Some Tricks for the Single Crystal Growth of Small Molecules

Prof. Dr. Bernhard Spingler

spingler@chem.uzh.ch

Updated excerpt of a talk held at the 30th European Crystallographic Meeting in Basel, Switzerland on the 29th August 2016
Table of Contents

– Currently used techniques for single crystal growth of small molecules
– Vapor diffusion
– Layering technique
– Copper radiation
– Summary and conclusions
– Acknowledgements
Importance of single crystal growth

- Prerequisite for single crystal X-ray analysis, a fast analytical method that yields the three dimensional arrangement of the elements within the crystal.

- Despite many technical advances, be it on the instrumental (X-ray beam, detector) or on the theoretical side:

  Still **single** crystals needed, ideally with dimensions of about 0.05*0.05*0.2 mm$^3$

- Crystal polymorphs also play an extremely crucial role in terms of processing, bioavailability, stability, regulatory affairs, and intellectual property protection.[1]

Techniques for single crystal growth

- In liquid phases: Main goal to achieve supersaturation that is followed by nucleation/crystal growth
- Sublimation sometimes also helpful
- Achievement of supersaturation:
  - cooling (from hot oil bath, in fridge or deep freezer)
  - reduction of solvent amount (evaporation, forgotten NMR tubes...)
  - change of solvent (vapor diffusion / layering)
- From pure liquids at low temperature

A. J. Blake [www.nottingham.ac.uk/~pczajb2/growcrys.htm](http://www.nottingham.ac.uk/~pczajb2/growcrys.htm)
Problems for a single crystal analysis

- Chemical purity / identity (e.g. crystals from $^{99m}$Tc chemistry)
- Oils / Powders
- Microcrystalline / too small
- Intergrown / twinned
- Not diffracting despite fair size (phase transition?)
- Not solvable
- Not enough data (too weak reflections)
- Residual electron density too high / “unreasonable” electron density
How to overcome the problem of “bad” crystals

– Common situation: crystallographer wants better crystals

– Despite many references, it is not always clear how to optimize the crystals

– Often only a few milligrams available!

⇒ No systematic study of the solubility

A. J. Blake, www.nottingham.ac.uk/~pczajb2/growcrys.htm
P. D. Boyle, www.xray.ncsu.edu/GrowXtal.html
**Vapor diffusion**

- About 4 mg of substance are dissolved in about 0.5 ml solvent in the inner container.

- About 2.5 ml of antisolvent (normally having a boiling point 5-10 °C **higher** than solvent) are placed in outer container.

- Wait for days, or a few weeks!

- If (anti)solvents have equilibrated and nothing happened, unscrew vial a bit:
  → evaporation experiment from a solvent mixture.

Influence of the boiling points

Antisolvent (e.g. diethylether) has a lower boiling point than solvent:

Antisolvent has a higher boiling point than solvent:

# Initial solvent choice for vapor diffusion

Use information gained during synthesis/purification!

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Antisolvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>tetrahydrofuran</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>methylformate</td>
<td>cyclopentane or hexane (dries out)</td>
</tr>
<tr>
<td>methylene chloride</td>
<td>cyclopentane</td>
</tr>
<tr>
<td>ethanol</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>methanol</td>
<td>hexane or tetrahydrofuran</td>
</tr>
<tr>
<td>acetonitrile</td>
<td>tetrahydropyran</td>
</tr>
<tr>
<td>acetone</td>
<td>chloroform</td>
</tr>
<tr>
<td>water</td>
<td>dioxane</td>
</tr>
</tbody>
</table>
Optimization of the vapor diffusion

Improving crystal form/size:

Substitute solvent and antisolvent with other solvents of the same class having similar dielectric constants.

For example:

<table>
<thead>
<tr>
<th>Solvent</th>
<th>B.p. °C / ( \varepsilon )</th>
<th>Antisolvent</th>
<th>B.p. °C / ( \varepsilon )</th>
</tr>
</thead>
<tbody>
<tr>
<td>methylene chloride</td>
<td>40   8.93</td>
<td>cyclopentane</td>
<td>49   1.97</td>
</tr>
<tr>
<td>1,1,1-trichloro-ethane</td>
<td>74   7.24</td>
<td>cyclohexane</td>
<td>81   2.02</td>
</tr>
<tr>
<td>1,2-dichloroethane</td>
<td>84   10.4</td>
<td>methylcyclohexane</td>
<td>101  2.02</td>
</tr>
</tbody>
</table>
An extremely simplified flow chart

Guess solubility in different solvents, resp. crystallinity after solvent evaporation

- **Initial crystallization experiments**
  - Crystalline
    - Use similar solvents
  - Powder
    - Use S/AS with higher bp's
  - Oil
    - Use different classes of S/AS

- **Further crystallization experiments**

Big and good enough single crystals

Change T, technique, chemistry
Layering I

– About 4 mg of substance are dissolved in ~0.5 ml of a dense solvent and put in a cheap NMR tube.

– With an extra-long Pasteur pipette, a 0.5 cm high protection layer of pure solvent is carefully layered above.

– The lighter antisolvent is carefully layered above, until the NMR tube is full.
Layering II

– Rather high boiling solvents are used (e.g. dibutyl ether than diethyl ether).
– Different densities of the solvent and the antisolvent are needed. Take change of density due to solute into account!
– Experiment takes more time to equilibrate (several weeks!).
– Once started the experiment is difficult to modify.
– Difficult optical crystal evaluation, if they grow at the bottom of the NMR tube (or when falling down during retrieval attempts).
Excerpt from a table with 107 solvents

<table>
<thead>
<tr>
<th>Sum formula</th>
<th>Name</th>
<th>b.p. °C</th>
<th>δ</th>
<th>ϵ</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₆H₁₂O₂</td>
<td>t-Butyl acetate</td>
<td>95</td>
<td>0.867</td>
<td>5.67</td>
</tr>
<tr>
<td>C₆H₅Cl</td>
<td>Chlorobenzene</td>
<td>132</td>
<td>1.106</td>
<td>5.69</td>
</tr>
<tr>
<td>C₅H₁₀O₂</td>
<td>Ethyl propanoate</td>
<td>99</td>
<td>0.892</td>
<td>5.76</td>
</tr>
<tr>
<td>C₄H₈O₂</td>
<td>Ethyl acetate</td>
<td>77</td>
<td>0.900</td>
<td>6.08</td>
</tr>
<tr>
<td>C₅H₁₀O₂</td>
<td>Butyl formate</td>
<td>106</td>
<td>0.889</td>
<td>6.10</td>
</tr>
<tr>
<td>C₄H₈O₂</td>
<td>Methyl propanoate</td>
<td>80</td>
<td>0.915</td>
<td>6.20</td>
</tr>
<tr>
<td>C₂H₄O₂</td>
<td>Acetic acid</td>
<td>118</td>
<td>1.045</td>
<td>6.20</td>
</tr>
<tr>
<td>C₄H₈O₂</td>
<td>Propyl formate</td>
<td>81</td>
<td>0.906</td>
<td>6.92</td>
</tr>
<tr>
<td>C₅H₁₀O</td>
<td>2-Methyltetrahydrofuran</td>
<td>78</td>
<td>0.855</td>
<td>6.97</td>
</tr>
<tr>
<td>C₃H₆O₂</td>
<td>Methyl acetate</td>
<td>57</td>
<td>0.934</td>
<td>7.07</td>
</tr>
<tr>
<td>C₆H₁₁O₃</td>
<td>Diethylene glycol dimethyl ether</td>
<td>162</td>
<td>0.943</td>
<td>7.23</td>
</tr>
<tr>
<td>C₂H₃Cl₃</td>
<td>1,1,1-Trichloroethane</td>
<td>74</td>
<td>1.339</td>
<td>7.24</td>
</tr>
<tr>
<td>C₄H₁₀O₂</td>
<td>Ethylene glycol dimethyl ether</td>
<td>85</td>
<td>0.869</td>
<td>7.30</td>
</tr>
<tr>
<td>C₄H₈O</td>
<td>Tetrahydrofuran</td>
<td>65</td>
<td>0.889</td>
<td>7.52</td>
</tr>
<tr>
<td>CH₂Br₂</td>
<td>Dibromomethane</td>
<td>97</td>
<td>2.497</td>
<td>7.77</td>
</tr>
<tr>
<td>C₂H₂Cl₈</td>
<td>1,1,2,2-Tetrachloroethane</td>
<td>131</td>
<td>1.541</td>
<td>8.50</td>
</tr>
<tr>
<td>C₃H₆O₂</td>
<td>Ethyl formate</td>
<td>54</td>
<td>0.917</td>
<td>8.57</td>
</tr>
</tbody>
</table>

Selection criteria for these solvents:
- m.p. < 20°C
- b.p. > 30°C
- few with b.p. > 150°C
- stability
- toxicity
- cost

Example I

Recrystallization from acetonitrile versus tetrahydropyran

Single crystals from methanol versus tetrahydropyran

B. Spingler, P. M. Antoni
Example II

3-carbethoxyquinoline (vapor diffusion):

THF versus cyclohexane

Chloroform versus cyclohexane

Trichloroethylene versus heptane
Example II

<table>
<thead>
<tr>
<th>Formula</th>
<th>C₁₂H₁₁NO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>P-1</td>
</tr>
<tr>
<td>a [Å]</td>
<td>7.5975(5)</td>
</tr>
<tr>
<td>b [Å]</td>
<td>12.2026(7)</td>
</tr>
<tr>
<td>c [Å]</td>
<td>12.8137(8)</td>
</tr>
<tr>
<td>α [°]</td>
<td>61.607(6)</td>
</tr>
<tr>
<td>β [°]</td>
<td>77.247(5)</td>
</tr>
<tr>
<td>γ [°]</td>
<td>78.353(5)</td>
</tr>
<tr>
<td>Volume [Å³]</td>
<td>1012.74(11)</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Crystal size [mm³]</th>
<th>0.21 x 0.13 x 0.08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength [Å]</td>
<td>0.71073</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>5457 [R_{int} = 0.0402]</td>
</tr>
<tr>
<td>Reflections observed (&gt;2sigma(I))</td>
<td>2329</td>
</tr>
<tr>
<td>Completeness to theta</td>
<td>99.9 % to 29.13°</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9928 and 0.8558</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>5457 / 0 / 273</td>
</tr>
<tr>
<td>Goodness-of-fit on F²</td>
<td>0.810</td>
</tr>
<tr>
<td>Final R indices (I&gt;2sigma(I))</td>
<td>R₁ = 0.0479, wR₂ = 0.0782</td>
</tr>
<tr>
<td>Largest diff. peak and hole [e.Å⁻³]</td>
<td>0.163 and -0.268</td>
</tr>
</tbody>
</table>
Example III, different chemistry

R: OH, OEt, Ot-Bu
all did not crystallize.

But R: NHPh did.

Mo versus Cu radiation?

– Traditional knowledge [1]:
  • Mo for crystals with heavy elements
  • Cu for organic crystals (absorption challenges with heavy elements)

– However with new diffractometer and software systems, Cu became an important rescue option for weakly diffracting crystals of high quality containing only a few heavy elements and mainly light elements

Mo: 60 s exposure

Cu: 16 s exposure

hkl: 1 -10 -21; I/σ: 13

hkl: 1 -10 -21; I/σ: 105
Two examples of heavy metal containing structure measured with Cu radiation

<table>
<thead>
<tr>
<th>Empirical formula</th>
<th>$C_{39}H_{33.25}Cl_2F_6N_8O_{7.13}PRu$ [1]</th>
<th>$C_{44}H_{58}Br_2CoN_6O_8$ [2]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffractometer</td>
<td>SuperNova dual radiation CCD</td>
<td>SuperNova dual radiation CCD</td>
</tr>
<tr>
<td>Space group</td>
<td>$P-1$</td>
<td>$P-1$</td>
</tr>
<tr>
<td>Abs. coeff. (mm$^{-1}$)</td>
<td>5.465</td>
<td>5.664</td>
</tr>
<tr>
<td>Crystal size (mm$^3$)</td>
<td>$0.33 \times 0.08 \times 0.04$</td>
<td>$0.18 \times 0.04 \times 0.02$</td>
</tr>
<tr>
<td>Indep. reflections</td>
<td>15295 [$R_{int} = 0.0338$]</td>
<td>4532 [$R_{int} = 0.0218$]</td>
</tr>
<tr>
<td>Completeness to $\theta$</td>
<td>95.0 % to 66.97°</td>
<td>99.4 % to 66.97°</td>
</tr>
<tr>
<td>Max. and min. transm.</td>
<td>0.8110 and 0.6223</td>
<td>0.918 and 0.617</td>
</tr>
<tr>
<td>Fin. R ind. [$I &gt; 2 \sigma(I)$]</td>
<td>$R1 = 0.0760$, $wR2 = 0.2128$</td>
<td>$R1 = 0.0235$, $wR2 = 0.0586$</td>
</tr>
<tr>
<td>Fin. diff. $\rho_{\text{max}}$ (e$^-$/Å$^3$)</td>
<td><strong>1.356 and -1.533</strong></td>
<td><strong>0.544 and -0.324</strong></td>
</tr>
</tbody>
</table>

Disadvantages of Cu radiation

– Only till 0.8 Å resolution

– Big theta values to be covered mean 3 series of scans (→ longer measurement times)

– For cobalt containing compounds and weekly diffracting crystals: observation of X-ray fluorescence

Left: 6s exposure
2.55-0.95 Å

Right: 24s exposure
2.29-0.93 Å
What about Ga radiation?

- Melts at 29.8°C, but actually Ga rich alloys being used
- Liquid metal dissipates heat much quicker than solid one
- Most intensive microsource
- Wavelength 1.34 Å
- No cobalt fluorescence observed
- Air-conditioning needed for diffractometer room, as Ga source most stable for room temperature not varying more than 0.2°C

Ga jet with a Pixel detector


Setup at the University of Basel, Switzerland
Summary

– Stay with your system as long as it works!

– For optimization of unsatisfactory crystals:
  • Systematically explore the crystallization properties of a solvent class with a similar dielectric constant
  • use an anti-/solvent pair with an inversed polarity
  • change the technique
  • change the anions, add additives

– Do not be afraid of copper radiation, even if you have some heavy elements present!
Acknowledgements

– Prof. Dr. Dongwhan Lee (Seoul National University)
– Dr. Philipp M. Antoni (University of Zurich)
– Dr. Tonya Todorova (University of Zurich)
– Stefan Schnidrig (University of Zurich)
– Dr. Markus Neuburger (University of Basel)
– All the colleagues that have been discussing crystallization issues with me over the last 15 years
– University of Zürich
– Swiss National Science Foundation, BNF