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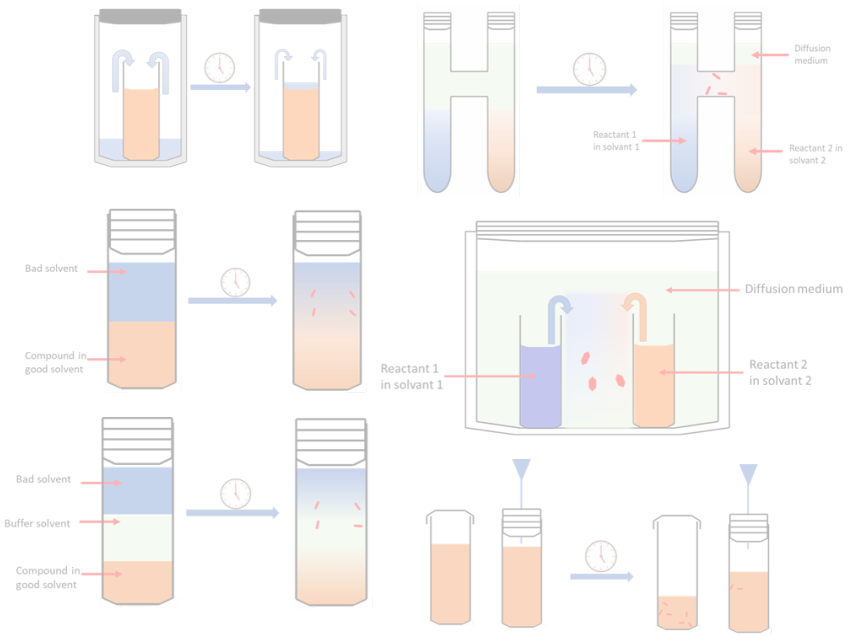

Guide for crystallization

Or How to make your crystallographer happy

Univerity of Fribourg
Chemistry Department
Dr. Aurélien Crochet

First, crystallization for SCXRD is different from recrystallization as purification technique. However, you could use the same technique. The most promising crystals for SCXRD are between 0.1 and 0.3 mm in at least two of the three dimensions, they are transparent and have sharp edges. **Crystallization is not a straight road, there are no hard and fast rules for growing such crystals**, the goal of this guide is to present you some techniques and give you some tips and tricks to maximize your chances of obtaining a good crystal.

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Disturbances

Disturbances during the crystal growth process are your worst enemy. These include bumping, swirling, vibrations (vacuum pump: Schlenk line, rotary evaporator…) temperature changes during the day (windows, heaters…), unstable fridge or cupboard and **YOU**. Indeed, **you must avoid the irrepressible desire to pick and check your vessel every day…**

Tips and Tricks: *For light sensitive compounds, it's better to place them in a stable cupboard or under a carton box than cover them with aluminum foil. You will have to unfold the aluminum to check them and it will create unnecessary vibration.*

Vessel

There is no universal glassware for crystallization, the vial used will depend on the quantity of compound that you want to use. You can use NMR tube, test tube, balloon, Erlenmeyer, HPLC vials, H-tube, Schlenck, watch glass, petri dish plate… the two important rules are: Clean vessel and able to be covered or closed. **Keep the container covered** so that no dust or hair or dirt can enter and cause crystallization.

Figure 1: example of useable glassware[i]

Nucleation

Crystallization process occurs on nucleation sites, it seems to be promising to have plenty of nucleation sites, means plenty of crystals; but the goal is to obtain crystals of suitable size for SCXRD rather than many smaller crystals (crystallization ≠ recrystallization). The fewer nucleation site will result in fewer crystals with larger size.

What is a nucleation site? Dust, undissolved material, extraneous particles, fibers (paper, filter, tissue, hair…) are all potential nucleation sites, so take care to filter your sample before attempting crystallization (if possible, without filter paper) and **make sure that your vessel is clean and free of dust**. Be also careful to grease residue.

Time

Growing crystals requires two essential virtues: Patience and perseverance! You can play on so many parameters (concentration, solvent(s), vessel, techniques…) that it can take weeks to test all possibilities. **Don't give up after your first attempts fail**. Crystallization is part of the experimental science, so make experiments. There is no given recipe that will always work - you rather need to find the "good conditions" for every family of new compounds.

And don't forget, don't be part of the possible disturbance, do not touch your vessel every five minutes, it takes time.

If possible, let your crystallizations essays in the back of the fume hood, fridge, or bench for several days.

Labelling

It could seem a bit stupid to say it but **label all your experiments** and use techniques to preserve the labelling. You will not be able to remember all your crystallization parameters after one week, you must be able to reproduce them, you have a lab book for the synthesis, so why not one for crystallization?

Tips and Tricks: *use permanent pen and cover the label with transparent tape. Take note of every single condition (solvent(s), amounts, quality (dry, distilled, wet, analytical or whatever), provider, T°, date,....)*

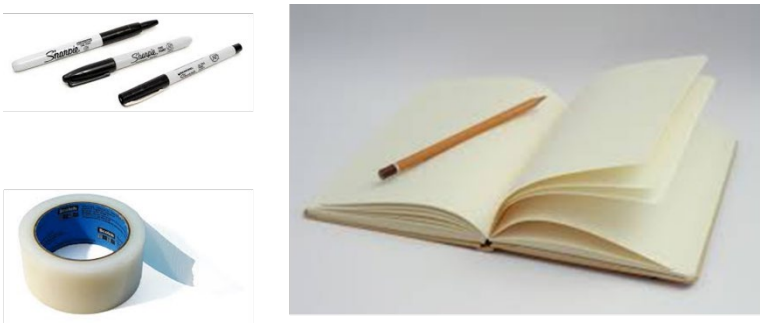

Figure 2: Additional materials needed[ii]

Quantity

To the question: *How much sample do I need to use?* I would answer **enough, but not too much**… I know that it is not helpful, but if you proceed to a small calculation, you will understand:

If we consider crystal size of 0.2 x 0.2 x 0.2 mm for a volume of 0.008 mm^3 = 8x10^{18} $Å^3$.
If we go for a cubic unit cell of 12 x 12 x 12 Å, then the volume of this unit cell is 1728 $Å^3$.
Therefore, in this crystal we have ~4.63x10^{15} unit cells with 8 molecules per unit cell, it means that we have ~3.7x10^{16} molecules in the crystal. It means only ~6.15x 10^{-8} moles in the crystal. If the molecular weight of the compound is around 250g.mol^{-1}, you need to form this crystal 0.015 mg of compound… for ONE good crystal size…

More than one crystal grows in the vessel so more material is needed, if you have 100 crystals with a mean size of 0.2x0.x0.2, you will have crystallized only ~1.5 mg of your compound… and all the compound in solution didn't crystallize !!!

Tips and Tricks: *in evaporation approach, one traditional concentration is the one use for NMR (2-10 mg in 0.6-1 mL) so Keep your NMR tube.*

Purity

Once again, crystallization for SCXRD experiment is not done to purify your compound. A minimum purity of 80-90 % is recommended before attempting to grow XRD samples. Protonated amine bases or urea derivatives crystallize much better than most of the organic compounds, so if your compounds contain one or the other, you have a large risk to crystallize it before your compound and give a protonated amine base to your crystallographer. So check the pH of your solutions !

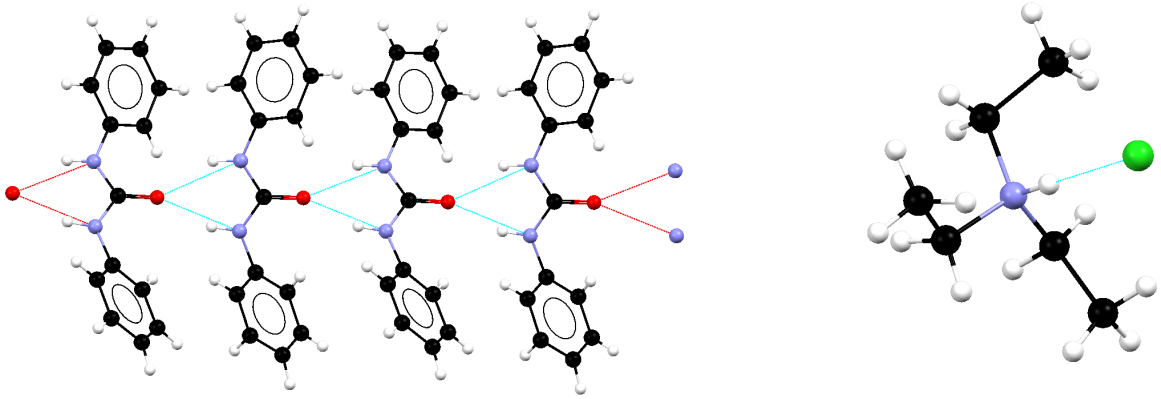

Figure 3: Crystal structure of diphenyl urea and triethylammonium chloride. Resp. CSD code: DPUREA03 and ETAMCL03.

Solvent

There is no universal or magic solvent! Solvent choice is one of the biggest issues in crystallization, obviously, you need to know the solubility of your sample. **Your compound needs to be soluble in the chosen solvent but not too much.** If the solvent is too good for

your compound, this one will not crystallize and you will obtain a supersaturated solution, which will result to small crystals or powder.

You can use any solvent but keep in mind that crystallization takes time, a too fast process generally gives poor crystal quality or worse powder or amorphous compound. This is why, generally it is better to avoid very volatile solvent such as: DCM, acetone, chloroform… for evaporation approach.

Hydrogen bonding is particularly 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.

From a crystallographic point of view:

Avoid highly volatile solvents (DCM, chloroform, acetone, diethyl ether…) if the lattice contains some solvent as they have a tendency to leave easily, it can turn to a degradation of the crystal. Unfortunately, these solvents give often suitable crystals by slow evaporation.

If possible, avoid long alkyl chains as solvent, when the solvent is trapped in the lattice, these cause disorders. They could have many conformations and therefore all atoms are not in the same place throughout the lattice.

For some people, benzene seems to give good luck to generate X-ray quality crystals. Most of the time the solvent didn't co-crystallized with the compound. However, the possible holes in the lattice could be filled by this aromatic solvent stabilized by pi-pi interactions. Benzene should however be avoided, use toluene instead - it will be less disordered if included in the structure. For organic complexes, ethyl acetate seems to be a good choice as solvent.

Techniques

Again, there are no universal or magic techniques. This list of techniques is not exhaustive and can be completed by your creativity. All these techniques can be adapted to inert conditions.

Slow evaporation

Evaporation is the easiest technique, you just let your solvent evaporate **slowly** but this method does not, generally, give the best crystal quality as crystallization occurs only when a small amount of solvent remains. It results to a twinning or aggregations of crystals.

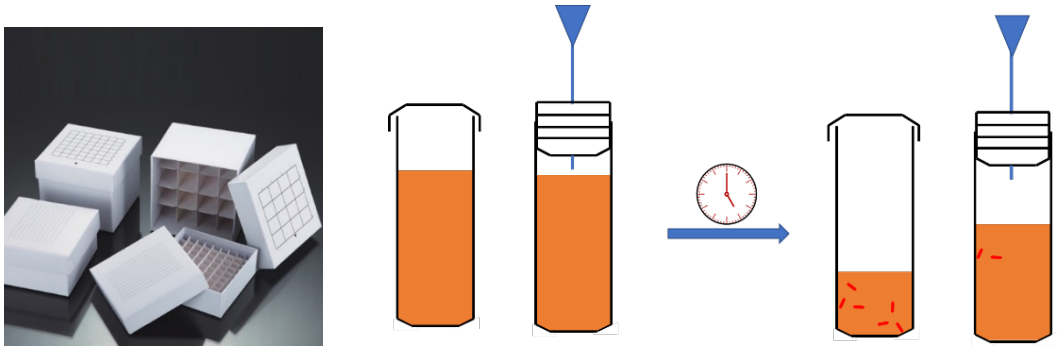

Figure 4: Example of compartment boxes[ii] and evaporation approach.

Evaporation rate can be slowed down by controlling the open area or/and by cooling the solution; In all cases, keep the solution clean by covering it, an easy option is to use aluminum foil or a rubber septum with an inserted needle.

Tips and Tricks: *in order to avoid falling off your vial, you can put them in a special box with compartment or just in a beaker.*

NMR tube can be used for crystallization after being used for their "normal" purpose. The caps fit tight enough to keep dirt out and allows a slow evaporation of the solvent.

Tips and Tricks: *You could also place your vessel in the fridge to slow down evaporation, or in a desiccator with desicants.*

Oil case: If you obtain an oil by evaporation, this could be, not due to an impure compound, but due to the too high solubility of the compound in the solvent chosen for the evaporation. So, try with a less good solvent.

Slow cooling

This is a standard recrystallization method, but this method can work very well; remember the rule: soluble hot, insoluble cold. Contrary to recrystallization, we want to form crystals very slowly. We don't care if some of the compound stays in solution. It means that after heating of your sample, you will not run to the fridge, but try to limit the cooling of the sample. For this, you can for example place the vial into another container like Styrofoam box, a jar filled with cotton, a Dewar…. if you have the possibility, you can also use a control temperature system to regulate the cooling of the sample or let your sample in the oil bath.

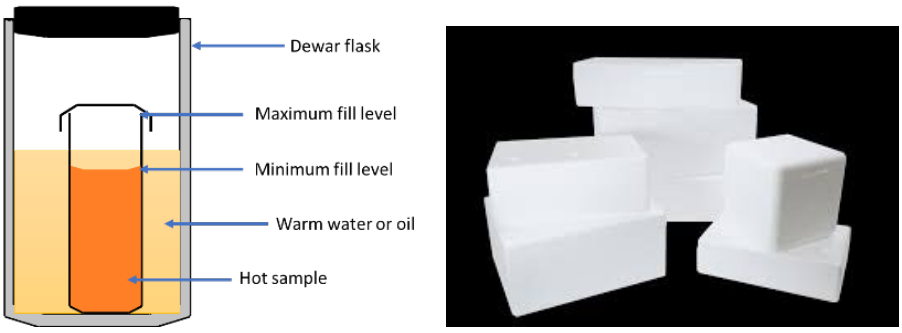

Figure 5: Slow cooling in Dewar and an alternative to Dewar: Styrofoam boxes[ii]

Variations around slow evaporation and cooling:

If these two approaches didn't give satisfactory results, they can be extended to a binary or tertiary solvent system. The used of multiple solvents is that they can promote or inhibit the growth of some crystal faces. They can be incorporated in the crystal lattice; they can affect the crystal packing and change the morphology of the crystals. However, solvents should have similar boiling points so that they can evaporate at approximate the same rate and the polarity should not be too different in order to avoid phase separation with the addition of the compound, you don't want to perform an extraction.

Sublimation

For this, the sample must be heated under reduced pressure until it vaporizes, the vapor will deposit on a cold surface of the system. One example is the cold finger system.

Figure 6: Cold finger and an example of setup: a watch glass used as cold surface[i]

Solvent layering

This approach is based on a simple concept: the compound is soluble in one solvent and not in the other one. Dissolve your compound in a good solvent and then layer the second one very carefully on the top of the second one. The two solvents must have enough difference in properties that an interface develops between the two solvents. Do not move the vessel after setting up the crystallization.

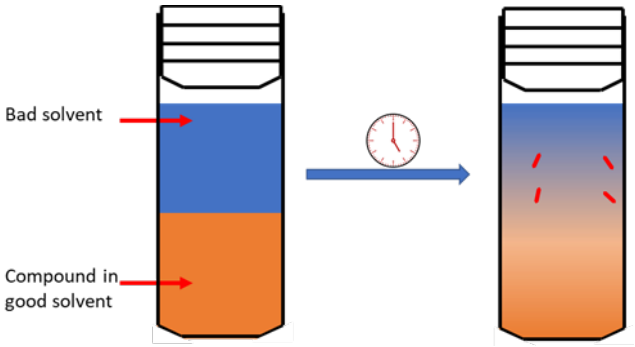

Figure 7: Solvent layering

Tips and Tricks: *A third solvent can be used to create an interface or a buffer and slowdown the diffusion rate i.e. benzene. You can also freeze the first solvent before adding the second one*

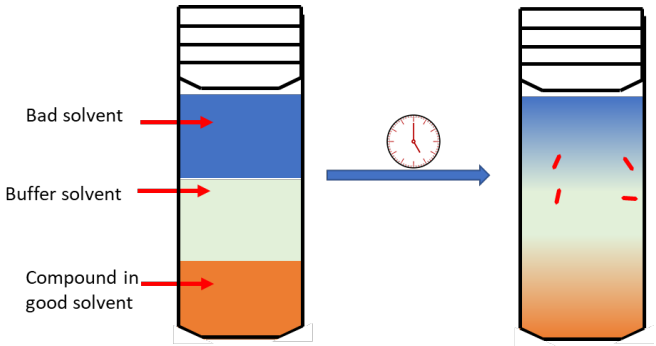

Figure 8: Solvent layering with 3 solvents

Tips and Tricks: *Check the density of your solvent in order to know in which order you must add them (Table 2).*

In this technique, the two solvents could also not be miscible, the crystallization will occur on the interface.

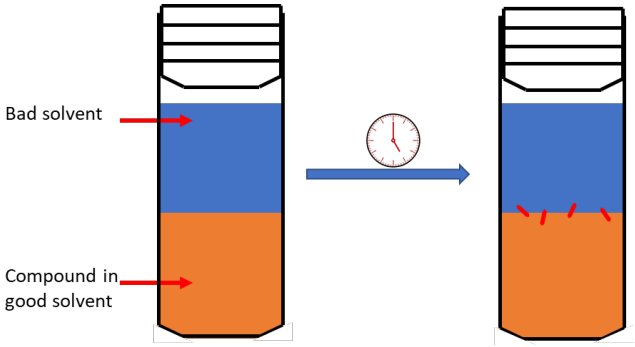

Figure 9: Solvent layering with non-miscible solvents

Vapor diffusion

This approach is, probably, the best method to use, it's a good choice if you have only milligrams of your compound. The vapor diffusion approach is done by dissolving your compound in a small vial, then placing this inner vial inside a larger vial that contains a small volume of a volatile solvent in which your sample is insoluble. The system is now closed and let free of disturbance.

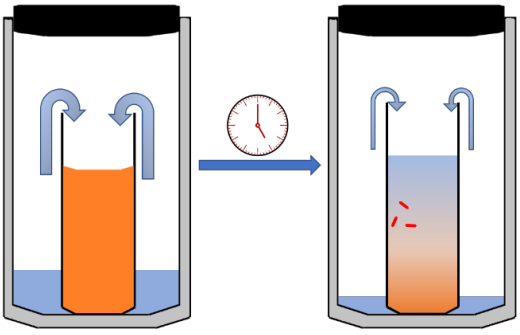

Figure 10: Vapor diffusion

It is important to notice that:

1- The inner vial should not touch the vertical surface of the outer vial in order to avoid any capillary forces.
2- The total volume of solvent (in and out the inner vial) should not exceed the maximum volume of the inner vial or the experiment will turn to solvent diffusion technique.
3- The bad solvent must have a higher vapor diffusion coefficient than your good solvent. For this look Table 2:.

Tips and Tricks: *You could also place your vessel in the fridge to slowdown diffusion.*

Table 1: Classical combination of solvent for vapor diffusion

Solvent	Antisolvent
Tetrahydrofuran	Cyclohexane
Methylformate	Cyclopentane or hexane (dries out)
Methylene chloride	Cyclopentane
Ethanol	Cyclohexane
Methanol	Hexane or tetrahydrofuran
Acetonitrile	Tetrahydropyran
Acetone	Chloroform
Water	Dioxane
Benzene	Diethyl Ether
Toluene	THF

Reactant diffusion

This technique, a variation of solvent layering approach, is perfectly designed for crystallization of complexes. Dissolve your compound in two different vials and immerge them in a third solvent, if the formed compound is insoluble in this third solvent, he will crystallize.

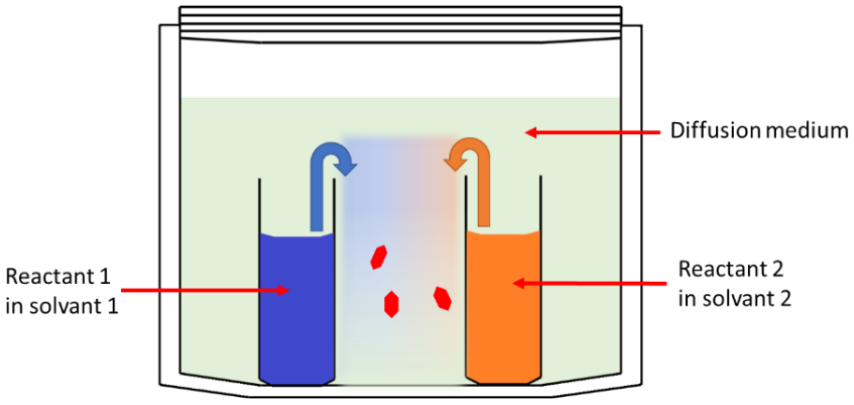

Figure 11: Reactant diffusion

Tips and Tricks: *In this approach, you can also use H-tube*

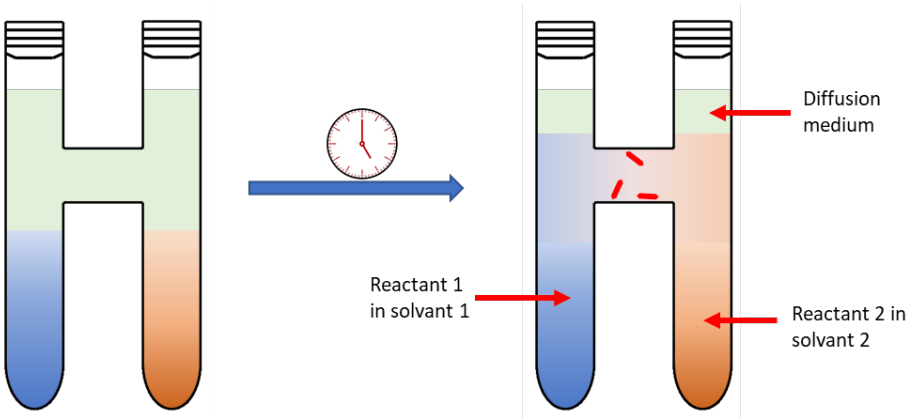

Figure 12: Reactant diffusion in H-tube

Convection

This technique is perfectly fitting with extremely poorly soluble compounds. The idea is that substances dissolved in the hotter part of the container, diffuse to a colder part where crystallization occurs. It is important in this case to:

1- Pack well the insoluble sample at bottom of the vessel (we don't want to have swimming non crystalline solid)
2- Have a slow heating in order to avoid movement of the insoluble part (again same purpose)
3- Have slow convection, in order to have an optimum nucleation (if it's too fast you will not have crystal growth)

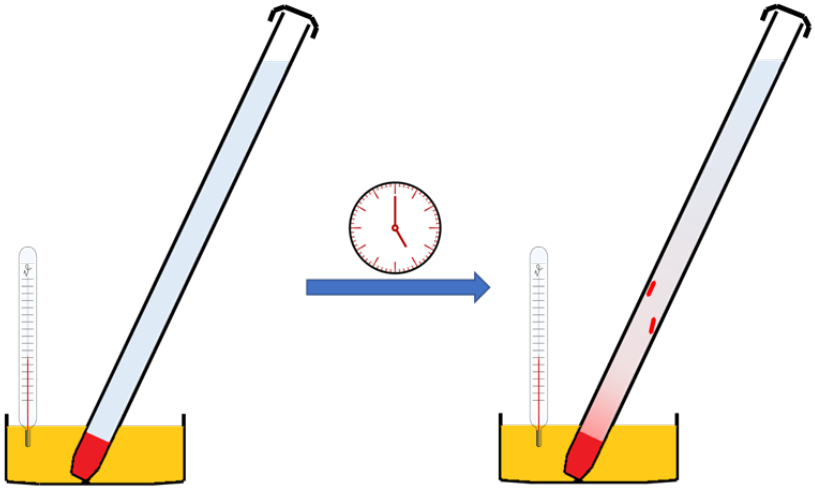

Figure 13: Convection in Pasteur pipette

One classical apparatus for this is the Thiele tube (it was used for the melting point determination) but a sealed Pasteur pipette, or a test tube (depending of the quantity of starting compound) can be used in an oil bath if the tubes are placed with an angles of 45° to slowdown the convection and avoid the formed crystal falling to the bottom part of the tube.

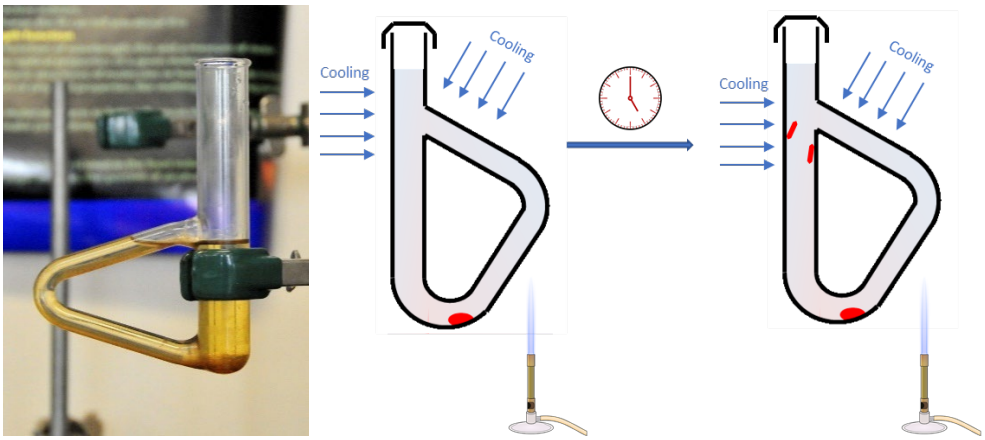

Figure 14: Thiele tube[i] and Convection in Thiele tube

Alternative methods:

Plenty of alternative methods exist to help you to crystallize your compound.

Counterions or ionization

It's probably one of the easiest methods, if none of the classical ones work. The goal is to exchange the problematic counter ion, Et_4N^+, Bu_4N^+, BF_4^-, and PF_6^-, by counter ions that are usually ordered are triflate, BPh_4^-, Me_4N^+, $(Ph_4P)_2N^+$, and Ph_4As^+.

In case of neutral compound, protonation or deprotonation can be done to generate a salt which may crystallize with the help of stronger H-bonding.

Co-crystal

In some case, the addition of triphenylphosphine oxide (TPPO) as a co-crystallant can be helpful. In the case of proton donator compound, it plays the role of proton acceptor.

Seeding

This approach consists of adding of microcrystal to the crystallization solution. It seems that it can also work with a crystal of a similar compound. But there is a major problem with this technique, you need to have an extremely good crystal for seeding.

Melting

If your compound can melt, melt it and let it crystallize. This can be done with a melting point tube or a microscope slide. The major problem with this technique is that it gives generally bad crystal quality as most of the time the crystals obtained are embedded to each other's.

Gel

Probably the most exotic technique present here. The idea is to use the permeability of a gel to induce crystallization. This techniques works very well when the form compound is insoluble, i.e. PbI_2. For more details look in the related literature.

What to do when I have crystals?

When you will obtain crystals, **it's important to never, and <u>when I say never, I mean NEVER</u>, dry your crystal**. And by drying, I include removing the mother liquor, or rinse them

with a bad solvent. Some structure includes solvent in the lattice and if the crystal is not surrounded anymore by the mother liquor, the trapped solvent will leave the lattice and a decomposition of the structure can occur.

Figure 15: examples of twinning[i], inclusion[i] or both[iii]

If you have a microscope, check them; but **how to recognize a good crystal?** A good crystal should be transparent with nicely defined edges and regular smooth faces and they should reflect light under certain angles. A good crystal should not have defaults, lines, differences in colors.

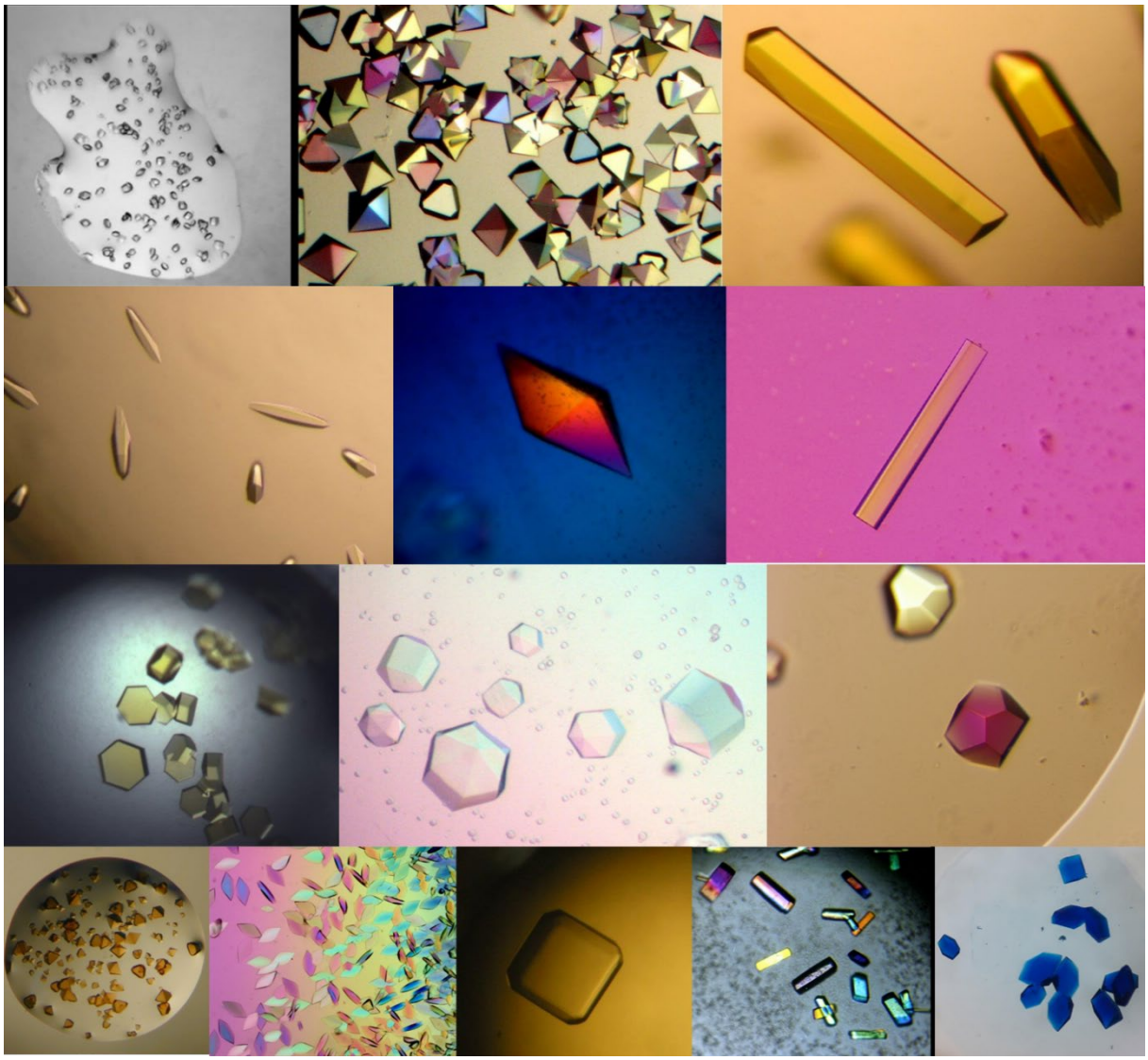

Figure 16: examples of good crystals, source: Hampton Research[iii]

When your crystals are cloudy, have cracks, have inclusion (bubbles, smaller crystals, powder…) or intergrown crystal they should be rejected. In case of crystals which look like bird's feather, a fern leaf, a dandelion seed or a star, you don't have single crystals. Very thin plate-like crystals or needle-like, you could be in the presence of stacked microcrystals and it could be difficult for non-experienced eye to determine the crystal quality.

Figure 17: examples of solidified oil and dendritic solidification

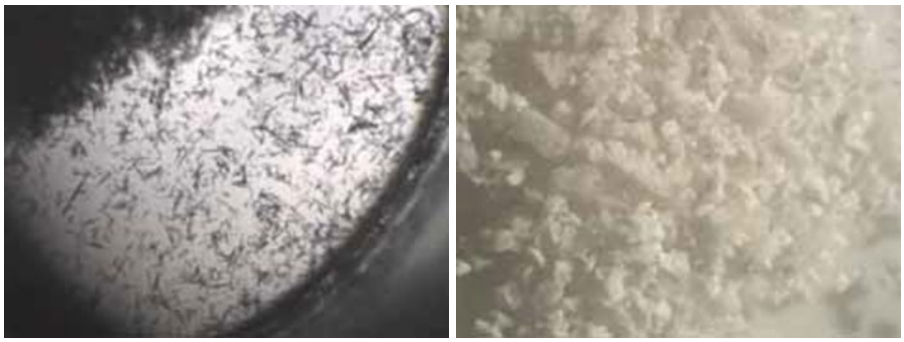

Figure 18: examples of microcrystalline and powder sample

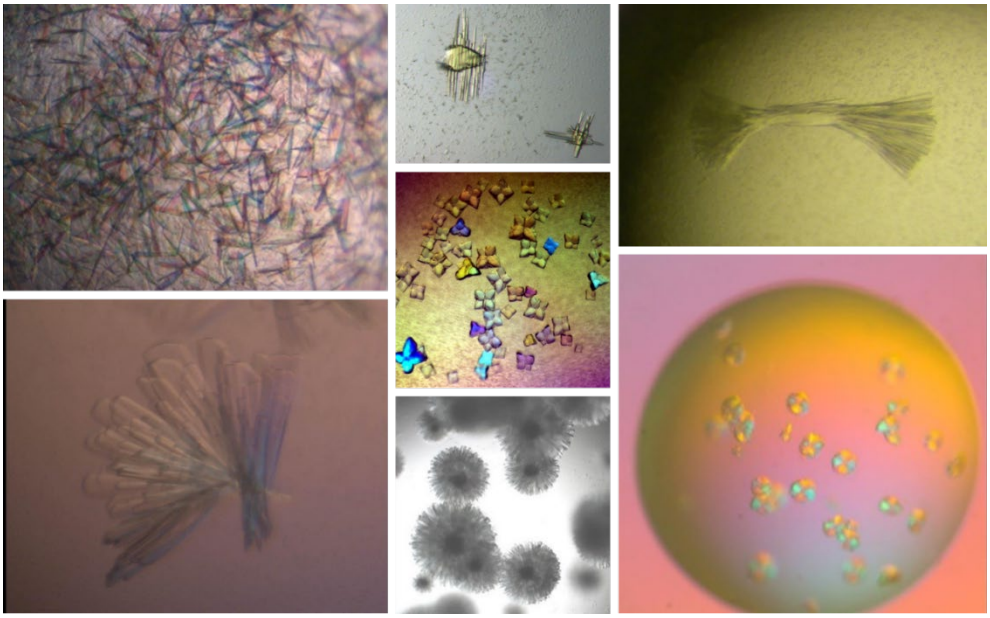

Figure 19: examples of bad crystals, source: Hampton Research[iii]

If you have a doubt, contact your crystallographer.

Summary

- Solvent: You need to have a good solvent but not too good.
- Nucleation: Only few nucleation sites, so cover or close your vessel.
- Mechanics: Choose carefully the location, you must avoid disturbances.
- Time: the longer it takes to grow the crystals, the better they are. Coffee time crystallization generally didn't give good crystal

And remember: Patience, Patience, Patience

Good Luck!

Table 2: Properties of some solvents.

Solvent	Boiling point (°C)	Melting point (°C)	Density	Solubility in H_2O[1] (g/100g)	Relative polarity[2]	Vapor pressure @ 20°C (hPa)	Dipole moment (D)	Dielectric constant	Viscosity (10^{-3} Pa s)
acetic acid	118	16.6	1.049	M	0.648	15.3	1.68	6.2	1.12
acetone	56.2	-94.3	0.786	M	0.355	240	2.85	21	0.30
acetonitrile	81.6	-46	0.786	M	0.460	97	3.5	37.5	0.34
acetyl acetone	140.4	-23	0.975	16	0.571	3	3.0	23	
2-aminoethanol	170.9	10.5	1.018	M	0.651	0.53	2.3 - 2.6	37.7	20.8
aniline	184.4	-6.0	1.022	3.4	0.420	0.4	1.6	6.8	3.8
anisole	153.7	-37.5	0.996	0.10	0.198	3.5*	1.4	4.3	
benzene	80.1	5.5	0.879	0.18	0.111	101	0	2.3	0.60
benzonitrile	205	-13	0.996	0.2	0.333	12	4.1	26	1.27
benzyl alcohol	205.4	-15.3	1.042	3.5	0.608	0.094*	1.7	13	5.47
1-butanol	117.6	-89.5	0.81	7.7	0.586	6.3	1.7	17.5	2.59
2-butanol	99.5	-114.7	0.808	18.1	0.506	18.3*	1.7	17.3	3.1
i-butanol	107.9	-108.2	0.803	8.5	0.552	10.5*	1.8	17.9	6.68
2-butanone	79.6	-86.3	0.805	25.6	0.327	105	2.8	18.6	0.41
t-butyl alcohol	82.2	25.5	0.786	M	0.389	41	1.7	12.4	3.35
carbon disulfide	46.3	-111.6	1.263	0.2	0.065	400	0	2.6	0.36
carbon tetrachloride	76.7	-22.4	1.594	0.08	0.052	120	0	2.3	0.90
chlorobenzene	132	-45.6	1.106	0.05	0.188	12	1.54	5.7	0.75
chloroform	61.2	-63.5	1.498	0.8	0.259	210	1.0	4.8	0.54
cyclohexane	80.7	6.6	0.779	0.005	0.006	104	0	2	0.89
cyclohexanol	161.1	25.2	0.962	4.2	0.509	1.2	1.9	15	
cyclohexanone	155.6	-16.4	0.948	2.3	0.281	5	2.9	15	2.00
di-n-butylphthalate	340	-35	1.049	0.0011	0.272				16.6
1,1-dichloroethane	57.3	-97.0	1.176	0.5	0.269	240	1.8	10	
1,2-dichloroethane	83.5	-35.4	1.235	0.87	0.327	79*	1.8	10.4	0.78
diethylamine	56.3	-48	0.706	M	0.145	260	1.2	3.8	0.32

Solvent	Boiling point (°C)	Melting point (°C)	Density	Solubility in H_2O[1] (g/100g)	Relative polarity[2]	Vapor pressure @ 20°C (hPa)	Dipole moment (D)	Dielectric constant	Viscosity (10^{-3} Pa s)
diethylene glycol	245	-10	1.118	M	0.713	0.027	2.3	31.8	30.2
diglyme	162	-64	0.945	M	0.244		1.9	7.23	1.88
dimethoxyethane (glyme)	85	-58	0.868	M	0.231		1.7	7.3	1.1
N,N-dimethylaniline	194.2	2.4	0.956	0.14	0.179				
dimethylformamide (DMF)	153	-61	0.944	M	0.386	3.5	3.8	37	0.80
dimethylphthalate	283.8	1	1.190	0.43	0.309				
dimethylsulfoxide (DMSO)	189	18.4	1.092	M	0.444	0.61*	3.9	46.7	2.00
dioxane	101.1	11.8	1.033	M	0.164	41	0.4	2	1.18
ethanol	78.5	-114.1	0.789	M	0.654	59	1.7	24	1.08
ether	34.6	-116.3	0.713	7.5	0.117	587	1.25	4.3	0.22
ethyl acetate	77	-83.6	0.894	8.7	0.228	97	1.78	6.0	0.43
ethyl acetoacetate	180.4	-80	1.028	2.9	0.577	0.78*			
ethyl benzoate	213	-34.6	1.047	0.07	0.228		2.0	6.0	
ethylene glycol	197	-13	1.115	M	0.790	0.092*	2.3	37.7	16.1
glycerin	290	17.8	1.261	M	0.812		2.7	42.5	934
heptane	98	-90.6	0.684	0.0003	0.012	48	0	1.9	0.39
1-heptanol	176.4	-35	0.819	0.17	0.549		1.7	12	6.0
hexane	69	-95	0.655	0.0014	0.009	160	0	1.9	0.29
1-hexanol	158	-46.7	0.814	0.59	0.559	0.22*		12.5	0.59
methanol	64.6	-98	0.791	M	0.762	128	1.6	33	0.54
methyl acetate	56.9	-98.1	0.933	24.4	0.253	220	1.7	6.68	0.36
methyl *t*-butyl ether (MTBE)	55.2	-109	0.741	4.8	0.124	250*	1.4	2.6	0.36
methylene chloride	39.8	-96.7	1.326	1.32	0.309	475	1.6	9.0	0.42
1-octanol	194.4	-15	0.827	0.096	0.537		1.7	10.3	7.4

Solvent	Boiling point (°C)	Melting point (°C)	Density	Solubility in H_2O[1] (g/100g)	Relative polarity[2]	Vapor pressure @ 20°C (hPa)	Dipole moment (D)	Dielectric constant	Viscosity (10^{-3} Pa s)
pentane	36.1	-129.7	0.626	0.004	0.009	573	0	1.84	0.23
1-pentanol	138.0	-78.2	0.814	2.2	0.568	2.2*	5.7	14	3.5
2-pentanol	119.0	-50	0.810	4.5	0.488	6.1*	1.7	13.7	3.5
3-pentanol	115.3	-8	0.821	5.1	0.463	8.8*	1.7	13.3	
2-pentanone	102.3	-76.9	0.809	4.3	0.321	35.4*	2.7	15.4	0.50
3-pentanone	101.7	-39.8	0.814	3.4	0.265	37.7*	2.8	17.0	
1-propanol	97	-126	0.803	M	0.617	21*	1.68	22	1.95
2-propanol	82.4	-88.5	0.785	M	0.546	44	1.66	19	2.07
pyridine	115.5	-42	0.982	M	0.302	20	2.2	13	0.88
Tetrahydrofuran (THF)	66	-108.4	0.886	30 or M[5]	0.207	200	1.63	7.5	0.46
toluene	110.6	-93	0.867	0.05	0.099	29	0.36	2.4	0.55
water	100.00	0.00	0.998	M	1.000	17.5	1.85	80.1	0.89
water, heavy	101.3	4	1.107	M	0.991	15	1.84	78.3	1.10
p-xylene	138.3	13.3	0.861	0.02	0.074	15	0	2.27	0.65

[1] M = miscible. [2] The values for relative polarity are normalized from measurements of solvent shifts of absorption spectra and were extracted from Christian Reichardt, *Solvents and Solvent Effects in Organic Chemistry*, Wiley-VCH Publishers, 3rd ed., **2003.** [3] Snyder's empirical eluant strength parameter for alumina. Extracted from Reichardt, page 495. [4] Threshold limits for exposure. Extracted from Reichardt, pages 501-502. [5] https://pubchem.ncbi.nlm.nih.gov/compound/tetrahydrofuran#section=Solubility; * 25°C

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