Cooling Crystallization of Indomethacin: Effect of Supersaturation, Temperature and Seeding on Polymorphism and Crystal Size Distribution

Chandrakant Ramkrishna Malwade, and Haiyan Qu

Org. Process Res. Dev., Just Accepted Manuscript • DOI: 10.1021/acs.oprd.8b00078 • Publication Date (Web): 25 May 2018

Downloaded from http://pubs.acs.org on May 28, 2018

Just Accepted

“Just Accepted” manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides “Just Accepted” as a service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. “Just Accepted” manuscripts appear in full in PDF format accompanied by an HTML abstract. “Just Accepted” manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are citable by the Digital Object Identifier (DOI®). “Just Accepted” is an optional service offered to authors. Therefore, the “Just Accepted” Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the “Just Accepted” Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these “Just Accepted” manuscripts.
Cooling Crystallization of Indomethacin: Effect of Supersaturation, Temperature and Seeding on Polymorphism and Crystal Size Distribution

Chandrakant R. Malwade*, Haiyan Qu

Department of Chemical Engineering, Biotechnology and Environmental Technology,
University of Southern Denmark, 5230 Odense M, Denmark
For Table of Contents only

![Graph](image_url)

- α-IMC product
- γ-IMC product
- Temperature (°C)
- Solubility (g/100g solvent)

Legend:
- α-IMC
- γ-IMC
ABSTRACT

In this work, effect of crystallization parameters i.e., supersaturation, seeding, and temperature on polymorphism and crystal size of a non-steroidal anti-inflammatory drug, indomethacin (IMC), was investigated. Firstly, several crystallization solvents (ethanol, methanol, ethyl acetate, acetone, acetonitrile, and dichloromethane) were screened through the measurement of IMC solubility at different temperatures. This was followed by the investigation of IMC nucleation through measurement of induction times in selected solvents at two supersaturations. Finally, seeded cooling crystallization of IMC in ethanol was performed with different process parameters to investigate the influence on polymorphism and crystal size distribution. Remarkably long induction time was observed for IMC in ethanol and ethyl acetate solutions, while shorter induction time was observed in acetone. Cooling crystallization of IMC from ethanol confirmed that the supersaturation, operating temperature and seeding does affect the polymorphism as well as crystal size distribution of IMC. Fine needle shaped crystals of metastable α-IMC were obtained at 5 °C with high supersaturation even in presence of γ-IMC seeds, while rhombic plates like crystals of thermodynamically stable γ-IMC were obtained in remaining experiments. The amount of seed loading only marginally influenced the crystal growth rate and median particle diameter (d50). Particle size analysis of crystals obtained showed bimodal distribution in all experiments and larger median particle diameter was observed at 15 °C with high supersaturation.

KEYWORDS Indomethacin, induction time, solubility, crystallization, polymorphism, crystal size distribution
1. Introduction

Most of the processes for manufacturing of active pharmaceutical ingredients (APIs) involve crystallization as a purification technique for the intermediates or APIs. In addition to the unique capability of delivering high purity crystalline product, the advantage of using crystallization for purification of APIs also includes the ability to engineer final product properties such as polymorphism, crystal size, and shape to the desired level. Although amorphous form of APIs possess better dissolution properties, crystalline form is mostly preferred for the development of various formulations due to the stability issues associated with amorphous form. Amorphous form of an API is a metastable form and tends to crystallize upon storage, thereby changing its physical properties leading to change in bioavailability. However, most of the crystalline APIs exhibit polymorphism where an API can exist in several crystal forms having different arrangement of molecules in unit cell. It is a very well established fact that the selection of a suitable polymorph is crucial for the development of drug products as the polymorphs may have significantly different physicochemical properties e.g., dissolution rate, solubility and thereby the bioavailability and stability. A notorious example of an antiviral drug ritonavir, where a batch of this drug had to be withdrawn from the market due to appearance of less soluble polymorph, illustrates the importance of polymorphism in pharmaceutical industry. Several reports of polymorphs of APIs exhibiting significantly different physical and chemical properties have been mentioned in the literature. Similarly, the particle size and shape of APIs are crucial quality attributes that are known to influence the downstream processing, formulation processes and drug product attributes such as rate of drug release, bioavailability etc. Therefore, the control of polymorphism, particle size and shape during crystallization of APIs assume great significance for the pharmaceutical industry. These quality attributes of APIs are often very
sensitive to the crystallization process parameters such as method and degree of supersaturation (cooling, evaporation, anti-solvent addition etc.), operating temperature, solvent, impurities, agitation etc.\textsuperscript{3,7–11} Therefore, a thorough understanding of crystallization process parameters and their impact on critical quality attributes of APIs is required in order to produce consistent and desired quality APIs. The objective of this work is twofold: firstly, to investigate the interplay between crystallization process parameters and quality attributes of APIs; secondly, to understand the mechanisms involved in the crystallization process.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{crystal_packing_diagram.png}
\caption{A crystal packing diagram of $\gamma$-IMC (left) and $\alpha$-IMC (right).}
\end{figure}

Indomethacin (IMC), a non-steroidal anti-inflammatory drug, is selected in this work as a model compound. IMC is known to exhibit five polymorphs and several solvates.\textsuperscript{12} However, out of the five polymorphs, only $\alpha$-IMC and $\gamma$-IMC are obtained most commonly. These two IMC polymorphs are monotropically related to each other: $\alpha$-IMC (mp 149–154 °C) being the metastable and $\gamma$-IMC (mp 158–161 °C) being thermodynamically the most stable. Distinct crystal packing diagrams of $\alpha$-IMC and $\gamma$-IMC owing to their different physical and chemical properties are shown in Fig. 1. An asymmetric unit of $\alpha$-IMC contain three IMC molecules; two molecules hydrogen bonded through carboxylic acid groups and third molecule attached to the dimer via hydrogen bond between its carboxylic acid group and one of the amide carbonyls of the dimer as shown in Fig. 1.\textsuperscript{13} On the contrary, $\gamma$-IMC has two IMC molecules hydrogen bonded...
through carboxylic acid groups. It has also been reported that γ-IMC crystals exist as rhombic plates while α-IMC crystals has fine short needle like shape.\textsuperscript{14} There are several reports illustrating the differences in physical and chemical properties such as solubility, dissolution rate, reactivity, morphology and stability of IMC polymorphs.\textsuperscript{13–16} Only preliminary crystallization studies about IMC have been carried out previously, which clearly indicates strong dependence of process parameters on polymorphic and morphological outcome.\textsuperscript{14,17,18} In the present work, effect of crystallization process parameters such as solvent, temperature, supersaturation, seeding with γ-IMC etc. on the polymorphism and particulate properties of IMC is investigated. This work is organized in the following order: firstly, solvents of varying polarity were screened via measurement of IMC solubility at different temperatures followed by characterization of IMC nucleation through measurement of induction times in the selected solvents. Finally, seeded cooling crystallization of IMC was performed from ethanol. Crystallization experiments were performed at different supersaturations, temperature, and γ-IMC seed loads were used to induce crystallization. PAT tools such as ATR-FTIR probe was employed to monitor desupersaturation.

2. Materials and methods

2.1 Chemicals

Ethanol of TechniSolv® grade (purity ≥ 99.5%) purchased from VWR Chemicals was used in this work. Methanol, acetone, ethyl acetate, acetonitrile, and dichloromethane of CHROMASOLV™ grade (purity ≥ 99.9%) obtained from Honeywell Specialty Chemicals were used in this work. Indomethacin (1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid) of purity ≥ 99% purchased from Shanghai Hungsun Chemical Co., Ltd. was used after recrystallization step. Ultra-pure MiliQ water obtained from Purelab Chorus (ELGA) water purifier system was used.
2.2 Characterization of Indomethacin

Re-crystallized IMC was analyzed with X-ray powder diffraction (XRPD) and compared with the calculated XRPD patterns of γ-IMC (INDMET01) and α-IMC (INDMET02) obtained from The Cambridge Structural Database (CSD) as shown in Fig. 2.\textsuperscript{19} The α-IMC was produced from re-crystallized IMC as per the procedure mentioned by Kaneniwa et al.,\textsuperscript{20} which involved crystallization of IMC from hot solution in methanol by adding water as an antisolvent. Comparison of the XRPD patterns of re-crystallized IMC and α-IMC produced from it showed very good agreement with the calculated patterns of γ-IMC and α-IMC, respectively. Rigaku MiniFlex600 benchtop X-ray diffractometer equipped with Cu-Kα radiation source operating at 40 kV and 15 mA, a graphite monochromator, and NaI scintillation detector was used for analysis of samples. Samples were scanned from 5° to 50° 2Θ angle with a step size of 0.02 at the speed of 10°/min. IMC polymorphs were also characterized by FT-Raman spectroscopy and Simultaneous Thermal Analysis (STA). An FT-Raman spectrometer (MultiRam) from Bruker equipped with Nd:YAG laser sample excitation source (1064 nm) and a liquid nitrogen cooled Germanium diode detector was used for analysis. The samples were scanned in the wavenumber range of 3500 - 500 cm\(^{-1}\) with spectral resolution of 4 cm\(^{-1}\). Raman spectra of both IMC polymorphs provided in supplementary information clearly show the distinct features of α- and γ-IMC and good agreement with the literature data.\textsuperscript{21,22} Simultaneous thermal analyzer (STA 449 F3 Jupiter\textsuperscript{®}) from NETZSCH-Gerätebau GmbH was used for characterization of IMC polymorphs. The samples were heated from room temperature up to 200 °C in an alumina (Al\(_2\)O\(_3\)) crucible at heating rate of 10 °C/min. Thermograms of IMC polymorphs (Supporting information) confirms the melting point of γ- and α-IMC as 158.6 and 152.4 °C, respectively. It is also obvious that both polymorphs melts cleanly without any degradation and the
thermodynamically stable (γ-IMC) form has a higher melting point than the metastable form, thereby confirming their monotropic relationship.

![XRPD patterns of γ-IMC and α-IMC](image)

**Figure 2.** Measured and calculated XRPD patterns of γ-IMC and α-IMC.\(^{19,23}\)

### 2.3 Experimental methods

#### 2.3.1 Solubility measurement of Indomethacin

Organic solvents representing alcohol, ketone, ester, halogenated alkane, and nitrile class were considered for screening. Classical isothermal technique was used to measure the solubility of γ-IMC in ethanol, methanol, ethyl acetate, acetonitrile, acetone, and dichloromethane. The solubility was measured at 15, 25, 35, and 45 °C except for dichloromethane, where it was measured up to 35 °C due to the lower boiling point. The procedure for solubility measurement involved preparation of a suspension with excess amount of solute in 2 mL solvent contained in a 10 mL glass vial. Sealed vials were maintained at constant temperature under magnetic stirring for 24 h to attain equilibrium. Detailed description of the apparatus used for measurements is provided by Malwade et al.\(^{24}\) At the end of equilibrium, saturated solution was separated from excess solid phase with a syringe and 0.2 µm PTFE filters, appropriately diluted, and analyzed with High Pressure Liquid Chromatography (HPLC) to determine the concentration of IMC. The
solid phase was analyzed with XRPD to ascertain any possible phase change during solubility measurement. Four repetitions were performed for each measurement. Solubility of α-IMC was measured in ethanol at 15, 25, 35, and 45 °C. However, the transformation of metastable α-IMC into stable γ-IMC during solubility measurement was observed as reported earlier in the literature. Therefore, samples were equilibrated for 2 h to avoid transformation, which was confirmed by XRPD analysis of excess solid phase. Thus, the measured solubility of α-IMC can be referred as kinetic solubility instead of equilibrium solubility.

2.3.2 Induction time measurement of Indomethacin

Nucleation behaviour of IMC in ethanol, acetone, and ethyl acetate was determined through the measurement of induction times. An EasyMax102 workstation from Mettler-Toledo AutoChem, Inc. equipped with two 100 mL reactors, overhead stirrer and solid state thermostat enabled cooling/heating jacket as shown in Fig. 3 was used for measuring the induction times of IMC. Nucleation of IMC was detected through monitoring the turbidity of solution with Crystal Eyes turbidity probe from HEL group, UK. An ATR-FTIR (ReactIR 15) probe from Mettler-Toledo AutoChem, Inc. equipped with AgX probe interface (6 mm × 1.5 m Fiber), DiComp (Diamond) probe tip, and liquid nitrogen cooled MCT detector was also used for detecting nucleation of IMC in solution. Induction time of IMC was measured in ethanol, acetone and ethyl acetate at two temperatures (5 and 15 °C) as shown in Table 1. Corresponding supersaturations ($S = c/c^*$), calculated as the ratio of starting concentration ($c$) to the equilibrium concentration ($c^*$) of γ-IMC at studied temperatures, are also shown in Table 1. Three repetitions were performed for each measurement. Procedure included preparation of 100 mL solution of IMC with initial concentration in respective solvents shown in Table 1 and cooling down to the target temperature at 10 °C/min under constant stirring of 300 rpm. Saturated solutions of IMC in ethanol and ethyl
acetate were prepared at 45 °C, while in acetone at 35 °C due to its lower boiling point. Before cooling down to target temperature, solutions were heated 5 °C above saturation temperature in order to obtain clear solution. The time between the clear solution reaching the target temperature and the appearance of detectable nuclei was considered as induction time.

![Figure 3. Experimental set up used for induction time and crystallization experiments.](image)

**Table 1.** Experimental plan and operating parameters for induction time measurements.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Scale (mL)</th>
<th>Stirring speed (rpm)</th>
<th>Initial concentration (g/100g solvent)</th>
<th>Temperature ($S^*$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>100</td>
<td>300</td>
<td>5.1</td>
<td>5 °C (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.4</td>
<td>15 °C (3.2)</td>
</tr>
<tr>
<td>Acetone</td>
<td>100</td>
<td>300</td>
<td>16.5</td>
<td>5 °C (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.5</td>
<td>15 °C (1.8)</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>100</td>
<td>300</td>
<td>6.5</td>
<td>5 °C (2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6.5</td>
<td>15 °C (1.7)</td>
</tr>
</tbody>
</table>

* $S^*$ – Supersaturation calculated as ratio of initial concentration to the equilibrium concentration of γ-IMC.

**2.3.3 Crystallization of Indomethacin**
Crystallization of IMC was performed by preparing solution with certain concentration at a high temperature and then cooling down the solution to the studied temperature, 5 and 15 °C respectively. The experimental plan is shown in Table 2. For each temperature, two supersaturations were used as shown in Table 2. In order to induce crystallization of IMC, cooling crystallization experiments were seeded with γ-IMC. Seed loads equivalent to 1, 2, and 4% of the difference between starting concentration and equilibrium concentration (ΔC) were used for each supersaturation. Seeds of γ-IMC were prepared by recrystallization of IMC from acetonitrile and consisted a sieve fraction of range 71–125 µm. The concentration of IMC during seeded cooling crystallization was monitored with ATR-FTIR probe. The procedure included preparation of a fixed concentration solution of IMC in ethanol in an EasyMax reactor and maintained at 45 °C to ensure complete dissolution of solute. The solution was then cooled down to the target temperature at 10 °C/min and dry seeds of γ-IMC were added to the solution after 10 min of temperature reaching the target. Constant overhead stirrer speed of 300 rpm was maintained throughout the experiments. IMC crystals obtained at the end of the experiment were dried and analyzed with XRPD as well as particle size analyzer (LS 13 320 Laser Diffraction Particle Size Analyzer, Beckman Coulter, Inc.) to determine the polymorph and crystal size distribution, respectively.

**Table 2.** Experimental plan and operating parameters for crystallization of IMC from ethanol.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Temperature (°C)</th>
<th>Initial concentration (g/100g solvent)</th>
<th>Supersaturation (S)</th>
<th>Seed load (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>3.5</td>
<td>2.65</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>6.0</td>
<td>4.8</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>3.5</td>
<td>1.75</td>
<td>1</td>
</tr>
</tbody>
</table>
2.3.4 Calibration of ATR-FTIR

Concentration of IMC during crystallization experiments was monitored online by using ATR-FTIR probe. For the purpose, ATR-FTIR probe was calibrated with different concentrations of IMC in ethanol at 5 and 15 °C. A constant temperature approach was used where IMC solution of a known concentration is successively diluted by addition of known amount of ethanol at constant temperature. The background scan in air was collected at room temperature before starting the calibration process. Air was chosen over pure solvent as a background due to high sensitivity of IR spectra of solvent to the temperature variations. The spectra were collected in the wavenumber range of 3000 – 650 cm\(^{-1}\) with resolution of 8 cm\(^{-1}\). Each spectrum consisted of 256 scans. Two separate multivariate Partial Least Square (PLS) calibration models were developed from 22 FTIR spectra of IMC solution of varying concentration at 5 and 15 °C by using iC IR™ software from Mettler-Toledo AutoChem, Inc. Among the collected spectra during calibration, 70% were used as a training set while the remaining were used as a test set. The model was constructed by selecting the spectral region, 1750–1650 cm\(^{-1}\) containing the signals from carboxyl and benzoyl carbonyl (>C=O) group stretching in IMC. The spectra were baseline corrected by using cubic spline interpolation, which subtracts a polynomial fitted to the points selected on baseline from original spectrum, before using in the calibration model.

3. Results and discussion

3.1 Solvent screening through solubility of Indomethacin
Solubility of γ-IMC measured in ethanol, methanol, acetonitrile, acetone, and dichloromethane is shown in Fig 4. Solubility values at 5 °C are extrapolated from the regression model fitted to the experimental data. It is obvious from the figure that the solubility of γ-IMC is highest in acetone while lowest solubility was observed in acetonitrile. Moreover, linear behavior of IMC solubility with respect to temperature was observed in all solvents except ethanol and methanol, where exponential dependency on temperature was observed. The solubility values for IMC in ethanol and methanol were almost same. The product yield in cooling crystallization strongly depends on the slope of the solubility curve; higher slope is always desirable in order to obtain higher yields. In case of acetonitrile, the slope of IMC solubility curve is not very significant meaning that the product yield of a cooling crystallization would be rather low. Measured solubility values of IMC corresponds very well with previously reported solubility data.29 XRPD analysis of excess IMC recovered after solubility measurement confirmed formation of solvates in acetone, methanol, and dichloromethane at all temperatures, while no phase change was observed in other solvents. XRPD patterns of solid phase analysis during solubility measurements are provided in supplementary data. The solvents ethanol, acetone and ethyl acetate were considered for further investigation of nucleation behavior based on solubility values.
Solubility of metastable α-IMC was also measured in ethanol. Measured solubility of α-IMC is shown in Fig. 5 along with solubility of thermodynamically stable γ-IMC. During solubility measurement, solvent mediated transformation of α-IMC into γ-IMC was observed in agreement with previous reports.\(^{20}\) It was also found that the rate of transformation increase with increasing temperature and the transformation is complete in 18 h at 25 °C. Therefore, the solubility samples were agitated for only 2 h to avoid transformation. It is clear from Fig. 5 that the kinetic solubility of α-IMC is higher than γ-IMC at all temperatures, thereby confirming that the two polymorphs of IMC are monotropically related to each other.
**Figure 5.** Solubility of $\alpha$- and $\gamma$-IMC in ethanol at different temperatures.

### 3.2 Induction time of Indomethacin

Exemplary results from induction time measurement of IMC in ethanol at 5 °C (Supersaturation ($S$) = 4) containing temperature, turbidity and IMC concentration profile measured with ATR-FTIR probe are shown in Fig. 6. It is evident that the supersaturation was generated by rapid cooling of IMC solution from 45 °C to 5 °C and maintained at 5 °C until nucleation. Turbidity and IMC concentration of supersaturated solution was constant until nucleation of IMC happened at 168 min as indicated by an arrow in Fig. 6. The detection of nuclei formed was also confirmed with visual inspection. Upon nucleation of IMC at 168 min, the turbidity of solution started increasing and IMC concentration started decreasing marginally until 190 min indicating slower primary nucleation rate. However, the respective increase and decrease in turbidity and IMC concentration appears to be faster onwards 190 min, which might be due to the inducement of secondary nucleation, growth of primary nuclei or combination of both. A large clump consisting of fine needle shaped crystals was obtained at the end of experiment. Analysis of these crystals with XRPD, FT-Raman spectroscopy, and DSC confirmed crystallization of metastable $\alpha$-IMC. Exemplary results from induction time measurement of IMC in acetone at 15 °C...
(corresponding supersaturation ($S = 1.81$) are shown in Fig. 7. It is evident from the concentration and turbidity profile that the nucleation of IMC occurs in 17 min after the solution is cooled down to 15 °C. Thus, relatively shorter induction time of IMC was observed in acetone. Moreover, the nucleation kinetics appears to be very fast from the sharp increase in turbidity and decrease in IMC concentration. A dense slurry of fine needle shaped crystals was obtained at the end of experiment. The crystals were analysed with XRPD and TG-DSC. XRPD patterns clearly showed additional peaks compared to $\alpha$- and $\gamma$-IMC indicating formation of acetone solvate. TG-DSC analysis of the crystals confirmed formation of a non-stoichiometric acetone solvate. The thermogram of crystals clearly showed an endothermic peak corresponding to the desolvation of acetone at 85.7 °C followed by crystallization of a mixture of $\alpha$- and $\gamma$-IMC as evident from two exothermic peaks corresponding to their melting temperatures. In case of ethyl acetate, no nucleation of IMC was observed even after waiting until 5 h for both supersaturations used during induction time measurements. Table 3 summarizes the averaged results of IMC induction time measurements in all experiments. As expected, an increase in induction time of IMC in ethanol and acetone is observed with decrease in driving force i.e. supersaturation. The stochastic nature of nucleation is also evident from the different induction times obtained in the repeated experiments. Longer induction times of IMC in ethanol conforms to the previously reported results\textsuperscript{17}, where it has been attributed to the strong hydrogen bonding between IMC and ethanol molecules, thereby posing higher energy barrier for nucleation. Possible solute-solvent interactions have been discussed below in order to justify the nucleation behaviour of IMC in ethanol, acetone and ethyl acetate.
Figure 6. Results of induction time measurement of IMC in ethanol at 5 °C.

Figure 7. Results of induction time measurement of IMC in acetone at 15 °C.

Table 3. Results from induction time measurement of IMC in all solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>No. of repetitions</th>
<th>Induction time (min)</th>
<th>Solid form</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 °C</td>
<td>15 °C</td>
</tr>
<tr>
<td>Ethanol</td>
<td>3</td>
<td>108 ± 10.6</td>
<td>161 ± 10.26</td>
</tr>
<tr>
<td>Acetone</td>
<td>4</td>
<td>4.50 ± 4.09</td>
<td>14.0 ± 3.60</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Solute-solvent interactions
As described by Davey et al., a nucleation process can be considered as a two step process where the solute molecules pre-assemble themselves into clusters with a structure that is similar to the prospective crystal form followed by the growth of this assembly. For this to happen in a crystallization from solution, firstly the non-covalent interactions between solvent and solute molecules (hydrogen bonding, van der Waals forces, π-π interactions etc.) needs to be broken, which may require varying amount of energy depending upon the strength of such interactions. The strength of such interactions reportedly vary with different solvents. Several other studies describes the importance of non-covalent solute-solvent interactions influencing the outcome of crystallization processes. In case of IMC nucleation, specifically α-IMC, three hydrogen bonded molecules of IMC needs to orient to form an asymmetric unit of crystal structure as shown in Fig. 1. IMC molecule with hydrogen bond acceptor and donor count of 4 and 1, respectively has strong tendency to form intramolecular as well as intermolecular hydrogen bonds. Ethanol molecules with one hydrogen bond donor and acceptor each have the ability to engage all hydrogen bond acceptors as well as donor of IMC molecule as shown in Fig. 8. Thus, ethanol-IMC hydrogen bonding interactions in solution offers a barrier for self assembling of IMC molecules to form crystal nuclei, which might be the reason for remarkably longer induction times of IMC in ethanol. Ethyl acetate with two hydrogen bond acceptor count is capable of forming hydrogen bond only with carboxylic group of IMC as shown in Fig. 8, however, it has been reported to interact with π system of indole ring. Additionally, it is also capable of forming C-H….. π interactions with benzoyl chloride π system through the ethyl group of ether. These ethyl acetate-IMC interactions might be hindering the assembling of IMC molecules to form pre-assemblies that leads to formation of crystal nuclei. Hydrogen bonding of acetone molecule with carboxyl group of IMC molecule is also shown in Fig. 8. Despite
possibility of forming hydrogen bonded dimers with IMC, very short induction times of IMC were observed in acetone. In this case, intramolecular (IMC-IMC) non-covalent interactions might be dominating due to relatively high concentration of IMC in acetone solution (Fig. 7) leading to faster nucleation of IMC-acetone solvate.

Figure 8. Possible solute-solvent interactions between IMC-ethanol (1), IMC-ethyl acetate (2), IMC-acetone (3).

3.3 Crystallization of IMC from Ethanol

3.3.1 Effect of supersaturation and seeding at 5 °C

Desupersaturation profiles from seeded cooling crystallization of IMC from ethanol at low supersaturation ($S = 2.65$) and particle size distributions of the obtained products are shown in Fig. 9. It is evident from figure that the IMC concentration starts decreasing after addition of γ-IMC seeds at 50 min indicating the consumption of supersaturation for the growth of seed crystals or secondary nucleation. Desupersaturation profiles for all seed loads clearly indicate very slow consumption of supersaturation meaning the slow crystal growth or nucleation rate. XRPD analysis of the crystals obtained at the end of experiment confirmed the polymorph as γ-IMC.
Figure 9. Results from seeded cooling crystallization of IMC from ethanol at 5 °C and low supersaturation (left); particle size distribution of obtained crystals (right).

Moreover, the effect of seed mass on the rate of supersaturation consumption is clearly visible from the desupersaturation profiles and shows that the increase in seed mass (increase in surface area) slightly increase the supersaturation consumption rate. The particle size analysis of the crystals obtained for all seed loads showed bimodal distribution as shown in Fig. 9 (right). The median diameter (d50) of crystals decreases slightly with increasing seed mass. In order to get insight into the crystal morphology, the obtained crystals were analyzed with scanning electron microscope (SEM). An SEM image of the crystals obtained for 1% seed load is shown in Fig. 10, which clearly indicates the rhombic plate like morphology of γ-IMC. Furthermore, it also shows the smaller particles that might be the fragments of larger plates as the plate like crystals are more prone to breakage. However, the presence of seed crystals in the solution may induce the secondary nucleation as well. Therefore, bimodal particle size distribution obtained during the experiments can be attributed to the growth of seed crystals and either secondary nucleation, breakage or combination of both.
Results of seeded cooling crystallization of IMC from ethanol at high supersaturation \((S = 4.8)\) containing desupersaturation profiles and an SEM image of crystals obtained for 1% seed load are shown in Fig. 11. Similar to the experiments at low supersaturation, IMC concentration started decreasing after addition of seeds at 80 min as shown in desupersaturation profiles. However, after 20 to 30 mins of seeds addition, a sudden crystallization of IMC was observed, as it is apparent from the sharp decrease in the IMC concentration. A dense slurry of IMC crystals was collected, dried and analyzed with XRPD as well as scanning electron microscope. An SEM image of the crystals shown in Fig. 11 (right) confirms the morphology of crystals as fine short needles. XRPD analysis confirmed the polymorph of obtained crystals as metastable \(\alpha\)-IMC despite seeding the experiment with stable \(\gamma\)-IMC. Similar observation was observed in our previous work for the crystallization of nitrofurantone from an acetone-water solution, where seeding the supersaturated solution with the polymorphic form I of nitrofurantone monohydrate couldn’t produce form I, instead the polymorphic form II was crystallized.\(^{41}\) According to Desiraju,\(^{39}\) the crystallization of polymorphs in such situations may follow kinetically favored
path, which can be achieved relatively faster due to lower activation barrier leading to the crystallization of metastable form.

**Figure 11.** Results from seeded cooling crystallization of IMC from ethanol at 5 °C and high supersaturation (left); an SEM image of crystals obtained for 1% seed load (right).

### 3.3.2 Effect of supersaturation and seeding at 15 °C

Desupersaturation profiles for seeded cooling crystallization experiments at 15 °C with low and high supersaturation followed similar trends as shown earlier for 5 °C i.e., slow consumption of supersaturation after addition of seeds and marginal increase in the rate with increasing seed mass. However, thermodynamically more stable \( \gamma \)-IMC was obtained in all experiments at 15 °C. The particle size distribution of crystals obtained for low supersaturation (left) and high supersaturation (right) at 15 °C is shown Fig. 12. Nearly unimodal distribution of \( \gamma \)-IMC crystals resulting from the growth of seed crystals is obtained at low supersaturation. Moreover, the effect of seed mass on mean diameter seems to be negligible. On the contrary, a bimodal distribution of \( \gamma \)-IMC crystals resulting from growth of seed crystals and either breakage, secondary nucleation or combination of both is obtained at high supersaturation. The effect of
seed mass is not very significant at low supersaturation; however, larger median particle
diameter is obtained with seed loadings of 2% and 4% at high supersaturation.

![Particle size distribution](image)

**Figure 12.** Particle size distribution of γ-IMC obtained with low supersaturation (left) and high
supersaturation (right) at 15 °C.

The polymorphic and morphological outcomes of seeded cooling crystallization of IMC from
ethanol at all experimental conditions investigated in this work are summarized in Table 4. It is
evident that the crystallization process parameters does influence the particulate properties of
IMC. The crystallization course for all seeded cooling crystallization experiments are projected
on solubility diagram of IMC polymorphs as shown in Fig. 13. It is evident that the super cooled
solutions for high supersaturation at 5 °C and 15 °C (point 1 and 2, respectively) are
supersaturated in terms of both, α-IMC and γ-IMC, polymorphs. According to Ostwald’s rule of
stages, the metastable α-IMC is expected to crystallize first in such situations followed by
solvent mediated transformation into more stable γ-IMC. In case of crystallization at 15 °C,
addition of γ-IMC seeds altered the outcome while it didn’t influence the outcome at 5 °C with
high supersaturation. For crystallization experiments with low supersaturation at 5 °C and 15 °C
(point 3 and 4, respectively), although a marginal supersaturation was achieved for α-IMC,
addition of \( \gamma \)-IMC seeds dictated the outcome. The existence of a critical supersaturation between the two levels used in this work for crystallization of IMC at 5 °C above which presence of \( \gamma \)-IMC seeds do not dictate the outcome is apparent from the results. Present work clearly establishes the fact that the outcome of a crystallization process can be engineered through careful crafting of the crystallization process parameters to produce consistent and desired quality APIs.

**Table 4.** Summary of results from seeded cooling crystallization of IMC in ethanol.

<table>
<thead>
<tr>
<th>Properties</th>
<th>5 °C</th>
<th>15 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low S (2.65)</td>
<td>High S (4.8)</td>
</tr>
<tr>
<td>Polymorph</td>
<td>( \gamma )</td>
<td>( \gamma )</td>
</tr>
<tr>
<td>Median particle Diameter – d50 (µm)</td>
<td>76</td>
<td>72</td>
</tr>
</tbody>
</table>

**Figure 13.** Projection of crystallization course of IMC from ethanol on solubility diagram. Point 1 and 3 - high and low supersaturation at 5 °C; point 2 and 4 - high and low supersaturation at 15 °C, respectively.

4. **Conclusion**
Solubility of \( \gamma \)-IMC was measured in different solvents at temperatures ranging from 15 °C to 45 °C with the aim of selection of crystallization solvents followed by measurement of IMC induction times in selected solvents. In order to establish the thermodynamic boundaries associated with both polymorphs of IMC, the solubility of \( \alpha \)-IMC was also measured in ethanol. Highest solubility of \( \gamma \)-IMC was observed in acetone while lowest was in acetonitrile. Solid phase analysis of excess solute during solubility measurements confirmed formation of solvates in acetone, methanol, and dichloromethane at all temperatures studied. Longer induction times of IMC were observed in ethanol, while shorter induction times were observed in acetone, from which IMC-acetone solvate was nucleated. Metastable \( \alpha \)-IMC was nucleated from ethanol, while no nucleation was observed from ethyl acetate at studied temperatures and supersaturations. Analysis of possible IMC-solvent non-covalent interactions suggests the role of hydrogen bonding and C-H...\( \pi \) hydrogen bonding in delaying the nucleation from ethanol and inhibiting nucleation from ethyl acetate, respectively. Results from cooling crystallization of IMC from ethanol seeded with \( \gamma \)-IMC suggests that the supersaturation, temperature, and seeding does influence the polymorphism and morphology of IMC. Thermodynamically stable \( \gamma \)-IMC crystals with well-defined rhombic plate like shape were obtained in all experiments except at 5 °C with high supersaturation (4.8), where \( \alpha \)-IMC crystals with fine needle like shape were obtained. At 5 °C with high supersaturation, the crystallization of IMC follows kinetically favored path leading to the crystallization of metastable \( \alpha \)-IMC despite seeding with \( \gamma \)-IMC. In terms of crystal size distribution, larger size particles were obtained at 15 °C with high supersaturation (2.6). Bimodal distribution of crystal size obtained in all experiments was the result of seed crystal growth, secondary nucleation, breakage or combination of secondary nucleation and breakage. Thus, this work confirms the effect of operating parameters on the outcome of IMC crystallization from
ethanol in terms of polymorphism and morphology and highlights the importance of operating the crystallization process within the design space in order to obtain desired outcome.

ASSOCIATED CONTENT

Supporting Information. FT-Raman spectra showing distinct features of carbonyl group vibrations in α- and γ-IMC, thermograms showing melting point and melting enthalpy of α- and γ-IMC, a thermogram of IMC-acetone solvate, XRPD patterns of IMC-acetone and IMC-dichloromethane solvates.

AUTHOR INFORMATION

Corresponding Author

*Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Campusvej 55, 5230 Odense, Denmark. E-mail: crm@kbm.sdu.dk; Phone: +45 65508669

Funding Sources

Danish Council for Independent Research (DFF) financed this work with grant ID: DFF-6111-00077B.

ACKNOWLEDGMENT

Authors would like to thank Danish Council for Independent Research (DFF) for financing this work with grant ID: DFF-6111-00077B.

REFERENCES


Dev. 2017, 21 (6), 855–865.


(26) Saal, C.; Peterit, A. C. Optimizing Solubility: Kinetic versus Thermodynamic Solubility


(34) Tulashie, S. K.; Lorenz, H.; Malwade, C. R.; Seidel-Morgenstern, A. Ternary Solubility Phase Diagrams of Mandelic Acid and N-Methylephedrine in Chiral Solvents with


(42) Davey, R. J.; Allen, K.; Blagden, N.; Cross, W. I.; Lieberman, H. F.; Quayle, M. J.;
Righini, S.; Seton, L.; Tiddy, G. J. T. Crystal Engineering – Nucleation, the Key Step.