you get the caps.

Table of vapor pressures.

The lower number (solvent) will diffuse into the higher number (solvent).

Solvent	Vapor pressure
Water	21.0
Diethyl ether	34.6
Pentane	36.1
Dichloromethane	40.7
Acetone	56.5
Chloroform	61.3
Methanol	64.1
Hexane	68.7
Ethyl acetate	77.1
Ethanol	78.4
Benzene	80.1
Acetonitrile	81.8
Heptane	98.4
Toluene	110
Octane	125

Literature to crystal growing

"*Crystal Growing*", Peter G. Jones, *Chemistry in Britain*, *17* (**1981**) 222-225.

See WWW site by Dr Paul D. Boyle, http://xray.chem.uwo.ca/crystal_growing/GrowXtal.html

Crystals and Crystal Growing, Alan Holden and Phylis Singer, Anchor Books-Doubleday, New York, **1960**

The Growth of Single Crystals, R. A. Laudise, Solid State Physical Electronics Series, Nick Holonyak, Jr. *Editor*, Prentice-Hall, Inc., **1970**

Protein Crystallization Techniques, Strategies, and Tips, Terese M. Bergfors, International University Line, **1999-2000**, ISBN 0-9636817-5-3

Hampton Research Catalog, many good discussions regarding crystal growth and crystal growing. http://www.hamptonresearch.com/

"*Some thoughts about the single crystal growth of small molecules"* B. Spingler, S. Schnidrig, T. Todorova and F. Wild Cryst. *Eng. Comm.*, **2012**, *14*, 751-757.

"*Chemistry and Crystal Growth*" J. Hulliger *Angew. Chem. Int. Ed. Engl*. (1994) 33, 143-162. *Angew. Chem.* (1994) 106, 151-171
