Crystal Transformations and Crystallisation Methodologies: Polymorphic Transformations of Piracetam

Anthony Maher B.Sc.

Ph.D Thesis

Supervisors: Prof. B. K. Hodnett, Prof. Å. C. Rasmuson & Dr. D. M. Croker

A thesis submitted for the degree of Doctor of Philosophy to the Faculty of Science and Engineering, University of Limerick, Ireland.

Submitted: July 2013
Declaration

This thesis is the original work of the author and due reference and acknowledgement has been made, when necessary, to the work of others. No part of this thesis has been accepted for any degree and is not being concurrently submitted for any other award.

__________________________

Anthony Maher
Acknowledgements

First and foremost, I would like to express my sincerest gratitude and appreciation to my co-supervisors, Prof. Kieran Hodnett, Prof. Åke Rasmuson and Dr. Denise Croker, for their guidance and encouragement during the course of this work. Their willingness to discuss results and put forward suggestions was vital to the quality of the project.

Thanks to Dr. Colin Seaton for his work on molecular modelling of the transformations. It complimented the experimental results very well. Also thanks to Dr. Sarah Hudson for the SS-NMR characterisation of the polymorphs of piracetam at Waterford Institute of Technology.

Many thanks to the members of the Solid State Pharmaceutical Cluster (SSPC) group at UL. Specific thanks to Dr. Aine Munroe and Dr. Marcus O’Mahony and Donal Mealey, who undertook similar projects to me, for the many discussions on each other’s work and results.

Thanks to the staff in the Materials and Surface Science Institute at UL. To Dr. Wynette Redington for training on the XRD and DSC; Dr. Gordon Armstrong for training on the ATR-FTIR; Dr. Calum Dickinson for training on the SEM; and Dr. Serguei Belochapkine for his help in putting together the set-up for the in-situ microscopy.

Many thanks to the Irish Research Council for Science Engineering and Technology (IRCSET) for funding the first three years of the project and to the SSPC thereafter.

Thanks to my family, Dad, Mom, Greta, Loretta, Jean and Joey! Without their support I would not have had the opportunity to do a Ph.D and get through it. I will, at last, ‘come off my parents back!’ And finally my girlfriend Megan, thanks for the support and putting up with the bad moods when experiments didn’t go to plan!
Abstract

The Ph.D project detailed in this thesis investigated the phenomena of crystallisation and polymorphism, focusing on 2-oxo-1-pyrrolidine acetamide (piracetam) as the model compound. The three polymorphs of practical relevance were isolated via different methods, and characterised using numerous analytical techniques. Solubility data was obtained between 5 and 50 °C for Form II and Form III in organic solvents. It was found that the solubility values correlated positively with solvent polar characteristics from a qualitative point of view; an increase in solubility was observed with increasing solvent polarity and solvent acidity. The metastable Form II has a slightly higher solubility than the stable Form III.

A thorough investigation of the polymorphic transformations of the system was carried out, employing a combination of off-line and in-situ techniques. The solution mediated polymorphic transformation from Form II to Form III was investigated by monitoring the solution and solid phases. The effect of factors such as solvent, temperature, agitation, excess solid mass and specific surface area of the solid phase were examined. Solvent, temperature and agitation were all found to alter the transformation rate significantly due to their impact on the kinetics of the system. Increases in temperature and agitation increased the rate of the transformation. Indirectly, due to its impact on solubilities, temperature also influenced the driving force for the transformation. Solvent was found to affect the transformation rate both in terms of the solubility of piracetam in a particular solvent and the solvent-solute interactions. An insight into the mechanism of the transformation was also obtained. The transformation is governed by the nucleation and growth of the stable form, with nucleation likely to take place on the faces of the metastable form crystals.

The solid state polymorphic transformations of piracetam were examined. Form II and Form III were each observed to transform directly to Form I upon heating, while Form I consistently transformed to Form II when cooled. Form II transforms to Form I at a slightly lower temperature than Form III. The transformation of both polymorphs to Form I was observed to cause physical cracking of the crystals as well as changing the optical properties. The molecular rearrangements required for the transformation from Form I to Form II were found to be more energetically favourable than those required for the transformation to Form III. The transformation from the metastable Form II to the stable Form III was not observed in the solid state. The true thermodynamic transition points of the three enantiotropically related polymorphs were probed in detail.
Figure 1: Optical Micrograph of a metastable Form II piracetam (rough dissolving crystal) undergoing a solution mediated polymorphic transformation to Form III (smooth growing crystal) in methanol at 25 °C.

**Keywords:** Polymorphism, solubility, characterization, analytical techniques, polymorphic transformations, solution mediated polymorphic transformations, solid state polymorphic transformations, piracetam, crystallization, nucleation, van’t Hoff enthalpy of solution.
Publications

This thesis is based in part on the following journal publications.


in a range of Organic Solvents” Poster presentation at 42nd British Association for Crystal Growth Conference, University College London.


Notation

A  Pre-exponential or collision factor in nucleation rate equation
a  Activity
\(a_{eq}\)  Activity of a saturated solution
A  Solubility regression coefficient
B  Solubility regression coefficient
C  Solubility regression coefficient
c  Mass concentration
\(c^*\)  Mass concentration at equilibrium
\(C_s\)  Solubility
\(E_a\)  Activation energy for nucleation
G  Gibbs free energy
\(G_{liq}\)  Free energy of melting
\(\Delta G\)  Free energy difference between two phases
\(\Delta G_{crit}\)  Free energy of formation of a critical nucleus under homogeneous condts.
\(\Delta G_{crit}^c\)  Free energy of formation of a critical nucleus under heterogeneous condts.
\(\Delta G_s\)  Free energy difference between the surface and the bulk of the crystal
\(\Delta G_v\)  Free energy diff. between the solute in the crystal and the solute in solution
\(\Delta G_t\)  Free energy change per unit volume
\(\Delta G_{tr}\)  Activation free energy for the transport resistance encountered by the solute for volume diffusion from the bulk solution to the nucleus
\(\Delta H\)  Enthalpy difference between two phases
\(\Delta H^H_{sol}\)  van’t Hoff enthalpy of solution
J  Homogeneous nucleation rate
J’  Heterogeneous nucleation rate
k  Boltzmann constant
\(m_{empty}\)  Mass of vial + cap
\(m_{liq}\)  Mass of vial + cap + saturated solution
\(m_{dry}\)  Mass of vial + cap + dried solid
\(N_0\)  Number of solute molecules per unit volume
\(\eta\)  Dynamic Solution viscosity
R  Gas constant
r  radius
\(r_c\)  Radius of critical nucleus
\( S \)  Supersaturation Ratio
\( \Delta S \)  Entropy difference between two phases
\( T \)  Temperature
\( x \)  Mole fraction concentration
\( x^* \)  Mole fraction concentration at equilibrium
\( \mu \)  Chemical potential
\( \Delta \mu \)  Difference in chemical potential between two phases
\( \mu_0 \)  Standard potential
\( \mu_s \)  Chemical potential of a molecule in solution
\( \mu_c \)  Chemical potential of a molecule in the bulk of the crystal
\( \mu_{eq} \)  Chemical potential of the system at equilibrium
\( \gamma \)  Interfacial energy
\( \Phi \)  Heterogeneous nucleation factor
\( \theta \)  Contact angle in heterogeneous nucleation

**Abbreviations, Acronyms**

- **ATR-FTIR**: Attenuated total reflectance fourier transform infrared
- **CIF**: Crystal information file
- **CSD**: Cambridge structural database
- **DSC**: Differential Scanning Calorimetry
- **EtOH**: Ethanol
- **GMM**: Molecular mass
- **HT-XRD**: High temperature X-ray diffraction
- **IR**: Infrared
- **RHS**: Right hand side
- **R1**: Solution contained only pure solvent at \( t_0 \)
- **R2**: Solution was saturated at \( t_0 \)
- **R3**: Solution was supersaturated at \( t_0 \)
- **SEM**: Scanning electron microscopy
- **SMPT**: Solution mediated polymorphic transformation
- **SSPT**: Solid state polymorphic transformation
- **t_0**: Starting point of experiment
- **wrt**: with respect to
- **XRD**: X-ray diffraction
- **TP**: Transition point
- **MP**: Melting point
- **D**: Density
- **Z**: Number of molecules per unit cell
Presentation of Thesis

This thesis has been prepared in the style “Thesis by Publication.” The first part of the thesis outlines the reasons for undertaking the work, the main experiments carried out and results obtained. The second part includes the journal publications associated with the work. These include additional results information, mainly molecular modelling which was utilised to compliment the experimental findings. This work was carried out by Dr. Colin Seaton in the Materials and Surface Science Institute at the University of Limerick. Table 1 below documents the manuscripts and the journals in which it has been published. The corresponding current ISI Impact Factor of the respective journal and status of the publication is also included.

<table>
<thead>
<tr>
<th>Publication</th>
<th>Journal</th>
<th>Impact Factor</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Journal of Chemical &amp; Engineering Data</td>
<td>1.693</td>
<td>Published</td>
</tr>
<tr>
<td>II</td>
<td>Journal of Chemical &amp; Engineering Data</td>
<td>1.693</td>
<td>Published</td>
</tr>
<tr>
<td>III</td>
<td>Crystal Growth &amp; Design</td>
<td>4.720</td>
<td>Published</td>
</tr>
<tr>
<td>IV</td>
<td>Crystal Growth &amp; Design</td>
<td>4.720</td>
<td>Published</td>
</tr>
<tr>
<td>V</td>
<td>Crystal Growth &amp; Design</td>
<td>4.720</td>
<td>Manuscript in Preparation</td>
</tr>
</tbody>
</table>
# Contents

Declaration................................................................................................................................. ii
Acknowledgements.................................................................................................................... iii
Abstract........................................................................................................................................ iv
Publications................................................................................................................................... vi
Notation........................................................................................................................................ vii
Abbreviations, Acronyms ......................................................................................................... viii
Presentation of Thesis................................................................................................................ ix

## Chapter 1. Introduction ............................................................................................................... 2

1.1 Scope................................................................................................................................... 3
1.2 Objectives............................................................................................................................ 4
1.3 Practical Relevance ............................................................................................................ 4

## Chapter 2. Theory ................................................................................................................... 7

2.1 Solubility, Supersaturation & Crystallisation ....................................................................... 8
2.1.1 Nucleation ..................................................................................................................... 9
2.1.2 Growth .......................................................................................................................... 12
2.2 Polymorphism & Polymorphic Transformations ................................................................ 13
2.3 Piracetam ........................................................................................................................ 18

## Chapter 3. Materials & Experimental Methodologies ............................................................ 24

3.1 Materials ............................................................................................................................ 24
3.2 Techniques ......................................................................................................................... 25
3.2.1 X-ray Diffraction (XRD) ............................................................................................. 25
3.2.2 Differential Scanning Calorimetry (DSC) .................................................................. 27
3.2.3 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) .. 28
3.2.4 Scanning Electron Microscopy (SEM) ....................................................................... 28
3.2.5 Optical Microscopy ...................................................................................................... 29
3.2.6 In-situ Infrared Analysis ............................................................................................. 30
3.3 Experimental Methodologies............................................................................................ 31
3.3.1 Isolation of the Polymorphs of Piracetam .................................................................. 31
3.3.2 Solubility of FII(6.403) and FIII(6.525) ..................................................................... 32
3.3.3 Solution Mediated Polymorphic Transformation: FII(6.403) to FIII(6.525) ................. 33
3.3.4 Solid State Polymorphic Transformations of Piracetam ............................................ 34

## Chapter 4. Results .................................................................................................................. 36
Chapter 1. Introduction

Crystallisation can be defined as a phase change in which a solid crystalline product is obtained from solution (Myreson 2002). It is an age old technology extensively employed as a method of separation, isolation and purification. The true crystal comprises a rigid repeating lattice of molecules, atoms or ions, the locations of which are characteristic of the substance (Mullin 2001). Many active pharmaceutical ingredients (API’s) exist in the crystalline solid state due to reasons of stability and ease of handling during the various stages of drug development (Vippaguntaa et al. 2001). The two main steps involved in crystallisation are nucleation and growth. However, since the understanding of these processes is not very complete, particularly the element involving the formation of the nucleus, control of the size, shape and form of the solid product of a crystallisation can be most challenging (Cardew and Davey 1985). Different theories on nucleation exist; the most commonly accepted being the classical nucleation theory which is utilised in understanding the crystallisation processes of this project (Kashchiev and van Rosmalen 2003).

Polymorphism in crystalline solids is defined as materials having the same chemical composition but different lattice structure orientations and/or different molecular conformations. Therefore, different polymorphs will have different physico-chemical properties (Vippaguntaa et al. 2001, Bernstein et al. 1999). These properties, including stability, solubility and habit have a major influence on which polymorph of the system will be employed as the API (Yu et al. 2003). They have a considerable influence on the choice of solvents and the course of operation in solvent-based processes such as crystallisation, also affecting the formulation and tableting processes, as well as the therapeutic response of the drug once administered. While the crystallisation of an API in the correct form is an important step in the manufacture of a drug product, the stability of that form in the final product and during its shelf life is just as critical. Instances of disappearing and appearing polymorphs are not uncommon in the pharmaceutical industry. One such case is ritonavir, the API in Norvir (Abbott Laboratories, Chicago). A more stable polymorph (Form II), identified after the product was on the market, had an adverse effect on the bioavailability of the drug (Bauer et al. 2001). Differences in properties between polymorphs in a system has led to extremely tight regulation of polymorphism, resulting in the filing of an API requiring it to be produced as a particular polymorph in most cases.

Like crystallisation, polymorphism is far from completely understood. Some researchers in the field believe that all compounds are potentially polymorphic, with the number of discovered polymorphs being proportional to the amount of time spent investigating the particular compound (Kuhnert-Brandstaetter 1975). Polymorphic transformations are of particular interest in this study, principally
solution mediated and solid state transformations. The stability hierarchy of the polymorphs in a particular system is determined by the relative Gibbs free energy of each polymorph. The lower the free energy of the polymorph the more thermodynamically stable it is. Under a certain set of changes in environmental conditions (e.g. temperature, pressure etc.) a polymorph will become thermodynamically unstable, meaning that another more stable polymorph exists. Whether the transformation to the more stable polymorph occurs or not, and the timescale of the transformation, depends on the kinetics of the system. Therefore, polymorphic transformations are often a trade-off between the kinetics and the thermodynamics of the system.

According to Ostwald’s rule of stages, a bi-morphic compound will crystallise as its metastable polymorph before transforming into the stable polymorph. The transformation process has the same basic requirements as crystallisation; nucleation and growth. In addition, the withdrawal of the metastable phase from the system needs to transpire. The presence of the metastable form is sometimes recognised as playing a role in the nucleation of the stable phase (Croker and Hodnett 2010).

1.1 Scope

The main focus of this Ph.D. project is on the role of polymorphism and polymorphic transformations within the phenomenon of crystallisation. The model polymorphic compound 2-oxo-1-pyrrolidine acetamide (Piracetam) was investigated in the solid state as well as in the presence of up to eight solvents. The requirement of pure polymorphic samples to characterise and then investigate relevant polymorphic transformations provided an initial focus on crystallisation techniques, as well as gaining an insight into the stability hierarchy of the polymorphs in the piracetam system and the environmental conditions that favour each of the polymorphs. Cooling a solution at a constant rate was the most commonly utilised method of evoking crystallisation, producing a resulting crystalline form(s).

The polymorphic transformations of the piracetam system have been identified in the literature. However, a comprehensive study, showing an in-depth analysis and interpretation has not been reported. In this project the solution mediated polymorphic transformation (SMPT) and three solid state polymorphic transformations (SSPT’s) are examined in detail. On-line monitoring of the solution phase and visualisation of the solid phase and off-line analysis of the solid phase enabled such investigation to be carried out.
The study also includes a detailed evaluation of the influence of numerous processing conditions on the kinetics of the SMPT. Experimental procedure involves, in particular, different conditions of temperature, solvent, agitation, excess solid mass and specific surface area of the solid phase. In addition, the work also includes a series of experiments for obtaining an understanding of the mechanistic features of the transformations, with a view to establishing the rate limiting features and the means by which the transformations initiate and go to completion. Attention was placed on the three main steps involved as outlined above (nucleation and growth of the stable phase and the dissolution of the metastable phase), with particular emphasis on the nucleation site of the stable form. Molecular level analysis of these steps was not incorporated. However, it is hoped that the information presented in this thesis will form the basis of a study detailing such a level of analysis on the polymorphic transformations of piracetam.

1.2 Objectives

The initial objectives involve comprehending the fundamental requirements for crystallisation to occur, achieving competency in controlling crystallisation experiments and establishing a suitable set of analytical techniques to analyse both the crystallisation process and the resulting products. Thereafter, a more in-depth understanding of both the solution mediated and solid state polymorphic transformations of the model compound is sought, specifically focusing on the effect of processing conditions and the mechanistic features of the transformations.

1.3 Practical Relevance

During the course of this Ph.D, a 5 month placement was undertaken in a leading pharmaceutical company which experienced polymorph issues in a plant process. The API, produced by a seeded cooling crystallisation, was filed as the metastable polymorph of the system and several batches had failed due to the presence of the stable polymorph. It was established that the seeding regime was insufficient and ran the risk of spontaneous crystallisation of the stable polymorph. Also holding periods at elevated temperatures, in contact with the solvent, during production promoted the initiation of the SMPT from the metastable to the stable polymorph. With recommendations from the work completed as part of the placement, no polymorph failures were experienced in the most recent campaign. This placement was clear evidence of the importance of understanding
polymorphism in the control and management of crystallisation processes in the pharmaceutical industry.
Chapter 2. Theory

Most organic and inorganic compounds of pharmaceutical relevance can exist in one or more crystalline forms. Crystalline solids have long range orderly arranged units, in which the structural units, known as unit cells, are repeated regularly and indefinitely in three dimensions (Davey and Garside 2000). Obviously, the crystallisation of an API is a very important step in the manufacture of a drug product, especially when the final drug product is in a solid dosage form. All crystal structures can be grouped into one of seven possible crystal systems (Figure 2.1) that are defined by the relationships between the individual dimensions, $x$, $y$, and $z$, of the unit cell and between the individual angles, $\alpha$, $\beta$, and $\gamma$ (Brittain and Bryn 1999, Bryn et al. 1999).

![Figure 2.1: The seven crystal systems (Mullin 2001)](image)

In this chapter, the conditions required for crystallisation to occur are introduced. There are two steps to every crystallisation event. Firstly, nucleation of the solid phase must occur and thereafter, the growth of this solid phase to its final size shapes the crystallisation product. Nucleation is the critical step, since without it growth is not a factor.
2.1 Solubility, Supersaturation & Crystallisation

The most basic methods of generating supersaturation include cooling, antisolvent addition, evaporation and reactive solution crystallisation. If the supersaturation generated in solution is significant enough, crystallisation will occur. Figure 2.2 presents a standard solubility curve. When an undersaturated/saturated solution is brought into a region, by cooling in Figure 2.2, where it contains a higher proportion of solute than the equilibrium solubility at that temperature, it is said to be supersaturated. The metastable zone represents a region, under a certain set of conditions (cooling rate, agitation, volume etc.) where the solution remains homogeneous in a supersaturated state; i.e. nucleation does not occur or is very slow. If the solution is seeded in this region, growth of the seed will occur as the solution concentration reverts back towards equilibrium along the solubility curve. Cooling further across the metastable zone width boundary results in spontaneous nucleation, which is then followed by growth during the crystallisation process.

The thermodynamic driving force for nucleation is the difference in chemical potential between the solute in the supersaturated solution, $\mu$, and the solute as a pure solid, the latter equal to that of a saturated solution, $\mu^*$. The driving force can be expressed in terms of activities, $a$, and mole fraction concentrations, $x$, as:

$$\Delta\mu = \mu - \mu^* = RT\ln \left( \frac{a}{a^*} \right) = RT\ln \left( \frac{x}{x^*} \right) \approx RT\ln \left( \frac{x}{x^*} \right) = RT\ln S \quad [2.1]$$
Commonly the ratio of activity coefficients, $\gamma/\gamma^*$, is assumed to be close to unity, but in many cases of crystallization of organic compounds this assumption appears to be adopted primarily for reasons of convenience (Svärd and Rasmuson 2013).

Different expressions exist in the literature for the representation of supersaturation ($S$). During the course of this work the supersaturation ratio is used to quantify the level of supersaturation in a system (Mullin 2001).

$$S = \frac{c}{c^*} \approx \frac{x}{x^*} \tag{2.2}$$

Therefore, a very useful starting point in understanding a system is to obtain concrete solubility data so that the equilibrium conditions can be compared to the experimental conditions in the calculation of the driving force for a crystallisation process. Solubility usually has a major dependence on temperature. Solubility increases with temperature in almost all systems. One of the few cases where solubility is known to decrease with increasing temperature is cerium (III) sulphate.

The van’t Hoff enthalpy of solution can be determined from the slope of the solubility curve in a so-called van’t Hoff plot by plotting the mole fraction solubility ($\ln x$) against the reciprocal of temperature (K).

$$\Delta H_{\text{soln}}^{\text{vH}} = -R \frac{\ln x}{d(T^{-1})} \tag{2.3}$$

### 2.1.1 Nucleation

The process of the formation of nanoscopically small molecular clusters of a crystalline phase, large enough to be thermodynamically stable, in a liquid solution is called nucleation (Kashchiev and van Rosmalen 2003). Figure 2.3 classifies the different types of nucleation into two header categories, primary and secondary. Secondary nucleation is nucleation by mechanisms that require the presence of parent crystals in the solution. Instead of the nucleation of a new crystal phase, the generation of nuclei of the same crystal phase occurs in the vicinity of the seeded crystals. Primary nucleation, the creation of a new phase, can be divided into two distinct circumstances; homogeneous, referring to nucleation of a crystal phase in a homogeneous solution, and heterogeneous, referring to nucleation of a new crystal phase catalysed by the presence of the
surface of a foreign body. These foreign entities can range from impurities such as dust, to vessel walls, to crystals of a different phase to the one nucleating.

Figure 2.3: An outline of the different types of nucleation (Mullin 2001).

The thermodynamic description of the nucleation process, outlined by researchers in the 1930s and 1940s (Gibbs 1948), is based on the condensation of a vapour to a liquid with this treatment extended to crystallisation from melts and solutions. This classical nucleation theory is based upon the idea of molecular addition of the solute molecules creating a stable nucleus. At some point, referred to as the critical size, the cluster of molecules becomes stable enough to continue to grow as a crystal. Below this critical size the molecules will dissemble into solution again. The free energy required in the formation of a nucleus ($\Delta G_{nuc}$) can be expressed as:

$$\Delta G_{nuc} = \Delta G_s + \Delta G_v$$  \[2.4\]

In terms of crystallisation from solution, $\Delta G_s$ describes the free energy difference between the surface and the bulk of the crystal and $\Delta G_v$ denotes the free energy difference between the solute in the crystal and the solute in solution. The solid state is more stable than the solution; therefore, $\Delta G_v$ becomes negative by an amount proportional to the volume of the deposition, thereby favouring growth. The introduction of a solid-liquid interface is not energetically favourable and $\Delta G_s$, being positive, increases the free energy by an amount proportional to the surface area of the cluster. As outlined in eq 2.5, both terms depend on the size of the cluster in terms of its radius ($r$) and assuming a perfect sphere:

$$\Delta G = 4\pi r^2 \gamma + \frac{4}{3}\pi r^3 \Delta G_v$$  \[2.5\]

Where $\Delta G_v$ is the free energy change per unit volume and $\gamma$ is the interfacial energy of the crystal in solution. Because the two terms on the right hand side are of opposite sign and depend on $r$ differently, a balance between the two results in a maximum in the free energy of formation referred to as $\Delta G_{crit}$ (Figure 2.4), with a critical nucleus radius ($r_c$). At small radii ($r < r_c$), the $\Delta G_s$ (+) term dominates, causing an initial increase in free energy urging small clusters to dissolve. When $r >$
\( r_c \Delta G_v \) dominates and makes it more energetically favourable for the cluster to grow. Therefore, the critical size, representing the minimum size of a stable nucleus, can be estimated when \( d\Delta G_{\text{nuc}}/dr = 0 \):

\[
r_c = \frac{-2\gamma}{\Delta G_v}
\]

[2.6]

The critical size is known to decrease with increasing supersaturation (Davey and Garside 2000). Introducing \( r_c \) into eq 2.5, the critical free energy of the nucleus is:

\[
\Delta G_{\text{crit}} = \frac{4\pi\gamma r_c^2}{3}
\]

[2.7]

Figure 2.4: Free energy diagram for nucleation (Erdemir et al. 2009).

The nucleation rate, communicated as the number of stable nuclei formed per unit time per unit volume, can be expressed as an Arrhenius equation:

\[
J = A \exp \left( -\frac{\Delta G_{\text{crit}}}{kT} \right)
\]

[2.8]

Where \( A \) is the pre-exponential factor and \( k \) is the Boltzmann constant \((1.3805 \times 10^{-23} \text{ J.K}^{-1})\). Substituting the Gibbs-Thomson relationship for a non-electrolyte:

\[
\ln S = \frac{2\gamma}{kT}\nu
\]

[2.9]

into eq 2.1 gives a relationship for the free energy of the critical radius:

\[
\Delta G_{\text{crit}} = \frac{16\pi\gamma^3\nu^2}{3(kT\ln S)^2}
\]

[2.10]
giving a rate equation for homogeneous primary nucleation:

$$J = A \exp \left( \frac{16\pi y^2 v^2}{3kT^2(\ln\lambda)^2} \right)$$

[2.11]

In the case of heterogeneous primary nucleation, the free energy associated with the formation of a critical nucleus, $\Delta G'_{\text{crit}}$, is less than that associated with homogenous nucleation, $\Delta G_{\text{crit}}$. In homogeneous nucleation, all solute molecules in solution are potential nucleation sites while only those solute molecules in solution that are in contact with the propagating foreign phase are potential nucleation sites in heterogeneous nucleation. The rate equation for this type of nucleation has been expressed as (Gu et al. 2001):

$$J' = A. \exp \left[ -\frac{16\pi y^2 v^2 \phi}{3kT^2(\ln\lambda)^2} \right]$$

[2.12]

where $\phi$ is the heterogeneous nucleation factor, with a value between 0 and 1 based on the contact angle, $\theta$, between the nucleating phase and the propagating surface.

### 2.1.2 Growth

Once a stable nucleus has been formed, the next phase of crystallisation is growth to the final crystal size. Numerous theories on the mechanism of growth exist in the literature. One such model, the 2-D nucleation theory is based upon adsorption of molecules on a layer by layer basis as the crystal grows. It requires nucleation of each layer on the crystal as it increases in size. However, some crystals are known to grow at supersaturations as low as 0.01% (Markov 2003). Such a low driving force is not strong enough to generate 2-D nucleation on the face of the crystal. This issue was resolved with the Burton, Cabrera, Frank theory of crystal growth via a spiral mechanism (Burton et al. 1951). It was concluded that growth of crystals under low supersaturation can only be explained by the presence of defects on the crystal surface. When an imperfection terminates at the surface of the crystal with a dislocation, growth takes place at the steps along the edge of the dislocation. Therefore 2-D nucleation is not required after the initial stable nucleus is formed. Many expressions for the growth rate of a surface growing in this manner exist in the literature (Davey and Garside 2000, Mullin 2001, Markov 2003, Burton et al. 1951).

The rate of growth is affected by numerous parameters including the supersaturation, temperature, presence of impurities, surface area available for growth and the affinity of the solute molecule for the solvent. Two desolvation processes must occur during the addition of each solute molecule to the crystal lattice during the nucleation and growth processes. Firstly, desolvation of the solute
molecule in solution before it binds to the lattice must occur, and secondly, desolvation of the site on the lattice where the solute molecule will bind to must occur.

### 2.2 Polymorphism & Polymorphic Transformations

Many drugs exist in the crystalline solid state due to reasons of stability and processability during the various stages of drug development. Crystalline solids can exist in the form of polymorphs, solvates or hydrates. Polymorphic crystalline solids are of interest in this study. The relative stability of the various polymorphs of a given compound is determined by the respective Gibbs free energies ($\Delta G$) of the different forms. The enthalpy difference ($\Delta H$) of the system minus the product of the temperature ($T$) times the entropy difference ($\Delta S$) of the system equates to the Gibbs free energy:

$$
\Delta G = \Delta H - T \Delta S
$$

[2.13]

The most stable polymorph in a system, under a certain set of conditions, will have the lowest free energy while the least stable polymorph will have the highest. Based on the thermodynamic properties of a specific compound, depending upon whether one polymorph can transform reversibly to another or not, its polymorphs are classified as either enantiotropes or monotropes (Henck and Uhnert-Brandstatter 1999). In Figure 2.5 A is presented a simple bi-morphic system, in which, at lower temperatures polymorph I is stable and polymorph II is metastable. A reversible transition between polymorphs is observed at a definite transition temperature below the melting point, inferring that the system is enantiotropic. In a monotropic system (Figure 2.6 A), a transition in the stability of the polymorphs below the melting point is not observed. The free energy relationship between the polymorphs of a system is reflected in the solubility data of the system. In Figure 2.5 B, the solubility of the more stable polymorph I is lower than polymorph II at lower temperatures. At higher temperatures, polymorph II becomes stable and has a lower solubility. At the transition temperature, the solubility of both polymorphs is identical. In the case of a monotropic system, polymorph I is more stable at all temperatures below the melting temperature, and accordingly it has a lower solubility at all temperatures (Figure 2.6 B). Burger and Ramberger have described a series of rules based on thermodynamic data like melting point and enthalpy of melting for the assignment of a given polymorphic pair as enantiotropic or monotropic which also aid in the determination of the relative stability of polymorphs (Rodriguez-Spong et al. 2004, Bernstein 2002).
Moving from an undersaturated region under the solubility curves in Figure 2.6 B, by decreasing temperature, the solution becomes supersaturated with respect to the stable polymorph I initially. Thermodynamically speaking, the more stable polymorph is favoured to crystallise before the metastable polymorph. However, while thermodynamics establish the stability hierarchy of the various solid states, once a metastable domain is encountered, kinetic pathways will determine which form will be crystallised and for how long it will survive (Cardew and Davey 1985). The starting point for a discussion of the kinetic factors is the traditional energy-reaction diagram as shown in Figure 2.7. $G_{SS}$ represents the free energy per mole of a solute in the solution at the particular level of supersaturation which crystallises into one of two forms, polymorph I and II with respective free energies $G_I$ and $G_{II}$, $E_{aI}$ and $E_{aII}$, the activation energies associated with the formation of a nucleus of critical size for each polymorph needs to be overcome for crystallisation of that polymorph to occur.
As the level of supersaturation increases, the nucleus critical size and accordingly the height of the activation energy barrier decreases (Davey and Garside 2000). For a solution with a particular level of supersaturation, according to Ostwald’s rule of stages, the critical size required for the metastable polymorph II to nucleate is smaller than that for polymorph I, and accordingly the activation free energy for nucleation of II is lower and the kinetics of the system will favour crystallisation of this polymorph.

![Free Energy Diagram of Polymorph System](image)

**Figure 2.7:** A schematic representation of the free energy diagram of a polymorph system, with a corresponding concentration-time profile.

Thermodynamics favour the most stable polymorph, while kinetics often favour the metastable polymorph (Bernstein et al. 1999). The solute in the supersaturated solution in Figure 2.7 has a free energy, $G_{SS}$ (left), and a corresponding solution concentration (right). Crystallisation results in a reduction in the free energy of the system as the crystal is formed. As mentioned above, nucleation of polymorph II is often kinetically favoured, while the thermodynamic driving force for the crystallisation of polymorph I is greater. Therefore, the metastable polymorph II crystallises initially, resulting in a decrease in free energy and a corresponding decrease in solution concentration. Over time, the thermodynamics of the system will result in the transformation to the stable polymorph I, again resulting in a decrease in free energy and solution concentration. The rate of this transformation is affected thermodynamically by the relative difference in free energy between the two polymorphs, and kinetically by factors including temperature, solvent etc. The thermodynamic driving force for the transformation can be calculated from the solubility difference between the polymorphs using eq 2.1 where $x$ and $x^*$ are the mole fraction solubilities of the metastable and stable polymorph respectively.

Exaining the kinetic section of the nucleation rate equation, pre-exponential factor ($A$), gives an insight into the effect of processing conditions on SMPT’s. $A$ is a complex function:
\[ A = N_0 4\pi (r_c)^2 \left( \frac{kT}{h} \right) \exp \left( \frac{\Delta G_T}{kT} \right) \]  

Where \( N_0 \) is the number of solute molecules per unit volume, \( 4\pi (r_c)^2 \) is the surface area of the assumed to be spherical critical nucleus, \( kT/h \) is a frequency factor and \( \Delta G_T \) is the activation free energy for the transport resistance encountered by the solute for volume diffusion from the bulk solution to the nucleus. It is obvious that processing conditions such as solvent and temperature have a major influence on \( A \).

Polymorphic transformations can proceed via a number of different means (Davey et al. 1985, Cardew and Davey 1985); the principal two being through solution mediated and solid state mechanisms. Numerous different examples of both types exist in the literature. Under a certain set of conditions a polymorph can exist in a metastable region, provided the stable polymorph is not present and the kinetics of the system do not favour the transformation. Nucleation of the stable form sets the transformation in motion, making it the critical step. In an extensive review, Croker and Hodnett (2010) observed that in SMPT’s, nucleation of the stable polymorph tends to occur on, or at, the surface of the existing polymorph. In a special case called epitaxial nucleation, there is a structural relationship between the two polymorphs leading to a well-defined orientation for the epitaxially nucleating form on the surface of the stable form (Stoica et al. 2005). Numerous examples of epitaxial nucleation of a metastable polymorph on the stable form have been reported (Boistelle et al. 1981, Boerrigter et al. 2002 Courvoisier et al. 2003).

The nucleation of the stable \( \beta_L \) glutamic acid was proved to be facilitated by the presence of the surface of the metastable \( \alpha_L \) form. Additives, which selectively adsorbed to the surface of the metastable form, prevented nucleation of the stable form (Cashell et al. 2005). Further studies confirming the surface mediated nature of the transformation found that the transformation rate was faster when a smaller particle size of the \( \alpha_L \) glutamic acid was employed, indicating that the rate of nucleation of the \( \beta_L \) form increases concurrently with surface area of the metastable form (Scholl et al. 2006). In the case of the transformation from form 1 to form 2 of 2,6-Dihydroxybenzoic acid, a higher local supersaturation on the (002) face of form 1 encouraged heterogeneous nucleation of form 2 on this face (Davey et al. 2001, Davey et al. 2002).

Su et al. (2010) investigated the effect of different amounts (3 to 5 g) of the metastable \( \alpha \) form of D-mannitol in 100-mL of saturated 17% w/w ethanol-water at 27 °C on the transformation to the \( \beta \) form. Higher loadings resulted in slightly longer transformation times to the \( \beta \) form. This was attributed to a constant rate of crystal growth and therefore, higher loadings were believed to cause an extended growth phase. However, if nucleation of the stable phase is facilitated by the presence
of a surface, as it is thought to be in most cases (Croker and Hodnett 2010), nucleation has a specific rate per unit surface area of the metastable phase. Hence, different loadings of the metastable phase should not affect the overall transformation time as long as the material has the same surface area per unit mass.

At 20 °C, γ glycine was detected in the solid phase of the metastable α form after 3 hr in an 81 g/l sodium chloride aqueous solution, and the transformation was completed in 16 hr. At 37 °C, the transformation had begun after 1.5 hr and was completed within 7 hr (Yang et al. 2008). Numerous other examples of the effect of temperature on polymorphic transformations exist in the literature. Molecular movement increases with temperature, and the interfacial energy between solid-liquid boundary layer is expected to be lower at higher temperatures (Mullin 2001, Davey et al. 2002). This is crucial in both the dissolution of the metastable crystals and the nucleation and growth of the stable phase. Since the solubility of a compound generally increases with temperature, the likelihood of molecular collisions occurring for the formation of critical nuclei increases with temperature. The concentration in solution and the interfacial energy also vary greatly with solvent choice, thereby having an effect on the nucleation rate. Solvent choice also affects the molecular solvent-solute interactions, having a knock-on effect on the desolvation processes which need to occur during the transfer of solute molecules from the solution to the solid phase.

The effect of scale up on transformation rates has also been extensively examined. Form 1 to form 2 of 2,6-Dihydroxybenzoic acid in toluene was found to have a faster transformation rate on a smaller scale, where collisions between the metastable crystals with vessel walls and the agitation device can help propagate nucleation of the stable form (Davey et al. 2002). It was also reported that nucleation of the stable form can be facilitated by employing a magnetic stirrer as opposed to an overhead impellor. The magnetic stirrer is thought to work like a mill at the base of the vessel, increasing the attrition of the metastable crystals. In a similar way, the rate of agitation in a vessel will have an effect on the transformation rate.

In order to establish the rate determining step (s) and gain an understanding of the growth and dissolution rate constants which dominate the kinetics of the transformation, the solution concentration as well as the composition of the solid phase is monitored during the transformation (Cardew and Davey 1985).

Solid-state polymorphic transformations involve molecular rearrangement of a metastable crystal structure into a more stable crystal structure while remaining in the solid state (Verma and Krishna 1966). Byrn et al. (1999) extended the four-step mechanism for solid-state chemical reactions to suggest that solid–solid phase transitions involve four steps:
• molecular loosening of the intermolecular forces of the metastable phase
• formation of an intermediate disordered solid solution
• formation of new intermolecular bonds leading to nucleation of the new solid phase
• growth of the new stable phase

In an enantiotropic system there may or may not be a region across the transition point where a metastable polymorph can exist under a certain set of conditions in a region where an alternative polymorph is thermodynamically more stable. Kawakami et al. (2005) published a detailed study including numerous examples of systems of both types.

Examples of this type of polymorphic transformation exist in the literature. Using fourier transform raman spectroscopy the SSPT from Form III to Form I carbamazepine was examined (O’Brien et al. 2004). Monitoring the intensity of two C-H bending modes, during in-situ analysis at 130, 138, 140 and 150 °C allowed the transformation from prism shaped form III crystals to whisker like Form I to be scrutinised. The transformation was found to proceed via a solid-gas-solid mechanism. The solid state transformation from FI to FII caffeine was monitored via Atomic Force Microscopy (Kishi and Matsuoka 2010). FII was observed to nucleate on the surface of FI and subsequently grow inwards through the original crystal. As remarked in an review by Herbstein (2006): “there are relatively few papers about the actual transition directly viewed by microscopic techniques in order to infer the mechanism, and not many about changes in crystal structure as the system passes through the transition.” Beckham et al. (2008) noted that gaining a direct molecular level insight into the dynamic events occurring during solid state polymorphic transformations is outside of the scope of current experimental capabilities, and thus there is little definitive evidence for any particular mechanism. During the course of this project molecular modelling was carried out, by Dr. Colin Seaton, to compliment the experimental results and gain a greater understanding of the governing mechanisms of the transformations examined.

2.3 Piracetam

2-oxo-1-pyrrolidine acetamide (Piracetam) (Figure 2.8) is a nootropic drug, which is an agent that acts on cognitive dysfunction without causing sedation or stimulation (Winbald 2005). Cognitive dysfunction is one of the main symptoms accompanying ageing, stroke, head injury and neurodegenerative diseases such as Alzheimer’s (Gualtieri et al. 2002). Piracetam is a cognition enhancer, which is a class of drugs that facilitate attentional abilities and acquisition, storage and retrieval of information, but do not change the rate of progression of neurodegeneration (Parnetti et
Throughout the literature there is some confusion over the nomenclature of the different polymorphs. In this work the system used for naming polymorphs is simply the numerical value of the $a$-lattice parameter in angstroms, which appears in the corresponding Cambridge Structural Database (CSD) file (Allen 2002), added to the polymorph label.

Table 2.1 shows some of the properties of the different polymorphs and also the different nomenclature systems seen in the literature. The existence of three distinct polymorphs of piracetam was first reported by Pavlova et al. (1979). Under the new naming convention these polymorphs are FI(6.747), FII(6.403) and FIII(6.525). The polymorphs were identified by Infrared spectroscopy and X-ray diffraction. The crystal structure of these three polymorphs has been determined (Fabbiani et al. 2005). FI(6.747) and FIII(6.525) are obtained in high pressure (> 0.5 GPa) conditions only, and were not observed during the course of this work (Fabbiani et al. 2005, Fabbiani et al. 2007). FI(6.747) and FIII(6.525) are monoclinic while FII(6.403) is triclinic (Toscani 1998).

**Table 2.1: New naming convention and some properties of the five polymorphs of piracetam.**

<table>
<thead>
<tr>
<th>New Name proposed</th>
<th>FI(6.747)</th>
<th>FII(6.403)</th>
<th>FIII(6.525)</th>
<th>FIV(8.9537)</th>
<th>FV(6.3903)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorph</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>V</td>
</tr>
<tr>
<td>space group</td>
<td>P2$_1$/n</td>
<td>PⅠ</td>
<td>P2$_1$/n</td>
<td>P2$_1$/c</td>
<td>PⅠ</td>
</tr>
<tr>
<td>a (Å)</td>
<td>6.747</td>
<td>6.403</td>
<td>6.525</td>
<td>8.9537</td>
<td>6.3903</td>
</tr>
<tr>
<td>b (Å)</td>
<td>13.418</td>
<td>6.618</td>
<td>6.440</td>
<td>5.4541</td>
<td>6.2932</td>
</tr>
<tr>
<td>c (Å)</td>
<td>8.090</td>
<td>8.556</td>
<td>16.463</td>
<td>13.610</td>
<td>8.6450</td>
</tr>
<tr>
<td>Beta (°)</td>
<td>99.01</td>
<td>102.39</td>
<td>92.19</td>
<td>104.93</td>
<td>113.680</td>
</tr>
<tr>
<td>Z</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cell Volume (Å$^3$)</td>
<td>723.361</td>
<td>348.508</td>
<td>691.286</td>
<td>642.199</td>
<td>314.262</td>
</tr>
<tr>
<td>Symmetry Cell Setting</td>
<td>Monoclinic</td>
<td>Triclinic</td>
<td>Monoclinic</td>
<td>Monoclinic</td>
<td>n/a</td>
</tr>
<tr>
<td>D (g.cm$^{-3}$)</td>
<td>1.306</td>
<td>1.351</td>
<td>1.371</td>
<td>1.47</td>
<td>1.502</td>
</tr>
<tr>
<td>Pressure</td>
<td>Ambient</td>
<td>Ambient</td>
<td>Ambient</td>
<td>0.4 GPa</td>
<td>0.9 GPa</td>
</tr>
<tr>
<td>CCDC Ref Code</td>
<td>BISMEV03</td>
<td>BISMEV</td>
<td>BISMEV01</td>
<td>BISMEV04</td>
<td>BISMEV08</td>
</tr>
<tr>
<td>Pavlova et al. 1979/1983</td>
<td>III$_p$</td>
<td>I$_p$</td>
<td>II$_p$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fabbiani et al. 2005/2007</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td>V</td>
</tr>
<tr>
<td>Toscani et al. 1998</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Pavlova et al. (1983) crystallised piracetam from various solvents and solvent/solute ratios, with the results documented in Table 2.2.

Table 2.2: Crystal forms obtained by crystallisation of piracetam from different solvent systems (Pavlova et al. 1983).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Solvent/Solute ratio</th>
<th>Crystalline Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>3.0 ml.g⁻¹</td>
<td>III</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.0 ml.g⁻¹</td>
<td>III</td>
</tr>
<tr>
<td>n-Butanol/water (9:1 v/v)</td>
<td>4.0 ml.g⁻¹</td>
<td>III</td>
</tr>
<tr>
<td>n-Butanol/water (9.5:0.5 v/v)</td>
<td>4.0 ml.g⁻¹</td>
<td>II</td>
</tr>
<tr>
<td>n-Butanol</td>
<td>4.0 ml.g⁻¹</td>
<td>II</td>
</tr>
<tr>
<td>p-Dioxane</td>
<td>10.0 ml.g⁻¹</td>
<td>II</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>10.0 ml.g⁻¹</td>
<td>II</td>
</tr>
<tr>
<td>Chloroform</td>
<td>25.0 ml.g⁻¹</td>
<td>II</td>
</tr>
<tr>
<td>1,2-Diacetoxyethane</td>
<td>8.0 ml.g⁻¹</td>
<td>I</td>
</tr>
</tbody>
</table>

In a similar study carried out by Dematos et al. (2007), cooling crystallisations in numerous solvents were monitored by in-situ XRD analysis. Figure 2.9 shows the polymorphs that were present in each of the solutions during the cooling process. It can be seen that none of the solvent systems finished up with a pure product of an individual polymorph except nitromethane. The other three systems finished with mixtures of FII(6.403) and FIII(6.525). These studies show that the kinetics of the system are affected significantly by the solvent choice, supersaturation and temperature, leading to the crystallisation of different polymorphs under different conditions.
The existence of both FII(6.403) and FIII(6.525) during the ageing phases of this work could be explained by the polymorphs stability and structural similarities. There are some discrepancies in the literature as to whether FII(6.403) or FIII(6.525) is the thermodynamically stable polymorph. In the 1970’s and 1980’s, Pavlova et al. (1979 and 1983) failed to establish which was the stable polymorph, reporting that FII(6.403) and FIII(6.525) transform to FI(6.747) in the range 130 to 140 °C. In 1994 using thermomicroscopy, Differential Scanning Calorimetry (DSC) and a binary mixture stability study, Kuhnert-Brandstaetter et al. (1994) found that FIII(6.525) is stable and FII(6.403) is metastable at ambient temperature, while at higher temperatures FI(6.747) is the stable polymorph, with all three enantiotropically related. Based on the assumption that piracetam is a trimorphic system, in 1996, Ceolin et al. built a semi-quantitative pressure-temperature phase diagram from topological rules (Ceolin et al. 1992) indicating that FII(6.403) was the stable polymorph under ambient conditions and transformed to FI(6.747) at a higher temperature than FIII(6.525). In 2011, detailed analysis was carried out by Picciochi et al. (2011) using DSC, solution calorimetry and combustion calorimetry. The stability hierarchy between FII(6.403) and FIII(6.525) under ambient conditions and the transition temperatures to FI(6.747) were established to be in agreement with Kuhnert-Brandstetter et al. (1994). The main results of the Kuhnert-Brandstatter et al. (1994) study are summarised in Table 2.3.
<table>
<thead>
<tr>
<th>Polymorph Name</th>
<th>FI(6.747)</th>
<th>FII(6.403)</th>
<th>FIII(6.525)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production</strong></td>
<td>Heating of FII or FIII</td>
<td>Out of dioxane/out of form I at 75% relative humidity</td>
<td>Out of methanol</td>
</tr>
<tr>
<td>Melting Point (°C)</td>
<td>152.5</td>
<td>140.5</td>
<td>140</td>
</tr>
<tr>
<td>Density (g.cm(^{-3}))</td>
<td>1.304</td>
<td>1.351</td>
<td>1.371</td>
</tr>
<tr>
<td>Heat of Fusion (J.mol(^{-1}))</td>
<td>25.7</td>
<td>28.7</td>
<td>29.3</td>
</tr>
<tr>
<td>Entropy of Fusion (J.mol(^{-1}).K(^{-1}))</td>
<td>60.3</td>
<td>69.3</td>
<td>70.9</td>
</tr>
<tr>
<td>Heat of Transition to form I (kJ.mol(^{-1}))</td>
<td>-</td>
<td>+3.0</td>
<td>+3.8</td>
</tr>
<tr>
<td>First IR-Peak (cm(^{-1}))</td>
<td>3360</td>
<td>3350</td>
<td>3345</td>
</tr>
<tr>
<td>Thermodynamic Stability at 20 °C</td>
<td>&lt; FII</td>
<td>&lt; FIII</td>
<td>Stable</td>
</tr>
</tbody>
</table>

Looking at Table 2.3 the differences in the heat of fusion, entropy of fusion and heat of transition between FII(6.403) and FIII(6.525) are very small. In all cases the values for FIII(6.525) are just slightly higher than those for FII(6.403). The fact that the energy data reported for FII(6.403) is similar to that of FIII(6.525) reflects the reported high relative stability of FII(6.403) to FIII(6.525).

Ceolin et al. (1996) carried out studies on the isothermal (25 °C) solid-state transformation of FI(6.747) into FII(6.403) as a function of time. The transformation was monitored by XRD analysis. The transformation occurred spontaneously after about two hours at room temperature. This pattern would indicate that the system follows Ostwald’s Rule of Stages. The unstable FI(6.747) was present at the start and it transformed to the nearest metastable state which can be reached with loss of free energy, namely FII(6.403). The reason that FII(6.403) did not transform to FIII(6.525) is probably because FII(6.403) has such a high relative stability when compared with the stable FIII(6.525) as indicated by Kuhnert-Brandstatter et al. (1994).

No solubility data for any of the polymorphs of piracetam has previously been reported in the literature. Some preliminary studies on the solid-state polymorphic transformation from FI(6.747) to FII(6.403) has been published as outlined above, but very little information on the other polymorphic transformations, both solid-state and solution mediated, has been reported.
Chapter 3. Materials & Experimental Methodologies

A condensed summary of the experimental method is given in the present section. For a more detailed description, see experimental sections in paper I through V.

3.1 Materials

Piracetam, complying with European Pharmacopoeia 6.5 quality and purity standards, was supplied by UCB Pharma SA (CAS Number: 7491-74-9, Batch Number: 09G06-B93. The Certificate of Analysis states that the batch complies with the IR, HPLC and solution appearance tests. It also complies with heavy metals limit of < 10 ppm, sulphated ash of < 0.1 %, water content of < 0.1% and the purity is 100 +/-2 %.). The solvents utilised during the course of the project and some of their properties from corresponding Material Safety Data Sheets (MSDS) are outlined in Table 3.1.

Table 3.1: The solvents used during the course of the project, along with their properties. GMM; Gram Molecular Mass, T_m; Melting Temperature, T_bp; Boiling Point.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Acronym</th>
<th>GMM (g.mol⁻¹)</th>
<th>T_m (°C)</th>
<th>T_bp (°C)</th>
<th>Density (g.cm⁻³)</th>
<th>Viscosity (cP = mPa .s)</th>
<th>CAS registry number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>MeOH</td>
<td>32.04</td>
<td>-97</td>
<td>65</td>
<td>0.792</td>
<td>0.544</td>
<td>67-56-1</td>
</tr>
<tr>
<td>Ethanol</td>
<td>EtOH</td>
<td>46.07</td>
<td>-114</td>
<td>78</td>
<td>0.789</td>
<td>1.074</td>
<td>64-17-5</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>1-Prop</td>
<td>60.1</td>
<td>-126</td>
<td>98</td>
<td>0.803</td>
<td>1.938</td>
<td>71-23-8</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>2-Prop</td>
<td>60.1</td>
<td>-89</td>
<td>83</td>
<td>0.786</td>
<td>2.073</td>
<td>67-63-0</td>
</tr>
<tr>
<td>Isobutanol</td>
<td>Isobut</td>
<td>74.12</td>
<td>-102</td>
<td>107</td>
<td>0.802</td>
<td>3.950</td>
<td>87-83-1</td>
</tr>
<tr>
<td>Acetone</td>
<td>Acet</td>
<td>58.08</td>
<td>-94</td>
<td>57</td>
<td>0.791</td>
<td>0.306</td>
<td>67-64-1</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>1,4-Diox</td>
<td>88.11</td>
<td>11.8</td>
<td>101</td>
<td>1.033</td>
<td>1.370</td>
<td>123-91-1</td>
</tr>
<tr>
<td>Water</td>
<td>H₂O</td>
<td>18.02</td>
<td>0</td>
<td>100</td>
<td>0.998</td>
<td>1.000</td>
<td>-</td>
</tr>
</tbody>
</table>
3.2 Techniques

Since the differences between the polymorphs of a system can be very slight, it is important to employ a wide range of analytical techniques in order to identify the most suitable for polymorph characterisation and sample composition determination. The main techniques utilised are outlined.

3.2.1 X-ray Diffraction (XRD)

XRD is a qualitative and quantitative technique that is used for fingerprint characterisation of crystalline materials and their crystal structures. The diffraction pattern produced contains a range of peaks of different relative intensities at specific angles of diffraction, which is unique to a specific crystal structure. Crystal phases can be identified from these diffraction patterns.

Sample preparation involved grinding using a mortar and pestle and flattening of the sample on a silicon crystal zero-background disc using a glass slide. A Phillips PANalytical X’Pert MPD Pro PW3064 Sample Spinner was employed with a Cu Kα source (λ = 1.5418 Å) applied over the range 8 to 35°2θ, equipped with a nickel filter and a 0.5 ° divergence slit. A step size of 0.017°2θ, a count time of 33 s per step and a scan speed of 0.064°2θ/ s was employed. The generator was set to 40 kV and 35 mA. The samples were rotated at 4 rpm. Data analysis was completed using X’Pert HighScore Plus software (PANalytical).

High Temperature-XRD (HT-XRD) utilised an Anton Paar HTK1200 furnace. Samples were heated at 60 °C/min from room temperature (22 °C) to 90 °C, and then at 5 °C/min from 90 to 130 °C. The program was set up to hold the heating ramp at various temperatures and obtain XRD patterns. XRD patterns were also collected at room temperature before starting the heating profile and upon returning to room temperature after heating and up to 24 hr after.

During the course of this work a lot of emphasis was placed on the SMPT from FII(6.403) to FIII(6.525) and ex-situ XRD analysis was commonly employed to monitor the solid phase, a method of quantifying the polymorphic composition of samples of FII(6.403) and FIII(6.525) was devised. Numerous peak parameters of XRD patterns, such as intensity and area, have been employed as a means of quantifying the composition of polymorphic mixtures (Konotoyannis et al. 1997, Suryanarayanan 1989 & 1990, Bugay et al. 1996). Large variations in line shape resulting from differences in particle size can affect peak intensity, whereas peak area is more invariant to particle size differences (Campell et al. 2002). Diffraction patterns from the eleven calibration scans were analysed quantitatively, using X’Pert HighScore Plus software, by calculating peak areas and
intensities and relating to the percentage weight composition of the respective polymorphs. In agreement with the literature it was found that relative peak areas gave the most accurate results for the quantification analysis. The default settings used in X'Pert HighScore Plus were; minimum peak significance of 1.00, minimum tip width of 0.01°2θ, maximum tip width of 1.00°2θ, Peak base width of 2.00°2θ. Figure 3.1 shows the results of a ratio of the (101) FII(6.403) peak area at 16.0°2θ to the (014) FII(6.525) peak area at 25.8°2θ peak area, according to eq 3.1, plotted against the sample weight per cent composition of FIII(6.525).

\[
\% \text{ Area } FIII(6.525) = \left( \frac{FIII(6.525)\text{Peak Area}}{FII(6.403)\text{Peak Area}+FIII(6.525)\text{Peak Area}} \right) \times 100 \tag{3.1}
\]

An \(R^2\) value of 0.9986 was observed for the fit illustrating a good fit between calculated amounts and actual amounts present. The calibration curve for FII(6.403) was prepared in a similar manner by taking a ratio of the area of the FII(6.403) peak to the area of both the FII(6.403) and FIII(6.525) peaks. The calibration was found to be accurate to ±3 % with a number of mixtures where the composition was known. Analysis of standards with small amounts of FIII(6.525) present in FII(6.403) established that FIII(6.525) could be detected at levels as low as 0.5 %.

Figure 3.1: Calibration curve illustrating the relationship between the mass of FIII(6.525) and the calculated % area of FII(6.525) in a mixture of FII(6.403) and FIII(6.525), as measured by XRD.
The molecular visualisation and modelling software program, Mercury 3.0, was employed to compare and contrast the three polymorphs and generate theoretical XRD patterns for each form. The program reads Crystal Information Files (CIF’s) supplied by the CSD and automatically generates an XRD pattern for that crystal structure. The following assumptions are taken in generating patterns in Mercury 3.0.

- The Lorentz-polarisation correction assumes a laboratory X-ray source. No absorption is simulated. Fixed slit widths are assumed. No background is included.
- All non-hydrogen atoms are assumed to have isotropic atomic displacement parameters ($U_{iso}$) of 0.05 Å$^2$. Hydrogen atoms for which 3D coordinates are available are taken into account and assigned $U_{iso}$ values of 0.06 Å$^2$.
- The powder pattern simulator takes site occupation factors into account. This corrects the patterns generated for disordered structures read from CIF and SHELX Res files.
- All reflections have a symmetric pseudo-Voight peak shape with a full width half maximum of 0.1 $^\circ$θ, corresponding to medium resolution laboratory data.
- The (000) reflection is excluded.
- The default 2$\theta$ resolution is 50.0 $^\circ$, which, for the default CuK$\alpha$$_1$ radiation, corresponds to a direct space resolution of 3.0 Å.

3.2.2 Differential Scanning Calorimetry (DSC)

DSC is a technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. In this work heat flux DSC was used to analyse different thermal events, including melting and transition points. It was found to be the most accurate technique in establishing the temperature of the events.

Sample preparation involved grinding the crystals of interest using a mortar and pestle. 4 - 6 mg was added to the sample aluminium pan, and an aluminium cap was sealed onto the pan. The DSC was calibrated to run at the desired heating rate and subsequently, a baseline run was subtracted from the sample run in the output. A PerkinElmer instruments Pyris1 DSC was used.
3.2.3 Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR)

In this work ATR-FTIR was used to characterise the polymorphs of piracetam. As the name suggests Infrared Spectroscopy deals with the infrared region of the electromagnetic spectrum. Infrared spectra are the result of transitions between quantised vibrational energy states in a molecule ranging from the simple coupled motion of the two atoms of a diatomic molecule to much more complex motion of each atom in a large polyfunctional molecule. When the frequency of the IR beam matches the vibrational frequency of the bond, absorption occurs. The FTIR used in this work was equipped with an attenuated total reflection (ATR) accessory, which measures the changes that occur in a totally internally reflected IR beam when the beam comes in contact with the sample. The samples were prepared by grinding and then clamping a thin portion to the crystal of the ATR. The IR spectrum from 500 – 4000 cm\(^{-1}\) was collected and compared to the data in the literature to identify which polymorph (s) was present in the sample. A PerkinElmer precisely Spectrum 100 FTIR Spectrometer with a PerkinElmer precisely Universal ATR Sampling Accessory was used in this work.

3.2.4 Scanning Electron Microscopy (SEM)

SEM was employed as one method of visualising the habit of the crystal samples during this project. A beam of electrons, produced by the heating of a metallic filament, travels vertically down through the column of the microscope while under vacuum. The beam makes its way through the electromagnetic lenses which focuses and directs the beam down towards the sample. Once the beam hits the sample, backscattered electrons are reflected from the sample to detectors, converting them to a signal that is sent to a viewing screen producing an image. Sample preparation involves spreading the crystals of interest across one side of a double-sided sticky carbon tab. The other side is adhered to a stub that fits the holder in the SEM. To prevent the crystals from charging in the SEM, they are then coated with gold. The stub with the sample is placed in a vacuum chamber with a tungsten coil containing a piece of gold. The coil is heated until it vaporises the gold and coats the sample. The stub with the sample is then placed in the holder in the SEM and a vacuum is generated in the chamber before the imaging begins. A JEOL CarryScope Scanning Electron Microscope JCM-5700 was used.
3.2.5 Optical Microscopy

Optical Microscopy allowed on-line visualisation of crystal habits during the polymorphic transformations of piracetam, both solution mediated and solid-state. The set-up for the SMPT in methanol consisted of a 5 ml jacketed glass vessel viewed under a Sony HDR350VE video recorder with a 5X lens attached, as shown in Figure 3.2.

Figure 3.2: Experimental set-up for the observation of the SMPT from FII(6.403) to FIII(6.525) in methanol.

The set-up for the solid-state polymorphic transformations of piracetam employed a Linkam Scientific Instruments Ltd. TMS 94 temperature controller to heat the sample on the Optical Microscope (Zeiss Axioscope AxioImager MAT Reflected-Light Microscope) as shown in Figure 3.3. The optical microscope was also employed for monitoring the SMPT in the 5 ml jacketed glass vessel.
3.2.6 *In-situ* Infrared Analysis

A Mettler-Toledo ReactIR iC10 was used to monitor solution concentration during experiments. Employing mid-infrared spectroscopy (650-2000 cm\(^{-1}\)) and ATR technology, it collected IR spectra of the solution phase in a vessel. Experiments of this nature were carried out on a 130 g scale, to facilitate use of the IR probe. An un-calibrated measurement of the solution concentration was obtained by recording the absorption of the carbonyl stretch (1700 cm\(^{-1}\)) of piracetam in solution. The peak area is proportional to the concentration and was internally standardised by the peak area of a solvent peak. An example of one of these scans of a piracetam/2-propanol solution is shown in Figure 3.4, with the piracetam and 2-propanol peaks of interest highlighted.

Figure 3.3: Experimental set-up for the observation of solid-state polymorphic transformations of piracetam.
3.3 Experimental Methodologies

The principal experiments of the project involved the isolation and characterisation of the polymorphs of piracetam. Subsequently, where possible, solubility data was obtained. The focus then turned to investigating the polymorphic transformations of the system; both solution mediated and solid state. The mechanisms of the transformations were investigated as well as examining the effect of processing conditions.

3.3.1 Isolation of the Polymorphs of Piracetam

The commercial form of piracetam is FIII(6.525), the thermodynamically stable polymorph under ambient conditions. For all experiments, the commercial form was recrystallised into the desired form. In this section, the methodology of preparing each polymorph is briefly outlined. Extensive assessment of the polymorphic purity of each material was carried out after isolation using some characterisation techniques outlined above.

Heating of FII(6.403) or FIII(6.525) to 130 °C in the solid state resulted in the transformation to FI(6.747). This transformation is detailed in the sections 4.4 and 5.4 on the SSPT’s of piracetam. FI(6.747) can also be prepared by crash cooling crystallisation from a highly supersaturated solution of 1,4-dioxane, with very low levels of agitation, harvesting the crystals immediately.
FII(6.403) can be prepared via two routes; the SSPT from FI(6.747) under ambient conditions, which is dealt with in sections 4.4 and 5.4, and cooling crystallisation from 1,4-Dioxane in a HEL PolyBLOCK Parallel Synthesis reactor. Piracetam (11 g), dissolved in 200 ml 1,4-dioxane at 98 °C with agitation of 200 rpm was cooled to 20 °C at a rate of 1 °C/min. FII(6.403) was harvested by vacuum filtration after 24 hr and dried in an oven at 45 °C. Ten grams of FII(6.403) was isolated giving a yield of 91 %.

Firstly, FIII(6.525) can be prepared by cooling crystallisation from methanol. FIII(6.525) was produced using a HEL PolyBLOCK Parallel Synthesis reactor. Eighty grams of piracetam were dissolved in 200 ml methanol at 60 °C in a HEL PolyBLOCK Parallel Synthesis reactor with agitation of 200 rpm. The solution was then cooled to 10 °C at a rate of 1 °C/min. FIII(6.525) was harvested by vacuum filtration after 24 hr and dried in an oven at 45 °C. A 74 % yield gave 59 g FIII(6.525).

Secondly, since FIII(6.525) is the thermodynamically stable polymorph of piracetam, FII(6.403) transforms into FIII(6.525) in solution in some organic solvents. As shown in sections 4.3 and 5.3, the transformation rate is faster in polar solvents, with higher solubilities, and at higher temperatures.

3.3.2 Solubility of FII(6.403) and FIII(6.525)

The experimental setup for the solubility measurements in the project consisted of a thermostatic water bath (Grant GR150 with S38 stainless steel water bath; 38 L; 690-300-200 mm; @ 37 °C; stability ± 0.005 °C and uniformity ± 0.02 °C) with a serial magnetic stirrer plate placed on the base. Test tubes (150x25 mm) with a Teflon-coated magnetic stirrer were charged with each solvent and placed in a rack in the water bath which was set at the desired temperature. After 1 hr (temperature of solvent had equilibrated with the temperature of the water bath) excess solid of the desired polymorph was added to each test tube. The test tubes were agitated at 500 rpm.

Solubility data for the stable FIII(6.525) was obtained by gravimetric determination after an equilibration time of 48 hr over the temperature range, 5 – 50 °C, at increments of 5 °C, in five solvents; methanol, ethanol, 2-propanol, acetone and 1,4-dioxane. Data was also obtained for FIII(6.525) in isobutanol:water 95:5 (v/v) over the range 5 – 30 °C.

The stability of the metastable FII(6.403) in contact with different solvents, at different temperatures was examined prior to obtaining solubility data. It was established that the solubility of FII(6.403) could be measured at the point when FIII(6.525) was first detected in the solid phase using XRD analysis, or after a 48 hr equilibration time when the transformation did not begin within that time frame. Slurry samples, taken from the tubes using a disposable pasteur pipette, were vacuum filtered for approx. 90 seconds, drying the solids sufficiently for ex-situ XRD analysis. Solubility data
was obtained gravimetrically for FII(6.403) over the range 5 – 50 °C in four solvents; ethanol, 2-propanol, acetone and 1,4-dioxane. Solubility data was also obtained for FII(6.403) and FIII(6.525) in 1-propanol at 30 and 50 °C.

In the cases of both FII(6.403) and FIII(6.525) the solubility was determined by transferring samples of the clear saturated solution (approx 4 mL) from each test tube to three 50·25 mm clean dry weighed vials (mass of dry vial + cap = \( m_{\text{empty}} \)) using a preheated syringe. A 0.2 µm, 15 mm membrane diameter syringe filter was attached to the head of the syringe before the saturated solution was passed into the vials to ensure there was no suspended solid present. Caps were put on the vials immediately after solution had been filtered into them, in order to prevent solvent evaporation, and then re-weighed (mass = \( m_{\text{liq}} \)). The caps were then removed and the solvent was allowed to evaporate at room temperature in the fume hood for approximately 1 week. At this point only a solid residue remained in the vials and the drying process was completed by placing the vials in an oven at 60 °C for 3 days (Lenton Thermal Designs oven). The vials were then allowed to return to room temperature in the fume hood before reweighing with their caps (mass = \( m_{\text{dry}} \)). All weighing was carried out using a Mettler Toledo AX054 with a weighing capacity of up to 520 g and readability of 0.1 mg. A typical mass of solid residue was approx 0.1 g. The solubility of each polymorph in each solvent, \( C_s \), could then be calculated as shown in eq 3.2, expressed as g piracetam/g solvent.

\[
C_s = \frac{(m_{\text{dry}} - m_{\text{empty}})}{(m_{\text{liq}} - m_{\text{dry}})} \tag{3.2}
\]

### 3.3.3 Solution Mediated Polymorphic Transformation: FII(6.403) to FIII(6.525)

The FII(6.403) to FIII(6.525) SMPT was examined on three scales: 5 g, 25 g and 130 g. On the 5 g scale, analysis involved visualisation of the crystals during the transformation. Ex-situ XRD analysis of the solid phase was used to monitor the 25 g scale, while the 130 g scale employed in-situ infrared analysis of the solution phase in conjunction with ex-situ XRD analysis of the solid phase.

The experimental set-up on the 5 g scale consisted of a temperature controlled jacketed glass vessel as shown in Figure 3.2. FII(6.403) crystals, prepared by cooling crystallisation from 1,4-dioxane, were added to methanol solutions with different levels of saturation at 25 °C. The crystals were then monitored by one of two apparatuses; either the video recorder with 5X lens attached, as shown in Figure 3.2, or under magnification of 5X on the optical microscope. In the former case, a video of the
transformation was recorded, while in the latter case an image was captured every three minutes. The time scale for these transformations was approx. 20 hr. The resulting images were processed in Google Picasa 3™ to give a final video of the transformation process. The transformation was monitored until all traces of FII(6.403) had disappeared and the FIII(6.525) crystals had grown to their final size. The products of these experiments were analysed using XRD analysis to confirm the composition was indeed FIII(6.525).

The set-up for the transformation experiments on the 25 g scale was identical to that of the solubility studies. The experiments were monitored via ex-situ XRD analysis until the SMPT from FII(6.403) to FII(6.525) had gone to completion. Different factors that are known to affect the rate of SMPT’s were examined:

- Temperature: Every 5 °C from 5 to 50 °C.
- Solvent: Methanol, ethanol, 1-propanol, 2-propanol, isobutanol:water 95:5 (v/v), acetone, 1,4-dioxane.
- Agitation rate: 0, 200, 500, 1000 and 1350 rpm.
- Amount of solid to be transformed: 5.6 g FII(6.403)/kg ethanol, 21.6 g/kg, 38.0 g/kg, 54.0 g/kg.

The large scale experiments (130 g) were carried out in a 250 mL temperature controlled glass jacketed vessel. The solution phase was monitored via in-situ infrared analysis in conjunction with ex-situ XRD analysis of the solid. This type of analysis gave an insight into the governing mechanism of the transformation. At 30 and 50 °C, the effect of temperature on the SMPT was analysed using these techniques on a 130 g scale. The effect of pre-treatment of the loaded FII(6.403), manipulating the surface area per unit mass, or the specific surface area, of FII(6.403) in the vessel was also investigated.

### 3.3.4 Solid State Polymorphic Transformations of Piracetam

The transformations from FII(6.403) and FII(6.525) to FII(6.747) were investigated by heating samples of each polymorph, while the transformation from FII(6.747) to FII(6.403) upon cooling was also examined. The transformations were analysed using DSC, HT-XRD, Hot-Stage Optical Microscopy and thermal analysis in an oven, characterising the transformations and identifying the true thermodynamic temperature of the thermal events in both cases. Mixtures of both polymorphs were also examined.
Chapter 4. Results

4.1 Characterisation of the Polymorphs of Piracetam

Figures 4.1, 4.2 and 4.3 show the XRD patterns of FI(6.747), FII(6.403) and FIII(6.525) respectively compared to the corresponding theoretical profiles generated from the CIF files in the CSD (Allen 2002). Each experimental pattern is identical to the corresponding theoretical pattern, and no additional peaks are observed, demonstrating pure samples at the resolution of the technique (± 0.5 %).

Figure 4.1: The experimental (Exptl) FI(6.747) XRD pattern, prepared by heating FII(6.403) to 140 °C, compared to the theoretical pattern from the CSD.

Figure 4.2: The experimental (Exptl) FII(6.403) XRD pattern, prepared by cooling crystallisation from 1,4-Dioxane, compared to the theoretical pattern from the CSD.
Figure 4.3: The experimental (Exptl) FIII(6.525) XRD pattern, prepared by cooling crystallisation from Methanol, compared to the theoretical pattern from the CSD.

The ATR-FTIR spectra for FII(6.403) and FIII(6.525) is shown in Figure 4.4. The most obvious regions unique to each polymorph are located from 1100 to 1250 cm\(^{-1}\) and 2850 to 3050 cm\(^{-1}\) as outlined by Pavlova et al. (1979) and Kuhnert-Brandstaetter et al. (1994) A triplet and doublet of equal intensity are observed in these regions in FII(6.403), while one peak is more intense than the others in the case of FIII(6.525). Characterisation of FII(6.403) and FIII(6.525) via DSC is discussed in detail in sections and 5.4 below.

Figure 4.4: ATR-FTIR spectra for pure FII(6.403) and FIII(6.525) piracetam, with the regions unique to each polymorph highlighted.
SEM and Optical Microscopy were used to view the habit of each of the polymorphs when isolated by cooling crystallisation. Figure 4.5 shows an optical micrograph of FI(6.747) (left) and SEM images of FII(6.403) (middle) and FIII(6.525) (right). FI(6.747) and FII(6.403) have similar rod like habits, with well-defined faces. FIII(6.525) on the other hand, has a chunky hexagonal shaped habit with well-defined faces.

![Figure 4.5: An optical micrograph of FI(6.747) (left) crystallised from 1,4-Dioxane immediately after crash cooling, and SEM images of FII(6.403) (middle) and FIII(6.525) (right) harvested from 1,4-Dioxane and Methanol respectively, 24 hr after cooling crystallisation.](image)

### 4.2 Solubility of FII(6.403) and FIII(6.525)

The solubility is represented in terms of mole fraction on total basis for FIII(6.525) and FII(6.403) in Figure 4.6 and 4.7 respectively. It is clear that the solubility of piracetam increases with temperature. The solubility decreases as the number of carbons in the n-alcohols increases from methanol to ethanol to 2-propanol, which indicates a decreasing solubility with decreasing solvent polarity and hydrogen bond donor ability. 1,4-dioxane and acetone are relatively non-polar solvents when compared to alcohols and accordingly piracetam is much less soluble in these solvents. In addition, 1,4-dioxane and acetone are aprotic solvents, and cannot hydrogen bond to the proton accepting carbonyl oxygen of the solute molecule. Alcohols are protic and can interact not only with the hydrogen bond donating amino groups but also with the carbonyl oxygen of the solute. The water content in the isobutanol:water solvent mixture increases the solubility of piracetam significantly, as a result of the increase in polarity of the mixture.
4.3 Solution Mediated Polymorphic Transformation: FII(6.403) to FIII(6.403)

The result of these experiments was a series of XRD patterns of samples collected as the SMPT from FII(6.403) to FIII(6.525) took place. Figure 4.8 is a quantitative output of the composition of the solid
phase during the transformation in repeated runs at 25 °C in ethanol, indicating that the data is reproducible.

The point at which the stable form is first detected in the solid phase, the best estimate of nucleation of the stable FIII(6.525), is interpreted as the induction time of the transformation. The total time for completion of the transformation after the addition of the excess FII(6.403), determined as the moment when the composition of the solid phase is 100 % FIII(6.525), is expressed as the transformation time.

4.3.1 Effect of Processing Conditions on the Transformation

The induction and transformation times of the experiments on a 25 g scale in ethanol are presented in Figure 4.9, over the temperature range 5 to 50 °C. It is obvious that the rate of transformation increases with temperature. The induction time decreases from 43 hr at 5 °C to 2.5 hr at 50 °C, while the transformation time decreases from 93 hr at 5 °C to 6.75 hr at 50 °C.
The effect of solvent is outlined in Figure 4.10 at 25 °C, where the percentage of FII(6.403) in the solid phase during the transformation in each of the solvents is shown. Solvent has a major effect on the transformation rate, with the transformation time range spanning from less than 10 hr in methanol to almost 200 hr in 2-propanol. The reason 1,4-Dioxane is not documented is that the transformation to the stable form was not observed to take place in this solvent even at the highest temperature (50 °C) in the time frame examined (300 hr).
The combined effect of temperature and solvent on the transformation is presented in Figure 4.11, where the inverse of the induction time for the transformation is plotted against temperature in the solvents examined.

![Image](image.png)

**Figure 4.11:** The inverse of the induction time for the transformation from FII(6.403) to FIII(6.525) plotted against temperature in a range of solvents from 5 – 50 °C. ♦ methanol; ❁ isobutanol:water 95:5 (v/v); ■ ethanol; × 2-propanol; ● acetone.

The rate of agitation was found to have a significant effect on the transformation rate. With no agitation the transformation time in methanol at 25 °C was 27.5 hr. The periodic sampling of the solid phase provided slight agitation, therefore it would be expected that the transformation would have taken longer without this agitation. A clear trend is observed in Figure 4.12; as the level of agitation increases from 200 rpm to 1350 rpm, the transformation to the stable form occurs in a shorter time frame.
Figure 4.12: Percentage of FII(6.403) in the solid phase during the investigation of agitation levels on the transformation. × (purple), 1350 rpm; ▲ (green), 1000 rpm; ■ (red), 500 rpm; ♦ (blue), 200 rpm.

As outlined in section 3.3.3 the SMPT was carried out on a number of different scales. The resulting effect agrees with data reported in the literature (Davey et al. 2001 & 2002). At 30 °C the mass of solids to solvent ratio on the 25 g scale (0.020) is slightly lower than the 130 g scale (0.024). The induction time and transformation time in ethanol on the smaller scale are 9 and 21 hr respectively, while on the larger scale these values are 17 and 30 hr. The equipment surface area for collision per unit volume of suspension, which helps propagate nucleation of the stable form, is higher on the smaller scale. Also, the magnetic stir bar agitator on the smaller scale increases the extent of attrition of the crystals, compared to the overhead impeller. The influence of scale on the induction and transformation times is less pronounced at 50 °C but a similar trend is observed.

4.3.2 Mechanistic Features of the Transformation

There are three steps involved in the SMPT from FII(6.403) to FIII(6.525): nucleation of FIII(6.525), growth of FIII(6.525) and dissolution of FII(6.403).

Figures 4.13 & 4.14 show portions of the transformation in ethanol at 50 & 30 °C respectively, as monitored via in-situ infrared analysis of the solution phase and ex-situ XRD analysis of the solid phase. The equilibrium saturation of the metastable polymorph is reached within a short period of time after adding FII(6.403). In both cases FIII(6.525) is not detected in the solid phase for a considerable amount of time, while the concentration in solution remained at the solubility of
FII(6.403) even with significant amounts of FIII(6.525) in the solid phase. The governing mechanism of the transformation appears to be the same across the temperature range of this study. Comparing the transformation times in both cases again illustrates the effect of temperature on the transformation.

Figure 4.13: A portion of the Concentration-Time profile and polymorphic composition of the solid phase during the transformation, FII(6.403) \( \rightarrow \) FIII(6.525), at 50 °C in ethanol (R1). Blue; Concentration of Piracetam in Solution. Red ■; Percentage FII(6.403) in solid phase. Green ▲; Percentage FIII(6.525) in solid phase. Increase in concentration after 2.7 hr corresponds to liquid nitrogen addition to the ReactIR iC10 to keep the detector cool.

Figure 4.14: A portion of the Concentration-Time profile and polymorphic composition of the solid phase during the transformation, FII(6.403) \( \rightarrow \) FIII(6.525), at 30 °C in ethanol (R1). Blue; Concentration of Piracetam in Solution. Red ■; Percentage FII(6.403) in solid phase. Green ▲; Percentage FIII(6.525) in solid phase.
The dissolution of FII(6.403) firstly involves rounding of the ends of the rods, before the ‘stripping’ of layers of FII(6.403) along the length of the crystals (Figure 4.15). Moving from top to bottom, FII(6.403) crystals were agitated until equilibrium was achieved in 2-propanol – piracetam solutions of varying levels of saturation from 0.95 to 0.70, with respect to FIII(6.525). The resulting harvested crystals showed different levels of dissolution. The dissolution features can also be seen in Video 1 and in the initial stages of the Video 2 of the solution mediated transformation in Appendix B.
Figure 4.15: SEM images of FII(6.403) of varying levels of dissolution in 2-propanol. Levels of dissolution increases from first to last.

In Figure 4.16 is shown the absolute concentration of FIII(6.525) in the solid phase during the transformation experiments with different loadings of FII(6.403). The induction and transformation times appears to be similar in all four experiments, indicating that the number of nucleation points of FIII(6.525) increases accordingly with the available surface area of FII(6.403).
Figure 4.16: Absolute Concentration of FIII(6.525) in the solid phase during the investigation of the effect of different FII(6.403) loadings on the transformation in ethanol at 40 °C.  ♦ (blue), 5.6 g FII(6.403).kg⁻¹ ethanol in excess; ■ (red), 21.6 g.kg⁻¹ in excess; ▲ (green), 38.0 g.kg⁻¹ in excess; ● (purple), 54.0 g.kg⁻¹ in excess.

The effect of the amount of surface area of FII(6.403) available for nucleation of FIII(6.525) on the transformation time was further examined by carrying out three experiments, in which the total loading of FII(6.403) was the same in all three but the surface per unit mass of FII(6.403) was significantly higher in one (R1) compared to the other two (R2 & R3). The rate of the transformation in the pre-saturated (R2) and pre-supersaturated (R3) solutions was observed to be very similar, while the rate was slightly faster in the R1 experiment (Figure 4.17). The concentration in solution drops from the solubility of FII(6.403) after 5.2 hr in R1, while the plateau at the solubility of FIII(6.525) is reached after 6.7 hr. In R2 and R3 these events occur approximately one hour later. Examining the composition of the solid phase, 100 % FIII(6.525) is attained approx. one hour earlier in the case of R1 compared to the other two.

47
Figure 4.17: The Concentration-Time profiles and percentage FII(6.403) in the solid phase during the transformations, FII(6.403) → FIII(6.525), at 50 °C in ethanol with different starting solution concentrations. Blue (R1); Starting solution of pure ethanol, Red (R2); Starting solution of ethanol saturated w.r.t. FIII(6.525), Green (R3); Starting solution of supersaturated ethanol ($\sigma = 1.1$, w.r.t. FIII(6.525)). Blue ▲ (R1); % FII(6.403) in Pure ethanol starting solution experiment; Red ■ (R2); % FII(6.403) in saturated ethanol starting solution experiment; Green ● (R3); % FII(6.403) in supersaturated ethanol.

The mechanism of the transformation was further examined on a 5 g scale using in-situ microscopy, allowing for visualisation of the changes in crystal habit. Video 2, in Appendix B, allows visualisation of the changes in crystal habit during the transformation on a 5 g scale using in-situ microscopy. A sample of the images during one of these experiments is presented in Figure 4.18, with some of the FIII(6.525) nucleation points highlighted in red.
4.4 Solid State Polymorphic Transformations of Piracetam

4.4.1 Transformation of FII(6.403) or FIII(6.525) to Fl(6.747)

FII(6.403) and FIII(6.525) are known to transform to Fl(6.747) at elevated temperatures (Kuhnert-Brandstaetter et al. 1994, Picciochi et al. 2011, Ceolin et al. 1996, Fabbiani et al. 2005 & 2007). Variation in the composition of samples of FII(6.403) and FIII(6.525) during the transformation to Fl(6.747) in HT-XRD analysis is shown in Figure 4.19 & 4.20. Corresponding peaks for the original polymorphs in the samples disappear during heating, and are replaced by the peaks representative of Fl(6.747).
DSC provides a more accurate estimation of the temperature of the thermal event. The scans obtained for FII(6.403) and FIII(6.525) with a heating rate of 5 °C/min are presented in Figure 4.21, with the properties summarised in Table 4.1. The first endothermic peak on each of the scans represents the transformation to FI(6.747). The sharp endothermic peak seen at approximately 154 °C represents the melting of FI(6.747).
Figure 4.21: DSC scans of pure FII(6.403) (red) and FIII(6.525) (blue) with a heating rate of 5 °C/min. Endothermic peaks indicating the transformation to FI(6.747) and then melting of FI(6.747).

Table 4.1: DSC data for FII(6.403) and FIII(6.525) at a heating rate of 5 °C/min.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Event</th>
<th>Peak Onset (°C)</th>
<th>Peak Centre (°C)</th>
<th>ΔH_{trans} (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FII(6.403)</td>
<td>Transformation to FI(6.747)</td>
<td>109</td>
<td>117</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>Melting of FI(6.747)</td>
<td>150</td>
<td>154</td>
<td>25.4</td>
</tr>
<tr>
<td>FIII(6.525)</td>
<td>Transformation to FI(6.747)</td>
<td>116</td>
<td>123</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>Melting of FI(6.747)</td>
<td>150</td>
<td>154</td>
<td>25.5</td>
</tr>
</tbody>
</table>

In Figure 4.22 is shown the changes to the crystals during the transformation of FIII(6.525) to FI(6.747) as visualised using hot-stage optical microscopy. The FI(6.747) nucleation point is highlighted in red in the relevant image. A video of the transformation is included in Appendix B. The transformation of FII(6.403) to FI(6.747) showed similar visual features.
Figure 4.22: A series of optical micrographs showing the visual changes to the FIII(6.525) crystal during the transformation to FI(6.747).

Mixtures of FII(6.403) and FIII(6.525) were analysed using HT-XRD (Figure 4.23) and DSC (Figure 4.24) in order to determine whether or not the FII(6.403)-FIII(6.525) thermodynamic transition point could be achieved experimentally and if the transformation from the metastable FII(6.403) to stable FIII(6.525) occurred in the solid state. The only transformation observed was directly from the original polymorph mixture to FI(6.747).
Figure 4.23: Theoretical XRD patterns of FII(6.403) (red), FIII(6.525) (orange) and FII(6.747) (blue) from CSD compared to XRD patterns of the FII(6.403):FIII(6.525) 25:75 sample collected at 25 °C, 105 °C and 110 °C.

Figure 4.24: DSC scans of FII(6.403) (red) and FIII(6.525) (blue) and mixtures of both; heating rate of 10 °C/min. FII(6.403):FIII(6.525) 25:75 (purple), FII(6.403):FIII(6.525) 50:50 (green), FII(6.403):FIII(6.525) 75:25 (orange). All scans show two endothermic peaks indicating the transformation to FII(6.747) at in the range 110 to 123 °C and a melting of FII(6.747) at 152 °C.

DSC scans of FII(6.403) and FIII(6.525) at heating rates of 10 °C/min and higher consistently showed a small endothermic peak at 139 and 138 °C respectively. These peaks, shown in Figure 4.25 in a section of the DSC scans of both polymorph, are believed to represent the melting of trace amounts of the original polymorph in the sample, which have not transformed to FII(6.747).
Figure 4.25: A section of the DSC scans of pure FII(6.403) (red) and FIII(6.525) (blue) with a heating rate of 10 °C/min. Endothermic peaks indicating, firstly, the transformation to FII(6.747), the melting of trace amounts of the original polymorph, and finally melting of FII(6.747).

4.4.2 Transformation of FII(6.747) to FII(6.403)

Figure 4.26 shows that FII(6.747) transforms to FII(6.403) upon cooling. FII(6.747) consistently transformed to FII(6.403), and not FIII(6.525). However the transformation did not occur at the same temperature as the transformation in the opposite direction. Figure 4.27 shows a DSC scan of the transformation of FIII(6.525) to FII(6.747) upon heating (blue). The sample was then cooled to room temperature but the transformation from FII(6.747) to FII(6.403) was not observed. The HT-XRD analysis shows that this transformation occurs after a few hours at room temperature.
Figure 4.26: Theoretical XRD patterns of Fl(6.747) and FlI(6.403) from CSD compared to XRD patterns of the transformed FlII(6.525) sample when returned to room temperature (RT) (22 °C), 4 and 24 hours after RT was reached.

Figure 4.27: DSC scan of pure FlII(6.525) with a heating rate of 10 °C/min from 20 to 135 °C (blue) with an endothermic peak indicating the transformation to FlI(6.747). After reaching 135 °C, the temperature was held for 5 min and then decreased to 20 °C at 5 °C/min (red).
Chapter 5. Analysis & Discussion

5.1 Molecular Analysis of the Polymorphs of Piracetam

Analysis of the polymorphs on a molecular level was carried out using the Mercury 3.0 software program. Some of the properties of the polymorphs are already shown in the introduction section. Each molecule in the crystal structure of all three polymorphs contributes to four hydrogen bonds. In all three polymorphs, there are two sets of identical hydrogen bonds making up the four in total. In the case of FII(6.403) and FIII(6.525) a dimer bond formation with another piracetam molecule consumes two of the hydrogen bonds. This formation extends throughout the crystal structure in chains, with each dimer linked to the next and previous dimer via the remaining two hydrogen bonds. In the case of FI(6.747) the four bonds of each molecule are connected to four separate molecules in a plate like formation. The properties of these bonds are outlined in Table 5.1, according to the labelling in Figure 5.1.

Table 5.1: The bond lengths and angles of the hydrogen bonds associated with the molecules of piracetam in the three polymorphs. Atom labels correspond to labels in Figure 5.1.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Property</th>
<th>Units</th>
<th>FI(6.747)</th>
<th>FII(6.403)</th>
<th>FIII(6.525)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 – N2</td>
<td>Length</td>
<td>Å</td>
<td>2.894</td>
<td>2.942</td>
<td>2.945</td>
</tr>
<tr>
<td>N2 – O1</td>
<td>Length</td>
<td>Å</td>
<td>2.928</td>
<td>2.963</td>
<td>2.971</td>
</tr>
<tr>
<td>O2 – N2 – O2</td>
<td>Angle</td>
<td>°</td>
<td>146.11</td>
<td>87.12</td>
<td>86.07</td>
</tr>
<tr>
<td>O1 – N2 – O2</td>
<td>Angle</td>
<td>°</td>
<td>98.38</td>
<td>128.04</td>
<td>125.08</td>
</tr>
</tbody>
</table>

The main feature of the crystal structure in FII(6.403) and FIII(6.525), the hydrogen bonded dimer chain, is shown along the (010) and (100) planes in Figures 5.2 and 5.3 respectively. In the case of FII(6.403) each chain is identical, while every second chain in FIII(6.525) is identical to the FII(6.403) chains. The molecules in every other chain of FIII(6.525) are rotated 180° along the (010) and (001) planes. The crystal structure of FI(6.747) is significantly different from FII(6.403) and FIII(6.525), with shorter hydrogen bonds. The alternative crystal structure of FI(6.747), compared to FII(6.403) and FIII(6.525) infers it’s at higher temperature (Kuhnert-Brandstaetter et al. 1994, Picciochi et al. 2011, Ceolin et al 1996).
Figure 5.2: FII(6.403) (left) and FIII(6.525) (right) molecular packing viewed along the b-axis or (010) plane.

Figure 5.3: FII(6.403) (top) and FIII(6.525) (bottom) molecular packing viewed along the a-axis or the (100) plane.
FII(6.403) grows fastest along the \( \alpha \)-axis, corresponding to the rod like habit of the crystals. It is interesting that the angles at the end of the rods are not right angles (Figure 4.5). As can be seen in Figure 5.2, the dimer chains are tilted slightly in relation to the \( \alpha \)-axis, when looking along the \( b \)-axis. Figure 5.4 shows an extension of the dimer chains viewed down along the \( b \)-axis, with planes cutting the \( a \) ((100) & (200)) and \( c \) ((001) & (002)) axis’ highlighted. These planes represent the possible side and end faces of the crystals, depending on the chemical entities that are present at the surfaces. It is thought that when the crystal grows in the \( \alpha \) direction and reaches its final size, it results in an end face that is not at a right angle to the length of the crystal. The theoretical and experimental angle values are identical (78 & 112 \(^\circ\)).

![Figure 5.4: The molecular packing of FII(6.403) piracetam viewed along the \( b \)-axis or the (010) plane. Included are the (100) and (200) planes with cut the \( \alpha \)-axis and the (001) and (002) planes which cut the \( c \)-axis.](image)

### 5.2 Solution & Solid Phase Analysis

The range of analytical techniques employed for analysis of solution and solid phase composition ensured a comprehensive examination of the relevant samples. *In-situ* mid IR analysis of the solution phase is fast becoming a very popular method of monitoring the solution concentration. There are
examples in the literature where the system was calibrated and arbitrary values for the actual concentration in solution were taken (Ó’Ciaradhá et al. 2012). In this study, it was employed qualitatively. The area under the C = O piracetam peak at 1700 cm\(^{-1}\) was monitored and internally standardised by a solvent peak. A decrease in the intensity of the solvent peak would be expected with changes of the concentration of piracetam in solution during SMPT’s. However, since the difference in solubility between the two polymorphs of interest in this study is very small, the decrease in intensity of the solvent peak had a negligible effect.

XRD was the technique most commonly employed for analysis of solid samples. Theoretical patterns generated from CIF files using Mercury 3.0 were used as pure standards for comparison of samples. Sample preparation and analysis was uncomplicated and time efficient allowing for fast and accurate analysis. Sample preparation played a major role in the amount of preferred orientation observed in the resulting XRD patterns. Preferred orientation is the result of non-random orientation of the crystals with respect to the incoming X-ray beam. The habit of the crystals is the main contributing factor. Platelets or needle shaped crystals, observed in the polymorphs of piracetam, tend to lie preferentially on the basal planes, resulting in the X-ray beam hitting a particular face of the crystal much more than the others. Preferred orientation effects make polymorph identification from the XRD patterns more difficult and can mask impurities which are present at low levels. Therefore sample preparation involved grinding of the samples with a mortar and pestle to reduce the preferred orientation effects. The limit of detection for any of the three polymorphs in sample mixtures was found to be 1 %. Even though ATR-FTIR is perceived to have a lower detection limit than XRD, the FTIR spectra for FII(6.403) and FIII(6.525) were not as distinctive as the respective XRD patterns.

SEM and Optical Microscopy gave a visual representation of samples, aiding in analysis of the habit of the polymorphs and the position of different polymorphs in a mixture. Because SEM samples required gold coating to prevent charging, this technique was only used in off-line mode. Advantages of SEM over other types of microscopy include a larger depth of field and higher resolution. Optical Microscopy was used in off-line mode as well as on-line in monitoring the SSPT’s of the system. A Sony HDR350VE video recorder with a 5X lens attached, as shown in Figure 3.2, was used to visualise the changes in crystal habit during the SMPT in a 5 ml temperature controlled glass jacketed vessel. DSC was utilised in the characterisation of the polymorphs as well in monitoring the SSPT’s of the system. Compared to HT-XRD and Hot-Stage Optical Microscopy, it provides a more accurate identification of the temperature of the thermal event. Used in conjunction with each other, these three techniques give a thorough overview, monitoring the composition of the sample, the visual changes and identifying the temperature of the event.
Comprehensive analysis using a range of analytical techniques ensured that pure batches of the relevant polymorphs were prepared for the subsequent studies.

### 5.3 Solubility of FII(6.403) and FIII(6.525)

Since FIII(6.525) is known to be the thermodynamically stable polymorph in the range of this study, its solubility in any solvent could simply be measured after an equilibration time. In the case of the metastable FII(6.403), it had to be guaranteed that on one hand sufficient time for equilibrium to be reached was allowed, and on the other hand the solid form present was still the metastable form. Accordingly, both the solution and solid phases were monitored, via *in-situ* mid IR and *ex-situ* XRD analyses respectively, in solvents at temperatures spanning the range to be examined. Figure 5.5 shows the case of 2-propanol at 50 °C. Very soon after the addition of the FII(6.403) to the solvent a plateau in solution concentration is reached above the solubility of the stable FIII(6.525). This plateau is maintained for approx. 63 hr. The solution is in equilibrium with FII(6.403) in the solid phase for the first 30 hr of this plateau. Therefore, the author is confident that this solution concentration represents the solubility of FII(6.403). FIII(6.525) is detected in the solid phase after 30 hr. However, the solution concentration remains at the solubility of FII(6.403) until the composition of the solid phase is predominantly FIII(6.525) (> 80 %). In measuring the concentration of piracetam in solution at the point where FIII(6.525) is first detected in the solid phase, the author is confident that the solubility of FII(6.403) has been reached, while the transformation to the stable form has not yet resulted in a decrease in the solution concentration from the solubility of FII(6.403).
Figure 5.5: A Portion of the Concentration-Time profile and Polymorphic Composition of the Solid Phase during the Transformation, FII(6.403) → FIII(6.525), at 50 °C in 2-propanol. ■ (red); Percentage FII(6.403) in Solid Phase. ▲ (Green); Percentage FIII(6.525) in Solid Phase. Blue; Concentration of Piracetam in Solution (Peak Area Ratio).

As expected, the solubility of FII(6.403) was found to be slightly higher than FIII(6.525) in all solvents at all temperatures, reflecting it’s metastability. In Figure 5.6 is shown the solubility of FII(6.403) and FIII(6.525) in 2-propanol.

Figure 5.6: Mole Fraction Solubility curves for FII(6.403) (■) and FIII(6.525) (♦) in 2-Propanol over the Temperature Range 5 – 50 °C.
As given by Table 5.2, the ratio of the mole fraction solubility of FII(6.403) to FIII(6.525) is weakly dependent on the solvent and decreases slightly with increasing temperature; from an average of 1.151 at 5 °C to 1.026 at 50 °C. A detailed study on 55 polymorphic compounds showed a ratio between the highest and lowest solubility polymorphs to have an average of 1.7, with a maximum at 4.7 and a minimum close to unity (Pudipeddi & Serajuddin 2004). Since solubility reflects the stability hierarchy of polymorphs in a system, and such a small difference in free energy is reported between FII(6.403) and FIII(6.525) (Kuhnert-Brandstaetter 1994, Pavlova 1979, Pavlova et al. 1983), it is not surprising that the solubility ratio is at the lower end of this scale. Because of a fast transformation rate in methanol, especially at higher temperatures, it was not possible to obtain reliable solubility data in this solvent.

Table 5.2: The mole fraction solubility ratio between FII(6.403) and FIII(6.525) in four solvents.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Ethanol</th>
<th>2-Propanol</th>
<th>Acetone</th>
<th>1,4-Dioxane</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.118</td>
<td>1.174</td>
<td>1.161</td>
<td>n/a</td>
</tr>
<tr>
<td>10</td>
<td>1.115</td>
<td>1.118</td>
<td>1.149</td>
<td>n/a</td>
</tr>
<tr>
<td>15</td>
<td>1.101</td>
<td>1.092</td>
<td>1.133</td>
<td>1.095</td>
</tr>
<tr>
<td>20</td>
<td>1.081</td>
<td>1.089</td>
<td>1.119</td>
<td>1.080</td>
</tr>
<tr>
<td>25</td>
<td>1.053</td>
<td>1.068</td>
<td>1.093</td>
<td>1.071</td>
</tr>
<tr>
<td>30</td>
<td>1.050</td>
<td>1.060</td>
<td>1.074</td>
<td>1.057</td>
</tr>
<tr>
<td>35</td>
<td>1.038</td>
<td>1.041</td>
<td>1.061</td>
<td>1.052</td>
</tr>
<tr>
<td>40</td>
<td>1.029</td>
<td>1.033</td>
<td>1.049</td>
<td>1.040</td>
</tr>
<tr>
<td>45</td>
<td>1.031</td>
<td>1.030</td>
<td>1.042</td>
<td>1.030</td>
</tr>
<tr>
<td>50</td>
<td>1.025</td>
<td>1.022</td>
<td>1.029</td>
<td>1.026</td>
</tr>
</tbody>
</table>

In Figure 5.7 is presented the van’t Hoff plot of the mole fraction solubility of FII(6.403) in four solvents. The van’t Hoff enthalpy of solution can be calculated from the slope of this plot according to eq 2.8.
Figure 5.7: A van’t Hoff plot of the Mole Fraction Solubility ($\ln(x)$) of FII(6.403) in Different Solvents against Temperature ($T$) with a Straight Line fitted to the Data in each Solvent. ■, ethanol; ×, 2-propanol; ●, acetone; ▲, 1,4-dioxane.

The corresponding values for FII(6.403) and FIII(6.525) in each of the solvents are presented in Table 5.3. In all solvents the van’t Hoff enthalpy of solution is slightly lower for FII(6.403) compared to FIII(6.525).

Table 5.3: Estimated van’t Hoff enthalpy of solution ($\Delta H^{\text{soln}}_{\text{vH}}$) values for FII(6.403) and FIII(6.525) in the four solvents examined in the solubility study.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>FII(6.403) $\Delta H^{\text{soln}}_{\text{vH}}$ (kJ/mol)</th>
<th>FIII(6.525) $\Delta H^{\text{soln}}_{\text{vH}}$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>31.5</td>
<td>33.1</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>33.9</td>
<td>36.0</td>
</tr>
<tr>
<td>Acetone</td>
<td>26.4</td>
<td>28.5</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>30.3</td>
<td>31.8</td>
</tr>
</tbody>
</table>

Regression equations, eq 5.1 and 5.2, were fitted to the solubility data in order to estimate the melting temperature of FII(6.403) and FIII(6.525), and estimate the thermodynamic transition point between the polymorphs (Nordstrom & Rasmuson 2009, Grant et al. 1984). The regression equations were successfully fitted to the solubility data using Microsoft Office Excel 2010 and Matlab 6.5. $R^2$ values of greater than 0.995 were obtained.
\begin{align*}
\ln(x) &= A + BT \\
\ln(x) &= AT^{-1} + B + CT
\end{align*}

[5.1]  
[5.2]

Determination of melting temperatures of \text{FII}(6.403) and \text{FIII}(6.525) is difficult because of the enantiotropic relationship of both polymorphs to \text{FI}(6.747), which results in the transformation to \text{FI}(6.747) in the range 108 to 126 °C. Pavlova \textit{et al.}\textsuperscript{11} stated that the melting points are in the region of 145 to 153 °C. At increasing temperature, solubility data should gradually approach the conditions at the melting temperature where \(\ln(x) = 0\) and the van’t Hoff enthalpy of solution equals the melting enthalpy \textit{(Nordstrom & Rasmuson 2009)}. Hence, eq 5.1 and 5.2 can be used to estimate the melting points of \text{FII}(6.403) and \text{FIII}(6.525). Table 5.4 shows data for each solvent. The transition point between \text{FII}(6.403) and \text{FIII}(6.525) can be estimated by calculating the intersection point between the equations of the respective polymorphs in each solvent.

\textbf{Table 5.4: Estimated Melting Temperatures for FII(6.403) and FIII(6.525) and Estimated Transition Temperatures Calculated by Extrapolation of eq 5.1 and eq 5.2 in each Solvent, along with Reported Data.}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Eq 1: (\ln(x) = A + BT)</th>
<th>Eq 2: (\ln(x) = AT^{-1} + B + CT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Melting Point (°C)</td>
<td>Transition Temperature (°C)</td>
</tr>
<tr>
<td>\text{FII}(6.403)</td>
<td>126</td>
<td>56</td>
</tr>
<tr>
<td>\text{FIII}(6.525)</td>
<td>123</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>129</td>
<td>53</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>181</td>
<td>58</td>
</tr>
<tr>
<td>Acetone</td>
<td>178</td>
<td>62</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>170</td>
<td>62</td>
</tr>
<tr>
<td>Mean</td>
<td>153</td>
<td>57</td>
</tr>
<tr>
<td>Literature</td>
<td>140.7</td>
<td>100 – 138</td>
</tr>
<tr>
<td>\textit{Kuhntert-Brandstaetter 1994, Picciochi et al 2011}</td>
<td>140.2</td>
<td>100 – 138</td>
</tr>
</tbody>
</table>

\textit{notes:}

-
When eq 5.1 is fitted to the data, the melting point of FII(6.403) is higher than that of FIII(6.525) in all four solvents, while for eq 5.2 the melting point of FIII(6.525) is predicted to be higher in ethanol and 2-propanol. Since solubility data proved that FIII(6.525) is the stable polymorph under ambient conditions and an enantiotropic relationship exists between FII(6.403) and FIII(6.525) (Kuhnert-Brandstaetter 1994, Picciochi et al 2011) it becomes apparent that FII(6.403) should have a higher melting point than FIII(6.525). While the predicted melting points in the individual solvents show a certain level of variation, the averaged values are in the range outlined in the literature. Collecting data for a number of solvents increases the accuracy of the predications. The mean of the predicted values in the four solvents using eq 5.2 is within 5 °C of the melting points proposed by Kuhnert-Brandstaetter et al. (1994), while the mean for eq 5.1 is in the range outlined by Pavlova at al. (1983). The error in the predictions is approximately equal to the error reported for the predictions by Nordström and Rasmuson (2009).

Figure 5.8 shows the van’t Hoff plot for the solubility of both polymorphs in ethanol, with straight lines (eq 5.1) extrapolated to the melting points. The calculated transition temperature depends on the solvent and the equation but appears to be in the range 62 ± 10 °C. Since FIII(6.525) was predicted to have a higher melting point than FII(6.403) in ethanol and 2-propanol when eq 5.2 is fitted to the data, it follows that no transition point between the two polymorphs is predicted. In Figure 8 the transition point between FII(6.403) and FIII(6.525), where the two lines intersect each other (56 °C), is very close to the limit of the solubility study (50 °C). Thermal analysis found that the enantiotropic transition point between the FII(6.403) and FIII(6.525) is located in the range 100 – 138 °C (Kuhnert-Brandstaetter 1994, Picciochi et al 2011). Clearly the transition point predictions are not accurate in this case. The fact that the solubility difference between the two polymorphs is very small, and the temperature range of the solubility study is far removed from the actual transition and melting points makes it difficult to predict the transition point accurately in the case of piracetam. However, the method is useful in estimating such parameters when they are experimentally unavailable.
Figure 5.8: Mole Fraction Solubility (ln(x)) curves for FII(6.403) (♦) and FIII(6.525) (■) in Ethanol with fitted eq 5.1 extrapolated to the melting points.

5.3 Solution Mediated Polymorphic Transformation: FII(6.403) to FIII(6.403)

5.3.1 Effect of Processing Parameters on the Transformation

Using the solubility data of the polymorphs in ethanol for each experimental temperature the values of the thermodynamic driving force for the transformation (eq 2.1) are presented in Table 5.5, along with the induction time for the transformation.
Table 5.5: The thermodynamic driving force ($RT\ln S$) for the transformation over the temperature range 5 to 50°C along with the induction time for nucleation of FIII(6.525).

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$RT\ln S$ (J/mol)</th>
<th>Induction Time (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>259</td>
<td>43</td>
</tr>
<tr>
<td>10</td>
<td>256</td>
<td>31</td>
</tr>
<tr>
<td>15</td>
<td>230</td>
<td>23</td>
</tr>
<tr>
<td>20</td>
<td>191</td>
<td>15.5</td>
</tr>
<tr>
<td>25</td>
<td>129</td>
<td>11</td>
</tr>
<tr>
<td>30</td>
<td>123</td>
<td>9</td>
</tr>
<tr>
<td>35</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>40</td>
<td>74</td>
<td>5</td>
</tr>
<tr>
<td>45</td>
<td>81</td>
<td>3</td>
</tr>
<tr>
<td>50</td>
<td>66</td>
<td>2.25</td>
</tr>
</tbody>
</table>

In Figure 5.9, the induction time is plotted against the driving force. In accordance with the data in Figure 4.9 and Table 5.5, the induction time increases with increasing driving force, which at first is counter intuitive. However, the explanation is that the transformation driving force cannot be altered, without changing the temperature. Increasing temperature obviously increases the kinetics sufficiently to counteract the effect of the decreasing driving force.
The combined effect of temperature and solvent is documented in Figure 5.10, illustrating a trend of increasing concentration of piracetam in solution corresponding to faster transformation times. The higher the solubility of piracetam, regardless of temperature, the faster the rate of the transformation to the stable form. Piracetam is most soluble in methanol at 50 °C and accordingly, this experiment shows the shortest induction time. The transformation time results showed an identical pattern to the induction times. The kinetics of the transformation are affected greatly by temperature and solvent. Since supersaturation was found to be almost independent of solvent, the effect of solvent on the SMPT could be easily investigated (Figure 5.10). A representation of the pre-exponential ($A$) in the nucleation rate equation is given in eq 5.3. It increases with temperature directly through increasing solute concentration, which is also varied by the solvent choice, primarily because of an increased molecular attachment frequency.

$$A \propto \left( \frac{C \tau_0^3}{\eta} \right) \quad [5.3]$$

Where $C$ is the solute concentration and $\eta$ is the solution viscosity. The viscosity of the solution decreases with increasing temperature, also leading to an increase in $A$. Therefore nucleation of FII(6.525), which is a critical step in the transformation, is more likely to occur.
One solvent was consistently found to disagree with the trend of increasing solubility corresponding to faster transformation rates. This is illustrated in Figure 5.11 where the five solvents are compared at 30 °C. Concentrating on the pure alcohols methanol (red), ethanol (blue) and 2-propanol (grey), the time taken to reach the induction time of the transformation increases with decreasing solubility as the polarity of the solvent decreases. The water content in the isobutanol:water mixture increases the solubility of piracetam in the solvent mixture dramatically and accordingly we see the induction time for the transformation reduced significantly. Focusing on acetone and 2-propanol, we can see that the trend outlined is reversed. Piracetam is less soluble in acetone (purple) when compared to 2-propanol (grey), however the induction time for the transformation is significantly shorter in acetone, indicating a faster transformation rate. This was the case at all temperatures examined.
Figure 5.11: A plot of the natural log of the inverse of the induction time for the transformation against the solubility of FIII(6.525) in the five solvents at 30°C.

The rate of dissolution of FII(6.403) in 2-propanol was found not to be significantly slower than in the other solvents, despite a much higher viscosity. Therefore, the possibility of a lag time before nucleation of FIII(6.525) existing while the solution becomes supersaturated with respect to the stable form is eliminated. 1-propanol was then examined with a view to establishing if the positioning of the alcohol group on the carbon chain has an effect on the transformation. The solubility of FII(6.403) and FIII(6.525) was measured in 1-propanol at 30 and 50 °C and the transformation was also examined at these temperatures. The results can be seen in Figure 5.12 and Table 5.6. 1-propanol was found to be consistent with the trend of solubility of piracetam correlating with transformation rates. The solubility of piracetam is only slightly higher in 1-propanol when compared to 2-propanol, but the induction time for the transformation is much shorter. This shows that the positioning of the alcohol group on the carbon chain of the solvent does not affect the solubility as much as it affects the transformation rate. Molecular modelling, carried out by Dr. Colin Seaton in conjunction with the work in this project, showed that a stronger interaction between the solute and solvent molecules is observed in the case of 2-propanol compared to the other solvents, thereby resulting in a slower transformation rate by hindering nucleation and growth of FIII(6.525) during the transformation. These interactions show a hydrogen bond ‘bridging’ of the amide group of the piracetam molecule, which is vital in the crystal structure of FIII(6.525), thereby retarding the desolvation processes of the solute from the solution phase and at the lattice incorporation site.
Figure 5.12: A plot of the inverse of the induction time at 30 °C for the transformation against the solubility of FIII(6.525) in the six solvents at 30 °C.

Table 5.6: Results for the solubility of FII(6.403) and FIII(6.525) as well as the transformation in 1-propanol and 2-propanol at 30 and 50 °C.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>30 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-propanol</td>
<td>1.31 x 10^{-2}</td>
<td>1.28 x 10^{-2}</td>
<td>18</td>
</tr>
<tr>
<td>2-propanol</td>
<td>1.09 x 10^{-2}</td>
<td>1.03 x 10^{-2}</td>
<td>70</td>
</tr>
<tr>
<td>50 °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.22 x 10^{-2}</td>
<td>3.11 x 10^{-2}</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>2.73 x 10^{-2}</td>
<td>2.67 x 10^{-2}</td>
<td>15</td>
</tr>
</tbody>
</table>

Increasing the agitation rate was found to increase the transformation rate. The movement of piracetam molecules in solution increases with agitation, thereby increasing the number of molecular collisions. This again increases the kinetic portion of the nucleation rate equation (A) leading to faster transformation times.

### 5.3.2 Mechanistic Features of the FII(6.403) to FIII(6.525) SMPT

The dissolution of FII(6.403) appears to mirror the ‘unwrapping / unzipping’ of the dimer chains of molecules which extend throughout the crystal structure of FII(6.403). Figure 4.15 indicates that the
FII(6.403) ends and edges lose the sharp features of the faces initially with dissolution. Thereafter, steps can be seen along the length of the rods. It is thought that this is a result of the exposed molecules at the ends of the dimer chain formation being stripped off into solution. This stripping then extends along the length of the rod and new steps are developed behind it. In the early to middles stages of dissolution the length of the rod does not decrease hugely, but the width does decrease significantly. This would correspond to the dimer chains getting stripped away, making the rod narrower. It is not until the latter stages that the rod gets significantly shorter and the crystal dissolves completely (Video 1 in Appendix B).

Figures 4.13 & 4.14 give an understanding of the rate determining steps in the transformation. At 50 °C (Figure 4.13), after 3 hours FIII(6.525) is detected for the first time indicating that nucleation is a limiting factor in the transformation. The amount of FIII(6.525) increases slowly in the initial stages. In the first hour after detection the amount of FIII(6.525) increases to 10 %, while in the second and third hours increases of 40 and 50 % respectively are observed until the transformation has gone to completion. The overall growth of FIII(6.525) appears to be limited by the surface area of FIII(6.525) available and the rate increases as the crystal size increases after nucleation. The concentration in solution remained at the solubility level of FII(6.403) even with significant amounts FIII(6.525) in the solid phase ( > 80 %). After approx. 5.2 hr a decrease in the concentration in solution corresponding to the transformation is observed. The concentration plateau corresponding to the saturation level of FIII(6.525) in ethanol was reached after 6.7 hours while the composition of the solid phase was 100 % FIII(6.525) approx. 1 hour earlier. This indicates that the dissolution rate of FII(6.403) is faster than the growth of FIII(6.525), and is not limiting in the transformation. A similar pattern of events is observed in Figure 4.14 at 30 °C implying that the governing mechanism of the transformation appears to be the same across the temperature range of this study.

In an extensive review, Croker & Hodnett (2010) observed that in solution-mediated polymorphic transformations, nucleation of the stable polymorph tends to occur on, or at, the surface of the existing polymorph. In experiments where the amount of solid to be transformed is changed by adding different masses of the same solid material, we would expect the transformation time to be constant based on an identical specific surface area. The plot documenting the absolute amount of FII(6.403) in the solid phase during the transformation (Figure 4.16) showed a similar transformation time in all experiments, even though the tube with the highest loading contained nine times more solid FII(6.403) to be transformed. A plot documenting the percentage of FII(6.403) in the solid phase during the four transformation experiments (Figure 5.13), indicates that the transformation rate is a little faster in the experiments with lower loadings of FII(6.403). The reason
for this is thought to be that the surface area per unit mass, or specific surface area of FII(6.403) to be transformed is higher in the lower loaded tubes since the dissolution of the added solids is more pronounced as shown in Table 5.7.

Table 5.7: Influence of solids loading on the rate of transformation. Concentrations and amounts of FII(6.403) added to 25 g of ethanol in each tube at 40 °C during the investigation.

<table>
<thead>
<tr>
<th>Test Tube</th>
<th>C, FII(6.525) (g FII/kg EtOH)</th>
<th>Saturation Level before t₀ wrt FII (σ)</th>
<th>Amount of FII(6.403) added at t₀ (g FII/kg EtOH)</th>
<th>% Dissolution of added FII(6.403)</th>
<th>Excess to be transformed (g FII/kg EtOH)</th>
<th>Excess to be transformed (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>79.2</td>
<td>1</td>
<td>8.0</td>
<td>30</td>
<td>5.6</td>
<td>0.24</td>
</tr>
<tr>
<td>2</td>
<td>79.2</td>
<td>1</td>
<td>24.1</td>
<td>17</td>
<td>20.0</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>79.2</td>
<td>1</td>
<td>40.2</td>
<td>7</td>
<td>37.4</td>
<td>1.50</td>
</tr>
<tr>
<td>4</td>
<td>79.2</td>
<td>1</td>
<td>56.3</td>
<td>4</td>
<td>54.0</td>
<td>2.16</td>
</tr>
</tbody>
</table>

The supersaturation associated with the transformation at 50 °C in ethanol is 1.025 with respect to FIII(6.525), and the transformation time was approx. seven hours. For comparison, a few separate
nucleation experiments have been performed. Homogenous solutions of supersaturations ranging from 1.03 to 1.06 with respect to FIII(6.525), at 50 °C, showed no nucleation after 20 days. This suggests that nucleation of the stable form during the transformation is facilitated by the surface of the metastable form.

The effect of the amount of surface area of FII(6.403) available for nucleation of FIII(6.525) on the transformation rate was further examined as outlined in Figure 5.14 and Table 5.8. Since each run contained the same mass of solids to be transformed after equilibrium saturation of FII(6.403) was reached, and equilibrium was reached from different starting solution concentrations, the extent of dissolution and number of the particles of FII(6.403) in each run was different. The number of particles of FII(6.403) per unit mass was orders of magnitude higher in R1, leading to a significantly higher specific surface area in this experiment compared to the other two.

Figure 5.14: A schematic of the concentration-time profiles associated with the methodologies for the introduction of FII(6.403) into ethanol at 50 °C. Blue (R1), Addition to Pure Solvent; Red (R2), Addition to a Solution Saturated w.r.t. FIII(6.525); Green (R3), Addition to a Solution Supersaturated w.r.t. both polymorphs.
Table 5.8: Concentrations and amounts of FII(6.403) added to 130 g of ethanol during the investigations of the transformation at 50 °C.

<table>
<thead>
<tr>
<th>Preparation Method</th>
<th>$C_i$ (g FII.kg$^{-1}$ EtOH)</th>
<th>Piracetam in homogenous solution (g)</th>
<th>Saturation Level before wrt FII $t_0$ ($\sigma$)</th>
<th>Amount of FII(6.403) added at $t_0$ (g)</th>
<th>Concentration after $t_0$ (g FII.kg$^{-1}$ EtOH)</th>
<th>% Dissolution of FII(6.403)</th>
<th>Excess available to be transformed (g FII.kg$^{-1}$ EtOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>125</td>
<td>0.00</td>
<td>0</td>
<td>19.5000</td>
<td>150</td>
<td>85</td>
<td>22</td>
</tr>
<tr>
<td>R2</td>
<td>125</td>
<td>16.2240</td>
<td>1</td>
<td>3.2760</td>
<td>150</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>R3</td>
<td>125</td>
<td>17.8464</td>
<td>1.1</td>
<td>1.6536</td>
<td>150</td>
<td>-72</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 4.17 shows the progression in the three experiments in terms of the concentration in solution and the percentage composition of the solid phase. Upon the addition of FII(6.403) at $t_0$, the concentration reaches the equilibrium saturation of the metastable form in all three experiments. In the case of the experiment prepared via R1, 85 % of the added material is dissolved. Assuming that each particle of FII(6.403) dissolves to a similar extent and disregarding breakage, the number of particles when FII(6.403) equilibrium saturation is reached should be the same as the number added to the vessel at $t_0$. A significantly smaller mass of FII(6.403) was added to the solution that was saturated with respect to FIII(6.525) at $t_0$ (R2), and less again was added in the case of R3. Only 13 % dissolution of the material added in R2 occurred, while growth of the added FII(6.403) crystals to R3 increased the solid mass by 72 %. The early ex-situ XRD analysis of this experiment confirmed that no FIII(6.525) had nucleated during this desupersaturation phase.

Since an equal mass of FII(6.403) is present at the point where FII(6.403) equilibrium is reached, and the number of particles in R1 is orders of magnitude greater than in R2 and R3 the specific surface area of the metastable form is significantly higher in the case of R1.

The rate of the transformation in the pre-saturated (R2) and pre-supersaturated (R3) solutions is very similar, while the rate is faster in the R1 experiment. The concentration in solution begins to drop from the solubility of FII(6.403) after 5.2 hr in R1, while the plateau at the solubility of FIII(6.525) is reached after 6.7 hr. In R2 and R3 these events occur approximately one hour later. When one examines the composition of the solid phase, 100 % FIII(6.525) is attained approx. one hour earlier in the case of R1. The curves can be broken into two sections: the initial decrease, associated with nucleation, followed by the significant drop associated with growth and the completion of the transformation. Nucleation is detected earlier in R1. As outlined earlier,
nucleation is thought to be surface mediated and it appears that the higher specific surface area of the metastable phase in R1 compared to R2 and R3 results in a higher number of nucleation points, thereby causing the initial stages of the transformation to proceed at a faster rate. The second section of the curves, associated with growth and the completion of the transformation have a similar rate in all three experiments.

Adding to the theory of surface mediated nucleation outlined above, in each case viewed by in-situ microscopy, nucleation of FIII(6.525) was associated with the surface of the metastable FII(6.403). Table 5.9 outlines the location on the surface of the FII(6.403) rods, according to Figure 5.15, where nucleation of FIII(6.525) was observed. In addition to these points, on three occasions a very small FII(6.525) crystal was observed floating away from the FII(6.403) mother crystal, before growing to its final size out in the solution. This indicates that the interaction between the new and mother crystal is not very strong, especially since no agitation was employed in these experiments.

![Figure 5.15: The habit of a FII(6.403) crystal with the five observed nucleation points of FIII(6.525) highlighted.](image)

Table 5.9: A documentation of the nucleation points of FIII(6.525) on the surface of FII(6.403), based on Figure 5.15, during the SMPT as viewed by in-situ microscopy.

<table>
<thead>
<tr>
<th>FIII(6.525) Nucleation Position</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Nucleation Events</td>
<td>10</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

The most common location for nucleation is on the slow growing faces along the length of the crystals. This is hardly surprising given that these are the big faces of the crystals with the largest surface area. However, the side edges of the FII(6.403) rods, which are thought to be the (002)/(001) faces based on the molecular analysis in Section 5.1, appear to be more favourable to nucleation of FIII(6.525) than the top and bottom (200)/(100) faces of the crystal, as viewed in Figure 5.15. Two different habits of FIII(6.525) were observed in this work. The hexagonal well faceted crystal resulted
when a single clean nucleation site was observed. In a few instances, almost always in the case of position 5 of Figure 5.15, a cluster of nucleation points occurred in very close proximity to each other, resulting in numerous different nuclei growing as one crystal. This resulted in a crystal that defaulted from the hexagonal habit and grew as a rough cube with a large number of stepped faces.

Since nucleation of the stable FIII(6.525) was observed to be surface mediated with respect to the metastable FII(6.403), the question of epitaxial crystallisation arose. As demonstrated in section 5.1, the crystal structures of FII(6.403) and FIII(6.525) appear to be similar, which would indicate that FII(6.403) surfaces may form a viable template for epitaxial nucleation and growth of FIII(6.525). However, in all transformation experiments viewed by optical microscopy, the affinity between the nucleating FIII(6.525) crystal and surface of the metastable FII(6.403) did not appear strong enough to indicate epitaxial growth. Even in this unagitated system, there were numerous examples of FIII(6.525) crystals moving away from the surface of the FII(6.403) crystals as soon as they became visible under the microscope.

A noticeable feature on all of the optical microscopy experiments is that the ratio of the number of FIII(6.525) crystals at the end of the transformation compared to the number of FII(6.403) crystals at the start of the transformation is very low, much less than a 1:1 ratio. This adds to the hypothesis that nucleation of FIII(6.525) is one of the limiting factors of the transformation. The growth of FIII(6.525) appears to go on for some time after all of the FII(6.403) crystals have dissolved completely. Along with the in-situ mid IR analysis of the transformation showing the concentration in solution decreasing after the solid phase has transformed to the stable form (Figure 4.13 & 4.14), this indicates that growth of the stable form is also a rate determining step in the transformation. Ostwald ripening was observed when the solution and FIII(6.525) crystals produced during the transformation were left for significant amounts of time (> 2 weeks). It was noticed that the smaller crystals dissolved and resulted in fewer but larger FIII(6.525) crystals in the vessel.

5.4 Solid State Polymorphic Transformations of Piracetam

5.4.1 Thermal Transformation of FII(6.403) or FIII(6.525)

In the case of the FII(6.403) sample (Figure 4.19), XRD patterns collected in the range from room temperature to 100 °C were identical to the theoretical FII(6.403) pattern. At 105 °C a mixture of FII(6.747) and FII(6.403) peaks were observed indicating the transformation is underway. At 110 °C, all FII(6.403) peaks have disappeared and the transformation to FII(6.747) has gone to completion.
The HT-XRD analysis of the thermal transformation of FIII(6.525) in Figure 4.20 showed similar results.

In all DSC scans collected in this study, FII(6.403) transformed at a slightly lower temperature than FIII(6.525), verifying the conclusions of the HT-XRD data, agreeing with the information reported by Kuhnert-Brandstaetter et al. (1994) and Picciochi et al. (2011). Employing a 1 °C/min heating rate, the transformation temperature onset was observed at 103.8 °C for FII(6.403), and 108.3 °C for FIII(6.525). These values are expected to be closer to the true thermodynamic transition temperature than those reported in the literature. Holding samples for 5 days at a series of temperatures in advance of the transition temperature from DSC analysis facilitated a more accurate determination. An oven at a set temperature was used for this investigation as it is not practical to program DSC and HT-XRD experiments to hold at elevated temperatures for prolonged periods of time, with XRD analysis carried out immediately after the holding period. The transition from FII(6.403) to FI(6.747) was observed at 98 °C, while the FIII(6.525) to FI(6.747) transition was observed at 102 °C. In fact, binary mixtures, on a 130 g scale, of both polymorphs and 1,4-dioxane showed that the true temperature of the thermodynamic FII(6.403) – FI(6.747) transition point is no higher than 90 °C and the thermodynamic FIII(6.525) – FI(6.747) transition point is no higher than 95 °C.

Nucleation of FI(6.747) was observed as a shadow that appeared at the top of the crystal at 109 °C during the Hot-Stage Optical Microscopy investigation in Figure 4.22. This shadow then extended throughout the whole crystal and changed it from being optically transparent to opaque. The habit of the crystal does not change during the transformation but a major crack, extending from the point where the shadow was first seen, splits the crystal in two. Other cracks can also be seen appearing and extending during the transformation. The visual properties of the crystal did not change between 120 °C and 152 °C. In the range of 152 to 153 °C, the now FI(6.747) crystal melted. Repeated runs of FIII(6.525), as well as FII(6.403) showed similar changes in optical properties and cracking of the crystal during the transformation, without changing the outer habit.

It was decided to investigate whether the appearance of the cracks in the crystal was a direct result of the molecular rearrangement during the transformation or merely caused by the stress of heating. A FI(6.747) crystal, analysed immediately after isolation by crash cooling from a highly supersaturated 1,4-Dioxane solution, was subjected to the same heating profile. No cracks were seen in the crystal during the heating profile and the crystal melted in the range 152 to 153 °C, indicating the cracking of the crystal during the transformation may be caused by the stress of the molecular rearrangement. The density, as determined by gas comparison pycnometry (Kuhnert-Brandstaetter et al. 1994), at room temperature of FI(6.747) (1.304 g/cm³) is lower than FIII(6.525)
(1.371 g/cm³) so it is feasible that the cracking observed during the transformation is caused by the change in density between the two polymorphs.

The HT-XRD and DSC analysis implied that FIII(6.525), when heated, appears to transform directly to F(6.747) and not via FII(6.403). To avoid any nucleation energy barrier that may exist, samples of FIII(6.525) were seeded with FII(6.403), by grinding the mixture, in order to establish whether or not the FII(6.403)-FIII(6.525) transition temperature is lower than that of the FIII(6.525)-F(6.747) transition. Also, although it is known to occur in solution (Dematos et al. 2007), the transformation from metastable FII(6.403) to the stable FIII(6.525) was not observed in the solid state after storage for one year under ambient conditions. Samples of FII(6.403) were seeded with FIII(6.525) by grinding both phases together, again to overcome the nucleation barrier and investigate if this transformation occurs in the solid state.

In every mixture analysed HT-XRD did not show any inter-conversion between FII(6.403) and FIII(6.525), only direct transformations to F(6.747). DSC analysis of these samples agrees, in that the FII(6.403)–FIII(6.525) transition point appears to be at a higher temperature than the FIII(6.525)–F(6.747) transition and the transformation from FII(6.403) to FIII(6.525) does not occur in the solid state. As mentioned earlier FII(6.403) transforms to F(6.747) at a slightly lower temperature than FIII(6.525). While the peaks are skewed slightly towards the transition temperature of the polymorph present in higher proportion in Figure 4.24, a single smooth peak in this region indicated that the only transformation that occurred was directly to F(6.747). In all samples the transformation product, F(6.747), melts with an onset at 152 °C.

The mixtures of FII(6.403) and FIII(6.525) held at 95 °C for 1 week did not show any transformation, supporting the gathered evidence that the FII(6.403)-FIII(6.525) transition temperature is above the transition point of both polymorphs to F(6.747). FII(6.403) was observed to transform to FIII(6.525) at temperatures from room temperature to 85 °C via a solution mediated mechanism, confirming that the relationship between the two is monotropic in this range and the transition point lies above the transition point of both polymorphs to F(6.747). It also confirms that the transformation from the metastable FII(6.403) to the stable FIII(6.525) does not materialise in the solid state.

It has been reported that trace amounts of FIII(6.525) sometimes persist during DSC analysis after the bulk of the sample has transformed to F(6.747), as evident by the presence of a small endothermic peak at 139 – 140.2 °C in addition to the melting of F(6.747) (Kuhnert-Brandstaetter et al. 1994, Picciochi et al. 2011). No such data has been reported for FII(6.403). Thermomicroscopy estimated melting points are available for FII(6.403) (140.7 °C) and FIII(6.525) (140.2 °C). Employing a heating rate of 10 °C/min in the current study, DSC scans showed suspected melting points for FII(6.403) and FIII(6.525) (Figure 4.25). Very small endothermic peaks were observed at 139.0 °C for
FII(6.403) and 138.3 °C for FIII(6.525). It is thought that these peaks represent the melting of trace amounts of the samples that did not transform to FI(6.747). The difference between the transition temperature of FII(6.403) and FIII(6.525) to FI(6.747) and the proposed melting points of FII(6.403) and FIII(6.525) is 20 – 30 °C. Using a heating rate of 10 °C/min this temperature span is achieved within 2 – 3 min. It is feasible that the entirety of the sample has not transformed to FI(6.747) in this time frame, and when 138 – 139 °C is reached any traces of the original polymorph remaining in the sample melt. In fact, the Hot Stage Optical Microscopy studies showed that the transformation to FI(6.747) does not appear to go to completion instantaneously but rather takes a number of minutes. When heating rates of 1 and 2 °C/min were employed the proposed melting peaks of FII(6.403) and FIII(6.525) were not observed, presumably because there was sufficient time for the transformation of the sample to FI(6.747) to go to completion.

5.4.2 Thermal Transformation of FI(6.747)

Ceolin at al. (1996) reported that FI(6.747) transforms to FII(6.403) at ambient conditions. The composition of samples of FII(6.403) and FIII(6.525) that had transformed to FI(6.403) were monitored after the samples had been cooled to room temperature. Figure 4.26 shows the composition of an originally FIII(6.525). After transformation to FI(6.747) by heating, the sample was cooled and monitored by XRD analysis. It is still completely FI(6.747) immediately when cooled to room temperature. Four hours later the transformation to FII(6.403) has begun, while the XRD of the sample 24 hours later shows the transformation to FII(6.403) was complete. There appears to be a region below the transition point between FI(6.747) and FII(6.403) where FI(6.747) exists as a metastable polymorph. Figure 4.27 shows a DSC scan of FIII(6.525) (unbroken) heated to 135 °C with the transformation to FI(6.747) occurring as expected with an endothermic peak. The cooling profile of FI(6.747) to 20 °C (broken) does not contain any thermal event, indicating that the transformation to FII(6.403) does not occur at the same temperature in the opposite direction.

The transformation to FII(6.403) did not occur during 5 day holding periods of FI(6.747) at temperatures from 100 °C down to 38 °C, confirming that FI(6.747) can exist in a metastable state. Only after holding at 30 °C did the transformation occur. This shows that the activation energy barrier for the transformation is significant, as the transformation does not happen in a practical time frame until the temperature has reached almost 60 °C beyond the thermodynamic transition point. As FI(6.747) transforms to FII(6.403) and not FIII(6.525), it appears that the system follows Ostwald’s rule of stages. When leaving the unstable phase (FI(6.747)), the system does not seek out the most stable phase (FIII(6.525)), but rather the nearest metastable phase (FII(6.403)) which can be reached with a loss of free energy (Nyvlt 2006).
Figure 5.16 shows an extension of the semi-schematic energy–temperature diagram proposed by Kuhnert-Brandstaetter et al. (1994) with experimental data for different thermal events that occur included. Table 5.10 compares the previously reported temperatures of the thermal events to those established in this study (Kuhnert-Brandstaetter et al. 1994, Ceolin et al. 1996, Picciochi et al. 2011). In this work transition temperatures 20 to 40 °C lower than outlined previously are reported for the transformation of FII(6.403) and FIII(6.525) to FI(6.747) using DSC analysis.

DSC showed the melting point of FII(6.403) to be 139.0 °C, while that of FIII(6.525) was 138.3 °C. Because FII(6.403) transforms to FI(6.747) at a lower temperature than FIII(6.525) and melts at a slightly higher temperature than FIII(6.525), it confirms enantiotropy and a transition point between FII(6.403) and FIII(6.525) is located inside the range 90 °C and 138 °C. There is less than 1°C difference between the melting points of FII(6.403) and FIII(6.525), while there is approx. 5 °C between the transition point of both polymorphs to FI(6.747). Therefore, it is thought that the FII(6.403)-FIII(6.525) transition point is closer to their melting points than to the transition point of both polymorphs to FI(6.747).

Figure 5.16: Energy-Temperature diagram of the three polymorphs of piracetam with temperatures of different thermal events included.
Table 5.10: Comparison of the temperatures of previously reported transitions of the three polymorphs of piracetam to those obtained in this study. Kuhnert-Brandstaetter et al. 1994 DSC\textsuperscript{1A}; Kuhnert-Brandstaetter et al. 1994 Thermomicroscopy\textsuperscript{1B}; Picciochi et al. 2011\textsuperscript{3}; Ceolin et al. 1996\textsuperscript{2}; PS, Present Study.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{onset}}$ (°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>108\textsuperscript{1A}</td>
<td>120\textsuperscript{1A}</td>
<td>152.5\textsuperscript{1A}</td>
<td>140.5\textsuperscript{1A}</td>
<td>140\textsuperscript{1A}</td>
<td></td>
</tr>
<tr>
<td>109.7\textsuperscript{3}</td>
<td>121.4\textsuperscript{3}</td>
<td>151/151.5\textsuperscript{3}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>126\textsuperscript{2}</td>
<td>119\textsuperscript{2}</td>
<td>153\textsuperscript{2}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 90 PS</td>
<td>&lt; 95 PS</td>
<td>150 PS</td>
<td>139.0 PS</td>
<td>138.3 PS</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 6. Conclusions

The polymorphs of piracetam can be produced by cooling crystallisation: FI(6.747) and FII(6.403) from 1,4-dioxane, under differing supersaturations and cooling rates, and FIII(6.525) from methanol. They can also be isolated using alternative methods via polymorphic transformations. The polymorphs are distinguishable from each by a number of analytical techniques, with a combination of techniques recommended to confirm the purity of batches to be used in subsequent studies.

The solubility of piracetam is greatly affected by temperature, increasing six fold between 5 and 50 °C. The solubility also increases with solvent polarity and the hydrogen bonding capacity of the solvent. As the polarity and acidity of the alcohol decreases through methanol, ethanol and 2-propanol with the increasing number of carbons in the aliphatic chain, so too does the solubility of piracetam in these solvents. Acetone and 1,4-dioxane are relatively non-polar when compared to the n-alcohols, and accordingly the solubility is much lower in these solvents.

The solubility of the metastable FII(6.403) is only slightly higher than that of the stable FIII(6.525) at all the temperatures, reflecting the relatively small stability difference between the two polymorphs. The ratio of the mole fraction solubility of FII(6.403) to FIII(6.525) is weakly dependent on the solvent and decreases slightly with increasing temperature; from an average of 1.151 at 5 °C to 1.026 at 50 °C.

The choice of solvent has a major effect on the kinetics of the transformation, adjusting both the induction time and the transformation time. A general trend exists where the higher the solubility of piracetam in a solvent, the faster the transformation rate was in that particular solvent. Molecular modelling indicates that stronger solvent-solute interactions are observed in the case of 2-propanol compared to the other solvents, thereby decreasing the transformation rate by retarding nucleation and growth of FIII(6.525) during the transformation. These interactions involve ‘bridging’ of the amide group of the piracetam molecule, which is vital in the crystal structure of FIII(6.525).

Along with the level of mixing in the vessel, the kinetics of the transformation are also affected significantly by temperature. The rate of transformation increases quite strongly with increasing temperature, in spite of a decreasing driving force. Nucleation and growth of the stable form are the rate determining steps of the transformation. It was established that nucleation of the stable phase occurs on the faces of the metastable face, and the number of nuclei generated is approximately proportional to the available surface area.
An investigation of the transition points between the enantiotropically related FI(6.747), FII(6.403) and FIII(6.525) showed that the temperatures reported in the literature are up to 40 °C removed from the true thermodynamic transition point. As with the SMPT, kinetics play a major role in determining when the transition occurs. FI(6.747) can exist as the unstable polymorph in the region from 90 °C to below 38 °C, before consistently transforming to the metastable FII(6.403). The FII(6.403) – FIII(6.525) transition temperature was established to be located above the transition of both polymorphs to FI(6.747) (90 & 95 °C), and below their melting points (139 and 138 °C respectively). The transformation from the metastable FII(6.403) to the stable FIII(6.525) does not occur in the solid state.
References


Suryanarayanan, R. (1989) Determination of the relative amounts of anhydrous carbamazepine (C_{15}H_{12}N_{2}O) and carbamazepine dihydrate (C_{15}H_{12}N_{2}O.2H_{2}O) in a mixture by powder X-ray diffractometry, Pharm. Res., 6, 1017-1024.


Appendix A
Introduction

The crystallization of an active pharmaceutical ingredient is a very important step in the manufacturing of a drug product. Crystalline molecular solids, either pure or in solvate form, can exist in different crystal arrangements. Polymorphism in crystalline solids is defined as materials having the same chemical composition but different lattice structure orientations and/or different molecular conformations and, therefore, different physicochemical properties. The difference in properties (e.g., solubility) between different polymorphs of a drug has led to a tight regulation of polymorphism in the pharmaceutical industry, as they have a direct impact on drug substance processability, drug product manufacturability, and drug product quality or performance, including stability, dissolution, and bioavailability.

As solubility and drug dissolution rate are related to drug processing in the body, a lot of work has been carried out investigating the effect of polymorphism on solubility and dissolution. Solubility data are very important for any polymorphic system, as they give information on the relative stability of the polymorphs in that system. The most stable polymorph in a system will have the lowest solubility, while the least stable polymorph will have the highest solubility, irrespective of the solvent.

2-Oxo-1-pyrrolidine acetamide (piracetam) (Figure 1) is a nootropic drug, which is an agent that acts on cognitive dysfunction without causing sedation or stimulation. Cognitive dysfunction is one of the main symptoms accompanying aging, stroke, head injury, and neurodegenerative diseases such as Alzheimer’s. Throughout the literature there is some confusion over the nomenclature of the different polymorphs. In this work the system used for naming polymorphs is simply the form number followed by the a lattice parameter reported for the particular polymorph in the Cambridge Crystallographic Data Centre (CCDC) in parentheses, so that Form III is referred to below as FIII(6.525). The reference code for the polymorphic entries of piracetam in the CCDC is BISMEV01, while the entry that corresponds to FIII(6.525) is BISMEV01.

Five polymorphs of piracetam have been reported, but two of these [FIV(8.9537) and FV(6.3903)] are obtained in high pressure (> 0.5 GPa) conditions only. Another polymorph, F(6.747), is only seen when F(6.403) or FIII(6.525) are heated to 400 K and then quenched to room temperature. However, F(6.747) transforms back to F(6.403) within a few hours at room temperature and so is not of practical relevance. The other two polymorphs, F(6.403) and FIII(6.525), have been identified and structurally characterized under ambient conditions. Conflicting views on the stability hierarchy of the polymorphs in the system are presented in the literature. While it is well agreed that F(6.747) is the stable polymorph at higher temperatures, there is some confusion as to which polymorph is stable at lower temperatures. Analysis of the polymorphs in solution and in the solid state suggest that FIII(6.525) is the thermodynamically stable polymorph at lower temperatures until it transforms to F(6.747) at approximately 393 K. This is in agreement with the work published by Kuhnert-Brandstaetter et al. To date, there are no published solubility data for the polymorphs of piracetam. In this study, the solubility of FIII(6.525) was determined in a range of five solvents, at increments of 5 K, over the range of (278 to 323) K.

![Figure 1. Molecular structure of piracetam.](image-url)
Experimental Section

Piracetam was supplied by UCB Pharma SA and complies with European Pharmacopeia standards (CAS Number: 7491-74-9). The five solvents listed in Table 1 were obtained from Sigma-Aldrich and were used without further purification. FIII(6.525) was produced using a HEL PolyBLOCK parallel synthesis reactor. Eighty grams of piracetam were dissolved in 200 mL of methanol at 333 K with agitation of 200 rpm. The solution was then cooled to 283 K at a rate of 1 K·min⁻¹. Solids were sampled periodically before harvesting the FIII(6.525) crystals by vacuum filtration after 24 h. X-ray diffraction (XRD) analysis (Phillips PANalytical X’Pert MPD Pro with PW3064 sample spinner) was used to analyze the composition of the sampled solids. The solids were dried in an oven at 318 K. Fifty-nine grams of FIII(6.525) were obtained per batch and characterized by XRD, optical microscopy (Zeiss Axioscope Axio Imager MAT reflected-light microscope), scanning electron microscopy (SEM, JEOL CarryScope scanning electron microscope JCM-5700), attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR, PerkinElmer precisely Spec- trum 100 FT-IR spectrometer with a PerkinElmer precisely Universal ATR sampling accessory) and differential scanning calorimetry (DSC, PerkinElmer instruments Pyris 1 differential scanning calorimeter).

The experimental setup for the solubility measurements of FIII(6.525) consisted of a thermostatic water bath (Grant GR150 with S38 stainless steel water bath; 38 L; 690 × 300 × 200 mm; @ 310 K; stability ± 0.005 K and uniformity ± 0.02 K) with a serial magnetic stirrer plate placed on the base. Five test tubes (150·25 mm) with a Teflon-coated magnetic stirrer were charged with each of the five solvents and placed in a rack in the water bath which was set at the desired temperature. After 1 h (temperature of solvent had reached the temperature of the water bath) excess FIII(6.525) was added to each of the five test tubes. The test tubes were agitated at 500 rpm for at least 48 h to allow the equilibrium concentration to be reached. The stirring was then turned off, and excess FIII(6.525) was allowed to settle for 2 h. Samples of the clear saturated solution (approx 4 mL) were transferred from each of the test tubes to three 50 × 25 mm clean dry weighed vials (mass of dry vial + cap = m_empty) using a preheated syringe. A 0.2 µm, 15 mm membrane diameter syringe filter was attached to the head of the syringe before the saturated solution was passed into the vials to ensure that there was no suspended solid present. Caps were put on the vials immediately after solution had been filtered into them, to prevent solvent evaporation, and then reweighed (mass = m_empty). The caps were then removed, and the solvent was allowed to evaporate at room temperature in the fume hood for approximately 1 week. At this point only a solid residue remained in the vials, and the drying process was completed by placing the vials in an oven at 333 K for 3 days (Lenton Thermal Designs oven). The vials were then allowed to return to room temperature in the fume hood before reweighing with their caps (mass = m_dry). All weighing was carried out using a Mettler Toledo AX054 with a weighing capacity of up to 520 g and readability of 0.1 mg. A typical mass of solid residue was approximately 0.1 g. The solubility of FIII(6.525) in the solvent, C_s, could then be calculated as shown in eq 1, expressed as g FIII(6.525)/g solvent. The solubility was measured at every 5 K in the temperature range (278 to 323) K. Three samples were taken for each solvent at each temperature. The freezing point of 1,4-dioxane is 284.8 K so the solubility of FIII(6.525) in this solvent was not measured below 288 K.

\[
C_s = \frac{(m_{\text{dry}} - m_{\text{empty}})}{(m_{\text{aq}} - m_{\text{dry}})}
\]

At each temperature as the clear saturated solution sample was taken from the test tube, a sample of the excess solid was also taken. XRD was carried out on the solid sample to verify that the composition was indeed FIII(6.525). The reproducibility of the solubility results was examined in methanol by repeating the solubility measurements at each temperature in a separate test tube.

An experiment was carried out to examine the time it took for the equilibrium saturation to be reached in methanol at 298 K. Samples of the clear solution were taken every hour for 12 h and after that once every 12 h up to 48 h. The samples were dried in the same manner as outlined, and the resulting curve showed that the solubility point was reached after (5 to 6) h. The concentration in solution then remained at that same value for the duration of the analysis.

The drying procedure used in this work was verified by weighing certain amounts of solvents (methanol and acetone) and FIII(6.525) into vials and subjecting them to the drying procedure. The vials were then reweighed and the percentage weight loss calculated. FIII(6.525) showed a percentage weight loss of 0.04 %. This drop in the weight may be a result of any residual moisture that is present during storage evaporating off during drying. No detectable residue was found after the drying of the pure solvents.

Results and Discussion

XRD demonstrated the commercial piracetam to consist mostly of FIII(6.525) but with traces of FIII(6.403). Pure FIII(6.525) was crystallized by cooling crystallization in methanol. Figure 2 shows the XRD of the pure FIII(6.525) crystals that were harvested after 24 h from methanol. This XRD pattern is identical to that of the FIII(6.525) entry in the CCDC. Looking at the XRD of the crystals harvested after 1 h 20 min

Table 1. List of Solvents Used along with Their Specification and CAS Registry Number

<table>
<thead>
<tr>
<th>solvent</th>
<th>specification/mass %</th>
<th>CAS No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>ACS reagent, &gt; 99.8 %</td>
<td>67-56-1</td>
</tr>
<tr>
<td>ethanol</td>
<td>ACS reagent, &gt; 99.5 %</td>
<td>64-17-5</td>
</tr>
<tr>
<td>2-propanol</td>
<td>ACS reagent &gt; 99.5 %</td>
<td>67-63-0</td>
</tr>
<tr>
<td>1,4-dioxane</td>
<td>spectrophotometric grade, &gt; 99 %</td>
<td>123-91-1</td>
</tr>
<tr>
<td>acetone</td>
<td>ACS reagent, &gt; 99.5 %</td>
<td>67-64-1</td>
</tr>
</tbody>
</table>

Figure 2. Theoretical XRD patterns of FIII(6.403) and FIII(6.525) from CCDC compared to the XRD patterns of the crystals harvested from methanol after 1 h 20 min and 24 h.
in Figure 2, some FII(6.403) peaks are present, such as the (1,0,1) peak at 16.0° and the (0,1,1) peak at 18.5°. All peaks related to FII(6.403) have clearly disappeared after 24 h agitation at 283 K, at which time pure FIII(6.525) was harvested.

After isolation, FIII(6.525) was characterized. The FTIR spectrum obtained is shown in Figure 3. The regions unique to FIII(6.525) are (1100 to 1250) cm⁻¹ and (2850 to 3050) cm⁻¹ as outlined by Pavlova¹³ and Kuhnert-Brandstaetter et al.¹⁴ The DSC scan in Figure 4, using a heating rate of 5 K·min⁻¹, shows that FIII(6.525) is stable until heated to 393 K where it transforms to FI(6.747) with an endothermic peak. The melting of FI(6.747) gives an endothermic peak at approximately 425 K. It is the stable polymorph of piracetam from 393 K to its melting point (425 K). The SEM images of the FIII(6.525) crystals from methanol after 24 h in Figure 5 shows that the crystals have a hexagonal habit, with very well-defined faces.

Table 2 gives the mean values of the three samples of the experimental results obtained for the solubility of FIII(6.525) in the five solvents over the range of (278 to 323) K. The standard deviation between the three samples taken at each temperature for each solvent is also shown in brackets. The standard deviation is quite low (less than 1.50×10⁻³ in all cases) indicating little variance and reproducible results. The solubility graph for FIII(6.525) in the five solvents of choice is presented in Figure 6. The solubility in methanol increases from 7.96×10⁻² g FIII(6.525)/g solvent at 278 K to 48.04×10⁻² g FIII(6.525)/g solvent at 323 K. As can be seen in all five solvents analyzed in this work, the solubility of FIII(6.525) in the solvents increases with temperature. By examining Figure 6 and Table 2, it can be said that the solubility of FIII(6.525) in methanol (one carbon) is greater than that of ethanol (two carbons), which is greater than that of 2-propanol (three carbons) across the range of the study. The solubility decreases as the number of carbons in the n-alcohols increases, which reflects a decreasing solubility with decreasing solvent polarity and hydrogen bond donor ability. 1,4-Dioxane and acetone are relatively nonpolar solvents when compared to alcohols, and accordingly FIII(6.525) is much less soluble in these solvents. In addition, 1,4-dioxane and acetone are aprotic solvents and cannot hydrogen bond to the proton-accepting carbonyl oxygen of the solute molecule. Alcohols are protic and can interact not only with the hydrogen bond donating amino groups but also with the carbonyl oxygen of the solute.

Along with the low standard deviation values, Figure 6 shows that the solubility results are reproducible. The solubility determination was repeated at all temperatures in methanol. As can be seen on the graph, the two solubility curves for FIII(6.525) in methanol are almost identical, indicating that the data obtained is reliable.

Figure 7 shows a van’t Hoff plot where the mole fraction solubility (ln(x)), eq 2, of FIII(6.525) in the five solvents is plotted against temperature:

\[
\ln(x) = \ln \left( \frac{n_{\text{FIII}(6.525)}}{n_{\text{FIII}(6.525)} + n_{\text{solvent}}} \right)
\]  

(2)

where n is the number of moles. In addition it is noted that the van’t Hoff enthalpy of solution:
Table 2. Solubility Data (C_s) (g FIII(6.525)/g Solvent) and Standard Deviation in Brackets (Std. Dev.) Obtained for FIII(6.525) in the Five Solvents over the Range (278 to 323) K.

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Methanol C_s (std. dev.)</th>
<th>Methanol C_s Repeat (std. dev.)</th>
<th>Ethanol C_s (std. dev.)</th>
<th>2-Propanol C_s (std. dev.)</th>
<th>1,4-Dioxane C_s (std. dev.)</th>
<th>Acetone C_s (std. dev.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>278</td>
<td>7.96 × 10^{-2} (1.17 × 10^{-2})</td>
<td>7.98 × 10^{-2} (1.32 × 10^{-2})</td>
<td>1.61 × 10^{-2} (2.12 × 10^{-2})</td>
<td>0.73 × 10^{-2} (1.02 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>283</td>
<td>9.72 × 10^{-2} (1.80 × 10^{-2})</td>
<td>9.87 × 10^{-2} (1.02 × 10^{-2})</td>
<td>2.05 × 10^{-2} (7.87 × 10^{-2})</td>
<td>0.91 × 10^{-2} (2.21 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>288</td>
<td>11.95 × 10^{-2} (9.80 × 10^{-3})</td>
<td>12.00 × 10^{-2} (1.21 × 10^{-2})</td>
<td>2.65 × 10^{-2} (1.26 × 10^{-2})</td>
<td>1.20 × 10^{-2} (1.16 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>293</td>
<td>14.81 × 10^{-2} (1.96 × 10^{-2})</td>
<td>14.82 × 10^{-2} (1.74 × 10^{-2})</td>
<td>3.24 × 10^{-2} (2.27 × 10^{-2})</td>
<td>1.50 × 10^{-2} (1.78 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>298</td>
<td>17.96 × 10^{-2} (1.19 × 10^{-2})</td>
<td>18.01 × 10^{-2} (1.53 × 10^{-2})</td>
<td>4.08 × 10^{-2} (7.90 × 10^{-2})</td>
<td>1.90 × 10^{-2} (4.18 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>303</td>
<td>21.60 × 10^{-2} (1.95 × 10^{-2})</td>
<td>21.58 × 10^{-2} (1.03 × 10^{-2})</td>
<td>5.11 × 10^{-2} (1.29 × 10^{-2})</td>
<td>2.45 × 10^{-2} (1.33 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>308</td>
<td>26.31 × 10^{-2} (1.32 × 10^{-2})</td>
<td>26.26 × 10^{-2} (1.01 × 10^{-2})</td>
<td>6.31 × 10^{-5} (3.94 × 10^{-5})</td>
<td>3.12 × 10^{-2} (2.69 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>313</td>
<td>32.30 × 10^{-2} (6.01 × 10^{-3})</td>
<td>32.29 × 10^{-2} (1.43 × 10^{-3})</td>
<td>7.92 × 10^{-5} (1.19 × 10^{-5})</td>
<td>4.00 × 10^{-5} (8.49 × 10^{-5})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>318</td>
<td>39.47 × 10^{-2} (1.06 × 10^{-2})</td>
<td>39.39 × 10^{-2} (1.04 × 10^{-2})</td>
<td>9.82 × 10^{-5} (1.25 × 10^{-5})</td>
<td>5.13 × 10^{-2} (1.14 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
<tr>
<td>323</td>
<td>48.04 × 10^{-2} (3.16 × 10^{-3})</td>
<td>48.01 × 10^{-2} (1.50 × 10^{-2})</td>
<td>12.48 × 10^{-2} (1.19 × 10^{-2})</td>
<td>6.49 × 10^{-2} (2.46 × 10^{-2})</td>
<td>0/0/0/0/0</td>
<td>0/0/0/0/0</td>
</tr>
</tbody>
</table>

“n/a” refers to temperatures where the solubility of FIII(6.525) could not be measured as it was below the freezing point of the particular solvent.

\[ A \times \text{Temperature}^2 + B \times \text{Temperature} + C \]  
\[ \Delta H^{\text{soln}} = -R \frac{d \ln x}{dT} \]  
\[ \ln(x) = A + BT \]  
\[ \text{In the present work, both equations were successfully fitted to the solubility data using Microsoft Office Excel 2003 and Matlab 6.5. } \]
\[ R^2 \text{ values of greater than 0.9990 were obtained for the data in every solvent. The obtained coefficient values for eq 4 are given in Table 3.} \]
\[ \text{Since FIII(6.525) transforms to Fl(6.747) at about 393 K, the determination of melting data for FIII(6.525) becomes uncertain. Pavlova et al.} \]
\[ \text{evaluated a series of “regression equations” for the correlation of experimental solubility data and for extrapolation to the melting point. For the latter purpose,} \]
\[ \text{the two most successful equations were:} \]
\[ \text{Table 3. Calculated } A \text{ and } B \text{ Coefficients for a Regression Equation (eq 4) Fitted to the Solubility Data Obtained for FIII(6.525) in Each Solvent in the Range (278 to 323) K.} \]

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Equation ( \ln(x) = A + BT )</th>
<th>( A )</th>
<th>( B )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>-14.609</td>
<td>0.0381</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>-17.571</td>
<td>0.0444</td>
<td></td>
</tr>
<tr>
<td>2-Propanol</td>
<td>-19.198</td>
<td>0.0482</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>-17.143</td>
<td>0.0382</td>
<td></td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>-18.377</td>
<td>0.0412</td>
<td></td>
</tr>
</tbody>
</table>

Nordström and Rasmuson\(^{15}\) evaluated a series of “regression equations” for the correlation of experimental solubility data and for extrapolation to the melting point. For the latter purpose, the two most successful equations were:
the melting temperature of FIII(6.525). In all solvents except for 2-propanol, the predicted melting temperature is within approximately ±20 K of the true value, with the average being 402 K. This level of error is approximately equal to the error reported for the predictions by Nordström and Rasmussen. The Equation 4 is a straight line. While the melting temperatures predicted using the straight line are not as good as those for eq 5, the mean (414 K) is closer to the true value. The melting enthalpies were calculated using the predicted melting temperatures, and in this instance the values obtained were almost double the value published by Kuhnert-Brandstaetter et al., (29.3 kJ mol⁻¹), and there is a significant variation especially in the data estimations by eq 5.

Conclusion

FIII(6.525) piracetam displays a wide range of solubility in the five solvents studied. The solubility of FIII(6.525) is influenced by the polarity of the solvent in question and the hydrogen-bonding capacity of the solvent. In this work it was shown that FIII(6.525) is most soluble in the alcohol with the shortest aliphatic chain, methanol. As the polarity and acidity of the alcohol decrease through ethanol and 2-propanol with the increasing number of carbons, so too does the solubility of FIII(6.525) in these solvents. Acetone and 1,4-dioxane are relatively nonpolar when compared to the n-alcohols, and accordingly the solubility of FIII(6.525) is much lower in these solvents. By fitting certain regression equations to the solubility data according to Nordström and Rasmussen, the approximate melting temperature can be estimated for FIII(6.525) piracetam, by extrapolation to ln(x) = 0.

Acknowledgment

The authors thank Aine Munroe (Solid State Pharmaceuticals Cluster, Materials and Surface Science Institute, University of Limerick) for her polymorph naming scheme which can be applied to any polymorphic system.

Literature Cited

(8) Munroe, A. Polymorphic Transformations of Sulphatiazole; University of Limerick: Limerick, Ireland, 2010.
(a) The reference code for the polymorphic entries of piracetam in the CCDC is BISMEV, while the entry that corresponds to FIII (6.525) is BISMEV01.

Received for review April 21, 2010. Accepted September 27, 2010. A.M. gratefully appreciates funding from an Irish Research Council for Science, Engineering and Technology (IRCSET) scholarship as well as the Solid State Pharmaceutical Cluster (SSPC).
Solubility of the Metastable Polymorph of Piracetam (Form II) in a Range of Solvents

Anthony Maher,* Åke C. Rasmuson, Denise M. Croker, and Benjamin K. Hodnett

Solid State Pharmaceuticals Cluster, Materials and Surface Science Institute and Department of Chemical and Environmental Sciences, University of Limerick, Limerick, Ireland

ABSTRACT: The solid−liquid solubility of the polymorph known as Form II of 2-oxo-1-pyrrolidine acetamide (Piracetam) has been determined gravimetrically in different solvents. Form II is the metastable polymorph of piracetam at ambient conditions and has been isolated and characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). Monitoring the solution concentration and the polymorphic composition of the solid phase displayed that this metastable form has a sufficient lifetime when in contact with the solvents to allow measurement of its solubility over the temperature range (278 to 323) K. Four solvents are included: ethanol, 2-propanol, acetone, and 1,4-dioxane. The results show that the solubility of Form II increases with increasing solvent polarity and solvent acidity. Form II has a slightly higher solubility than the stable Form III in all solvents at all temperatures, but the solubility difference is very small. Since Form II is known to transform to Form I below its melting point, a set of regression equations which can be used to extrapolate solubility data to the melting point of Form II were applied to the collected data.

INTRODUCTION

The solubility of an active pharmaceutical ingredient (API) has a considerable influence on the choice of solvents and the course of operation in solvent-based processes such as crystallization. One of the most challenging tasks in any crystallization from solution is to control the solid phase that comes out of solution. Crystalline molecular solids, either pure or in solvate form, can exist in different crystal structures, having different physicochemical properties influencing drug dissolution rates and hence drug processing and bioavailability in the body. Therefore, a lot of work has been carried out investigating the effect of polymorphism on these parameters, and differences in properties between different polymorphs have led to tight regulation of polymorphs in the pharmaceutical industry. The solubility of respective polymorphs in a system increases with decreasing polymorph stability since at equilibrium the free energy of the solute in the solid phase is equal to the free energy of the solute in solution. Hence, a simple test of the stability hierarchy in a system is to obtain solubility data for the different polymorphs.

Solubility data for the metastable polymorph of a drug system are rarely reported, and in the chemical engineering literature the actual polymorph under investigation is not always clearly established. Scheme 1 shows a schematic of the scenario where the solubility of the metastable phase is reached before the transformation to the stable phase occurs. For a metastable form, the experimental procedure must, on one hand, ensure sufficient time for equilibration to be reached and, on the other hand, ensure that the solid form present still is the metastable form. Accordingly, for a proper determination of solubility of metastable forms, it is important to examine both the solution phase and the solid phase.

Piracetam (Figure 1) is a nontropic drug used to treat diseases associated with cognitive dysfunction. Throughout the literature, there is some confusion over the nomenclature of the different polymorphs. In this work, the system used for naming polymorphs has been outlined previously. The numerical value of the $a$-lattice parameter in angstroms, which appears in the corresponding Cambridge Crystallo-

Scheme 1. Schematic Concentration in Solution−Time Profile Displaying the Behavior of a Metastable Solid Phase in Contact with a Solvent in Which It Has a Finite Period of Stability
graphic Data Centre (CCDC) file, is added to the polymorph label. In the case of FII piracetam, the file name is BISMEV01, and the value is 6.403 Å, hence FII (6.403). In the case of FIII, the file name is BISMEV01, and the value is 6.525 Å, hence FIII (6.525). FII (6.403) has been reported to transform to FIII (6.525) in numerous solvents but not in the solid state. Previously, some confusion existed on the stability hierarchy between FII (6.403) and FIII (6.525) under ambient conditions. It was later established that FIII (6.525) was the stable polymorph but that the metastable FII (6.403) had almost the same stability.

Solubility data for FIII (6.525) have been reported in five solvents; to date, no solubility data for FII (6.403) have been published. In the present work, the solubility of FII (6.403) has been measured in four solvents (ethanol, 2-propanol, acetone, and 1,4-dioxane) over the temperature range (278 to 323) K, at increments of 5 K. The stability has been examined in detail in a previous study. Piracetam is known to have no enantiotropic transition in the range of this solubility study. Therefore, the solubility of the metastable FII (6.403) would be expected to be higher than the stable FIII (6.525) at each temperature. However, FII (6.747), FII (6.403), and FIII (6.525) are all enantiotropic with each other above the range of the solubility study. Both FII (6.403) and FIII (6.525) transform to FII (6.747) in the region (381 to 399) K. This transition ensures that the transformation to FII (6.747) is complete. The mortar was then transferred to a fume hood and left at room temperature (293 K) for one week to transform to FII (6.403), before XRD analysis was again carried out. The polymorphic purity of the FII (6.403) was further analyzed by Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) (PerkinElmer Spectrum 100 FT-IR Spectrometer with a PerkinElmer Universal ATR Sampling Accessory) and Differential Scanning Calorimetry (DSC) (PerkinElmer instruments Heat Flux Pyris 1 Differential Scanning Calorimeter) using a heating rate of 0.833 K s⁻¹.

### EXPERIMENTAL SECTION

FII (6.403) was isolated, characterized, and then used to determine the solubility by the gravimetric method in four organic solvents over the temperature range (278 to 323) K. The stability of the metastable form at the conditions of the experiments has been properly established.

**Materials.** Piracetam, complying with European Pharmacopoeia 6.5 quality and purity standards, was supplied by UCB Pharma SA (CAS Number: 7491-74-9, Batch Number: 09G06-B93; Certificate of Analysis states that the batch complies with the IR, HPLC, and solution appearance tests, and it also complies with a mass fraction of sulfated ash of <0.001, water content of <0.001, and purity of 1.000 ± 2). The solvents employed in this study, obtained from Sigma Aldrich and used without further purification, are outlined in Table 1.

**Preparation and Characterization of FII (6.403).** The metastable FII (6.403) has been prepared according to Scheme 2. FIII (6.525) (140 g) was prepared by cooling crystallization from methanol and ground using a mortar and pestle. The mortar was then covered with a clock glass and placed in an oven at 413 K for 3 days. The ground powder was agitated every 12 h using a preheated pestle. After 3 days, a sample was analyzed using X-ray Diffraction (XRD) (Phillips PANalytical X’Pert MPD Pro PW3064 Sample Spinner) to confirm that the transformation to FII (6.747) was complete. The mortar was then transferred to a fume hood and left at room temperature (293 K) for one week to transform to FII (6.403), before XRD analysis was again carried out. The polymorphic purity of the FII (6.403) was further analyzed by Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) (PerkinElmer Spectrum 100 FT-IR Spectrometer with a PerkinElmer Universal ATR Sampling Accessory) and Differential Scanning Calorimetry (DSC) (PerkinElmer instruments Heat Flux Pyris 1 Differential Scanning Calorimeter) using a heating rate of 0.833 K s⁻¹.

### Stability of FII (6.403) in Contact with Solvent.

The stability of FII (6.403) was investigated at 323 K in 2-propanol and ethanol, as well as at 303 K in ethanol, by analysis of the liquid-phase concentration as well as the solid-phase structural composition versus time. The experiments were performed in a 250 mL jacketed vessel controlled by a Lauda E305 recirculator and E300 controller and equipped with an overhead axial flow turbine impeller. The solvent (130 g), held at the desired temperature, was agitated at 270 rpm. A Mettler Toledo ReactIR iC10 with DiComp probe and silver halide fiber was configured before placing the probe tip approximately 1.5 cm under the level of the solvent in the jacketed vessel. An ATR-FTIR measurement was taken every 60 s in the range from (650 to 1800) cm⁻¹ using iCIR software. After a few scans, FII (6.403) was added to the solvent (Table 2).

The solution concentration was monitored by recording the infrared absorption of the carbonyl stretch (1700 cm⁻¹) of piracetam in solution. The peak area, as determined by Mettler-Toledo iCIR software, is proportional to the concentration and

![Figure 1. Molecular structure of piracetam.](image)

### Table 1. List of the Solvents Employed during the Study and Their Respective Purities

<table>
<thead>
<tr>
<th>solvent</th>
<th>purity/mass fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethanol</td>
<td>0.995 ± (0.005)</td>
</tr>
<tr>
<td>2-propanol</td>
<td>0.995 ± (0.005)</td>
</tr>
<tr>
<td>1,4-dioxane</td>
<td>0.990 ± (0.010)</td>
</tr>
<tr>
<td>acetone</td>
<td>0.995 ± (0.005)</td>
</tr>
</tbody>
</table>

*Expanded uncertainty is less than 2·10⁻³ g with a confidence interval of 0.95.*

### Table 2. Amounts of FII (6.403) Added to 130 g of Solvent during the Investigation on the Stability of FII (6.403) in Organic Solvents at (303 and 323) K

<table>
<thead>
<tr>
<th>solvent</th>
<th>temperature K</th>
<th>Cs FII (6.525) g 130 g solvent⁻¹</th>
<th>total amount of FII (6.403) g added</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-propanol</td>
<td>323</td>
<td>8.437</td>
<td>11.730</td>
</tr>
<tr>
<td>ethanol</td>
<td>323</td>
<td>16.224</td>
<td>19.500</td>
</tr>
<tr>
<td>ethanol</td>
<td>303</td>
<td>6.643</td>
<td>9.919</td>
</tr>
</tbody>
</table>

*Cs represents solubility. Expanded uncertainty of 2·10⁻³ g with a confidence interval of 0.95.*
is internally calibrated by dividing by the peak area of a solvent peak (950 cm\(^{-1}\) in 2-propanol and 875 cm\(^{-1}\) in ethanol). An example of one of these scans is shown in Figure 2. A decrease in the intensity of the solvent peak would be expected with changes of the concentration of piracetam in solution. However, since the difference in solubility between the two polymorphs of interest in this study is very small, the decrease in intensity of the solvent peak had a negligible effect.

The solid phase was monitored by analyzing samples via ex situ XRD. The samples, taken from the vessel using disposable Pasteur pipettes, were vacuum filtered for approximately 90 s. Since 2-propanol and ethanol do not have high boiling points, vacuum filtration dried the solids sufficiently for XRD analysis. The solids were then ground using a mortar and pestle and flattened on a silicon crystal zero-background disk using a glass slide. X-ray powder diffraction patterns of the samples were then obtained, and quantification of the polymorphic composition was carried out as outlined previously. Analysis of standards with small amounts of FIII(6.525) present in FII(6.403) established that FIII(6.525) could be detected at levels as low as 0.5 %. The composition of the solid phase was monitored throughout the transformation by isolating and analyzing approximately ten samples. The experiments were monitored until the concentration in the solution reached the solubility of FII(6.403).

**RESULTS AND DISCUSSION**

**Assessment of Structural Purity.** XRD patterns of samples taken during the production of FII(6.403) are compared to the corresponding theoretical patterns from the CCDC in Figure 3. Characterization was completed by ATR-FTIR (Figure 4) and DSC analysis (Figure 5).

**Solubility of FII(6.403) in Organic Solvents.** The solubility of FII(6.403) was determined gravimetrically from (278 to 323) K at 5 K intervals in ethanol, 2-propanol, acetone, and 1,4-dioxane in test tubes placed in a thermostatic water bath identical to that used previously in obtaining solubility data for FIII(6.525). After a 1 h equilibration of 30 g of each solvent in the test tubes at a set temperature, excess FII(6.403) was added to each test tube.

The solid phase in each tube was sampled periodically until FIII(6.525) was detected using ex situ XRD, at which point the solution phase was sampled for gravimetric determination of the FII(6.403) solubility using the same method as the FIII(6.525) solubility study. The fraction of FIII(6.525) present at the solubility determination point was no greater than 3 % at any of the temperatures analyzed in the study. At this level, the solution concentration is entirely governed by the presence of solid FII(6.403). In the cases where the transformation had not begun after 48 h, the solubility was measured at this point.

Figure 2. Infrared spectrum of absorbance against wavenumber (\(\lambda^{-1}\)) of piracetam in 2-propanol. C = O piracetam peak at 1700 cm\(^{-1}\) and 2-propanol peak at 950 cm\(^{-1}\).

Figure 3. XRD analysis of the transformation of FIII(6.525) to FII(6.747) by heating and then to FII(6.403) after cooling. Transformation direction: bottom to top. Included are the experimental (Exptl) XRD patterns of FIII(6.525) starting material (blue), FII(6.747) at 413 K (red), and FII(6.403) 1 week at room temperature, after heating finished (green), compared to the corresponding theoretical (Thr) patterns from the CCDC (broken lines).

Figure 4. Infrared spectrum of Pure FII(6.403), absorbance against wavenumber (\(\lambda^{-1}\)). Doublet of peaks of equal intensity in the region of (2850 to 3050) cm\(^{-1}\) and triplet of equal intensity in the region of (1100 to 1250) cm\(^{-1}\) are unique to the FII(6.403) polymorph.

FTIR (Figure 4) and DSC analysis (Figure 5). As indicated in the literature, FII(6.525) transforms to FII(6.747) in the range (392 to 395) K. FII(6.525) peaks such as the (100) and (012) peaks are not present when the mortar was removed from the oven, indicating the transformation to FII(6.747) has gone to completion. One week later under ambient conditions, the transformation to FII(6.403) has gone to completion. FII(6.747) peaks ((011) and (12-1) have disappeared, and the XRD pattern corresponds to the CCDC entry for FII(6.403).

With respect to the FTIR spectrum (Figure 4), the doublet of peaks of equal intensity in the region of (2850 to 3050) cm\(^{-1}\)
and triplet of equal intensity in the region of (1100 to 1250) cm$^{-1}$ are unique to the FII(6.403) polymorph.$^{1,13}$ The DSC data obtained for FII(6.403) are shown in Figure 5, with the calculated enthalpy and entropy of the phase transition reported in Table 3. As expected, FII(6.403) is seen transforming to FI(6.747) with the onset at 382 K and melting of FI(6.747) with an onset at 423 K. Heating rate of 0.833 K·s$^{-1}$.

Table 3. FII(6.403) to FI(6.747) and FIII(6.525) to FI(6.747) Transition Analysis$^a$

<table>
<thead>
<tr>
<th>Polymorph transition</th>
<th>$T_{onset}$</th>
<th>$T_{mid}$</th>
<th>$\Delta H$</th>
<th>$\Delta S$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FII(6.403) to FI(6.747)</td>
<td>382.0</td>
<td>393.3</td>
<td>3.37</td>
<td>8.61</td>
</tr>
<tr>
<td>FIII(6.525) to FI(6.747)</td>
<td>393.0</td>
<td>398.4</td>
<td>3.76</td>
<td>9.40</td>
</tr>
</tbody>
</table>

$^a$Onset and midpoint temperatures ($T$), enthalpy ($\Delta H$), and entropy ($\Delta S$) values for the transition calculated from the DSC scans of FII(6.403) in Figure 5 and FIII(6.525) from ref 9.

transforming to FI(6.747) with the onset of an endothermic peak at approximately 382 K. The onset for the melting of FI(6.747) then occurs at 423 K with another endotherm. FII(6.403) consistently transforms to FI(6.747) at a slightly lower temperature than FIII(6.525).$^9$ No traces of FI(6.747) or FIII(6.525) were identified in the outputs of any of the three techniques employed to analyze the batch of FII(6.403) produced for subsequent studies.

Stability of FII(6.403) in Contact with Solvent. Figure 6 shows the result of the investigation on the stability of FII(6.403) in 2-propanol at 323 K in terms of the concentration in solution and the composition of the solid phase. Very soon after the addition of the FII(6.403) to the solvent in the vessel at 323 K, a plateau in solution concentration is reached above the solubility of the stable FIII(6.525). This plateau is maintained for approximately 63 h. The solution is in equilibrium with FII(6.403) in the solid phase for the first 30 h of this plateau. Therefore, the author is confident that this solution concentration represents the solubility of FII(6.403). FIII(6.525) is detected in the solid phase after 30 h. However, the solution concentration remains at the solubility of FII(6.403) until the composition of the solid phase is predominantly FIII(6.525) (>80%).

In measuring the concentration of piracetam in solution in 2-propanol at 323 K at the point where FIII(6.525) is first detected in the solid phase, the author is confident that the solubility of FII(6.403) has been reached, while the transformation to the stable form has not yet resulted in a decrease in the solution concentration from the solubility of FII(6.403). By similar experiments in ethanol at 303 and 323 K, it was observed that equilibrium saturation of FII(6.403) was attained almost immediately after adding the solid phase to the vessel, while the solid phase was found to be stable for 2.5 h at 323 K and 17 h at 303 K. As in the case of 2-propanol, the concentration in solution remained at the equilibrium saturation of FII(6.403) until the composition of the solid phase is almost completely FIII(6.525).

Even at the highest temperature in 1,4-dioxane, FII(6.403) was found to be stable after 300 h. In this solvent, the solubility of FII(6.403) was determined after a 48 h equilibration time. The solubility of FII(6.403) was also determined after 48 h in acetone below 298 K and in 2-propanol below 303 K since it was found to be stable under these conditions for greater than 48 h. At higher temperatures in acetone and 2-propanol, and at all temperatures in ethanol, the solubility of FII(6.403) was determined immediately when FIII(6.525) was detected in the solid phase. It was hoped to measure the solubility of the metastable form in methanol also. However, in methanol, especially at higher temperatures, FII(6.403) was found to be very unstable, and reliable solubility data could not be obtained.

Solubility of FII(6.403) in Organic Solvents. The solubility results are presented in Table 4, as the mean value and the standard uncertainty of the three samples as grams of solute per gram of solvent on a solute-free basis. Solubility data could not be obtained in 1,4-dioxane below 288 K because the freezing point of the solvent is 284.8 K. The standard uncertainty is less than 2.31·10$^{-4}$ g·g$^{-1}$ in all cases, and the expanded uncertainty is less than 4.62·10$^{-4}$ with a confidence interval of 0.95 indicating little variance and reproducible results. The solubility graph for FII(6.403) in the four solvents is presented in Figure 7 with exponential curves fitted to the data. R$^2$ values of greater than 0.995 were obtained for all solvents. The solubility of FII(6.403) in all solvents increases with temperature. As with the case of FIII(6.525),$^9$ the solubility of FII(6.403) increases with increasing solvent polarity and solvent acidity. Piracetam is extremely soluble in water (>1g·g$^{-1}$ at room temperature); therefore, it was not practical to obtain solubility data in water. 1,4-Dioxane and acetone are aprotic solvents and cannot hydrogen bond to the proton-accepting carbonyl oxygen of the solute molecule.

Figure 5. DSC scan of pure FII(6.403). Endothermic peaks indicating the transformation to FI(6.747) with an onset at 382 K and melting of FI(6.747) with an onset at 423 K. Heating rate of 0.833 K·s$^{-1}$.

Figure 6. Portion of the concentration–time profile and polymorphic composition of the solid phase during the transformation of FII(6.403) $\rightarrow$ FIII(6.525), at 323 K in 2-propanol. $\bullet$, percentage FII(6.403) in the solid phase; $\bigtriangleup$, percentage FIII(6.525) in the solid phase; unmarked line, concentration of piracetam in solution (peak area ratio).
Ho solubility of FII(6.403) is slightly higher than that reported for van

range (278 to 323) K. This plot shows the logarithm of the mole fraction solubility on total solvent content for different temperatures. Alcohols are protic and can interact not only with the hydrogen bond donating amino group but also with the carbonyl oxygen of the solute.

In Figure 8 is presented the corresponding van’t Hoff plot, where the logarithm of the mole fraction solubility on total basis is plotted against the reciprocal temperature. The van’t Hoff enthalpy of solution can be calculated from the slope of this plot:

\[ \Delta H_{\text{soln}}^\text{vH} = -R \frac{d \ln x}{d \left( \frac{1}{T} \right)} \]

(1)

The corresponding values for FII(6.403) and FIII(6.525) in each of the solvents are presented in Table 5. In all solvents, the van’t Hoff enthalpy of solution is slightly lower for FII(6.403) compared to FIII(6.525).

In Figure 9, the FII(6.403) mole fraction solubility is compared with that of the stable FIII(6.525) in ethanol. The solubility of FII(6.403) is slightly higher than that reported for FIII(6.525) at all temperatures. As given by Table 4, the ratio of the mole fraction solubility of FII(6.403) to FIII(6.525) is weakly dependent on the solvent and decreases slightly with increasing temperature, from an average of 1.151 at 278 K to 1.026 at 323 K. A detailed study on SS polymorphic compounds showed a ratio between the highest and lowest solubility polymorphs to have an average of 1.7, with a maximum at 4.7 and a minimum at 1.0. Since solubility reflects the stability hierarchy of polymorphs in a system, and

![Figure 7. Solubility of FII(6.403) (Cs) versus temperature (T) in a range of solvents from (278 to 323) K. ■ ethanol; ○, 2-propanol; ● acetone; ▲, 1,4-dioxane. Exponential curves fitted to the data with R² values of greater than 0.995.](image)

![Figure 8. van’t Hoff plot of the mole fraction solubility (ln(x)) of FII(6.403) in different solvents against temperature (T) with a straight line fitted to the data in each solvent. ■ ethanol; ○, 2-propanol; ● acetone; ▲, 1,4-dioxane. Straight lines fitted to the data with R² values of greater than 0.995.](image)

Alcohols are protic and can interact not only with the hydrogen bond donating amino group but also with the carbonyl oxygen of the solute.

Table 4. Solubility Data, Cs (g FII(6.403)/g solvent⁻¹), Standard Uncertainty (Std. Uncert) Values of Three Samples for Each Solubility Value, and the Mole Fraction Solubility (Mol. Frn. Sol.) Ratio (FII(6.403)/FIII(6.525)) in the Four Solvents over the Range (278 to 323) K

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>Ethanol Cs (FII(6.403))</th>
<th>2-Propanol Cs (FII(6.403))</th>
<th>Acetone Cs (FII(6.403))</th>
<th>1,4-Dioxane Cs (FII(6.403))</th>
<th>Uncertainty</th>
</tr>
</thead>
<tbody>
<tr>
<td>278</td>
<td>1.80×10⁻² g/10⁻¹ g</td>
<td>1.04×10⁻² g/10⁻¹ g</td>
<td>1.11×10⁻² g/10⁻¹ g</td>
<td>1.08×10⁻² g/10⁻¹ g</td>
<td>1.11×10⁻² g/10⁻¹ g</td>
</tr>
<tr>
<td>323</td>
<td>3.89×10⁻² g/10⁻¹ g</td>
<td>1.04×10⁻² g/10⁻¹ g</td>
<td>1.11×10⁻² g/10⁻¹ g</td>
<td>1.08×10⁻² g/10⁻¹ g</td>
<td>1.11×10⁻² g/10⁻¹ g</td>
</tr>
</tbody>
</table>

Table 5. Estimated van’t Hoff Enthalpy of Solution (ΔHvH) Values for FII(6.403) and FIII(6.525) in the Four Solvents Examined in the Solubility Study

<table>
<thead>
<tr>
<th>Solvent</th>
<th>ΔHvH (kJ mol⁻¹)</th>
<th>ΔHvH (kJ mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>31.5</td>
<td>33.1</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>33.9</td>
<td>36.0</td>
</tr>
<tr>
<td>Acetone</td>
<td>26.4</td>
<td>28.5</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>30.3</td>
<td>31.8</td>
</tr>
</tbody>
</table>

"Expanded uncertainty of ΔHvH for FII(6.403) and FIII(6.525) is (5.4 and 5.3) kJ mol⁻¹ with a confidence interval of 0.95."
such a small difference in free energy is reported between 
FII(6.403) and FIII(6.525),\textsuperscript{11−13} it is not surprising that the 
 solubility ratio is at the lower end of this scale. 

As outlined in Figure 5 for FII(6.403) and previously in the 
case of FII(6.525),\textsuperscript{9} determination of melting temperatures of 
FII(6.403) and FII(6.525) is very difficult because of the 
transformation of these polymorphs to FII(6.747) in the range 
(381 to 399) K.\textsuperscript{13,15,16} Pavlova et al.\textsuperscript{12} estimated the melting 
points are in the region of (418 to 426) K, while Kuhnert-
Brandstätter et al.\textsuperscript{13} estimated the melting points of FII(6.403) 
and FIII(6.525) to be (413.7 and 413.2) K, respectively, using 
thermomicroscopy. At increasing temperature, solubility data 
should gradually approach the conditions at the melting 
temperature where ln(x) = 0 and the van’t Hoff enthalpy of 
solution equals the melting enthalpy.\textsuperscript{19} As with the solubility 
data obtained for FIII(6.525),\textsuperscript{9} regression equations (eq 2 and 
3) are fitted to the data of FII(6.403) with a view to estimating 
the melting point.\textsuperscript{19}

\[
\ln(x) = A + BT \tag{2}
\]

\[
\ln(x) = AT^{-1} + B + CT \tag{3}
\]

The regression equations (eqs 2 and 3)\textsuperscript{19} were successfully 
fitted to the solubility data using Microsoft Office Excel 2010 and 
Matlab 6.5, with the coefficients listed in Table 6. $R^2$ values of greater than 0.995 were obtained. Table 7 shows data for 
each solvent, along with mean values for each equation. When 
eq 2 is fitted to the data, the melting point of FII(6.403) is 
higher than that of FII(6.525)\textsuperscript{9} in all four solvents, while for eq 
3 the melting point of FIII(6.525) is predicted to be higher in 
ethanol and 2-propanol. Since solubility data proved that 

Table 6. Coefficients of Equation 2 and Equation 3 Fitted to 
the Solubility Data of FII(6.403) in Each of the Solvents 
Examined, Estimated with Microsoft Office Excel 2010 and 
Matlab 6.5

<table>
<thead>
<tr>
<th>solvent</th>
<th>eq 2: $\ln(x) = A + BT$</th>
<th>eq 3: $\ln(x) = AT^{-1} + B + CT$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>ethanol</td>
<td>-16.85</td>
<td>0.0422</td>
</tr>
<tr>
<td>2-propanol</td>
<td>-18.31</td>
<td>0.0455</td>
</tr>
<tr>
<td>1,4-dioxane</td>
<td>-17.75</td>
<td>0.0393</td>
</tr>
<tr>
<td>acetone</td>
<td>-16.20</td>
<td>0.0354</td>
</tr>
</tbody>
</table>

Table 7. Estimated Melting Temperatures for FII(6.403) and 
FIII(6.525)\textsuperscript{9} Calculated by Extrapolation of Equation 2 and 
Equation 3 in Each Solvent, Along with Reported Data\textsuperscript{a}

<table>
<thead>
<tr>
<th>solvent</th>
<th>eq 2: $\ln(x) = A + BT$</th>
<th>eq 3: $\ln(x) = AT^{-1} + B + CT$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>melting point/K</td>
<td>melting point/K</td>
</tr>
<tr>
<td>FII(6.403)</td>
<td>FIII(6.525)</td>
<td>FII(6.403)</td>
</tr>
<tr>
<td>ethanol</td>
<td>399</td>
<td>396</td>
</tr>
<tr>
<td>2-propanol</td>
<td>402</td>
<td>398</td>
</tr>
<tr>
<td>acetone</td>
<td>454</td>
<td>449</td>
</tr>
<tr>
<td>1,4-dioxane</td>
<td>451</td>
<td>446</td>
</tr>
<tr>
<td>mean</td>
<td>426</td>
<td>422</td>
</tr>
<tr>
<td>literature</td>
<td>413.7</td>
<td>413.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Expanded uncertainty for the melting point of FII(6.403) and 
FIII(6.525) with eq 2 is (52.0 and 50.5) K, respectively, and for eq 3 is 
(35.9 and 34.3) K, respectively, with a confidence interval of 0.95.

FIII(6.525) is the stable polymorph under ambient conditions 
and an enantiotropic relationship exists between FII(6.403) and 
FIII(6.525).\textsuperscript{13,16} it becomes apparent that FII(6.403) should 
have a higher melting point than FIII(6.525). While the predicted 
melting points in the individual solvents show a certain level of variation, the averaged values are in the range 
outlined in the literature. Collecting data for a number of 
solvents increases the accuracy of the predictions. The mean of 
the predicted values in the four solvents using eq 3 is within 5 K 
of the melting points proposed by Kuhnert-Brandstaetter et al.,\textsuperscript{13} while the mean for eq 2 is in the range outlined by Pavlova 
at al.\textsuperscript{12} The error in the predictions is approximately equal to 
the error reported for the predictions by Nordström and 
Rasmusson.\textsuperscript{19}

\section*{Conclusion}

The work shows that FII(6.403) can be prepared in the pure 
form and then distinguished from the other polymorphs of 
piracetam using analytical techniques, XRD, DSC, and ATR- 
FTIR, and that solubility can be determined by the gravimetric 
method in spite of the metastability. The solubility of piracetam 
FII(6.403) in four different solvents is shown to increase with 
temperature and solvent polarity. FII(6.403) is most soluble in 
the alcohol with the shortest aliphatic chain, ethanol. Acetone 
and 1,4-dioxane are relatively nonpolar when compared to the 
$\alpha$-alcohols, and accordingly the solubility of FII(6.403) is much 
lower in these solvents. As expected, the solubility of the 
metastable FII(6.403) was higher than that of the stable 
FII(6.525) in all solvents at all temperatures. However, there 
was not a dramatic difference between the solubilities, owing to 
the high relative stability of FII(6.403) compared to 
FIII(6.525). By fitting regression equations to the solubility 
data, an estimation of the melting temperature is obtained not 
far from previously reported values.

\section*{Author Information}

\textbf{Corresponding Author}

\textbf{A. Maher gratefully appreciates funding from an Irish Research Council for Science, Engineering and Technology (IRCSET) scholarship as well as the Solid State Pharmaceutical Cluster (SSPC).}

\textbf{Notes}

\textbf{The authors declare no competing financial interest.}
REFERENCES


III
Solution Mediated Polymorphic Transformation: Form II to Form III Piracetam in Ethanol

Anthony Maher,* Denise M. Croker, Åke C. Rasmuson, and Benjamin K. Hodnett

Solid State Pharmaceuticals Cluster, Materials and Surface Science Institute, Department of Chemical and Environmental Sciences, University of Limerick, Limerick, Ireland

ABSTRACT: The polymorphic transformation of the Piracetam (2-oxo-1-pyrrolidine acetamide) metastable form II to the stable form III has been investigated in ethanol, by ex-situ X-ray diffraction analysis of the solid phase combined with in situ infrared analysis of solution concentrations. The main factors studied were temperature, mass of solids loading, and pretreatment of the loaded solids. At increasing temperature, the solubility ratio decreases and, hence, the transformation driving force decreases. In spite of this, the nucleation induction time of the stable form and the time for completion of the transformation decrease with increasing temperature. The transformation rate is governed by the nucleation and growth of the stable form, with nucleation likely to take place on the faces of the metastable form crystals and the rate increasing with surface area of the metastable form.

INTRODUCTION

While the crystallization of an active pharmaceutical ingredient (API) in the correct form is an important step in the manufacture of a drug product, the stability of that form in the final product and during its shelf life is just as critical. Polymorphism, defined as the ability of a material to crystallize and exist in different crystal structures, is a phenomenon often observed in the pharmaceutical industry. In the case of ritonavir, the API in Norvir (Abbott Laboratories, Chicago), a more stable polymorph (form II) identified after the product was on the market, had an adverse effect on the bioavailability of the drug. Differences in properties between polymorphs in a system have led to tight regulation of polymorphism in the pharmaceutical industry.

Piracetam (Structure 1) is a nootropic drug used to treat diseases associated with cognitive dysfunction. Throughout the literature, there is some confusion over the nomenclature of the different polymorphs. The system employed in this work for naming polymorphs was outlined previously. The numerical value of the a-lattice parameter in angstroms, which appears in the corresponding Cambridge Crystallographic Data Centre (CCDC) file, is added to the polymorph label. In the case of FII piracetam, the file name is BISMEV, and the value of a is 6.403 Å, hence FII(6.403). In the case of FIII, the file name is BISMEV01 and a is 6.525 Å, hence FIII(6.525). As verified by solubility data, FIII(6.525) is the stable polymorph under ambient conditions, while FII(6.403) is metastable. Following Ostwald’s rule of stages, FII(6.403) has been reported to transform to FIII(6.525) in numerous solvents. However, this transformation does not occur in the solid state.

Solution mediated polymorphic transformations are composed of three stages; dissolution of the metastable phase, nucleation of the stable phase, and growth of the stable phase. Under certain conditions, a polymorph can exist in a metastable region, provided the stable polymorph is not present. Nucleation of the stable form “activates” the transformation, making it a critical step. In an extensive review, Croker et al. observed that, in solution-mediated polymorphic transformations, nucleation of the stable polymorph tends to occur on, or at, the surface of the existing polymorph. The nucleation of the stable βL glutamic acid was proved to be surface mediated in the presence of the metastable αL form. Additives, which selectively adsorbed to the surface of the metastable form, prevented nucleation of the stable form. Further studies confirming the surface mediated nature of the transformation by Scholl et al. found that the transformation rate was faster when a smaller particle size of the αL glutamic acid was employed, indicating that the rate of nucleation of the βL form increases concurrently with the surface area of the metastable
form. In the case of the transformation from form 1 to form 2 of 2,6-dihydroxybenzoic acid, a suspected higher local supersaturation on the (002) face of form 1 encouraged heterogeneous nucleation of form 2.2,19,20

In order to establish the rate determining step(s) and gain an understanding of the growth and dissolution rate constants which dominate the kinetics of the transformation, it is necessary to monitor the solution concentration as well as the composition of the solid phase during the transformation.21 A convenient measure of the transformation kinetics is the transformation time: defined as the time to total disappearance of the metastable polymorph in the solid material and growth of the stable form to its final size, corresponding to the plateau in the solution concentration at the solubility of the stable form.20

Numerous factors affect the rate of transformation in a specific solvent.21 At 20 °C, γ-glycine was detected in the solid phase of the metastable α-form after 3 h in an 81 g/L sodium chloride aqueous solution, and the transformation was completed in 16 h. At 37 °C, the transformation had begun after 1.5 h and was completed within 7 h.22 Numerous other examples of the effect of temperature on polymorphic transformations exist in the literature.20 Molecular movement is thought to increase with temperature, and the solid–liquid boundary layer is expected to be more easily overcome at higher temperatures.15,20 This is crucial in both the dissolution of the metastable crystals and the nucleation and growth of the stable phase. Since the solubility of a compound generally increases with temperature, the likelihood of molecular collisions occurring for the formation of critical nuclei increases with temperature.

Su et al.23 investigated the effect of different amounts (3–5 g) of the metastable α-form of β-mannitol in 100 mL of saturated 17% w/w ethanol–water at 27 °C on the transformation to the β form. Higher loadings resulted in slightly longer transformation times to the β form. This was attributed to a constant rate of crystal growth, and therefore, the higher loadings resulted in an extended growth phase. However, if nucleation of the stable phase is surface mediated, as it is thought to be in most cases,16 nucleation has a specific rate per unit surface area of the metastable phase.7,24 Hence, different loadings of the metastable phase should not affect the overall transformation time.

The effect of scale up on transformation rates has also been extensively examined. Form 1 to form 2 of 2,6-dihydroxybenzoic acid in toluene was found to have a faster transformation time.23 The effect of scale up on transformation rates has also been extensively examined. Form 1 to form 2 of 2,6-dihydroxybenzoic acid in toluene was found to have a faster transformation time.23

In this study the effect of temperature, amount of solid loading of FII(6.403), specific surface area of the metastable form, and scale of operation are examined.

**EXPERIMENTAL SECTION**

Piracetam, complying with European Pharmacopoeia 6.5 quality and purity standards, was supplied by UCB Pharma SA (CAS Number: 7491-74-9, Batch Number: 09G06-893. The Certificate of Analysis states that the batch complies with the IR, HPLC, and solution appearance tests. It also complies with a heavy metals limit of <0.0 ppm and sulphated ash of <0.1%, and the purity is 100 ±2%. The water content was found to be less than 0.1%. Ethanol (ACS reagent, ≥99.5%, CAS Number 64-17-5) from Sigma-Aldrich was used without further purification.

The method of producing pure FIII(6.403) has been outlined previously.10 FII(6.403) produced in this manner was used to investigate the solution mediated polymorphic transformation to FIII(6.525) in ethanol.

The solution mediated polymorphic transformation was investigated on 25 and 130 g scales. The smaller scale experiments were carried out to investigate the effect of temperature and mass loading on the transformation. The experimental setup consisted of a thermostatic water bath (Grant GR150; 38 L; 690 mm × 300 mm × 200 mm; @ 37 °C; stability ±0.005 °C, and uniformity ±0.02 °C) with a serial magnetic stirrer plate placed on the base. The effect of temperature was examined between 5 and 50 °C at increments of 5 °C, allowing for estimation of the time for nucleation of FIII(6.525) and the time of complete transformation. At each temperature, 25 g of ethanol was initially saturated with respect to FIII(6.525). An extra 0.5 g of FII(6.403) was then added. Of the extra FII(6.403) added, the difference in the amount of dissolution, across the temperature range, in reaching the solubility of FII(6.403) was small (10–16%). The transformation of FII(6.403) to the stable FIII(6.525) was monitored via ex-situ X-ray diffraction (XRD) analysis of the solid material, as outlined previously.10 Quantification of the polymorphic composition of each sample was carried out according to ref 25. The composition of the solid phase was monitored throughout the transformation via approximately 10 samples. Each experiment was carried out twice at each temperature. The first run gave an indication of the time scale for the transformation, while the second allowed for accurate identification of the time for nucleation of FIII(6.525) and the transformation time. The results from the second run are presented below.

The effect of mass loading of FII(6.403) on the rate of transformation was also investigated on a 25 g scale. Four 25 g piracetam/ethanol solutions saturated with respect to FIII(6.525) (79.2 g kg⁻¹) at 40 °C were prepared. The concentration of piracetam in each vessel was increased by different amounts, giving final concentrations ranging from 87.2 g kg⁻¹ to 135.5 g kg⁻¹. The solutions were agitated at 500 rpm. The transformation was monitored via ex-situ XRD analysis of the solid phase until the composition was 100% FIII(6.525). All experiments were repeated at each loading to verify the reproducibility of the data.

Larger scale experiments were performed at 30 and 50 °C to establish the rate-determining steps of the transformation. The experiments were performed in a 250 mL Jacketed Vessel controlled by a Lauda E305 recirculator and a E300 controller equipped with an overhead paddle impeller. To 130 g of ethanol held at 30 °C and agitated at 270 rpm was added 9.919 g of FII(6.403), bringing the concentration in the vessel to 76.3 g piracetam kg⁻¹ ethanol. In addition to the fact that the volumes and the agitation differ, in the larger scale experiments, there is enough room to investigate the solution mediated polymorphic transformation to FIII(6.525). The solutions were then cooled and held at 50 °C, allowing for estimation of the time for nucleation of FIII(6.525) and the time of complete transformation. At each temperature, 25 g of ethanol was initially saturated with respect to FIII(6.525). An extra 0.5 g of FII(6.403) was then added. Of the extra FII(6.403) added, the difference in the amount of dissolution, across the temperature range, in reaching the solubility of FII(6.403) was small (10–16%). The transformation of FII(6.403) to the stable FIII(6.525) was monitored via ex-situ X-ray diffraction (XRD) analysis of the solid material, as outlined previously.10 Quantification of the polymorphic composition of each sample was carried out according to ref 25. The composition of the solid phase was monitored throughout the transformation via approximately 10 samples. Each experiment was carried out twice at each temperature. The first run gave an indication of the time scale for the transformation, while the second allowed for accurate identification of the time for nucleation of FIII(6.525) and the transformation time. The results from the second run are presented below.

The effect of mass loading of FII(6.403) on the rate of transformation was also investigated on a 25 g scale. Four 25 g piracetam/ethanol solutions saturated with respect to FIII(6.525) (79.2 g kg⁻¹) at 40 °C were prepared. The concentration of piracetam in each vessel was increased by different amounts, giving final concentrations ranging from 87.2 g kg⁻¹ to 135.5 g kg⁻¹. The solutions were agitated at 500 rpm. The transformation was monitored via ex-situ XRD analysis of the solid phase until the composition was 100% FIII(6.525). All experiments were repeated at each loading to verify the reproducibility of the data.

Larger scale experiments were performed at 30 and 50 °C to establish the rate-determining steps of the transformation. The experiments were performed in a 250 mL Jacketed Vessel controlled by a Lauda E305 recirculator and a E300 controller equipped with an overhead paddle impeller. To 130 g of ethanol held at 30 °C and agitated at 270 rpm was added 9.919 g of FII(6.403), bringing the concentration in the vessel to 76.3 g piracetam kg⁻¹ ethanol. In addition to the fact that the volumes and the agitation differ, in the larger scale experiments, there is enough room to fit a Mettler-Toledo ReactIR IC10 ATR-FTIR probe, allowing for in-situ monitoring of the solution concentration.10 The composition of the solid phase was monitored by ex-situ XRD analysis. It was found that taking samples of the excess solid from the vessel had no effect on the concentration in solution. This experiment was repeated at 50 °C at a concentration of 150 g of FII(6.403) kg⁻¹ ethanol.

In the larger scale experiments, the effect of the specific surface area of FII(6.403) on the transformation was also investigated at 50 °C. The methodology is outlined in Scheme 1, schematically showing how the equilibrium saturation of FII(6.403) was reached from three distinct routes (R1, R2, and R3). Different amounts of FII(6.403) were added to each run, and homogeneous solutions were obtained at 60 °C in all three experiments. The solutions were then cooled and held at 50 °C, resulting in different levels of saturation (σ = 0 (R1), 1 (R2), 1.1 (R3) with respect to FIII(6.525)). The experiments were then started (t₀) when FII(6.403) was added to the homogeneous solutions with agitation of 270 rpm. The concentration of piracetam in ethanol
Scheme 1. Schematic of the Concentration—Time Profiles Associated with the Methodologies for the Introduction of FII(6.403) into Ethanol at 50 °C*.

*Blue (R1), addition to pure solvent; red (R2), addition to a solution saturated wrt FIII(6.525); green (R3), addition to a solution supersaturated wrt both polymorphs.

in all three runs after ζ, was 150 g·kg⁻¹, giving an equal mass of FII(6.403) (2.85 g) to be transformed after the equilibrium concentration of FII(6.403) was reached. Again, the transformation was monitored using in situ ATR-FTIR analysis of the solution phase and ex-situ XRD analysis of the solid phase.

**RESULTS AND DISCUSSION**

The result of the experiments investigating the effect of temperature on the transformation is a series of plots documenting the composition of the solid phase during the transformation at each temperature. Figure 1 shows the composition of the solid phase during the transformation in repeated runs at 25 °C in ethanol, indicating that the data is reproducible.

The point at which the stable form is first detected in the solid phase, the best estimate of nucleation of the stable form, is interpreted as the induction time of the transformation. The total time for completion of the transformation after the addition of the excess FII(6.403), determined as the moment when the composition of the solid phase is 100% FIII(6.525), is expressed as the transformation time. Figure 2 shows a portion of the transformation at 50 °C on the 130 g scale in terms of an FTIR concentration—time profile with the composition of the solid phase included. The equilibrium saturation of the metastable polymorph is reached, via R1 (Scheme 1), within a short period of time after adding FII(6.403). After 3 h, FIII(6.525) is detected for the first time, indicating that nucleation is a limiting factor in the transformation. The amount of FIII(6.525) increases slowly in the initial stages. In the first hour after detection, the amount of FIII(6.525) increases to 10%, while in the second and third hours, increases of 40 and 50% transformation, respectively, are observed until the transformation has gone to completion. The overall growth of FIII(6.525) is limited by the surface area of FIII(6.525) available, and the rate increases as the crystal size increases after nucleation. The concentration in solution remained at the solubility level of FII(6.403) even with significant amounts FIII(6.525) in the solid phase (∼80%). After approximately 5.2 h, a decrease in the concentration in solution corresponding to the end of the transformation is observed. The concentration plateau corresponding to the saturation level of FIII(6.525) in ethanol was reached after 6.7 h while the composition of the solid phase was 100% FIII(6.525) approximately 1 h earlier. This indicates that the dissolution rate of FII(6.403) is faster than the growth of FIII(6.525) and is not limiting in the transformation.

**Influence of Temperature.** A similar pattern of events is observed in Figure 3 at 30 °C, with the FII(6.403) equilibrium again reached via R1. As expected, the ratio of the area of the piracetam and ethanol peaks is much smaller as a result of the lower solubility at 30 °C. In addition, the lower temperature results in longer induction and transformation times. FIII(6.525) was first detected in the excess solid after 17 h, but the concentration in solution remains at the solubility point of FII(6.403) until 28 h, when the composition of the solid phase is mostly FIII(6.525). It then took 5 h for the solubility plateau of FIII(6.525) to be reached. Again nucleation and growth of the stable form are the limiting factors in the transformation, indicating that the governing mechanism of the transformation is the same across the temperature range of this study.
The effect of temperature on the transformation rate is further revealed in Figure 4. The induction and transformation times of the experiments on a 25 g scale, set up via R2, are presented over the temperature range 5–50 °C. Added FII(6.403): 0.5 g in excess of FIII(6.525) solubility.

Figure 4. A plot of the induction time (▲) and transformation time (■) for the transformation in ethanol on a 25 g scale, set up via R2, over the range 5–50 °C. Added FII(6.403): 0.5 g in excess of FIII(6.525) solubility.

The thermodynamic driving force (Δμ) and supersaturation ratio (S) initially driving the transformation can be expressed as follows:

$$\Delta \mu \approx RT \ln \left( \frac{x^*_{\text{FII}(6.403)}}{x^*_{\text{FIII}(6.525)}} \right) = RT \ln S$$

(1)

where \(x^*\) is the mole fraction solubility, \(R\) is the gas constant (8.314 J·mol\(^{-1}\)·K\(^{-1}\)), and \(T\) is temperature (K). Using the solubility data of the polymorphs\(^{15}\) for each experimental temperature, the values are presented in Table 1, along with the induction time for the transformation. The supersaturation decreases as the temperature increases.

In Figure 5, the induction time is plotted against the driving force (RT ln S).

First is counterintuitive. However, the explanation is that the transformation driving force cannot be altered without changing the temperature. Increasing temperature obviously increases the kinetics sufficiently to counteract the effect of the decreasing driving force.

Table 1. Supersaturation (S) \((x^*_{\text{FII}(6.403)}/x^*_{\text{FIII}(6.525)})\) and Thermodynamic Driving Force \((RT \ln S)\) for the Transformation over the Temperature Range 5–50 °C

<table>
<thead>
<tr>
<th>temp (°C)</th>
<th>supersat. ratio (S) ((x^<em>_{\text{FII}(6.403)}/x^</em>_{\text{FIII}(6.525)}))</th>
<th>RT ln S (J·mol(^{-1}))</th>
<th>induction time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.118</td>
<td>259</td>
<td>43</td>
</tr>
<tr>
<td>10</td>
<td>1.115</td>
<td>256</td>
<td>33</td>
</tr>
<tr>
<td>15</td>
<td>1.101</td>
<td>230</td>
<td>23</td>
</tr>
<tr>
<td>20</td>
<td>1.081</td>
<td>191</td>
<td>15.5</td>
</tr>
<tr>
<td>25</td>
<td>1.053</td>
<td>129</td>
<td>11</td>
</tr>
<tr>
<td>30</td>
<td>1.050</td>
<td>123</td>
<td>9</td>
</tr>
<tr>
<td>35</td>
<td>1.038</td>
<td>96</td>
<td>7</td>
</tr>
<tr>
<td>40</td>
<td>1.029</td>
<td>74</td>
<td>5</td>
</tr>
<tr>
<td>45</td>
<td>1.031</td>
<td>81</td>
<td>3</td>
</tr>
<tr>
<td>50</td>
<td>1.025</td>
<td>66</td>
<td>2.25</td>
</tr>
</tbody>
</table>

In accordance with the work of others,\(^{16}\) it is assumed that nucleation of the stable form during the transformation is promoted by the surface of the metastable form. This is supported by a few separate homogeneous nucleation experiments at supersaturations ranging from 1.03 to 1.06 with respect to FIII(6.525), at 50 °C, in which no nucleation occurred within 20 days. In comparison, in the transformation experiments at 50 °C, at the supersaturation ratio of 1.025 with respect to FIII(6.525), the transformation time was 6.75 h. The surface of FII(6.403) appears to facilitate the nucleation of FII(6.403)/FIII(6.525), i.e. the free energy associated with the formation of a critical nucleus, \(\Delta G_{\text{crit}}\), is less than that associated with homogeneous nucleation, \(\Delta G_{\text{hom}}\).\(^{16,24}\) Assuming a spherical nucleus, the rate equation for this type of nucleation can be expressed as follows:\(^{15,26,27}\)

$$J = \frac{16 \pi n^3 \gamma \Phi}{3 k^3 T^3 (\ln S)^2}$$

(2)

where \(A\) is the pre-exponential factor, \(\gamma\) is the interfacial tension, \(v\) is the molecular volume, \(\Phi\) is the heterogeneous nucleation factor, and \(k\) is the Boltzmann constant (1.3805 × 10\(^{-23}\) J·K\(^{-1}\)). The pre-exponential or collision factor \(A\) increases with temperature directly through increasing solute concentration and decreasing solution viscosity according to the following:


\[ A \propto \left( \frac{C T^{3/2}}{\eta} \right) \quad (3) \]

where \( C \) is the solute concentration and \( \eta \) is the solution viscosity. Hence, the explanation to Figure 5 is that the pre-exponential factor increases with increasing temperature, primarily because of an increased molecular attachment frequency, to an extent that more than enough compensates for the decreasing driving force.

**Influence of Solids Loading.** If the transformation process is governed by the surfaces available for nucleation of the stable form, the transformation time should depend on the available surface area per unit mass of the metastable form. In experiments where the amount of solid to be transformed is changed by adding different masses of the same solid material, we would expect the transformation time to be constant. In Figure 6 is shown the absolute concentration of FIII(6.525) in the solid phase during the transformation experiments with different loadings of FII(6.403) according to Table 2, prepared via R2. The induction and transformation times appear to be identical in all four experiments, indicating that the number of nucleation points of FIII(6.525) increases accordingly with the available surface area of FII(6.403).

A plot documenting the percentage of FII(6.403) in the solid phase during the four transformation experiments (Figure 7) indicates that the transformation rate is very slightly faster in the experiments with lower loadings of FII(6.403). The reason for this is that the surface area per unit mass of FII(6.403) to be transformed is higher, because during the preparation the dissolution of the added solids is more pronounced, as shown in Table 2. Since nucleation is assumed to occur on the faces of the FII(6.403) crystals and is approximately proportional to the solid surface area, there will be more FIII(6.525) nuclei per unit mass in the experiments with more dissolution of the seeds.

**Influence of Seed Pretreatment.** The effect of the amount of surface area of FII(6.403) available for nucleation of FIII(6.525) on the transformation time was further examined as outlined in Scheme 1 and Table 3.

Figure 8 shows the progression in the three experiments in terms of the concentration in solution and the percentage composition of the solid phase. Upon the addition of FII(6.403) at \( t_0 \) the concentration reaches the equilibrium saturation of the metastable form in all three experiments. In the case of the experiment prepared via R1, 85% of the added material is dissolved. Assuming that each particle of FII(6.403) dissolves to a similar extent and disregarding breakage, the number of particles when FII(6.403) equilibrium saturation is reached should be the same as the number added to the vessel at \( t_0 \). A significantly smaller mass of FII(6.403) was added to the solution that was saturated with respect to FIII(6.525) at \( t_0 \) (R2), and less again was added in the case of R3. Only 13% dissolution of the material added in R2 occurred, while growth of the added FII(6.403) crystals to R3 increased the solid mass by 72%. The early ex-situ XRD analysis of this experiment confirmed that no FIII(6.525) had nucleated during this desupersaturation phase.

Since an equal mass of FII(6.403) is present at the point where FII(6.403) equilibrium is reached, and the number of particles in R1 is orders of magnitude greater than that in R2 and R3, the surface area per unit mass, or the specific surface area, of the metastable form is significantly higher in the case of R1.

The rate of the transformation in the presaturated (R2) and presupersaturated (R3) solutions is very similar, while the rate is slightly faster in the R1 experiment. The concentration in solution begins to drop from the solubility of FII(6.403) after

Table 2. Influence of Solids Loading on the Rate of Transformation

<table>
<thead>
<tr>
<th>test tube</th>
<th>C, FII(6.403) (g)</th>
<th>sat. level before ( t_0 )</th>
<th>amt of FII(6.403) added at ( t_0 ) (g)</th>
<th>% dissoln of added FII(6.403)</th>
<th>excess to be transformed (g)</th>
<th>excess to be transformed (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>79.2</td>
<td>1</td>
<td>8.0</td>
<td>30</td>
<td>5.6</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>79.2</td>
<td>1</td>
<td>24.1</td>
<td>17</td>
<td>21.6</td>
<td>0.54</td>
</tr>
<tr>
<td>3</td>
<td>79.2</td>
<td>1</td>
<td>40.2</td>
<td>7</td>
<td>38.0</td>
<td>0.95</td>
</tr>
<tr>
<td>4</td>
<td>79.2</td>
<td>1</td>
<td>56.3</td>
<td>4</td>
<td>54.0</td>
<td>1.35</td>
</tr>
</tbody>
</table>

\(^a\) Concentrations and amounts of FII(6.403) added to 25 g of ethanol in each tube at 40 °C during the investigation.
Table 3. Concentrations and Amounts of FII(6.403) Added to 130 g of Ethanol during the Investigations of the Transformation at 50 °C

<table>
<thead>
<tr>
<th>C, FIII(6.525) (g FII·kg⁻¹ EtOH)</th>
<th>piracetam in homog soln (g)</th>
<th>sat. level before t₀ (wt FII) (g)</th>
<th>FII(6.403) added at t₀ (g)</th>
<th>conc after t₀ (g FII·kg⁻¹ EtOH)</th>
<th>% dissolution of FII(6.403) added at t₀</th>
<th>excess avail to be transformed (g FII·kg⁻¹ EtOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>0.00</td>
<td>0</td>
<td>19.5000</td>
<td>150</td>
<td>85</td>
<td>22</td>
</tr>
<tr>
<td>125</td>
<td>16.2240</td>
<td>1</td>
<td>3.2760</td>
<td>150</td>
<td>13</td>
<td>22</td>
</tr>
<tr>
<td>125</td>
<td>17.8464</td>
<td>1.1</td>
<td>1.6536</td>
<td>150</td>
<td>−72</td>
<td>22</td>
</tr>
</tbody>
</table>

5.2 h in R1, while the plateau at the solubility of FII(6.525) is reached after 6.7 h. In R2 and R3 these events occur approximately 1 h later. When one examines the composition of the solid phase, 100% FII(6.525) is attained approximately 1 h earlier in the case of R1. As outlined earlier, the transformation is thought to be surface mediated. Therefore, it is not surprising that it goes to completion in a shorter time frame in the run with a higher specific surface area of the metastable phase (R1) compared to R2 and R3. The fact that the transformation rate varies with specific surface area of FII(6.403) adds to the argument that nucleation of FII(6.525) is surface mediated.

The concentration–time profiles in these experiments show that equilibrium saturation of FII(6.403) was reached within minutes in all three cases, verifying that the systems are well mixed and dissolution is not limiting in the transformation.

**Influence of Scale.** Finally, the effect of the scale of operation agrees with data reported in the literature. At 30 °C the mass to solvent ratio on the 25 g scale (0.020) is slightly lower than that on the 130 g scale (0.024). The induction time and transformation time on the smaller scale are 9 and 21 h, respectively, while on the larger scale these values are 17 and 30 h. The likelihood of collisions between the crystals and the test tubes walls and agitator, which help propagate nucleation of the stable form, is higher on the smaller scale. Also, the magnetic stir bar agitator on the smaller scale increases the extent of attrition of the crystals, compared to the overhead impeller. The difference in induction and transformation times is not as great at 50 °C on the two scales, but a similar trend is observed.

**CONCLUSIONS**

This work shows that the rate of transformation from piracetam FII(6.403) to FII(6.525) is governed by nucleation and growth of the stable phase, and belongs to “scenario d” as outlined by O’Mahony et al. The results further show that the rate of transformation increases quite strongly with increasing temperature, in spite of a decreasing driving force. The rate of nucleation of FII(6.525) as well as the overall rate of transformation increases. This clearly points to the importance of the pre-exponential factor in the classical nucleation equation. This work clearly suggests that nucleation of the stable phase occurs on the faces of the metastable phase, and is approximately proportional to the available surface area. Hence, having the metastable phase in the form of smaller particles should result in a faster process.

**AUTHOR INFORMATION**

Corresponding Author

E-mail: anthony.maher@ul.ie. Tel.: +353 61 234159.

Notes

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

A.M. gratefully appreciates funding from an Irish Research Council for Science, Engineering and Technology (IRCSET) scholarship as well as the Solid State Pharmaceutical Cluster (SSPC).

**REFERENCES**


Investigation of the Solid-State Polymorphic Transformations of Piracetam

Anthony Maher,*† Colin C. Seaton,† Sarah Hudson,‡ Denise M. Croker,† Åke C. Rasmuson,† and Benjamin K. Hodnett†

†Solid State Pharmaceuticals Cluster, Materials and Surface Science Institute, Department of Chemical and Environmental Sciences, University of Limerick, Limerick, Ireland
‡Pharmaceutical and Molecular Biotechnology Research Centre, Waterford Institute of Technology, Waterford, Ireland

ABSTRACT: The solid-state polymorphic transformations of 2-oxo-1-pyrrolidine acetamide (piracetam) were investigated using a combination of off-line and online techniques: differential scanning calorimetry (DSC), high temperature X-ray diffraction (HT-XRD), thermal analysis, and hot-stage optical microscopy. Form II and Form III were each observed to transform directly to Form I upon heating, with Form II transforming at a slightly lower temperature. The transformation of both polymorphs to Form I was observed to cause physical cracking of the crystals as well as changing the optical properties. Form I consistently transformed to Form II when cooled. The molecular rearrangements required for the transformation from Form I to Form II were found to be more energetically favorable than those required for the transformation to Form III. The transformation from the metastable Form II to the stable Form III was not observed in the solid-state, while the Form II–Form III transition temperature was found to be higher than the transition temperature of both polymorphs to Form I.

INTRODUCTION

Pharmaceutical solids may undergo phase transformations resulting in undesirable dosage form performance. A polymorphic transformation occurs when a particular phase becomes unstable as a result of the prevailing environmental conditions. The relative stability of the various polymorphs of a given compound is determined by the respective Gibbs free energies (G) of the different forms. Possible polymorphic transformations are dictated by differences in free energy at the transition point associated with structural or compositional changes, with the most stable polymorph having the lowest free energy.

Polymorphic transformations can proceed via a number of different means.1,2 The transformations examined in this work involve the molecular rearrangement of a metastable crystal structure into a more stable crystal structure while remaining in the solid state.3 In enantiotropic systems, where polymorphic transformations are thermodynamically reversible, there may or may not be a metastable region across the transition point where a polymorph can exist under a set of conditions but where an alternative polymorph is thermodynamically more stable. Kawakami et al.4 published a detailed study including numerous examples of systems of both types. The kinetics of an enantiotropic transformation may be hindered if the activation energy for the transformation is large enough to present a barrier, thereby creating a metastable region where the original polymorph has a finite lifetime.4–6

Piracetam (Chart 1) is a nootropic drug, which is an agent that acts on cognitive dysfunction without causing sedation or stimulaton.7,8 Throughout the literature there is some confusion over the nomenclature of the different polymorphs. In this work the system used for naming polymorphs is outlined previously, with the α-lattice parameter from the Cambridge Structural Database (CSD) placed in brackets after the polymorph identification.9 For example, Form II is referred to below as FII(6.403). Five polymorphs of piracetam have been reported, but two of these (FIV(8.9537) and FV(6.3903)) are obtained in high pressure (>0.5 GPa) conditions only.10,11 Another polymorph, FI(6.747) is only seen when FII(6.403) or FIII(6.525) are heated to 130 °C in the solid-state.10,12,13 FI(6.747) transforms to FII(6.403) within a few hours under ambient conditions.10,11,12,13

Chart 1. Molecular Structure of Piracetam

Received: September 27, 2012
Revised: October 24, 2012
Published: November 5, 2012
Fi(6.747) is acknowledged as the least stable polymorph of the three at ambient temperature. However, there are some discrepancies in the literature as to whether Fi(6.403) or FiII(6.525) is the thermodynamically stable polymorph. In the 1970s and 1980s, Pavlova et al.15,16 failed to establish which was the stable polymorph, reporting that Fi(6.403) and FiII(6.525) transform to Fi(6.747) in the range 130–140 °C. In 1994 using thermomicroscopy, differential scanning calorimetry (DSC) and a binary mixture stability study, Kuhnert-Brandstätter et al.13 found that FiII(6.525) is stable and FiI(6.403) is metastable at ambient temperature, while at higher temperatures FiI(6.747) is the stable polymorph, with all three enantiotropically related. The proposed energy-temperature diagram indicates that FiI(6.403) transforms to FiI(6.747) at a lower temperature (110 °C) than FiIII(6.525) (120 °C). It suggests that both polymorphs transform directly to FiI(6.747) and the transition temperature between FiI(6.403) and FiIII(6.525) is above those of FiI(6.403) or FiIII(6.525) to FiI(6.747). On the basis of the assumption that piracetam is a trimorphic system, in 1996, Ceolin et al.12 built a semi-quantitative pressure—temperature phase diagram from topological rules17 indicating that FiI(6.403) was the stable polymorph under ambient conditions and transformed to FiI(6.747) at a higher temperature than FiIII(6.525). In 2011, detailed analysis was carried out by Picciochi et al.14 using DSC, solution calorimetry and combustion calorimetry. It found the stability hierarchy between FiI(6.403) and FiIII(6.525) under ambient conditions and the transition temperatures to FiI(6.747) to be in agreement with Kuhnert-Brandstätter et al.13 In this work, the solid-state transformations of piracetam are characterized via DSC, high temperature X-ray diffraction (HT-XRD), hot-stage optical microscopy and thermal analysis. It has been noted that the thermodynamic transition temperature on the free energy diagram may often be in advance of the observed transition temperature from the experimental analysis in the literature. Hence, efforts were made to establish the exact temperature of the thermodynamic transitions seen in the piracetam system.

As remarked in an review by Herstein: “there are relatively few papers about the actual transition directly viewed by microscopic techniques in order to infer the mechanism, and not many about changes in crystal structure as the system passes through the transition.” Beckham et al. noted that gaining a direct molecular level insight into the dynamic events occurring during solid-state polymorphic transformations is outside of the scope of current experimental capabilities, and thus there is little definitive evidence for any particular mechanism.19 In this study the physical changes to the crystals during the transformations to FiI(6.747) are visually observed and computational prediction modeling is employed to gain an understanding on a molecular level.

**EXPERIMENTAL SECTION**

Piracetam was supplied by UCB Pharma SA and complies with European Pharmacopoeia 6.5 standards (CAS Number: 7491-74-9, Batch Number: 09G06-893 Certificate of Analysis states that the batch complies with the IR, HPLC and solution appearance tests. It also complies with a heavy metals limit of <10 ppm, sulphated ash of ≤0.1%, water content of ≤0.1% and the purity is 100 ± 2%). FiI(6.403) and FiII(6.525) were produced by cooling crystallization from 1,4-dioxane (spectrophotometric grade, ≥99%, CAS Number: 123-91-1) and methanol (ACS reagent, ≥99.8%, CAS Number: 67-56-1) respectively, and used to investigate the solid-state polymorphic transformations of piracetam.

**Isolation and Characterization of FiI(6.403) and FiII(6.525).** FiI(6.403) was produced using a HEL PolyBLOCK Parallel Synthesis reactor. Piracetam (11 g) was dissolved in 200 mL of 1,4-dioxane at 98 °C with agitation of 200 rpm. The solution was cooled to 20 °C at a rate of 1 °C/min. FiI(6.403) was harvested by vacuum filtration after 24 h and dried in an oven at 45 °C, giving a yield of 91% (10 g). FiII(6.525) was isolated as outlined by Maher et al.20 The purity of the both polymorphs was confirmed by XRD (Phillips PANalytical XPert MPD Pro with PW3064 Sample Spinner), solid-state nuclear magnetic resonance spectroscopy (SS-NMR) (JEOL 400 MHz spectrometer with a broad band solid-state probe), scanning electron microscopy (SEM) (JEOL CarryScope scanning electron microscope JCM-7000) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) (PerkinElmer Spectrum 100 FT-IR spectrometer with a PerkinElmer Universal ATR Sampling Accessory).

Piracetam samples FiI(6.403) and FiII(6.525) were packed into 3.2 mm silicon nitride rotors for SS-NMR analysis.13 C CPMAS experiments with arrays to optimize contact times for cross-polarization were run using a 1H pulse width of 2 µs, a 13C pulse width of 3.46 µs, a spinning rate of 40 kHz, cross-polarization conditions of 1H at 40% and 13C at 50% with a ramp of 10% and T2PPM decoupling at 100%. 1H Double pulse experiments were run to obtain an average optimum relaxation delay for each sample. 13 C CPMAS spectra were collected using the optimized contact times and relaxation delays for each sample (Table 1).1 C Double pulse cross-polarization experiments were run to obtain a T1 relaxation time for each carbon in each sample.

**Analysis of Solid-State Polymorphic Transformations of Piracetam.** Pure FiI(6.403) and FiII(6.525) as well as mixtures of both polymorphs were analyzed using DSC. Ground samples (5–6 mg) in sealed aluminum pans were heated from 50 to 180 °C at 10 °C/min. A baseline run was carried out using two reference pans (empty sealed aluminum pans), and the baseline data were subtracted from that of the sample when collected. FiI(6.403) and FiII(6.525) were analyzed at heating rates of 1 to 500 °C/min, while samples of FiII(6.525) seeded with different amounts of FiI(6.403) (25%, 50%, 75%) were analyzed at heating rates of 10 and 20 °C/min. Further DSC experiments involved heating pure samples of FiI(6.403) or FiII(6.525) from 20 to 135 °C, holding for 5 min and then cooling to 20 °C. Heating/cooling rates of 5, 10, and 20 °C/min were employed.

HT-XRD was carried out on samples of FiI(6.403) and FiII(6.525) as well as mixtures of both polymorphs. The XRD was equipped with an Anton Paar HTK1200 furnace. A Cu Kα source (λ = 1.5418 Å), a nickel filter and a 0.5° divergence slit were employed. Data over the range 8 to 35°2θ was collected with a step size of 0.017°2θ, a count time of 33 s per step and a scan speed of 0.064°/s. The generator was set to 40 kV and 30 mA. The samples were rotated at 4 rpm. Data analysis was completed using XPert HighScore Plus software (PANalytical). Samples were heated at 60 °C/min from room temperature (22 °C) to 90 °C, and then at 5 °C/min from 90 to 130 °C. The program was set up to hold the heating and obtain an XRD pattern at 40 °C, 90 °C, 95 °C, 100 °C, 105 °C, 110 °C, 115 and 130 °C. Patterns were also collected at room temperature before starting the heating profile, upon returning to room temperature after heating and up to 24 h after. The furnace in the HT-XRD was calibrated using potassium nitrate/indium. However, due to thermal drift as one moves from the calibration temperature, DSC analysis is a more accurate method of establishing the true temperature of the thermal event.

FiI(6.403) and FiII(6.525) respectively (2.5 g), on clock glasses, covered by inverted clock glasses, were placed in an oven at 140 °C for 24 h. XRD analysis of a sample of each was employed to confirm the...
transformation to FII(6.747) had gone to completion in both samples. The samples were then cooled to 100°C in the oven. After holding for 5 days XRD analysis was again employed to examine the polymorphic composition of the samples. XRD analysis was carried out at 85, 65, 57, 50, 44, 38, and 30°C after holding at each temperature for 5 days. In a separate experiment, 2.5 g of FII(6.403) and FIII(6.525) on clock glasses, covered by inverted clock glasses, were placed in an oven at 95°C for 5 days. XRD analysis was employed to identify the polymorphic composition. The temperature was then increased and held for 48 h twice (98 and 102°C), analyzing the polymorphic composition of the samples after each holding period via XRD. Finally a 50:50 mix of FII(6.403) and FIII(6.525) was placed in the oven at 95°C for one week with XRD analysis carried out every 24 h. The temperature of the oven was monitored using a calibrated mercury thermometer.

A Linkam Scientific Instruments Ltd. TMS 94 temperature controller was used to heat the sample on the Optical Microscope (Zeiss Axioscope AxioImager MAT Reflected-Light Microscope). In separate runs, a crystal of FII(6.403) and FIII(6.525) was focused under the microscope (×5) and set to capture images periodically over time during the heating program. Extra images were collected in the region where the transformation was occurring. The temperature controller was programmed to heat the crystal from room temperature (22°C) to 90°C at 10°C/min, followed by heating to 120°C at 2°C/min, then to 145°C at 5°C/min and finally to 160°C at 2°C/min. The resulting images were processed in Google Picasa 3 to give a final video of the transformation process, with one included as Supporting Information. The hot-stage was calibrated with triphenylphosphine oxide, with a known melting range of 154–158°C. As mentioned above DSC gives a more accurate output of the thermal event temperature.

**Molecular Analysis.** Optimization of the total epitaxial interaction (normalized by the total number of unit cells in the system) between two crystal blocks was undertaken using the differential evolution global optimization21,22 implemented in the program DEX.23,24 Initial studies optimized a 2 × 2 × 2 block of FIII(6.525) onto the (100), (010), (001) and (10̅1) faces of 2 × 2 × 1 block FII(6.747). Upon identification of the surface with the strongest interaction energy, calculations between a 3 × 3 × 2 crystal block of FII(6.747) and a 3 × 5 × 1 crystal block of either FII(6.403) or FIII(6.525) were performed. The interaction energy was calculated using the force field described by No et al.25 using the atomic point charges calculated by fixing to the electrostatic potential calculated in the *ab initio* program orca (2.8.0)26 at the PBE-D3/def2-qzvp/PBE-D3/TZVP level.27–33 The trial structures were encoded as the vector \((u, v, w, \theta, \phi, \gamma)\), where \(u, v, w\) are the location of the overlayer relative to the crystal surface and \(\theta, \phi, \gamma\) are the Eularian angles of rotation of the overlayer. The control parameters of the DE algorithm \((K, F, G_{max}, N_p)\) were 0.9, 0.6, 2000, and 60. Boundary conditions were applied to each parameter \((-15 \leq u, v \leq 15\), \(-10 \leq w \leq 20\) Å and \(-180 \leq \theta, \phi, \gamma \leq 180\)).

**RESULTS AND DISCUSSION**

**Isolation and Characterization of FII(6.403) and FIII(6.525).** The products isolated from the crystallizations after 24 h, show a rod or needle like habit, with well-defined faces for FII(6.403) (Figure 1, left), while FIII(6.525) (Figure 1, right) is well faceted with a hexagonal habit. XRD analysis of the solid phases confirms the crystals identity by comparison with simulated patterns from the known crystal structures extracted from the CSD (Figures 2 and 3).9

The polymorphs were further characterized through SS-NMR analysis, while measurement of the T1 relaxation times for each carbon atom in the two polymorphs of piracetam were also undertaken (Table 2). The carbons (C1 to C6 from Figure 4) in FIII(6.525) take longer to relax, implying that they have lower mobility than in FII(6.403). The 13C CPMAS spectra of the two polymorphs are shown in Figure 5.

The spectra appear to be quite similar but upon close examination, it is clear that there are slight differences in the packing environment of the crystal structures which cause the
changes in NMR signal for the different polymorphs. Upon comparing polymorphs FII(6.403) and FIII(6.525), the C1 (C=O) carbon peak moves to a higher chemical shift in FIII(6.525), while all other carbon peaks move to lower chemical shifts. This indicates that the observed changes in carbon peak shifts between polymorphs are real differences, not systematic errors during data collection, and are due to differences in the chemical environment of each carbon atom in the two polymorphs.

Peak splitting, observed in the C6 carbonyl of FIII(6.525), and not in FII(6.403) is another difference in the spectra for the two polymorphs (Figure 6). The observed peak splitting in FIII(6.525) may be due to residual dipolar interactions from the quadrupolar 14N. These dipolar interactions are averaged out in the more mobile chemical environment of FII(6.403) (shorter relaxation times are also observed for this polymorph due to its more mobile chemical environment). Neither sample contains any trace of a C6 peak that overlaps with that which is characteristic of the other polymorph, inferring purity. Characterization of both polymorphs by ATR-FTIR analysis has been documented previously.20,34

Analysis of Solid-State Polymorphic Transformations of Piracetam. Transformation of FII(6.403) or FIII(6.525). FII(6.403) and FIII(6.525) are known to transform to FI(6.747) at elevated temperatures.12−16 Variation in composition during the transformation was monitored using HT-XRD (Figure 7 and Figure 8). In the case of the FII(6.403) sample, XRD patterns collected in the range from room temperature to 100 °C were identical to the theoretical FII(6.403) pattern. At 105 °C a mixture of FI(6.747) and FII(6.403) peaks was observed indicating the transformation is underway. At 110 °C, all FII(6.403) peaks have disappeared and the transformation to FI(6.747) has gone to completion.

Initial examination of the HT-XRD analysis would suggest that the transformation to FI(6.747) occurs at similar temperatures for FII(6.403) and FIII(6.525). However, close inspection of the data at 105 °C indicates that the transformation of FII(6.403) is more advanced, agreeing with the information reported by Kuhnert-Brandstaetter et al.13 and Picciochi et al.,14 that FII(6.403) transforms to FI(6.747) at a slightly lower temperature than FIII(6.525).

DSC provides a more accurate estimation of the temperature of the thermal event. The DSC scans obtained for FII(6.403) and FIII(6.525) with a heating rate of 5 °C/min are presented in Figure 9, with the properties summarized in Table 3. The first endothermic peak on each of the scans represents the
transformation to FI(6.747). The sharp endothermic peak seen at approximately 154 °C represents the melting of FI(6.747). In all DSC scans collected in this study, FII(6.403) transformed at a slightly lower temperature than FIII(6.525), verifying the conclusions of the HT-XRD work. Employing a 1 °C/min heating rate, the transformation temperature onset was observed at 103.8 °C for FII(6.403), and 108.3 °C for FIII(6.525). These values are thought to be closer to the true thermodynamic transition temperature than those reported in...
the literature, but when DSC is operated with a heating rate of less than 5 °C/min, the peak shape may be distorted. Holding samples for 5 days at a series of temperatures in advance of the transition temperature from DSC or HT-XRD analysis facilitated a more accurate estimation of the true thermodynamic transition temperature. An oven at a set temperature was used for this investigation as it is not practical to program DSC and HT-XRD experiments to hold at elevated temperatures for prolonged periods of time. XRD analysis was carried out immediately after the holding period on a preheated zero background disk to determine the composition of the sample. The transition from FIII(6.403) to FI(6.747) was observed at 98 °C, while the FIII(6.525) to FI(6.747) transition was observed at 102 °C. In fact, ex-situ XRD analysis of binary mixtures of FII(6.403) and 1,4-dioxane or FIII(6.525) and 1,4-dioxane showed that the true position of the thermodynamic FII(6.403)—FI(6.747) transition point is no higher than 90 °C and the thermodynamic FIII(6.525)—FI(6.747) transition point is no higher than 95 °C.

The transformation of FIII(6.525) to FI(6.747) was visualized using hot-stage optical microscopy (Figure 10). The transformation began as a shadow that appeared at the top of the crystal at 109 °C, which is highlighted in red. This presumably is the nucleation point of FI(6.747). This shadow then extended throughout the whole crystal and changed it from being optically transparent to opaque. The habit of the crystal does not change during the transformation but a major crack, extending from the point where the shadow was first seen, splits the crystal in two. Other cracks can also be seen appearing and extending during the transformation. The visual properties of the crystal did not change between 120 and 152 °C. In the range of 152–153 °C, the now FI(6.747) crystal melted. Repeated runs of FII(6.525), as well as FII(6.403), showed similar changes in optical properties and cracking of the crystal during the transformation, without changing the outer habit.

To investigate if the appearance of the cracks in the crystals was a direct result of the molecular rearrangement during the transformation or merely caused by the stress of heating, a FI(6.747) crystal, analyzed immediately after isolation by crushing from a highly supersaturated 1,4-dioxane solution, was subjected to the same heating profile. No cracks were seen in the crystal during the heating profile and the crystal melted in the range 152–153 °C, indicating the cracking of crystals during the transformation may be caused by the stress of the molecular rearrangement. The density, as determined by gas comparison pycnometry, at room temperature of FI(6.747) (1.304 g cm−3) is lower than FIII(6.525) (1.371 g cm−3), so it is feasible that the cracking observed during the transformation is caused by the change in density between the polymorphs.

**FII(6.403)—FIII(6.525) Transition Point and Melting Temperatures.** The analysis in the previous section showed that FIII(6.525), when heated, appears to transform directly to FI(6.747) and not via FII(6.403). This indicates that the thermodynamic FII(6.403)—FIII(6.525) transition point is higher than the FI(6.747)—FIII(6.525) transition temperature. To avoid any nucleation energy barrier that may exist, samples of FII(6.525) were seeded with FII(6.403), by grinding the mixture, in order to investigate whether or not the FII(6.403)—FIII(6.525) transition temperature is lower than that of the FIII(6.525)—FI(6.747) transition. Second, although it is known to occur in solution, the transformation from metastable FII(6.403) to the stable FIII(6.525) was not observed in the solid-state after storage for one year under ambient conditions. Samples of FII(6.403) were seeded with FIII(6.525) by grinding both phases together, again to overcome the nucleation barrier and investigate if this transformation occurs in the solid-state.

In both instances the HT-XRD analysis of these samples did not show any interconversion between FII(6.403) and FIII(6.525), only direct transformations to FI(6.747). DSC analysis of these samples (Figure 11) agrees, in that the FII(6.403)—FIII(6.525) transition point appears to be at a higher temperature that the FIII(6.525)—FI(6.747) transition, and the transformation from FII(6.403) to FIII(6.525) does not occur in the solid-state. As mentioned earlier FII(6.403) transforms to FI(6.747) at a slightly lower temperature than FIII(6.525). While the peaks are skewed slightly toward the transition temperature of the polymorph present in higher proportion, a single smooth peak in this region indicated that the only transformation that occurred was directly to FI(6.747). In all samples the transformation product, FI(6.747), melts with an onset at 152 °C.

The mixtures of FII(6.403) and FIII(6.525) held at 95 °C for 1 week did not show any transformation, supporting the gathered evidence that the FII(6.403)—FIII(6.525) transition temperature is above the transition point of both polymorphs to FI(6.747). FII(6.403) was observed to transform to...
FFI(6.747) at temperatures from room temperature to 85 °C via a solution mediated mechanism, confirming that the relationship between the two is monotropic in this range, and the transition point lies above the transition point of both polymorphs to FII(6.747). It also confirms that kinetic effects prevent the transformation from the metastable FII(6.403) to the stable FIII(6.525) materialising in the solid-state.

It has been reported that trace amounts of FIII(6.525) sometimes persist during DSC analysis after the bulk of the sample has transformed to FII(6.747), as evident by the presence of a small endothermic peak at 139−140.2 °C in addition to the melting of FII(6.747). No such data has been reported for FII(6.403). Thermomicroscopy estimated melting points for FII(6.403) (140.7 °C) and FIII(6.525) (140.2 °C). Employing a heating rate of 10 °C/min in the current study, DSC scans showed suspected melting points for FII(6.403) and FIII(6.525). Very small endothermic peaks were observed at 139.0 °C for FII(6.403) and 138.3 °C for FIII(6.525). It is thought that these peaks represent the melting of trace amounts of the samples that did not transform to FII(6.747).

The difference between the transition temperature of FII(6.403) and FIII(6.525) to FII(6.747) and the proposed melting points of FII(6.403) and FIII(6.525) is 20−30 °C. Using a heating rate of 10 °C/min this temperature span is achieved within 2−3 min. It is feasible that the entirety of the sample has not transformed to FII(6.747) in this time frame, and when 138−139 °C is reached any traces of the original polymorph remaining in the sample melt. In fact, the hot-stage optical microscopy studies above showed that the transformation to FII(6.747) does not appear to go to completion instantaneously but rather takes a number of minutes. When heating rates of 1 and 2 °C/min were employed, the proposed melting peaks of FII(6.403) and FIII(6.525) were not observed, presumably because there was sufficient time for the transformation of the sample to FII(6.747) to go to completion.

**Transformation of FII(6.747).** Ceolin at al. reported that FII(6.747) transforms to FIII(6.403) at ambient conditions. The composition of samples of FII(6.403) and FIII(6.525) that had transformed to FII(6.747) were monitored after the samples had been cooled to room temperature. Figure 12 shows the composition of an originally FIII(6.525) sample. After transformation to FII(6.747) by heating, the sample was cooled and monitored by XRD analysis. It was still completely FII(6.747) immediately when cooled to room temperature. Four hours later the transformation to FII(6.403) has begun, while the XRD of the sample 24 h later shows the transformation was complete.

There appears to be a region below the transition point between FII(6.747) and FII(6.403) where FII(6.747) exists as a metastable polymorph. Figure 13 shows a DSC scan of FIII(6.525) (unbroken line) heated to 135 °C with the transformation to FII(6.747) occurring as expected with an endothermic peak indicating the transformation to FII(6.747). After reaching 135 °C, the temperature was held for 5 min and then decreased to 20 °C at 5 °C/min (broken line).

FIII(6.525) (unbroken line) heated to 135 °C with the transformation to FII(6.747) occurring as expected with an endothermic peak. The cooling profile of FII(6.747) to 20 °C (broken line) indicates that the transformation back to FII(6.403) does not occur at the same temperature in the opposite direction.

The transformation to FII(6.403) did not occur during 5 day holding periods of FII(6.747) at temperatures from 100 °C down to 38 °C, confirming that FII(6.747) can exist in a metastable state. Only after holding at 30 °C did the transformation occur. The activation energy barrier for the transformation must be significant, as the transformation does not happen in a practical time frame until the temperature has
reached almost 70 °C beyond the thermodynamic transition point. As FI(6.747) transforms to FI(6.403) and not FIII(6.525), the system appears to follow Ostwald’s rule of stages. When leaving the unstable phase (FI(6.747)), the system does not seek out the most stable phase (FIII(6.525)), but rather the nearest metastable phase (FIII(6.403)) which can be reached with a loss of free energy. Interestingly, as viewed under the optical microscope, the opaque optical property of FIII(6.525), it con...

**Figure 14.** Semi-schematic energy-temperature diagram of the three polymorphs of piracetam with temperatures of different thermal events included.

DSC analysis showed the melting point of FI(6.403) to be 139.0 °C, while that of FIII(6.525) was 138.3 °C. Because FI(6.403) transforms to FI(6.747) at a lower temperature than FIII(6.525) and melts at a slightly higher temperature than FIII(6.525), it confirms enantiotropy and a transition point between FI(6.403) and FIII(6.525) is located inside the range 90 and 138 °C. There is less than 1 °C difference between the melting points of FI(6.403) and FIII(6.525), while there is approximately 5 °C between the transition point of both polymorphs to FI(6.747). Therefore, it is thought that the FI(6.403)→FIII(6.525) transition point is closer to their melting points than the transition point of both polymorphs to FI(6.747).

**Molecular Analysis.** The preference for FI(6.403) over FIII(6.525) during the cooling transformation may be due to an element of crystallographic match between the phases. To identify possible surface interactions between crystal phases, a series of molecular modeling studies were undertaken. Initially, optimization of crystal blocks of FI(6.403) or FIII(6.525) onto the dominant faces of FI(6.747) was carried out to identify any matching between the surfaces. These studies utilized a small-sized block and the strongest intermolecular interactions (defined in Experimental Section) occurred between the (001) face of FI(6.747) and the (001) faces of both FI(6.403) and FIII(6.525). Further studies on extended blocks were therefore only performed on this interface. While the unit cell parameters for the (001) faces of the three polymorphs (a, b, γ (I) = 6.725 Å, 13.250 Å, 90°; a, b, γ (II) = 6.403 Å, 6.618 Å, 88.91°; a, b, γ (III) = 6.525 Å, 6.440 Å, 90°) have similar values, no commensurate relationships between the values could be located using epicalc methodology, and so no long scale period interaction between the phases would be anticipated. The optimization of the larger crystal blocks identified a stronger interaction between FIII(6.525) and FI(6.747) (E = −9.67 kJ·mol⁻¹) compared to that between FI(6.403) and FI(6.747) (E = −7.45 kJ·mol⁻¹) due to the formation layers of hydrogen bonding in the FIII(6.525) case (Figure 15). However, the lack of a lattice match between the cells is reflected by the lengthening of these bonds for each repeat of the overlayer structure. The (001) surfaces of FI(6.403) and FIII(6.525) have the same qualitative arrangement of amide groups with slight differences in distances due to the differences in lattice parameters (Figure 16). Thus the chemical and crystallographic environment of the interface between the (001) faces of FI(6.403) and FIII(6.525) with FI(6.747) will be similar in both cases. Conversion from FI(6.747) to FI(6.403) or FIII(6.525) across the predicted interface would initially require the formation of the R₂(8) amide·amide dimer present in FI(6.403) and FIII(6.525). In both cases this can be achieved by internal rotation of the amide group and shift of the molecular layer (Figure 17). In the case of FI(6.747) to FIII(6.525), this requires breaking the hydrogen bond between the layers for a greater energy penalty. The dimer linked layers are packed differently in FI(6.403) and FIII(6.525). In the case of FI(6.403) a translation stacking of the layers occurs, while in FIII(6.525) they are stacked by a γ-glide operation. Because of this difference an increased number of piracetam molecules have to both shift and conformationally adjust during the transformation to...
Figure 15. Comparison of the optimized structures of (a) FII(6.403) and (b) FIII(6.525) onto the (001) surface of FI(6.747), both crystals viewed down the b-axis.

Figure 16. View onto the (001) of (a) FII(6.403) and (b) FIII(6.525) with the lengths of differing axes shown.

Figure 17. Comparison of the predicted intercrystal interfaces between (a) FI(6.747) and FII(6.403) and (b) FI(6.747) and FIII(6.525), showing the required changes to form the dimer motif observed in the two polymorphs.
FII(6.525) compared to FII(6.403) (Figure 18). Indeed every third layer of FI(6.747) would have to rotate 180° to undertake the transformation to FIII(6.525), while only isolated molecules have to adjust to convert to FII(6.403). Thus the combination of stronger hydrogen bonding and greater disruption of the crystal lattice suggests a greater kinetic barrier to the conversion of FI(6.747) to FIII(6.525). However, further studies are required to fully confirm this suggestion, such as molecular dynamics studies and experimental studies to fully confirm the atomic details of the transformation mechanism.

**CONCLUSIONS**

The purity of FII(6.403) and FII(6.525), isolated by cooling crystallization from 1,4-dioxane and methanol respectively, was verified using a number of techniques including XRD, DSC, SS-NMR and SEM. FII(6.403) was found to consistently transform to FI(6.747) at a lower temperature than FII(6.525). The temperatures reported for these transition points are expected to be closer to the true thermodynamic points, compared to the data reported in the literature. The transformation caused a physical cracking of the original crystal due to the molecular rearrangement, as well as changing of the optical properties from transparent to opaque. The transition between the polymorphs was found not to occur in the solid-state.

FI(6.747) was found to exist as the unstable polymorph in the region from 100 °C to below 38 °C, before transforming to the metastable FII(6.403). Molecular level analysis suggests that the transformation from FI(6.747) to FII(6.403) is kinetically favored over the transformation to FIII(6.525).

**ASSOCIATED CONTENT**

- Supporting Information

A video of the solid-state polymorphic transformation from FII(6.525) to FI(6.747) upon heating, as viewed by hot-stage optical microscopy, is included. This information is available free of charge via the Internet at http://pubs.acs.org/.

**REFERENCES**


**AUTHOR INFORMATION**

*E-mail: anthony.maher@ul.ie. Tel.: +353 61 234159.

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

A.M. gratefully appreciates funding from an Irish Research Council for Science, Engineering and Technology (IRCSET) scholarship as well as the Solid State Pharmaceutical Cluster (SSPC).
(32) The Ahlrichs (2d, 2p) polarization functions were obtained from the TurboMole basis set library under ftp.chemie.uni-karlsruhe.de/pub/basen, Ahlrichs R. and co-workers, unpublished.
Solution Mediated Polymorphic Transformation:
Form II to Form III Piracetam in Organic Solvents

*Anthony Maher*, Colin C. Seaton †, Denise M. Croker †, Åke C. Rasmuson †, and Benjamin K. Hodnett †

* † Solid State Pharmaceuticals Cluster, Materials and Surface Science Institute, Department of Chemical and Environmental Sciences, University of Limerick, Limerick, Ireland

*corresponding author email: anthony.maher@ul.ie

*Tel.: +353 61 234159

ABSTRACT

The solution mediated polymorphic transformation from Form II to Form III 2-oxo-1-pyrrolidine acetamide (piracetam) was investigated in seven organic solvents over the temperature range 5 – 50 °C. Increasing temperature increased the transformation rate. A general trend was observed; the higher the solubility of piracetam in a particular solvent, the faster the transformation to the stable form. However, this trend was reversed in acetone and 2-propanol. A hypothesis proposed that poor mixing in 2-propanol, associated with its high relative viscosity compared to the other solvents, causing a delay in the transformation to the stable form was proved inaccurate. The positioning of the alcohol group on the aliphatic chain in propanol was then examined and 1-propanol was found to agree with the trend observed with the other solvents. Molecular modelling demonstrated that 2-propanol forms stronger interactions with piracetam molecules in
solution compared to the other solvents, thereby retarding the nucleation and growth of FIII(6.525) during the transformation in this solvent.

INTRODUCTION

The crystal structures of five polymorphic forms of piracetam (Structure 1) have been published and collected in the Cambridge Structural Database (CSD) under the reference code BISMEV.\(^1\) Due to inconsistencies in the literature, the system used for naming polymorphs in this work is outlined by Maher et al.\(^2,3\), so that Form II is referred to below as FII(6.403) and Form III is referred to as FIII(6.525).

FII(6.403) is the metastable polymorph of piracetam and is known to transform to the stable FIII(6.525) in numerous organic solvents.\(^4,5,6,7\) Previous studies found nucleation and growth of FIII(6.525) to be the limiting steps of the transformation, over the dissolution of FII(6.403), and that nucleation is likely to take place on the surface of the metastable form.\(^8\) Before the growth phase in the transformation, nucleation of the stable FIII(6.525) must occur, making it the key step. The nucleation rate \((J)\) equation (eq. 1) is governed by three main variables: degree of supersaturation, \(S\); temperature, \(T\); and interfacial energy, \(\gamma;\)\(^6,9\)

\[
J = A. \exp \left[ -\frac{16\pi y^3v^2\phi}{3k^3T^3(LnS)^2} \right]
\]  

[1]

Where \(A\) is the pre-exponential or collision factor, \(v\) is molecular volume, \(\phi\) is the heterogeneous nucleation factor and \(k\) is the Boltzmann constant. Numerous studies have been carried out on
the effect of temperature on solution mediated polymorphic transformations. In general, higher temperatures increase the nucleation and growth kinetics through an increasing molecular thermal activity, caused by increasing solute concentration and decreasing interfacial energy.\textsuperscript{6,9,10,14} The supersaturation, which is governed by the free energy difference ($\Delta G$ in Scheme 1) between the polymorphs, represents the thermodynamic driving force for the transformation, and is independent of solvent.\textsuperscript{10} Since the solubility difference between the polymorphs is very small,\textsuperscript{2,3} so too is the driving force for the transformation.

\textbf{Scheme 1}. A qualitative illustration of the Free Energy diagram for the solution mediated polymorphic transformation from FII(6.403) to FIII(6.525) piracetam.

On the other hand, the kinetic activation energy barrier for nucleation of FIII(6.525) during the transformation, $E_a$, is dependent on numerous parameters, one being solvent.\textsuperscript{9} Employing a manipulation of the Arrhenius equation, the nucleation rate can be expressed as:

$$\ln(J) = -\frac{E_a}{RT} + \ln(A)$$  \[2\]
Where $R$ is the gas constant. Therefore, a plot of $\ln(J)$ against $T^{-1}$, under constant supersaturation, should give a straight line of slope $-E_a/R$, giving an estimation of the activation energy in each solvent.

The pre-exponential factor ($A$) of eq. 1 contains terms which are affected by the solvent of choice:

$$A = N_0 4\pi (r_c)^2 \left(\frac{kT}{h}\right) \exp\left(\frac{\Delta G_{tr}}{kT}\right)$$

Where $N_0$ is the number of solute molecules per unit volume, $4\pi (r_c)^2$ is the surface area of the assumed to be spherical critical nucleus, $kT/h$ is a frequency factor and $\Delta G_{tr}$ is the activation free energy for the transport resistance encountered by the solute for volume diffusion from the bulk solution to the nucleus. $N_0$, $kT/h$ and $\Delta G_{tr}$ will all be influenced strongly by solvent choice.

Solute-solvent interactions influence nucleation and growth in two ways; firstly, desolvation of the solvated solute molecules before integration into the crystal lattice, and secondly, in the removal of the solvent molecules which are adsorbed to the nucleus or crystal lattice. The stronger the interaction, the greater the hindrance to the nucleation and growth phases of the transformation. Solvent-solute interactions were found to be important in determining the rate of transformation from Form I to Form II sulfamerazine.\(^{11}\) Higher solubility values were observed in solvents with a strong hydrogen bond acceptor propensity, while slower transformation rates were observed in such solvents. Therefore, the transformation rate was determined by the balance between the solubility and the strength of the hydrogen bond interaction between the solvent and solute molecule. Another example of the effect of such interactions is the transformation rate from the C form to B form of stearic acid.\(^{12}\)
EXPERIMENTAL

FII(6.403), produced as previously outlined\textsuperscript{3}, was employed to examine the effect of solvent on the solution mediated polymorphic transformation to FIII(6.525) in seven solvents on a 25 g scale. Dissolution studies were carried out on a larger scale (130 g), allowing incorporation of an FTIR probe to monitor solution concentration. Computational modelling was employed in order to gain an understanding of the interactions between the piracetam molecules and respective solvent molecules.

Materials

Piracetam was supplied by UCB Pharma SA and complies with European Pharmacopoeia 6.5 quality and purity standards, (CAS Number: 7491-74-9, Batch Number: 09G06-B93. Certificate of Analysis states that the batch complies with the IR, HPLC and solution appearance tests. It also complies with a heavy metals limit of < 10 ppm, sulphated ash of < 0.1 % and the purity is 100 +/-2 %. The water content was found to be less that 0.1 %). Methanol, ethanol, 1-propanol, 2-propanol, isobutanol, 1,4-dioxane and acetone (ACS reagent grade) were obtained from Sigma-Aldrich. Except for isobutanol, where a solvent mixture of isobutanol:water 95:5 (v/v) was prepared, all solvents were used as obtained from the supplier.

Procedure

Test tubes equipped with a 12 x 4 mm Teflon coated magnetic stirrer were charged with 25 g of each of the solvents, placed in a water bath at a set temperature and agitated at 500 rpm. As an example, Table 1 refers to the experiments at 30 °C. FII(6.403) was added to each solvent (3\textsuperscript{rd} column), in order to saturate each solvent with respect to FIII(6.525). Homogenous solutions were obtained by agitating the solutions at 10 °C above the transformation experiment
temperature. The homogeneous solutions were then stabilised at the correct temperature, before adding 0.5 g FII(6.403) to each tube. This marked the starting point of the transformation study ($t_0$).

**Table 1.** Amounts of FII(6.403) added to 25 g of each solvent at 30 °C during the transformation experiments in the water bath.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>FIII(6.525) Solubility @ 30 °C (g/ g solvent)</th>
<th>FII(6.403) added to give solution saturated w.r.t. FIII(6.525) (g)</th>
<th>Total Amount of FII(6.403) added (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>21.60 x 10^{-2}</td>
<td>5.400</td>
<td>5.900</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>2.45 x 10^{-2}</td>
<td>0.613</td>
<td>1.113</td>
</tr>
<tr>
<td>Isobutanol:Water 95:5 (v/v)</td>
<td>8.50 x 10^{-2}</td>
<td>2.126</td>
<td>2.626</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.94 x 10^{-2}</td>
<td>0.235</td>
<td>0.735</td>
</tr>
<tr>
<td>1,4-Dioxane</td>
<td>0.42 x 10^{-2}</td>
<td>0.106</td>
<td>0.606</td>
</tr>
</tbody>
</table>

The solid phase was analysed over time via *ex-situ* XRD analysis as outlined previously.\(^6,8\) Slurry samples were vacuum filtered before XRD analysis of the solids. The fraction of each polymorph was estimated from the areas of characteristic peaks FII(6.403) and FIII(6.525) peaks. Detection limits for FIII(6.525) in FII(6.403) using XRD analysis were examined and it was found that FIII(6.525) could be detected at levels below 0.5 % in FII(6.403). The composition of the solid phase was monitored throughout the transformation by approximately 10 samples.

The transformation was examined between 5 and 50 °C in methanol, 2-propanol, acetone and 1,4-dioxane. The solubility of FIII(6.525) in these solvents along with ethanol has been
The transformation data in ethanol over the same temperature range has also been published. The solubility of FIII(6.525) in isobutanol:water 95:5 (v/v) was determined between 5 and 30 °C after a 48 hr equilibration time in an identical manner to that outlined in the previous solubility study, and the transformation was examined over the same temperature range. Transformation and solubility data were later obtained in 1-propanol at two temperatures, 30 and 50 °C. This experiment was carried out twice at each temperature in each solvent. An initial run gave a rough indication of the time required for FIII(6.525) nucleation and subsequent transformation completion, while subsequent runs allowed accurate identification of the points of interest. The results from the second run are presented. The reproducibility of these experiments was examined in acetone at 50 °C.

In a 250 mL jacketed vessel controlled by a Lauda E305 recirculator and E300 controller equipped with an overhead axial flow turbine impellor, 130 g solvent (ethanol, acetone & 2-propanol) was held at 15 °C and agitated at 270 rpm. A Mettler Toledo ReactIR iC10 with DiComp probe and silver halide fibre was employed to monitor the concentration in solution during dissolution rate studies in a similar fashion to previously. The amount of FII(6.403) added to each solvent was calculated to give an equal amount of excess solid after FII(6.403) equilibrium was reached.

The transformation was then examined in the 250 ml jacketed vessel in acetone and 2-propanol at 50 °C as outlined in Table 2. The transformation was monitored via ex-situ XRD analysis of periodic samples of the solid phase until completion. The transformation experiment was repeated in acetone.
Table 2. Amounts of FII(6.403) added to 130 g solvent in the jacketed vessel in the dissolution & transformation experiments at 15 & 50 °C respectively.

<table>
<thead>
<tr>
<th>Experiment Type/ Temperature (°C)</th>
<th>Solvent</th>
<th>Solubility of FIII(6.525) at temp (g / g solvent)</th>
<th>Scale Up to 130 g solvent (g)</th>
<th>Amount of FII(6.403) added (g)</th>
<th>Amount of Excess Solid (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolution/15</td>
<td>Ethanol</td>
<td>2.60 x 10^-2</td>
<td>3.38</td>
<td>4.99</td>
<td>1.61</td>
</tr>
<tr>
<td>Dissolution/15</td>
<td>2-Propanol</td>
<td>1.20 x 10^-2</td>
<td>1.56</td>
<td>3.17</td>
<td>1.61</td>
</tr>
<tr>
<td>Transformation/50</td>
<td>Acetone</td>
<td>2.00 x 10^-2</td>
<td>2.60</td>
<td>4.60</td>
<td>2.0</td>
</tr>
<tr>
<td>Transformation/50</td>
<td>Acetone Rpt</td>
<td>2.00 x 10^-2</td>
<td>2.60</td>
<td>4.60</td>
<td>2.0</td>
</tr>
<tr>
<td>Transformation/50</td>
<td>2-Propanol</td>
<td>6.49 x 10^-2</td>
<td>8.44</td>
<td>10.44</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The effect of agitation on the transformation was examined in methanol at 25 °C on a 25 g scale. 4.9 g FII(6.403) was added to each tube. Each tube was agitated at a different rate for the duration of the transformation (0, 200, 500, 1000, 1350 rpm), while the solid phase was monitored by ex-situ XRD analysis.

Computational Modelling

The molecular conformation of piracetam FIII(6.525) was extracted from the crystal structure and optimised in the ab initio program orca\textsuperscript{15} at the PBE-D3/def2-TZVPP level (AccOpt option).\textsuperscript{16-18} Molecular structures of the considered solvents were optimised at the same level of theory.

Molecular clusters of piracetam and selected solvent molecules were optimised using the differential evolution global optimisation algorithm\textsuperscript{19,20} to minimise the interaction energy between the molecules. The interaction energy was calculated using the AA-CLP forcefield\textsuperscript{21}. 
with atomic point charges generated by fitting the electrostatic potential calculated at the B2PLYP-D3/def2-TZVPP level using the RI approximation. To reduce the probability of stagnation during each optimisation run, the two populations strategy outlined in Ali et al. was applied.\textsuperscript{22} Trial structure were encoded as a vector of 6 parameters: the \(x, y, z\) location and \(\theta, \phi, \gamma\) orientation of the solvent molecule. Twenty-five independent calculations were performed for each dimer pair with the same DE parameters \((K, F, G_{\text{max}}, Np: 0.05, 0.2, 10000, 60)\).

**RESULTS AND DISCUSSION**

The transformation results were visualised as the change in composition of the solid phase against time. The repeatability of the transformation was confirmed and is outlined in Figure 1 for the acetone system. The induction time for the transformation refers to the first point of detection of the stable FIII(6.525) in the solid phase via \textit{ex-situ} XRD analysis.\textsuperscript{6} It is interpreted as being mainly governed by the time for nucleation of FIII(6.525). The transformation time is defined as the length of time, after \(t_0\), for the composition of the solid phase to become 100 \% FIII(6.525).\textsuperscript{6}
Plotting the inverse of induction time against temperature for all the solvents (Figure 2) shows that the induction time for the transformation decreases with increasing temperature. The transformation time had an identical pattern to the induction time in the different solvents over the temperature range. The transformation time in each experiment was approximately equal to double the induction time. 1,4-dioxane is not documented since the transformation to the stable form was not seen to take place in this solvent even at the highest temperature (50 °C) in the time frame examined (300 hr). A general trend is observed between the solubility of piracetam and the induction time for the transformation from FII(6.403) to FIII(6.525); the higher the concentration (Figure 3) of piracetam in solution, the faster the rate of the transformation to the stable form (Figure 2). This is supported by plotting the inverse of the induction time against the solubility of FIII(6.525) in the five solvents (Figure 4), which shows a general increase in transformation rate with increased solution concentration.
Figure 2. The inverse of the Induction Time for the transformation in five solvents over the range 5 – 50 °C. ♦, methanol; x isobutanol:water 95:5 (v/v); ■, ethanol; ●, acetone; ×, 2-propanol.

Figure 3. Solubility of FIII(6.525) versus temperature in a range of solvents from 5 – 50 °C. ♦, methanol; x isobutanol:water 95:5 (v/v); ■, ethanol; ×, 2-propanol; ●, acetone; ▲, 1,4-dioxane.
However, there are exceptions to the trend caused by both temperature and solvent choice. For example, the solubility is higher in the isobutanol:water 95:5 (v/v) at 30 °C than at 20 °C in methanol, but the induction time and transformation time are faster in methanol at 20 °C. The temperature difference is probably the main factor in this case. 2-propanol consistently deviates from the expected trend displaying a slower transformation rate than would be expected for its corresponding solubility. Figure 5 indicates that at 30 °C, 2-propanol has a much higher solubility than acetone but a longer induction time. It does not agree with the straight line that can be fitted to the points relating to the other solvents. This was the case at all temperatures examined. At lower temperatures the difference is of greater magnitude but due to time restraints it was not feasible to examine the induction time of the transformation in 2-propanol at 5 and 10 °C.
Figure 5. A plot of the natural log of the inverse of the induction time for the transformation against the solubility of FIII(6.525) in the five solvents at 30 °C.

During the study it was noticed that the level of mixing of FII(6.403) solids in the test tubes containing 2-propanol did not appear to be as uniform as in the other solvents. An investigation on the effect of the rate of agitation in identical transformation experiments in methanol found mixing to have a significant effect on the transformation rate. With no agitation the transformation time in methanol at 25 °C was 27.5 hr. The periodic sampling of the solid phase provided very slight agitation, therefore it would be expected that the transformation would have taken longer without this agitation. Figure 6 shows the percentage of FII(6.403) present in the solid phase during the transformation at the different agitation rates. A clear trend is observed; the better the mixing, the shorter the induction and transformation time. As the level of agitation increases from 200 rpm to 1350 rpm, the transformation to the stable form occurs in a shorter time frame. Firstly, higher agitation would reduce the lag before the solutions becomes
supersaturated with respect to FIII(6.525), thereby generating the conditions required for nucleation at an earlier time. However, since dissolution is not limiting in the transformation\(^6\), this should only be of major significance in the unmixed experiment. Secondly, the higher mixing would increase the probability of the molecules in solution coming together to nucleate as the stable phase. In this case the transformation is thought to be surface mediated\(^6\) so the important contacts are between piracetam molecules in solution and those attached to the surface of the metastable form. Poor mixing in the vessel will reduce the frequency of these contacts occurring.

Figure 6. Percentage of FII(6.403) in the solid phase during the investigation on the effect of mixing levels on the transformation. ×, 200 rpm; ▲, 500 rpm; ■, 1000 rpm; ♦, 1350 rpm.

The viscosity of 2-propanol (2.073 cP) is significantly higher than the other solvents (twice that of its nearest rival, ethanol), and so it was decided to investigate the rate of dissolution of FII(6.403) in the solvents in order to establish if the high relative viscosity of 2-propanol resulted
in a lag time before the solution became supersaturated with respect to FIII(6.525). The dissolution rate was determined using a Mettler Toledo ReactIR iC10 in 2-propanol and ethanol by monitoring the peak area ratio. It was not possible to examine the dissolution rate in acetone due to overlapping of solvent and solute peaks on the IR spectrum at 1700 cm\(^{-1}\).

The dissolution rates are very similar in the two solvents (Figure 7). FII(6.403) was added to both solvents at the 22 min mark and the equilibrium saturation of FII(6.403) was reached in both solvents less than 15 min after the addition of FII(6.403). Therefore we can conclude the high relative viscosity of 2-propanol does not cause a delay in the dissolution of FII(6.403) to equilibrium saturation. It was then decided to monitor the transformation in acetone and 2-propanol on the larger scale (130 g), where it is known that the system is well mixed, to verify the transformation to the stable form is indeed faster in acetone than in 2-propanol. It is clear that the transformation goes to completion in acetone (31 hr) much faster than in 2-propanol (66.5 hr) (Figure 8). The induction time for the transformation is 18 hr in acetone, as opposed to 35.5 hr in 2-propanol.

This proves that the rate of dissolution of FII(6.403) in 2-propanol is not decreased by the high relative viscosity of the solvent and a lag before nucleation of FIII(6.525) can occur does not exist. On the other hand, it is not known from these experiments if the wide viscosity range in the solvents examined has an influence on the nucleation or growth of FIII(6.525) (i.e. greater \(\Delta G_v\) to overcome the resistance encountered during volume diffusion from the bulk solution to the solid phase in a more viscous solvent).
Figure 7. Dissolution Profile for FII(6.403) in Ethanol (blue) and 2-Propanol (red) at 15 °C in jacketed vessel with probe tip located 1.5 cm under the level of the solution (FII(6.403) added at 22 min mark).

Figure 8. The fractions of FII(6.403) and FIII(6.525) in acetone and 2-propanol 50 °C. ♦, FII in acetone; ■, FIII in acetone; ▲, FII in 2-propanol; ×, FIII in 2-propanol.

Solubility and transformation data was then collected in 1-propanol at 30 and 50 °C with a view to establishing if the positioning of the alcohol group on the aliphatic chain has an effect on the
transformation rate. It was observed that 1-propanol is consistent with the trend of solubility correlating with transformation rates (Figures 9 and 10, Table 3). The solubility of piracetam is only slightly higher in 1-propanol when compared to 2-propanol, but the induction time for the transformation is much shorter. This indicates that the positioning of the alcohol group on the carbon chain of the solvent does not affect the solubility of piracetam in the solvent as much as it affects the rate of the transformation, perhaps suggesting different solvent-solute interactions.

**Figure 9.** A plot of the natural log of the inverse of the induction time for the transformation at 30 °C against the solubility of FIII(6.525) in the six solvents at 30 °C.
Figure 10. A plot of the natural log of the inverse of the induction time for the transformation at 50 °C against the solubility of FIII(6.525) in the five solvents at 50 °C.

Table 3. Results for the solubility (g polymorph.g solvent\(^{-1}\)) of FII(6.403) and FIII(6.525) as well as the transformation in 1-propanol and 2-propanol at 30 and 50 °C.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>FII(6.403)</th>
<th>FIII(6.525)</th>
<th>Transformation</th>
<th>FII(6.403)</th>
<th>FIII(6.525)</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mol Solubility</td>
<td>Frn Solubility</td>
<td>Induction Time (hr)</td>
<td>Mol Solubility</td>
<td>Frn Solubility</td>
<td>Induction Time (hr)</td>
</tr>
<tr>
<td>1-propanol</td>
<td>1.31 x 10(^{-2})</td>
<td>1.28 x 10(^{-2})</td>
<td>18</td>
<td>3.22 x 10(^{-2})</td>
<td>3.11 x 10(^{-2})</td>
<td>4.5</td>
</tr>
<tr>
<td>2-propanol</td>
<td>1.09 x 10(^{-2})</td>
<td>1.03 x 10(^{-2})</td>
<td>70</td>
<td>2.73 x 10(^{-2})</td>
<td>2.67 x 10(^{-2})</td>
<td>15</td>
</tr>
</tbody>
</table>

Since the induction time for the transformation is inversely proportional to the nucleation rate (\(J \propto t_{\text{ind}}^{-1}\)), the inverse of the induction time can be used as an estimate of the rate constant in eq. 2. Plotting ln\((t_{\text{ind}}^{-1})\) against \(T^{-1}\) in each solvent (Figure 11), the activation energy for nucleation of FIII(6.525) during the transformation can be estimated from the slope of each line, with the
assumption that the activation energy and driving force is constant across the temperature range of the study (Table 4).

**Figure 11.** A plot of the natural log of the inverse of the induction time for the transformation against temperature in the different solvents. ♦, methanol; x isobutanol:water 95:5 (v/v); ■, ethanol; +, 1-propanol; ×, 2-propanol; ●, acetone.

**Table 4.** Activation Energies for the transformation ($E_a$) (kJ.mol$^{-1}$) in the solvents examined.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$E_a$ (kJ.mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>42.8</td>
</tr>
<tr>
<td>Ethanol</td>
<td>48.0</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>51.6</td>
</tr>
<tr>
<td>2-Propanol</td>
<td>66.3</td>
</tr>
<tr>
<td>Isobutanol:Water 95:5 (v/v)</td>
<td>46.7</td>
</tr>
<tr>
<td>Acetone</td>
<td>49.4</td>
</tr>
</tbody>
</table>
The estimated activation energy is significantly higher in 2-propanol compared to the other solvents, implying that a greater barrier to the nucleation of FIII(6.525) during the transformation exists in 2-propanol.

**Molecular Modelling**

To investigate the strength of hydrogen bonding between the solvent and solute, a series of molecular clusters were generated between piracetam and the different solvent molecules. Initially only dimers were constructed, with the energy minimised using the differential evolution global optimisation algorithm (DE). Due to the stochastic nature of the DE algorithm, 25 independent runs were performed and the results collated (Table 5). Analysis of the runs identified three low energy clusters with the same hydrogen bonding motifs for the alcoholic systems (Figure 12). The bonding energies and preferential geometries vary as the alcohol is altered, with methanol and ethanol favouring the interaction with the ring carbonyl (minima 1), while 2-propanol has the strongest interaction acting as a bridging ligand over the amide group (minima 3). These calculations reveal that the strongest interactions are formed between 2-propanol and piracetam, supporting the suggestion that solvent-solute interaction is the cause of the retardation of nucleation and growth of FIII(6.525) during the transformation in 2-propanol. Calculations for methanol and 2-propanol with increasing numbers of solvent molecules indicate that this trend continues and 2-propanol molecules cluster strongly around the amide groups of piracetam, while methanol spreads around the whole molecule (Figure 13). This ‘bridging’ of the amide group by 2-propanol is significant because the main feature of the crystal structure of FIII(6.525) is a hydrogen bonding dimer formation between the amide groups of two piracetam molecules. Modelling of the interactions between piracetam with acetone or 1,4-dioxane shows
the lowest interaction energies and in both cases the solvent acts as a hydrogen bond acceptor to the amide hydrogens (Figure 14).

**Table 5:** Average Energy of low energy dimers between piracetam and solvent.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Minima 1 (kJ·mol⁻¹)</th>
<th>Minima 2 (kJ·mol⁻¹)</th>
<th>Minima 3 (kJ·mol⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>-57.71</td>
<td>-57.70</td>
<td>-56.84</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-64.17</td>
<td>-63.14</td>
<td>-60.37</td>
</tr>
<tr>
<td>1-propanol</td>
<td>-60.61</td>
<td>-60.82</td>
<td>-58.89</td>
</tr>
<tr>
<td>2-propanol</td>
<td>-69.25</td>
<td>N/A</td>
<td>-70.91</td>
</tr>
<tr>
<td>Acetone</td>
<td>-53.92</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>1,4-dioxane</td>
<td>-32.19</td>
<td>-31.93</td>
<td>-31.34</td>
</tr>
</tbody>
</table>

**Figure 12.** Comparison of the hydrogen bonding of the three minima located for alcoholic piracetam systems (a) Minima 1, (b) Minima 2 and (c) Minima 3.
**Figure 13.** Comparison of minima located for 3 (a) methanol and (b) 2-propanol molecules optimised around a piracetam molecule.

**Figure 14.** Comparison of the packing of the low energy solutions of (a) acetone and (b) 1,4-dioxane with piracetam.
CONCLUSIONS

The choice of solvent has a major effect on the transformation rate from FII(6.403) to FIII(6.525) piracetam, adjusting both the induction time and the transformation time. A general trend is observed; the higher the concentration of piracetam in solution, the faster the transformation rate. 2-Propanol does not conform to this trend, with piracetam having a higher solubility in 2-propanol compared to acetone, but a slower transformation rate. The high relative viscosity of 2-propanol does not decrease the dissolution rate of piracetam in that solvent. 1-propanol conforms to the trend outlined indicating that the positioning of the alcohol group on the carbon chain of the solvent molecule results in different solute-solvent interactions. A stronger interaction between the solute and solvent molecules is observed in the case of 2-propanol compared to the other solvents, thereby decreasing transformation rate in this solvent by retarding nucleation and growth of FIII(6.525). Also these interactions show a ‘bridging’ of the amide group of the piracetam molecule, which is vital in the crystal structure of FIII(6.525).

Temperature and agitation affect the transformation rate, with increases in both increasing the kinetic influence on the transformation and thereby, leading to shorter induction and transformation times.

REFERENCES


(4) Pavlova, A. W. *Pharmazie* 1979, 34, 449-450.


(17) F. Weigend, R. Ahlrichs, Phys. Chem. Chem. Phys. 2005, 7, 3297. The Ahlrichs (2d, 2p) polarization functions were obtained from the TurboMole basis set library under ftp.chemie.uni-karlsruhe.de/pub/basen;


Appendix B