The Importance of Impurity on Pharmaceutical Processes

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A thesis submitted for the degree of Doctor of Philosophy (Ph.D.)

Submitted to the University of Limerick, November 2019.
Solution crystallization processes are widely treated as binary systems consisting of a solute and a solvent. For real systems, additional components such as additives and impurities may significantly impact crystallization processes even when present in very small amounts. An understanding of the mechanistic role of additives and impurities is therefore essential to design and control crystallization processes. This thesis first describes the solubility and crystallization of pure active pharmaceutical ingredients (API’s) from solution. Subsequently, it discusses the thermodynamic, kinetic and crystallization effects, caused by impurities. Eventually, these knowledge were applied to optimize impurity removal processes by using a combined experimental-modelling approach to investigate a mother-liquor recycle operation and improve properties on the processability of API.

The gravimetric solubility method and how solubility models cope with industrially-relevant complex products belonging to the α-Thio-β-chloroacrylamide family which is a class of highly versatile synthetic intermediates was examined. One of the drawbacks of the gravimetric method is the evaporation of solvents which is due to elevated operating temperature or the volatile nature of the solvent itself. Solubility data at higher temperatures, beyond the atmospheric boiling point of solvents, allows for an increase in crystallization yield. A pressurized-synthetic methodology was presented as a new technique for determining high-temperature solubility data even beyond the atmospheric boiling point. With the gravimetric method in combination with HPLC analysis, the effect of impurities (4-nitrophenol and 4’-chloroacetanilide) on the solubility of paracetamol has been determined and modelled.

To study the effect of volume on the nucleation kinetics of paracetamol, an automated FBRM-method was applied to record induction times. The shear rate was rationalized
to be the part of the kinetic parameter that changes most significantly when changing the crystallizer type, up to a specific volume beyond which the effect becomes negligible. Induction time experiments were used in combination with the classical nucleation theory and demonstrated that the impurities employed reduced the nucleation rate. The impurities did not affect the solid–liquid interfacial energy but significantly reduced the kinetic factor.

The poor compression ability of paracetamol is well known. The crystal habit of paracetamol was altered in the present of structurally similar impurity (4’-chloroacetanilide) to improve the compaction behaviour of the paracetamol crystals. An experimental design space was developed and utilized to select the most important process parameters for impurity incorporation. As a result, it was feasible to accurately control the compressibility and the amount of 4’-chloroacetanilide in the solid phase of paracetamol by simply choosing the required alcohol as the solvent for crystallization.

In crystallization process, recycle of mother liquor allows for reduced waste and increased yield with complete control of the impurity concentration. A sequence of batch-cooling crystallization experiments was demonstrated to investigate how a mother liquor recycle operation affects the crystallization of paracetamol as a result of the gradual build-up of the impurity 4-nitrophenol. The results can be used as a guide to estimate the optimum mother liquor recycle conditions that would lead to reduced product and solvent waste and improved process efficiency.

The result of this thesis addresses a number of challenges in the crystallization of API’s and impurities and leads to improved impurity removal processes. To obtain high yield as well as specific crystal quality attributes while maintaining a control on impurities, techniques strategies including continuous crystallization with recycle and pressurized methods were developed. Furthermore, rational process control over the incorporation of impurities and additives allows for advanced manufacturing of products with tailored specifications.
List of Publications


* These authors contributed equally.
Hereby, I declare, as supervisor and corresponding author of the mentioned papers, that Leila Keshavarz, in an association with our colleagues, was responsible for the greater part of the work on solubility and induction time experiments, modelling, crystallization experiments and analysing the results and contributed to the lettering of the manuscripts.

[Signature]

Dr Patrick Frawley
(Supervisor and co-author of the papers)
Declaration

July 2019

The substance of this thesis is the original work of the author and due reference and acknowledgment has been made, where necessary, to the work of others. No part of this thesis has been submitted for any degree or award.

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This thesis is dedicated to my family and my friends for their endless support and love.
Acknowledgements

I would like to take this opportunity to thank those who helped make this thesis and my four years at the University of Limerick. I am grateful for everyone’s dedication and support throughout these four years. First and foremost, I thank my supervisor, Dr. Patrick Frawley for his great help during my PhD study and the thesis preparation. It was nice to work with him in a kind and helpful atmosphere. A very special thank you to my friends and my colleagues in the crystallisation group specially Dr. Brian De Souza and Dr. Renê R. E. Steendam for motivation and their help. Finally, I would like to express special thanks to the Solid State Pharmaceutical Centre (SSPC) and Science Foundation Ireland (SFI) for financial support and equipment through my PhD project.
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Nomenclature

Acronyms
ACE  Acetone
ACN  Acetonitrile
API  Active Pharmaceutical Ingredient
CA  4’-chloroacetanilide
DIW  Deionized Water
DSC  Differential Scanning Calorimetry
GC  Gas Chromatography
HPLC  High Performance Liquid Chromatography
IPA  Iso-Propyl Alcohol
LC  Liquid Chromatography
MSE  Mean Square Error
MSZW  Meta-Stable Zone Width
MW  Molecular Weight [g/mol]
NP  4-Nitrophenol
PA  Paracetamol
PSD  Particle Size Distribution
PTFE  Polytetrafluoroethylene
SEM  Scanning Electron Microscopy
UV  Ultraviolet
XRPD  X-Ray Powder Diffraction

Variables with units and symbols
\[ a_A \]  First Apelblat parameter
\[ a_2 \]  Solute activity
Margules binary interaction parameter

First Van-Laar interaction parameter

Second Apelblat parameter

Second Van-Laar interaction parameter

Third Apelblat parameter

Equilibrium concentration \( [\text{g}\text{sol}/\text{Kg}\text{solv}] \)

Exchange of heat capacity at constant pressure evaluated at melting point \( [\text{J/mol/K}] \)

NRTL binary interaction parameter

Corrected temperature-dependent NRTL binary interaction parameter

Exchange of enthalpy of fusion evaluated at melting point \( [\text{KJ/mol}] \)

Impurity content [mole fraction]

Boltzmann constant \( [\text{J/K}] \)

Molecular weight solute \([\text{g/mol}]\)

Molecular weight solvent \([\text{g/mol}]\)

Number of carbon atoms of solvent

Number of experimental points for each solvent

Pressure \([\text{Pa}]\)

Reference pressure \([\text{Pa}]\) (atmospheric condition)

Ideal gas constant \([\text{J/mol/K}]\)

Temperature \([\text{K}]\)

Triple-point temperature \([\text{K}]\)

Melting temperature of paracetamol \([\text{K}]\)

Molar volume of solute in the solid state \([\text{cm}^3/\text{mol}]\)

Molar volume of the i-th or j-th component \([\text{cm}^3/\text{mol}]\)

Solute molar fraction

Reference solute molar fraction (atmospheric condition)

Composition of HPLC mobile phase \([v/v\%]\)

NRTL non-randomness parameter

The alpha polymorphic form of 4-nitrophenol
\( \beta \)-NP \hspace{1cm} \text{The beta polymorphic form of 4-nitrophenol}

\( \gamma_2 \) \hspace{1cm} \text{Activity coefficient referred to the solute}

\( \lambda_{ij} \) \hspace{1cm} \text{Wilson binary interaction parameter}

\( \Lambda_{ij} \) \hspace{1cm} \text{Temperature-dependent Wilson binary interaction parameter}

\( \tau_{ij} \) \hspace{1cm} \text{Temperature-dependent NRTL binary interaction parameter}

\( \theta \) \hspace{1cm} \text{Set of binary interaction parameters for each activity equation model}
Introduction

Separation processes to reduce the level of impurities are consequently widely used in the chemical and pharmaceutical industry. Solution crystallization is a highly selective and scalable unit operation that is widely used to purify the desired product from its impurities (Moynihan & Horgan, 2017). From the pharmaceutical industry perspectives, impurity can carry significant health risks; strict regulations related to the quality of active pharmaceutical ingredients (API) are put in place by regulatory agencies such as Food and Drug Administration (FDA) in USA or European Medicines Agency (EMA) in Europe (Gorog, 2006) (Raillard, 2012). Hence quality aspects of product/API, such as bioavailability, toxicity, and stability, which can be influenced by impurities, additives and excipients that can be present in the system, are required to be considered in the study of industrial crystallization. In some cases impurities or additives can be added in order to improve the API behaviour (Goole et al., 2010) (García-Arieta, 2014).

The driving force in crystallization is supersaturation and solubility of API can increase or decrease in the presence of impurities. The influence of impurities/additives on the crystal formation, such as nucleation, growth or polymorphic transformation is known and a number of publications dealt with the subject. An impurity that acts as a nucleation inhibitor in one case may not necessarily be effective in another; or it may even act as an accelerator. No general rule applies and each case must be considered separately as impurity effect can be rationalized in terms of intermolecular interactions (Mullin, 2001). The impurity effect on growth in most cases is inhibition and it is explained as a result of surface adsorption mechanism (Myerson, 2002).
The effect of impurities on the crystal shape is an area of increasing interest in crystallization with considerable amount of experimental work looking into shape changes and purity of the product (Prasad et al., 2001) (Lim & Yao, 2009).

The model system chosen in this work is Paracetamol. PA is known as acetaminophen has three different polymorphic forms: form I, the stable form at room temperature, monoclinic Form I (Haisa et al., 1976) (Nicnols & Frampton, 1998); metastable orthorhombic Form II (Haisa et al., 1974) (Drebushchak & Boldyreva, 2004) and the highly unstable Form III (Perrin et al., 2009).

The monoclinic form is the thermodynamically stable polymorph at room temperature and, hence, is the commercially used form of paracetamol. A particular problem with the monoclinic form of paracetamol is that it displays poor compaction behaviour, that is, it resists compression into tablets (Femi-Oyewo & Spring, 1994). There is considerable interest in modifying the properties of paracetamol using different crystallization techniques or additives to improve the compaction behaviour of the crystals (Garekani et al., 2000).

In this thesis in order to study the effect of impurities on the different aspects of crystallization, first, a clear understanding of the fundamental of crystallization (thermodynamic and kinetic factors) for pure system has been studied. Solubility was examined to investigate the thermodynamic effect. Nucleation was examined to investigate the kinetic effect. Subsequently, systems include impurities were studied. Furthermore, in term of optimization, solubility and induction time data was used to develop and model solution crystallization processes with a mother liquor recycle operation.

An experimental and modelling approach to investigate the maximum fraction of mother liquor that could be recycled while still maintaining the desired crystal product specifications were used. The different aspects involved in thesis are outlined in fig. 1.
Aims and objectives of the research

The first step in the development of impurity removal strategies is to obtain fundamental data on how impurities affect crystallization. This data includes solubility, polymorphism, nucleation kinetics, crystal shape and crystal size distributions. Therefore, the first main aim of the thesis is to investigate the fundamental data for both pure and impure system. Furthermore, this data was used to develop the purification with mother liquor recycle and improving the Compressibility of API.

The aims and objectives of the research are listed as follows:

- To establish solubility data for paracetamol up to and beyond the atmospheric boiling point in pure solvents.
- Obtain the binary interaction coefficient of activity coefficient models include NRTL, Margules, Van Laar and Wilson for API’s and impurities in presence of a wide range of solvents.
- To provide a better understanding on the effect of crystallizer type and volume on the nucleation mechanism and kinetics.
- To characterise the temperature dependent solid-liquid properties (such as solubility and polymorphic screening) of two impurities of paracetamol.
• To determine the effects of the main impurities of paracetamol on the solubility, polymorphism, crystal shape, crystal size distribution and nucleation kinetics of paracetamol.
• To control the crystal size and shape of paracetamol in the presence of additive by applying different process parameters in order to increase the compressibility of paracetamol.
• To investigate the effects of impurity build up in the continuous crystallization with recycle of mother liquor.
• To optimise impurity removal processes by using a combined experimental-modelling approach to investigate a mother-liquor recycle operation.

Thesis Outline

This thesis comprises of ten chapters:

Chapter one reviews and discusses the relevant literature and concepts of crystallization processes, such as solubility, supersaturation, kinetics of crystallization and the effect of impurities.

In chapter two, the solubility of a key synthetic intermediate (N-4-methylphenyl-Z-3-chloro-2-(phenylthio) propenamide) has been measured in 12 pure solvents which were specifically selected for their potential utility in synthesis and isolation at scale.

In chapter three, a new nonintrusive technique (pressurized-synthetic method) was developed for determining high-temperature solubility data and paracetamol was used as a model active pharmaceutical ingredient to validate the methodology. The pressurized-synthetic methodology is presented as a new technique for determining high-temperature solubility data.

In Chapter four, induction times of crystallization of paracetamol in 2-propanol are described, which were acquired using an automated methodology involving the use of a focused beam reflectance measurement (FBRM) probe. This methodology is easily interchangeable between different crystallizers which allowed us to investigate the effects of scale-up on the kinetics of crystal nucleation of paracetamol from 2-
propanol in four different crystallizers, ranging from small magnetically stirred 10 mL solutions to overhead-stirred solutions of 680 mL.

The second part of the thesis focuses on the effect of impurities on crystallization and process engineering. The impact of structurally-related additives and impurities on active pharmaceutical ingredients is an essential yet poorly understood area. **Chapter five** describes the characterization of temperature dependent solid-liquid properties of 4-nitrophenol and 4′chloroacetanilide in four different alcohols and their effect as impurities on the crystallization and solubility of paracetamol.

**In chapter six,** the effect of two markedly different impurities 4-nitrophenol and 4′-chloroacetanilide on the solubility, nucleation and crystallization of paracetamol are described. This chapter comprehensively shows how different impurities impact the key crystallization mechanisms and properties of a pharmaceutical product.

**In chapter seven,** the effect of 4-nitrophenol on the nucleation of paracetamol was investigated and in the following, a simple model was used to describe the impurity build-up and to estimate the optimum mother liquor recycle fraction required to obtain the maximum achievable product yield while still maintaining the desired product specifications.

**Chapter eight** provides a conclusion and summarizes the work carried out in this thesis and provides an outlook on potential future work.
Chapter 1  Literature Review

1.1 Crystallization

Crystallization is one of the most widely used technologies in the chemical industry. In particular, the pharmaceutical and food sectors are utilizing crystallization for optimized separation, purification, and solid form selection. For example, crystallization is the most common method of formation of pharmaceutical solids for Active Pharmaceutical Ingredient (API) development. One of the most applied methods of crystallization is cooling crystallization which is achieved by reducing the solubility of the product in a saturated starting solution by cooling.

1.2 Solubility and supersaturation

Crystallization process mainly bases itself on the thermodynamic behaviour of the system. These characteristics include solubility, supersaturation and the metastable zone; which play a role in subsequent kinetics. An understanding of solubility is the starting point for crystallization design, forming a key aspect to decide the throughput and yield. Also, it plays a crucial role in the solvent selection system as well as the type of crystallization. The next step being, an understanding of metastable characteristics, refer to solutions that are supersaturated, yet are not ready to nucleate spontaneously.

The first step in industrial application of crystallization is often to examine equilibrium data and select appropriate mode of crystallization.
A typical solubility curve which determines the mode of crystallization to be employed, in order to crystallize a particular substance is shown in figure 1.1. Solubility refers to the maximum amount of solute that will dissolve in a given amount of solvent at a specified temperature and pressure, which show as solubility line in the figure 1.1. In most cases, the solubility increases with increasing temperature, although the rate of the increase varies. Because of the temperature dependence at the solubility, different techniques can be applied for crystallization, such as cooling crystallization, evaporation (J. W. Mullin, 2001).

There are three different types of solutions that will result depending on how much of a particular solute is dissolved in the solvent.

- **Unsaturated solution** (Point A) is in the stable region, contains less dissolved solute for a given temperature and pressure than a saturated solution and no possibility of crystallization.
- **Saturated solution** (point B) contains the maximum amount of dissolved solute for a given amount of solvent at a specific temperature and pressure.
- **Supersaturation solution** contains more dissolved solute than a saturated solution at the same temperature.
Metastable zone is the area between the solute temperature and the temperature at which spontaneous nucleation can occur in a short time (dash line). Point C is in Metastable zone that solution can remain in this area without spontaneous nucleation, while still growth is possible. The MSZW can also be characterized using Focused Beam Reflectance Measurement (FBRM). Two standard approaches are used to determine MSZW. In the polythermal method, the solution is cooled at a constant rate until nucleation is detected. For the isothermal method, a constant supersaturation is maintained until visible crystals are formed. The time between the generation of supersaturation and the formation of visible nuclei is defined as the induction time.

Davey and Garside (2000) explained the crystallization process based on chemical potentials. When a solution is in equilibrium with solid solution, the chemical potentials of the solute in solution, $\mu_{solution}$ and the solid phase, $\mu_{solid}$, are equal. The fundamental driving force for crystallization is the difference of the chemical potential at the substance in solution and solid states (Davey & Garside 2000):

$$\Delta \mu = \mu_{solution} - \mu_{solid} \quad (1.1)$$

The chemical potential is defined in terms of the standard potential and the activity, $a$.

$$\mu = \mu_0 - RT \ln a \quad (1.2)$$

The dimensionless driving force may be defined as:

$$S = \exp\left(\frac{\Delta \mu}{RT}\right) \quad (1.3)$$

A simple and widely used expression of the driving force is based on the difference of the actual and saturation concentration:

$$\Delta c = c - c^* \quad (1.4)$$

where $\Delta c$ means the concentration driving force, $C^*$ and $C$ are the actual concentration and solubility at a given temperature, respectively.
1.3 **Solubility measurement**

Solubility is a very important property for pharmaceutical industry. There are several widely used methods for solubility measurements, the most common methods are gravimetric and shake flask methods (Higuchi & Connors, 1965). In these methods, an excess of drug solute is added to the solubility medium and the phases are separated using filtration or centrifugation. Quantification of drug concentration in the saturated solution is measured by techniques such as ultraviolet (UV) spectrophotometric analysis or high-pressure liquid chromatography (HPLC). One of the drawbacks of these methods is at elevated temperature with volatile solvents, solubility measurements can be compromised by evaporation of the solvent.

In the case of the pharmaceutical cooling crystallization process, the difference in solubility as measured between upper and lower temperature bounds is by definition implicitly linked with the critical crystallization design metrics of yield, throughput and solvent usage. The providing of high and low temperature solubility data is therefore of upmost importance for industrial crystallization design. In general, is apparent that API solubility curves show the most marked gradients at elevated temperatures. Thus, small increases in the upper temperature can potentially lead to significant process improvements. Accurate high temperature solubility data is therefore imperative to designing crystallization processes; yet remarkably, to date, the provision of high temperature solubility data is notably absent from published literature.

1.3.1 **Gravimetric method**

The gravimetric method is one of the most accurate methods to determine solubility but it is time consuming. Also, this method is not ideal for the viscous solvents where separation of the excess solid saturated solutions is not achievable. In the gravimetric approach, the temperature of equilibration is fixed, and a saturated solution achieved by contacting excess solute with solvent. Equilibrium is approached asymptotically,
where upon a sample of the equilibrated solution is filtered and weighed. The solvent is then, gently evaporated thus allowing for the evaluation of residual crystalline material mass and solubility. Unavoidable cooling occurs despite efforts to minimise the time between sample collection, filtering and gravimetric weighing. Filtration of fine particles may not be entirely successful, with some particles passing to the sample of equilibrated solution. At elevated temperatures, and with volatile solvents, solubility measurements using either of these methods can be compromised by virtue of evaporation of the solvent. The maximum temperature for which solubility data can be obtained using both approaches is bounded by the atmospheric boiling point of the solvent. Due to the growing need to determine solubility, new devices and automated maintenance methods have been developed.

1.3.2 Synthetic method

The synthetic method which is so called laser monitoring technique (Jouyban & Fakhree, 2012) (Sheng et al., 2018) (Luo et al., 2016), last crystal disappearance method (Hao et al., 2005) is based on disappearance of the solid drug (from the mixture of solvent and drug) monitored by a laser beam. It is claimed that the synthetic method is much faster and more reliable than an analytical method.

1.3.3 Pressurized synthetic method

This method is a non-intrusive synthetic method for solubility determination is utilised in conjunction with a pressurised vessel. This provides an effective way to minimise evaporation, while allowing for the evaluation of solubility close to and in some cases beyond the atmospheric boiling temperature of the pure solvents. As mentioned, this method is based on the dissolution and consequent disappearance of the solute. The signal transmitted through the vessel was collected by a detector that decided the rate of temperature rise and estimated the equilibrium point of the given system on the basis of the signal change. The use of reactor pressurisation extends the capability of the synthetic method, providing an innovative way to determine
solubility even beyond the atmospheric boiling point of the solvent. The disappearance of drugs could be achieved by changing the temperature of a known amount of the solvent. The approach overcomes all the drawbacks previously listed for the gravimetric method. It does not require equilibrated sample collection, or filtration. The approach can be readily automated, and the methodology does not occupy resources of specialised instrumentation.

1.4 Nucleation

Crystal nucleation is an important parameter in solution crystallization as it affects many properties of the product including crystal shape, polymorphic form, and the crystal size distribution. Crystal nucleation involves the aggregation of dissolved molecules in the supersaturated solution into organized clusters. This process can be split into two main categories (Mullin, 2001); (1) primary nucleation, when no crystals are initially present in the solution, and (2) secondary nucleation, when crystals of the solute are already present or are deliberately added to the solution as seeds (fig. 1.2).

Nucleation, which occurs spontaneously in the pure solution, is homogeneous nucleation. The two commonly used theories of primary homogeneous nucleation are classical nucleation theory (CNT) and the two steps nucleation theory, which are both based on activation energy. The classical nucleation theory states that within saturated solution clusters of solute molecules are continuously forming and dissolving as a reversible process. The thermodynamic explanation of the theory is given by Gibbs (Gibbs, 1928). Formation of clusters effectively creates a surface that has energy penalty. Forming stable clusters is governed by the energy associated with its formation and growth.
As it is shown in Figure 1.3, the overall free energy difference between the solid particle and the solute in solution is the summation of the surface excess free energy and the volume excess free energy (Thanh et al., 2014):

$$\Delta G = \Delta G_V + \Delta G_S$$

(1.5)

The volume excess free energy can be written as:

$$G_V = -\frac{4\pi r^3 \Delta \mu}{3V}$$

(1.6)

while the surface excess free energy takes the form of:

$$G_S = 4\pi r^2 \gamma$$

(1.7)

where $r$ is the radius of particles, $V$ is the molecular volume, and $\gamma$ defines the interfacial surface tension.

The rate of nucleation for the CNT can be defined as the rate at which clusters grow to the critical nuclei size and become a stable crystal. A semi-empirical equation can be derived from an energy balance on a critical nucleus (Thanh et al., 2014) (Mullin, 2001):

$$J = A \exp\left(\frac{-\Delta G}{kT}\right)$$

(1.8)
where the rate constant is $A$, $k$ is the Boltzmann constant ($1.3805 \times 10^{-23}$ J/K) and the temperature is $T$. The two-step nucleation theory also explains homogeneous primary nucleation. According to the theory, formation of the nucleus is assumed to occur by the formation of a stable liquid cluster at higher density than the solution. The high concentration of molecules in this cluster favours the formation of the solid nuclei which then can grow in ordered crystalline structures (Davey et al., 2013). The rate of nucleation can be affected considerably by the presence of foreign particles (heterogeneous nucleation). The surface of the foreign particles allows adsorption of solute reducing the energy penalty associated with forming an effective surface, which means the value of the critical free energy difference presented in Figure 1.3 can be lowered (Mullin, 2001).

Thermodynamically, it is straightforward, when a solution is brought to a state of supersaturation for a given crystalline phase, for the system this structure becomes a more stable state than the pure solution, and nucleation becomes theoretically possible. Because of kinetics, the process of primary nucleation becomes much more uncertain, especially in those cases where there are impurities involved in the system (Svärd et al., 2013). One of the most accurate methods to determine nucleation

![Figure 1-3. Free energy curves of nucleation (Thanh et al., 2014).](image)
kinetics is through probability distributions of induction time crystallization experiments (Jiang & Ter Horst, 2011). To date, this approach has revealed new insight into the nucleation mechanism of many compounds (Davey et al., 2013).

One of the most important effects on the nucleation process is the crystallization volume. For example, results from polythermal metastable zone width experiments have shown that the probability of nucleation rate and volume increases, leading to a higher nucleation probability. In addition instead, the observed increase in the probability of nucleation with increasing volume in polythermal metastable zone width experiments is argued to be the result of inhomogeneous mixing, long equilibration times, large numbers of heterogeneous nucleation sites and a possibly high level of mechanical energy input (Bhamidi et al., 2017a). A link between mechanical energy input and induction times of crystal nucleation has been established (Xiao et al., 2017).

Understanding the effects of scale-up on the mechanism and kinetics of crystal nucleation is essential to control crystallization processes. Relating probability distributions form inductions times at constant supersaturation to the CNT is a powerful tool to reveal important kinetic and thermodynamic parameters that provide more insight into the nucleation process. However, it remains unclear how scale-up affects the nucleation mechanism and nucleation kinetics in solution crystallization processes.

1.5 Impurities

The manufacture of active pharmaceutical ingredients (API) often involves a synthetic route that requires multiple reactions to obtain the desired product. Reaction products typically consist of the desired compound together with impurities resulting from unreacted starting material or side reactions (Elder & Teasdale, 2015). Impurity control is particularly important in pharmaceutical manufacturing; as such compounds
can be toxic or may unfavourably affect the crystallization of the desired product (Sun et al., 2015) even when impurities are present in minute quantities (Schmidt et al., 2013) (Ottoboni et al., 2018).

### 1.5.1 The structurally related impurity in crystallization

During crystallization, molecules of the product assemble to form crystals. If the mismatch between impurity molecules and the crystal lattice is significant, the incorporation of an impurity molecule in the lattice becomes unfavourable whereas if the molecular difference in the structure of the desired product and impurity is small, the impurity molecule is more likely to incorporate into the crystal lattice (Nguyen et al., 2017).

Reactive disubstituted aromatic compounds are often used in the manufacture of pharmaceuticals to enable the required synthetic steps that lead to the desired product. However, the reactive nature of the intermediates also leads to the formation of organic impurities that are structurally similar to the target compound. This is for example reflected in the synthesis of paracetamol which mainly yields paracetamol together with trace amounts of unwanted 1,4-disubstituted aromatic impurities. The three main impurities of paracetamol (PA) are 4-nitrophenol (NP), 4-aminophenol (AP) and 4’-chloroacetanilide (CA) (Calinescu et al., 2012).

4-Aminophenol is the starting material in the final step of the synthesis of PA and is obtained through the reduction of NP. NP can crystallise as two polymorphic forms of which the $\beta$-form ($\beta$-NP) could undergo an irreversible transformation into the light unstable $\alpha$-form ($\alpha$-NP) at temperatures between 331 and 366 K (Coppers & Schmidt, 1965) (Wójcik & Mossakowska, 2006). CA is structurally the same as PA except that CA has a chlorine atom instead of an alcohol group at the 4-position. Only one crystalline form has been reported for CA (Naumov et al., 2007).

To date, the effect of a range of structurally-related compounds have been studied as additives on the crystallization of paracetamol (Hendriksen et al., 1998) (Prasad et al.,
2001) (Saleemi et al., 2013). These structurally related molecules might pose a considerable challenge for the downstream process such as filtration washing and drying.

### 1.5.2 Effect of impurity on the solubility

Impurities influence crystallization processes in several ways (Peng et al., 2014). The effect of impurities on the solubility of the target compound is essential for the design and use of solvent crystallization. The solubility of the product can change as a result of a change in the solute-solvent interfacial energy due to the presence of additional components. Consequently, the solubility of the product may decrease due to the presence of common ions or increase as a result of complex formation or the presence of foreign ions (Sangwal, 2007).

### 1.5.3 Effect of impurity on nucleation and growth

The challenges involved in controlling crystallization are significant, since the kinetic parameters of the process are strongly affected by several factors such as the presence of impurities. The presence of impurities in crystallization processes can affect crystal nucleation growth rates and crystal morphology (Moynihan & Horgan, 2017), (Thompson et al., 2004). The effect of foreign substances in the solution is system dependent. An impurity that acts as a nucleation inhibitor in one case may not necessarily be effective in another, or it may even act as an accelerator. No general rule applies and each case must be considered separately as the impurity effect can be rationalized in terms of intermolecular interactions (Mullin, 2001).

Impurities may influence the nucleation process by changing the solid-liquid interfacial energy (Pino-García & Rasmuson, 2004) and/or the kinetic factor or they may act as heterogeneous surfaces (Anwar et al., 2009). For instance, amphiphilic, polymeric, and surfactant additives were found to promote nucleation (Kim et al., 2013) (Bodnár et al., 2019) (Poornachary et al., 2016). In other studies, it was found
that additives and impurities did not significantly influence the interfacial energy but instead led to a reduction in the kinetics (Heffernan et al., 2018) (Pons Siepermann & Myerson, 2018). These findings were explained using the additional energy that is required to remove the impurity from the clustering process.

Paracetamol crystals are reported to be affected by the structurally similar impurity Metacetamol, as this impurity leads to increased nucleation times as well as wider metastable zone widths (Saleemi et al., 2013) (Mullin, 2001).

Crystal growth times can become much longer for systems in the presence of impurities (Heffernan et al., 2018). Impurities can influence crystal growth rates in a variety of ways, often as a result of the impurity being adsorbed onto specific growing faces and blocking the active growth sites and consequently modifying the crystal habit (Mullin, 2001) (Ukrainczyk et al., 2016).

1.5.4 Effect of impurity/additive on the compressibility

Additives can be used to influence the processability of pharmaceutical compounds by changing the intrinsic physical properties. In recent years, attentions on the changing morphology of pharmaceutical materials particles to desirable shape, size and surface area has long been actively increased because it has many advantages especially for improving physicochemical properties of APIs.

Paracetamol displays poor compaction behaviour and resists compression into tablets (Garekani & October, 1996) (Femi-Oyewo & Spring, 1994). There is considerable interest in modifying the properties of paracetamol using different crystallization techniques or additives to improve the compaction behaviour of the paracetamol tablet (Femi-Oyewo & Spring, 1994) (Garekani et al., 2000) (Thompson et al., 2004) (Kaialy et al., 2014). For example, Garekani prepared a sintered form of paracetamol crystals by crystallization from dioxane followed by a controlled drying process. The porous texture of desolvated crystals induced plasticity and improved the compressibility of paracetamol (Garekani et al., 2000).
1.6 Design of experiments (DOE)

Controlling or designing crystallization processes is very important to deliver the right purity, crystal form and yield, but also to produce crystals with a desired particle shape and size. These product properties can have a significant effect on the efficiency of downstream operations and product effectiveness, such as bioavailability or tablet stability for pharmaceutical compounds. Design of experiments (DOE) is an approach for systematically varying the governable input factors (such as cooling rate, seeding, and agitation) and observing the effects of these factors on the output product parameters (Hvalec et al., 2004). In chemical development, Design of Experiments (DOE) has become a reference method to speed up reaction optimization, since it determines the optimum processing conditions of a large number of reaction parameters in a small number of experiments.

The data resulting from a DoE study is used to build mathematical functions that best describe the relationship between the factors and the measured responses. These equations can be first, second, or higher order, depending on how the responses react to changes in the factors.

Mathematical model with $y=$response (e.g. yield), $x_{n}=$input factors, $\beta_{n}=$function coefficients and $e=$error.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + e$$ (1.9)

For system include the impurity, the impact of impurity on crystallization depends on parameters such as structure of impurity, concentration of impurity, solvent, agitation and cooling methods (Ukrainczyk et al., 2016).
1.7 **Mother liquor recycle**

After crystallization, the suspension is filtered resulting in pure product crystals as well as a mother liquor fraction. However, in addition to impurities, the mother liquor still contains the desired product (Wong et al., 2012). Moreover, the solvents that are part of the mother liquor could represent a source of environmental waste and may require disposal steps in some processes.

A reduction in mother liquor waste can be realized through a recycle operation, in which the mother liquor is used as part of the starting material for a new process. Through a recycle operation, high yields can be achieved while waste can be reduced at the same time. Improved yields and waste reduction have been realized, for example, in cooling single-stage continuous mixed-suspension, mixed-product removal (MSMPR) (Wong et al., 2012), antisolvent cooling single-stage continuous MSMPR (Tahara et al., 2015), multistage continuous MSMPR (Alvarez et al., 2011), and plug-flow processes (Cogoni et al., 2015).

The key challenge behind implementing a mother liquor recycle operation is to account for the gradual build-up of impurities in the solution. According to a simple mathematical model, the amount of impurities should stop to increase after a sufficiently large number of cycles have been conducted (Smith, 1997). The number of cycles that are required to reach a steady state impurity concentration is expected to depend on the amount of mother liquor that is recycled. However, it remains unclear how the fraction of mother liquor recycle affects the build-up of impurities in experimental work as no such mother liquor recycle studies have been conducted to the best of my knowledge.
Chapter 2

Brief description of the paper

In this study, the solubility of N-(4-methylphenyl-Z-3-chloro-2-(phenylthio) propenamide) (Z-1) in twelve solvents was measured by the gravimetric method and modelled with four different activity coefficient models. The methodology in this work can be used to determine the solubility of other compounds. The reported solubility results illustrate how the gravimetric solubility method and solubility models cope with industrially-relevant complex products such as Z-1. Z-1 belongs to the α-Thio-β-chloroacrylamide family which is a class of highly versatile synthetic intermediates due to the proximal location of diverse functional groups enabling different reaction types within a small skeleton.

URL: https://doi.org/10.1021/acs.jced.7b01011


Leila Keshavarz’s contribution: Performed gravimetric experiments and XPRD experiments. Conducted the modelling. Contributed to analysing the results. Contributed to the preparation, design and writing of the manuscript.
Solubility Measurement and Thermodynamic Modelling of an α-Thio-β-chloroacrylamide in Twelve Pure Solvents at Temperatures Ranging from (278.15 to 318.15) K

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Abstract

α-Thio-β-chloroacrylamides are of considerable synthetic utility due to their versatile reactivity profile enabling a diverse range of useful transformations. Availability of accurate and extensive solubility data and models is a prerequisite for advanced process optimization of such valuable pure synthetic intermediate compounds, in
Chapter 2

particular facilitating their isolation with a high degree of efficiency and control. As an illustrative example the solubility of one such derivative, N-(4-methylphenyl-Z-3-chloro-2-(phenylthio) propenamide) (Z-1), is described in the present work. Solubility data is reported in 12 pure solvents specifically selected for their potential utility in synthesis and isolation at scale. Solubility data are determined using the gravimetric method across a range of temperatures T= (278.15 to 318.15) K under pressure of 0.1 MPa. On a molar basis, the solubility of Z-1 at temperature T = 298.15 K was observed to follow the order: tetrahydrofuran > 1,2-dichloroethane > 2-methyltetrahydrofuran > butanone > acetone > ethyl acetate > methyl acetate > toluene > tert-butyl methyl ether > acetonitrile > 2-propanol > 2-methyl-2-butanol. The experimental solubility data were correlated by the modified Apelblat, Margules, Van-Laar, Wilson, and nonrandom two-liquid (NRTL) models. The NRTL model was found to result in the lowest error for 8 of the 12 solvents tested. In the case of acetonitrile, the Wilson model had a slightly lower mean square error of 3.52 × 10^-4 while for methyl acetate and 1,2-dichloroethane the Van-Laar model had the smallest mean square error of 1.47 × 10^-3 and 3.54 × 10^-4, respectively. The provision of solubility data and models for such a prized and versatile compound will assist with further development of continuous isolation strategies.

2.1 Introduction

α-Thio-β-chloroacrylamides are a class of highly versatile synthetic intermediates due to the proximal location of diverse functional groups enabling different reaction types within a small skeleton. The flexible synthetic utility of these heavily functionalized acrylamide compounds is well documented (Kissane & Maguire, 2011). Specifically, a wide array of transformations have been successfully applied to these compounds including nucleophilic substitution (Murphy et al., 2007), Diels–Alder reactions (Kissane et al., 2010a), 1,3-dipolar cycloadditions (Kissane et al., 2008) (Kissane et
al., 2010b), and oxidation of the sulfide group (Kissane et al., 2010c) (Kissane et al., 2010d), as summarized in Scheme 1.

Scheme 1. Reaction Pathways of α-Thio-β-chloroacrylamides.\(^a\)

\(^a\) R\(^1\) and R\(^2\) represent alkyl, aryl, or H substituents whereas R\(^3\) represents alkyl or aryl groups.

Until recently the key challenge associated with exploiting the synthetic potential of α-thio-β-chloroacrylamides has concerned the generation of appreciable quantities of material for process investigation. Preparation of α-thio-β-chloroacrylamides has typically involved the use of a three-step process (Tietze, 1996) involving a final reaction whereby a toluene solution of α-thioamide and N-chlorosuccinimide is subjected to a “hot plunge” by placing it in an oil bath at 363.15 K. This approach provides a consistent means of generating β-chloroacrylamides but only at scales of 1–10 g. The method suffers from several obstacles which constrain ease of scale-up. A recent work has focused on the development of a continuous process for the multigram, tunable production of β-chloroacrylamides (Dennehy et al., 2016). The optimized process utilizes flow chemistry as a key step enabling technology to overcome limitations of the batch manufacturing process such as heat removal due to exothermic reactions. The specific target product selected to exemplify the approach
was \(N\)-(4-methylphenyl-Z-3-chloro-2-(phenylthio) propenamide (Z-1)\) the prototypical compound studied extensively in the synthetic and mechanistic investigations (Kissane & Maguire, 2011). The chemical structure of Z-1 is shown in Figure 2.1.

![Chemical structure of \(N\)-(4-methylphenyl-Z-3-chloro-2-(phenylthio)propenamide (Z-1).](image)

Figure 2-1. Chemical structure of \(N\)-(4-methylphenyl-Z-3-chloro-2-(phenylthio)propenamide (Z-1).

Solvent crystallization is essential to the separation and isolation of Z-1 whether operating in batch or continuous mode. Thermodynamic calculations, based on accurate solubility data, are fundamentally important for designing and controlling crystallization. Yet solubility data for \(\beta\)-chloroacrylamides are notably absent in the literature, likely resultant from their limited availability. The use of the continuous process facilitates the ability to access sufficient quantities of material to enable such measurements.

This chapter reports the equilibrium solubility measurements of \(\alpha\)-thio-\(\beta\)-chloroacrylamide Z-1 in a range of selected pure organic solvents, over the temperature range 278.15–318.15 K, using the gravimetric approach under atmospheric conditions. Empirical, correlative thermodynamic and predictive modeling of the solubility data is presented. Each model is analyzed in terms of accuracy and quality of fit.
2.2 Solubility modeling

Thermodynamic modelling allows for representation of solubility data of solutes in pure solvents. Many models have been proposed to achieve such with differing levels of rigor, complexity, and accuracy. In this thesis, the solubility in the selected solvents at different temperatures are correlated with the modified Apelblat equation (Prausnitz et al., 1998), in addition to the Margules, Van-Laar, NRTL, and Wilson activity coefficient based models. Other models which are not specifically used here, but which are widely used to describe the solubility of a solute in different pure solvents include the perturbed-chain statistical associating fluid theory (PC-SAFT) modeling approach (Ruether et al., 2009) (Reschke et al., 2016) the Buchowski–Ksiazaczak ($\lambda$h) model, and the Combined Nearly Ideal Binary Solvent/Redich–Kister (CNIBS/R–K) model.

2.2.1 Modified Apelblat equation

The modified Apelblat equation is expressed in eq 2.1. It is used extensively in correlating the solute solubility in pure solvents. The model provides a simple empirical representation of solubility.

$$\ln C = a_A + \frac{b_A}{T} + c_A \ln T$$ (2.1)

In eq 2.1, C represents the equilibrium concentration in grams of solute per kilograms of solvent, at the temperature T expressed in Kelvin; the three adjustable parameters of the empirical solubility model are indicated as $a_A$, $b_A$, and $c_A$.

The conversion between molar solubility $x_2$ and mass solubility C and vice versa was performed using the following relationship:

$$C = \frac{1000 \times x_2 \text{MW}_{\text{Solute}}}{\text{MW}_{\text{Solvent}}(1-x_2)}$$ (2.2)
\[ x_2 = \frac{\frac{C}{MW_{\text{solute}}} - \frac{1000}{MW_{\text{solute}}}}{\frac{C}{MW_{\text{solvent}}}} \]  \quad (2.3)

### 2.2.2 Wilson models

Considering the fundamental (solid– liquid) phase equilibrium theory, the solubility of Z-1 in pure solvents can be expressed using a nonideal solid–liquid equilibrium equation defined as follows (Prausnitz, 1998):

\[ \ln(x_2 \gamma_2) = \frac{\Delta H_f}{R} \left( \frac{1}{T_t} - \frac{1}{T} \right) - \frac{\Delta C_P}{R} \left( \frac{1}{T_t} - \frac{1}{T} \right) - \frac{\Delta C_P}{R} \ln \left( \frac{T_t}{T} \right) \]  \quad (2.4)

where \( R \) is the universal gas constant, 8.314 J·K\(^{-1}\)·mol\(^{-1}\). \( \gamma_2 \) and \( \Delta H_{\text{fus}} \) are the activity coefficient and fusion enthalpy of the solute, respectively. \( T_t \) denotes the triple-point temperature; \( \Delta C_P \) is the difference of the heat capacity of a solute between the liquid state and the solid state. Generally, the \( \Delta C_P \) value is so minor that the terms containing \( \Delta C_P \) in eq 2.4 can be neglected because they are less important than the first term on the right side (Prausnitz, 1998). For solid–liquid equilibrium, small changes of pressure do not significantly affect equilibrium unless the pressure changes are very large (10–100 MPa) (De Souza et al., 2017). The triple-point temperature \( T_t \) is almost equal to the normal melting temperature, \( T_m \). So, eq 2.4 can be simplified to eq 2.5:

\[ \ln(x_2) = \frac{\Delta H_{\text{fus}}}{R} \left( \frac{1}{T_m} - \frac{1}{T} \right) - \ln(\gamma_2) \]  \quad (2.5)

Using on molecular considerations, Wilson (1964) presented the following expression for the excess Gibbs energy of binary solutions:

\[ \ln(\gamma_2) = -x_2 \left( \frac{A_{12}}{x_1 + A_{12}x_2} - \frac{A_{21}}{x_2 + A_{21}x_1} \right) - \ln(x_2 + A_{21}x_1) \]  \quad (2.6)

As can be seen, the Wilson equation has two adjustable parameters, \( A_{12} \) and \( A_{21} \), which are related to the pure component molar volumes and to characteristic energy differences by:
\[
\ln(\gamma_2) = \frac{v_j}{v_i} \exp \left( -\frac{\lambda_{ij}}{RT} \right)
\]  
(2.7)

where \(i \neq j\) and \(i,j = 1,2\) and \(v_i\) or \(v_j\) are the molar volumes of the \(i\)-th or \(j\)-th components in \(\text{cm}^3/\text{mol}\) and the \(\lambda\) represent energies of interactions. \(\Lambda_{ij}\) are cross interaction energy parameters (\(\text{J} \cdot \text{mol}^{-1}\)) between the components \(i\) and \(j\).

### 2.2.3 Margules & Van Laar models

Margules and Van Laar are two further activity coefficient based models, offering a more thermodynamically rigorous approach to solubility modelling, accounting for deviation from ideal solubility. In the case of Margules, the temperature dependence is considered explicitly as is shown in eq 2.8 below, while for Van-Laar the temperature dependence is implicit.

\[
\ln(\gamma_2) = \frac{A}{RT} (1 - x_2)^2
\]

(2.8)

A single adjustable parameter \(A\) is incorporated in the Margules model, while the Van-Laar model makes use of two adjustable parameters, as shown in eq. 2.9, namely \(A_{vl}\) and \(B_{vl}\).

\[
\ln(\gamma_2) = \frac{B_{vl}}{(1 + B_{vl}x_2x_1)^2}
\]

(2.9)

### 2.2.4 NRTL Model

Similar to the Wilson and the Van-Laar and Margules models, the NRTL (Renon & Prausnitz, 1969) model is also an activity coefficient equation which has been widely used in correlating and predicting of fluid phase equilibrium. The NRTL model is based on the molecular local composition concept, and is presented as eq 2.10.

\[
\ln(\gamma_2) = -x_2^2 \left[ \tau_{12} \left( \frac{G_{12}}{x_2 + G_{12}x_1} \right)^2 - \frac{\tau_{21}G_{21}}{(x_1 + G_{21}x_2)^2} \right]
\]

(2.10)
\[ G_{ij} = \exp(-\alpha \tau_{ij}) \text{ and } \tau_{ij} = \frac{g_{ij}}{RT} \]  

where \( i \neq j \) and \( i,j = 1,2 \). \( g_{ij} \) are model parameters relating to the cross interaction energy (J·mol\(^{-1}\)), \( \tau_{12} \) and \( \tau_{21} \), are interaction parameters specific to a particular pair of species and the parameter \( \alpha \) is a measure of the non-randomness of solution.

### 2.3 Experimental section

#### 2.3.1 Materials

\( N \)-(4-methylphenyl-Z-3-chloro-2-(phenylthio)propenamide) (Z-1) (CAS No. 162375-28-2) (up to 99\% pure by HPLC Analysis and H-NMR spectroscopy) was synthesized using both batch and continuous flow as recently outlined by University College Cork (Dennehy et al., 2016). H-and C- NMR spectra in addition to IR and mass spectra for Z-1 have already been reported (Kissane et al., 2008).

Powder X-ray diffraction was used in order to confirm the crystal structure of the synthesised material as being that of (Z-1). In addition, \(^1\)H NMR and \(^{13}\)C NMR spectroscopy was used to confirm the molecular structure of Z-1 (Supporting Information, Figure S2.1 and Figure S2.2).

Powder X-ray diffraction was further utilized in order to eliminate the possibility of a polymorphic transformation during the solubility experiments, across the full range of solvents considered. High temperature samples of solute were prepared through rapid filtration, followed immediately by X-ray diffraction analysis in order to mitigate against the risk of solvent-mediated polymorphic transformation during storage.

Powder diffraction data were collected on a Philips X’Pert-MPD PRO diffractometer (PW3064 sample spinner) with nickel filtered copper Cu K\( \alpha \) radiation \((\lambda = 1.5418 \text{ Å})\), run at 40 kV and 35 mA, \( 2\theta = 5–40^\circ \), with a step size of 0.02\( ^\circ \) 20 and a scan speed of 0.02\( ^\circ \) s\(^{-1}\). Samples were prepared by light grinding prior to placement in a sample holder and flattening with a glass slide. The possibility of a polymorph transformation occurring during grinding was eliminated by analyzing a sample before and after
grinding. Analysis of the spectra indicated no polymorphic transformation, with consistent peaks before and after equilibration indicative of $Z_{-1}$.

The 12 pure solvents and one solute utilised in the present work are listed in Table 2.1, alongside supplier and purity data. Each of the organic solvents selected, as shown in Table 2.1, were determined as potential candidates for further development of the $\alpha$-thio-$\beta$-chloroacrylamide continuous synthesis process, either for synthesis, isolation or both.

<table>
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<th>chemical name</th>
<th>CAS registry number</th>
<th>Source</th>
<th>mass fraction purity</th>
<th>additional purification</th>
<th>method for purity determination</th>
</tr>
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<td>162375-28-2</td>
<td>Synthesized</td>
<td>$\geq 0.97$</td>
<td>recrystallisation</td>
<td>HPLC$^a$</td>
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<tr>
<td>propanamid(Z-1)</td>
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<td>Acetone</td>
<td>67-64-1</td>
<td>Fisher Scientific</td>
<td>$\geq 0.998$</td>
<td>None</td>
<td>GC$^b$</td>
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</tr>
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</tr>
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</tr>
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<td>GC</td>
</tr>
</tbody>
</table>

$^a$ High Performance Liquid Chromatography $^b$ Gas Chromatography

2.3.2 Apparatus and Procedure

The procedure for determining the solubility was the same as the one used in the previous work (De Souza et al., 2017). Equilibrium solubility measurements of $Z_{1}$ in each of the selected organic solvents were determined using the gravimetric method at
temperatures ranging from 278.15 to 318.15 K. A thermostatic stainless steel water bath (Grant GR150; 38 L; stability ±0.005 K and uniformity ±0.02 K) with a serial magnetic stirrer plate placed on the base was employed for the gravimetric tests. A further verification reading of temperature was obtained using a calibrated PT-100 resistance thermometer. To reach the solid–liquid equilibrium, excess Z-1 was added to each solvent tube. The tubes were sealed to prevent solvent loss due to evaporation, following which the solution was stirred at 500 rpm for a minimum of 72 h using a PTFE coated magnetic stirrer. The remaining solid residue was left to settle for at least 12 h at constant temperature and without stirring, until the saturated solution was observed to be visibly clear. This was further verified using a laser light of 532 nm. The clear solution was then sampled using syringe of equivalent temperature and filtered into a preweighed dry glass vial using a 0.2 μm, PTFE membrane (15 mm diameter) syringe filter. Caps were placed on the vials immediately after solution addition in order to prevent solvent evaporation. The combined mass of vials and sample was measured without undue delay. The caps were removed, and the solvents were allowed to evaporate in a vacuum oven at 323.15 K over 24 h until only the solid residue of Z-1 was found to remain in the vials. All the masses were weighed using an analytical balance (Mettler Toledo AX054, weighing capacity up to 520 g, sensitivity ±0.1 mg).

Triplicate measurements were performed under the same conditions and the arithmetic average value was calculated. Furthermore, the relative standard uncertainty (estimated as 100% standard deviation/average value) was calculated. Further verification measurements of mass were taken 24 h after the initial mass measurements. All cases that showed negligible change in mass were noted thus indicating that the samples were completely dry. Validation of the experimental procedure was performed by measuring the solubility of paracetamol in various solvents. The determined solubility data and the literature values are presented in Table S1 of the Supporting Information, and the results show that the experimental solubility data agreed well with those in literature.
2.3.3 Thermal analysis

As already noted, the melting temperature $T_m$ of Z-1 was reported previously (Dennehy et al., 2016). In the present paper, the melting temperature was again confirmed and the enthalpy for Z-1 determined using the differential scanning calorimetry (DSC) (Pyris-Diamond, PerkinElmer) under nitrogen atmosphere. Before determination, the instrument was precalibrated with the reference material (indium). Accurately weighed samples of Z-1 were introduced into a DSC pan, and then heated at a rate of $5 \text{ K} \cdot \text{min}^{-1}$ under nitrogen flow. The temperature range considered was from 283.15 to 433.15 K. Standard uncertainties of the experiments were evaluated to be 0.5 K for temperature and 400 J·mol$^{-1}$ for the enthalpy of melting.

2.4 Results and discussion

2.4.1 Pure component properties

The acquired DSC result of Z-1 is shown graphically in Figure 2.2. It can be seen from the DSC curve of Z-1 that the melting temperature $T_m$ and the enthalpy of fusion $\Delta_{\text{fus}}H$ are 381.15 K and 25.78 kJ·mol$^{-1}$, respectively. In this work, the melting point is used as the onset point of the DSC curve which is the intersection of the extension of the baseline with the tangent at the point of greatest slope (inflection point) of the DSC curve. The melting temperature of Z-1 differs slightly from batch to batch as a result of minute differences in purity. The melting temperature used in this work falls within the melting temperature range of 379.15–381.15 K reported in the literature (Dennehy et al., 2016).
Chapter 2

2.4.2 Solubility data

The obtained mole fraction solubility ($x$) of Z-1 in acetone, acetonitrile, butanone, ethyl acetate, methyl acetate, tert-butyl methyl ether, tetrahydrofuran, toluene, 1,2-dichloroethane, 2-methyl-2-butanol, 2-methyltetrahydrofuran and 2-propanol over the temperature range from (278.15 to 318.15) K are tabulated in Table 2.2, and the plots of the solubility data of Z-1 in the selected solvents at different temperatures are shown in Figure 2.3.

Each solubility data point is a mean value of three measurements. Furthermore, the plots of $\ln(x)$ versus $1/T$ for Z-1 in twelve pure solvents at different temperatures are given in Figure 2.4. From Table 2.2 and Figure 2.3, it is found that the solubility of Z-1 increases with temperature for all the selected solvents.
Table 2-2. Experimental and calculated solubility of Z-1 with Apelblat, Margules, Van-Larr, Wilson and NRTL models across entire range of solvents at saturation temperature $T = (278.15$ to $318.15)$ K and pressure $P = 0.1$ MPa$^a$.

<table>
<thead>
<tr>
<th>T/K</th>
<th>C / g/Kg</th>
<th>$x^{exp}$</th>
<th>$x^{apbl}$</th>
<th>$x^{marg}$</th>
<th>$x^{vl}$</th>
<th>$x^{w}$</th>
<th>$x^{NRTL}$</th>
</tr>
</thead>
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<td>Acetone</td>
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<td></td>
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<td>0.0216</td>
<td>0.0208</td>
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<td>0.0202</td>
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<td>tert-butyl methyl ether</td>
<td>0.0068</td>
<td>0.0067</td>
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<tr>
<td></td>
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<td>tetrahydrofuran</td>
<td>0.0049</td>
<td>0.0045</td>
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<tr>
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<td>toluene</td>
<td>0.0035</td>
<td>0.0030</td>
<td>0.0027</td>
<td>0.0032</td>
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<tr>
<td></td>
<td></td>
<td>1,2-dichloroethane</td>
<td>0.0013</td>
<td>0.0011</td>
<td>0.0009</td>
<td>0.0010</td>
<td>0.0009</td>
</tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-methyl-2-butanol</td>
<td>0.0003</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.0003</td>
<td>0.0003</td>
</tr>
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<td></td>
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</tbody>
</table>
Across the full range of temperatures studied, the solubility of Z-1 was determined to be highest in tetrahydrofuran and lowest in 2-methyl-2-butanol. The solubility of Z-1 does not change significantly with temperature in 2-propanol, 2-methyl-2-butanol, tert-butyl methyl ether or acetonitrile. The remaining eight solvents would thus be preferred for application to cooling crystallization. In terms of yield, 1,2-dichloroethane, tetrahydrofuran, 2-methyltetrahydrofuran and butanone appear to be best candidates for cooling crystallisation.

The solubility of Z-1 at $T=298.15$ K follows the order tetrahydrofuran $>$ 1,2-dichloroethane $>$ 2-methyltetrahydrofuran $>$ butanone $>$ acetone $>$ ethyl acetate $>$ methyl acetate $>$ toluene $>$ tert-butyl methyl ether $>$ acetonitrile $>$ 2-propanol $>$ 2-methyl-2-butanol. The solubility of Z-1 is seen to be marginally lower in acetonitrile than tert-butyl methyl ether at temperatures $T>303$ K. At temperatures $T>308$K, the solubility of Z-1 is higher in toluene than methylacetate. Furthermore, at temperatures $T<289$K the solubility of Z-1 is higher in 2-methyltetrahydrofuran than 1,2-dichloroethane.
Figure 2-3. Solubility ($x$) of Z-1 versus temperature $T$ in each of the selected solvents: (◇) Acetone; (◆) Acetonitrile; (●) Butanone; (■) Ethyl Acetate; (○) Methyl Acetate; (□) tert-Butyl methyl ether; (▲) Tetrahydrofuran; (Δ) Toluene; (▽) 1,2-Dichloroethane; (+) 2-Methyl-2-Butanol; (X) 2-Methyltetrahydrofuran; (▼) 2-Propanol.

Figure 2-4. Experimental plots of $\ln (x)$ of Z-1 versus $1/ T$ in each of the selected solvents: (◇) Acetone; (◆) Acetonitrile; (●) Butanone; (■) Ethyl Acetate; (○) Methyl Acetate; (□) tert-Butyl methyl ether; (▲) Tetrahydrofuran; (Δ) Toluene; (▽) 1,2-Dichloroethane; (+) 2-Methyl-2-Butanol; (X) 2-Methyltetrahydrofuran; (▼) 2-Propanol.
From the data it appears that Z-1 is least soluble in protic solvents (i.e. 2-methyl-2-butanol and 2-propanol) that contain a hydrogen-donating group. The hydrogen-donating group of the solvent might prevent the hydrogen-donating amide group of Z-1 from forming hydrogen bonds with the solvent which would reduce the solubility of Z-1.

The solubility of Z-1 was found to be generally similar for solvents that have the same functional group. This is apparent for the solvents containing an alcohol group (i.e. 2-methyl-2-butanol and 2-propanol), a carboxylate group (i.e. methyl acetate and ethyl acetate) or a ketone group (i.e. acetone and butanone). However, no such correlation was found for the ethereal solvents as the solubility of tetrahydrofuran is almost twice as high as 2-methyltetrahydrofuran and more than ten times higher than tert-butyl methyl ether. The lower solubilities are possibly caused by the steric effects of the tert-butyl group in tert-butyl methyl ether and the methyl group in 2-methyltetrahydrofuran.

From the data summarized above rationalizing the order of the solubility values based on a single factor is clearly not feasible, as anticipated for a highly functionalized molecule. Group similarity, often recognized in the empirical rule “like dissolves like” is likely to play a fundamental role, as are van der Waals forces, polarity and so forth. Analysis of several properties including polarities, dipole moments and dielectric constants of the solvents indicate that the factors which determine solubility are complex and multifactorial.

### 2.4.3 Solubility correlation and calculation

The adjustable parameters of the modified Apelblat equation were obtained through regression and are summarized in table 2.3. The empirical model shows good agreement with the underlying data in the case of all solvents tested.
Activity coefficient models offer a more thermodynamically rigorous approach than empirical models, accounting for the deviation from ideal solubility. To calculate the binary interaction parameters utilized by these models, it was first necessary to calculate the experimental activity coefficients, based on the solid–liquid equilibrium equation, presented earlier as eq 2.4.

The activity coefficient can illustrate the solute–solvent intermolecular interactions. The ideal solubility is represented by the value 0 on the logarithmic activity coefficient scale, with higher values relating to lower solubility. In Figure 2.5, the highest values for $ln\gamma$ can be attributed to 2-propanol and 2-methyl-2-butanol which have the lowest solubilities. In the case of all solvents, with the exception of tetrahydrofuran, the solutions exhibit negative deviation from Raoult’s law.

Having established the experimental activity coefficients, activity coefficient models could be fitted to the experimental data.

The binary interaction parameters were estimated using the following mathematical optimization problem solved using the MATLAB nonlinear least-squares algorithm *nlinfit*:

$$
\min_{\theta} \sum_{i=1}^{N_p} \left( \frac{ln\gamma_{2,i|\text{mod}}(T, \theta) - ln\gamma_{2,i|\text{exp}}(T)}{\sigma_i^2} \right)^2
$$

(2.12)

Table 2-3. Parameters of the modified Apelblat equation and Root Mean Square Error (RMSE) as determined for Z-1 in different pure solvents.

<table>
<thead>
<tr>
<th>Apelblat Parameters</th>
<th>$a_A$</th>
<th>$b_A$</th>
<th>$c_A$</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>-5.12x10^2</td>
<td>1.94x10^4</td>
<td>78.5006</td>
<td>5.12x10^-6</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>-4.71x10^2</td>
<td>1.70x10^4</td>
<td>73.5204</td>
<td>5.89x10^-6</td>
</tr>
<tr>
<td>Butanone</td>
<td>-2.04x10^2</td>
<td>6.30x10^3</td>
<td>33.1566</td>
<td>1.47x10^-5</td>
</tr>
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<td>Ethyl Acetate</td>
<td>-2.37x10^2</td>
<td>7.42x10^3</td>
<td>38.0948</td>
<td>1.74x10^-5</td>
</tr>
<tr>
<td>Methyl Acetate</td>
<td>1.14x10^3</td>
<td>1.14x10^3</td>
<td>-14.1902</td>
<td>6.60x10^-6</td>
</tr>
<tr>
<td>tert-Butyl Methyl Ether</td>
<td>-3.07x10^2</td>
<td>1.11x10^4</td>
<td>48.0864</td>
<td>4.09x10^-5</td>
</tr>
<tr>
<td>Tetrahydrofuran</td>
<td>-1.98x10^2</td>
<td>6.97x10^3</td>
<td>31.6988</td>
<td>2.15x10^-5</td>
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<tr>
<td>Toluene</td>
<td>-2.39x10^2</td>
<td>7.11x10^3</td>
<td>38.6697</td>
<td>3.39x10^-5</td>
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<td>1,2-Dichloroethane</td>
<td>-5.88x10^1</td>
<td>-3.73x10^2</td>
<td>11.5256</td>
<td>7.31x10^-5</td>
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<tr>
<td>2-Methyl-2-Butanol</td>
<td>-3.47x10^2</td>
<td>1.07x10^3</td>
<td>54.9585</td>
<td>9.13x10^-6</td>
</tr>
<tr>
<td>2-Methyltetrahydrofuran</td>
<td>-3.42x10^2</td>
<td>1.27x10^3</td>
<td>53.5191</td>
<td>4.58x10^-6</td>
</tr>
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<td>2-Propanol</td>
<td>-1.95x10^2</td>
<td>5.58x10^3</td>
<td>31.4747</td>
<td>3.72x10^-6</td>
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</table>
Figure 2.5. Calculated logarithm of activity coefficient of Z-1 as a function of temperature at measured solubility data points in each of the selected solvents: (◇) Acetone; (◆) Acetonitrile; (●) Butanone; (■) Ethyl Acetate; (○) Methyl Acetate; (□) tert-Butyl methyl ether; (▲) Tetrahydrofuran; (△) Toluene; (▽) 1,2-Dichloroethane; (+) 2-Methyl-2-Butanol; (x) 2-Methyltetrahydrofuran; (▼) 2-Propanol.

Where $\theta$ is the set of binary interaction parameters to be estimated for each model and each solvent used, $N_p$ is the number of experimental points carried out for each solvent used, and the subscripts $mod$ and $exp$ are respectively referred to the model and the experimental activity coefficients.

Insertion of the binary coefficients, from Table 2.4, into the activity model equations allows for the prediction of molar solubility, which can then be converted to mass solubility as per eq 2.2.

The selection of the activity coefficient model is influenced both by the complexity of the model, and the quality of fit to the underlying data. In Table 2.2, the solubility derived from each activity coefficient model is directly compared to the underlying experiment data. The mean square error of each of the models is compared in Table 2.5.
Table 2-4. Estimated Binary Coefficients for Z-1 in Different Pure Solvent Using Margules ($A$), Van-Laar ($A_{vl}, B_{vl}$), Wilson ($\lambda_{12}, \lambda_{21}$), and NRTL ($g_{12}, g_{21}, \alpha$) Models

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Margules Model</th>
<th>Van-Laar model</th>
<th>Wilson model</th>
<th>NRTL model</th>
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<tr>
<td></td>
<td>$A$</td>
<td>$A_{vl}$</td>
<td>$B_{vl}$</td>
<td>$\lambda_{12}$</td>
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<td>Acetone</td>
<td>5.71 x10^3</td>
<td>0.38</td>
<td>2.69</td>
<td>3.53 x10^3</td>
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<tr>
<td>Acetonitrile</td>
<td>2.32 x10^3</td>
<td>0.43</td>
<td>1.09</td>
<td>3.09 x10^3</td>
</tr>
<tr>
<td>Butanone</td>
<td>1.53 x10^3</td>
<td>0.42</td>
<td>0.67</td>
<td>2.93 x10^3</td>
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<td>Ethyl Acetate</td>
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<td>0.48</td>
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<td>Methyl Acetate</td>
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<td>0.50</td>
<td>1.38</td>
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<td>tert-Butyl Methyl Ether</td>
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<td>1.40</td>
<td>2.22</td>
<td>89.6 x10^3</td>
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<td>Tetrahydrofuran</td>
<td>-7.58 x10^2</td>
<td>1.45x10^4</td>
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<td>Toluene</td>
<td>3.14 x10^3</td>
<td>0.41</td>
<td>1.53</td>
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<td>1,2-Dichloroethane</td>
<td>1.06 x10^4</td>
<td>0.19</td>
<td>0.56</td>
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<tr>
<td>2-Methyl-2-Butanol</td>
<td>8.52 x10^3</td>
<td>0.24</td>
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<td>1.55 x10^3</td>
</tr>
<tr>
<td>2-Methyltetrahydrofuran</td>
<td>1.23 x10^3</td>
<td>0.42</td>
<td>1.19</td>
<td>4.95 x10^3</td>
</tr>
<tr>
<td>2-Propanol</td>
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<td>0.46</td>
<td>3.54</td>
<td>7.92 x10^3</td>
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</table>

In the case of acetone, butanone, ethyl acetate, tert-butyl methyl ether, tetrahydrofuran, toluene, 2-methyltetrahydrofuran, 2-methyl-2-butanol, and 2-propanol the NRTL model was seen to have the lowest mean square error. The Wilson model was seen to have a slightly smaller error in the case of acetonitrile and the Van-Laar model had the lowest error in the case of methyl acetate and 1,2-dichloroethane. The Margules model was seen to have the largest error for all solvents with the exceptions of 2-methyltetrahydrofuran, where the Van-Laar model gave the highest error, and 2-propanol, where the Wilson model gave the highest error.
2.5 Conclusions

In this chapter, the solubility of N-(4-methylphenyl-Z-3-chloro-2-(phenylthio) propenamide) was measured in a variety of organic solvents screened as potentially useful in synthesis and crystallization. Across the entire range of solvents tested, solubility was seen to increase with temperature. The solubility at temperature $T = 298.15 \, \text{K}$ was found to follow the order from high to low, tetrahydrofuran > 1,2-dichloroethane > 2-methyltetrahydrofuran > butanone > acetone > ethyl acetate > methyl acetate > toluene > tert-butyl methyl ether > acetonitrile > 2-propanol > 2-methyl-2-butanol.

The modified Apelblat equation, Margules, Van-Laar, Wilson, and NRTL models were successful in fitting the data well; in particular the maximum mean square error of $6.05 \times 10^{-2}$ was seen using the Margules model for 2-methyl-2-butanol. The NRTL
model was found to result in the lowest error with mean square errors of the order $10^{-2}/10^{-5}$. In the case of tetrahydrofuran, the NRTL mean square error was almost 1000 times smaller than that of Margules. While some work remains to further explore the complex solubility behavior of Z-I in each of the solvents tested, the data will facilitate further development in terms of isolation of these highly prized β-chloroacrylamide compounds.

As mentioned before, one of the drawbacks of the gravimetric method is the limitation for measuring the solubility in the elevated temperature. Solubility data at higher temperatures beyond the atmospheric boiling point of solvents, allows for an increase in crystallization yield as higher temperatures can be reached during solution crystallization. In the next chapter, a new method introduced to overcome this limitation. Paracetamol was employed as API to test the method. No previous literature was sourced in which the solubility of paracetamol was established at a temperature in excess of the atmospheric boiling point of the solvent.
Supporting Information

Figure S2.1H-NMR spectrum of N-(4-methylphenyl-Z-3-chloro-2-(phenylthio)propenamide) (Z-1).

Figure S2.2. C-NMR spectrum of N-(4-methylphenyl-Z-3-chloro-2-(phenylthio)propenamide) (Z-1).
Two parameters of Apelblat for Acetone and methyl Acetate required revision. Updated values shown in bold in tables S2.1 and S2.2

Table S2.1 Updated Apelblat value for Acetone

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<td>$A$</td>
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<tr>
<td>$b$</td>
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<td>$C$</td>
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Table S2.2 Updated Apelblat value for Methyl Acetate

<table>
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<tbody>
<tr>
<td>$a$</td>
<td>-8264.466139</td>
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</tr>
<tr>
<td>$b$</td>
<td>113.63744</td>
<td></td>
</tr>
<tr>
<td>$C$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 3

Brief description of the paper

In this paper, a pressurized-synthetic methodology was used for the determination of the solubility of pure paracetamol in solvents beyond their atmospheric boiling point. This allows for an increase in crystallization yield as higher temperatures can be reached during solution crystallization. The data obtained from the pressurized-synthetic measurements was validated for solvents by comparison with gravimetric solubility data. The results demonstrated that there was no discernible discontinuity between data obtained through the gravimetric and synthetic approach. Binary interaction parameters for the pressurized-synthetic solubility data of paracetamol were derived and estimated for different activity coefficient models along with an empirical solubility equation. The solubility data obtained by the synthetic methodology demonstrated that the error associated with the extrapolation of solubility data from the gravimetric method was potentially significant.

URL: https://doi.org/10.1021/acs.jced.7b00118.


Leila Keshavarz’s contribution: Contributed to design experiments. Performed the pressurized synthetic experiments. Conducted the modelling. Measured the gravimetric solubility data and compared with the pressurized synthetic method. Utilized the method to measure the Metastable zone (MSZW). Investigated advantages and drawbacks of the new method. Contributed to writing of the manuscript.
The Pressurized-Synthetic methodology for Solubility Determination at Elevated Temperatures, with application to Paracetamol in Pure Solvents

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ABSTRACT

This paper describes a new nonintrusive method for the determination of high-temperature solubility data. Accurate high-temperature solubility data is vital to many industrial manufacturing processes such as cooling crystallization with direct implications for yield, throughput, and solvent usage. However, the provision of such data is notably absent from published literature for many active pharmaceutical ingredients. Pressurized-synthetic methodology is presented as a new technique for determining high-temperature solubility data. Paracetamol (acetaminophen) is used as a reference active pharmaceutical ingredient to validate the methodology. Solubility data determined using the pressurized-synthetic approach is reported for several pure solvents across a significantly extended temperature range. In the case of methanol, solubility data is obtained up to 354.15 K, above the atmospheric boiling point of the solvent, 337.65 K, and far in excess of the temperature range for which data exists in the literature, 268.15−303.15 K. The data obtained using the pressurized-synthetic method is validated against an extended gravimetric data set at temperatures up to the atmospheric boiling point for each solvent. Sensitivity studies were conducted to
determine the influence of factors such as temperature gradient on the ultimate solubility determination. A temperature-based standard deviation of 0.1 K was established for paracetamol in 2-propanol at 303.15 K, comparing favourably with the temperature-based equivalent standard deviation of 0.2 K for the gravimetric approach. Binary interaction parameters for the pressurized-synthetic solubility data are derived and estimated for four different activity coefficient models, namely Margules, Van-Laar, Wilson, and non-random two-liquid (NRTL), along with the empirical solubility equation of Apelblat. For each solvent, the quality of fit of each of the activity coefficient models is analysed. The NRTL model was found to best fit the experimental data for methanol, ethanol, 2-propanol, and acetone with mean square errors of $5.73 \times 10^{-5}$, $3.00 \times 10^{-4}$, $1.70 \times 10^{-4}$, and $7.35 \times 10^{-5}$, respectively. The pressurized-synthetic approach provides a nonintrusive, validated, and readily automated approach for the provision of valuable high-temperature solubility data that can be readily extended to binary and ternary systems.

### 3.1 Introduction

The solubility of bioactive compounds is of critical importance to the pharmaceutical industry. Not only is accurate solubility data vital in terms of quantifying drug absorption and bioavailability, it is also essential for the development of manufacturing operations such as separation, liquid extraction, and crystallization.

In the case of the pharmaceutical cooling crystallization process, the difference in solubility as measured between upper and lower temperature bounds is by definition implicitly linked with the critical crystallization design metrics of yield, throughput, and solvent usage. For maximizing yield and throughput of cooling crystallization while minimizing solvent usage, a wide temperature span is normally desired. The provision of high- and low-temperature solubility data is therefore of upmost importance for industrial crystallization design. In general, it can be seen that API solubility curves show the most pronounced gradients at elevated temperatures. Thus,
small increases in the upper temperature can potentially lead to significant process improvements. Accurate high-temperature solubility data is therefore imperative for designing crystallization processes; however, remarkably, the provision of high temperature solubility data is notably absent from published literature to date.

There are several widely used methods for thermodynamic solubility determination, the most common of which include the gravimetric and shake flask methods.1 In the gravimetric approach, the temperature of equilibration is fixed, and a saturated solution is achieved by contacting excess solute with solvent. Equilibrium is approached asymptotically, whereupon a sample of the equilibrated solution is filtered and weighed. The solvent is then gently evaporated, thus allowing for the evaluation of residual crystalline material mass and solubility. In the case of the shake flask (Higuchi & Connors, 1965), an excess of drug solute is added to the solubility medium and the phases are again separated using filtration or centrifugation. Quantification of drug concentration in the saturated solution is measured by techniques such as ultraviolet (UV) spectrophotometric analysis or high-pressure liquid chromatography (HPLC). Multiple drawbacks are associated with both gravimetric and shake flask methods. At elevated temperatures, and with volatile solvents, solubility measurements using either of these methods can be compromised by virtue of evaporation of the solvent. Unavoidable cooling occurs despite efforts to minimize the time between sample collection, filtering, and gravimetric weighing. Filtration of fine particles may not be entirely successful with some particles passing to the sample of equilibrated solution. Neither approach lends favorably to high-throughput screening studies. The maximum temperature for which solubility data can be obtained using both approaches is bound by the atmospheric boiling point of the solvent. In the case of UV or HPLC, samples may need to be diluted further for analysis, introducing the potential for measurement error. Regulation and maintenance of equilibrium temperature during analysis is a further potential concern. For viscous solvents in particular, settling of particles for analysis of clear solution may not occur.
This paper introduces a newly developed methodology for solubility determination, namely the pressurized-synthetic method. This nonintrusive method is based on the dissolution and consequent disappearance of the solute as monitored using laser instrumentation. The use of reactor pressurization extends the capability of the synthetic method, providing an innovative way to determine solubility even beyond the atmospheric boiling point of the solvent. The approach overcomes all the drawbacks previously listed for the gravimetric and shake flask methods. It does not require equilibrated sample collection or filtration. The approach can be readily automated, and the methodology does not occupy resources of specialized instrumentation.

For evaluating the pressurized-synthetic methodology, a reference Active Pharmaceutical Ingredient (API) with well-established solubility data was required. Paracetamol (also known as acetaminophen) was chosen for this purpose. Paracetamol is an important analgesic and antipyretic drug. As a benchmark compound, it has, and continues to be, widely applied in crystallization research (Mitchell et al., 2011) (Nagy et al., 2008) (Worlitschek & Mazzotti, 2004). A range of pure solvents are employed in the manufacturing of paracetamol; in this chapter solubility of paracetamol in eight of the most commonly used pure solvents are considered (methanol, ethanol, 1-pentanol, 1-propanol, 2-propanol, 1-butanol, water, and acetone).

There are several examples of solubility studies regarding paracetamol at low temperatures. No previous literature was sourced in which the solubility of paracetamol was established at a temperature in excess of the atmospheric boiling point of the solvent. Perhaps the most relevant of these studies is that of Granberg and Rasmuson (Granberg & Rasmuson, 1999) who determined the solubility of paracetamol in a range of pure solvents, using the gravimetric approach. The range of temperatures in this study was limited to 268.15-303.15 K. Likewise, Fernandez (Fernandez, 1999) gravimetrically determined the effect of solubility of paracetamol in ethanol for solution temperatures of 283.15 to 308.15 K, returning a slightly lower
solubility when compared to the Granberg and Rasmuson data. A further study by Granberg and Rasmuson (Granberg et al., 2001) examined the solubility of paracetamol of binary and ternary mixtures of water, acetone, toluene, again with a temperature range of 268.15 to 303.15 K.

Shakeel et al. (Shakeel et al., 2013) did present data covering a higher temperature range 298.15 K to 333.15 K for paracetamol using the shake flask method. However, the elevated temperature in this study was facilitated by the transcutol-water mixture, where transcutol has a high boiling point under atmospheric conditions of 475.15 K.

No previous literature was sourced in which the solubility of paracetamol was established at a temperature in excess of the atmospheric boiling point of the solvent.

The aims set out for this chapter were to (1) establish solubility data for paracetamol up to and beyond the boiling point in pure solvents using the new pressurized-synthetic methodology; (2) validate the approach with comparisons with earlier and new gravimetric data, if necessary; (3) provide thermodynamic modelling of the extended solubility data; and finally (4) perform a comparative evaluation of activity coefficient models in terms of quality of fit.

### 3.2 Materials and methods

#### 3.2.1 Materials

Paracetamol (BioXtra ≥99% NMR) was obtained directly from Sigma-Aldrich [CAS 103-90-2]. Paracetamol is known to exist in three polymorphic forms: monoclinic, the commercially available and room temperature relatively thermodynamically stable Form I, orthorhombic (Form II, metastable) and the highly unstable Form III which is rarely observed outside of fusion reactions. The crystal structures for both monoclinic and orthorhombic polymorphs have previously been reported in literature (Nicnols & Frampton, 1998). High temperature samples of solute were prepared through rapid filtration, followed immediately by X-ray diffraction analysis in order to mitigate
against the risk of solvent-mediated polymorphic transformation during storage. Powder X-ray diffraction was utilized as fig. 3.1 in order to confirm the crystal structure of the as received paracetamol as being that of the commercially used form I with characteristic peaks (2θ): 12.1 ± 0.2, 13.6 ± 0.2, 15.5 ± 0.2, 18.2 ± 0.2 and 26.6 ± 0.2.

Figure 3-1. Experimental powder X-ray diffraction pattern for monoclinic paracetamol (form I) as received.

X-ray diffraction was utilized to eliminate the possibility of a polymorphic transformation during the solubility experiments, across the full range of solvents considered. High-temperature samples of solute were prepared through rapid filtration followed immediately by X-ray diffraction analysis to mitigate the risk of solvent-mediated polymorphic transformation during storage. Powder diffraction data were collected on a Philips X’Pert-MPD PRO diffractometer (PW3064 sample spinner) with nickel-filtered Cu Kα radiation (λ = 1.5418 Å) run at 40 kV and 35 mA, 2θ = 5−40°, with a step size of 0.02° 2θ and a scan speed of 0.02° s⁻¹. Samples were prepared by light grinding prior to placement in a sample holder and flattening with a glass slide. The possibility of a polymorph transformation occurring during grinding was eliminated by analyzing a sample before and after grinding. Analysis of the
spectra indicated no polymorphic transformation, with consistent peaks before and after equilibration indicative of form I.

The eight solvents utilized in this work are listed in Table 3.1, alongside supplier and purity data, boiling points at atmospheric pressure and under pressurization (1.25 bar(g)). The water utilized was distilled, deionized and filtered (0.2 µm).

Table 3-1. Source and mass fraction purity of the materials used in solubility of paracetamol.

<table>
<thead>
<tr>
<th>chemical name</th>
<th>Source</th>
<th>mass fraction purity</th>
<th>analysis method</th>
<th>additional purification</th>
<th>boiling point, K (1atm)/(1.25bar(g))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Sigma-Aldrich</td>
<td>≥0.999</td>
<td>HPLC(^a)</td>
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<td>337.65 / 359.35</td>
</tr>
<tr>
<td>Ethanol</td>
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<td>≥0.999</td>
<td>HPLC</td>
<td>None</td>
<td>351.48 / 373.01</td>
</tr>
<tr>
<td>1-propanol</td>
<td>Sigma-Aldrich</td>
<td>≥0.999</td>
<td>HPLC</td>
<td>None</td>
<td>370.35 / 393.25</td>
</tr>
<tr>
<td>2-propanol</td>
<td>Sigma-Aldrich</td>
<td>≥0.999</td>
<td>HPLC</td>
<td>None</td>
<td>355.38 / 376.77</td>
</tr>
<tr>
<td>1-butanol</td>
<td>Sigma-Aldrich</td>
<td>≥0.995</td>
<td>HPLC</td>
<td>None</td>
<td>390.65 / 414.82</td>
</tr>
<tr>
<td>1-pentanol deionized water</td>
<td>Distilled</td>
<td>≥0.99</td>
<td>GC(^b)</td>
<td>None</td>
<td>410.65 / 437.67</td>
</tr>
<tr>
<td>Acetone</td>
<td>Sigma-Aldrich</td>
<td>≥0.998</td>
<td>HPLC</td>
<td>None</td>
<td>329.44 / 354.83</td>
</tr>
<tr>
<td>monoclinic paracetamol</td>
<td>Sigma-Aldrich</td>
<td>≥0.99</td>
<td>NMR(^c)</td>
<td>None</td>
<td>-</td>
</tr>
</tbody>
</table>

\( ^a\) All normal boiling point data listed were obtained from Sigma-Aldrich and estimated for elevated pressure using the Clausius–Clapeyron relation. \(^b\) High-performance liquid chromatography. \(^c\) Gas chromatography. \(^d\) Nuclear magnetic resonance spectroscopy.

### 3.2.2 Pressurized-Synthetic solubility determination

Suitable reactors were required to perform the pressurized solubility measurements. Three glass lined stainless steel pressure vessels were custom designed with a nominal capacity of 300 ml. These were fitted with flush mounted 15 bar pressure rated sight glasses to facilitate the synthetic laser measurement. The solvents are incompressible, so the pressure does not have effect on the volume of solvents. Vessel lids were mechanically fastened securely in place, with Viton seals, except in the case of acetone where EDPM seals were used for solvent chemical compatibility. The
pressure vessel lids incorporated PTFE sealed, compression fittings for PT-100
temperature gauge, chemically resistant 316 stainless steel pressure gauge and
pressure supply fittings. A pressure regulator was installed, in addition to a pressure
relief valve for safety reasons. For each solubility data point, predetermined masses of
solvent and solute were added to, and weighed in each reactor, using an analytical
balance (Mettler Toledo MS4002S, weighing capacity up to 4200 g, sensitivity +/-0.01 g).

To suspend the crystals in the solvent and further to minimize temperature and
concentration gradients during the experiments, each vessel was agitated by means of
a PTFEcoated magnetic stirrer bar driven by a serial magnetic stirrer plate (2mag
MIXdrive 1) with 40 W stirring power and a maximum stirring volume of 3,000 ml
per stir point. A maximum stir rate of 500 rpm was determined due to the potential for
formation of vortex and consequent air entrapment. A conservative 450 rpm was
adopted for the solubility experiments; more than sufficient to ensure full suspension
of paracetamol crystals.

The pressure vessels were sealed, purged and slowly pressurized with N2 to a pressure
of 1.25 bar (g), a pressure sufficiently high to ensure minimal evaporation of any of
the solvents used during the experiments. The pressure vessels were positioned within
a thermostatic stainless steel bath (Grant GR150; 38L; stability +/- 0.005 K and
uniformity +/- 0.02 K). For experiments up to 343.15 K, the bath was filled with
deionised water, and the surface covered with polypropylene spheres to avoid
evaporation and minimise thermal gradients. For higher temperature experiments
silicone oil was used to ensure appropriate thermal conduction while avoiding
evaporation.

For the determination of solubility, a continuous wave 635 nm red laser (MRL-III-
635L) was utilised as is commonly adopted in laser diffraction measurements. The
laser power was adjustable from 0-30mW. A low power setting of 4 mW was selected
to avoid excessive heating of the solution.
Silicon photodiode based laser detectors (Coherent UV/VIS Quantum Power Sensor, 325-1065nm, Calibration Uncertainty +/-2%, 5 µW to 100 mW wavelength dependant) were used to evaluate laser power and obscuration. The complete assembly of apparatus used for pressurized-synthetic solubility measurements is illustrated in fig. 3.2.

Figure 3.2. Schematic diagram of the experimental rig including temperature and pressure sensor, submersed magnetic stirring plate, thermostatic bath with temperature controller, 635 nm red laser, detector, magnetic stirring bar and polypropylene spheres.

The experimental procedure adopted was broadly based on the laser monitoring approach (Jouyban & Fakhree, 2012) (Luo et al., 2016) (Sheng et al., 2018). This approach is also referred to as the synthetic or last crystal disappearance method (Hao et al., 2005). The temperature of the reactors were slowly but progressively increased while monitoring obscuration of the laser beam by means of particle scattering. For each concentration of solute in solvent, a solubility temperature consistent with a sharp change in obscuration was detected as the particles dissolved. A representative sample result illustrating the sharp change in obscuration commensurate with the progressive, slow, and linear temperature increase of the reactor is shown in Figure 3.3 for the 0.05 molar fraction of paracetamol in 2-propanol.
Figure 3-3. Sample result demonstrating step change in laser obscuration as recorded for 0.05095 molar fraction of paracetamol in isopropanol, with temperature gradient of 0.3 K/h.

Temperature profiles were implemented using the Labwise control software based on the feedback of the external temperature probe positioned within the reactor. A sensitivity study was performed in order to evaluate the effect varying the temperature gradient, the results from which are shown below as fig. 3.4.

Figure 3-4. Sensitivity study investigating the influence of vessel temperature change gradient on the observed solubility point temperature measurements. The laser power measurement is normalized by the maximum laser power.
Chapter 3

The results of the sensitivity study indicated a high level of consistency in the solubility temperature measurements with a standard deviation of 0.1 K.

For maximizing accuracy and ensuring sufficient time for equilibration, a conservative 0.3 K/h temperature gradient was adopted, which is significantly slower than the maximum suggested 2 K/h value (Jouyban & Fakhree, 2012) for synthetic measurements.

To monitor laser obscuration, data points were recorded every ten seconds with the average and standard deviation of laser power over 100 measurements returned. All experiments were conducted in the presence of a fume hood.

3.2.3 Gravimetric solubility measurements

Equilibrium solubility measurements were conducted for paracetamol in each of the eight pure solvents. The focus of the gravimetric measurements was to extend the earlier dataset of (Granberg & Rasmuson, 1999) such as to allow comparison with synthetic solubility data at more elevated temperatures. A Grant GR150 thermostatic stainless steel bath was used with a magnetic stainless steel stirrer plate placed on the base. Verification of temperature was obtained using a calibrated PT-100 resistance thermometer.

For experiments up to 343.15 K, the bath was filled using deionised water insulated by a layer of polypropylene balls. For higher temperature gravimetric samples, high temperature silicone bath oil was used. The datasets of low boiling point temperature solvents, namely acetone and methanol were extended to 313.15 K, 2-propanol and ethanol extended to 343.15 K, while higher boiling point temperature solvents were extended to 343.15 K in 10 K increments.

Excess solute was added to the solvent, which were then sealed to prevent solvent loss by evaporation. The added amount should be enough to make a saturated solution in equilibrium with the solid phase. The solution was stirred at 500 rpm using Teflon-coated stirrer bars for a minimum of 72 h at constant temperature. Following this
time, the solid residue was allowed to settle for at least 12 h, without stirring, until the saturated solution was observed to be visibly clear. This was briefly further verified using a laser light of 532 nm. The clear solution was then sampled (approximately 10 ml) using a preheated syringe and filtered into pre-weighed dry glass vials using a 0.2 µm, PTFE membrane (15mm diameter) syringe filter.

The use of cap incorporating PTFE septum reduced the risk of solvent evaporation. The combined mass of vials and sample was measured without undue delay. The caps were then removed and the solvents were allowed evaporate slowly until only the solid residue of paracetamol was found to remain in the vials. In the case of volatile solvents, with a boiling point of < 393.15 K, drying was performed using a vacuum oven at 313.15 K for approximately one week, with a further verification measurement performed several days later to ensure the sample was completely dry. In the case of less volatile solvents, the temperature of the vacuum oven was increased from 313.15 K to 343.15 K after three days, and sustained for approximately one week before first mass measurement. A verification measurement was again performed several days later in order to ensure the sample was fully dry. All masses were weighed using an analytical balance (Mettler-Toledo AX054, weighing capacity up to 520 g, sensitivity +/-0.1 mg).

Triplicate measurements were performed under the same conditions and the arithmetic average value of solubility calculated. The estimated relative standard uncertainty based on repeat experiments (calculated as 100% standard deviation/average value) of the solubility measurement was less than 2% in each case. Negligible mass changes were noted between initial and verification measurements thus indicating that the samples were completely dry.
3.3 Theory

As noted earlier, the synthetic solubility studies conducted in this paper were carried out in pressurized vessels. The purpose of this pressurization was to control evaporation and furthermore to raise the boiling point of the solvent. It was not the intention of the study to directly affect the solubility of paracetamol in any of the pure solvents by means of the pressurization alone. Indeed, a theoretical demonstration was necessary to verify the appropriateness of negating the influence of pressure, as detailed below.

Early studies by Adam (Adams, 1931), it was recognized that only in exceptional cases is the solubility of solids in liquids influenced by pressure. These exceptional cases include: solution of the carbonates, sulphates, sulphides, fluorides and hydroxides of some alkalis, the alkaline earths and the heavy metals in water, especially where the saturated solutions are very dilute. Pressure in the order of 1000’s of atmospheres can significantly increase the solubility of these substances.

The variation of solubility with pressure for solids in pure liquids at equilibrium follows the following relationship (Adams, 1931):

\[
\frac{dx_2}{dP} = \frac{v_2 - v_2^s}{\frac{\partial \mu_2}{\partial x_2}}_{P,T}
\] (3.1)

Where \( x_2 \) is the molar fraction referred to the solute, \( P \) is the pressure in Pascals, \( v_2 \) is the partial volume of the solute in the solution and \( v_2^s \) its specific volume in the solid phase, both expressed in m\(^3\)/mol, and \( \frac{\partial \mu_2}{\partial x_2} \)\(_{P,T} \) is the rate of change of the chemical potential of the solute in the solution with a change of composition at the temperature and pressure of the experiment.

Considering the chemical potential change between solid and liquid solution and in solid phase (Granberg et al., 2001):

\[
\mu_2^L = \mu_2^s + kT \ln \frac{a_2}{a_{2,eq}} = \mu_2^s + kT \ln \frac{x_2 y_2}{x_{2,eq} y_{2,eq}}
\] (3.2)
where \( k \) represents the Boltzmann constant, equal to \( 1.38 \times 10^{-23} \) J/K, \( \gamma_2 \) is the activity coefficient of the solute in the liquid phase, \( T \) is the temperature in Kelvins and the subscript “eq” denotes the equilibrium conditions. Assuming the activity coefficients approximately equal to 1 for the sake of simplicity, it is possible to evaluate the change in chemical potential with a change in solution composition:

\[
\frac{d\mu_2}{dx_2}_{P,T} = \frac{kT}{x_2} \tag{3.3}
\]

Substituting eq. 3.3 to 3.1 and integrating, it leads to the following expression:

\[
\ln \frac{x_2}{x_2^*} = -\frac{v_2 - v_2^s}{kT}(P - P^*)
\]

\[
x_2 = \exp \left[ -\frac{v_2 - v_2^s}{kT}(P - P^*) \right] x_2^* \tag{3.4a-3.4b}
\]

Incorporating the pressure applied for the experiments (1.25 bar(g)) and a volume variation for polymorphic form I of paracetamol of \( 1.75 \times 10^{-6} \) m\(^3\)/mol at the melting temperature (442.2K) (Espeau et al., 2015), it is apparent that the term inside the exponential, relative to the atmospheric conditions in eq (3.4b), must have a magnitude order of \( O(10^{-19}) \) for the range of temperatures under consideration. This implies that the RHS of eq (3.4b) must tend to zero to balance the equation. Therefore, it can be concluded that the direct effect of pressure on solubility is negligible in the case of the present work.

### 3.4 Results and discussion

The pressurized-synthetic method, as well as the extended gravimetric data from the present work and Granberg and Rasmuson (Granberg & Rasmuson, 1999) solubility data for paracetamol as measured in each of the different pure alcohols is presented in Figure (3.5). No discernible discontinuity was observed between data obtained through the gravimetric and synthetic approaches. Each of the experimental data points represents a single concentration measurement. Given that the synthetic
solubility curves were not known in advance and were estimated by extrapolation of existing data, some non-uniformity in the distribution of pressurized-synthetic data points is present. The pressurized-synthetic solubility is shown correlated using the NRTL model, which was found to have the best fit of the four activity coefficient models used for the thermodynamic modelling of solubility.

Using the pressurized-synthetic approach, solubility data for paracetamol in methanol was obtained up to 354.15 K, well beyond the atmospheric boiling point of methanol (337.65 K). The availability of such high temperature solubility data overcomes the need for extrapolation with implicit error. For instance, in the case of applying the Apelblat correlation to the published data for paracetamol in methanol, an 11.2% error was calculated at the highest temperature measured (354.15 K) when compared to pressurized-synthetic measurements.

As previously noted, in the case of cooling crystallisation, operation beyond the boiling up can enhance yield, throughput and solvent usage. Consider a cooling crystallisation of paracetamol in methanol; cooling from the atmospheric boiling point of methanol 337.65 K to the lowest temperature solubility point available (268.15 K) results in a theoretical yield of 80.2%. Increasing the upper temperature limit to 354.15 K facilitates an improvement in yield; which is increased to 87.5%. Indeed, further yield enhancements may be gained through extension of this method to temperatures in excess of those considered in the present work. Crystallisation is a late-stage process in pharmaceutical manufacture wherein the costs of the precursors have largely been absorbed. Therefore the economic impact of process intensification can be very significant on an industrial scale. These benefits illustrate the clear need for high temperature solubility data in excess of solvent atmospheric boiling points.

In order to validate the pressurized-synthetic method, a comparison between gravimetric and pressurized-synthetic derived solubility data was required. A direct comparison of the gravimetric derived solubility data with the NRTL modelled pressurized synthetic data is shown in Figure (3.6) for the alcohols.
Figure 3.5. Solubility of paracetamol C versus temperature $T$ in each of the alcohols as determined using (○) Published literature values (Granberg & Rasmuson, 1999); (▲) Pressurized-Synthetic Data and (□) Extended Gravimetric Dataset. Pressurised-synthetic data is shown fitted using NRTL model.
Figure 3-6. Comparison of gravimetric and pressurized-synthetic solubility results at temperatures up to the boiling point of each alcohol. The straight line represents an ideal correlation with gravimetric results, and the point x and y axis coordinates are obtained directly from gravimetric data and best-fit NRTL interpolation of synthetic solubility results, respectively.
Figure 3-7. Solubility of paracetamol C versus temperature T in each of the non-alcohols as determined using (○) Published literature values (Granberg & Rasmuson, 1999); (▲) Pressurized-Synthetic Data and (□) Extended Gravimetric Dataset. Pressurized-synthetic data is shown fitted using NRTL model.

The data shown in Figure (3.6), in general, show excellent agreement between the solubility data obtained using the gravimetric and pressurized-synthetic methods. This is particularly true in the case of higher boiling point alcohols such as 1-butanol and 1-pentanol. Lower boiling point temperature alcohols such as ethanol, 1-propanol, and 2-propanol returned a marginally higher solubility using the pressurized-synthetic method at temperatures close to the atmospheric boiling point. This was anticipated in advance given the potential for error of the gravimetric approach at elevated temperatures.

In the case of the non-alcohols, solubility data for acetone was obtained up to a maximum of 323.15 K. The aggressive nature of hot acetone necessitated the provision of ethylene propylene diene monomer (EPDM) rather than Viton seals.

One of the drawbacks of the synthetic and the pressurized-synthetic method is that the solute must be freely suspended within the solvent and physically obscure the laser path. Among all the solvents methanol has the highest solubility and solubility of water is significantly less than all other solvents tested. Despite vigorous agitation, a significant mass of paracetamol was found to be suspended as froth on the surface of the water. As such, it was not possible to obtain accurate solubility data for
paracetamol in water using the pressurized-synthetic approach. In the case of gravimetric testing, even with limited mixing equilibrium is ultimately reached with the opportunity to sampling the clear solution between the paracetamol froth and settled crystals below. Additional gravimetric test results were taken to extend the solubility curve for paracetamol in water to 353.15 K as shown in figure (3.7).

Table 3.2 presents the full dataset of pressurised-synthetic results in both mole fraction $x_2$ and as $g_{\text{solute}}/kg_{\text{solvent}}$ (solute free basis) at measured saturation temperature, under a pressure of 1.25 bar (g). Also presented is the calculated mole fraction solubility of paracetamol in each of the solvents using four activity coefficient models and the empirical modified Apelblat equation. Table 3.3 provides the extended set of gravimetric data for each solvent. For some data points, the mole fraction solubilities in Tables 3.2 and 3.3 are identical. In such cases, the concentration of paracetamol in pure solvent utilized for the pressurized-synthetic measurements was informed by the preceding gravimetric results.
Table 3-2. Pressurized-Synthetic and calculated Mole Fraction Solubility of Paracetamol as calculated using Margules, Van-Laar, Wilson, NRTL and Modified Apelblat Equation across entire range of solvents at saturation temperature $T$ and pressure $P = 1.25$ bar(g).

<table>
<thead>
<tr>
<th>T/K</th>
<th>C / g/Kg</th>
<th>$x^{exp}$</th>
<th>$x^{apd}$</th>
<th>$x^{Marg}$</th>
<th>$x^{VL}$</th>
<th>$x^W$</th>
<th>$x^{NRTL}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>293.45</td>
<td>297.81</td>
<td>0.0594</td>
<td>0.0613</td>
<td>0.0633</td>
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<td>303.55</td>
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<tr>
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<td>323.35</td>
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<td>0.2004</td>
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Methanol

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<th>$x^{apd}$</th>
<th>$x^{Marg}$</th>
<th>$x^{VL}$</th>
<th>$x^W$</th>
<th>$x^{NRTL}$</th>
</tr>
</thead>
<tbody>
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*aStandard uncertainties $u$ are $u(T) = 0.02$ K, $u_r(P) = 0.05$ bar, $u_r(x_2) = 2\%$. 
Table 3-3. Extended Gravimetric dataset of solubility of paracetamol in pure solvents expressed in both mole fraction $x$ and as $g_{\text{solute}}/kg_{\text{solvent}}$ (solute free basis) at temperature $T$.

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*Standard uncertainties $u$ are $u(T) = 0.02$ K, $u(P) = 0.05$ bar, $u(x_2) = 2\%$.

The conversion between molar solubility $x_2$ and mass solubility $C$ and vice versa was performed using the relationship:

$$C = \frac{1000 x_2 \cdot MW_{\text{solute}}}{MW_{\text{solvent}} (1-x_2)} \quad (3.5)$$
\[ x_2 = \frac{c}{c + \frac{1000}{MW_{Solute} + MW_{Solvent}}} \]  

(3.6)

### 3.4.1 Solubility Modeling

Many methods have been proposed to describe the solubility of a solute in different pure solvents; for instance, the perturbedchain statistical-associating fluid theory (PC-SAFT) modeling approach has been widely applied for the correlation and prediction of solubility even at elevated temperatures (Ruether & Sadowski, 2009) (Reschke et al., 2016). In this work, the solubility of paracetamol in the selected solvents at different temperatures is correlated with the modified Apelblat equation (Prausnitz, 1998), and Margules, Van-Laar, NRTL and Wilson activity coefficient-based models. The modified Apelblat equation is expressed in eq 3.7.

\[ \ln C = a_A + \frac{b_A}{T} + c_A \ln T \]  

(3.7)

where \( a_A, b_A, \) and \( c_A \) are adjustable equation constants; \( C \) represents the equilibrium concentration in grams of solute per kilograms of solvent at temperature \( T \) expressed in kelvin.

where \( C \) represents the equilibrium concentration in grams of solute per kilograms of solvent at temperature \( T \) expressed in kelvin; the three adjustable parameters of the empirical solubility model are indicated as \( a_A, b_A, \) and \( c_A \). The values of these parameters were found for each solvent through regression and are summarized in Table 3.4. The empirical model shows good agreement with the underlying experimental data in the case of all solvents tested.
Table 3-4. Apelblat Parameters and Root Mean Square Error (RMSE) Calculated for Each Solvent.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>a_A</th>
<th>b_A</th>
<th>c_A</th>
<th>RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>-193.9</td>
<td>7236</td>
<td>30.79</td>
<td>0.0020</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-193.3</td>
<td>7303</td>
<td>30.55</td>
<td>0.0034</td>
</tr>
<tr>
<td>1-propanol</td>
<td>-129.2</td>
<td>4322</td>
<td>20.97</td>
<td>0.0021</td>
</tr>
<tr>
<td>2-propanol</td>
<td>-156.6</td>
<td>5545</td>
<td>25.06</td>
<td>0.0014</td>
</tr>
<tr>
<td>1-butanol</td>
<td>-170.4</td>
<td>6393</td>
<td>26.93</td>
<td>0.0029</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>-105.0</td>
<td>3207</td>
<td>17.26</td>
<td>0.0011</td>
</tr>
<tr>
<td>Acetone</td>
<td>-270.4</td>
<td>10300</td>
<td>42.20</td>
<td>0.0128</td>
</tr>
<tr>
<td>Water</td>
<td>-341.6</td>
<td>13280</td>
<td>52.64</td>
<td>3.7x10^-5</td>
</tr>
</tbody>
</table>

Although the correlation of Apelblat shows good agreement with the underlining experimentally derived solubility data, the correlation is empirical in nature rather than thermodynamically derived. Activity coefficient models, such as Margules, Van-Laar, Wilson and NRTL, offer a more thermodynamically rigorous approach, accounting for deviation from the ideal solubility. For calculating the binary interaction parameters utilized by these models, it was first necessary to calculate the experimental activity coefficients based on the solubility data.

The experimental activity coefficients were calculated using a nonideal solid-liquid equilibrium equation defined as (Prausnitz, 1998):

\[
\ln(x_2\gamma_2) = \frac{\Delta H_f}{R} \left( \frac{1}{T_l} - \frac{1}{T} \right) - \frac{\Delta C_p}{R} \left( \frac{1}{T_l} - \frac{1}{T} \right) - \frac{\Delta C_p}{R} \ln \left( \frac{T_l}{T} \right) \tag{3.8}
\]

In which the exchange of enthalpy (\(\Delta H_f\)) and heat capacity at constant pressure (\(\Delta C_p\)) have been assumed constant and evaluated between the equilibrium condition and the melting point, both evaluated at the melting temperature \(T_m\). Using different scanning calorimetric analyses (Hojjati & Rohani, 2006), measured the thermal properties of paracetamol to be \(T_m = 442.2\) K, \(\Delta H_f = 28.1\) kJ/mol, and \(\Delta C_p = 99.6\) J mol\(^{-1}\) K\(^{-1}\) (at the fusion temperature).

Through insertion of the enthalpy, melting temperature, and heat capacity values into eq 3.8, and given that the experimental solubility values \(x_2\) and temperatures are
known, it is possible to evaluate the experimental activity coefficients by rewriting eq 3.8 for $\gamma_2$. The ideal solubility of paracetamol may also be estimated (setting $\gamma_2 = 1$).

Plots of the logarithm of experimental activity coefficients of Paracetamol in alcohol and non-alcohol based solvents are given in Figure 3.8a and b respectively. Referring to eq. 3.8, it is apparent that at any given temperature, the activity coefficient is inversely proportional to the measured solubility. The ideal solubility is represented by the value 0 on the logarithmic activity coefficient scale, with higher values relating to lower solubility. Increasing chain length in alcohols, as is shown in Figure 3.8a, results in greater activity coefficient values and consequently reduced solubility. Activity coefficient trends for all solvents are shown to remain relatively flat, or decrease slightly with increasing temperature.

![Figure 3-8. Logarithm of experimental activity coefficients of paracetamol at selected temperatures in alcohols (a) and in water and acetone (b).](image)

The highest activity coefficients are observed with water, a high polar solvent. Physically, this can be explained by the significant influence of the aromatic ring and methyl group in paracetamol on surrounding water molecules, despite the fact that the alcohol and amide groupings in the paracetamol molecule are also polar, facilitating the formation of hydrogen bonds.
Having established the experimental activity coefficients, activity coefficient models could now be fitted to the experimental data. The four models selected are described in Table 3.5. Utilization of each of the models is commonly found in the literature. They can be differentiated by such factors as complexity and the inherent nature of the temperature dependency.

### Table 3-5. Activity Coefficient Models Used in the Present Work Complete with Their Respective Number of Parameters and Temperature Dependency Information

<table>
<thead>
<tr>
<th>model</th>
<th>equation*</th>
<th>number of parameters</th>
<th>temperature dependency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margules</td>
<td>$\ln \gamma = \frac{A}{RT_i}(1 - \varepsilon_i)^2$</td>
<td>1 $[\varepsilon_i]$</td>
<td>explicit</td>
</tr>
<tr>
<td>Van-Laar</td>
<td>$\ln \gamma = \frac{R \varepsilon_i}{R + \frac{\lambda_{ij}}{\lambda_{ij} + \lambda_{ij}}}$</td>
<td>2 $[\lambda_{ij}, \lambda_{ij}]$</td>
<td>implicit</td>
</tr>
<tr>
<td>Wilson</td>
<td>$\ln \gamma = -\varepsilon i \left(\frac{\lambda_{ij}}{\varepsilon_i + \lambda_{ij}} - \frac{\lambda_{ij}}{\varepsilon_i + \lambda_{ij}}\right)$</td>
<td>2 $[\lambda_{ij}, \lambda_{ij}]$</td>
<td>explicit</td>
</tr>
<tr>
<td>NRTL</td>
<td>$\ln \gamma = i \left(\frac{G_i}{\varepsilon_i + G_i} + \frac{\varepsilon_i G_i}{(\varepsilon_i + G_i)^2}\right)$</td>
<td>3 $[\varepsilon_i, \eta_i, \eta_j]$</td>
<td>explicit</td>
</tr>
</tbody>
</table>

Where $\theta$ is the set of binary interaction parameters to be estimated for each model and each solvent used, $Np$ is the number of experimental points carried out for each solvent used, and the subscripts mod and exp are respectively referred to the model and the experimental activity coefficients.

The binary interaction parameters were estimated using the following mathematical optimization problem solved using the MATLAB nonlinear least-squares algorithm nlinfit:

$$\min \sum_{i=1}^{Np} \left(\ln \gamma_{2,i}^{\text{mod}}(T, \theta) - \ln \gamma_{2,i}^{\text{exp}}(T)\right)^2$$

(3.9)

Where $\theta$ is the set of binary interaction parameters to be estimated for each model and each solvent used, $Np$ is the number of experimental points carried out for each solvent used, and the subscripts mod and exp are respectively referred to the model and the experimental activity coefficients.

Insertion of the binary coefficients, from Table 3.6 into the activity model equations of Table 3.5 allows for the prediction of molar solubility, which can then be converted...
to mass solubility as per eq 3.5. Returning to Table 3.2, the solubility derived from each of the activity coefficient model is directly compared to the experimental data. The selection of the activity coefficient model is influenced both by the complexity of the model, and the quality of fit to the underlying data.

Table 3-6. Estimated binary coefficients for paracetamol in different pure solvents using Margules, Van-Laar, Wilson and NRTL models.

<table>
<thead>
<tr>
<th>solvent</th>
<th>Margules $A$</th>
<th>$A_{ij}$</th>
<th>$B_{ij}$</th>
<th>$\lambda_{ij}$</th>
<th>$\lambda_{2j}$</th>
<th>$g_{ij}$</th>
<th>$g_{2j}$</th>
<th>$\alpha$</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>127.62</td>
<td>17.695</td>
<td>-0.085</td>
<td>549.958</td>
<td>1.46x10$^3$</td>
<td>1.50x10$^4$</td>
<td>-7.77x10$^3$</td>
<td>0.1627</td>
</tr>
<tr>
<td>ethanol</td>
<td>624.49</td>
<td>0.4312</td>
<td>0.0903</td>
<td>587.877</td>
<td>614.984</td>
<td>1.84x10$^4$</td>
<td>-1.07x10$^4$</td>
<td>0.1019</td>
</tr>
<tr>
<td>1-propanol</td>
<td>1.28x10$^3$</td>
<td>0.5542</td>
<td>0.2805</td>
<td>1.897x10$^3$</td>
<td>-531.1754</td>
<td>8.86x10$^3$</td>
<td>-5.83x10$^3$</td>
<td>0.1033</td>
</tr>
<tr>
<td>2-propanol</td>
<td>1278.31</td>
<td>0.6022</td>
<td>0.2429</td>
<td>1.000x10$^3$</td>
<td>377.5298</td>
<td>12597.57</td>
<td>-7409.39</td>
<td>0.1257</td>
</tr>
<tr>
<td>1-butanol</td>
<td>1.72x10$^3$</td>
<td>0.6497</td>
<td>0.6108</td>
<td>3.35x10$^3$</td>
<td>-7.48x10$^2$</td>
<td>1.56x10$^3$</td>
<td>-45.657</td>
<td>-2.94</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>2.16x10$^3$</td>
<td>0.802</td>
<td>0.733</td>
<td>3.67x10$^3$</td>
<td>-1.61x10$^2$</td>
<td>1.67x10$^3$</td>
<td>1.49x10$^2$</td>
<td>-9.468</td>
</tr>
<tr>
<td>acetone</td>
<td>1.69x10$^3$</td>
<td>1.037</td>
<td>0.1632</td>
<td>-558.174</td>
<td>3740.595</td>
<td>11752.49</td>
<td>-5241.13</td>
<td>0.2406</td>
</tr>
<tr>
<td>water</td>
<td>8.84x10$^3$</td>
<td>3.799</td>
<td>0.2622</td>
<td>4.990x10$^3$</td>
<td>4.871x10$^3$</td>
<td>11912.37</td>
<td>-1477.12</td>
<td>0.5141</td>
</tr>
</tbody>
</table>

The selection of activity coefficient model is influenced by the quality of fit of the model to the underlying data as well as the complexity of the model. Although the quality of fit of each activity coefficient model is solvent-dependent, across all solvents it is evident that the greatest deviation between experimental and model results occurs in all plots at the extremes of the temperature measurements. Further points in this temperature space could potentially reduce these discrepancies. Indeed, the methodology adopted in the current work facilitates further extension of the temperature range, thus potentially increasing the robustness of prediction within the current temperature range.

In seven of the eight solvents examined the Margules model is shown to have the largest mean square error (MSE) (Table 3.7). The NRTL model has the smallest MSE.
for methanol, ethanol, 2-propanol and acetone; Wilson for 1-pentanol and water; Van-Laar for 1-propanol and 1-butanol.

Table 3.7. Mean Squared Error (MSE) of the fitted models for each solvent.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Margules</th>
<th>Van-Laar</th>
<th>Wilson</th>
<th>NRTL</th>
<th>$\frac{MSE_{\text{max}}}{MSE_{\text{min}}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>0.0076</td>
<td>0.0051</td>
<td>0.0015</td>
<td>5.73x10^{-5}</td>
<td>132.63</td>
</tr>
<tr>
<td>ethanol</td>
<td>0.00363</td>
<td>1.8x10^{-3}</td>
<td>2.21x10^{-3}</td>
<td>3.00x10^{-4}</td>
<td>12.10</td>
</tr>
<tr>
<td>1-propanol</td>
<td>2.30x10^{-4}</td>
<td>8.98x10^{-5}</td>
<td>2.33x10^{-4}</td>
<td>2.27x10^{-4}</td>
<td>2.59</td>
</tr>
<tr>
<td>2-propanol</td>
<td>7.11x10^{-4}</td>
<td>3.2882x10^{-4}</td>
<td>5.1931x10^{-4}</td>
<td>1.7021x10^{-4}</td>
<td>4.18</td>
</tr>
<tr>
<td>1-butanol</td>
<td>0.00193</td>
<td>1.28535x10^{-4}</td>
<td>1.80466x10^{-4}</td>
<td>5.4535x10^{-4}</td>
<td>15.07</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>0.0022128</td>
<td>3.3026x10^{-4}</td>
<td>2.63919x10^{-4}</td>
<td>2.9387x10^{-4}</td>
<td>7.58</td>
</tr>
<tr>
<td>acetone</td>
<td>0.003760</td>
<td>1.0249x10^{-4}</td>
<td>1.9164x10^{-4}</td>
<td>7.3511x10^{-5}</td>
<td>51.15</td>
</tr>
<tr>
<td>water</td>
<td>0.05501</td>
<td>3.8x10^{-3}</td>
<td>4.206x10^{-4}</td>
<td>6.4695x10^{-4}</td>
<td>130.95</td>
</tr>
</tbody>
</table>

3.5 Conclusions

Solubility measurement is essential to pharmaceutical process development, particularly in crystallization where elevated temperatures can lead to a significant reduction in operating costs. The methodology presented in this paper presents a novel approach for the determination of solubility data, in a temperature range in which the standard gravimetric method would fail or lack in accuracy. Such high temperature data is particularly important in the case of cooling crystallization where the difference in solubility between upper and lower temperature limits is implicitly associated with yield and industrial efficiency. The error associated with extrapolation of solubility data was demonstrated to be potentially significant (11.2% error), using the modified Apelblat model for paracetamol in methanol and extrapolating from 303.15 to 354.15 K.

The data obtained from the pressurized-synthetic measurements were validated for all solvents with the exception of water by comparison with gravimetric data. No discernible discontinuity was observed between data obtained through the gravimetric
and synthetic approaches. Excellent agreement was seen for higher boiling point solvents at elevated temperatures, whereas for more volatile solvents, the pressurized-synthetic approach indicated a marginally higher solubility when compared to that of the gravimetric approach as was expected.

The solubility data obtained for all pure solvents was modelled using the modified Apelblat empirical equation, as well as Margules, Van-Laar, NRTL, and Wilson activity coefficient models. The NRTL model was shown to have the smallest mean square error for methanol, ethanol, 2-propanol, and acetone.

The methodology presented in this paper is an important extension of the synthetic method which allows non-intrusive access to valuable high temperature solubility measurements. The method can be readily automated. Care must be taken when applying the method to ensure appropriate suspension of solute in solvent. When applying the methodology, one should be aware of the potential for polymorphism at elevated temperatures, and if applying to crystallization, one should carefully consider the maximum solids loading.

In a supersaturated solution, solute molecules may aggregates or clusters and size fluctuates with time. At a certain point, the solution may undergo primary nucleation and stable crystal nuclei appear in the solution. The exact appearance of these clusters and the mechanism of their formation, growth and dissolution, are unknown. So in the next chapter an attempt to understand the nucleation mechanism of paracetamol is made.
Supporting Information

In addition to solubility measurement, the pressurized synthetic method is able to measure the metastable zone width (MSZW). The MSZW is a region bounded by the solubility curve and the metastable limit (MSL) which spontaneous nucleation occurs. Therefore, the nucleation point, where particles become detectable, can be measured as the laser power drops considerably. In chapter three, the pressurized synthetic method was applied for measuring the solubility of paracetamol in presence of different solvents. Here, phenacetin as API was used in order to investigate the capability of this method to detect the metastable zone limit. The experimental procedure adopted was broadly based on the laser monitoring approach similar to method used Lasentec Focused Beam Reflectance (FBRM) (Barrett & Glennon, 2002). The rate of temperature change was 0.05 K/min and a stirring rate of 450 rpm was adopted for the experiments. The results of pressurized-synthetic data for phenacetin as measured in each of the different alcohols are shown in fig. S3.1. Table S5.3 shows the data set of pressurized-synthetic results in g solute/kg solvent at the measured saturation temperature under a pressure of 2 bar (g) and the values of the Apelblat parameters for each solvent summarized in table S3.2.
Figure S3.1. Solubility of phenacetin versus temperature T for IPA (■) and MeOH (□), data are fitted using the Apelblat model. Metastable limit data versus temperature T for IPA (▲) and MeOH (△).

Table S3.1. Pressurized-Synthetic Metastable Limit, Solubility and Calculated Solubility of Phenacetin as Calculated Using Apelblat

<table>
<thead>
<tr>
<th></th>
<th>2-Propanol</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T (K)</td>
<td>$C_{exp}$ (g/kg)</td>
<td>$C_{cal}$ (g/kg)</td>
<td>MSL (K)</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>295.92</td>
<td>29.43</td>
<td>23.14</td>
<td>287.4</td>
</tr>
<tr>
<td></td>
<td>303.19</td>
<td>44.12</td>
<td>35.57</td>
<td>293.44</td>
</tr>
<tr>
<td></td>
<td>313.81</td>
<td>73.53</td>
<td>65.97</td>
<td>308.32</td>
</tr>
<tr>
<td></td>
<td>317.29</td>
<td>88.23</td>
<td>80.56</td>
<td>309.03</td>
</tr>
<tr>
<td></td>
<td>320.98</td>
<td>102.94</td>
<td>99.42</td>
<td>314.96</td>
</tr>
<tr>
<td></td>
<td>330.72</td>
<td>161.76</td>
<td>172.05</td>
<td>323.66</td>
</tr>
<tr>
<td></td>
<td>336.37</td>
<td>220.59</td>
<td>235.42</td>
<td>332.97</td>
</tr>
<tr>
<td></td>
<td>349.54</td>
<td>516.67</td>
<td>482.83</td>
<td>344.62</td>
</tr>
<tr>
<td></td>
<td>354.11</td>
<td>641.67</td>
<td>617.41</td>
<td>349.68</td>
</tr>
<tr>
<td></td>
<td>357.68</td>
<td>750.23</td>
<td>746.18</td>
<td>354.06</td>
</tr>
<tr>
<td>Methanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T (K)</td>
<td>$C_{exp}$ (g/kg)</td>
<td>$C_{cal}$ (g/kg)</td>
<td>MSL (K)</td>
</tr>
<tr>
<td></td>
<td>282.38</td>
<td>50.32</td>
<td>51.86</td>
<td>277.86</td>
</tr>
<tr>
<td></td>
<td>296.50</td>
<td>93.75</td>
<td>92.63</td>
<td>292.29</td>
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<tr>
<td></td>
<td>315.34</td>
<td>229.01</td>
<td>231.13</td>
<td>310.63</td>
</tr>
<tr>
<td></td>
<td>325.68</td>
<td>400.11</td>
<td>403.16</td>
<td>320.64</td>
</tr>
<tr>
<td></td>
<td>341.10</td>
<td>966.25</td>
<td>973.11</td>
<td>337.32</td>
</tr>
</tbody>
</table>
Table S3.2. Apelblat formula values for Methanol and 2-Propanol models

<table>
<thead>
<tr>
<th>solvent</th>
<th>Apelblat parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Methanol</td>
<td>-433.1</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>-149.9</td>
</tr>
</tbody>
</table>

**Effect of evaporation and pressure on the solubility**

In chemistry, vapor pressure is the pressure that is exerted on the walls of a sealed container when a substance in it evaporates (converts to a gas). To find the vapor pressure at a given temperature, use the Clausius-Clapeyron equation:

$$\ln \left( \frac{P_1}{P_2} \right) = \frac{\Delta H_{vap}}{R} \left( \frac{1}{T_2} - \frac{1}{T_1} \right)$$

The formula used for calculating vapor pressure given a change in the vapor pressure over time. Where $\Delta H_{vap}$ is The enthalpy of vaporization of the liquid, $R$ is the real gas constant, or 8.314 kJ/mol, $T_1$ is The temperature at which the vapor pressure is known (or the starting temperature.), $T_2$ is The temperature at which the vapor pressure is to be found (or the final temperature.) and $P_1$ and $P_2$ are The vapor pressures at the temperatures $T_1$ and $T_2$, respectively.

To investigate the error associate with the effect of the evaporation by pressure, vapor pressure for Methanol is calculated that is the most volatile solvent between alcohols:

If starting temperature ($T_1$) is 298.15k, $P_1=1$ atm and $\Delta H_{vap}$ is 35.2 kJ/mol. If the temperature increases to the 348 K (10 degree higher than boiling temperature), this implies that vapor pressure for methanol would be $P_2=1.002$ atm. Therefore, it can be concluded that the effect of pressure on the evaporations and therefore solubility is negligible in the case of the present work.
Chapter 4

Brief description of the paper

In this paper, the nucleation kinetic parameters for the pure paracetamol in 2-propanol in different supersaturations using an automated methodology involving the use of a focused beam reflectance measurement (FBRM) probe is described. The effect of system volume on the nucleation probability was evaluated in combination with four different crystallization volumes (small magnetically stirred 10 mL and overhead-stirred solutions of 85ml, 340ml and 680ml). The resulting probability distributions of nucleation showed that the highest nucleation rates were obtained in magnetically stirred crystallization experiments. Shear rate was rationalized to be the part of the kinetic parameter that changes most significantly.


URL: https://doi.org/10.1021/acs.cgd.9b00490

Leila Keshavarz’s contribution: Prepared the set up and contributed in designing the experiments. Performed the induction time measurement experiments using FBRM. Contributed to preparation, design and writing of the manuscript.
Effects of Scale-Up on the Mechanism and Kinetics of Crystal Nucleation

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† Equal contribution

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ABSTRACT

Insight into nucleation kinetics and other nucleation parameters can be obtained from probability distributions of induction time measurements in combination with the classical nucleation theory. In this work, induction times of crystallization were recorded using a robust and automated methodology involving a focused beam reflectance measurement probe. This methodology is easily interchangeable between different crystallizers which allowed us to investigate the effects of scale-up on the kinetics of crystal nucleation of paracetamol from 2-propanol in four different crystallizers, ranging from small magnetically stirred 10 mL solutions to overhead-stirred solutions of 680 mL. The nucleation rate was an order of magnitude faster in the magnetically stirred crystallizer as compared to the crystallizers involving overhead stirring. The thermodynamic part of the nucleation rate expression did not significantly change the nucleation rate, whereas the kinetic nucleation parameter was found to be the rate-determining process when the crystallization process was scaled-up. In particular, the shear rate was rationalized to be the part of the kinetic parameter that changes most significantly when the crystallization process was scaled-up. The effect of shear rate on the nucleation kinetics decreases with increasing volume and plateaus when the volume becomes too large. In this work, the nucleation mechanism
was also investigated using the chiral sodium chlorate system. These experiments showed that the single nucleus mechanism is the underlying nucleation mechanism in all four tested crystallization setups when supersaturation remains the same. When the supersaturation was changed continuously through cooling, crystallization was driven by a multinucleus mechanism. The automated and robust method used to measure induction times can easily be extended to other crystallizers, enabling the measurement of induction times beyond small crystallizer volumes.

### 4.1 Introduction

Solution crystallization is a widely used process in the production of high purity compounds including pharmaceuticals, agrochemicals, and fine chemicals (Horst et al., 2015). Crystal nucleation is crucial process during crystallization because it may influence the crystal size distribution, purity of crystal product and polymorphism. Crystal nucleation is an important parameter in solution crystallization as it affects many product properties including crystal shape, polymorphic form, chiral form, and the crystal size distribution of the product. Nucleation is a stochastic process for unseeded crystallization processes involving low supersaturation levels or for small crystallizer volumes. Droplet-based methods, double pulse methods, and stirred small volume solutions methods can be utilized to capture the stochastic nature of nucleation and to estimate nucleation kinetics (Xiao et al., 2018). Due to the presence of stirring, the kinetic data obtained from the stirred small volume solution method reflects industrial crystallization processes more accurately as opposed to the other approaches involving stagnant solutions.

Nucleation rates can be used in combination with nucleation theories to reveal more insight into the nucleation mechanism (Davey et al., 2013). Probability distributions of induction times from isothermal induction time experiments can be used in conjunction with the classical nucleation theory (CNT) to estimate the nucleation rate and other nucleation parameters (Jiang & Horst, 2011). Using constant
supersaturations, the deterministic CNT approach can be linked to a probabilistic stationary Poisson process. The theory assumes that a clustering process at a fixed supersaturation involves attachment/detachment transition attempts of monomers, in which the rate of transition probability depends on both the attempt frequency and the probability of success for each attempt. Moreover, the use of the Poisson process means that nuclei are formed independently from each other. This assumption holds if nucleation proceeds through a single nucleus mechanism (SNM) where a single nucleus is formed and grows until detection or undergoes secondary nucleation until detection. The SNM has been observed in cooling crystallization experiments involving chiral (Buhse et al., 2000) and polymorphic compounds (Kulkarni et al., 2014) but it remains unclear if this mechanism holds in induction time experiments, especially in large volumes.

One of the most important effects on the nucleation process is the crystallization volume. With increasing crystallization volume, the attempt frequency for the attachment/detachment of monomers to form clusters increases and with that the probability of nucleation increases. Polythermal metastable zone width experiments have indeed shown that the probability of nucleation increases with increasing volume (Kadam et al., 2011) (Kadam et al., 2012). These results have been explained from the perspective of the volume dependency in CNT. However, in polythermal metastable zone width experiments the probability of nucleation does not depend on the attempt frequency for the attachment/detachment of monomers to form clusters but rather depends on the probability of success for each attempt, which is volume independent (Bhamidi et al. 2017). The underlying probability function is based on constant supersaturation and cannot be used to estimate nucleation kinetics from polythermal metastable zone width experiments (Bhamidi et al., 2017) (Liu, 2013). Instead, the observed increase in the probability of nucleation with increasing volume in polythermal metastable zone width experiments is argued to be the result of inhomogeneous mixing (Kadam et al., 2012), long equilibration times, large numbers of heterogeneous nucleation sites and a possibly high level of mechanical energy
input (Bhamidi et al., 2017). A link between mechanical energy input and induction times of crystal nucleation has been established (Jin & Rasmuson., 2013).

Understanding the effects of scale-up on the mechanism and kinetics of crystal nucleation is essential to control crystallization processes. Relating probability distributions form inductions times at constant supersaturation to the CNT is a powerful tool to reveal important kinetic and thermodynamic parameters that provide more insight into the nucleation process. However, it remains unclear how scale-up affects the nucleation mechanism and nucleation kinetics in solution crystallization processes.

In this chapter the effect of scale-up on the nucleation mechanism and nucleation kinetics in isothermal crystallization experiments is reported. A robust methodology was used to automatically measure induction times using a focused beam reflectance measurement (FBRM) probe. Induction times were acquired for paracetamol in 2-propanol for small volume magnetic stirred crystallization experiments as well as for three different overhead stirrer-type crystallizers. The resulting induction times were used to estimate the nucleation rates and the corresponding nucleation parameters for each crystallizer which revealed the most important factors that influence the nucleation kinetics when the crystallization process is scaled up. In addition, experiments were carried out to test whether nucleation proceeds through a single nucleus mechanism (SNM) or a multiple nuclei mechanism. The compound used to investigate this was chiral sodium chlorate. If nucleation proceeds through an SNM, the first crystal formed through primary nucleation would be of single chirality and subsequent secondary nucleation would result in chiral-pure product crystals. If nucleation proceeds through a multiple nuclei mechanism, both chiral forms nucleate through primary nucleation and both chiral forms would eventually be obtained as crystals. Overall, the results provide a better understanding on the effect of crystallizer type and volume on the nucleation mechanism and kinetics which contribute to an increased level of process control.
4.2 Theoretical description

This section describes how the previously reported theoretical framework (Jiang & Ter Horst, 2011) (Xiao et al., 2017) has been utilized in the current work to attain the nucleation rate and nucleation parameters. At a constant supersaturation $S$ and at a constant temperature $T$, the theoretical probability $P^*$ that at least one nucleus has formed in a total volume $V_t$ after an induction time $t_i$ can be expressed as

$$ P^*(t_i) = 1 - \exp(-J V_t t_i) \quad (4.1) $$

The total volume $V_t$ is expressed in $m^3$ and is defined as the combined volume of solvent and solute. The nucleation rate $J$ is the frequency of appearance of supernuclei per unit volume ($m^{-3}s^{-1}$) and the induction time $t_i$ is expressed in seconds.

Equation 4.1 relies on a stationary Poisson process (Bhamidi et al., 2017b) and assumes that nuclei are formed independently from each other until the detection point. This assumption holds if nucleation proceeds through a single nucleus mechanism (SNM) where a single nucleus is formed and grows or undergoes secondary nucleation at which point nucleation detection becomes possible. In the first part of this work experiments were carried out involving chiral crystals to get an indication on whether a SNM would hold in experiments.

It has been assumed that, when the solution temperature reached the steady set temperature for the induction time experiments, the supersaturation would remain the same. This point in time is defined as the start time $t=0$ of the experiment (fig. 4.1). After the start time $t=0$, monomers in the supersaturated solution reversibly agglomerate to form unstable prenucleation clusters. The time required for one of these clusters to become sufficiently stable is stochastic and is defined as the induction time $t_i$. The stable nucleus grows during a growth time $t_g$ until it becomes sufficiently large to undergo secondary nucleation. The time when the first single crystal undergoes secondary nucleation is the detection time $t_D$. For the magnetically stirred experiments, the detection time $t_D$ is the time when crystal formation was
optically observed through recorded images. An FBRM probe was used in the experiments involving overhead stirring (Mitchell et al., 2011). The detection time $t_D$ was identified as the start of an exponential increase in FBRM counts (fig. 4.1). The accuracy of detecting the onset of crystallization in cooling crystallization experiments using FBRM and using video imaging is comparable (see fig. S4.2) (Fujiwara et al., 2002). Secondary nucleation in stirred small volume solutions is assumed to proceed through a mechanical attrition process where a single crystal breaks into many fragments after coming into contact with the stirrer (Kadam et al., 2011). In larger volumes however, crystals are less likely to hit the impeller (Tyrrell et al., 2018) and small crystals could undergo secondary nucleation through a nuclei breeding mechanism instead, in which molecular aggregates nucleate after coming into contact with a crystal (Anwar et al., 2015) (Buhse et al., 2000). Experimental evidence for nuclei breeding was observed for the paracetamol model system used in the present study (De Souza et al., 2016).

![Figure 4-1](image.png)

**Figure 4-1.** The total FBRM counts (dotted line) as a function of time for a single induction time experiment. The detection time $t_D$ of the first crystal is determined to be at the time when the exponential increase in crystal counts starts, which is calculated using an exponential fit (solid line).
The induction time $t_i$ in eq (4.1) can be calculated from the detection time $t_D$ and the growth time $t_g$ as follows

$$ t_i = t_D - t_g \quad (4.2) $$

The method of determining the growth time $t_g$ does not significantly influence the determination of the nucleation rates (Xiao et al., 2017). In this work, the growth time $t_g$ as a parameter from fitting eq. 4.1 to the experimental data was estimated. The average induction time $t_{avg,i}$ is defined through:

$$ t_{avg,i} = \frac{\sum t_i}{N} \quad (4.3) $$

For $N$ isolated experiments, the induction time probability $P(t)$ of which crystals are detected at time $t$ is defined as:

$$ P(t_i) = \frac{N(t_i)}{N} \text{ for } t_i = t_s \ldots t_l \quad (4.4) $$

in which $N(t_i)$ is the number of experiments in which nucleation occurred after time $t_i$. Experiments involving low supersaturations $S$ are typically associated with long induction times which are unable to be captured when they exceed the total experimental time. Failing to capture such long induction times leads to a higher degree of uncertainty in estimating nucleation kinetics (Xiao et al., 2017). In this work, the experimental setup to start a new induction time experiment was programmed only when crystallization has started. This way, all induction times were captured.

According to a previous study (Xiao et al., 2017), the most accurate way to estimate the nucleation rate $J$ is by minimizing the sum of squared differences between model $P^*$ (eq. 4.1) and experiments $P$ (eq. 4.4) according to

$$ \phi_J = \sum_{t_i}^{t_f} (P^*(t_i) - P(t_i))^2 \quad (4.5) $$
In addition to the nucleation rate $J$, other parameters can be estimated by measuring the nucleation rate at different supersaturation ratios, where the supersaturation $S$ is defined as

$$
S = \frac{C}{C_H}
$$

(4.6)

in which $C$ is the total concentration in g per kg solvent and $C_H$ is the concentration in g per kg solvent that would be achieved from $t = 0$ when supersaturation $S$ remains the same.

According to the Classical Nucleation Theory (CNT), the dependence of the nucleation rate $J$ on the supersaturation ratio is:

$$
J = AS\exp\left(-\frac{B}{\ln^2 S}\right)
$$

(4.7)

A fit of the linearized form of eq. 4.7 through a least-squares approach yields parameter $A$ from the intercept and parameter $B$ from the slope. Parameter $A$ represents the kinetic factor and according to eq. 4.8 depends on the Zeldovich factor $z$, which accounts for the probability of clusters that are larger than the critical nucleus size to dissolve rather than grow (Brandel & Ter Horst, 2015) the attachment frequency $f^*$ of building units to the nucleus as well as the concentration $C_0$ of nucleation sites (Xiao et al., 2017) (De Souza et al., 2016).

$$
AS = zf^*C_0
$$

(4.8)

When the attachment frequency $f^*$ of building units to the nucleus depends on interfacial transfer (Davey et al., 2017), the following expression can be derived

$$
f^* = \lambda A^*D \frac{X_1}{d}
$$

(4.9)

where $\lambda$ is the sticking coefficient which is the fraction of molecules that collide and attach to the nucleus, $A^*$ is the surface area of the nucleus, $D$ is the diffusion coefficient, $X_1$ is the concentration of building units in solution and $d$ is the diameter of the building unit. Parameter $B$ in eq 4.7 represents the thermodynamic parameter $B$. 
and accounts for the energy barrier for nucleation expressed as the heterogeneous nucleation work $W^*$ through

$$\frac{W^*}{k_B T} = \frac{B}{\ln S}$$

(4.10)

4.3 Experimental section

4.3.1 Materials and Equipment

Paracetamol (98.0–102.0%), sodium chlorate (≥99.0%), and 2-propanol (anhydrous, 99.5%) were purchased from Sigma-Aldrich and used as received. Deionized water was used as a solvent in the experiments involving sodium chlorate. Small 20 mL scintillation flasks and three different Mettler-Toledo work stations (Easymax 102, Easymax 402 and Optimax 1001) were used to study the crystallization processes (fig. 4.2). A thermostatic stainless steel water bath (Grant GR150; 38 L; stability ± 0.005 K, and uniformity ± 0.02 K) with a serial magnetic stirrer plate placed on the base was used for the small 20 mL magnetically stirred experiments. For these experiments, crystallization was detected using a Logitech Quickcam USB camera (Liu & Rasmuson, 2013). In the larger overhead-stirred crystallizers, the temperature in the Mettler-Toledo workstations was controlled using solid-state thermostats. A Mettler-Toledo Focused Beam Reflectance Measurement (FBRM) G400 probe was used for the detection of the onset of crystallization in the experiments involving overhead stirring. Each experiment involving overhead stirring was controlled using iControl in conjunction with an FBRM probe, a temperature probe and an overhead stirrer. Downward pitched-blade stirrer heads were used and the stirrer speed was set to ensure sufficient mixing without creating a vortex. DynoChem software from Scale-up Systems was used to calculate the stirrer speed used in the overhead crystallizers whilst maintaining the same power input per unit mass. The volume $V$ of solvent, the stirring method, the diameter of the stirrer head and the stirrer speeds used in each crystallizer are shown in Table 4.1.
Table 4-1. Overview of volume $V$ of solvent, stirring type, diameter of the stirrer and the stirring speed used in each of the crystallizers.

<table>
<thead>
<tr>
<th>Crystallizer</th>
<th>$V$ [mL]</th>
<th>Stirring Type</th>
<th>Diameter [mm]</th>
<th>Stirring Speed [rpm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scintillation Flask</td>
<td>10</td>
<td>Magnetic</td>
<td>18</td>
<td>350</td>
</tr>
<tr>
<td>Easymax 102</td>
<td>85</td>
<td>Overhead</td>
<td>25</td>
<td>267</td>
</tr>
<tr>
<td>Easymax 402</td>
<td>340</td>
<td>Overhead</td>
<td>38</td>
<td>400</td>
</tr>
<tr>
<td>Optimax 1001</td>
<td>680</td>
<td>Overhead</td>
<td>45</td>
<td>346</td>
</tr>
</tbody>
</table>

Figure 4-2. Schematic overview of the crystallizers used in this study. In the crystallizers with overhead stirrers, a temperature probe and FBRM probe were used.

4.3.2 Single nucleus experiments

A solution of sodium chlorate in deionized water was prepared with concentration $C=1130$ g/kg which has a solubility temperature $T$ of about 31 °C (Mullin, 2001). The solution was prepared in one of the crystallizers in combination with the corresponding conditions outlined in Table 4.1. The solution was heated to $T=51$ °C where it remained at that temperature for 30 min to ensure complete dissolution of all crystals as indicated by FBRM. The solid-free solution was brought to a crystallization temperature at a rate of 3 K/min. After an induction time $t_i$ at which point crystallization occurred, a sample of the slurry was taken from the crystallizer.
and was immediately analysed using a polarized microscope to identify the chiral identity of the crystals. The polarizer was set to 0° and the analyser was set to 90°. By slightly turning the analyser clockwise as viewed by the observer, crystals that appeared darker were dextrorotatory (D) whereas crystals becoming lighter were laevorotatory (L) (Abrahams, et al., 1977).

### 4.3.3 Induction time experiments

A solution of paracetamol in 2-propanol was prepared in the crystallizer in combination with the corresponding conditions outlined in Table 4.1. Supersaturation ratios of 1.6, 1.8, 2.0 and 2.2 were used in the experiments involving overhead stirring. In the experiments involving magnetic stirring, a supersaturation ratio of 2.2 resulted in immediate nucleation upon cooling which prevented us to obtain induction time data at this supersaturation rate. Therefore, supersaturation ratios $S$ of 1.4, 1.6, 1.8, 2.0 were used in the vials with magnetic stirring. The supersaturation ratios were calculated using solubility data reported in literature (De Souza et al., 2017). The solution was heated to 10 °C above the theoretical solubility temperature where it stayed for 30 min to ensure complete dissolution of all crystals. For the experiments involving magnetic stirring, the vials were placed in a thermostated waterbath which was set at a temperature of $T=5 \, ^\circ C$. Once the solution in the vials reached the set temperature $T$, the experiment was started at $t = 0$. After crystallization, the vials were reheated to dissolve the crystals and the experimental procedure was repeated. For the experiments involving overhead stirring, the solid-free solution was brought to a temperature of $T=15 \, ^\circ C$ at a rate of 3 K/min, where it remained until crystallization occurred, as indicated by FBRM (Fig. 4.1). Mettler-Toledo iControl software was programmed to repeat this induction time experiment by triggering the next experiment at the point when the FBRM detected a minimum of 10 crystals.

Different crystallization temperatures were used in the magnetically stirred and overhead-stirred crystallizers. A difference in crystallization temperature did not lead
to significantly different induction times in our experiments (Supporting Information, Figure S 4.3).

4.4 Results

First, the results regarding the SNM experiments are described which support the use of the simplified CNT equations to describe nucleation kinetics in our isothermal induction time experiments. Next, the results from the isothermal induction time experiments involving paracetamol with different supersaturation ratios in different crystallizer volumes are described. In the final section, the scale-up effect on the estimated kinetic parameters is discussed.

4.4.1 Nucleation mechanism

One of the key assumptions for using the simplified probability eq. 4.1 to model experimental induction time data is that crystallization should proceed through a single nucleus mechanism (SNM). So far it remains unclear if the SNM applies to induction time experiments and whether it holds at larger crystallization volumes. To assess if the SNM is present in the crystallizers, isothermal induction time experiments were conducted involving sodium chlorate. Sodium chlorate is achiral in solution but is chiral in the solid state which means that a single crystal of sodium chlorate is either dextrorotatory (D), or its mirror image, laevorotatory (L). Despite their difference in optical rotation, all other physical-chemical properties are the same which means that primary nucleation would give either an L or D form with equal probability. If crystallization of sodium chlorate proceeds through the SNM, only one of the two chiral forms would crystallize through primary nucleation after which it undergoes secondary nucleation to give many crystals that have the same chiral form as the first crystal (Fig. 4.3). If the SNM holds, the final suspension should consist of crystals having the same chiral form. However, if multiple nuclei are formed, both
chiral forms will crystallize prior to secondary nucleation and the final suspension will as a result contain crystals of both chiral forms.

Figure 4-3. Schematic overview of nucleation mechanisms of a chiral compound in an isothermal induction time experiment starting from a solid-free supersaturated solution (left). The top shows the SNM in which one crystal is formed through primary nucleation which undergoes secondary nucleation to give a suspension of only one chiral form (black). The bottom shows when multiple nuclei are formed through primary nucleation and undergo secondary nucleation to give a suspension which consists of both chiral forms (black and white).

Crystallization experiments of sodium chlorate in water were conducted in the four different crystallizers tested in this work. Each isothermal induction time experiment resulted in the crystallization of many crystals that were all of the same chiral form (fig. 4.4a). This result was reproducible across all tested crystallizers. Theoretically, it would be possible for a system to evolve from a racemic to a chiral-pure system through Viedma ripening (Söğütoglu et al., 2015). However, previous attempts to induce Viedma ripening in overhead-stirred crystallizers failed due to a lack of crystal breakage (Steendam & Ter Horst, 2017) (Steendam & Ter Horst, 2018). The crystals in our experiments were taken from the suspension immediately after the onset of crystallization, and therefore, it is unlikely that Viedma ripening would have led to the observed chiral-pure crystals.
In the magnetically-stirred crystallization experiments crystal proliferation was likely the result of contact-induced secondary nucleation and crystal breakage (Söğütoğlu et al., 2015). In the experiments involving overhead stirring, crystal breakage is less pronounced (Tyrrell et al., 2018) and shear-effects are more likely to result in secondary nucleation through nuclei breeding (De Souza et al., 2016).

In successive crystallization runs the same solution was used in order to test whether all of the crystals and clusters dissolve into monomers or if some clusters survive the heating step which would then result in a history of solution effect (Steendam & Ter Horst, 2018). In the successive crystallization runs, the history of solution effect was not observed as pure L or D crystal populations were randomly obtained. A history of solution effect would otherwise result in the deterministic crystallization of only one of the two chiral forms.

Interestingly, a mixture of both chiral forms was observed only in experiments when crystallization occurred during cooling (Figure 4.4b). During cooling, the supersaturation continues to increase after the first crystal has been formed, facilitating enough driving force for subsequent primary nucleation events. In isothermal experiments on the other hand, the first nucleation event leads to a reduction in supersaturation and the chance that a subsequent primary nucleation event would occur is small.

These results show that the SNM controls the crystallization of sodium chlorate in isothermal induction time experiments in each of the crystallizers used in this study. Although this does not directly imply that the SNM controls crystallization of other compounds as well, it does provide more support to use eq. 4.1 to estimate nucleation kinetics using isothermal induction times in the crystallizers used in this study.
Figure 4.4. The solution temperature \( T \) (solid line) and total FBRM count (dashed line) as a function of time for an experiment with a solvent volume \( V \) of 680 mL in which crystallization occurred during constant temperature (a) and during cooling (b). The insets show micrographs of the resulting suspension viewed between crossed polarizers, in which the different chiral forms can be distinguished by dark and bright colours. The scale bar represents 1 mm.

### 4.4.2 Nucleation probability of paracetamol

The isothermal induction time method in combination with FBRM was used to acquire probability distributions for the crystallization of paracetamol from 2-propanol. Video images and an FBRM probe were utilized to determine the induction times in the small scale magnetically stirred crystallizer and in the overhead-stirred crystallizers, respectively. This time difference was insignificant as the induction times were several orders of magnitude longer. The induction times obtained in all experiments were stochastic in nature as there was no order between the induction time and the experiment number (fig. S4.2).

Different crystallization temperatures were used in the magnetically stirred and overhead-stirred crystallizers. A difference in crystallization temperature did not lead to significantly different induction times in our experiments (fig. S4.3).

The induction times \( t_i \) for all experiments as a function of volume \( V \) of solvent and supersaturation ratio \( S \) are plotted in Figure 4.5a. The probability distribution becomes wider with decreasing supersaturation ratio \( S \). In experiments involving the smallest
tested volume $V$ of 10 mL, the probability distribution is the widest in combination with supersaturation ratio $S=1.4$. This wide distribution is mainly the result of a few induction times $t_i$ that were significantly longer than the other induction times measured under those conditions. Long induction times were often not measured in previous work as those experimental protocols were programmed to start a new induction time experiment after a set time (Xiao et al., 2017). As a result, any induction time that takes longer than the set time will not be measured. On the other hand, in the experiments the experimental protocol was programmed to start a new induction time experiment only when crystals were detected. This way all induction times were captured. Experiments involving the largest volume $V$ of 680 mL resulted in a comparatively narrow probability distributions across all tested supersaturation ratios.

The induction time $t_i$ in the experiments is estimated to be the start time of the onset of crystallization (Figure 4.1). The onset of crystallization is due to the formation of a single crystal, according to the SNM. The detection of a single crystal is volume dependent and can be considered as $1/V$, in which $V$ is the volume of solution per single crystal. Based on these grounds, stochastic models predict that the average induction time $t_{\text{avg},i}$ (eq. 4.3) linearly decreases with increasing volume $V$ (Kubota, 2012). This linear relationship is indeed reflected in the experiments (Figure 4.5b). Furthermore these results show that the volume-dependent average induction time $t_{\text{avg},i}$ becomes more constant with increasing supersaturation ratios $S$. This is in agreement with the published deterministic models (Kubota, 2012) and shows that an increase in supersaturation ratio $S$ leads to the nucleation process to become more deterministic.
4.4.3 Nucleation rate and parameter estimation

The probability distributions of induction times for paracetamol in 2-propanol for each supersaturation ratio $S$ and volume $V$ are fitted to eq. 4.1 and are shown in Figure 4.6. Eq. 4.1 was used to estimate the nucleation rate $J$ whereas eq. 4.7 was used to determine the kinetic parameter $A$ and thermodynamic parameter $B$ for each supersaturation ratio $S$ and volume $V$.

The nucleation rate $J$, expressed as the number density per time, is plotted in Figure 4.7a for different supersaturation ratios $S$ and different volumes $V$. As expected, the nucleation rate $J$ increases with increasing supersaturation ratio $S$ in all experiments. The highest nucleation rates $J$ were acquired from experiments involving the smallest tested volume $V$ of 10 mL in which the nucleation rates were an order of magnitude faster than the nucleation rates obtained in larger volumes. This difference in nucleation rate can be attributed to the method of stirring, as the magnetically-stirred small scale experiments induced significantly higher kinetics (kinetic parameter $A$, Figure 4.6) as compared to the experiments involving overhead stirring. In experiments involving overhead stirring the nucleation rate differences between the different crystallizer volumes were less apparent. In the overhead stirred experiments,
the nucleation rate $J$ was the slowest in crystallizer volume $V$ of 340 mL whereas the highest nucleation rates were obtained in the smallest volume of 85 mL.

Figure 4-6. Probability distributions $P(t)$ of the induction times $t_i$ [s] measured at supersaturation ratios $S=1.4$ ($\times$), 1.6 ($\blacksquare$), 1.8 ($\blacktriangle$), 2.0 ($\bullet$) and 2.2 ($\blacklozenge$) in crystallization volumes $V$ of 10 mL (a), 85 mL (b), 340 mL (c) and 680 mL (d). Parameter $A$ is expressed in m$^3$s$^{-1}$. The solid lines are fits of eq 4.1 to the experimental data.
Discussion

From a theoretical viewpoint, the nucleation rate $J$ expressed as the number density per time, does not change when only the volume is changed (Kubota, 2015). However, in the experiments differences in nucleation rates $J$ were observed (Figure 4.7a). To understand what factors causes these differences, the nucleation rate expression should be viewed from its thermodynamic and kinetic parts.

The thermodynamic part $B$ of the nucleation rate, which can be expressed as the nucleation work $W^*/k_bT$ as per eq. 4.10, was found to be similar across the different crystallization volumes $V$. In all experiments, the nucleation work $W^*/k_bT$ decreased with increasing supersaturation ratio $S$ and decreased with volume $V$ in the order of $340 \text{ mL} > 85 \text{ mL} > 10 \text{ mL} > 680 \text{ mL}$. The highest nucleation work $W^*/k_bT$ was obtained in experiments involving volume $V$ of $340 \text{ mL}$, causing the nucleation rate $J$ under those conditions to become the slowest (Figure S4.4). However, no other apparent correlation between the thermodynamic parameter and the nucleation kinetics $J$ could be established. The significant differences between stirring method,
volume and nucleation rate \( J \) in magnetic and overhead stirred crystallization experiments are not reflected in the thermodynamic part of the nucleation process. Therefore, the thermodynamic parameter does not significantly affect the nucleation kinetics when the crystallization experiment is scaled-up, provided that the method of stirring remains the same. These results are in line with the results reported in literature in which the thermodynamic parameter remained unchanged in experiments with different shear- and stirring rates (Liu & Rasmuson, 2013).

The low values for the thermodynamic parameters estimated in this work are of the same order of magnitude as values reported for other crystallization systems (Brandel & Ter Horst, 2015) (Kulkarni et al., 2013) (Sullivan et al., 2014). However, these low thermodynamic barriers for nucleation still led to comparatively low nucleation rates in overhead-stirred experiments due to low values for kinetic parameter \( A \), which are significantly lower than the magnetically stirred experiments and lower than the values measured from 1.5 mL magnetically stirred crystallization vials used in the literature. Estimated values for the kinetic parameters are of the same order of magnitude as the kinetic parameters found in a Taylor–Couette cell, in which mixing proceeds mostly through shear forces rather than agitation (Liu & Rasmuson, 2013). A relative low level of agitation in overhead-stirred crystallizers in combination with low values for thermodynamic parameter \( B \) might explain why the nucleation rates are low.

The kinetic part \( A \) of the nucleation rate, expressed through eq. 4.8 as \( zfC^0 \) in Figure 4.7b, shows that the kinetic value depends on the crystallizer volume \( V \) and that these trends for the most part mirror the nucleation rate order in Figure 4.7a. The concentration \( C^0 \) of nucleation sites, which is a measure for heterogeneous particles, is often assumed to become higher with increasing volume \( V \) (Bhamidi et al., 2017b). Although the concentration \( C^0 \) of nucleation sites could increase with increasing volume \( V \), this effect is overruled by other kinetic factors as there is no correlation between the concentration of nucleation sites and the volume. Furthermore, it can be assumed that the number of clusters that decay rather than grow, which is expressed
as the Zeldovich factor $z$, does not change when the crystallizer type and volume is changed. Therefore, the only kinetic variable that is expected to change when the volume $V$ of the crystallization experiment is changed is the attachment frequency $f^*$ of building units to the nucleus. According to Eq. 4.8, the attachment frequency $f^*$ depends on constants that are related to the nucleus, which are expected to remain the same when the crystallizer volume $V$ is changed. Eq. 4.9 also depends on the diffusion coefficient $D$ which would change when the crystallizer $V$ is changed. High diffusion coefficients $D$ could originate from high shear values. The highest shear values are expected in the magnetic stirred crystallization experiments due to the comparatively high stirring speeds in combination with small volumes and this effect is reflected in the high values of $z f^* C_0$ in Figure 4.7b. For the overhead-stirred crystallizers, the analytical probes might act as baffles which increase the nucleation rate as a result of shear forces (Liu et al., 2015). The highest shear rates are expected to be present in the smallest crystallizer in which the analytical probes are closest to the impeller (fig. 4.2). This was observed experimentally as a small vortex which was present only in the smallest overhead crystallizer as a result of the proximity of the FBRM with respect to the impeller. For a crystallizer volume $V$ of 340 mL and larger, the distance between the analytical probes and stirrer becomes larger and the nucleation rate $J$ remains the same, which suggests that the diffusion coefficient $D$ might have reached its limit. The limit of the shear effect is expected to be compound and solvent specific as different crystallization systems exhibit different flow properties and shear characteristics.

Overall, these results show that the nucleation rate, expressed as the number density per time, does not depend on the crystallizer volume but on kinetic effects that most likely originate from shear values. One of the expected effects from high shear rates is a high degree of agglomeration of pre-nucleation clusters (Bhamidi et al., 2017b). However, cluster agglomeration of sodium chlorate would result in agglomeration of both chiral forms which would in turn result in crystals of both chiral forms (Kubota, 2012). This was not observed in the induction time experiments involving sodium
chlorate as only chiral-pure crystals were obtained. Therefore, shear-induced effects could instead result in a higher degree of molecular alignment or locally higher levels of supersaturation. A detailed understanding of fluid dynamics on a molecular level is needed to understand the complex kinetic nature of crystal nucleation, which is outside the scope of this study.

These results show that probability distributions from induction times can be used to estimate nucleation kinetics, even at crystallizer volumes up to 680 mL. The SNM controls crystallization in such isothermal induction time experiments and enables the use of the simplified CNT probability distribution. Magnetically stirred crystallization experiments result in significantly higher nucleation rates as compared to experiments involving overhead stirring due to differences in kinetic effects. Therefore, results obtained from magnetically stirred small scale experiments should be treated as an approximation for larger scale experiments, especially regarding kinetic effects. Acquiring data from large scale experiments in this study was feasible using an automated FBRM approach in which induction times were recorded in a simple and robust fashion. This approach is easily transferrable and requires only a single reactor. However, this method also requires a considerable amount of time and resources to record induction times, and therefore, fewer induction times could be recorded which led to a less accurate parameter estimation as compared to experiments involving many induction times (Xiao et al., 2017).

Overall, the presented data revealed that the small changes observed in nucleation rates in overhead-stirred experiments could be due to differences in shear effects. As a consequence, it is important to understand the details of hydrodynamics in order to achieve a high level of control over crystal nucleation kinetics.
4.6 Conclusions

Induction times were recorded for paracetamol using a robust and automated methodology involving an FBRM probe that can be applied to different crystallizers. The resulting probability distributions of nucleation showed that the highest nucleation rates were obtained in magnetically stirred crystallization experiments, whereas the different overhead stirred crystallization experiments involving larger volumes resulted in nucleation rates that were more similar to each other. The thermodynamic part of nucleation did not significantly influence the nucleation rate, whereas the kinetic part did when the crystallizer volume changed. In particular, the shear rate was rationalized to be the part of the kinetic parameter that changes most significantly when the type of crystallizer is changed. Despite these differences, experiments involving the chiral sodium chlorate showed that the single nucleus mechanism controls isothermal crystallizations across all tested crystallizers. These results provide a better understanding on how the nucleation mechanism and kinetics changes when the crystallization process is scaled-up.

After studying fundamental of crystallization of pure system (solubility and nucleation), the study will expand to the effect of impurity on the different aspect of crystallization. Structurally-related impurities considerably affect the crystallization of API’s. The first step in the development of the impurity removal strategies is to obtain fundamental data such as solubility, polymorphism, nucleation kinetics, crystal shape and crystal size distributions. Such data is essential to control and predict the yield, form, shape and purity of the product. Next chapter discuss about the thermodynamic properties of impurities of paracetamol and effect of them on the paracetamol solubility and its shape.
Figure S4-1. A plot of $\ln(J/S)$ versus $1/\ln(S)^2$ is depicted in for experiments with volumes $V$ of 10 mL (a), 85 mL (b), 340 mL (c) and 680 mL (d). The lines are exponential fits to the data.
Figure S4-2. Detection times $t_D$ measured for different experiment numbers $N$ at supersaturation ratio $S=2.0$ in a crystallization volume $V$ of 340 mL using a webcam (filled bars) and an FBRM (open bars).

Figure S4-3. Probability distributions $P(t)$ of the induction times $t_i$ [s] measured at supersaturation ratio $S=1.8$ in a crystallization volume $V$ of 340 mL at a fixed crystallization temperature of 15 °C (■) and 25 °C (●).
Figure S4-4. The nucleation work $W^* / k_B T$ plotted as a function of supersaturation ratio $S$ for experiments with volumes $V$ of 10 mL (♦), 85 mL (●), 340 mL (■) and 680 mL (▲). The lines are plots of eq. 2.19 from the main text.

Table S4.1. Nucleation Rates $J$ for different supersaturation ratios $s$ and volumes $V$ with 90% confidence limits.

<table>
<thead>
<tr>
<th>Supersaturation Ratio $S$</th>
<th>Volume $V$ [mL]</th>
<th>$J$ [$m^{-3} s^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>10</td>
<td>26.48 ± 1.34</td>
</tr>
<tr>
<td>1.6</td>
<td>10</td>
<td>27.04 ± 1.65</td>
</tr>
<tr>
<td>1.8</td>
<td>10</td>
<td>136.2 ± 3.60</td>
</tr>
<tr>
<td>2.0</td>
<td>10</td>
<td>232.7 ± 7.80</td>
</tr>
<tr>
<td>1.6</td>
<td>85</td>
<td>2.39 ± 0.34</td>
</tr>
<tr>
<td>1.8</td>
<td>85</td>
<td>2.27 ± 0.20</td>
</tr>
<tr>
<td>2.0</td>
<td>85</td>
<td>4.26 ± 0.76</td>
</tr>
<tr>
<td>2.2</td>
<td>85</td>
<td>6.83 ± 1.31</td>
</tr>
<tr>
<td>1.6</td>
<td>340</td>
<td>0.37 ± 0.01</td>
</tr>
<tr>
<td>1.8</td>
<td>340</td>
<td>0.63 ± 0.08</td>
</tr>
<tr>
<td>2.0</td>
<td>340</td>
<td>1.41 ± 0.12</td>
</tr>
<tr>
<td>2.2</td>
<td>340</td>
<td>3.18 ± 0.25</td>
</tr>
<tr>
<td>1.6</td>
<td>680</td>
<td>10.76 ± 0.21</td>
</tr>
<tr>
<td>1.8</td>
<td>680</td>
<td>1.01 ± 0.19</td>
</tr>
<tr>
<td>2.0</td>
<td>680</td>
<td>1.78 ± 0.27</td>
</tr>
<tr>
<td>2.2</td>
<td>680</td>
<td>0.60 ± 4.04</td>
</tr>
</tbody>
</table>
Chapter 5

Brief description of the paper

This work describes the characterisation of temperature-dependent solid-liquid properties of 4-nitrophenol and 4’-chloroacetanilide in four different alcohols and their effect as impurities on the crystallisation of paracetamol. The same approach as used in chapter three was applied to obtain and model the solubility data of the impurities of paracetamol. An increase in the difference in solubility between impurity and paracetamol is expected to lead to an increase in the efficiency of separation of the impurity from paracetamol through crystallisation. The crystal shape of paracetamol did not change as a result of the 4-nitrophenol impurity which suggests that the impurity did not selectively inhibit the crystal growth of a specific crystal face. On the other hand, the molecular similarity between 4’-chloroacetanilide and paracetamol resulted in face specific crystal growth inhibition giving rise to needle-shaped crystals of paracetamol. The results represent fundamental information for the development of crystallization strategies for the removal of impurities.


URL: https://doi.org/10.1016/j.jct.2019.02.004

Leila Keshavarz’s contribution: Carried out literature reviews which identify the gap in the data associated with impurities of paracetamol. Performed the gravimetric and XPRD experiments. Conducted the modelling. Contributed to analyse the results. Contributed to preparation, design and writing of the manuscript.
Thermodynamic properties of Paracetamol Impurities 4-nitrophenol and 4′-chloroacetanilide and the impact of such Impurities on the Crystallisation of Paracetamol from solution

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ABSTRACT

The impact of structurally-related additives and impurities on active pharmaceutical ingredients is an essential yet poorly understood area. This work describes the characterisation of temperature-dependent solid-liquid properties of 4-nitrophenol and 4′chloroacetanilide in four different alcohols and their effect as impurities on the crystallisation of paracetamol. The solubility of 4-nitrophenol appeared to be significantly higher than paracetamol whereas the solubility of 4′chloroacetanilide was lower than paracetamol. The solubility difference between the impurities could be rationalised based on their molecular structure and hydrogen bonding interactions. The solubility data was modelled using empirical and thermodynamic models. Recrystallisation of paracetamol from solutions containing the highly soluble 4-nitrophenol impurity resulted in small uniformly sized high purity paracetamol crystals whereas the presence of the poorly soluble 4′chloroacetanilide impurity...
induced the formation of large needle shaped crystals of paracetamol. These differences in crystallisation are a consequence of the solubility difference and the different functional groups of paracetamol and its impurities. Overall this study serves as fundamental information for the development of crystallisation approaches for the purification of paracetamol from its main impurities.

5.1 Introduction

Reactive disubstituted aromatic compounds are often used in the manufacture of pharmaceuticals to enable the required synthetic steps that lead to the desired product. However, the reactive nature of the intermediates also leads to the formation of organic impurities that are structurally similar to the target compound. This is for example reflected in the synthesis of paracetamol which mainly yields paracetamol together with trace amounts of unwanted 1,4-disubstituted aromatic impurities. The three main impurities of paracetamol (PA) are 4-nitrophenol (NP), 4-aminophenol (AP) and 4’-chloroacetanilide (CA) (Figure 5.1) (Badea & Vla, 2012).

Figure 5-1. The chemical structures of paracetamol and its three main impurities.

PA is known as acetaminophen has three different polymorphic forms: form I, the stable form at room temperature, monoclinic Form I (Haisa et al., 1976); metastable orthorhombic Form II (Drebushchak & Boldyreva, 2004) and the highly unstable Form III (Perrin et al., 2009). AP is an intermediate in the manufacture of dyes and is used as a reducing agent in photography. The solubility of AP in aqueous solutions is reported and it was found that it readily oxidises upon exposure to air. AP is the starting material in the final step of the synthesis of PA and is obtained through
Chapter 5

reduction of NP. NP can crystallize as two polymorphic forms of which the β-form (β-NP) could undergo an irreversible transformation into the light unstable α-form (α-NP) at temperatures between 331 and 366 K (Coppens & Schmidt, 1965) (Wojcik & Mossakowska, 2006). CA is structurally the same as PA except that CA has a chlorine atom instead of an alcohol group at the 4-position. Only one crystalline form has been reported for CA (Naumov et al., 2007).

The levels of impurities in pharmaceutical products should strictly kept below a specified amount in order to guarantee the desired biological effect of the pharmaceutical and to avoid undesired side effects such as a change in polymorphism or chirality of the product (Pilaniya et al., 2010) (Llinàs & Goodman, 2008) (Steendam et al., 2013). An efficient purification technique to remove impurities is solvent crystallisation as the crystalline lattice of the target compound is typically able to selectively incorporate target molecules in preference over impurities (Schmidt & Jones, 2013). Important process parameters in solvent crystallization, including the metastable zone width and nucleation rates, rely on the solubility of the compound. Thermodynamic calculations based on solubility data of impurities and the effect of impurities on the solubility of the target compound is therefore essential for the design and use of solvent crystallization. The presence of ionic impurities generally leads to an increase in the solubility of inorganic salts (Sangwal, 2007) (Peng et al., 2014). However, the effects of organic impurities on the solubility of organic pharmaceuticals is more complex and less understood. PA is one of the most widely used model systems in crystallization research yet the impurity effect on the solubility of PA remains unreported. Solubility measurements for PA impurities is challenging as these compounds can form different polymorphs and are prone to undergo oxidation and decomposition. The solubility of NP in water (Jaoui et al., 2002) (Achard et al., 1996) and ethanol (Carrick, 2005) is reported but is unclear which polymorph was used in those studies. Except for the aqueous solubility of AP and PA, no solubility data is currently reported for AP, NP or CA to the best of our
knowledge. Moreover, it is unclear if the polymorphism of the impurities is affected by solution crystallization and whether impurities influence the crystallization of PA.

This chapter describes the solubility of the main impurities of PA and their effect on the solubility of PA. First, a systematic solid-state investigation was carried out to determine the polymorphic nature of the solids used in this study. In addition, solution phase analysis was conducted to determine the stability of the impurities in solution. Secondly, equilibrium solubility measurements are reported for α-NP and CA in four different alcohols (ethanol, 2-propanol, 1-pentanol and 1-butanol) over the temperature range 278.15 to 318.15 K. In previous solubility measurements of pure PA the same solvents were used and were therefore chosen in the present study for comparison (De Souza et al., 2017). The solubility data was analysed using empirical and thermodynamic models. Finally, the effect of the impurities on the crystal morphology and purity of PA from solution crystallisation experiments are described.

The results reported herein represent fundamental information for the design of solvent crystallization strategies that can be used for the purification of PA from its main impurities.

5.2 Thermodynamic modelling

The solubility of the impurities NP and CA and the solubility of PA in the presence of NP and CA in the selected solvents as a function of temperature are correlated with the empirical modified Apelblat equation, which is defined as

\[
\ln(x_2) = a_A + \frac{b_A}{T} + c_A \ln(T)
\]

(5.1)

where \(x_2\) is the solute molar fraction at temperature \(T\) in Kelvin and \(a_A\), \(b_A\) and \(c_A\) are the three adjustable parameters which can be obtained through regression (Prausnitz et al. 1986).
The Margules, Van Laar, Wilson and NRTL models are used to describe the solubility of the pure compounds NP and CA. These activity coefficient models account for deviations from ideal solution behaviour and use a solid-liquid equilibrium equation

\[
\ln(x_2\gamma_2) = \frac{\Delta H_{\text{fus}}}{R} \left(\frac{1}{T_t} - \frac{1}{T}\right) - \frac{\Delta C_P}{R} \ln\left(\frac{T_t}{T}\right) - \frac{\Delta C_P}{R} \left(\frac{1}{T_t} - \frac{1}{T}\right) - \frac{\Delta C_P}{R} \ln\left(\gamma_2\right) \tag{5.2}
\]

where \(\gamma_2\) is the activity coefficient, \(\Delta H_{\text{fus}}\) the fusion enthalpy of the solute, \(T_t\) the triple-point temperature, \(\Delta C_P\) the difference of the heat capacity of a solute between the liquid- and solid state and \(R\) is the universal gas constant (8.314 J·K\(^{-1}\)·mol\(^{-1}\)) (Prausnitz et al. 1986). Due to the small value of \(\Delta C_P\) with respect to the first term on the right side of equation, \(\Delta C_P\) can be neglected (Prausnitz et al. 1986). The triple-point temperature \(T_t\) is nearly equal to the melting temperature \(T_m\) of the compound and therefore equation 5.2 can be simplified to

\[
\ln(x_2) = \frac{\Delta H_{\text{fus}}}{R} \left(\frac{1}{T_m} - \frac{1}{T}\right) - \ln(\gamma_2) \tag{5.3}
\]

The experimental activity coefficients \(\gamma_2\) can be calculated by inserting the experimental solubility values \(x_2\) measured at temperature \(T\), the enthalpy of fusion \(\Delta H_{\text{fus}}\) and the melting temperature \(T_m\) into equation 5.3. The ideal solubility can be calculated by setting \(\gamma_2 = 1\).

The experimental activity coefficients \(\gamma_2\) were correlated to the Margules, van Laar, Wilson and NRTL models. The Margules model considers the temperature dependence explicitly and is expressed as

\[
\ln(\gamma_2) = \frac{A}{R T} (1 - x_2)^2 \tag{5.4}
\]

where \(A\) is an adjustable parameter.

In the van Laar equation, the temperature dependence is implicit and the van Laar equation can be written as

\[
\ln(\gamma_2) = \frac{B_{vl}}{\left(1 + \frac{B_{vl} x_2}{A_{vl} x_1}\right)^2} \tag{5.5}
\]

where \(A_{vl}\) and \(B_{vl}\) are the adjustable van Laar parameters and \(x_i\) is the mole fraction of component \(i\).

The Wilson model can be expressed as
\[
\ln(y_2) = -x_2 \left( \frac{\Lambda_{12}}{x_1 + \Lambda_{12} x_2} - \frac{\Lambda_{21}}{x_2 + \Lambda_{21} x_1} \right) - \ln(x_2 + \Lambda_{21} x_1) \quad (5.6)
\]
in which
\[
\Lambda_{ij} = \frac{v_j}{v_i} \exp\left(-\frac{\lambda_{ij}}{RT}\right) \quad \text{with } i \neq j \text{ and } i, j = 1, 2 \quad (5.7)
\]
and where \(v_i\) is the molar volume of the solute and \(v_j\) that of the solvent. \(\Lambda_{ij}\) are the cross-interaction energy parameters (J·mol\(^{-1}\)) between the components \(i\) and \(j\).

The NRTL model considers that the local concentration around a molecule is different from the concentration in the bulk. The NRTL equation is expressed as
\[
\ln(y_2) = x_1^2 \left[ \left( \tau_{12} \frac{G_{12}}{x_2 + x_1 G_{12}} \right)^2 + \frac{\tau_{21} G_{21}}{(x_1 + x_2 G_{21})^2} \right] \quad (5.8)
\]
in which
\[
G_{ij} = \exp(-\alpha \tau_{ij}) \quad \text{with } i \neq j \text{ and } i, j = 1, 2 \quad (5.9)
\]
and
\[
\tau_{ij} = \frac{g_{ij}}{RT} \quad \text{with } i \neq j \text{ and } i, j = 1, 2 \quad (5.10)
\]
where \(g_{ij}\) (J·mol\(^{-1}\)) are the model parameters which relate to the cross-interaction energy and where \(\alpha\) is the parameters that account for the non-randomness of the solution.

The parameters of each activity coefficient model were estimated by using the nonlinear least-squares algorithm \textit{nlinfit} in Matlab to solve
\[
\min_{\theta \in \mathbb{R}} \sum_{i=1}^{N} \left[ \ln(y_{2,i})_{\text{mod}}(T, \theta) - \ln(y_{2,i})_{\text{exp}}(T) \right]^2 \quad (5.11)
\]
where \(\theta\) represents the parameters to be estimated for each model, each compound and each solvent used, \(N\) refers to the number of data points and where \(y_{2,i}\)\(_{\text{mod}}\) and \(y_{2,i}\)\(_{\text{exp}}\) refer to the model and experimental activity coefficients respectively. The activity coefficients \(\gamma_2\) described in the results are the experimental activity coefficients.

Conversion from solute molar fraction \(x_2\) to mass-fraction solubility \(C\) was carried out through
\[
C = \frac{1000 x_2 M_w\text{Solute}}{((1-x_2) M_w\text{Solvent} + x_2 M_w\text{Solute})} \quad (5.12)
\]
5.3 Experimental section

5.3.1 Chemicals

The chemicals used in this study, together with their suppliers, mass fraction purity and method for purity determination are summarised in Table 5.1. All the chemicals were used as received without further purification. The methods employed by the supplier for the determination of the purity of PA include infrared absorption, ultraviolet absorption, thin-layer chromatography, melting point, titration and residue on ignition tests. Using the HPLC measurements, it was established that no detectable amounts of AP, NP or CA were present in the supplied PA.

Table 5-1.CAS registry number, supplier, mass fraction purity and method for purity determination of the chemicals used for measuring solubility of impurity of paracetamol

<table>
<thead>
<tr>
<th>chemical name</th>
<th>CAS-registry number</th>
<th>Supplier</th>
<th>mass fraction purity</th>
<th>Analysis method</th>
</tr>
</thead>
<tbody>
<tr>
<td>paracetamol (PA)</td>
<td>103-90-2</td>
<td>Sigma-Aldrich</td>
<td>0.98-1.02</td>
<td>several, see main text</td>
</tr>
<tr>
<td>4-aminophenol (AP)</td>
<td>123-30-8</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.99$</td>
<td>HPLC$^a$</td>
</tr>
<tr>
<td>4'-chloroacetanilide (CA)</td>
<td>539-03-7</td>
<td>Sigma-Aldrich</td>
<td>0.97</td>
<td>GC$^b$</td>
</tr>
<tr>
<td>4-nitrophenol (NP)</td>
<td>100-02-7</td>
<td>Alfa Aesar</td>
<td>0.99</td>
<td>GC</td>
</tr>
<tr>
<td>deionized water</td>
<td>7732-18-5</td>
<td>Distilled</td>
<td>-</td>
<td>none</td>
</tr>
<tr>
<td>Ethanol</td>
<td>64-17-5</td>
<td>Honeywell</td>
<td>$\geq 0.99$</td>
<td>GC</td>
</tr>
<tr>
<td>Methanol</td>
<td>67-56-1</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.99$</td>
<td>GC</td>
</tr>
<tr>
<td>2-propanol</td>
<td>67-63-0</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.99$</td>
<td>GC</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>71-41-0</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.99$</td>
<td>GC</td>
</tr>
<tr>
<td>1-butanol</td>
<td>71-36-3</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.99$</td>
<td>GC</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$ dibasic solution</td>
<td>7558-79-4</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.99$</td>
<td>Titration</td>
</tr>
<tr>
<td>phosphoric acid</td>
<td>7664-38-2</td>
<td>Sigma-Aldrich</td>
<td>$\geq 0.85$</td>
<td>Titration</td>
</tr>
</tbody>
</table>

$^a$High-Performance Liquid Chromatography. $^b$Gas Chromatography
5.3.2 Thermal analysis

The melting temperature $T_m$ and the enthalpy of fusion $\Delta H_f$ were determined using a PerkinElmer Pyris-Diamond differential scanning calorimetry (DSC) instrument. The instrument was pre-calibrated by using the onset temperature for indium. A precisely weighted DSC sample of CA was inserted into the DSC and heated from (298.15 K to 468.15 K) at a rate of 10 K/min under a nitrogen flow. Standard uncertainties of the experiments were evaluated to be 0.5 K for temperature and 400 J·mol$^{-1}$ for the enthalpy of melting. The onset temperature of melting, which was obtained by taking the inflection point of the DSC curve, was used as the melting temperature $T_m$ as per the recommendation from Gesellschaft für Thermische Analyse (GEFTA) and the International Confederation for Thermal Analysis and Calorimetry (ICTAC).

5.3.3 Solid state characterization

XRPD measurements were conducted to establish the polymorphic nature of the chemicals. Crystal samples were lightly ground into a fine powder and measured on a PANalytical EMPYREAN diffractometer using Bragg–Brentano geometry and an incident beam of Cu K-Alpha radiation ($\lambda = 1.5406 \text{ Å}$). Scans were performed at room temperature on a spinning silicon sample holder with a step size of 0.013° 2θ and a step time of 68 s.

To establish which polymorph of each compound was present in our solubility experiments, XRPD analysis was carried out of the solid phase samples taken from the equilibrium solubility measurements. After equilibration for 24 h at a fixed temperature, about 5 mL of the suspension was subjected to vacuum filtration. The residual crystals were completely dried at the same temperature and were measured using XRPD. The experimental XRPD patterns were compared with reference XRPD patterns which were obtained using Mercury by converting single crystal data taken from the Cambridge Structural Database (CSD).
5.3.4 HPLC analysis

The HPLC method for detecting the ratio of PA to its impurities reported in literature has been adapted to this work (Kamberi et al., 2004). An Agilent 1260 Infinity Quaternary LC was used in combination with a ZORBAX eclipse XDB-C18 column (4.6x150 mm, 3.5µ). A 0.01 M sodium phosphate buffer was prepared through the dilution of a 0.5 M phosphate buffer using deionised water (18.2 Ω) after which the buffer was brought to pH=3 using phosphoric acid. The 0.01M sodium phosphate buffer was used in combination with methanol as the mobile phase. The flow rate was set to 1.000 mL/min, the column temperature to 20 °C and the injection volume used was 5 µL. 2 mL Amber borosilicate glass vials were used for the HPLC samples. A stock solution was prepared for each compound which was subsequently diluted into a concentration range \( C_R \). Triplicate measurements were carried out for each sample and the resulting low relative standard deviation of <1% indicated that the HPLC method was sufficiently accurate. The peak heights measured over the entire concentration range fell well below the ultraviolet (UV) detection limit of the HPLC and the absorption peaks of the different compounds did not overlap. The peak area, which was averaged over three measurements of the same sample, was plotted against 10 different concentrations and resulted in a linear calibration curve \( R^2 > 0.999 \) for each compound, as shown in the Supporting Information (Figure S5.2).

The solute obtained from the gravimetric methodology was dissolved in methanol and the resulting solution was diluted until the concentration was within the concentration range \( C_R \) of the calibration series. The ratio of paracetamol to impurities and from that, the actual concentration of paracetamol (PA) in the solute, was calculated from the acquired peak areas in conjunction with the calibration curves. The concentration range \( C_R \) of the calibration series, composition of the mobile phase \( X \), the wavelength \( \lambda \) of detection and the retention times \( t_R \) for each compound are summarised in supporting information (Table S5.1).
5.3.5 Solubility determination

The solubility of CA and NP in different solvents (ethanol, 2-propanol, 1-butanol and 1-pentanol) at 8 different temperatures (278.15 to 318.15 K, with steps of 5 K) was determined using equilibrium gravimetric solubility measurements. For a single solubility data point, one of the tested solvents was added to a glass vial containing an excess of solids of either CA or NP. The gravimetric method has been used and validated by us in previous works and the same protocol was used in the current work (De Souza et al., 2017) (De Souza et al., 2018).

As per the NIST guidelines, the standard uncertainties \( u(C) \) and \( u(x_2) \) were determined by estimating the standard deviation of the three samples of each solubility data point. For each data point, the relative standard uncertainties \( u_r(C) \) and \( u_r(x_2) \) were calculated by dividing the standard uncertainties over the average solubility. The herein reported relative standard uncertainties for a specific compound were obtained by taking an average of the relative standard uncertainties of all data points for that specific compound.

5.4 Results & Discussion

In this work, a polymorphic screening is first described that shows which polymorph of each compound is used in this study. Next the solubility data of NP and CA as a function of temperature in different solvents is described which in the following section is modelled. In the final two sections the influence of NP and CA as impurities on the solubility and crystallisation of PA are described.

5.4.1 Polymorphic screening

XRPD studies were conducted to establish the polymorphic nature of PA, NP and CA used in this study. The XRPD patterns were compared with the known polymorphic
forms in literature (Supporting Information S5.3-S5.6) and the results are summarized in Table 5.2.

PA was obtained as Form I from the supplier and remained stable in solubility experiments. The crystalline form of CA obtained from the supplier was the same as the one reported in literature (Naumov et al., 2007). Although two forms are reported for NP, only α-NP was present in the starting material. It is reported in literature that a polymorphic transformation from α-NP to β-NP is not possible (Wojcik & Mossakowka, 2006). Indeed, in the experiments no such polymorphic transformation was observed across the full temperature range and solvents studied. During the experiments however observe a colour change of α-NP crystals from yellow to red which reportedly results in insignificant changes in crystal structure and molecular dynamics (Wojcik & Mossakowka, 2006). Figure S5.6 shows similar XRPD patterns of α-NP crystals before and after being exposed to visible light for 48 h.

Table 5-2. The polymorphic form of each compound determined from samples obtained from the supplier and experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Obtained From</th>
<th>Polymorphic Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA</td>
<td>Literature</td>
<td>I</td>
</tr>
<tr>
<td>PA (Starting Material)</td>
<td>Sigma Aldrich</td>
<td>I</td>
</tr>
<tr>
<td>PA + 10 mol% CA</td>
<td>Experiments</td>
<td>I</td>
</tr>
<tr>
<td>PA + 10 mol% NP</td>
<td>Experiments</td>
<td>I</td>
</tr>
<tr>
<td>CA</td>
<td>Literature</td>
<td>CA*</td>
</tr>
<tr>
<td>CA (Starting Material)</td>
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<td>CA</td>
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<td>CA</td>
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<tr>
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<td>Literature</td>
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<td>Experiments</td>
<td>α-NP</td>
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</table>

*No polymorphic forms of CA are reported in literature or were found in this study.

5.4.2 Solid-Liquid equilibria of CA and α-NP

The mass-fraction solubility $C$ of CA and α-NP as a function of temperature $T$ in all four tested solvents is tabulated in the Table S5.2 and S5.3 respectively and plotted in
Figure 5.2. The solubility of each compound was found to increase with increasing temperature in all four tested alcohols. Solution studies of both compounds involving HPLC measurements across the full temperature range and solvent range showed no signs of degradation of the compounds.

Figure 5-2. The mass solubility \( C \) of CA (a) and \( \alpha \)-NP (b) versus temperature \( T \) in ethanol (▲), 2-propanol (♦), 1-butanol (■) and 1-pentanol (●). The data is fitted using the NRTL model.

The experimentally determined mass-fraction solubility of NP in ethanol was compared with literature values (Figure 5.3). The data show that the mass-fraction solubility is similar within the temperature range (20-45 °C). The variations in mass-fraction solubility outside the temperature range (20-45) °C might be explained by the difference in methodology for solubility determination. In the literature report, solubility was measured by monitoring the disappearance of crystals whereas the approach in this work involved isothermal equilibrium measurements. In addition, the literature report does not mention which polymorph of NP was used and the variations in mass-fraction solubility might be the result of measurements on different polymorphs.
The mass fraction of both CA and α-NP across the full temperature range follows the order ethanol > 2-propanol > 1-butanol > 1-pentanol. The solubility decreases monotonically with increasing number $n$ of carbon atoms in the alkyl chain of the solvent (Figure 5.4). Solvents with longer alkyl chain lengths exhibit lower polarity and have more difficulty forming hydrogen bonds with the solute, overall leading to a lower solubility. The solubility of α-NP increases almost linearly with increasing number $n$ of carbon atoms in the alkyl chain of the solvent. The solubility of CA appears to increase exponentially with increasing number $n$ of carbon atoms in the alkyl chain of the solvent. Such a relationship was also found for PA (Granberg & Rasmuson, 1999) which is very similar to CA in terms of molecular structure. The relative high solubility of α-NP could be attributed to its nitro group which, like the alcohol group, forms hydrogen bonds with the solvent molecules. Moreover, the electron withdrawing nature of the nitro group is expected to enhance the hydrogen-donating capability of the alcohol group which could further enhance hydrogen bonding and with that the solubility of NP in alcohols.
Figure 5-4. The mass-fraction solubility (C) of ●, CA and ■, α-NP versus the number n of carbon atoms in the alkyl chain of the solvent at temperature \( T = 55 \, ^\circ\text{C} \).

### 5.4.3 Thermodynamic modelling of CA and α-NP

For the description of the solubility of CA and α-NP using the thermodynamic models, experimental activity coefficients \( \gamma_2 \) were needed which were calculated using the melting temperature \( T_m \) and the enthalpy of fusion \( \Delta H_{\text{fus}} \). The melting temperature \( T_m \) and the enthalpy of fusion \( \Delta H_{\text{fus}} \) of α-NP is 381 K and 12 kJ mol\(^{-1}\) respectively (Wojcik & Mossakowska, 2006). It has been found through DSC measurements that the melting temperature \( T_m \) of CA, determined as the onset of melting, is 448.80 K. This value corresponds to some of the reported literature values (Fisher et al., 1959) (Crounse, 1951), although most of the published melting temperatures \( T_m \) of CA fall within the temperature range of (451.15-452.15) K (Wang, 2008) (Southwick et al., 1956) (Eshghi et al., 1953) (Pearson et al., 1953). The reported melting temperature range might have been determined from peak temperatures, as in the analysis the peak temperature would result in 451.88 K. For the estimation of experimental activity coefficients \( \gamma_2 \) was used the onset of melting of 448.80 K.

The enthalpy of fusion \( \Delta H_{\text{fus}} \) of CA reported in literature corresponds to 40.75 kJmol\(^{-1}\) which was measured through the depression of the freezing point of the amine by
benzene, dibromobenzene, or benzil in a Beckmann apparatus. In the experiments, DSC to obtain an enthalpy of fusion $\Delta H_{\text{fus}}$ of CA of 27.14 kJmol$^{-1}$ was used, which is similar to the enthalpy of fusion of 27.6 kJ•mol$^{-1}$ of the structurally-related PA (Mota et al., 2009). The difference between the experimental value in this work and the literature value may be due to the difference in measurement technique.

Plots of the natural logarithm of the experimental activity coefficients $\gamma_2$ for CA and $\alpha$-NP as a function of temperature $T$ are shown in Figure 5.5a. From eq 5.2 it follows that the activity coefficient $\gamma_2$ is inversely proportional to the measured solubility. Therefore, the poorly soluble CA exhibits higher activity coefficients $\gamma_2$ as compared to the highly soluble $\alpha$-NP. The larger deviation of CA from ideal solubility ($\ln(\gamma_2) = 0$) could be explained by its more complex chemical structure as compared to NP. The activity coefficients $\gamma_2$ exhibit a trend in relation to solvent type. The order of activity coefficients $\gamma_2$ as a function of solvent can be explained by the chain length of the solvent, with short-chain alcohols resulting in stronger hydrogen bonding which result in larger deviations from ideal solubility. The activity coefficients $\gamma_2$ for pure PA range between 0.2 and 0.8 (De Souza et al., 2017b) and are therefore similar to the activity coefficients $\gamma_2$ of CA. This could be explained by the similarities in terms of molecular structure.
The calculated activity coefficients were used to model the experimental solubility data using four activity coefficient models. The estimated binary coefficients of each thermodynamic model and the Apelblat parameters are reported in Table S5.4 and S5.6. The quality of fit of thermodynamic models to the experimental data is expressed as the mean square error (MSE) which is tabulated in the Table S5.4 and S5.7 and the inverse of the MSE is plotted in Figure 5.5b. For each impurity, the quality of fit of the model to the experimental data increases in the order Margules < van-Laar < Wilson < NRTL < Apelblat. This order can be explained by the number of adjustable parameters used in each of the models where more adjustable parameters lead to a better fit. The number of adjustable parameters is the same for the van-Laar model and the Wilson model, yet the temperature dependency is implicit in the van-Laar model which results in a poorer fit as compared to the Wilson model in which the temperature dependence is explicit. For CA the quality of fit of the NRTL model increases with increasing solubility. A similar trend can be observed for α-NP in
combination with the Margules, van-Laar and Wilson models. For the other models, no apparent trend was observed.

5.4.4 Comparison of CA and α-NP of paracetamol

Figure 5.6 shows the difference in solubility between α-NP and PA (positive values) and CA and PA (negative values) as a function of temperature $T$ in each of the alcohols. An increase in the difference in solubility between impurity and PA (i.e. a larger deviation from 0 in Figure 5.6) could increase the efficiency of separation of the impurity from PA through crystallization.

The mass-fraction solubility of CA is lower than that of PA and the difference increases in the order 1-pentanol < 1-butanol < 2-propanol < ethanol. Furthermore, the solubility difference increases with increasing temperature in all solvents tested. The most efficient separation conditions to remove CA from PA are expected to involve high temperatures and short chain alcohols (i.e. ethanol). The relative low solubility of CA could be ascribed to its lack of having an alcohol group which in PA notably enhances the solubility due to its hydrogen bond donating capability.

The mass-fraction solubility $C$ of α-NP is significantly larger than the mass-fraction solubility of PA in all tested solvents. Such large differences in solubility suggest that separation of α-NP from PA should be efficient. The difference in mass-fraction solubility between NP and PA increases in the order 1-pentanol < 1-butanol < ethanol < 2-propanol. Therefore, the most suitable solvent to remove α-NP from PA would be 2-propanol.

The similarity between CA and PA in terms of molecular structure (Figure 5.1) is reflected in the similar solubility of each compound. On the other hand, the molecular structure of α-NP differs significantly from PA and hence its solubility is markedly different.
Figure 5-6. The difference between the concentration of α-NP and PA (positive values) and CA and PA (negative values) as a function of temperature $T$. Solubility data of PA was used from literature. From left to right, the bars represent the solubility difference in 1-pentanol (blue), 1-butanol (green), 2-propanol (red) and ethanol (orange).

5.4.5 Crystallisation of paracetamol in the presence of impurities

The effect of the impurities on the crystal shape of PA was analysed using scanning electron microscopy (SEM). In the absence of impurities, cooling crystallisation of PA at temperature $T=15 \, ^{\circ}\text{C}$ from 2-propanol resulted in an apparent wide size distribution of tabular crystals (Figure 5.7a). Cooling crystallisation of PA from 2-propanol in the presence of 0.10 mole fraction of α-NP resulted in an apparent narrow size distribution of small tabular crystals (Figure 5.7b).
The crystal shape of PA did not change as a result of the α-NP impurity which suggests that the impurity did not selectively inhibit the crystal growth of a specific crystal face. On the other hand, the molecular similarity between CA and PA resulted in face specific crystal growth inhibition giving rise to needle-shaped crystals of PA (Figure 5.7c). These changes in crystal habit were also observed in combination with 0.001 mole fractions of impurities. Despite these dramatic changes in crystal shape, the presence of the impurities did not change the polymorphic form of PA Form I across all the solvents and temperature tested in this work (Figure S5.3). Therefore, although the impurities inhibit the crystal growth rate, the intermolecular interactions between molecules in PA Form I are sufficiently strong to retain its polymorphic form.

In the presence of 0.10 mole fraction of α-NP, cooling crystallisation experiments of PA typically resulted in product crystals containing >0.99 mole fraction of PA. The significant difference in functional groups between α-NP and PA prevents incorporation of α-NP molecules into the growing PA crystal lattice. In addition, α-NP is much more soluble in alcohols than PA (Figure 5.6) and recrystallization of PA from α-NP therefore proceeds in a highly selective manner, giving highly pure PA products crystals. Therefore, purification of PA from α-NP proceeds efficiently as is reflected in recent study where mother liquor fractions were recycled in crystallisation experiments (Keshavarz et al., 2018).

On the other hand, crystallisation experiments of PA in the presence of 0.10 mole fraction of CA resulted in product crystals with lower purities in the order of 0.98 mole fraction of PA. Due to the structural similarity between CA and PA, CA is expected to easily incorporate into the crystal lattice of PA. In addition, CA has a similar solubility as compared to PA in alcohols and recrystallization of PA from CA was therefore found to be challenging.
5.5 Conclusions

The solubility of 4-nitrophenol (NP) and 4′-chloroacetanilide (CA) increases with increasing temperature and solvent order ethanol > 2-propanol > 1-butanol > 1-pentanol. The quality of fit of the tested thermodynamic models to the experimental data increases in the order Margules < van-Laar < Wilson < NRTL < Apelblat. The solubility of α-NP is significantly higher than the solubility of paracetamol (PA) whereas the solubility of CA is slightly lower than that of PA. Recrystallisation of PA in the presence of α-NP resulted in small and uniform pure PA crystals whereas recrystallisation of PA in the presence of CA resulted in large needle-shaped PA crystals contaminated with CA. These results can be rationalised by the molecular similarities and solubility differences between the impurities and the target compound. Overall this study provides fundamental information on the solid-liquid properties of structurally-related impurities of PA that are essential for the design of solution crystallisation strategies for the purification of PA from its main impurities.

As mentioned before, solvent crystallization is an efficient purification technique to remove impurities as the crystalline lattice of the target compound is typically able to selectively incorporate molecules of the target compound in preference over impurities (Schmidt & Jones, 2013). Important process parameters in solvent crystallization, including the metastable zone width and nucleation rates, and solubility of the compound are affected by the impurities.

However, the effect of organic impurities on the solubility and nucleation kinetics of organic compounds is far less established. Impurities also play a critical role in defining key physical product properties including flowability and compressibility which are important in subsequent formulation processes. Therefore, next chapter investigates the effect of impurities of paracetamol on the different aspects of crystallization and properties of the paracetamol crystal.
Supporting Information

DSC Data

Figure S5-1. DSC Thermogram of CA. The onset temperature of melting, obtained by taking the inflection point, was used as the melting temperature $T_{m}$.

HPLC Data

Table S5-1. HPLC conditions for the compounds studied in this work, including phosphate buffer / methanol composition $x$ of mobile phase, wavelength $\lambda$ of detection, retention time $t_r$, and concentration range $C_R$.

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Figure S5-2. HPLC calibration lines in which the concentration $C$ is plotted versus the peak area $A$ for a) PA, b) NP, and d) CA. The equation for the linear calibration line and the quality $R^2$ of its fit is shown in each graph.
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XRPD Data

Figure S5-3. XRPD Patterns of PA. a) After solubility measurements in the presence of 0.10 mole fraction of CA, b) after solubility measurements in the presence of 0.10 mole fraction of NP, c) raw material from Sigma Aldrich and d) Form I (CSD structure refcode HXACAN01).

Figure S5-4. XRPD Patterns of CA. a) Representative sample after solubility measurements, b) raw material from Sigma Aldrich and c) CSD structure refcode CLACTN.
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Figure S5-5. XRPD Patterns of NP. a) Representative sample after solubility measurements, b) raw material from Alfa Aesar, c) α-NP (CSD structure refcode NITPOL01) and d) β-NP (CSD structure refcode NITPOL).

Figure S5-6. XRPD Patterns of α-NP crystals before (bottom) and after (top) being exposed for 48 hours to visible light. The insets show photographs of the crystals.
### Solubility Data of Impurities

Table S5-2. Experimental mass-fraction solubility $C$, mole fraction solubility $x^{\text{exp}}$ and calculated mole fraction solubility data of CA in four different alcohols at saturation temperature $T$ and pressure $P = 0.1$ MPa.

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<th>$C$ [g/kg]</th>
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<th>$x^{\text{Marg}}$</th>
<th>$x^{\text{YLC}}$</th>
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Chapter 5

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<tr>
<td>288.15</td>
<td>30.405</td>
<td>561.22</td>
</tr>
<tr>
<td>293.15</td>
<td>34.112</td>
<td>582.47</td>
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<tr>
<td>298.15</td>
<td>38.711</td>
<td>609.68</td>
</tr>
<tr>
<td>303.15</td>
<td>43.265</td>
<td>628.75</td>
</tr>
<tr>
<td>308.15</td>
<td>50.696</td>
<td>650.18</td>
</tr>
<tr>
<td>313.15</td>
<td>58.409</td>
<td>660.48</td>
</tr>
<tr>
<td>318.15</td>
<td>66.130</td>
<td>679.05</td>
</tr>
<tr>
<td>323.15</td>
<td>74.642</td>
<td>699.55</td>
</tr>
<tr>
<td>328.15</td>
<td>83.528</td>
<td>705.52</td>
</tr>
</tbody>
</table>

*Standard uncertainty for temperature is \( u(T) = 0.2 \text{ K} \). Type A relative standard uncertainties for pressure and mass-fraction solubility and mole fraction solubility are \( u_r(p) = 0.05 \), \( u_r(C) = 0.0185 \) and \( u_r(x_2) = 0.0156 \), respectively.

Table S5-3. Experimental mass-fraction solubility \( C \) and mole fraction solubility \( x^{\text{exp}} \) and calculated mole fraction solubility data of \( \alpha \)-NP in four different alcohols at temperature \( T \) and pressure \( P = 0.1 \) MPa.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>1-Butanol</th>
<th>2-Propanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.15</td>
<td>23.267</td>
<td>532.26</td>
</tr>
<tr>
<td>283.15</td>
<td>26.636</td>
<td>552.14</td>
</tr>
<tr>
<td>288.15</td>
<td>30.405</td>
<td>561.22</td>
</tr>
<tr>
<td>293.15</td>
<td>34.112</td>
<td>582.47</td>
</tr>
<tr>
<td>298.15</td>
<td>38.711</td>
<td>609.68</td>
</tr>
<tr>
<td>303.15</td>
<td>43.265</td>
<td>628.75</td>
</tr>
<tr>
<td>308.15</td>
<td>50.696</td>
<td>650.18</td>
</tr>
<tr>
<td>313.15</td>
<td>58.409</td>
<td>660.48</td>
</tr>
<tr>
<td>318.15</td>
<td>66.130</td>
<td>679.05</td>
</tr>
<tr>
<td>323.15</td>
<td>74.642</td>
<td>699.55</td>
</tr>
<tr>
<td>328.15</td>
<td>83.528</td>
<td>705.52</td>
</tr>
</tbody>
</table>
1. Standard uncertainty for temperature is $u(T) = 0.2$ K. Type A relative standard uncertainties for pressure, mass-fraction solubility and mole solubility are $u(p)=0.05$, $u(C)=0.0051$ and $u(x_2)=0.0033$.

## Modelling Data

Table S5.4. Apelblat Parameters and Mean Square Error (MSE) for Each Solubility System.

<table>
<thead>
<tr>
<th>solute</th>
<th>solvent</th>
<th>$a_A$</th>
<th>$b_A$</th>
<th>$c_A$</th>
<th>MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>ethanol</td>
<td>-5.6796 × 10^1</td>
<td>2.5280 × 10^2</td>
<td>9.0833</td>
<td>2.0213 × 10^-5</td>
</tr>
<tr>
<td>CA</td>
<td>2-propanol</td>
<td>-1.5919 × 10^2</td>
<td>4.9285 × 10^3</td>
<td>2.4305 × 10^4</td>
<td>1.8292 × 10^-6</td>
</tr>
<tr>
<td>CA</td>
<td>1-butanol</td>
<td>-1.0570 × 10^2</td>
<td>2.5457 × 10^3</td>
<td>1.6351 × 10^4</td>
<td>4.9189 × 10^-6</td>
</tr>
<tr>
<td>CA</td>
<td>1-pentanol</td>
<td>-9.0249 × 10^1</td>
<td>1.8314 × 10^3</td>
<td>1.4081 × 10^4</td>
<td>2.8515 × 10^-5</td>
</tr>
<tr>
<td>α-NP</td>
<td>ethanol</td>
<td>-1.4472</td>
<td>-7.6476×10^2</td>
<td>5.3483×10^-1</td>
<td>4.9799×10^-5</td>
</tr>
<tr>
<td>α-NP</td>
<td>2-propanol</td>
<td>3.0708×10^1</td>
<td>-2.1149×10^2</td>
<td>-4.3046</td>
<td>1.2214×10^-4</td>
</tr>
<tr>
<td>α-NP</td>
<td>1-butanol</td>
<td>-3.8500×10^1</td>
<td>8.9547×10^2</td>
<td>6.0448</td>
<td>4.2390×10^-4</td>
</tr>
<tr>
<td>α-NP</td>
<td>1-pentanol</td>
<td>-3.7694×10^1</td>
<td>8.7787×10^2</td>
<td>5.9087</td>
<td>1.4614×10^-5</td>
</tr>
</tbody>
</table>
Table S5.5. Estimated Binary Coefficients for CA in Four Different Alcohols Using Margules, van Laar, Wilson and NRTL Models.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Margules</th>
<th>van Laar</th>
<th>Wilson</th>
<th>NRTL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$A \times 10^3$</td>
<td>$A_{VL} \times 10^7$</td>
<td>$B_{VL} \times 10^4$</td>
<td>$\lambda_{12} \times 10^6$</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.400</td>
<td>6.568</td>
<td>5.682</td>
<td>1.438</td>
</tr>
<tr>
<td>2-propanol</td>
<td>1.325</td>
<td>5.012</td>
<td>5.191</td>
<td>4.470</td>
</tr>
<tr>
<td>1-butanol</td>
<td>0.8954</td>
<td>0.9757</td>
<td>3.533</td>
<td>0.8044</td>
</tr>
<tr>
<td>1-pentanol</td>
<td>0.5653</td>
<td>0.1204</td>
<td>2.259</td>
<td>0.6740</td>
</tr>
</tbody>
</table>

Table S5.6. Estimated Binary Coefficients for $\alpha$-NP in Four Different Alcohols Using Margules, van Laar, Wilson and NRTL Models.

| Solvent  | Margules | van Laar | Wilson | NRTL | | | | | | |
|----------|----------|----------|--------|------| | | | | | | |
|          | $A \times 10^2$ | $A_{VL} \times 10^2$ | $B_{VL}$ | $\lambda_{12} \times 10^{-5}$ | $\lambda_{21} \times 10^{-3}$ | $g_{12} \times 10^{-6}$ | $g_{21}$ | $\alpha \times 10^6$ |
| Ethanol  | -5.601   | 1.710    | -5.440$\times 10^{-2}$ | 7.312 | -4.280 | 1.426 | -1.417 | 0.1179 |
| 1-pentanol | 1.488    | -1.096   | 1.017$\times 10^{-2}$ | 3.098 | -2.261 | 1.927 | -1.914 | 9.105 |

Table S5.7. Mean Squared Errors (MSE) of the Fitted Thermodynamic Models for Each Solvent and Solute.

<table>
<thead>
<tr>
<th>solute</th>
<th>solvent</th>
<th>Margules</th>
<th>van Laar</th>
<th>Wilson</th>
<th>NRTL</th>
<th>MSE$<em>{max}$/MSE$</em>{min}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>ethanol</td>
<td>3.7806$\times 10^2$</td>
<td>2.5415$\times 10^2$</td>
<td>6.3107$\times 10^3$</td>
<td>1.0449$\times 10^4$</td>
<td>3.6182$\times 10^2$</td>
</tr>
<tr>
<td>CA</td>
<td>2-propanol</td>
<td>4.8002$\times 10^2$</td>
<td>4.3607$\times 10^2$</td>
<td>1.6110$\times 10^2$</td>
<td>2.1622$\times 10^4$</td>
<td>2.2200$\times 10^2$</td>
</tr>
<tr>
<td>CA</td>
<td>1-butanol</td>
<td>4.0655$\times 10^2$</td>
<td>3.4473$\times 10^2$</td>
<td>1.2943$\times 10^2$</td>
<td>1.7189$\times 10^4$</td>
<td>2.3652$\times 10^2$</td>
</tr>
<tr>
<td>CA</td>
<td>1-pentanol</td>
<td>3.5145$\times 10^2$</td>
<td>3.3224$\times 10^2$</td>
<td>1.2726$\times 10^2$</td>
<td>1.7624$\times 10^4$</td>
<td>1.9942$\times 10^2$</td>
</tr>
<tr>
<td>$\alpha$-NP</td>
<td>ethanol</td>
<td>7.1766$\times 10^3$</td>
<td>1.3303$\times 10^3$</td>
<td>2.9108$\times 10^4$</td>
<td>4.1233$\times 10^4$</td>
<td>2.4655$\times 10^1$</td>
</tr>
<tr>
<td>$\alpha$-NP</td>
<td>2-propanol</td>
<td>8.2794$\times 10^3$</td>
<td>2.5874$\times 10^3$</td>
<td>1.3751$\times 10^3$</td>
<td>9.7714$\times 10^4$</td>
<td>8.4731</td>
</tr>
<tr>
<td>$\alpha$-NP</td>
<td>1-butanol</td>
<td>1.1196$\times 10^2$</td>
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<td>1.6522$\times 10^3$</td>
<td>5.9021$\times 10^4$</td>
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</tr>
<tr>
<td>$\alpha$-NP</td>
<td>1-pentanol</td>
<td>1.3732$\times 10^2$</td>
<td>1.2356$\times 10^2$</td>
<td>2.7941$\times 10^3$</td>
<td>5.2836$\times 10^4$</td>
<td>2.5990$\times 10^1$</td>
</tr>
</tbody>
</table>
Chapter 6

Brief description of the paper

In the first part of this paper, the effect of impurities on the solubility and nucleation kinetics of paracetamol has been studied. In the second part of this paper, the incorporation of 4’chloroacetanilide into the solid phase of paracetamol was investigated. 4’chloroacetanilide is not a hazardous substance and as such may be used as an additive. The presence of 4’chloroacetanilide in the solid phase of paracetamol improved processability properties of paracetamol. Therefore to control the amount of 4’chloroacetanilide in product, an experimental design space was developed and utilized to show that solvent was the most effective process parameters for 4’chloroacetanilide incorporation. Process control over the incorporation of additives allows for advanced manufacturing of products with tailored specifications.


URL: https://doi.org/10.1021/acs.cgd.9b00490

Leila Keshavarz’s contribution: Examined the solubility of paracetamol in presence of impurities. Performed the DOE experiments. Contributed to design, preparation and editing of manuscript. Submitted the manuscript.
**Influence of Impurities on the Solubility, Nucleation, Crystallization and Compressibility of Paracetamol**

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**ABSTRACT**

The striking ability of impurities to significantly influence crystallization processes is a topic of paramount interest in the pharmaceutical industry. Despite being present in small quantities, impurities tend to considerably change a crystallization process as well as the final crystalline product. In the present work, the effect of two markedly different impurities 4-nitrophenol and 4′-chloroacetanilide on the solubility, nucleation, and crystallization of paracetamol is described. In the first part of this work, the fundamentals are outlined and show that, although each impurity led to a small increase in solubility of paracetamol, their effect as a nucleation inhibitor was much more pronounced. Induction time experiments were used in conjunction with the classical nucleation theory to show that the impurities did not affect the solid–liquid interfacial energy but instead significantly reduced the kinetic factor, overall resulting in reduced nucleation rates. Intriguingly, both impurities influenced the solubility and nucleation of paracetamol in a similar fashion despite their
significant differences in terms of molecular structure, solubility, and ability to incorporate into the crystal structure of paracetamol. In the second part of this work, the incorporation of 4′-chloroacetanilide into the solid phase of paracetamol was investigated. The presence of 4′-chloroacetanilide in the solid phase of paracetamol significantly increased the compressibility of paracetamol, resulting in improved processability properties of paracetamol. The compressibility efficiency of paracetamol could be controlled using the amount of incorporated 4′-chloroacetanilide. Therefore, an experimental design space was developed and utilized to select the most important process parameters for impurity incorporation. Intriguingly, the number of carbon atoms in the aliphatic chain of the alcohol solvent strongly correlated to the impurity incorporation efficiency. As a result, it was feasible to accurately control the compressibility and the amount of 4′-chloroacetanilide in the solid phase of paracetamol by simply choosing the required alcohol as the solvent for crystallization. Thus, the present work comprehensively shows how different impurities impact the key crystallization mechanisms and properties of a pharmaceutical product. Rational process control over the incorporation of impurities and additives allows for advanced manufacturing of products with tailored specifications.

6.1 Introduction

Solution crystallization processes are widely treated as binary systems consisting of a solute and a solvent. For real systems, additional components such as additives and impurities are typically present in minute amounts. Such additional components may significantly impact crystallization processes even when present in very small amounts (Sangwal, 2007) (Schmidt et al., 2013). An understanding of the mechanistic role of additives and impurities is therefore essential to design and control crystallization processes. Impurity control is particularly important in pharmaceutical
manufacturing, as such compounds can be toxic or may unfavourably affect the crystallization of the desired product (Sun et al., 2015) (Singh, 2018).

Impurities influence crystallization processes in several ways (Peng et al., 2014) (Song & Colfen, 2011). For instance, the solubility of the product can change as a result of a change in the solute-solvent interfacial energy due to the presence of additional components. Consequently, the solubility of the product may decrease due to the presence of common ions or increase as a result of complex formation or the presence of foreign ions (Sangwal, 2007). Impurities furthermore may influence the nucleation process by changing the solid-liquid interfacial energy (Pino-García & Rasmuson, 2014) and/or the kinetic factor or they may act as heterogeneous surfaces (Sangwal, 2007) (Anwar et al., 2011). For instance, amphiphilic, polymeric and surfactant additives were found to promote nucleation (Kim et al., 2013) (Bodnár et al., 2019) (Poornachary et al., 2016). In other studies it was found that additives and impurities did not significantly influence the interfacial energy but instead led to a reduction in the kinetics (Heffernan et al., 2018) (Pons Siepermann & Myerson, 2018). These findings were explained using the additional energy that is required to remove the impurity from the clustering process. Finally, additives can be used to influence the processability of pharmaceutical compounds by changing the intrinsic physical properties. For example, additives were found to increase the compressibility of paracetamol which enabled enhanced direct compression resulting in stronger tablets (Kaialy et al., 2014) (Garekani et al., 2000).

Paracetamol (PA, Figure 6.1) is widely used as an over-the-counter analgesic and antipyretic painkiller. Three polymorphic forms of PA have been reported, of which Form I is the most stable form at room temperature (Haisa et al., 1976) (Drebushchak & Boldyreva, 2004) (Perrin et al., 2009). To date, the effect of a range of structurally-related compounds has been studied as additives on the crystallization of PA (Hendriksen et al., 1998) (Prasad et al., 2001) (Saleemi et al., 2013) (Nguyen et al., 2017). However, it remains unclear how the main impurities 4-nitrophenol (NP) and 4’-chloroacetonilide (CA) affect the solubility, nucleation and crystallization of PA.
(Figure 6.1). The synthesis of paracetamol typically involves the reduction of NP into 4-aminophenol which in turn is used as the final precursor to paracetamol. NP can be obtained through the p-nitrochlorobenzene route, in which unreacted p-nitrochlorobenzene forms impurity CA parallel to the desired synthesis route (Board,, 2004). CA is not a hazardous substance and as such may be used as an additive. Understanding the mechanisms by which additional compounds influence crystallization is of great interest to the scientific community and would allow for more controlled and robust crystallization processes (Sun et al., 2015).

In this chapter the influence of impurities NP and CA on the crystallization of PA is reported. In the first part of this chapter, the effect of NP and CA on the solubility and crystal nucleation of PA is described. Equilibrium solubility experiments in combination with high pressure liquid chromatography (HPLC) analysis were used to determine the solubility increase of PA as a result of impurity NP and CA. The crystal nucleation mechanisms were elucidated by combining the data of more than 1000 isothermal induction time experiments with the classical nucleation theory (CNT). In the second part of this chapter, the ability of impurity CA to incorporate into the solid phase of PA was studied. Compressibility studies were performed for pure PA crystals as well as PA crystals doped with CA. The compressibility of PA significantly depended on the amount of CA in the crystal structure. Therefore, a statistical fractional factorial design approach was utilized to determine which factors most significantly controlled the impurity incorporation process (Sato et al., 2015). The type of solvent was one of the most important factors that affected the impurity incorporation and as such was investigated in more detail by testing alcohols with varying carbon chain lengths as solvents for crystallization.
Figure 6-1. Molecular structures of paracetamol (PA) and impurities 4- nitrophenol (NP) and 4'-chloroacetanilide (CA).

6.2 Experimental section

6.2.1 Materials

PA (98.0−102.0%) was purchased from Sigma Aldrich, whereas NP (99%) and CA (98+%) were obtained from Alfa Aesar. The solvents methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 1-pentanol, 1-hexanol, and 1-octanol were obtained from Sigma-Aldrich. HPLC-grade water was obtained using a PURELAB flex 3 Purification instrument. All chemicals and solvents were used as received. Seed crystals of PA were acquired through recrystallization of PA from methanol at a slow cooling rate of 0.1 °C/min at a stirring rate of 300 rpm in a Mettler Toledo Easymax 402 setup. The seed crystals were sieved, and a size range of 180–250 μm was used in the experiments.

The solids obtained at the end of the experiments were analysed using powder X-ray diffraction (PXRD) to ensure that no polymorphic transformations occurred. A PANalytical EMPYREAN diffractometer with Bragg–Brentano geometry, and an incident beam of Cu K-Alpha radiation (λ = 1.5406 Å) was used for the PXRD measurements. A spinning silicon sample holder was used, and the analysis was performed at room temperature with a step size of 0.013° 2θ and a step time of 68 s.
6.2.2 Solubility Determination

The solubility of PA in the presence of impurities was determined through a previously reported gravimetric method in combination with HPLC analysis (De Souza et al. 2017). For these experiments, 2-propanol was used as a representative solvent because of its low cost in combination with favourable environmental, health and safety properties (Capello et al., 2007). A solid mixture consisting of 2.0 g of PA and 5 mol% of either NP or CA was added to 2-propanol. The resulting suspension was heated until the solids were completely dissolved after which the solution was brought down to the solubility temperature for solubility determination. The suspension was stirred for a minimum of 24 h after which three samples from the filtered solid-free supernatant were removed, weighted and dried. The resulting solids were analysed using HPLC to determine the ratio of PA to NP or CA.

6.2.3 Induction Time Measurements

A solution of PA in 2-propanol was prepared with supersaturation ratios $S=1.4$, 1.6, 1.8 and 2.0. In order to minimize variations between the eight vials, a stock solution was prepared in 100 mL 2-propanol. 1 mol% of either CA or NP was added to the stock solution. The supersaturation ratios $S$ were calculated with solubility data reported in literature (Granberg, 1999). Crystallization was carried out at a temperature $T$ of 5 °C. For supersaturation ratios $S=1.4$, 1.6, 1.8 and 2.0 the stock solutions were heated for half an hour at 30 °C, 40 °C, 50 °C and 50 °C, respectively. The stock solution was filtered using Whatman filter paper (25 µm pore size) to remove particulates larger than 25 µm. 10 mL was taken from the stock solution and transferred using a 5 mL micropipette into 20 mL vials. The vials were heated for another half hour at their respective temperatures and were subsequently placed in a thermostated waterbath (Grant GR150; 38L; stability ±0.005 K; uniformity ±0.02 K) which was set at a temperature of 5 °C. The time required to reach the set temperature was recorded and used as the start time of the experiment. Each vial contained a 10
mL solution and a cross-shaped stirrer bar (ø=18 mm) and the vials were closed with a screw cap. The vials were placed on a submersible stirrer plate (2mag MIXdrive 1) and the stirrer speed was kept constant at 350 rpm. The experiments were recorded with a Logitech Quickcam USB camera. Stirring continued until the clear solution turned opaque, which indicated the onset of crystallization. The time difference between the onset of crystallization and the solution reaching the set temperature was taken as the crystallization time.

### 6.2.4 Crystallization experiments

The cooling crystallization experiments for the fractional factorial design were carried out in a Mettler Toledo Easymax 402 setup. Mixing was performed using an overhead stirrer with a downward pitched-blade stirrer (ø = 25 mm). The stirring rate was set at either 300 rpm or 400 rpm. PA (62.8 g) and CA (1.4 g, 2 mol%) were mixed with 2-propanol (235.8 g) and heated to 70 °C for an hour to ensure complete dissolution of the solids. Once dissolved, the temperature $T$ of the solution was decreased to 15 °C during a cooling time of either 550 min or 61 min to reach a supersaturation ratio $S=2.82$. For the experiments in ethanol, PA (98.9 g) and CA (2.3 g, 2 mol%) were mixed with ethanol (235.8 g). The same process parameters used in the 2-propanol experiments were applied in the ethanol experiments. For the experiments in ethanol the supersaturation ratio was $S=2.5$.

The cooling method was programmed to proceed either linearly or through temperature cycles (fig. 6.2). Both cooling methods were tested as a linear cooling approach is perhaps more commonly applied in industry whereas temperature cycles lead to changes in product properties and possibly higher product purities (Z. Wu, Yang, & Wu, 2016). Seed crystals (2 wt%) were added at a temperature of $T=50$ °C in the seeded experiments. After the cooling programme was finished, the suspension was mixed at a temperature $T=15$ °C for a minimum of 48h to ensure complete desupersaturation. The suspension was subsequently filtered and the solids were dried.
in an oven set at a temperature $T=50$ °C. A sample of the resulting dried solids was analysed using HPLC to determine the ratio of PA to CA.

Experiments to study the effect of different alcohols were performed in 20 mL vials equipped with cross-shaped stirrer bars ($\phi=18$ mm). The vials were closed with a screw cap and placed on a submersible stirrer plate (2mag MIXdrive 1) inside of a thermostated waterbath (Grant GR150; 38L; stability ±0.005 K; uniformity ±0.02 K). The applied stirring rate was 500 rpm. PA and CA (2 mol%) were combined with each alcohol and the suspensions were mixed at 10 °C above their saturation temperatures at 40 °C for 1 h to ensure complete dissolution of the solids. The temperature of the resulting clear solutions was reduced during a cooling time of 550 min to a crystallization temperature that would induce supersaturation ratio $S=1.7$ for all experiments.

Crystals for the compressibility studies were obtained from cooling crystallization in a Mettler Toledo Easymax 402 setup. PA (62.8 g) was combined with either 1 mol% or 2 mol% of CA in 2-propanol (235.8 g). A clear solution was obtained by stirring the suspension at 400 rpm at a temperature $T=70$ °C for 1 h. Once dissolved, the temperature of the solution was linearly reduced to 15 °C during a cooling time of 61
min. Stirring continued at a temperature $T=15 \, ^\circ\text{C}$ for a minimum of 48 h to ensure complete desupersaturation after which the solids were isolated through filtration, dried at temperature $T=50 \, ^\circ\text{C}$ and used for the compressibility study. A small sample was taken from the solids and subjected to HPLC analysis.

### 6.2.5 HPLC Analysis

The ratio of PA to either 4-Nitrophenol (NP) or 4-Chloroacetilide (CA) was determined using a high pressure liquid chromatography (HPLC) methodology based on previous studies (Keshavarz et al., 2018) (Steendam et al., 2019). An Agilent 1260 Infinity Quaternary LC instrument was used that was equipped with a ZORBAX eclipse XDB-C18 column (4.6 × 150 mm, 3.5 μ). The mobile phase consisted of a 0.01 M sodium phosphate buffer (pH=3) and methanol in a ratio of 0.8/0.2 (v/v) or 1/1 (v/v), for PA/CA or PA/NP, respectively. For the 0.8/0.2 (v/v) mobile phase, the retention times for PA and CA were 1.48 min and 2.10 min, respectively. Using the 1/1 (v/v) mobile phase, the retention times of 1.72 min and 4.58 min were assigned to PA and NP, respectively. The applied flowrate was 1 mL/min, the column temperature was set to 20 °C and the injection volume was 5 μL. PA and CA were measured at a wavelength of 254 nm whereas NP was measured at a wavelength of 310 nm. The solid phase samples were dissolved in methanol and added to 2 mL amber borosilicate glass vials. Calibrations lines of at least 10 concentration points were measured for PA, CA and NP and each concentration was measured in triplicate. The resulting low standard deviation of <1% for each sample reflects the accuracy of the analytical procedure. Using the linear calibration lines, which are reported in the literature (Steendam et al., 2019), it was feasibly to determine the ratio of PA to either NP or CA of unknown samples.

### 6.2.6 Compressibility studies

The compaction behaviour of the materials was studied using a powder flow rheometer (Freeman Technologies FT4) which was used to measure the
compressibility (Pishnamazi et al., 2019). A vented piston was used as a standard measurement method in order to compact powder by applying a normal stress. During the test, the range of normal stress was varied between 1 and 2-4-6-8-10-12-15 kPa.

6.3 Results and discussion

The first part of the results describes the fundamentals as to how impurities CA and NP influence the solubility and crystal nucleation of PA. In these experiments, 2-propanol was used as the solvent. Impurity CA incorporates into the solid phase of PA whereas NP remained in solution and did not affect the solid phase of PA (Steendam et al., 2019) (Ottoboni et al., 2018). In the second part of the results, the ability of CA to incorporate into the solid phase of PA was used to tailor the solid phase of PA and to improve its product properties. A statistical design approach was utilized to learn which process factors control the incorporation of impurity CA and with that the compressibility of PA.

6.3.1 Fundamentals

6.3.1.1 Solubility

Both PA and NP may form different polymorphs whereas only one form of CA has been reported to date. In a previous study it was established that Form I of PA and the α-form of NP were used and both forms remained stable across a temperature range of 5-55 °C in various alcohols (Steendam et al., 2019). The same conditions were used in the present study and XRPD analyses showed that the initial polymorphic form of the compounds was retained throughout the experiments. The solubility of CA was found to be slightly lower than the solubility of PA whereas NP has an approximate 10-fold higher solubility than PA (Steendam et al., 2019).

In the current work, the solubility $C^*$ of PA in the presence of either 5 mol % of NP or 5 mol % of CA was determined across a temperature range of 5–55 °C. Fig. 6.3 shows that the solubility of PA slightly increased due to the presence of CA or NP.
across the tested temperature range. Despite the significant differences between NP and CA in terms of solubility and molecular structure, each compound increased the solubility of PA in a similar way. A similar small solubility increase of PA was observed for additives metacetamol and acetanilide (Nguyen, 2017). Overall, the presence of either NP or CA did not significantly affect the solubility of PA.

![Graph showing solubility of PA in the presence of NP and CA](image)

Figure 6-3. Solubility $C^*$ of PA in the presence of 5 mol% NP (●) and 5 mol% CA (■) in 2-propanol as a function of temperature $T$. The line represents the solubility of pure PA in 2-propanol calculated using the Apelblat parameters reported in literature (De Souza et al., 2017).

### 6.3.1.2 Crystal nucleation

The influence of impurities NP and CA on the crystal nucleation of PA in 2-propanol was investigated using induction time measurements in combination with the classical nucleation theory (CNT) (Jiang & Ter Horst, 2011) (Steendam et al., 2018) (Xiao et al., 2017). Fig. 6.4 a-c shows probability distributions of induction times $t_i$ for pure PA and PA in the presence of either 1 mol% of NP or 1 mol% of CA for different supersaturation ratios $S$. The supersaturation ratio $S$ is defined as

$$S = \frac{c}{c^*}. \quad (6.1)$$
where $C$ is the total concentration and $C^\ast$ is the equilibrium solution concentration at the set temperature, both expressed in g per kg solvent. For each supersaturation ratio $S$, the dataset was fitted to the following equation

$$P(t_i) = 1 - \exp(-JVt_i)$$

(6.2)

where $P$ represents the probability that at least one nucleus has formed in volume $V$ and where $J$ represents the nucleation rate in m$^3$.s$^{-1}$. On average, the quality of fit of eq. 6.2 to the data was sufficiently high ($R^2 = 0.96$). The induction time $t_i$ of a nucleus could not be measured directly but could be estimated as follows:

$$t_i = t_c - t_g$$

(6.3)

where $t_c$ is the time of crystallization and $t_g$ the growth time of a nucleus. The growth time $t_g$ of a nucleus was taken as a parameter from fitting eq. 6.1 to the experimental data. The time required to reach the set temperature of 5 °C after placing the vials in the waterbath starting from a temperature 30 °C, 40 °C and 50 °C was 211 s, 230 s and 243 s, respectively. These cooling times were subtracted from the detection time to obtain the time of crystallization $t_c$.

The nucleation rate $J$ is plotted in fig. 6.5a as a function of supersaturation ratio $S$ for the pure and impure systems. Except for supersaturation ratio $S=1.6$, the nucleation rate $J$ of PA is significantly inhibited by both impurities CA and NP. Previous studies showed that the structurally-related impurities metacetamol and $p$-acetoxycacetanilide also inhibit the nucleation rate of PA (Prasad et al., 2001) (Saleemi et al., 2013). On average and across the tested supersaturation ratio $S$ range, the nucleation rate $J$ of PA was reduced by a factor of 1.9 or 2.6 due to the presence of 1 mol% impurity CA or NP, respectively. Therefore, NP inhibits the nucleation rate $J$ of PA more efficiently than CA. Both impurities inhibit the development of the PA clusters, which is reflected by the longer growth times $t_g$ of the impure nuclei with respect to pure PA (fig. 6.5b). With increasing supersaturation ratios $S$, the factor between the nucleation rate $J$ and growth time $t_g$ of the pure and impure systems increases.
Figure 6-4. Probability distributions $P(t)$ of induction times $t_i$ measured at supersaturation ratios $S = 1.4$ (■), 1.6 (●), 1.8 (▲) and 2.0 (▼) in 2-propanol for pure PA (a) and PA in the presence of 1 mol% CA (b) and 1 mol% NP (c). A plot of $\ln(J/S)$ versus $1/\ln(S)^2$ is depicted in (d) for the pure PA experiments (◄) as well as the experiments involving impurities CA (♦) and NP (►). From the linear fits, parameters $A$ and $B$ from eq. 2.20 were estimated and are shown in Figures a-c with 95% confidence limits.
Figure 6-5. Nucleation rate $J$ (a) and the growth time $t_g$ of a nucleus (b) as a function of supersaturation ratio $S$ for pure PA (■) and PA in the presence of 1 mol% CA (▲) and 1 mol% NP (●) in 2-propanol. The nucleation rate $J$ data points contain 95% confidence limits and the lines are plots of eq. 8.4.

The nucleation rate expression was inspected in more detail to understand the mechanism by which the impurities inhibit the development of the nuclei. According to the CNT, the nucleation rate $J$ depends on the supersaturation ratio $S$, kinetic parameter $A$ and thermodynamic parameter $B$, as shown in eq. 6.4

$$J = A S \exp \left( - \frac{B}{\ln 2 S} \right)$$

(6.4)

A fit of the linearized form of eq. 6.4 through a least-squares approach yield parameter $\ln (A)$ from the intercept and parameter $B$ from the slope (Figure 6.4d). The linear fits to the impure systems ($R^2 = 0.93$ for CA and 0.96 for NP) were better than the linear fit to the pure system ($R^2 = 0.74$).

The values for parameters $A$ and $B$ of each system are shown in the corresponding plots in fig. 6.4. The differences between the values of thermodynamic parameter $B$ of each system are virtually the same, showing that the presence of either CA or NP does not significantly change the interfacial energy of the crystal/solution interface of the system. In contrast, the presence of either CA or NP significantly lowered the kinetic parameter $A$ by a factor of 1.5 or 1.6, respectively. Kinetic parameter $A$ can be described through the CNT as
with $z$ representing the Zeldovich factor which accounts for clusters that decay rather than grow into stable crystals, $f^*$ accounting for the attachment frequency of monomers to the nucleus and $C_0$ accounting for the concentration of nucleation sites (Davey et al., 2013). Nucleation sites are typically considered as heterogeneous surfaces that promote heterogeneous nucleation.

As discussed in a previous study, the Zeldovich factor $z$ depends on the supersaturation ratio, Boltzmann constant, temperature, molecular volume in the crystal, and the interfacial energy, and these values are unlikely to change due to the presence of impurities (Bodnár et al., 2019). Instead, impurities may interact with nucleation interfaces, effectively lowering the concentration of nucleation sites $C_0$ for PA. The nature of the nucleation sites is unknown and therefore the exact mechanism by which heterogeneous nucleation occur remains elusive. One way of obtaining more insight into the mechanism by which nucleation sites induce nucleation is by controlling the nucleation sites using well-defined templates (Kulkarni et al., 2014).

The second parameter in eq 6.5 that may be influenced by the presence of impurities is the attachment frequency of building units to the nucleus $f^*$, which can be expressed through

$$f^* = \lambda A^* D \frac{X_1}{d}$$

where $\lambda$ is the sticking coefficient that accounts for building units near the cluster which are not incorporated into the nucleus, $A^*$ is the nucleus surface area, $D$ is the diffusion coefficient, $X_1$ is the concentration of building units, and $d$ is the diameter of the nucleus. The diffusion coefficient $D$ is perhaps the most important parameter to be affected by impurities as it depends on the activation energy $E$ through

$$D = D_0^{-E/RT}$$

in which $R$ is the gas constant, $D_0$ the diffusion constant and $T$ the absolute temperature (Davey et al., 2013). The activation energy $E$ accounts for the energy
barrier required for a molecule to undergo desolvation and/or to undergo a conformational change during incorporation of a growing nucleus.

It was previously found using HPLC that impurity NP does not incorporate into the crystal structure of PA (Keshavarz et al., 2018) (Steendam et al., 2019). The inability of NP to incorporate into the crystal structure of PA may be due to its strong hydrogen bond forming capability which would lead to strong hydrogen bonds with solvent 2-propanol, as evidenced by the high solubility of NP in alcohols. Therefore, impurity NP may affect the conformational change of PA molecules into the growing nucleus and/or influence the desolvation process of PA. A previous report explained the inhibiting effect of impurities on kinetic parameter \( A \) to a higher energy barrier that needed to be passed in order to remove the impurities from a growing nucleus (Poornachary et al., 2016). This extra energy requirement may be considered as the activation energy \( E \) in eq. 6.7 and may explain the inhibiting effect of NP on PA as well. Furthermore in related work, the inhibiting effect of a dilute hydrogen-bonding additive on the nucleation of a small molecule was explained using the two-step nucleation theory, in which the impurity inhibits the kinetic ordering of the transition of a high-density cluster into an ordered crystal structure (Siepermann & Myerson, 2018). The same principles may apply to the inhibiting effects of NP on PA as a decrease in kinetic parameter \( A \) was observed in the present work as well. Both the diffusion coefficient \( D \) in the CNT as well as the kinetic ordering parameter in the two-step nucleation theory may be influenced by impurities and therefore it remains unclear through which mechanism nucleation proceeds in the present work.

In the case of impurity CA, the mechanism by which the impurity inhibits nucleation of PA is strikingly different to NP and previous findings. This is because impurity CA incorporates and remains in the crystal structure of PA. The OH-group in PA is a key functional group in PA Form I as it acts as a hydrogen bond donor and acceptor, leading to hydrogen bonds with two different neighbouring molecules (Naumov et al., 1998). However, impurity CA lacks an OH-group and therefore restricts the possibilities of new PA building units to incorporate into the growing nucleus.
Therefore, more time is needed for PA building units to adopt the required limited configuration possibilities to incorporate into the nucleus, leading to a higher activation energy $E$ as a result of CA. On the other hand, the inhibiting effect may also be explained using the two-step nucleation theory as CA may affect the kinetic ordering of a high-density cluster into an ordered crystal structure, thereby lowering kinetic parameter $A$. As with impurity NP it remains elusive whether nucleation in the present work proceeds through the CNT or through a liquid-like intermediate in the two-step nucleation theory.

### 6.3.2 Tailoring product properties

#### 6.3.2.1 Compressibility

Compressibility is an important parameter for pharmaceutical formulations that can influence tabletability of powders and the properties of prepared tablets such as hardness and disintegration time (Pishnamazi et al., 2019). The density of powder is considered as a function of applied normal stress and compressibility is typically described as a function of density variability. Therefore, compressibility is the volume reduction of powders as a result of the applied normal stress.

PA Form I, which has been used throughout this study, is known for its poor flowability and capping tendency upon compression. One approach to increase the compressibility of pharmaceuticals is through the use of additives, as the presence of additives typically leads to less efficient crystal packing and more open crystal structures. Such an open structure would favour direct compression, resulting in stronger tablets. Polymer additives have been used in previous studies to enhance the compressibility of PA (Kaialy et al., 2014) (Garekani et al., 2000).

In the present work, the small molecule CA was used to increase the compressibility of PA. Figure 6.6 shows the compressibility as a function of normal stress for pure PA crystals as well as PA crystals doped with either 0.47 mol% or 0.73 mol% CA.
Interestingly, the presence of only a minute amount of CA induced a striking increase in compressibility with respect to pure PA. At an applied stress of 15 kPa, the compressibility of pure PA was only 7.26% whereas it significantly increased to 16.63% and 28.16% due to the presence of 0.47% and 0.73% CA, respectively. The incorporation of CA into the solid phase of PA also led to a significant change in crystal habit (Figure 6.6). Instead of tabular crystals, impurity CA induced the formation of needle-shaped crystals of PA. Thus, impurity CA can be used to significantly favour the compressibility of PA, even when present in a very small amount.

### 6.3.2.2 Parameter screening

As illustrated in the previous paragraph, the compressibility of PA significantly depends on the amount of CA that is present in the solid phase of PA. Accurately controlling the amount of CA that becomes incorporated into the solid phase of PA is therefore essential to tailor the desired product specifications of PA. The applied
Experimental conditions typically control the incorporation of impurities into the solid phase of a product (Ukrainczyk et al., 2016). However, it is unclear how the wide variety of experimental factors influence the incorporation of CA into the solid phase of PA. Therefore, a series of cooling crystallization experiments were performed to determine which factors most significantly control the impurity incorporation of CA into PA. The five factors that were investigated were the type of cooling method (linear or temperature cycles, fig. 6.2), cooling time (61 min or 550 min), stirring rate (300 rpm or 400 rpm), seeding (yes or no) and solvent type (2-propanol or ethanol). Instead of testing all the possible combinations of these factors, a fractional factorial approach in Minitab software was utilized to design a $2^{5-1}$ work space involving 16 experiments with resolution V.

The input and output of the 16 tested experiments are tabulated in Table 6.1 and a Pareto chart (calculated using Minitab 18) in fig. 6.7 shows the effects from the tested factors and their combinations on the percentage of PA in the solid phase. The direction on how the parameters affect the purity of PA is shown in the main effects plot in fig. 6.8.

Shown in the Pareto chart is Lenth’s pseudo standard error (PSE) at 0.4387, which separates the main effects from effects caused by random error. In the present work, the type of solvent, seeding and their combination have a significant effect on the purification of PA whereas the cooling method, cooling time and stirring rate are close to- or lower than Lenth’s PSE. Therefore, the cooling method, cooling time and stirring parameters were not investigated further in this study.
Table 6-1. Overview of the experiments designed using a fractional factorial approach.\(^a\)

<table>
<thead>
<tr>
<th>entry</th>
<th>cooling method</th>
<th>cooling rate [time min]</th>
<th>stirring rate [rpm]</th>
<th>seeding</th>
<th>solvent</th>
<th>PA [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>linear</td>
<td>61</td>
<td>300</td>
<td>no</td>
<td>ethanol</td>
<td>99.10</td>
</tr>
<tr>
<td>2</td>
<td>cycles</td>
<td>61</td>
<td>300</td>
<td>no</td>
<td>2-propanol</td>
<td>99.22</td>
</tr>
<tr>
<td>3</td>
<td>linear</td>
<td>550</td>
<td>300</td>
<td>no</td>
<td>2-propanol</td>
<td>99.43</td>
</tr>
<tr>
<td>4</td>
<td>cycles</td>
<td>550</td>
<td>300</td>
<td>no</td>
<td>ethanol</td>
<td>99.08</td>
</tr>
<tr>
<td>5</td>
<td>linear</td>
<td>61</td>
<td>400</td>
<td>no</td>
<td>2-propanol</td>
<td>99.27</td>
</tr>
<tr>
<td>6</td>
<td>cycles</td>
<td>61</td>
<td>400</td>
<td>no</td>
<td>2-propanol</td>
<td>99.37</td>
</tr>
<tr>
<td>7</td>
<td>linear</td>
<td>550</td>
<td>400</td>
<td>no</td>
<td>ethanol</td>
<td>99.37</td>
</tr>
<tr>
<td>8</td>
<td>cycles</td>
<td>550</td>
<td>400</td>
<td>no</td>
<td>2-propanol</td>
<td>99.37</td>
</tr>
<tr>
<td>9</td>
<td>linear</td>
<td>61</td>
<td>300</td>
<td>yes</td>
<td>2-propanol</td>
<td>99.31</td>
</tr>
<tr>
<td>10</td>
<td>cycles</td>
<td>61</td>
<td>300</td>
<td>yes</td>
<td>ethanol</td>
<td>96.45</td>
</tr>
<tr>
<td>11</td>
<td>linear</td>
<td>550</td>
<td>300</td>
<td>yes</td>
<td>ethanol</td>
<td>99.20</td>
</tr>
<tr>
<td>12</td>
<td>cycles</td>
<td>550</td>
<td>300</td>
<td>yes</td>
<td>2-propanol</td>
<td>99.31</td>
</tr>
<tr>
<td>13</td>
<td>linear</td>
<td>61</td>
<td>400</td>
<td>yes</td>
<td>ethanol</td>
<td>98.00</td>
</tr>
<tr>
<td>14</td>
<td>cycles</td>
<td>61</td>
<td>400</td>
<td>yes</td>
<td>2-propanol</td>
<td>98.84</td>
</tr>
<tr>
<td>15</td>
<td>linear</td>
<td>550</td>
<td>400</td>
<td>yes</td>
<td>2-propanol</td>
<td>99.58</td>
</tr>
<tr>
<td>16</td>
<td>cycles</td>
<td>550</td>
<td>400</td>
<td>Yes</td>
<td>ethanol</td>
<td>98.00</td>
</tr>
</tbody>
</table>

\(^a\) Experimental details are described in the experimental section.\(^b\) The output is the percentage PA in the solid phase.

The type of solvent was the statistically most significant effect at a confidence level higher than 80%, closely followed by seeding. Interestingly, the use of seed crystals led to an average product purity of 98.59% as compared to an average product purity of 99.28% in unseeded experiments. The addition of seed crystals leads to a large crystal surface area across the bulk solution and a rapid depletion of supersaturation which may favour the incorporation of the CA impurities into the solid phase of PA. Without seed crystals, crystallization proceeds locally through primary nucleation followed by a slow depletion of supersaturation, possibly favouring the crystal growth of PA over impurity CA.
Figure 6-7. Pareto chart of the effects from the tested factors and their combinations, where the response is the percentage PA in the solid phase. Lenth’s PSE is 0.4387 which separates the most likelihood effects from effects caused by random error. Effects that pass the vertical dashed line at 0.6475 are statistically significant at a confidence level of 80%.

Figure 6-8. Main effects plots showing the direction the parameters affect the mean purity of PA.

### 6.3.2.3 Solvent selection

On the basis of the analysis from the previous section, the purity of PA is mainly affected by solvent selection. Therefore, the effect of solvent type on the purity of PA was explored in more detail. Water as well as eight different alcohols that varied in the number $n$ of carbon atoms in the alkyl chain length was tested as solvents for recrystallization experiments. The dependence of the PA solid phase purity on the
number $n$ of carbon atoms in the alkyl chain length is plotted in fig. 6.9. Intriguingly, the incorporation of CA into the solid phase of PA strongly depends on the number $n$ of carbon atoms in the alkyl chain length of the solvent as the purity of PA becomes higher with increasing alcohol chain length. The crystal growth rate is possibly significantly reduced in alcohols with longer alkyl chain lengths. The reduced crystal growth rates may favour incorporation of PA over CA, resulting in higher purities of PA.

The data for 1-propanol and 2-propanol slightly deviates from the observed general trend. This may be due to experimental errors as each data point represents a single experiment. Nevertheless, a clear relationship between the purity of PA and the number $n$ of carbon atoms in the aliphatic chain of the alcohol solvent is apparent.

Thus, the amount of CA in the solid phase of PA can straightforwardly be controlled by solvent selection. The present work shows a correlation between the number $n$ of carbon atoms in the aliphatic chain of the alcohol solvent and purity of a pharmaceutical product for the first time to the best of my knowledge. An increase in
the number \( n \) of carbon atoms in the aliphatic chain of the alcohol solvent leads to an increase in PA purity. These results show that solvent selection can be used to accurately tailor the compressibility of PA as the amount of impurity in the solid phase of PA strongly impacts its compressibility.

### 6.4 Conclusions

Although significantly different in molecular structure and solubility, both impurity NP and CA slightly increased the solubility of PA and significantly reduced its nucleation rate in a similar fashion. The solid-liquid interfacial energy of PA remained unaffected by the impurities whereas the kinetic parameter was inhibited. The presence of CA in the solid phase of PA led to a striking increase in its compressibility resulting in favourable product specifications. An increase in the number of carbon atoms in the aliphatic chain of the alcohol solvent led to an increase in PA purity. Solvent selection could be used to tailor the product properties as shorter chain alcohols led to more efficient impurity incorporation and higher compressibility.

Maximizing chemical yield as well as process efficiency while minimizing losses of product to liquors is a major objective for economic and environmental aspects. When a product precipitates from a reaction mixture, or in crystallization, there are generally losses of product to the liquors. Usually, to increase the yield of the process, cooling crystallization experiments carried out with manual solids recycle (Li et al., 2016). Building up the impurity is one of the limitations of the recycle of mother liquor. It remained unclear how to seek the optimum process conditions to increase product yield while at the same time control the build-up of impurities.

With having acquired the fundamental data on the impurities, it is possible to develop a mother liquor recycle operation for the crystallization of paracetamol contaminated with impurity 4-nitrophenol (next chapter).
Supporting Information

Another additive that was studied in order to investigate the effect on the compressibility of paracetamol was phenacetin (Phen). Paracetamol was subjected for crystallization in the absence and presence of phenacetin as an additive. The cooling crystallization experiments were carried out in the Easymax 402 setup. Paracetamol (75.96 g) and phenacetin (2.2 g, 2.8 mol %) were mixed with 2-propanol (240 g) and heated to 70 °C for an hour to ensure complete dissolution of the solids. Once dissolved, the temperature of the solution was decreased to 5 °C with a cooling rate of 0.9 °C/min to reach a supersaturation ratio $S = 4$. Fig. S6.1 shows the compressibility as a function of normal stress for pure PA crystals as well as PA crystals with 2.8 mol % Phen. Interestingly, the presence of only small amount of phenacetin induced a significant increase in compressibility in compare to pure PA. At an applied stress of 15 kPa, the compressibility of pure PA was only 4.25%, whereas it significantly increased to 15.2% due to the presence of 2.8 mol % Phen. The incorporation of Phen into the solid phase of PA also led to a significant change in crystal habit (fig S6.1).

* The results in this section are intended for publication.
Figure S6.1. (Left) Compressibility as a function of normal stress and (right) SEM images of the starting materials of pure PA crystals (■) and PA crystals containing 2.8 mol % Phen(▲) which were used for the compressibility study. The size bar in images represents 500 μm.

**Tablets Preparation**

Direct compaction method is used to make tablets for each formulation. A single-punch tablet press (Gamlen Tableting GTD-1 D series) is carried out for tablets preparation. In order to make tablets, 100 mg of each blend is considered to compact in a 6 mm die. The tablet press was set at fixed load mode with the load of 400 kg, and the compaction rate was fixed at 180 mm/min.

**Tablet Hardness**

In order to study the effect of compressibility on tablet properties, tablet hardness is measured using a tablet hardness tester (Pharma Test PTB311E). Tablet hardness is an important test for tablet characterization, which determine the mechanical strength of the tablets and influence on tablet disintegration time and drug release rate.

The physical properties of materials in the tablet formulation are effect on tablet hardness, as it is shown in fig S6.2. The graph displays the tablet hardness of different formulations.
Generally, lower hardness equals to higher porosity. Therefore, the lower hardness and higher porosity of the PA+Phen tablet is due presumably to the structural differences between PA crystal with and without additive. Moreover, insignificant difference between hardness of both formulations doesn’t have unfavorable effect on the strength of PA tablet with Phen.
Chapter 7

Brief description of the paper

Fundamental thermodynamic data was used to develop and model solution crystallization processes with a mother liquor recycle operation. Impurity build-up is one of the limitations when the mother liquor is recycled in sequential or continuous crystallization processes. In this work, the removal of paracetamol impurity (NP) was achieved and a recycle operation was developed and modelled that allowed for reduced waste and increased yield with complete control of the impurity concentration. With the approach developed in this work, a rational design of mother recycle can be applied to a continuous crystallization process in order to estimate the optimum mother liquor recycle conditions that would lead to reduced product and solvent waste and improved process efficiency.


URL: https://doi.org/10.1021/acs.oprd.8b00308

Leila Keshavarz’s contribution: Contributed to design the experiments. Performed the crystallization experiments. Contributed to compare the experimental results with a mathematical model from literature. Contributed to design, preparation and editing of manuscript. Submitted the manuscript.
Impact of Mother Liquor Recycle on the Impurity Build-Up in Crystallization Processes

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ABSTRACT

Reusing the mother liquor fraction in crystallization processes significantly increases the product yield but also leads to an accumulation of impurities. In this work, it has been investigated that how a mother liquor recycle operation affects the crystallization of paracetamol as a result of the gradual buildup of the impurity 4-nitrophenol. The results show that the rate of impurity buildup decreases with increasing number of cycles until the amount of the impurity remains the same. The number of cycles required to reach the steady state increases when larger fractions of mother liquor are reused. A simple model was used to describe the impurity buildup and to estimate the largest possible mother liquor recycle fraction required to obtain the maximum achievable product yield while still maintaining the desired product specifications. The presented work shows how to optimize mother liquor recycle conditions that will lead to enhanced process efficiency by reducing product and solvent waste.
7.1 Introduction

The manufacture of active pharmaceutical ingredients (API) often involves a synthetic route that requires multiple reactions to obtain the desired product. Reaction products typically consist of the desired compound together with impurities resulting from unreacted starting material or side reactions (Elder et al., 2015). Impurities can exhibit undesirable biological effects and may also influence the properties of the desired product, even when impurities are present in minute quantities (Steendam et al., 2013) (Schmidt & Jones, 2013) (Ottoboni et al., 2018) (Sangwal, 2007). Therefore, the FDA and ICH impose strict rules to confine the presence of impurities in pharmaceutical products (Teasdale et al., 2017). Separation processes to reduce the level of impurities are consequently widely used in the chemical- and pharmaceutical industry.

Solution crystallization is a highly selective and scalable unit operation that is widely used to purify the desired product from its impurities (Moynihan & Horgan, 2017). After crystallization, the suspension is filtered resulting in pure product crystals as well as a mother liquor fraction. However, in addition to impurities, the mother liquor still contains the desired product (Wong et al., 2012). Moreover, the solvents that are part of the mother liquor could represent a source of environmental waste and may require disposal steps in some processes.

A reduction in mother liquor waste can be realized through a recycle operation, in which the mother liquor is used as part of the starting material for a new process. Through a recycle operation, high yields can be achieved while waste can be reduced at the same time. Improved yields and waste reduction have been realized, for example, in cooling single-stage continuous mixed-suspension, mixed-product removal (MSMPR) (Wong et al., 2012) (Vartak & Myerson, 2017) antisolvent cooling single-stage continuous MSMPR, (Tahara & Myerson, 2015) multistage continuous MSMPR (Alvarez et al., 2011), and plug-flow processes (Cogoni et al., 2015).
The key challenge behind implementing a mother liquor recycle operation is to account for the gradual build-up of impurities in the solution. According to a simple mathematical model, the amount of impurities should stop to increase after a sufficiently large number of cycles have been conducted (Smith, 1997). The number of cycles that are required to reach a steady state impurity concentration is expected to depend on the amount of mother liquor that is recycled. However, it remains unclear how the fraction of mother liquor recycle affects the build-up of impurities in experimental work as no such mother liquor recycle studies have been conducted to the best of our knowledge.

In the present work, we used an experimental and modelling approach to investigate the maximum fraction of mother liquor that could be recycled while still maintaining the desired crystal product specifications. The target API investigated was paracetamol that was contaminated with the impurity 4-nitrophenol. The results show that the amount of impurity stops building up after a sufficiently large number of cycles have been carried out and that reaching this steady state depends on the fraction of mother liquor that is recycled. The increasing amount of impurity did not affect the product quality, as indicated by the unchanging product purity, crystal size distribution (CSD), and particle shape. Overall the presented results demonstrate that a link between experiments and a model enables the approximation of optimized mother liquor recycle conditions that lead to a significant increase in product yield while still maintaining the desired product specifications.

7.2 Experimental section

7.2.1 Materials and Equipment

Paracetamol (98.0–102.0%) and methanol (HPLC grade, 99%) were purchased from Sigma-Aldrich, whereas 4-nitrophenol (99%) and 2-propanol (analytical grade, 99.97%) were acquired from Alfa Aesar and Fisher Scientific, respectively. All of the
chemicals were used as received. The crystallization experiments were carried out in a Mettler-Toledo Easymax 402 workstation in combination with a stainless steel temperature probe and an overhead stirrer with a downward pitched-blade stirrer (ø = 25 mm).

7.2.2 Experimental procedure

A sequence of batch-cooling crystallization experiments was carried out to investigate the mother liquor recycle effect on the build-up of impurities. The start-up experiment (n=0) involved the crystallization of initial material SM₀, resulting in paracetamol crystals P and mother liquor ML (fig. 7.1). The starting material in subsequent cycles (n ≥ 1) consisted of fraction x of the mother liquor from the previous experiment n-1 and a fraction 1-x of fresh starting material SM. The concentration of starting material SM was higher than the concentration of initial material SM₀ used in the start-up phase as it was necessary to compensate for the loss of product as a result of the removal of product P from the start-up experiment. The design of the experiment mimics a continuous crystallization experiment as a continuous approach is often initiated through a batch process followed by a fixed continuous feed of starting material and recycle stream (Tahara et al., 2015). The yield Y represents the amount of crystalline paracetamol P obtained from the process relative to the amount of paracetamol used as starting material. The total yield Yₜ is defined as the percentage of combined amount of paracetamol crystallized from each experiment relative to the combined amount of paracetamol and 4-nitrophenol used in each experiment.

7.2.3 Start-up experiment (n=0)

The first experiment of the recycle sequence n=0 involved the cooling crystallization of starting material SM₀, which consisted of 62.25 g of paracetamol, 2.97 g of 4-nitrophenol (4.55 wt%) and 235.8 g of 2-propanol. As a result, a concentration C of paracetamol was 264 g/kg solvent.
Figure 7-1. Schematic overview of the experimental process. In cycles \( n \geq 1 \), shown within brackets, a fixed concentration of starting material \( SM \) was used in combination with a fraction \( x \) of the mother liquor \( ML \) from the previous experiment.

The suspension was stirred at 400 rpm and the temperature of the suspension was increased to 70 °C which is 10 °C higher than the theoretical solubility, as calculated using the reported solubility data (De Souza et al., 2017). Stirring was continued at 70 °C for 1 hour to ensure complete dissolution after which the temperature was reduced to 15 °C at a rate of 0.9 °C/min during which crystallization occurred. The suspension was subsequently stirred for approximately 30 hours at a temperature of 15 °C to enable complete desupersaturation after which the solids were separated from the solution through vacuum filtration. During the desupersaturation period, the solution concentration was monitored by regularly taking samples for gravimetric analysis to ensure that a solid-liquid equilibrium had been reached.

### 7.2.4 Mother liquor recycle experiments \((n \geq 1)\)

After the start-up experiment, subsequent mother liquor recycle experiments \((n \geq 1)\) involved the use of starting material \( SM \) which consisted of a fraction \( x \) of the mother liquor \( ML \) from the previous experiment \((n-1)\). In order to induce crystallization at 15 °C, it was essential that the initial paracetamol concentration \( C \) in starting material
was 264 g/kg solvent. For the calculations, the total solution concentration \(C\) of paracetamol in the mother liquor was set to a fixed value of 120 g/kg solvent for all experiments. Furthermore, it was assumed that all of the impurity remained in the solution and that no solvent evaporation occurred. With these simplifications, it was possible to complete the mass balance and to calculate the second fraction of SM required to start the new experiment with \(C = 264\) g/kg.

### 7.2.5 Gravimetric Analysis

The total solution concentration of paracetamol and 4-nitrophenol was determined through gravimetric analysis. A sample from the suspension was filtered using a 0.2 μm PTFE membrane (φ=15 mm) syringe filter and the mother liquor was collected in a pre-weighted glass vial. The vial was closed immediately and the combined weight of the solvent, solute, vial and cap was recorded. Subsequently, the vial was placed in an oven set at a temperature of 50 °C for longer than 24 hours to evaporate the solvent. The remaining weight of the solids was recorded and used to calculate the solution concentration. The masses were weighed using an analytical balance (Mettler Toledo AX054, sensitivity ±0.1 mg).

### 7.2.6 XRPD Analysis

X-ray powder diffraction (XRPD) measurements were carried out to determine the polymorphism of the paracetamol and 4-nitrophenol crystals. Samples of the crystals taken after each experiment were gently ground, and the resulting powder was measured using a PANalytical EMPYREAN diffractometer with a Bragg–Brentano geometry in combination with an incident beam of Cu Ka radiation (\(\lambda = 1.5406\) Å). The sample was placed on a spinning silicon sample holder, and the scans were conducted at room temperature. A step time of 68 s and a 20 step size of 0.013° were applied. The obtained experimental XRPD patterns were compared with reference XRPD patterns calculated with the Mercury software using single-crystal data from the Cambridge Structural Database.
7.2.7 HPLC Analysis

An Agilent 1260 Infinity quaternary LC system was used in combination with a ZORBAX Eclipse XDB-C18 column (4.6 mm × 150 mm, 3.5 μm) to calculate the ratio of paracetamol to 4-nitrophenol in the solid and liquid samples. The mobile phase consisted of a 0.01 M sodium phosphate buffer of pH 3 and methanol in a 0.15/0.85 (v/v) ratio, respectively. The flow rate was set to 1.000 mL/min and the column temperature to 20 °C, and the injection volume ranged between 1 and 5 μL, depending on the concentration of the sample. Each solid sample was dissolved in methanol and measured in triplicate, and the resulting low relative standard deviation of different concentrations C to obtain a linear calibration curve (R² > 0.999) for each compound (Figure 7.2).

![HPLC calibration lines](image)

Figure 7-2 HPLC calibration lines in which the concentration C is plotted versus the peak area A for (a) paracetamol and (b) the impurity 4- nitrophenol. The measurements for paracetamol and 4-nitrophenol were carried out at wavelengths of 254 and 310 nm, respectively.

7.2.8 Crystal size and shape analysis

Scanning electron microscope (SEM) images were taken using a JEOL Carryscope. The crystalline samples on the sample holders were coated with a thin layer of gold before analysis. The crystal size distribution (CSD) of the samples were analysed using a Malvern Mastersizer 3000 in combination with a wet dispersion unit. A 1
gram sample of dried crystalline product was dispersed in cyclohexane and introduced into the flow cell of the Mastersizer unit. In order to ensure a sufficiently mixed suspension, a stirring speed of 2,500 rpm was applied. Sonication was not applied as no significant agglomeration was observed by SEM. A refractive index of $n=1.619$ and $n=1.426$ were used for paracetamol and cyclohexane respectively. An absorption of 0.1 and density $\rho = 1.33 \text{ g/cm}^3$ for paracetamol were used, as per the CAS datasheet. The laser alignment was adjusted and a stable background signal was recorded before addition of the sample. Three measurements of each sample were performed and the average values were used in this work.

7.3 Results and discussion

7.3.1 Paracetamol/4-Nitrophenol process parameters

The model system used in the present work involves the purification of paracetamol from its main impurity 4-nitrophenol through solution crystallization from 2-propanol. Paracetamol can crystallize in three different polymorphic forms, whereas 4-nitrophenol can crystallize in two different forms (Haisa et al. 1976) (Wojcik & Mossakowska, 2006). In this work, paracetamol form I was used in combination with the alpha form of 4-nitrophenol, based on the CSD reference codes HXACAN01 and NITPOL01 respectively. No polymorphic transformations were encountered throughout the experimental procedure. To induce crystallization, the temperature of the solution was brought down from 70 °C to a steady temperature of 15 °C. Fig. 7.3 shows that across the temperature range of 5–55 °C, the solubility of 4-nitrophenol is about 10 times higher than paracetamol, indicating that 4-nitrophenol remains in solution. In addition, gravimetric solubility measurements showed that the solubility of paracetamol did not significantly increase by the presence of up to 9.3 wt% of 4-nitrophenol at a temperature of 15 °C (Steendam et al., 2019).
The purity of the crystalline paracetamol product $P$ was >99.8% after crystallization, which could be maintained in combination with impurity levels up to 5 g (7.4 wt%) of 4-nitrophenol in solution. Therefore it appears that 4-nitrophenol did not incorporate into the crystal structure of paracetamol, which is in line with data reported in literature (Ottoboni et al., 2018). The CSD and particle shape of the paracetamol product crystals did not significantly depend on the amount of 4-nitrophenol in solution (fig. 7.4a). An increase in the amount of 4-nitrophenol in solution leads to smaller crystals but this shift stops when the amount of 4-nitrophenol becomes larger than 3.72 g or 5.64 wt% (fig. 7.4b).

The only process parameter that appears to be significantly affected by the amount of 4-nitrophenol was the time required to reach solid-liquid equilibrium. The total solution concentrations as a function of time for cooling crystallization experiments involving different amounts of 4-nitrophenol are shown in fig. 7.5 and illustrate that the time required to reach solid-liquid equilibrium is longer when the amount of 4-nitrophenol in solution becomes larger. The 4-nitrophenol impurity is slowing down the desupersaturation process by possibly inhibiting the crystal growth of paracetamol. For operation window in this work it was necessary to limit the crystallization time to 10 hours, as the crystallization experiments were conducted only overnight. From fig. 7.5 it can be derived that a solid-liquid equilibrium can be achieved within 10 hours for experiments involving 5 g of 4-nitrophenol or lower. Therefore, the maximum allowable amount of impurity in this crystallization process is set to be 5 g (7.4 wt%) of 4-nitrophenol. Therefore this as a criterion to define the threshold for the amount of impurity in experiments was used. In the following paragraph an approach to determine the recycle conditions which result in the maximum achievable yield in combination with the maximum impurity of 5g is described. In the following paragraph an approach to determine the recycle conditions that result in the maximum achievable yield in combination with the maximum impurity presence of 5 g.
Figure 7-3. The solubility $C$ of paracetamol (■) and 4-nitrophenol (●) as a function of temperature $T$ in 2-propanol. Solubility data was taken from literature.

Figure 7-4. a) CSD’s of product crystals $P$ obtained from experiments in the presence of 2.97 g (solid line), 3.46 g (dashed line) and 3.72 g (dotted lines) of 4-nitrophenol. b) SEM image of product crystals $P$ obtained from an experiment in the presence of 2.97 g of 4-nitrophenol. The scale bar represents 200 μm.
Figure 7-5. The solution concentration $C$ of paracetamol and 4-nitrophenol plotted as a function of time $t$ for crystallization experiments involving 1.48 g (■), 2.08 g (●), 2.97 g (▲), 4.17 g (▼) and 5.96 g (♦) of 4-nitrophenol. Time 0 is the time that the temperature of the suspension remained steady at 15 °C.

The time required to reach solid-liquid equilibrium is estimated by an arrow.

### 7.3.2 Impurity build up

A sequence of batch cooling crystallization experiments was carried out to determine the build-up of impurities in the mother liquor. Initial material $SM_0$ consisted of paracetamol and 2.97 g (4.55 wt%) 4-nitrophenol. Recrystallization of initial material $SM_0$ proceeds through cooling crystallization according to

$$SM_0 \rightarrow P + ML_{l,0}$$

(7.1)

where $P$ is the crystalline product of paracetamol and $ML_{l,0}$ is the amount of impurities in the mother liquor from the first experiment $n=0$. The mother liquor also contains the solvent and paracetamol but these terms are omitted for simplicity. The yield of product $P$ in the first step was 60% and the crystals were of 99.98% purity. After a wash step involving cold water, the crystal purity increased to 100% indicating that the impurity did not incorporate in the crystals but were present as a
result of solvent evaporation. The high crystal purity shows that virtually all of the 2.97 g of the 4-nitrophenol in the starting material retained in solution.

A fraction \(x\) of the impurities in the mother liquor from the first experiment was used in the second experiment \(n=1\) according to

\[
SM + xML_{l,0} \rightarrow P + xML_{l,0} + ML_{l,1}
\]  
(7.2)

which after crystallization and filtration resulted in a mother liquor fraction that consisted of impurities from experiment \(n=0\) (i.e. \(xML_{l,0}\)) and the impurities from experiment \(n=1\) (i.e. \(ML_{l,1}\)).

Because product \(P\) was removed from the experiment through crystallization, the resulting solution concentration of the mother liquor was too low for subsequent cycles. To compensate for the concentration difference, a fixed high concentration of starting material \(SM\) was used in subsequent \(n \geq 1\) cycles. The initial concentration of starting material \(SM\) in the experiments was increased by reducing the amount of fresh solvent. An alternative approach to increase the initial concentration is to concentrate the mother liquor recycle stream (Vartak & Myerson, 2017) (Alvarez et al., 2011). Reducing the amount of fresh solvent is more desirable than concentrating the mother liquor recycle stream as it allows one to recycle the solvent and to avoid additional concentrating steps.

After the second experiment, fraction \(x\) of the mother liquor from the preceding experiment \((n=1)\) was used in the third experiment \((n=2)\) according to

\[
SM + x^2ML_{l,0} + xML_{l,1} \rightarrow P + x^2ML_{l,0} + xML_{l,1} + ML_{l,2}
\]  
(7.3)

The amount of 4-nitrophenol impurities in each experiment are the same (i.e. \(ML_{l,0} = ML_{l,1} = ML_{l,2}\) etc.). Therefore, after \(n\) cycles, the total amount of impurities \(ML_{l}\) in the mother liquor can be expressed as

\[
ML_{l} = x^nML_{l,0} + x^{n-1}ML_{l,1} + \cdots + ML_{l,n} = ML_{l,0} \sum_{j=0}^{n} x^j
\]  
(7.4)
Where $ML_I$ represents the total amount of impurity in the mother liquor expressed in grams, where $ML_{I,0}$ is the amount of impurity in the mother liquor in experiment $n=0$, which is 2.97 g of 4-nitrophenol in the experiments.

Fig. 7.6 shows the amount of impurity 4-nitrophenol in the mother liquor as a function of the number $n$ of cycles in which a fraction $x=0.3$, 0.5 or 0.7 of mother liquor from the previous experiment was used as part of the starting material for the following experiment. Both the experimental data as well as a plot of eq. 7.4 are shown. The results in fig. 7.6 show that the amount of impurity in the mother liquor significantly increases in the first few cycles after which the impurity build-up slows down and eventually stops, reaching a steady state.

Fig. 7.6 furthermore shows that the experimental build-up of impurities in solution can be estimated using eq. 9.4. The experimental amount of impurity was calculated assuming that no solvent evaporation occurred during filtration. If solvent evaporation occurred, this would result in smaller estimated experimental values for the amount of impurity. In experiment $n=2$ for recycle fraction $x=0.3$ it was found, based on gravimetric analysis, that solvent evaporation had taken place. Therefore, the experimental amount of impurity after experiment $n=2$ was higher than the predicted values.

Fig. 7.7 shows the theoretical impurity build-up as a function of the number $n$ of cycles in combination with different recycle fractions $x$ of mother liquor. Experiments involving a recycle fraction of up to $x=0.7$ would lead to a steady state within approximately 10 cycles. On the other hand, more than 15 cycles would be required to reach a steady state for experiments involving a recycle fraction of $x=0.9$.

The impurity build-up changes when different fractions $x$ of mother liquor are reused. This can be mathematically expressed considering the normalized impurity profile $ML_{I,\text{norm}} = ML_I / ML_{I,n}$, in which eq. 7.4 can be rewritten as

$$ML_{I,\text{norm}} = \sum_{0}^{n} x^n = \frac{1 - x^{n+1}}{1 - x} \quad (7.5)$$
Figure 7-6. a) The amount of impurity $ML_i$ in the mother liquor versus the number $n$ of cycles in which a $x=0.3$ (●), $x=0.5$ (■) and $x=0.7$ (▲) fraction of mother liquor was used from the previous experiment $n-1$. Each data point represents a single crystallization experiment and the lines are plots of eq. 7.4.

Figure 7-7. Estimated amount of impurity in the mother liquor $ML_f$ vs the number $n$ of cycles in which a fraction $x=0.3$ (●), $x=0.5$ (■), $x=0.7$ (▲) or $x=0.9$ (♦) of mother liquor was used.
If an infinite number of mother liquor recycle experiments were to be carried out (i.e. when \( n \to \infty \)), the process enters a steady state and eq. 7.5 simplifies into

\[
ML_{I,\infty} = \frac{1}{1-x} \times ML_{I,0}
\]

(7.6)

where \( ML_{I,\infty} \) is the amount of impurity in the mother liquor in steady state and \( ML_{I,0} \) is the amount of impurity in initial material \( SM_0 \). Thus, by knowing the amount of impurity in the initial material it becomes possible to estimate the impurity build-up as a function of recycle fraction \( x \) and the number \( n \) of cycles. A plot of eq. 7.6 for the paracetamol / 4-nitrophenol system is shown in fig. 7.8 together with the experimental mass of impurity in the mother liquor after \( n \) cycles in which different fractions \( x \) of mother liquor were recycled. Fig. 7.8 shows that more cycles are required to reach a steady state when a larger mother liquor recycle fraction \( x \) is used. The \( y \)-intercept of the line in Figure 7.8 denotes the amount of impurity in the starting material.

As described before, a maximum recycle fraction through which a maximum yield could be achieved, while still limiting the amount of 4-nitrophenol to 5 g was sought. The impurity limit of 5 g was set to be the criterion in this work as impurity quantities above this value would lead to crystallization processes becoming longer than 10 hours, as the 4-nitrophenol was found to inhibit the time to reach solid-liquid equilibrium (fig. 7.5). Figure 7.9 shows that such threshold conditions involve a recycle fraction of \( x=0.55 \), which results in a steady state with 5 g of impurity in the solution and a yield of 75%. Without a mother liquor recycle operation, the product yield was around 60%.

Overall the presented work provides a strategy to seek optimum mother liquor recycle conditions that facilitate a maximum achievable yield while maintaining a controlled level of impurities. The presented model is expected to apply to other mother liquor recycle processes, provided that the impurities remain in solution. The aspects that could differ among crystallization systems are the process parameters described in
section 7.3.1 and the y intercepts of the figures in section 7.3, as these values are system-specific.

Figure 7-8. a) The amount of impurity in the mother liquor $ML_I$ versus the recycle fraction $x$ after $n=0$ (O), 1 (∆), 2 (▽), 3 (◊) and 4 (×) cycles. The line is a plot of eq. 7.6 and represents the amount of impurity in steady state $n \rightarrow \infty$.

Figure 7-9. The amount of impurity in the mother liquor $ML_I$ (open symbols) and total product yield $Y_t$ (filled symbols) plotted as a function of recycle fraction $x$ after $n=0$ (○), 1 (∆), 2 (▽), 3 (◊) and 4 (□) cycles. The dashed line depicts the maximum permitted amount of impurity in the mother liquor and the solid line represents the amount of impurity in steady state estimated through eq. 7.6 for $n \rightarrow \infty$. 
By increasing the recycle fraction of mother liquor, a significant reduction in solvent and solute waste can be realized. The presented experiments and model demonstrate that an easy approach can be applied to optimize crystallization processes involving mother liquor recycle steps, which enables increased efficiency in crystallization processes.

### 7.4 Conclusion

A simple model was used to estimate the impurity build-up as a result of a mother liquor recycle operation. The experimental proof of principle for this model was demonstrated for the paracetamol / 4-nitrophenol system. The results show that a larger fraction of mother liquor recycles requires more cycles to reach a steady state. The experimental results were modelled from which the steady state impurity level for different mother liquor recycle fractions was estimated. The approach described herein also enabled the estimation of the optimum recycle fraction needed to achieve the highest possible product yield from a crystallization process. The results are expected to be applicable to other crystallization systems and as such can be used as a guide to estimate the optimum mother liquor recycle conditions that would lead to reduced product and solvent waste and improved process efficiency.
Chapter 8  General Conclusion and Future Work

A fundamental understanding of crystallization is required to facilitate precise process product control. The pressurized synthetic solubility methodology was used to provide solubility data at higher temperatures beyond the boiling point of solvents. The solubility of paracetamol with impurities was used for understanding the effect of impurities on nucleation kinetics of paracetamol. With detailed solubility data and models on paracetamol, nucleation kinetics at different crystallizer volumes, different supersaturations and different impurity levels was determined.

Eventually, this fundamental data was utilized for the development of crystallization strategies for the removal of impurities and improve compressibility of paracetamol.

In the paper I, the gravimetric solubility data on a compound developed by University College Cork was obtained and modelled using empirical and thermodynamic models. The temperature dependent solubility data for a β-chloroacrylamide derivative (Z-1) in twelve solvents was acquired. These compounds are a class of highly versatile synthetic intermediates due to the proximal location of diverse functional groups enabling different reaction types. The solubility results allow for advanced process optimization of this valuable synthetic intermediate and illustrate how the solubility and solubility models cope with industrially-relevant complex products such as Z-1. The NRTL model was found to result in the lowest error for 8 of the 12 solvents tested.

Paper II introduced a novel method named synthetic solubility methodology which provided solubility data at higher temperatures beyond the boiling point of solvents. This allows for an increase in crystallization yield as higher temperatures can be reached during solution crystallization. The high-pressure synthetic solubility methodology has been used for the determination of the solubility of pure paracetamol in solvents beyond their boiling point. In the case of methanol, solubility data is
obtained up to 354.15 K (the atmospheric boiling point of the methanol is 337.65 K), and far in excess of the temperature range for which data exists in the literature, 268.15–303.15 K. The data obtained using the pressurized-synthetic method is validated against an extended gravimetric data set at temperatures up to the atmospheric boiling point for each solvent. Sensitivity studies were conducted to determine the influence of factors such as temperature gradient on the ultimate solubility determination. A temperature-based standard deviation of 0.1 K was established for paracetamol in 2-propanol at 303.15 K, comparing well with the temperature-based equivalent standard deviation of 0.2 K for the gravimetric approach. The experimental solubility data were correlated by the modified Apelblat, Margules, Van-Laar, Wilson, and nonrandom two-liquid (NRTL) models. The NRTL model was found to best fit the experimental data for most of solvents used in this paper.

In paper III, the nucleation mechanism was also investigated using the chiral sodium chlorate system. Small 20 mL flasks and three different Mettler-Toledo work stations (Easymax 102, Easymax 402 and Optimax 1001) were used to study the crystallization processes in this chapter. These experiments showed that the single nucleus mechanism is the underlying nucleation mechanism in all four tested crystallization setups when supersaturation remains the same. In this chapter induction times were recorded using either a webcam or a focused beam reflectance measurement (FBRM) probe and the data was used in combination with the classical nucleation theory to determine the nucleation kinetics. This approach was used to determine the scale-up effects of nucleation kinetics in solution crystallization processes. The probability distribution for nucleation widened with decreasing supersaturation and smaller crystallizer volumes. The thermodynamic part of the nucleation rate expression did not significantly change the nucleation rate, whereas the kinetic nucleation parameter was found to be the rate-determining process when the crystallization process was scaled-up. In particular, the shear rate was rationalized to be the part of the kinetic parameter that changes most significantly when the
crystallization process was scaled-up. The effect of shear rate on the nucleation kinetics decreases with increasing volume and plateaus when the volume becomes too large.

Paper IV focused on the solubility of impurities 4-nitrophenol and 4’-chloroacetanilide. The gravimetric solubility method has been conducted in this paper. The solubility of 4-nitrophenol was significantly higher than paracetamol whereas the solubility of 4’-chloroacetanilide was lower than paracetamol based on their molecular structure and hydrogen bonding interactions. Recrystallisation of paracetamol from solutions containing the highly soluble 4-nitrophenol impurity resulted in small uniformly sized high purity paracetamol crystals whereas the presence of the poorly soluble 4’-chloroacetanilide impurity induced the formation of large needle shaped crystals of paracetamol. These differences in crystallisation are a consequence of the solubility difference and the different functional groups of paracetamol and its impurities.

Paper V examined the impact of impurities 4-nitrophenol and 4’-chloroacetanilide on the polymorphism and solubility of paracetamol. Furthermore, the impact of the impurities on the nucleation kinetics of paracetamol were determined and modelled and the mechanism by which this proceeds has been studied. Both impurities influenced the solubility and nucleation of paracetamol in a similar fashion despite their significant differences in terms of molecular structure, solubility and ability to incorporate into the crystal structure of paracetamol. In the first part of this paper, the fundamentals are outlined and show that although each impurity led to a small increase in solubility of paracetamol, their effect as a nucleation inhibitor was much more pronounced. Induction time experiments were used in conjunction with the classical nucleation theory to show that the impurities did not affect the solid-liquid interfacial energy but instead significantly reduced the kinetic factor, overall resulting in reduced nucleation rates. In the second part of this paper, the incorporation of 4’-chloroacetanilide into the solid phase of paracetamol was investigated. The presence of 4’-chloroacetanilide in the solid phase of paracetamol significantly increased the
compressibility of paracetamol, resulting in improved processability properties of paracetamol. The compressibility efficiency of paracetamol could be controlled using the amount of incorporated 4'-chloroacetanilide. Therefore, an experimental design space was developed and utilized to select the most important process parameters for impurity incorporation. Solvent was recognized as the most important parameter. Intriguingly, the number of carbon atoms in the aliphatic chain of the alcohol solvent strongly correlated to the impurity incorporation efficiency. As a result, it was feasible to accurately control the compressibility and the amount of 4'-chloroacetanilide in the solid phase of paracetamol by simply choosing the required alcohol as the solvent for crystallization. Thus, the present work comprehensively shows how different impurities impact the key crystallization mechanisms and properties of a pharmaceutical product.

With the fundamental thermodynamic data, it was possible to develop and model solution crystallization processes with a mother liquor recycle operation. In paper VI, the removal of paracetamol impurities was achieved and a recycle operation was developed and modelled that allowed for reduced waste and increased yield with complete control of the impurity concentration. Mother liquor recycle experiments were carried out by employing sequential batch experiments in an Easymax crystallizer. The model system used in this paper was paracetamol contaminated with the impurity 4.55 wt % 4-nitrophenol and 2-propanol. The results show that a larger fraction of mother liquor recycles requires more cycles to reach a steady state. The approach described herein also enabled the estimation of the optimum recycle fraction needed to achieve the highest possible product yield from a crystallization process. A threshold recycle fraction (50%) was chosen through which a maximum yield could be achieved while still limiting the amount of 4-nitrophenol to 5 g (impurity quantities above this value would cause the crystallization process to become longer than 10 h). The results of the predicted model were in good agreement with the experimental values. Quantification of the amount of impurity with respect to paracetamol was determined through HPLC.
Future work

- The pressurized-synthetic approach applied for paracetamol and phenacetin in this work can be applied for other API’s, moreover this method can be readily extended to binary and ternary systems.

- The data of solubility of Z-1 forms a benchmark for further research involving solution crystallization of Z-1.

- The results from the solubility of paracetamol in presence of impurities can lead to improved impurity removal processes.

- For increasing the compressibility of paracetamol, crystallization of paracetamol was carried out in presence of two needle shaped additives. Therefore, the potential of using other additives during the crystallization of paracetamol should be investigated.

- In this study, the compressibility and tablet hardness of paracetamol crystallized in the presence of additives was investigated. Therefore, it would be useful to measure the effect of additives on other tableting properties such as disintegration and dissolution time.

- With the approach for recycling the mother liquor developed in this project, a rational design of mother recycle can be applied to a continuous crystallization process in order to control the product specifications while at the same time reducing product waste.
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Appendix 1A

To Whom It May Concern,

We the undersigned are writing this letter to stipulate the role of Leila Keshavarz in the preparation and submission of the following manuscript.


URL: https://doi.org/10.1021/acs.jced.7b01011

Leila Keshavarz’s contribution: Performed gravimetric experiments and XPRD experiments. Conducted the modelling. Contributed to analysing the results. Contributed to the preparation, design and writing of the manuscript.

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To Whom It May Concern,

We the undersigned are writing this letter to stipulate the role of Leila Keshavarz in the preparation and submission of the following manuscript.


URL: [https://doi.org/10.1021/acs.jced.7b00118](https://doi.org/10.1021/acs.jced.7b00118).

**Leila Keshavarz’s contribution:** Contributed to design experiments. Performed the pressurized synthetic experiments. Conducted the modelling. Measured the gravimetric solubility data and compared with the pressurized synthetic method. Utilized the method to measure the Metastable zone (MSZW). Investigated advantages and drawbacks of the new method. Contributed to writing of the manuscript.

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To Whom It May Concern,

We the undersigned are writing this letter to stipulate the role of Leila Keshavarz in the preparation and submission of the following manuscript.


URL: https://doi.org/10.1021/acs.cgd.9b00490

Leila Keshavarz’s contribution: Prepared the set up and contributed in designing the experiments. Performed the induction time measurement experiments using FBRM. Contributed to preparation, design and writing of the manuscript.

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Appendix 4A

To Whom It May Concern,

We the undersigned are writing this letter to stipulate the role of Leila Keshavarz in the preparation and submission of the following manuscript.


URL: https://doi.org/10.1016/j.jct.2019.02.004

Leila Keshavarz’s contribution: Carried out literature reviews which identify the gap in the data associated with impurities of paracetamol. Performed the gravimetric and XPRD experiments. Conducted the modelling. Contributed to analyse the results. Contributed to preparation, design and writing of the manuscript.

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*Equal contribution.
Appendix 5A

To Whom It May Concern,

We the undersigned are writing this letter to stipulate the role of Leila Keshavarz in the preparation and submission of the following manuscript.


URL: https://doi.org/10.1021/acs.cgd.9b00490

Leila Keshavarz’s contribution: Examined the solubility of paracetamol in presence of impurities. Performed the DOE experiments. Contributed to design, preparation and editing of manuscript. Submitted the manuscript.

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* L.K. and R.R.E.S. made an equal contribution.
Appendix 6A

To Whom It May Concern,

We the undersigned are writing this letter to stipulate the role of Leila Keshavarz in the preparation and submission of the following manuscript.


URL: https://doi.org/10.1021/acs.oprd.8b00308

Leila Keshavarz’s contribution: Contributed to design the experiments. Performed the crystallization experiments. Contributed to compare the experimental results with a mathematical model from literature. Contributed to design, preparation and editing of manuscript. Submitted the manuscript.

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