

Crystallization via Colloidal Dispersion: For The Difficult Cases

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Introduction

Many different techniques are available for crystallization of a dissolved solute, such as a Drug Candidate. However, they all involve creating an oversaturated solution from which the solute crystallizes. Most often the nucleation kinetics for a small organic molecule is such that the right conditions for crystallization is relatively easy to obtain by e.g. cooling, evaporation, or addition of an appropriate anti-solvent. In some cases, however, the nucleation kinetics of the crystallization process is very slow, most often observed for larger complex organic structures. It may then be very difficult to create the right environment for crystallization since the competing oiling-out process is more rapid than the desired crystallization process. The result is very often that "all" crystallization trials performed by the experimentalist result in an irreversible "oiling-out" of the solute.

For these special cases with slow nucleation kinetics we have developed a crystallization technique we call crystallization via colloidal dispersion (CCD). In the CCD method an extremely high super-saturation is generated, of a kinetically relatively stable dispersion, thereby offering ideal conditions for crystallization of solutes with slow nucleation kinetics. The principles for CCD method is outlined in figure 1.

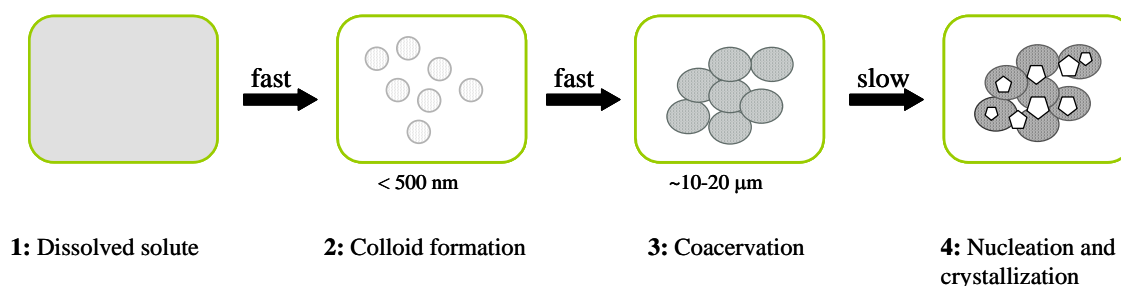


Figure 1: Crystallization via colloidal dispersion

The CCD system is composed of the substance and normally three solvents: a good solvent, an anti-solvent and a solvent modifier (poor solvent). We have used the CCD method in many different examples to generate the first crystals of complex drug candidates and also in some examples as a method to obtain new polymorphs. Once the first crystals

have been obtained it is often possible to go back and develop a more traditional crystallization process, more suitable for large scale manufacturing, by using the CCD obtained crystals as seed.

In one recent example described herein the drug candidate, LTB₄, was for the first time crystallized using the CCD method.

Leukotriene B₄ (LTB₄) is a twenty carbon tetra-unsaturated fatty acid (figure 2). LTB₄ has been reported to be an oil in its isolated form.¹ LTB₄ has great pharmaceutical potential but its use as therapeutic agents² is made difficult by insufficient stability and short shelf life, furthermore, as an oil LTB₄ is not suitable for solid formulations.³

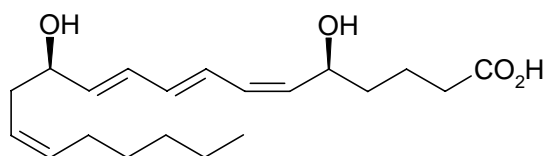


Figure 2: LTB₄

Results and discussion

Step 1: Solubility determination

A quick screen of solubility of LTB₄ was performed and appropriate good solvents, anti-solvents and solvent modifiers were selected based on the results (table 1).

Table 1: Solubility of LTB₄

Very soluble Good Solvents	Slightly soluble Solvent modifier	Not soluble Anti solvents
Methanol Ethanol IPA Acetone MiBK Ethyl acetate Isopropyl acetate Acetonitrile ^t Butyl-methyl ether Dichloromethane THF	Toluene	Water Heptane

¹ Corey, E. J.; Marfat, A.; Goto, G.; Brion, F. *J. Am. Chem. Soc.* **1980**, *102*, 7984; Nicolaou, K. C.; Abe, Y. U.S Patent 5110949, 1992.

² Samuelsson, B., Funk, C.D. *J Biol Chem*, **1989**, *264*, 19469

³ Karol Horvath WO2007113239

Step 2: Solvent selection

In the second step appropriate solvent combinations and temperature affording solid precipitate was looked for. The experiments were performed on an object glass placed under a light microscope equipped with plane polarized light and the experiments were continuously monitored. A droplet of LTB4 diluted with ethanol was combined with anti-solvent and solvent modifier until observable opalescence was reached, indicative of colloid formation. The good solvent was then either allowed to evaporate, in case of high boiling anti-solvent, or more anti-solvent was added. The solvent combination affording solid precipitate or solid residue upon evaporations was selected for evaluation on mL-scale. A solid amorphous precipitate of LTB 4 was obtained from MTBE/Toluene/Heptane at 10 °C.

Step 3: Crystallization screening

The aim of the third step was to screen for conditions affording crystalline precipitate. The experimental conditions generating the solid precipitate was repeated at mL-scale with the difference that once the coacervated state was reached the system was allowed to equilibrate for 24 hours. After 24 h the mL-sample was inspected under a light microscope and the conditions were gently pushed by addition of more anti-solvents. First crystallization of LTB4 was obtained after an additional 24 h. Crystallized LTB4 was isolated by filtrations and stored cold.

Step 4: Crystallization method development

The final step involved finding a crystallization method suitable for larger scale manufacturing. The first crystals obtained from the CCD screen were used as seed, which greatly facilitated the method development. A suitable method for large-scale manufacturing was developed affording crystallized LTB4 in an 87 % yield.⁴

Solid-state analysis of LTB4

The crystals of LTB4 were fibre-like with a thickness of less than 5 µm and a length of 100 – 150 µm (Figure 3).

⁴ Karol Horvath unpublished results



Figure 3: LTB4

The crystallinity of precipitated LTB4 was confirmed by an XRPD analysis (Figure 4).

The crystalline solid form of LTB4 was shown to have considerably improved stability, in spite of a rather low melting point (Figure 4), as compared to the concentrated oil. Furthermore DVS measurement demonstrated that LTB4 is relatively non-hygroscopic in its crystallized form.

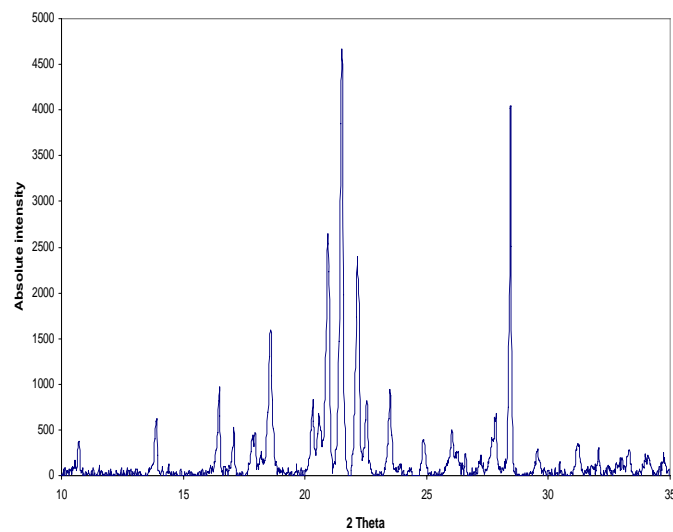


Figure 4: X-ray diffraction of LTB4