Study of polymorphic crystallization : characterization, nucleation, transformation and in situ monitoring

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**STUDY OF POLYMORPHIC CRYSTALLIZATION:
CHARACTERIZATION, NUCLEATION,
TRANSFORMATION AND IN SITU MONITORING**

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SCHOOL OF CHEMICAL & BIOMEDICAL ENGINEERING

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Summary

Polymorphism is a highly significant aspect in the quality control of pharmaceutical and fine chemical products. Polymorphs (different crystalline forms of the same compound) can have great impact on the physicochemical properties of drug substances. Hence, controlling a final product with reproducible desired polymorph is of paramount importance. Polymorphic crystallization is a complicated process involving multiple mechanisms, which demand a comprehensive consideration of thermodynamics, kinetic factors and crystal features of these polymorphs.

This dissertation presents a detailed study on structural characterization, nucleation, crystallization and transformation of two polymorphic compounds, namely, glycine and *p*-aminobenzoic acid. A variety of in situ techniques including Raman spectroscopy, real time microscopy and attenuated total reflectance Fourier transform infrared spectroscopy have been applied to investigate crystallization and transformation mechanism of the model compounds.

Nucleation of glycine was performed in aqueous solutions with the presence of additive, sodium chloride. The occurrence of two common polymorphs of glycine (α -form and γ -form) is found to be strongly affected by degree of supersaturation and additive. It is illustrated that the nucleation behavior of the two polymorphs is related to their respective packing arrangements and specific interactions with the additive. The solution-mediated transformation from α -form to γ -form was conducted under various operating conditions. In situ observations of both solid phase and liquid phase during the transformation using Raman spectroscopy, FTIR and real time microscopy provide valuable insights into the mechanism underlying the polymorphic crystallization. It is shown that sodium chloride can preferentially promote the

formation of γ -form and significantly accelerate the transformation. According to the results from nucleation and transformation experiments, nucleation mechanisms of both polymorphs from aqueous solutions were proposed.

In addition, unseeded and seeded cooling crystallization of α -form glycine were carried out to investigate the effects of cooling mode and seeding on the product properties. The width of metastable zone was determined as a function of cooling rate using focused beam reflectance measurement. In situ FTIR spectroscopy was used to track real time solute concentration and hence supersaturation profiles throughout the cooling crystallization while a Malvern Mastersizer was used to measure the final crystal size distribution. Experimental results suggest that appropriate supersaturation control through the manipulation of cooling and seeding parameters could effectively improve the product crystal size distribution.

In the enantiotropic system of *p*-aminobenzoic acid (PABA), the solution-mediated transformation was studied for determination of relative stability of two polymorphs in aqueous solutions. Temperature dependence of transformation rate and transformation direction offers a reasonable estimation of the enantiotropic transition temperature. The solid state transition of β -form PABA to α -form was probed by in situ hot stage microscopy and Raman spectroscopy. The transition kinetics was described by solid-state reaction kinetic models in which the transition rate was quantified by measuring the changes in polymorphic composition. Effects of cooling modes on polymorph selection of PABA were also investigated.

The systematic structural characterization combined with in situ study of polymorphic crystallization and transformation could greatly facilitate understanding of polymorphic crystallization mechanism and process dynamics.

Nomenclature

Abbreviation

AFM	Atomic force microscopy
API	Active pharmaceutical ingredients
BCF	Burton, Cabrera, and Frank model
CSD	Cambridge Structural Database
CSD	Crystal size distribution
DSC	Differential scanning calorimetry
EM	Electron microscopy
FBRM	Focused beam reflectance measurement
FDA	US Food and Drug Administration
FTIR	Fourier transform infrared
IGC	Inverse gas chromatography
IR	Infrared spectroscopy
LM	Light microscopy
MSZW	Metastable zone width
NIRM	Near-infrared microscopy
NMR	Nuclear magnetic resonance
PABA	<i>p</i> -aminobenzoic acid
PEG	Polyethylene glycol
PXRD	Powder X-ray diffraction
rmsd	root-mean square deviations
SEM	Scanning Electron Microscopy
SSNMR	Solid-state nuclear magnetic resonance

TGA Thermal gravimetric analysis

Symbols

A	Pre-exponential factor
b	Cooling rate
c^*	Equilibrium saturation concentration
$\Delta c_{\max}$	Maximum supersaturation
C	Solution concentration
C_P	Heat capacity
C^*	Solubility
ΔG^*	Free energy barrier
ΔG_c^*	Free energy barrier for nucleation
G	Gibbs free energy
G	Growth rate
G_0	Partial molar free energy
G^*	Maximum free energy
$\Delta G_{\text{Surface}}$	Surface excess free energy
ΔG_{Volume}	Volume excess free energy
$\Delta_{\text{diss}} G$	Dissolution Gibbs free energy
H	Enthalpy
$\Delta_{\text{diss}} H$	Enthalpy of dissolution
J	Nucleation rate
k	Boltzmann's constant
k_n	Nucleation rate constant
k_T	Rate constant

K_{BCF}	BCF model parameter
n	Nucleation order,.
n	Unitless exponent
r	Mean radius of the nucleus
S	Entropy
t	Time
t_{ind}	Induction time
t_f	Batch time
T	Temperature
T_m	Melting points
ΔT_{max}	maximum supercooling
T_t	Transition temperature
T_i	Initial temperature
T_f	Final temperature
R	Gas constant
$\Delta_{diss}S$	Entropy of dissolution
x	Mole fraction of solute in the solvent

Greek Letters

α	Transformed fraction
θ	Bragg angle
γ_{st}	Interfacial free energy
σ	Supersaturation
v	Molecular volume

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Chapter 1 Introduction

1.1 Background

Polymorphism is the phenomenon that a compound exists in two or more crystalline phases with different arrangements and/or conformations of molecules in the crystal lattice (Brittain, 1999; Bernstein, 2002; Hilfiker, 2006). Polymorphs of the same substance are chemically identical and indistinguishable in vapor and solution phase. However, they have different internal structures and display different solid-state characteristics such as physicochemical properties and pharmaceutical performances. The variation in physical properties including crystal morphology, crystal size, solubility, hardness, melting point, stability and hygroscopicity exert great influences on the manufacturability and bioavailability of the compound.

According to McCrone's statement (1965), every compound has potential to form different polymorphic forms. The widespread existence of polymorphism in organic crystals is now attracting a growing attention and the investigation on polymorphism has become an important topic in a large variety of fields, especially in fine chemical and pharmaceutical research. Given the fact that polymorphism may have an impact on drug product bioavailability, the Food and Drug Administration (FDA) requires pharmaceutical manufacturers to consistently produce the approved polymorphic form of a drug substance (Byrn et al., 1995; Raw et al., 2004).

The main structural differences among polymorphic forms are due to different molecular packings and/or molecular conformations in the crystal lattice. There are two typical types of polymorphs including packing polymorphism and conformational polymorphism. Packing polymorphism represents those polymorphs in which the molecules with similar conformation pack into different crystal structures.

Conformational polymorphism is the most commonly observed type (Bernstein, 2002), which refers to the phenomenon wherein molecular conformations of a given compound are different in each polymorphic structure.

Crystallization is an important unit operation for the preparation of solid pharmaceutical products. As a polymorphic compound has the possibility of crystallizing into more than one crystal structure and appearance or disappearance of a polymorph is often highly dependent on operating conditions, the effective identification of polymorphs and rational control of polymorphs of active pharmaceutical ingredients (APIs) are of great importance in the pharmaceutical industry. The crystallization process of polymorphs is composed of competitive nucleation followed by crystal growth of polymorphs, and polymorphic transformation from a metastable form to a more stable one. Hence, the selective preparation of a desired polymorph requires extensive knowledge of unambiguous polymorph identification, thermodynamic and kinetic properties of the polymorphic system, mechanism of each elementary step in the crystallization process, and controlling factors for formation of the desired polymorph.

1.2 Objectives

The main objectives of this research are to establish the effective strategy for screening the possible polymorphs, and to enhance understanding of nucleation, crystallization and transformation behaviors of the model compounds by means of various in situ and off-line analytical techniques. The study includes physical characterization, real-time observations of solid-state and solution behavior of polymorphs and prediction of molecular conformation/packing. The emphases are given on identifying and characterizing the possible polymorphs, investigating the

effects of various operating conditions on selective crystallization of different polymorphs, analyzing the crystal structures of individual polymorphic forms and their relative stability and studying the occurrence of different polymorphs and polymorphic transformation between polymorphic forms from different solutions. Based on nucleation, crystallization and transformation behaviors according to the variation of operating parameters, the nucleation mechanism of stable and metastable polymorphs is proposed. The kinetic data obtained from the in situ measurements will be used to control and optimize the polymorphic crystallization processes.

1.3 Outline of the dissertation

Chapter 2 is literature review. This chapter provides the theoretical background on polymorphism and polymorphic crystallization. The basis of the thermodynamics and kinetic aspects of polymorphism is presented. The chapter also introduces various characterization techniques, which have been used in the investigation on polymorphs. In addition, the chapter provides a review of the literature on the crystallization of two model compounds (glycine and *p*-aminobenzoic acid) employed in this study.

In Chapter 3, the unambiguous identification and characterization methodologies of two polymorphs of glycine (α -form and γ -form) were established, which are the basis for the subsequent studies. The solubility of the two forms in various aqueous solutions was measured. The dependence of solubility on temperature was modeled using Van't Hoff equation. In addition, the chapter also shows the effect of electrolyte additives on the solubility of glycine.

Chapter 4 first focuses on nucleation behavior of the two polymorphs of glycine in aqueous solutions with and without additives. The nature of polymorphic forms that nucleate from solutions with different supersaturations and NaCl concentrations was

examined. Next, solution-mediated transformation from α -form to γ -form was studied by using various in situ techniques. Furthermore, the influence of process variables, such as additive, temperature, seed properties, stirring rate and ultrasound on the transformation was investigated.

In chapter 5, metastable zone width (MSZW) for α -form glycine in aqueous solutions was characterized using Lasentec focused beam reflectance measurement (FBRM). It was found that metastable zone width (MSZW) for glycine increases with cooling rate and its increase depends on initial concentration. The chapter also examines the effects of cooling profiles and seed loadings on the crystal size distribution of final products. Two commonly used cooling profiles (linear cooling and optimal cooling modes) were examined. In situ solute concentration and supersaturation during the cooling crystallization were measured by in situ FTIR. This will provide a guideline to control the suitable supersaturation and therefore produce crystals with desired quality.

Chapter 6 extends and applies the established strategy to investigate polymorphic transformation of two polymorphs of *p*-aminobenzoic acid (PABA) in solution and in solid-state. The solution-mediated transformation was identified and quantified by in situ Raman spectroscopy using fiber optic probe. The enantiotropic transition temperature in water was estimated through an analysis of the transformation kinetics. The kinetics of solid-state transition was studied through in situ measurement of the transition rate. Cooling crystallization was performed under different conditions to selective produce the two polymorphs. In situ Raman and FTIR were applied to measure the solid composition and solute concentration throughout the crystallization processes.

Chapter 7 gives the concluding remarks on the present work and suggests some

promising future research directions.

In summary, the results from this research are expected to improve our understanding of polymorphic crystallization. Especially, in situ process monitoring with the knowledge of thermodynamic and kinetic properties of the polymorphic system could provide critical information for the design of crystallization processes to obtain desired polymorphic products.

Chapter 2 Literature Review

2.1 Theoretical background

2.1.1 Thermodynamics of polymorphism

In terms of thermodynamics of a polymorphic system, one of the key questions is the relative stability of different polymorphs and thermodynamic relationship among these polymorphs at different temperatures, pressures and other conditions (Coquerel, 2006b). The relative stability of polymorphs can be examined by the difference in Gibbs free energy, G . Under given conditions (temperature and pressure), the polymorph which is the thermodynamically stable form has lower Gibbs free energy with respect to other polymorphs, and all other polymorphs are unstable or metastable. The metastable form is one that is thermodynamically unstable and has a thermodynamic tendency to reduce its free energy through transformation to a more stable one. However, the metastable form is sometimes pharmaceutically desirable because it can have higher bioavailability and better manufacturability than the stable form.

Gibbs free energy–temperature phase diagram is commonly used to describe thermodynamic behavior of polymorphs. The Gibbs free energy is a thermodynamic function defined by

$$G = H - TS \quad (2.1)$$

where H is enthalpy, it relates to the intermolecular energy in crystals; T is temperature; S is entropy which is related to the molecular disorder and lattice vibrations in crystals. From the expression for the Gibbs free energy the partial derivative with respect to

temperature is

$$\left[\frac{\Delta G}{\Delta T} \right]_P = -S \quad (2.2)$$

A typical energy–temperature diagram of a crystalline solid using the Gibbs relationship is given in Figure 2.1. Because S is always positive as a result of Third law of thermodynamics, the free energy (G) is constantly decreasing with increasing temperature. The enthalpy is increasing with temperature since the slope of curve $H = H(T)$ is the heat capacity (C_P). The free energy and its temperature dependence can be obtained using calorimetric method (melting point and enthalpy of fusion), or temperature dependence of solubility (enthalpy of solution from van't Hoff plot) (Gu and Grant, 2001). The energy–temperature diagram is a very useful tool to characterize polymorphic systems and to determine the phase relationship among various polymorphs.

Figure 2.1 Energy–temperature diagram (Haleblan and McCrone, 1969; Grunenberg et al., 1996).

2.1.2 Thermodynamic relationships between polymorphs

The stability relationship between a pair of polymorphs may be enantiotropic and monotropic. For an enantiotropic system, there is a transition temperature (T_t) at which the polymorphs are equally stable and in principle they can undergo reversible interconversion. A pair of polymorphs has a monotropic relationship if one polymorph is always more stable than the other one at all temperatures below the melting point of both polymorphs. As a compound has several polymorphic forms, there can be monotropic and enantiotropic relationships in the system between different polymorphs. The free energy diagrams shown in Figure 2.2 are helpful to understand the difference between monotropy and enantiotropy.

Figure 2.2a represents the energy temperature diagram for a monotropic system. As illustrated in the diagram, the free energy of polymorph I is always higher than that of polymorph II over the entire temperature range below $T_{m,I}$. This means form II is always more stable than form I beneath the melting point. Therefore, polymorph I can undergo a spontaneous transformation into polymorph II. In Figure 2.2b, there is a transition point (T_t), at where the Gibbs free energies of the two forms are identical, i.e., the two polymorphs are in equilibrium with each other. Below T_t , polymorph II is the stable form because the free energy of form II is lower than that of form I and vice versa at temperatures above T_t . These diagrams provide a great deal of information about the thermodynamic relationships of polymorphs and exist broad theoretical and practical applications.

Figure 2.2 Gibbs free energy–temperature curves for a monotropic system (a) and an enantiotropic system (b). (G , Gibbs free energy; liq, liquid; T_m , melting points of polymorphs I and II; T_t , the transition temperature.)

Moreover, a number of thermodynamic rules have been developed to predict the thermodynamic relationships of polymorphic pairs (Burger and Ramberger, 1979). Table 2.1 provides a summary of various thermodynamic rules that have been developed based on empirical observations to determine the thermodynamic relationships of polymorphic pairs. The three most useful and applicable rules are heat

of transition, heat of fusion, and density rules (Burger and Ramberger, 1979).

Heat of transition rule

This rule states that if an endothermic transition is observed between two crystal forms at a particular temperature and the thermodynamic transition point lies below this temperature, it can be concluded that the two polymorphs are enantiotropically related. If an exothermic phase transition is observed at a particular temperature, and there is no thermodynamic transition point at a higher temperature, then the two polymorphs are monotropically related (Burger and Ramberger, 1979).

Heat of fusion rule

In an enantiotropic system the higher melting polymorph will have the lower heat of fusion. If the higher melting polymorph has a higher heat of fusion the two polymorphs are monotropically related. If the melting points of the polymorphs differ by more than 30 °C, the rule may not be valid and the higher melting polymorph could have the higher heat of fusion in an enantiotropic system. In such cases it should be preferred to use other rules as guidelines.

Density rule

The energetically most stable packing of molecules in a crystal should have the strongest intermolecular interactions and have the highest density. Therefore, at absolute zero the most stable polymorph will have the highest density. Crystals whose packing structures are dominated by van der Waals interactions usually obey this rule. However, in some cases, the packing of crystals is controlled by other interactions, such as hydrogen bonds, the lower density polymorphic form may be the most stable form.

Table 2.1 Relative thermodynamic relationships between two polymorphs.

	Monotropism	Enantiotropism	
		$< T_t$	$> T_t$
Heat of transition	exothermic	exothermic	endothermic
Heat of fusion	$I > II$		$II > I$
Heat capacity	$II > I$		$I > II$
Entropy	$II > I$		$I > II$
Entropy of fusion	$I > II$		$II > I$
Solubility ^a	$II > I$	$I > II$	$II > I$
Density	$I > II$		$II > I$
Physical stability	$I > II$	$II > I$	$I > II$

I, higher melting form; II, lower melting form; $< T_t$, below the transition point; $> T_t$, above the transition point.

^a in given solvents.

2.1.3 Kinetic factors governing the formation of polymorphs

The kinetic factors governing the nucleation of polymorphs are illustrated by the free energy versus reaction coordinate diagram in Figure 2.3. G_0 is the partial molar free energy of a solute in a supersaturated solution. The diagram shows that there is a transition from the initial state G_0 to two different crystalline products forms I and II, in which form I is more stable than form II ($G_I < G_{II}$). Each reaction pathway passes through a transition state, where exists a maximum free energy (G^*) between the initial and final state.

The nature of polymorph that first nucleates from solution depends on the free energy barrier corresponding to ΔG^* ($G^* - G_0$) in Figure 2.3. Although the stable form I may have the greater thermodynamic driving force for the nucleation than form II ($G_I < G_{II}$), form II may nucleate at a faster rate than form I due to its lower energy barrier

($\Delta G^*_{II} < \Delta G^*_{I}$). This behavior is commonly observed in experimental practice, and this has been referred to Ostwald's Law of Stages, which basically states that a metastable form appears first from solution instead of the stable form. However, it has been observed that Ostwald's law of stages is not universally valid, and the appearance of solid phases depends on both thermodynamic and kinetic factors under the link between molecular assemblies and crystal structure (Bernstein, 1999).

Figure 2.3 Schematic diagram of the reaction coordinate for crystallization in a dimorphic system to show the activated barriers for the formation of polymorphs I and II (Bernstein, 2002).

2.2 Polymorphic crystallization

Crystallization from solution is an important separation and purification unit operation used throughout the pharmaceutical, chemical and food industries. Crystallization is the formation process of solid crystals from a homogeneous solution and consists of two main processes, nucleation and crystal growth. The nucleation refers to the formation of critical nucleus in the supersaturated solution. The next step,

crystal growth of the nuclei, leads to form macroscopic crystals. Nucleation and crystal growth steps together determine the nature of polymorph that eventually crystallizes from the solution. However, nucleation is the most critical step that determines the crystallization of different polymorphic form.

2.2.1 Nucleation

Nucleation mechanism can be divided into two main categories: primary nucleation and secondary nucleation. Primary nucleation refers to nucleation in bulk solutions (homogeneous nucleation) or that induced via random impurities (heterogeneous nucleation). The presence of foreign materials different from the crystallizing solute may serve as a nucleation substrate, which can decrease the activation energy barrier of nucleation. In the industrial crystallization practice, heterogeneous nucleation is commonly encountered. Secondary nucleation requires the presence of crystal seeds that promote the nucleation process.

Reduction in free energy is the thermodynamic driving force for nucleation. The overall excess free energy (ΔG) between tiny solid particles of solute and the solute in solution is equal to the sum of the surface excess free energy ($\Delta G_{\text{Surface}}$) and the volume excess free energy (ΔG_{Volume}). The volume free energy term favors the aggregation of molecules and the surface free energy term favors the dissolution of molecular clusters.

$$\begin{aligned}\Delta G &= \Delta G_{\text{Surface}} + \Delta G_{\text{Volume}} \\ &= 4\pi r^2 \gamma_{\text{st}} + \left(\frac{-4\pi r^3 kT \ln \sigma}{3\nu} \right)\end{aligned}\quad (2.3)$$

where r is the mean radius of the nucleus, k is Boltzmann's constant, T is absolute

temperature, γ_{st} is the interfacial free energy between the nucleus and the supersaturated solution, σ is the supersaturation, v is the molecular volume. In the crystallization process, σ is defined as the ratio of actual solute concentration in the supersaturated solution to that in the saturated solution.

$$\sigma = \frac{C}{C^*} \quad (2.4)$$

C^* is also known as the solubility of solute at certain temperature in a given solution.

Figure 2.4 represents the free energy diagram for a spherical nucleus as a function of the radius. It shows that the total free energy attains a maximum at $r = r_c$, at which the surface free energy is balanced with the volume free energy. At this point, the nucleus is a critical nucleus. The radius of the nucleus is the critical size of nucleus

$$r_c = -\frac{2\gamma_{st}}{\Delta G_v} \quad (2.5)$$

If the size of the nuclei is larger than the critical size, crystal growth will occur. Otherwise, the nuclei will dissolve. The critical free energy or the free energy barrier for nucleation (ΔG_c^*) can be calculated as

$$\Delta G_c^* = \frac{4\pi\gamma_{st}r_c^2}{3} = \frac{16\pi\gamma_{st}^3v^2}{3(kT\ln\sigma)^2} \quad (2.6)$$

The nucleation rate (J) can be expressed by an Arrhenius type of expression

$$J = A \exp\left(\frac{-\Delta G_c^*}{kT}\right) = A \exp\left(-\frac{16\pi\gamma_{st}^3v^2}{3k^3T^3\ln\sigma^2}\right) \quad (2.7)$$

where A is the pre-exponential factor.

Figure 2.4 Free energy diagram of nucleation.

2.2.2 Crystal growth

Once the solute molecules have formed larger than the critical size small particles, which we have called nuclei, the crystal growth for these nuclei will be spontaneous. Crystal growth is of great practical importance in crystallization processes because the growth rate of crystal faces and overall growth rate of a crystal have great impact on the product quality. Crystal growth in solutions is a complex process involving multiple steps and factors. Crystal growth theory has been reviewed in the literatures (Nývlt et al., 1985; Myerson, 1993). In these theories, it is assumed that the molecules are adsorbed on the crystal surface into kinks, steps and faces (Davey and Mullin, 1974; Kubota and Mullin, 1995; Mersmann, 2001). The crystals grow in a layer-by-layer mode and each time a step on the crystal sweeps over the surface, a new one has to be nucleated on the freshly completed crystal layer (Hurle, 1994; Levi and Kotrla, 1997). Burton, Cabrera, and Frank proposed the classical BCF model (Burton et al., 1951), which states that diffusion on the crystal surface is the rate-controlling step in crystal

growth and the growth rate of a crystal is related to the generation rate of steps at screw dislocation sites on its surface (Mohan and Myerson, 2002; Lu and Ulrich, 2005). This model has been widely employed for the modeling crystal growth kinetics in solutions with low supersaturations. According to BCF theory, the growth rate G can be written as (Mersmann, 2001)

$$G_{BCF} = \frac{K_{BCF1}}{K_{BCF2}} \sigma^2 \tanh\left(\frac{K_{BCF2}}{\sigma}\right) \quad (2.8)$$

where parameter K_{BCF1} is a function of temperature, number of growth units, activation energy and retardation factor, while parameter K_{BCF2} is temperature dependent and can be attributed to adsorption control. The BCF growth model is often simplified by

$$G_{BCF} \propto \sigma^g \quad (2.9)$$

where $g = 1$ at high supersaturation levels, $g = 2$ at low supersaturation levels.

2.2.3 Nucleation and crystal growth in polymorphic system

The preferable formation of a particular polymorph from solutions is the outcome of competitive nucleation and growth between different polymorphs. The selective crystallization of desired polymorphs is a highly complex process, which strongly depends on various thermodynamic and kinetic factors. Therefore, polymorphic crystallization requires careful control of the crystallization conditions to achieve the target product quality. There are a large number of methods that may be used for the control of polymorphism (Kitamura, 2002; Schroer and Ng, 2003), in which the use of additives and seeding have proved to be successful strategy for polymorph control.

Addition of impurities or additives to induce the nucleation and crystal growth of a desired polymorphic form or inhibit the nucleation and crystal growth of an undesired

polymorphic form has been successfully applied to achieve polymorph selectivity. Sonoda et al. (2006) reported the selective crystallization of metastable form IV of tolbutamide from an aqueous solution of 2,6-di-*O*-methyl- β -cyclodextrin (DM- β -CD). It was explained in their work that the formation of tolbutamide/DM- β -CD complexation inhibited the nucleation of the stable form I and thus stabilized the metastable form IV.

Seeding techniques have significant effects on polymorphic crystallization. Seeding a solution with desired crystals to induce the nucleation and crystal growth of target crystalline products may approve to be a convenient and efficient method for crystallization processes. Beckmann et al. (1998) investigated the effect of seeding on the crystallization of the metastable modification A of abecarnil. Pure A form was successfully obtained through the optimization of seeding and cooling rate.

The controlling factors of polymorphic crystallization have been summarized by (Kitamura, 2002), as shown in Figure 2.5 Controlling factors for polymorphic crystallization.

Figure 2.5 Controlling factors for polymorphic crystallization.

2.2.4 Polymorphic transformation

Polymorphic stability in biomolecules and pharmaceutical compounds is of considerable scientific interest and industrial importance. The polymorphic transformations are also critical issue in the chemical and pharmaceutical industry, where unpredicted polymorphic transformation of product can have significant effect on its physical and chemical properties, and eventually modify its activity and bioavailability. The polymorphic transformations occur through four different mechanisms: solid state, melt mediated, interface mediated and solution mediated transformation (Sato, 1993; Zhang et al., 2004).

Solid-state transformation

Solid-state phase transformations take place without passing through intervening transient liquid or vapor. Solid-state transformation is thought to occur via a positional rearrangement of the ions or molecules in a metastable crystal structure to achieve a new, more stable structure. Generally, the kinetics of transformation via solid-state phase is influenced by various operating conditions, presence of crystalline defects, particle size distribution and impurities (Linol and Coquerel, 2007). As a result, kinetic study of solid-state polymorphic transformation is of particular importance. The kinetic study of solid-state transformation of pharmaceutical drugs using a variety of characterization techniques has been very commonly reported in the literatures, such as carbamazepine (Brittain, 2004), theophylline (Matsuo and Matsuoka, 2007), sulfanilamide (Sheridan and Anwar, 1996), and other compounds (Urakami and Beezer, 2003). Various solid-state reaction models were applied to describe the experimental results and to determine possible mechanisms of solid-state transitions under different operating conditions.

Melt-mediated transformation

Melt-mediated transformation occurs via crystallization of the more stable form after melting the less stable form. The compound achieves its transformation through heating-cooling cycles. The actual process involves (a) melting of the less stable form, (b) nucleation and growth of the more stable forms, and (c) mass transfer in the liquid formed by melting of the less stable form (Sato, 2001). The course of the transformation via melt-mediated mechanism is affected by heating/cooling rate, crystal particle size, impurities and etc. In industrial crystallization, the melt-mediated crystallization is applied to the tempering process, which proceeds the formation of the more stable polymorph by fluctuating the temperature around the melting points of the less stable form.

Interface-mediated transformation

In interface-mediated polymorphic transformation, the phase transition occurs via an interfacial layer of solution between the stable form and the metastable form (Sato, 2001). This kind of transformation can be confused with both solid-state transformation and solution-mediated transformation. It differs from the other two transformations in that the phase transition occurs via a thin layer of solvent between the stable form and the metastable form in a single crystal and mass transfer at the interface plays a dominant role.

Solution-mediated transformation

According to “Ostwald's Rule of Stages”, in a polymorphic crystallization process a metastable form with higher solubility appears first from solution, and it then dissolves and transforms into a stable form with the lowest solubility by the “solution-mediated” mechanism. Therefore, solution-mediated transformation plays an

important role in polymorphic crystallization. The solution-mediated transformation operates when crystals of metastable form are suspended in the saturated solution. The driving force for this type of transformation is the difference in solubility between the stable and metastable form. That is, when the solution concentration is between the solubility of the metastable and the stable form, the stable form precipitates at the expense of the supersaturation of the metastable form. The solution-mediated transformation comprises three main steps: (1) dissolution of the metastable form; (2) nucleation of the stable form and (3) crystal growth of the stable form. Nucleation or crystal growth of the stable form is usually the rate-determining step. Any factor that affects each step involved in the transformation will influence the overall transformation process. These factors include temperature, solubility difference, agitation, solvent, seed crystals, additive, impurity, interfaces, etc.

2.2.5 New approaches for polymorphic crystallization

Generally, only one specific polymorphic phase of a given pharmaceutical compound will meet the product specifications. Therefore, a reproducible or reliable crystallization of the desired polymorph is particularly important in industry. Traditional methods, where conditions such as temperature, solvent, pH values, supersaturation, cooling rate, evaporation rate, seeding, impurity are modified during crystallization, have been extensively applied for discovery and selective production of polymorphs of pharmaceutical compounds. Recently, new approaches have been developed to direct polymorph selectivity and probe the mechanism for preferential crystallization of a particular polymorph. These approaches include laser induced nucleation (Zaccaro et al., 2001; Sun et al., 2008), crystallization with tailor-made soluble additives (Weissbuch et al., 2004), heterogeneous nucleation by foreign

particles or surfaces (i.e. polymer induced nucleation (Lang et al., 2002; Su et al., 2007), self-assembled monolayers (Küther et al., 1998; Aizenberg et al., 1999)), crystallization in capillaries (Chyall et al., 2002; Childs et al., 2004), solvent drop grinding (Trask et al., 2004; Trask et al., 2005), epitaxial growth (Saijo et al., 1977), confinement in porous materials (Ha et al., 2004), supercritical fluid crystallization (Beach et al., 1999), etc.

Nonphotochemical laser-induced nucleation (NPLIN), a phenomenon in which intense laser pulses induce supersaturated solutions to nucleate, has been applied to control polymorphism. It is reported that selective nucleation between α - and γ -forms of glycine can be achieved by switching between linear and circular polarization in the irradiation of supersaturated solution of glycine. Particularly, plane polarized laser light gives rise to the crystallization of the thermodynamically more stable but kinetically less favorable γ -polymorph of glycine. It has been hypothesized that the applied electric-field could induce alignment of molecules or groups of molecules into solute clusters and aid the cluster to organize into a crystal-like entity (Matic et al., 2005).

Effects of soluble additives on polymorph selection have been widely studied (He et al., 2001; Cashell et al., 2005; Kelleher et al., 2007). These studies focus on the choice and design of additives to stabilize the desired polymorph. The “tailor-made” additives, which are defined as the additive of molecular structure similar to the structure of corresponding substrate molecule, attract considerable interest. In the presence of tailor-made additives, the incorporation of additives into the crystal lattice of the host substance may alter the physical and chemical properties of the crystals and even modify the crystal characteristics with stereoselective target. The selective crystallization of the trimorphic crystals of glycine in aqueous solution with the

presence of α -amino acids is reported (Torbeev et al., 2005). The presence of enantiopure α -amino acids: phenylalanine, methionine and tryptophan, in the crystallizing glycine solutions induces changes in the morphology of α -form, leading to the formation of pyramidal instead of bipyramidal crystals. Increased amount of racemic α -amino acids inhibits both the α - and β -forms of glycine and induces precipitation of the γ -form. Another example of the application of additive to stabilize the metastable form is flufenamic acid (FFA) (Lee et al., 2008). It is found that the metastable form V of FFA can be crystallized and stabilized by using ammonium chloride as an additive in a water–ethanol mixture. From various characterization and structural analysis of FFA crystals, it is observed that form V has different conformation from more stable form I. It is explained that all hydrogen bonding sites on form I are exposed to interact with the ammonia chloride and this interaction inhibit the growth and/or nucleation of form I, leading to preferential formation of form V. To conclude, the application of tailor-made additives provides an opportunity to modify crystal morphology and to produce desired polymorphic forms.

Heterogeneous nucleation, the process by which nucleation occurs due to foreign particles or surfaces that are not chemically identical to the nucleated material, has been used to direct crystallization of many pharmaceutical compounds (Price et al., 2005). In this process, the selective nucleation and the oriented growth of polymorphic crystals are proposed to occur by the alignment of their lattice parameters. In particular, nucleation on self-assembled monolayers and polymer-induced nucleation have great potential for the discovery and selective production of polymorphs.

Self-assembled monolayers (SAM) (Love et al., 2005) and Langmuir-Blodgett (LB) (Kuzmenko et al., 2001) films have been demonstrated to be a convenient technique for monolayer or multilayer adsorption of molecules to the well-defined

functionalized surfaces. The major advantage of this method is that monolayer or multilayer is formed spontaneous by immersing a solid substrate into a solution containing the desired species with an appropriate functional group. The most popular SAMs is long-chain adsorbed thiols on gold substrata. Over the past decades, SAMs have been employed to modify crystal morphology and to control polymorphism and selective orientation of crystals. For instance, gold self-assembled multilayers of L-2-amino-N-{-[2-(2-amino-3-phenyl-propionylamino)-ethyl]disulfanyl}-ethyl-3-phenyl-propionamide (L-AAPP) were employed for the selective crystallization of L-glutamic acid. It was found that the metastable α -form of L-glutamic acid was stabilized on designed SAM surfaces (Dressler and Mastai, 2007). Hiremath et al. (2005) reported that gold-thiol SAMs of substituted 4'-X-mercaptobiphenyls (X = H, I, and Br) directed the nucleation and growth of three different polymorphs of 1,3-bis(m-nitrophenyl) urea (MNPU). These three forms, which grow concomitantly from solution, could each be selectively resolved with high phase purity on the prepared SAMs. Through analyzing long-range lattice matching and complementary functional group interactions on the SAM/crystal interfaces, they rationalized the selective crystallization of each form on the specific SAM template.

The polymer-induced heteronucleation approach provides an attractive alternative for the selective production of crystal polymorphs. A successful example is the application of polymers for polymorph selection of carbamazepine and acetaminophen reported by Lang et al. (2002). From a library of 84 different polymers, they found previously inaccessible form IV of carbamazepine crystallized from methanol in the presence of hydroxypropyl cellulose, poly(4-methylpentene), poly(R-methylstyrene) or poly(p-phenylene ether-sulfone). Similarly, orthorhombic and monoclinic forms of acetaminophen can be isolated by using certain polymers. Polymer-induced nucleation

can be easily and successfully employed to direct nucleation of different polymorphs, in which polymers act as an additional diversity element to affect the crystallization outcome.

2.3 Structural characterization techniques for polymorphs

In order to determine whether a crystalline is a new polymorph, series of characterization studies are carried out to analyze the chemical contents and crystal structures (Coquerel, 2006a). One may subdivide these analytical techniques into four groups: crystallography, spectroscopy, microscopy and thermal techniques (Yu et al., 1998). Crystallographic techniques comprise single crystal X-ray crystallography and powder X-ray diffraction (PXRD). Spectroscopic techniques, such as infrared spectroscopy (IR), Raman spectroscopy and solid-state nuclear magnetic resonance (SSNMR), provide powerful window to the composition of the sample. Microscopic analysis including light microscopy (LM) and electron microscopy (EM) distinguishes polymorphs through optical and morphological properties. Thermal techniques involve examining the thermodynamic properties and identifying the relative stability between polymorphs. The techniques which have been available for the examination of polymorphs include those listed in Table 2.2.

Table 2.2 Analytical techniques used in polymorph investigations.

<i>X-ray crystallography</i>	Powder X-ray diffraction
	X-ray single-crystal diffraction
<i>Spectroscopy</i>	Fourier transform infrared (FTIR)
	Raman spectroscopy
	Solid state nuclear magnetic resonance (SSNMR)
<i>Thermal methods</i>	Thermal gravimetric analysis (TGA)
	Differential scanning calorimetry (DSC)
<i>Microscopy</i>	Scanning electron microscopy (SEM)
	Atomic force microscopy (AFM)
	Hot-stage Microscopy (HSM)
<i>Density measurements</i>	Flotation
	Pyknometry
	Dilatometry

2.3.1 X-ray Diffraction

Single crystal X-ray diffraction is the most powerful approach for determining the crystal and molecular structures. This technique is the traditional method of identifying polymorphs, and it provides a direct characterization to establish the crystal structures of different polymorphs. However, the requirement for high quality single crystal samples limits the scope of this technique, as it is often impractical to prepare single crystals of sufficient size and purity for structural determination (Yu et al., 1998).

Powder X-ray diffraction (PXRD) is currently the most common tool for identifying polymorphic forms because every polymorph produces its unique diffraction pattern. The PXRD technique captures the three-dimensional scattering in a two-dimensional plot. Crystallographic detail for the crystal structures can be visualized and verified by comparing the measured pattern to the structures from the

Cambridge Structural Database (CSD, Cambridge Crystallographic Data Centre, CCDC, Cambridge, UK). Furthermore, PXRD is probably the most useful method for the quantitative analysis of mixtures of polymorphs. Recently, several investigators have explored the use of in situ X-ray diffraction to monitor the crystallization behavior of polymorphs (Blagden et al., 2003).

2.3.2 Spectroscopy

Spectroscopy techniques have played an important role in characterization and identification of pharmaceutical solids. IR and Raman spectroscopy provide fundamental information on molecular structure and conformation of various systems. Changes in dipole moment of molecules cause IR adsorption, whereas changes in polarizability associated with molecular vibrations result in Raman scattering. Thus, these techniques complement one another.

Fourier transform infrared (FTIR) spectroscopy has been proved to be a robust technique to identify solid-state polymorphic form. Traditional sample preparation for FTIR spectroscopy is grinding with an infrared transparent matrix (i.e. KBr and Nujol mulls) and pressing to form a pellet. For some compounds, the compression of KBr pellets can cause polymorphic transformation. This drawback of FTIR makes it unsuitable to study the polymorphic system exhibiting both grinding and compression induced transformation.

Raman spectroscopy is based on the detection of the scattering of incident radiation of light from molecules. Different polymorphs may have different molecular packing and intermolecular bonding, producing differences in electron distribution in the molecules, resulting in unique Raman spectra. In contrast to FTIR spectroscopy, Raman spectroscopy has tremendous advantages in investigations of polymorphism.

Sample preparation for Raman analysis is not necessary and thus the likelihood of inducing phase changes through processing is reduced (Taylor and Zografi, 1998). Raman spectroscopy coupled with a fiber optic probe provide the capability of in situ studies of polymorphism. This is of great importance in understanding the crystallization and transformation mechanism. In addition, Raman spectroscopy is compatible with both in water and behind glass, which makes Raman ideal for in situ monitoring crystallization process in aqueous solutions. Raman spectroscopy is now widely used in the chemical and pharmaceutical industry.

Solid-state NMR (SSNMR) has been employed to investigate polymorphism by probing chemical environments of atoms in solid-state. The most widely used SSNMR technique for pharmaceutical compounds is ^{13}C CP/MAS NMR. ^{13}C spectra show chemical shifts that are more sensitive to the details of structure than are proton shifts. SSNMR also provides information of crystallographically inequivalent sites in a unit cell. Furthermore, SSNMR is well-suited for investigating amorphous solids that are usually too trivial to be detected by XRD.

2.3.3 Microscopy

The purpose of microscopic techniques is to study optical properties of solid crystals. Polymorphs often have different optical properties and display different morphologies. The crystal size, shape and crystal growth and dissolution of polymorphs can be observed by using optical and scanning electron microscopy (SEM). Hot-stage microscopy (HSM) is a powerful tool for studying thermally induced events, such as melting point, polymorphic conversions, desolvation and sublimation. Coupling HSM with differential scanning calorimetry (DSC) can help interpret complicated melting/recrystallization behavior and polymorphic

transformation. Other types of microscope are used to probe the physicochemical properties include atomic force microscopy (AFM), FT-infrared and Raman microscopy, near-infrared microscopy (NIRM), acoustic microscopy and X-ray microscopy.

2.3.4 Thermal techniques

Commonly used thermal methods are DSC and thermal gravimetric analysis (TGA). The technique of DSC involves monitoring heat difference in heat flow between a sample and a reference during heating or cooling cycle. Thermodynamic data obtained by this method allow the determination of stability relationships between polymorphs based on Burger-Ramberger rules (Burger and Ramberger, 1979). TGA involves the measurement of mass loss during heating as a function of temperature. It is a useful tool to determine suspected solvate and to determine the decomposition temperature of the compound. DSC and TGA separately or together can be used to distinguish polymorphs according to the thermal behavior they undergo during heating.

2.3.5 Inverse gas chromatography

Inverse gas chromatography (IGC) is a gas phase technique for characterizing surface and bulk properties of solid materials. IGC is principally the reverse of a conventional gas chromatography (GC). The IGC column is packed with the solid material of interest over which is flowed various gas probes, the retention of which provides physicochemical information of the solid material. IGC has emerged as a suitable and pragmatic technique for characterizing surface properties and determining particulate surface energy (Gumbleton, 2007). IGC analysis is a valuable

complementary tool for characterization of powder samples as it can reveal subtle difference in surface properties. The application of IGC to polymorph characterization has been described (Tong et al., 2002; Tong et al., 2005). Some surface thermodynamic properties, such as surface free energy and surface entropy, were calculated to determine the surface intermolecular interactions. These parameters of different polymorphs were compared to find a relation between surface properties and polymorph stability.

2.4 Model compounds

The model compounds in this study are glycine and *p*-aminobenzoic acid (PABA).

2.4.1 Polymorphism in glycine

Glycine is the simplest amino acid, and is essential for the formation of heme, creatine, collagen, nucleic acids, bile acids, and other amino acids. As an important model system in biophysics and biochemistry, glycine has received considerable attention in polymorphism studies. Glycine has three polymorphs under ambient conditions (denoted forms α , β and γ) (Albrecht and Corey, 1939; Marsh, 1958; Jönsson and Krick, 1972; Power et al., 1976; Krick et al., 1980; Destro et al., 2000; Perlovich et al., 2000; Towler et al., 2004), and two polymorphs at high pressures (denoted forms δ and ϵ) (Dawson et al., 2005). In this work, we focus on the study of forms α and γ .

The basic molecular structure is the same for all the three polymorphs. Their crystal structures differ in intermolecular hydrogen bonds that are formed between NH_3^+ groups and COO^- groups. The cell constants for α , β and γ -forms are shown in Table 2.3. The growth of α -form is based on hydrogen bonded dimers of zwitterionic

glycine ($^+\text{NH}_3\text{CH}_2\text{COO}^-$). In the β -form, individual parallel layers are linked by hydrogen bonds forming a three-dimensional network. The γ -form is built from helical head-to-tail chains, in which zwitterions are linked to form three-dimensional network.

Table 2.3 Crystallographic data of the three polymorphs of glycine.

Polymorph	Form α	Form β	Form γ
Space group	P2 ₁ /n	P2 ₁	P3 ₂
a (Å)	5.10	5.09	7.037
b (Å)	11.97	6.27	7.037
c (Å)	5.46	5.38	5.483
β (°)	111.8	113.19	120
Z	4	–	3
Habit	prismatic	plate	bipyramid

a, b, c, β , lattice constants; Z , the number of molecules contained in unit cell.

The relative stability at ambient conditions of the three polymorphs is $\gamma > \alpha > \beta$. The γ -form is the thermodynamically stable form. It is typically produced from aqueous solutions with high or low pH values (i.e., pH above 8 or below 4) or with addition of additives (i.e., sodium salts). In addition, other methodologies reported to obtain the γ -form of glycine include application of polarized laser radiation (Garetz et al., 2002; Zaccaro et al., 2001) or dc electric field (Aber et al., 2005), evaporation of thin film of neutral aqueous solution at glass surface (Xu and Harris, 2007) or microporous membranes (Profio et al., 2007), from emulsions, microemulsions, and lamellar phases by surfactants (Allen et al., 2002), etc. The α -form is commercially available form and is kinetically favored in aqueous solution close to the isoelectric point (pI 5.97). It is generally taken for granted that the preferential crystallization of α -form from aqueous solution is that glycine molecules exist mainly as hydrogen

bonded cyclic dimers in aqueous solution. The conditions correlated with crystallization of γ -form may hinder the dimer formation, thus inhibiting the spontaneous nucleation of the α -form. β -form, the least stable polymorph, can be obtained from mixed water-alcohol solvents. The β -form is monotropically related to the other two polymorphs and it can transform to the α -form or γ -form in solution or humid air. The α -form and γ -form are enantiotropically related with a solid transition from γ -form to α -form at temperature around 170 °C. The α -to- γ transformation occurs in solution (Ito et al., 2005; Yang et al., 2008) or at high humidity (Sakai et al., 1992; Srinivasan, 2008). Some of these stability relationships among the three polymorphs of glycine are summarized in Figure 2.6.

Figure 2.6 Polymorphic conversion between the three polymorphs of glycine.

2.4.2 Polymorphism in *p*-aminobenzoic acid

Para-aminobenzoic acid (PABA) is an essential nutrient for several micro-organisms and it is an important active ingredient for the production of sunscreens, perfumes, dyes, esters, folic acid and its derivatives (Tripathi et al., 1996; Chignell et al., 1980). The PABA molecule consists of an aromatic ring, an electron

donating substituent, NH_2 , and an electron withdrawing substituent, COOH . Two different polymorphs of PABA have been reported in literature (α and β -form). The crystal structure data and crystal habit of the two polymorphs are summarized in Table 2.4. It has been reported that the polymorphs α and β exhibit an enantiotropic behavior with a transition temperature of around 20 °C (Gracin et al., 2004). It is proposed that the crystal structure of the α -form is based on dimers formed by the self-association of the carboxylic acid groups. PABA exists mainly as hydrogen-bonded dimers in the supersaturation solutions, which explains the preferential nucleation of the α -form. The β -form only crystallizes at the very slow controlled rate of supersaturation generation or from solutions that do not favor the dimers formation. The influence of ultrasound on polymorphism of PABA has been studied by Gracin et al. (2005). They found that sonication selectively favors the appearance of the β -form. It is explained that ultrasound disturbs the building up of the dimers in the solution and thus favors the crystallization of the β -polymorph.

Table 2.4 Crystal structure data and crystal habit of the two polymorphs of PABA.

Polymorph	Form α (Lai and Marsh, 1967)	Form β (Gracin and Fischer, 2005)
Space group	$P2_1/n$	$P2_1/c$
a (Å)	18.551	6.275
b (Å)	3.860	8.55
c (Å)	18.642	12.80
β (°)	93.56	108.30
Z	8	4
Habit	needle	prismatic

a , b , c , β , lattice constants; Z , the number of molecules contained in unit cell.

Chapter 3 Characterization and thermodynamic study of polymorphs of glycine

3.1 Introduction

Two common polymorphs of glycine (α -form and γ -form) were systematically investigated in this work. The major purposes of this chapter are to identify and characterize the two polymorphs with various techniques and to examine their solubility in different solutions. The solid-state structures of the α -form and γ -form show quite different packing arrangements, which differ from each other in the pattern of hydrogen bonds (Yu et al., 2002; Zaccaro et al., 2001). Packing diagrams of the two polymorphs are shown in Figure 3.1. In the structure of α -form, zwitterionic glycine molecules are linked through N–H $\cdots$ O bonds into hydrogen-bonded dimers. These dimers are packed to form mirror-related double layers. The γ -form is built from helical chains, in which head-to-tail glycine molecules link with each other to form a three-dimensional network.

Figure 3.1 Configuration of (a) the glycine zwitterion, (b) the dimer growth unit of α -form (Yu et al., 2002) and (c) the head-to-tail chain of γ -form (Williams, 2004).

Solubility data is indispensable for crystallization from solution. The knowledge of solubility of different polymorphs not only is a matter of theoretical interest but also allows one to carry out polymorphic crystallization in a more rational manner than happens at random. Many studies have been carried out on the measurement of solubility of glycine in aqueous solutions with various types and concentrations of electrolytes (Gatewood and Rousseau, 1994; Carta and Tola, 1996; Pradhan and Vera, 2000; Ramasami, 2002; Ferreira et al., 2005) and alkanols (Orella and Kirwan, 1991; Gude et al., 1996). However, to our knowledge, most of these investigations only concern glycine but not different polymorphs specially. For further understanding of solution thermodynamics of glycine in aqueous solutions, the present work takes into account more comprehensive and detailed measurements of solubility of the two polymorphs of glycine in various aqueous solutions. These results will be helpful to establish the strategy for rational design of separation and purification processes of glycine by crystallization.

3.2 Experimental

3.2.1 Materials

Glycine (99%) and sodium chloride (99.5%) were purchased from Sigma-Aldrich (Singapore). Methanol (>99.8 mol %), polyethylene glycol (PEG200, (>99.8 mol %), isopropanol (>99.8 mol %), hydrochloric acid (37%) and sodium hydroxide (99%) were obtained from Merck (Singapore) and used to prepare the buffer solutions. The ultra pure deionized water was obtained through a Millipore ultrapure water system (Milli-Q Gradient A10 System).

3.2.2 Preparation of α -form and γ -form of glycine crystals

The α -form of glycine was recrystallized from aqueous solution. The γ -form crystals were obtained by quench cooling method. To produce the γ -form, a saturated glycine solution with the presence of sodium chloride was prepared at 60 °C. The resulting solution was filtered and then quench-cooled in a refrigerator at -18 °C. Once the sample was frozen, it was allowed to anneal at room temperature. Then the slurry was filtered and the solids were dried in a vacuum oven at 60 °C for 12 h.

3.2.3 Powder X-ray diffraction

Powder XRD was performed at room temperature using a Bruker D8 Advance diffractometer with Cu-K α radiation source ($\lambda = 1.5406 \text{ \AA}$) at 40 mA, 40 kV. Powder samples were packed in an aluminum sample holder and scanned with diffraction angle 2θ from 5 to 50° at a step size of 0.05° for one second.

3.2.4 Vibrational spectroscopy

FTIR spectra were obtained under ambient conditions with a Digilab FTS-3000 FTIR spectrometer by using KBr in a palletized form. All spectra were collected in the range of wavenumber from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} .

Raman spectra were collected via a Renishaw inVia Raman spectroscopy equipped with RenCam CCD detector employing a 785 nm diode laser. Spectra obtained in the range of 300 to 4000 cm^{-1} were analysed using Renishaw WiRE 2.0 software.

3.2.5 Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA)

DSC measurements were carried out using a Mettler Toledo DSC 822^e together with the STAR–software. Samples of 5–7 mg were placed in a standard 40 μ L aluminum pan and then sealed with a perforated aluminum cover, and an empty aluminum pan was used as a reference. A heating rate of 10 $^{\circ}$ C/min was used over 25–350 $^{\circ}$ C temperature range for glycine samples. The instrument was calibrated with an indium standard.

TGA was performed with a Shimadzu TGA-50 instrument, using ceramic pans with about 5 mg of sample, at a heating rate of 10 $^{\circ}$ C/min under nitrogen flux.

3.2.6 Microscopy

The crystal morphology was observed by an Olympus BX51 microscope with an attached CCD video camera (Olympus, Japan). The images were recorded and analyzed by the analySISTM software.

3.2.7 Scanning electron microscopy (SEM)

The morphology of crystalline form was also investigated using a JSM-6700F Field Emission SEM (Jeol, Japan). A small amount of samples were scattered on double-sided adhesive carbon tabs mounted on SEM stubs and coated with Au/Pd in a Cressington 208 sputter coater (Pelco International, Redding, CA).

3.2.8 Focused beam reflectance measurement (FBRM)

Focused beam reflectance measurement (FBRM) is a powerful technique developed by Lasentec to perform in situ particle size measurements. This technique has been widely used in monitoring of crystallization process. The FBRM system is based on a high-speed scanning laser beam focused on the outside of a sapphire window (as shown in Figure 3.2). As a laser beam scans across a particle, the duration of backscattered light is measured and converted into a particle chord length. One major advantage of this technique is its in situ monitoring of particle size distribution and population trend of particles in suspension. The superiority of FBRM in sensitively tracking the onset of dissolution and nucleation makes it especially suitable for measuring the solubility and metastable zone width.

Figure 3.2 FBRM probe.

3.2.9 Solubility measurements

The solubility was measured by a gravimetric method. The experiments were

carried out in a 50 mL jacketed glass crystallizers equipped with a magnetic stirrer bar. The temperature of the crystallizer was controlled by circulating water from a Julabo programmable circulator FP50-ME. For each measurement, a certain amount of solvent or solvent mixtures was added into the crystallizer and stabilized at a measured temperature. An excess amount of pure polymorphic crystals of glycine (α -form and γ -form) was then added into the prepared stirred solvent. The solution was kept stirring at least 10 h to establish equilibrium at the constant temperature without the occurrence of polymorphic transformation. Equilibrium between solids and solution was determined by measuring the variation in solution concentration. Then the equilibrium suspension was settled down circa 4 h and aliquots of 5 mL were withdrawn by using a syringe and filtered through a 0.2 μ m PTFE filter (Whatman). The filtered clear solution was placed into a glass Petri dish (100 x 15 mm, previously weighed) and weighed. Then the solution was completely dried in a drying oven at 60 °C for circa 3 days. The solubility was calculated by determining the weight of dried solid and evaporated solvent. The mass of additives in the solution was taken into account in calculating the solubility. Considering the solution-mediated transformation between the two forms, the polymorph of solids after each measurement was checked and analyzed by XRD. The mass of glycine solid was measured by using a precise balance (Mettler Toledo, AB203-S) with a resolution of ± 0.1 mg. All experiments were repeated at least five times. The estimated accuracy of the solubility values based on error analysis and repeated observations was within 2.0 % for each measurement.

3.3 Results and discussion

3.3.1 Solid state characterization

3.3.1.1 Powder x-ray diffraction (PXRD)

Figure 3.3 PXRD patterns for α -form and γ -form of glycine.

Figure 3.3 shows typical XRD patterns for the prepared crystals of the two glycine polymorphs. These two polymorphs of crystals were identified based on their characteristic peaks as compared to those reported by other researchers (Cao et al., 2002; Bhat and Dharmaparakash, 2002; Moolya et al., 2005). Form α shows a characteristic peak at 2θ angle of 19.2° , while the γ -form displays characteristic peak at 25.3° which is not present in the form α .

3.3.1.2 Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA)

The experimental DSC and TGA traces for the two polymorphs are shown in Figures 3.4 and 3.5. The DSC curve of the α -form shows a single endothermic peak around 237 °C, which is due to the sublimation and decomposition of solids. Besides the peak of sublimation and decomposition around 235 °C, the DSC trace of γ -form shows another small endothermic peak around 171 °C, which is corresponding to the solid-state transformation from γ -form to α -form. This result is in agreement with the reported work (Bhat and Dharmaprakash, 2002). The forms α and γ are hence enantiotropically related according to the “heat of transition rule” (Burger and Ramberger, 1979).

The TGA curves of the two forms show no mass loss before 200 °C, which eliminate the possibility of hydrate or solvate formation. The major weight loss between 210 and 250 °C is due to the sublimation and decomposition of solids, which can be confirmed by the sharp endothermic peaks in the DSC curves.

Figure 3.4 DSC curves for α -form and γ -form of glycine.

Figure 3.5 TGA curves for α -form and γ -form of glycine.

3.3.1.3 Fourier transform infrared (FTIR) spectroscopy

Figure 3.6 FTIR spectra of α -form and γ -form of glycine.

IR spectra in the range of 500–4000 cm^{-1} for the forms α and γ are illustrated in Figure 3.6. The spectra of the two polymorphs show significant differences. The comparative spectral dissimilarities are highlighted in the region of 800–1000 cm^{-1} . The characteristic peaks at 910 cm^{-1} for α -form, 930 cm^{-1} for γ -form and a common peak at 890 cm^{-1} for both forms were selected for the identification.

3.3.1.4 Raman spectroscopy

Figure 3.7 Raman spectra of solid-state α -form and γ -form of glycine.

Raman spectra of the two pure polymorphs of glycine analyzed using Renishaw's inVia Raman microscope are given in Figure 3.7. It clearly shows that the characteristic Raman peaks for the α -form are at 3007 (CH_2 asymmetric stretching), 2972 (CH_2 symmetric stretching), 1456 (CH_2 bend), 1325 (CH_2 wag) and 893 (CC stretching) cm^{-1} , while the Raman bands for the γ -form are located at 2999 (CH_2 asymmetric stretching), 2963 (CH_2 symmetric stretching), 1439 (CH_2 bend), 1338 (CH_2 wag) and 894 (CC stretching) cm^{-1} , respectively (Goryainov et al., 2006; Baran and Ratajczak, 2005). Hence, the α -form has several distinguishable different features from those of the γ -form. It is noticeable that the α -form possesses a unique peak at

1325 cm^{-1} , while a band with a bigger intensity with two shoulders at 1338 and 1323 cm^{-1} is observed for the γ -form. The marked differences in Raman spectra of the two polymorphs are mainly due to different molecular packing in the crystal lattice of each polymorph. Table 3.1 summarizes the main characteristic bands with corresponding assignments for the α -form and γ -form, respectively. These Raman spectral differences make it possible for the two glycine polymorphs to be identified and quantified.

Table 3.1 Peak positions in the Raman spectra and tentative assignments for the α -form and γ -form of glycine.

Raman shift (cm^{-1})		Assignment
α -form	γ -form	
492	503	COO^- rock + NH_3^+ torsion
697	687	COO^- bend
893	894	C-C stretch
1035	1049	C-N stretch
	1323	CH_2 twist
1325	1338	CH_2 wag
1412	1399	COO^- sym. stretch
1456	1439	CH_2 bend
1506	1500	NH_3^+ sym. deform.
1569	1593	COO^- asym. stretch
2972	2963	CH_2 sym. stretch
3007	2999	CH_2 asym. stretch

3.3.1.5 Scanning electron microscopy (SEM)

Figure 3.8 Scanning electron micrographs of form α and form γ of glycine.

The morphology of prepared crystals of the two polymorphs was observed by SEM. As shown in Figure 3.8, the α -form shows prismatic shape while the γ -form crystals exhibit as bipyramids. The visual difference in morphology of the two forms thus makes it applicable to identify them by crystal shape.

3.3.1.6 Polarized light microscopy

Figure 3.9 Polarized light microscopy images of form α and form γ of glycine.

The crystal morphology observed by optical microscopy of the two polymorphs is shown in Figure 3.9. The α -form shows prismatic shape while form γ displays bipyramidal morphology.

3.3.2 Solubility

3.3.2.1 Solubility of α -form and γ -form in pure water

The solubility profiles of form α and form γ in water as a function of temperature are shown in Figure 3.10. It can be seen that the solubility of both forms increases with temperatures. The α -form has a higher solubility than the γ -form over the entire studied temperature range of 5–60 °C, which indicates that the γ -form is the thermodynamically stable form and the α -form is the metastable form at ambient temperature. Table 3.2 lists the solubility of the two polymorphs of glycine in water at different temperatures along with their standard deviation (numbers after the $\pm$ signs).

Figure 3.10 Solubility of α -form ($\blacklozenge$) and γ -form ($\circ$) in water.

Table 3.2 Solubility of α -form and γ -form in water.

α -form		γ -form	
T (°C)	Solubility (mg/mL)	T (°C)	Solubility (mg/mL)
5.0	141.1 ± 1.8	5.0	127.1 ± 1.1
12.5	166.4 ± 1.5	14.0	154.6 ± 0.6
20.0	198.5 ± 0.7	20.0	176.8 ± 1.0
25.0	225.9 ± 1.5	25.0	201.7 ± 0.9
28.5	241.2 ± 0.2	30.0	225.1 ± 0.5
33.0	259.1 ± 0.5	37.0	260.2 ± 0.6
36.0	282.5 ± 1.2	42.0	285.5 ± 0.8
46.0	333.8 ± 1.1	50.0	335.5 ± 1.3
50.0	353.5 ± 1.2	57.0	374.8 ± 1.9
55.0	384.0 ± 1.5	61.0	398.7 ± 1.6

The van't Hoff equation relates the logarithm of mole fraction of a solute in an ideal solution as a linear function of the reciprocal of the absolute temperature

$$\ln x = -\left(\frac{\Delta_{\text{diss}}H}{RT}\right) + \left(\frac{\Delta_{\text{diss}}S}{R}\right) \tag{3.1}$$

where x is the mole fraction of solute in the solvent, $\Delta_{\text{diss}}H$ and $\Delta_{\text{diss}}S$ are the enthalpy and entropy of dissolution, T is the corresponding absolute temperature, and R is the universal gas constant. The values of $\Delta_{\text{diss}}H$ can be regarded as a reflection of the nature of intermolecular interactions. By linear least-squares fitting of the van't Hoff equation to the experimentally obtained solubility data, the enthalpy and entropy of dissolution can be obtained from the slope of the solubility curve and the intercept with the y axis, respectively. Figure 3.11 shows van't Hoff plot of the logarithm of mole fraction solubility of the two forms versus reciprocal absolute temperature, the dissolution enthalpies of α -form and γ -form are therefore calculated to be (14.50 ± 1.1)

and (15.22 ± 0.9) kJ/mol, respectively. The dissolution entropies of α -form and γ -form are determined to be (23.77 ± 0.6) and (25.38 ± 0.2) J/mol/K, respectively. Gibbs free energy is an important thermodynamic function for expressing the relative polymorphic stability under defined temperature and pressure. The dissolution Gibbs free energy ($\Delta_{\text{diss}}G$) is related to dissolution enthalpy ($\Delta_{\text{diss}}H$) and dissolution entropy ($\Delta_{\text{diss}}S$) by the following fundamental thermodynamic relation

$$\Delta_{\text{diss}}G = \Delta_{\text{diss}}H - T\Delta_{\text{diss}}S \quad (3.2)$$

Thus, we obtained the difference in Gibbs free energy $\Delta\Delta G$ between the two polymorphs at

$$\Delta\Delta G = \Delta_{\text{diss}}G_{\gamma} - \Delta_{\text{diss}}G_{\alpha} \quad (3.3)$$

Using the $\Delta_{\text{diss}}H$ and $\Delta_{\text{diss}}S$ values determined at a typical experimental temperature of 298.15 K, we calculate $\Delta\Delta G = 0.24$ kJ/mol. This result shows that the thermodynamically more stable γ -form has a higher energy of dissolution than the less stable α -form.

When the solubility lines of two forms are extended backwards, they intersect at approximately 169.0 ± 4 °C, which is assumed to be the transition point between the two polymorphs. As revealed by DSC, the solid sample undergoes the polymorphic conversion from γ -form to α -form at 171 °C. These results confirm that the polymorphs α and γ possess an enantiotropic nature with a transition temperature at about 170 °C.

Figure 3.11 Van't Hoff plots of logarithm mole fraction solubility of α -form ($\blacklozenge$) and γ -form ($\circ$) as a function of the reciprocal of the absolute temperature in water.

3.3.2.2 Solubility of α -form and γ -form in aqueous solutions of alcohols

Organic solvents have been widely used as antisolvents to precipitate and crystallize amino acids from aqueous solutions. The solubility behavior of glycine in binary aqueous solutions of methanol, PEG200 and isopropanol is reported here. The solubility of α -form and γ -form of glycine in the mixtures of water with methanol, PEG200 and isopropanol as a function of antisolvent composition at 20 °C are presented in Figure 3.12 and also listed in Table 3.3 and Table 3.4. It can be seen that the solubility of both forms significantly decreased with increasing the concentration of methanol, PEG200 and isopropanol. Generally, hydrophilic amino acids (e.g., glycine and DL-serine) have poor solubility in alcohol or other organic solvents (Luk and Rousseau, 2006). It has been recognized that two major forces, electrostatic and hydrophobic interactions, may usually affect the solubility behavior of amino acids

(Mo et al., 2006). With an increase in the alcohol composition, hydrophobic interactions between glycine and alcohol could be promoted, which leads to decreased solubility of glycine. Even though increased electrostatic repulsion among glycine molecules could also contribute to solubility, it is offset by the promoted hydrophobicity of glycine molecules and therefore results in a decrease of the solubility.

For practical application, the experimental solubility data of glycine and solvent compositions are correlated by the following polynomial equation

$$S_c = A + Bw + Cw^2 + Dw^3 \quad (3.4)$$

where S_c is the solubility (g of solute/kg of solvent) of glycine; w refers to the initial mass fraction of the antisolvent in the total solvent (i.e. water + methanol); and A, B, C and D denote the regression curve coefficients. The regression curve coefficients and the root-mean square deviations (rmsd) defined by Equation 3.5 are listed in Table 3.5.

$$\sigma = \left[\sum_{i=1}^n \frac{(S_c - S_i)^2}{n-1} \right]^{1/2} \quad (3.5)$$

where S_c stands for the calculated values by Equation 3.5 and n is the number of experimental points.

Figure 3.12 Solubility of α -form ($\blacklozenge$) and γ -form ($\circ$) of glycine in (a) methanol+water, (b) PEG200+water and (c) isopropanol+water solutions at 20 °C; the line (---) represents the solubility calculated from Equation 3.4.

Table 3.3 Solubility of α -form in aqueous methanol (1), PEG200 (2) and isopropanol (3) solutions at 20 °C.

Methanol		PEG200		Isopropanol	
w_1 (%)	Solubility (g/kg of solvent)	w_2 (%)	Solubility (g/kg of solvent)	w_3 (%)	Solubility (g/kg of solvent)
0	198.9 ± 0.7	0	198.9 ± 0.7	0	198.9 ± 0.7
5.0	169.9 ± 0.3	8.58	164.8 ± 0.9	5.1	161.3 ± 0.6
10.0	142.1 ± 0.6	14.4	139.5 ± 0.5	11.9	112.5 ± 0.5
15.2	116.2 ± 1.1	22.9	109.9 ± 1.1	20.1	76.5 ± 1.0
19.1	96.9 ± 1.2	29.6	83.47 ± 0.8	30.2	45.5 ± 0.9
26.1	66.50 ± 0.9	35.8	61.52 ± 1.3	45.8	21.7 ± 1.2
31.7	50.57 ± 1.4	41.3	46.04 ± 1.7	60.5	8.1 ± 1.0
36.2	42.55 ± 1.3	48.4	25.45 ± 1.8	79.8	1.4 ± 1.1
44.4	28.02 ± 0.8	54.6	13.86 ± 1.8		
51.9	14.79 ± 1.1	68.4	4.33 ± 1.6		
60.4	8.82 ± 1.3				
70.0	6.61 ± 1.5				
100	2.4 ± 1.7				

Table 3.4 Solubility of γ -form in aqueous methanol (1), PEG200 (2) and isopropanol (3) solutions at 20 °C.

Methanol		PEG200		Isopropanol	
w_1 (%)	Solubility (g/kg of solvent)	w_2 (%)	Solubility (g/kg of solvent)	w_3 (%)	Solubility (g/kg of solvent)
0	177.0 ± 1.0	0	177.0 ± 1.0	0	177.0 ± 1.0
11.0	119.5 ± 0.4	14.5	128.6 ± 1.3	7.2	119.5 ± 0.9
14.7	102.0 ± 0.6	20.5	101.8 ± 1.4	15.7	83 ± 1.1
23.9	62.76 ± 1.1	30.1	65.75 ± 1.7	24.5	50.6 ± 1.0
35.0	34.93 ± 1.6	37.7	43.42 ± 1.6	36	26.3 ± 1.2
47.2	14.46 ± 1.4	45.6	25.16 ± 1.6	47.2	11.2 ± 1.1
63.8	4.02 ± 1.9	52.2	11.62 ± 1.5	63.8	4.01 ± 1.3
100	1.1 ± 0.8	59.2	6.62 ± 1.7		
		64.9	2.35 ± 1.9		

Table 3.5 Parameters of the model Equations 3.4 and 3.5.

	α -form			γ -form		
	Methanol	PEG200	Isopropanol	Methanol	PEG200	Isopropanol
$A \times 10^{-2}$	2.02	1.98	1.99	1.79	1.78	1.74
B	-6.95	-3.80	-8.37	-6.39	-3.40	-7.80
$C \times 10^2$	8.17	-1.64	12.9	7.60	-2.43	12.9
$D \times 10^4$	-3.21	4.44	-7.00	-2.99	5.46	-8.00
rmsd	0.0153	0.0049	0.0168	0.0196	0.0076	0.0260

3.3.2.3 Solubility of α -form and γ -form in aqueous solutions with different additives

In the past decades, considerable efforts have been devoted to investigate the phase behavior and molar properties of amino acids in aqueous solutions with electrolytes (Gatewood and Rousseau, 1994; Carta and Tola, 1996; Pradhan and Vera,

2000; Ramasami, 2002; Ferreira et al., 2005). Among the studies of amino acids in aqueous electrolyte solutions, some interesting solution behaviors have been found. It has been recognized that the presence of an electrolyte in solutions of amino acids greatly affects their solubility (Lu et al., 2006; Yang et al., 2008), activity coefficient (Khoshkbarchi and Vera, 1996; Mortazavi-Manesh et al., 2003), density (Ninni and Meirelles, 2001;) and enthalpy of the solutions (Larson et al., 1977; Palecz, 2000; Liu et al., 2005). A variety of thermodynamic models have been utilized to describe the phase behavior and molar properties of amino acid solutions considering the interactions between the amino acids and electrolyte solvent (Khoshkbarchi and Vera, 1996; Kirkwood, 1934; Chen et al., 1982). Kirkwood et al. (Kirkwood, 1934; Kirkwood, 1939) developed two models to describe the electrostatic interactions between amino acid and electrolyte in water-electrolyte-amino acid systems. Ferreira and coworkers (Ferreira et al., 2004; Ferreira et al., 2005; Ferreira et al., 2007) used the Pitzer-Simonson-Clegg equations to correlate the activity coefficients and predict qualitatively amino acid solubility in aqueous KCl and Na₂SO₄ solutions. The modified electrolyte NRTL model was applied by Chen et al. (Chen et al., 1982) to calculate the activity coefficients of amino acids in electrolyte solutions. These theories have given considerable quantitative predictions of solubility of amino acids in aqueous electrolyte solutions with emphasis on both experimental design of optimal separation and purification of amino acids and theoretical understanding of behavior of amino acids in electrolyte solutions. However, the predictions of such models ultimately depend on the detailed solubility data and specific interaction parameters. Also, the thermodynamic properties of amino acids in aqueous electrolyte solutions are much less well understood due to the complexity of amino acid–electrolyte interactions.

In this work, the solubility of glycine in aqueous solutions with various electrolyte additives were measured using the analytical gravimetric method. In contrast to prior works the present study takes into account more comprehensive data of different polymorphs of glycine in aqueous solutions considering the physical-chemical properties are different for the different polymorphs. Due to the relatively fast solution-mediated transformation of the metastable form α to the stable form γ in the presence of electrolytes, it is difficult to measure the reliable solubility of the α -form in electrolyte aqueous solutions, especially in solutions with high concentration of electrolyte additives.

Previous work (Lu et al., 2006) shows that addition of sodium chloride has a significant influence on the solubility of glycine and its oligopeptides. Figure 3.13 shows the dependence of solubility of γ -form on temperature and NaCl concentration. A general trend is that the solubility of γ -form increases with increasing temperature in the whole studied NaCl concentrations. At a given temperature, the solubility of γ -form increases with an increase of NaCl concentration, indicating a salting-in effect of NaCl on glycine solubility. However, the solubility variation is very slight as the NaCl concentration is higher than 80 mg/mL.

Figure 3.13 Solubility of γ -form glycine in aqueous solutions at different NaCl concentrations and temperatures.

Table 3.6 presents the experimental solubility data of γ -form in the presence of NaNO_3 , KCl and Na_2SO_4 at various concentrations at 25 °C. It can be seen that the three additives increase the solubility of γ -form over the whole range of concentration studied, indicating the salting-in effect of these salt additives on glycine.

Table 3.6 Solubility of γ -form glycine in aqueous solutions of NaNO_3 , KCl and Na_2SO_4 .

Additive concentration (mg/mL)	Solubility of γ -form (mg/mL)		
	KCl	NaNO ₃	Na ₂ SO ₄
0	201.7	201.7	201.7
10	228.7	226.8	244.0
40	262.3	259.6	275.2
80	273.6	308.9	284.6
120	278.0	359.1	291.3

Generally, the influence of electrolytes on the solubility of amino acids depends on the specific interactions between the amino acid and the electrolyte (Larson et al., 1977; Mo et al., 2006). The interaction may be a sum of different potentials such as direct electrostatic, hydrogen bonding, van der Waals, etc (Lu et al., 2006). Amino acids are well-known to exist in the form of zwitterions with double charges ($\text{NH}_3^+-\text{R}-\text{COO}^-$) in aqueous solution and solid state. The zwitterionic amino acid dissolved in aqueous solution is prone to interact with each other with participation of electrolytes through specific interactions. As such, when glycine is dissolved in the aqueous solution with the presence of NaCl, the formed ion-dipole pairs between NaCl and glycine can shield the hydrophobic interactions between the hydrocarbon skeleton of glycine molecules and contribute to the concentration change of glycine (Khoshkbarchi and Vera, 1997). The higher the concentration of electrolyte ions, the stronger the interactions. The solution phase equilibria between the various forms of glycine molecules are shown in Scheme 3.1. Therein, Gly(s) and Gly(aq) represent the glycine molecules in solid and aqueous phases, respectively, $\text{Gly}^+(\text{aq})$ represents zwitterionic glycine, and $\text{Gly}^+(\text{aq})$ and $\text{Gly}^-(\text{aq})$ represent the positively and negatively charged forms of glycine.

Scheme 3.1 Ionic equilibria in glycine solutions.

In general, amino acids appear in three ionic forms (cationic, zwitterionic, and anionic form) according to different pH values of the solution. Max et al. (Max et al., 1998) reported the distribution of different ionic forms of glycine as a function of pH. Three ionic forms, cationic form (pH 0–5), zwitterions (pH 0–12.5) and anionic form (pH 7–14), were observed for glycine in aqueous solution according to the pH values. In acid solutions, amino acids have net positive charge because the amino group is protonated with the presence of H^+ , while in alkaline solution they have negative charge because the carboxyl loses its proton with the presence of OH^- . At isoelectric point, the amino acids exist in the form of zwitterions ($NH_3^+-R-COO^-$), which is the most stable at the isoelectric point (pI) value. As pH is varied, the relative concentration of charged amino acid molecules rises. This results in an increase in solubility with increasing or decreasing pH values. The solubility of γ -form in water as a function of pH value at temperatures of 20 and 25 °C are listed in Table 3.7 and depicted in Figure 3.14. At 20 and 25 °C, a similar trend of solubility with varying pH values is observed. The pH–solubility profiles show a U-shape characteristic with a minimum solubility occurring at the pH value near the pI of glycine (pH 5.9). In the pH range of 3 to 10, the pH effect on glycine solubility is relatively small. However, some discrepancies between our data and literature values are observed at lower and higher pH values (Carta and Tola, 1996).

In the reported polymorphic system of glycine, the γ -form is the most stable and least soluble form at ambient conditions (Yang et al., 2008). Therefore, we verified our experimental data at lower and higher pH values by the crystal disappearance method to avoid the possible oversaturation. This method is based on sequentially adding required acidic and basic buffer solutions to the stirred slurry at a certain temperature. The solubility is determined by detecting the disappearance point of the particles

suspended in the solution. A Lasentec S400A FBRM was used to detect the onset of dissolution of particles. In Figure 3.14, these results are compared with those previously measured by the gravimetric method. A good agreement of these experimental data is observed.

Table 3.7 Solubility of form γ at various pH values and temperatures.

Solubility of γ -form (mg/mL)			
pH	20 °C	pH	25 °C
0.35	302.2	0.35	318.4
1.04	221.2	1.04	246.5
2.11	203.4	2.11	228.7
2.78	186.7	3.11	213.0
4.50	181.4	4.42	208.3
5.50	177.8	6.04	201.0
7.40	176.8	7.40	201.7
8.50	184.0	8.89	203.1
9.90	190.2	10.73	211.4
10.73	197.8	11.37	217.4
11.37	201.0	12.53	232.4
12.59	215.1	12.89	246.4
13.60	251.3	13.37	263.0
		13.70	283.6

Figure 3.14 Solubility of γ -form at various pH values and temperatures: ▲, 20 °C (gravimetric method); +, 20 °C (crystal disappearance method); ○, 25 °C (gravimetric method); ×, 25 °C (crystal disappearance method).

3.4 Summary

The two polymorphs (α -form and γ -form) of glycine were studied in this work. Physical methods including XRD, thermal analysis and spectroscopic techniques were used to fully characterize the two forms. The solubility of the two forms in different aqueous solutions were measured using the analytical gravimetric method. The results of DSC and solubility experiments show an enantiotropic relationship between the two forms. The solubility of both forms in aqueous organic solvent mixtures were found to decrease with the increase of concentration of methanol, PEG and isopropanol. The solubility of γ -form in aqueous solutions of NaCl, KCl, NaNO₃ and Na₂SO₄ was measured at different additive concentrations. The addition of these additives increased the solubility of γ -form, which is assumed to be a result of the interactions between

glycine molecules and electrolytes. The pH dependence of solubility of the γ -form in water was measured in the pH range of 0.35 to 13.7. The solubility profiles show a U-shape with the minimum solubility at the isoelectric point (pH 5.9). These results can contribute a better understanding of the thermodynamic behavior of glycine in electrolyte and non-electrolyte aqueous solutions.

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Chapter 4 Effect of process variables on nucleation and transformation of glycine polymorphs

4.1 Introduction

A survey of literature reveals that the metastable α -form can be obtained spontaneously from pure aqueous glycine solution under ambient conditions (Albrecht and Corey, 1939; Allen et al., 2002; Dawson et al., 2005). The spontaneous nucleation of form α from pure aqueous solutions is the typically kinetically controlled crystallization process. It has been reported that in pure aqueous solutions the α -form probably nucleates faster than the γ -form (Chew et al., 2007; Lee et al., 2008). The crystallization of the γ -form often requires special conditions, such as addition of electrolytes (Towler et al., 2004; Yang et al., 2008), application of plane-polarized laser light or DC electric field (Garetz et al., 2002; Zaccaro et al., 2001; Aber et al., 2005), slow evaporation from microporous membranes (Profio et al., 2007), etc. The polymorphic crystallization includes the formation of molecular growth units, the assembly of these growth units into nuclei, the growth of crystallites and polymorphic transformation from a metastable form to a stable form (Gu et al., 2001). Any factor that affects each step involved in the crystallization process will influence the polymorph of final products. Therefore, the nucleation of different polymorphs and the kinetics of polymorphic transformation under various operating conditions have to be fully understood.

One of the focuses of this chapter is to study the nucleation behavior of the two polymorphs of glycine with the presence of additive, sodium chloride. Secondly, the solution-mediated transformation from α -form to γ -form was investigated using various in situ analytical measurement techniques (Raman, ATR-FTIR and real-time

microscopy). Effects of operating factors such as additive, temperature on the transformation kinetics were studied.

4.2 Experimental

4.2.1 Nucleation experiments and induction time measurements

Nucleation experiments were carried out to investigate the occurrence domain of each polymorph with respect to different initial concentrations of glycine and additive concentrations. A suspension of the desired amount of glycine and additives was heated to above 5 °C of the equilibrium temperature and kept for 1 h with stirring. The solution was then “crash” cooled at required nucleation temperature and left to spontaneously crystallize. After the observation of initial nucleation, the slurry was immediately and rapidly filtered. The solid filter cake was analyzed by FTIR or Raman for their polymorphic form.

The time elapses from the creation of supersaturation until crystals grow to a detectable size is noted as the induction time, t_{ind} . Induction time is often used as a macroscopic measurement of the nucleation time, defined as the time that the creation of supersaturation until critical nuclei form (Kuldipkumar et al., 2007). Thus, the measurement of induction time could give important information for nucleation and crystal growth. The induction time for nucleation is a function of many parameters. In a certain solution, it is strongly dependent on the driving force (supersaturation) and the stirring rate. The techniques used for induction time measurements include scattered laser light (Bishop et al., 1992; Lancia et al., 1999; Prisciandaro et al., 2001), solution conductivity (Lyczko et al., 2002), turbidimetry (Feng et al., 2006), absorbance measurements (Kabdaszli et al., 2006), fluorescence (Crosio and Jullien, 1992), nuclear magnetic resonance (Mikol et al., 1991), electronic microscopy

(Michinomae et al., 1999), etc. In this work, FBRM was used to measure the induction time. The reported induction time, t_{ind} , is the average of at least three independent experiments.

4.2.2 In situ monitoring of solution-mediated transformation

In this work, solution-mediated transformation of glycine from the α -form to γ -form was studied under various conditions. The transformation experiments were carried out utilizing a 1 L cylindrical jacketed curved-bottom glass crystallizer built in a Mettler Toledo LabMax Automatic Laboratory Reactor system. In situ attenuated total reflectance Fourier transform infrared (ATR-FTIR) and Raman spectroscopy were used to measure solute concentration and solid-phase composition, respectively. A schematic diagram of the experimental setup is given in Figure 4.1. The stirring speed and temperature of crystallizer were automatically recorded and controlled by a PC-based data acquisition. The saturated solutions with respect to α -form with or without additives were prepared at the required temperature. When the concentration and temperature of the solution were steady, a certain amount of α -form crystals was added into the prepared solution.

A 785nm RamanRXN1 analyzer from Kaiser Optical Systems, Inc. (KOSI) with an immersion fiber-coupled MR probe (12" length x 1/2" diameter) together with HoloReact software was used to in situ quantitatively detect the solid phase composition of the two polymorphs of glycine. Raman spectra were collected with two accumulations of 10 s exposure time. The Raman spectra of pure forms α and γ in slurry in the range of 300–1700 cm^{-1} were analyzed using the KOSI Raman spectrometer equipped with a probe. The results are shown in Figure 4.2. As compared to Figure 3.7, it can be observed that some characteristic peaks for α -form and γ -form

overlap the solution peaks. The comparative spectral differences in the region of $1300\text{--}1360\text{ cm}^{-1}$ are highlighted. The baseline integrated areas of this region were used to develop calibration model and to monitor the transformation process. A group of binary mixtures with the two polymorphic forms in various mass ratios were prepared and charged to the presaturated aqueous solutions of glycine at $20\text{ }^{\circ}\text{C}$. Raman spectra for these standard samples were acquired. HoloGRAMS software (Kaiser Optical Systems, Inc, Ann Arbor, MI) was employed for base-lined integration of Raman bands. The calibration plot based on the normalized peak areas was obtained automatically by the software. As shown in Figure 4.3, the linear relationship between the relative integrated areas and the fraction of γ -form can be observed. These results will be used for quantitative analysis.

Figure 4.1 Experimental setup.

Figure 4.2 Raman spectra of the slurry for α -form and γ -form in aqueous solution (from in-situ probe Raman).

To consider the influence of temperature, solute concentration and solvent composition on the Raman characteristic peak, measurements were performed in the solution for various temperatures (20–40 °C), solute concentrations (200–350 mg/mL) and solvents (with/without additives). The results indicated that the relative integrated areas in the region of 1300–1360 cm^{-1} from solution are independent of temperature, solute concentrations and solvents.

Figure 4.3 Calibration curve of Raman spectra for the concentration of form γ in the slurry mixtures.

Apart from in-situ Raman, FTIR was used as off-line technique to quantitatively characterize the concentration of forms α and γ in solid phase. A group of binary mixtures with both polymorphic forms in various mass ratios were prepared and mixed with KBr to a mass concentration of 1 wt.%. Approximately 100 mg of sample was compressed to form a 1 cm diameter pellet in an evacuated die at 10 tons for 2 min. Figure 4.4 illustrates the IR spectra of the binary mixtures in the range of 860 to 960 cm^{-1} . Forms α and γ have a similar absorbance at about 890 cm^{-1} , which was chosen as the common peak as a reference. The concentrations of α -form and γ -form in the prepared samples were determined using the characteristic peaks of α -form at 910 cm^{-1} and γ -form at 930 cm^{-1} . For easier quantitative analysis of α - and γ -forms, dimensionless method was applied to develop the calibration curve. And the following ratio factor X was used

$$X = \frac{I_{910,\alpha} \times I_{890,\gamma} \times I_{930,S}}{I_{910,\alpha} \times I_{890,\gamma} \times I_{930,S} + I_{890,\alpha} \times I_{930,\gamma} \times I_{910,S}} \quad (4.1)$$

where $I_{890,\alpha}$ and $I_{890,\gamma}$ are the heights of common peak at 890 cm^{-1} for pure α -form and pure γ -form, respectively; $I_{910,\alpha}$ and $I_{910,S}$ are the heights of peak at 910 cm^{-1} for pure α -form and a sample; $I_{930,\gamma}$ and $I_{930,S}$ are the heights of peak at 930 cm^{-1} for pure γ -form and the sample. A calibration curve was constructed by plotting the factor X against the concentration of form γ in the prepared mixtures. As shown in Figure 4.5, the plot gives a straight line exhibiting good linearity, which suggests that the simple approach applied in this work for the quantification of the polymorphic mixture of glycine via FTIR is practical.

Figure 4.4 FTIR spectra in the range of 860 to 960 cm^{-1} for the standard mixtures with different concentrations of γ -form: (a) $0\text{ wt.}\%$; (b) $10\text{ wt.}\%$; (c) $20\text{ wt.}\%$; (d) $30\text{ wt.}\%$; (e) $40\text{ wt.}\%$; (f) $50\text{ wt.}\%$; (g) $70\text{ wt.}\%$; (h) $100\text{ wt.}\%$.

Figure 4.5 FTIR calibration curve for the concentration of form γ in the mixtures.

Prior to determining the glycine concentration in solution during the transformation, a linear calibration curve was constructed based on the concentration against FTIR absorbance. A series of standard glycine solutions with different concentrations from 100 to 250 mg/mL were prepared at 20 °C. Representative ATR-FTIR spectra of aqueous glycine solutions collected in the wavenumber range of 1200 to 1800 cm^{-1} are shown in Figure 4.6. The spectra show marked change in intensity with increasing concentration of glycine. Height of the peak at 1332 cm^{-1} , corresponding to the CH_2 wagging, was used to establish the calibration curve as shown in Figure 4.7.

The ultrasound generator is an ultrasonic processor (130 W Vibra-Cell, Sonics & Materials, Inc.) with a 13 mm diameter titanium alloy probe at a frequency of 20 kHz. The ultrasound power was varied via adjusting the amplitude setting. In this study, 30% and 60% of the maximum amplitude were chosen for comparison. The periodic ultrasound radiation was set via an independent on/off pulser.

Figure 4.6 FTIR spectra in the wavenumber range of 1200 to 1800 cm⁻¹ for the various standard glycine aqueous solutions. From bottom to top: 100 mg/mL; 125 mg/mL; 150 mg/mL; 175 mg/mL; 200 mg/mL; 225 mg/mL; 250 mg/mL.

Figure 4.7 Calibration of IR absorbance of the peak at 1332 cm^{-1} against the glycine concentrations in water at $20\text{ }^{\circ}\text{C}$.

4.3 Results and discussion

4.3.1 Effect of additives on the nucleation of glycine polymorphs

4.3.1.1 Nucleation behavior of the two polymorphs with the presence of NaCl

The occurrence domain of each respective polymorph with respect to different NaCl concentrations and supersaturations (defined as the ratio between initial solute concentration and solubility) were investigated. The initial nuclei were collected as soon as a few tiny particles appeared from the clear supersaturated solution and analyzed by FTIR or Raman for their polymorphic form. Results from the nucleation experiments are summarized in Table 4.1 and Figure 4.8. At nucleation temperature of $25\text{ }^{\circ}\text{C}$, it is clearly shown that the polymorph of nuclei depends on both supersaturation of glycine and NaCl concentration. At a certain supersaturation, the α -form

spontaneously nucleates from water or solutions with low NaCl concentrations, while the γ -form nucleates preferentially at high NaCl concentrations. It seems that the presence of additive NaCl and NaCl concentration show significant influence on the nature of polymorphs nucleate from aqueous solutions. The nucleation from aqueous solutions around isoelectric point usually gives α -form although γ -form is the stable form. Based on these observations, it is possible that the nucleation kinetics of the two polymorphs correlate with the solution speciation as well as the differences in the crystal structures of the two forms, and the nucleation mechanisms of glycine from solutions with and without NaCl may not be the same.

Table 4.1 Results of the nucleation experiments from solutions with various NaCl concentrations and degrees of supersaturation, σ .

Supersaturation, $\sigma_{25^\circ\text{C}}$	NaCl concentration			
	water	40 mg/mL	80 mg/mL	120 mg/mL
1.2	α	α	γ	γ
1.3	α	α	$\alpha+\gamma$	γ
1.5	α	α	α	γ
1.9	α	α	α	γ
2.0	α	α	α	γ
2.1	α	α	α	$\alpha+\gamma$
2.3	α	α	α	$\alpha+\gamma$
2.42	α	α	α	α
2.55	α	α	α	α

Figure 4.8 Nucleation behavior of the two polymorphs at various NaCl concentrations and initial concentrations of glycine.

Although there is no generally accepted mechanism for the nucleation of crystals from solution, many scientists agree that the nucleation involves the formation of liquid-like clusters of solute molecules in solution with ordered structure. This especially dominates the polymorphic systems (Zaccaro et al., 2001; Gracin and Rasmuson, 2004; Towler et al., 2004). The arrangement of solute molecules in the clusters present in solution determines the polymorphic form of crystals preferentially crystallize from solution. The solid-state structures of the α -form and γ -form show quite different packing arrangements, which differ from each other in the pattern of hydrogen bonds (Yu and Ng, 2002; Zaccaro et al., 2001). In the structure of α -form, zwitterionic glycine molecules are linked through $\text{N-H}\cdots\text{O}$ bonds into hydrogen-bonded dimers to form mirror-related double layers. The γ -form is built from helical chains, in which head-to-tail glycine molecules link with each other to form a three-dimensional network.

The effect of pH and additives on the growth of polymorphic glycine crystals have

been demonstrated and discussed (Yu and Ng, 2002; Towler et al., 2004). However, most of the interpretation was based on the examination of final crystals with relatively larger sizes and the possible transformation of α -form to γ -form was neglected. From this viewpoint, we conducted the above nucleation experiments and proposed the packing pathways of the two forms as shown in Figure 4.9. In pure water, two zwitterionic glycine molecules are linked together as a dimer through strong hydrogen bonding. As previously discussed, hydrogen-bonded dimers are the elemental growth units for the α -form. During the crystallization, the hydrogen-bonded dimers are packed to form mirror-related double layers in the crystal lattice (Yu and Ng, 2002), as shown in Figure 4.9a. However, the hydrogen bonded sequence of the dimers could be disrupted in the presence of electrolytes, which is due to Coulombic interactions occurring between the ions and carboxylic acid groups or amino groups of glycine. As a result, the tendency of spontaneous nucleation of α -form can be decreased when NaCl is employed as the additive.

Figure 4.9 The proposed packing pathways of the forms α (a) and γ (b).

The γ -form is built from polar chains (Zaccaro et al., 2001), in which all molecules exhibit the same orientation (Figure 4.9b). The investigation on nonphotochemical laser-induced nucleation of glycine polymorphs suggested that the electric field can induce partial alignment of glycine molecules and hence resulting in selective nucleation of γ -form (Zaccaro et al., 2001). The presence of electrolytes may also contribute an ionic atmosphere to charge and align the glycine molecules to head-to-tail chains. Taking all these considerations into account, the preferential nucleation of γ -form in the presence of electrolytes is probably due to a modification of molecular arrangements resulting from the Coulombic interactions between the electrolytes and glycine molecules. The crystallization of charged glycine molecules can result in direct nucleation of γ -form.

On the other hand, the results in Table 4.1 and Figure 4.8 show that the nucleation of two polymorphs is also strongly affected by the supersaturation (initial concentration of glycine). At NaCl concentrations higher than 40 mg/mL, the form γ is prone to nucleate from the solutions with low supersaturation, whereas the form α preferentially crystallizes at high supersaturation. For example, in the solution with NaCl concentration of 120 mg/mL and initial glycine concentration of 642 mg/mL, the α -form nucleates initially and then transforms to the stable γ -form rapidly. At a given NaCl concentration, the relative concentrations of charged and uncharged glycine molecules are closely related to the supersaturation. As discussed above, the dimer-based structure of α -form consists of two uncharged glycine molecules. With increasing the supersaturation the relative concentration of charged glycine molecules decreases, so the formation of dimerized molecular sequence is still available even at high NaCl concentrations. We thus suggest that the competitive nucleation of α -form and γ -form from aqueous solution is directly associated with the concentration of formed glycine dimers and the concentration of charged glycine molecules, respectively. The formation of the dimerized α -conformer is still available when the present electric field is not strong enough to completely disrupt the dimerization of glycine molecules. That is why the α -form can crystallize as initial nuclei at high supersaturation in the solution with high NaCl concentration in spite of very quick transformation to γ -form as final product.

4.3.1.2 Relative nucleation rate of the two polymorphs with the presence of NaCl

Another method to better understand the nucleation mechanism is the measurement of induction time (t_{ind}), which is defined as the time that elapses from the onset of supersaturation until the formation of critical nuclei to a detectable size. The

induction time is the sum of nucleation time (t_n) and growth time (t_g). With the assumption that the formation time of critical nuclei is much longer than the time taken by these nuclei to grow to measurable crystals, t_{ind} is inversely proportional to the nucleation rate, J . According to the classical nucleation theory, the nucleation rate is given by the following equation

$$J = A_n \exp\left(-\frac{16\pi\gamma^3\nu^2}{3k_B^3T^3(\ln\sigma)^2}\right) \quad (4.2)$$

where A_n is the pre-exponential coefficient, k_B is the Boltzmann constant, T is the absolute temperature, σ is the supersaturation, γ is the interfacial tension, and ν is the molecular volume of the solute. Therefore, the following relationship can be derived

$$\ln(t_{ind}) = A + B(\ln\sigma)^{-2} \quad (4.3)$$

Equation 4.3 suggests that at a given temperature, a plot of the natural logarithm of t_{ind} as a function of the square of the reciprocal of the natural logarithm of σ should give a straight line with the slope of $16\pi\gamma^3\nu^2/3k_B^3T^3$. As indicated in the Equation 4.2, T , σ and γ are the main factors dominate J or t_{ind} . Generally, the higher supersaturation leads to the shorter induction time. The dependence of t_{ind} on σ can be used to distinguish between the mechanisms of primary (homogeneous and heterogeneous) nucleation and to estimate some characteristic parameters (i.e. γ) of primary nucleation. The plot of $\ln(t_{ind})$ against $(\ln\sigma)^{-2}$ at 25 °C in 80 mg/mL NaCl solution is illustrated in Figure 4.10. Two distinct regions with different slopes can be linearly regressed from the experimental data. This change has been attributed to the change in nucleation mechanism from heterogeneous to homogeneous as supersaturation increases (Datta and Grant, 2005). The steeper line corresponds to homogeneous nucleation and generally, the homogenous nucleation rate has strong dependence on the

supersaturation. The nature of polymorph that nucleates is found to depend on the supersaturation of initial solution. At low σ the γ -form nucleates, at high σ the α -form nucleates, whereas both the α -form and γ -form nucleate simultaneously at intermediate σ . Therefore, the two straight lines in Figure 4.10 model the homogeneous nucleation of the two different polymorphs. The interfacial tension γ values obtained from the slopes of the straight lines represent the interfacial tensions between the supersaturated solution and the nucleus of the polymorph that nucleates respectively in each region. The interfacial tensions for the α -form and γ -form were calculated to be 8.15 and 5.47 mJ/m², respectively.

Figure 4.10 Plot of $\ln(t_{\text{ind}})$ versus $(\ln \sigma)^{-2}$ for the two polymorphs of glycine in the nucleation experiments carried in 80 mg/mL NaCl solutions at 25 °C.

Furthermore, the pre-exponential coefficients for the two polymorphs can be calculated from the intercept of straight lines in Figure 4.10. Therefore, the primary nucleation kinetics of each polymorph can be determined using Equation 4.2. Figure 4.11 shows the variation of the ratios of nucleation rates (J_{γ}/J_{α}) of the two polymorphs

at different supersaturations. For $J_\gamma/J_\alpha \rightarrow 1$ (around $\sigma = 1.3$), the nucleation probability of the two forms is nearly equal and the both forms nucleate simultaneously. At low supersaturation levels ($\sigma < 1.3$), the stable γ -form nucleates faster than the α -form. The nucleation rates for both polymorphs increase with increasing supersaturation ratio but at high supersaturation levels ($\sigma > 1.3$) the α -form nucleates at a higher rate than the γ -form.

Figure 4.11 Dependence of the ratio of the nucleation rates of the two polymorphs of glycine, J_γ/J_α , at 25 °C.

4.3.2 Solution-mediated polymorphic transformation of glycine

4.3.2.1 Effect of additives on the transformation behavior

To gain insight into the effect of additives on the crystallization of glycine, solution-mediated transformation experiments were performed in aqueous solutions with various additives. Solution-mediated transformation is usually driven by the

differences in solubility between the stable and metastable forms. The transformation typically involves three main stages. First, there is a dissolution of the metastable form into solution to increase the solute concentration. As a result of the solubility difference, the solution supersaturation with respect to the stable form leads to the nucleation of the stable form, which is followed by the crystal growth of the stable nuclei together with continuous dissolution of the metastable form.

The additive effects on the solution-mediated transformation of the α -form of glycine at 20 °C is shown in Figure 4.12. The amount of NaCl and KCl added was 80 mg/mL of water. The concentration of methanol and PEG was 20 wt.%. The suspension density was about 60 mg/mL. Over the course of the transformation, a decrease in the quantity of the metastable α -form was associated with a concurrent increase in the amount of the γ -form. The three different stages are distinguishable in Figure 4.12: (1) an initial period of induction, defined as the time required to detect a considerable proportion of the stable form, (2) an acceleratory period of rapid increase in conversion which is corresponding to the accelerated three-dimensional growth of crystals, and (3) a decay period indicating the completion of the crystallization process. The induction time for transformation, which is the time required for the formation of the γ -form nuclei, and transformation rate were different in different solvents. The transformation rates can be estimated from the slope of the acceleratory period of the transformation. The induction time and transformation rate for the transformation with different additives are summarized in Table 4.2.

Figure 4.12 Transformation behavior of the α -form in the presence of various additives.

Table 4.2 Induction time and transformation rate for the solution-mediated transformation of α -form to γ -form in the presence of various additives.

Additive	0	NaCl	KCl	methanol	PEG
Induction time (h)	33	1.5	1.2	36	>80
Transformation rate (wt.%/h)	7.5	11.9	10	2.7	–

It is clear that the α -form was transferred into the γ -form in water at a rather slow rate. The induction time was about 33 h while the complete transformation required circa 55 h and the reduction of α -form in solids show a typical sigmoidal profile. For comparison, we also investigated the influence of seeding with γ -form crystals on the transformation behavior of the α -form. In the experiment where the initial materials contained small fractions of the γ -form (5 wt.%), the transformation began with no induction time as a result of the secondary nucleation of the γ -form. With the addition

of NaCl and KCl, the transformation was accelerated significantly. The transformation can be completely achieved within 15 h and 20 h in NaCl and KCl solutions, respectively. Especially, the induction time for initiation of the transformation is profoundly affected by the presence of NaCl and KCl. These observations can be explained by, as discussed previously, the promotion effect of electrolytes on the nucleation of γ -form. By contrast, the transformation was retarded with the presence of methanol and PEG, particularly PEG, where the α -form remained unchanged even after 3 days. The retardation effect of methanol and PEG on the transformation from the α -form to the γ -form can be explained by the effect of additives on the solubility of glycine. As described in Section 3.3.2.2, methanol and PEG decreased the solubility of both α -form and γ -form. Also, solubility differences between the two polymorphs were decreased in the mixed solvents. As it has been discussed the solubility difference between the stable and metastable forms is the thermodynamic driving force for the solution-mediated transformation. Thus, the decrease in the solubility difference between the two polymorphs with the presence of methanol and PEG may be the primary cause of the reduction in the transformation rate of the α -form.

To understand the effect of the additive concentration on the transformation rate, different amounts of NaCl were added to the solution, and the results are shown in Figure 4.13. Overall, the transformation curve in NaCl solutions is very similar to that in pure water. The induction time and transformation rate are found to be sensitive to the NaCl concentration. The induction time is considered to be inversely proportional to the nucleation rate, which defined as the number of nuclei formed in solution per unit time and volume. The transformation rates were estimated from the slope of the acceleratory period of the transformation. As shown in Table 4.3, the induction time decreased significantly with increasing NaCl concentration. This probably indicates

that the nucleation of γ -form was induced with the presence of NaCl and thus the nucleation rate of γ -form was accelerated. The increase of transformation rate with increasing the NaCl concentration can be clearly seen from Table 4.3. However, transformation rate did not show significant change at NaCl concentrations lower than 120 mg/mL. These results suggest that the promotion effect of NaCl on the solution-mediated transformation may be mainly attributable to the promotion effect of NaCl on the nucleation of γ -form.

Figure 4.13 Effect of NaCl concentration on the transformation from α -form to γ -form at 20 °C.

Table 4.3 Induction time and transformation rate as a function of NaCl concentration for the solution-mediated transformation of α -form to γ -form.

NaCl concentration (mg/mL)	0	40	80	120
Induction time (h)	33	2.2	1.5	0.35
Transformation rate (wt.%/h)	7.5	8.1	11.9	31.0

Figure 4.14 shows the concentration change of glycine for the transformation measured by ATR-FTIR in both water and NaCl aqueous solutions. Three stages are distinguishable in these two cases. First, the solute concentration stayed at the solubility of the α -form until most of the suspended α -form crystals had transformed to the γ -form. The concentration reduced rapidly in the second stage and approached the solubility of γ -form. Finally, all the α -form transformed to the γ -form and the solute concentration kept at the solubility of the γ -form. Comparing the solute concentration change and solid composition change during the transformation, the whole transformation from α -form to γ -form can be split to three processes: the dissolution of α -form, the nucleation and crystal growth of γ -form. The starting point for the transformation occurs at heterogeneous nucleation of the γ -form at the expense of supersaturation of the α -form. As the γ -form crystals nucleate and grow, the solution goes into undersaturated zone with respect to the α -form. The α -form crystals further dissolve and its dissolution makes up the supersaturation for the γ -form to nucleate and grow. Since the solute concentration remains at the solubility of α -form for the period of nucleation and growth of γ -form and the concentration displays a rapid decrease at the end of the transformation, indicating fast dissolution of the α -form and relatively slow nucleation and growth of the γ -form. The rate-controlling step is thus considered to be the nucleation and growth of the γ -form.

Figure 4.14 Changes in glycine concentration in solution during the transformation. The lines I and II represent the solubility of α -form and γ -form in 80 mg/mL NaCl aqueous solution, while the lines III and IV represent the solubility of α -form and γ -form in water.

The differences in the crystal shapes of the α -form and γ -form are recognizable as shown in Figure 3.8 and Figure 3.9. In situ transformation observation was carried out with an optical microscopy. Figure 4.15 shows the real-time images taken during the transformation from α -form to γ -form in pre-saturated glycine solution with 80 mg/mL NaCl. The system was kept isothermally at 20 °C using heating/cooling stage under quiescent condition. After a few hours, the immersed prismatic α -form crystals dissolved gradually. Simultaneously, the γ -form (trigonal shape) crystals began to crystallize at the expense of the α -form. Finally, all the α -form crystals completely dissolved and the γ -form crystals continued to grow until the solute concentration reached the solubility of the γ -form.

Figure 4.15 Real-time microscope images taken during the solution-mediated transformation of glycine in the quartz cell at 20 °C: (a) before polymorphic transformation (prismatic α -form crystals); (b, c) halfway through the transformation; (d) after the transformation (trigonal pyramidal γ -form crystals).

The combined experimental techniques of in situ Raman, ATR-FTIR and microscopy allows insights into fundamental mechanisms of the solution-mediated transformation of glycine in aqueous solutions. The results from transformation experiments confirm that the γ -form is the stable form in aqueous glycine solutions. The transformation from the α -form to the γ -form is governed by dissolution of the α -form and nucleation and growth of the γ -form. The nucleation and growth of the γ -form is rate-controlling step due to the relatively slow crystallization kinetics. The induction time and transformation rate for the transformation are strongly affected by the addition of NaCl.

According to Ostwald's "Rule of stage", the classical polymorphic nucleation processes are generally occurred via the nucleation of kinetically favorable metastable

form and the thermodynamically controlled transformation from the metastable form to a stable form. From the clear in-situ observations with microscopy, it is worth noting that the γ -form can directly crystallize from the quiescent glycine solution with the presence of NaCl. The driving force for the heterogeneous primary and secondary nucleation and crystal growth of the γ -form is the supersaturation with respect to the γ -form, which is sufficient to induce the conversion of the disordered γ -conformers to crystalline phase. In other words, the γ -form can cross the energy barrier for the direct nucleation with the aid of additives. This result is also consistent with the proposed nucleation pathway that the homogeneous nucleation of γ -form is possible from bulk solution and the presence of NaCl preferentially promotes the crystallization of γ -form. Plausible nucleation processes for the two polymorphs are proposed based on the product distribution observed in this work. As shown in Scheme 4.1, k_1 and k_2 are the rate constants for the nucleation of α -form and γ -form, while k_3 is the transformation rate constant. In water and aqueous solutions with relative low concentration of charged glycine molecules, the metastable form α is known to appear more readily than the stable form γ , so the spontaneous nucleation of α -form is the typically kinetically controlled crystallization process. That is, $k_1 \gg k_2$, the formation of γ -form from water and solutions with low NaCl concentration is rate determined by the transformation from the α -form to the γ -form (k_3). While in the solutions with high concentration of charged glycine molecules, the nucleation of α -form is inhibited, that is, $k_2 > k_1$ so the direct nucleation of γ -form is available.

Scheme 4.1 Suggested pathway of nucleation.

4.3.2.2 Effect of temperature on the transformation behavior

Effect of temperature on the transformation behavior was studied by performing the transformation experiments at temperatures of 20, 30 and 37 °C. From Figure 4.16, the transformation rates show significant change with increasing the temperature from 20 to 37 °C. Since the operating temperatures are far away from the transition temperature, the solubility differences between α -form and γ -form at 20 and 37 °C are almost the same, that is, the effect of temperature on the thermodynamic driving force for the transformation can be negligible. On the other hand, molecular motion in crystals increases with increasing the temperature. Therefore, the increase of transformation rate with the increase of temperature is due to the kinetic effect.

Figure 4.16 Effect of temperature on the transformation from α -form to γ -form in 80 mg/mL NaCl aqueous solutions at 20, 30 and 37 °C.

4.3.2.3 Effect of seed properties on the transformation behavior

It has been expected that the particle size of seed crystals may have significant effects on polymorphic transformation. As the particle size decreases, the surface to volume ratio increases which may result in a greater dissolution rate. In this work, the effect of particle size of starting α -form seeds on the transformation kinetics was investigated. The α -form seeds with various sizes were prepared by sieving the products harvested from cooling crystallization of aqueous glycine solutions. Three ranges of particle size were studied: 25–40 μm , 50–100 μm and 200–300 μm . Figure 4.17 shows the transformation profiles under the different initial seed sizes in presaturated aqueous glycine solution with 80 mg/mL NaCl at 20 °C. It can be seen that the fastest transformation rate was obtained when the smallest size of α -form seeds was used. The transformation rate decreased with increasing seed size, e.g. when the

seed size was decreased from 200–300 μm to 25–40 μm , the transformation time was reduced from circa 14 h to less than 8 h. For the same seed amount, the smaller seed size provides larger number of crystals and higher specific surface area. This appears to result in a faster mass transfer and to help maintain the supersaturation with respect to the γ -form, thus generating greater overall transformation rate.

Grinding is a classical technique employed for seed crystal generation. Grinding is known to affect structure, composition and surface properties of particles, and to cause an increase in surface area due to fracturing the particles. Therefore, the α -form crystals were manually ground gently using a mortar and pestle, and then sieved to obtain size ranges of 25–40 μm , 50–100 μm and 200–300 μm . PXRD was used to confirm the solid state of the samples was unchanged. As shown in Figure 4.18, it can be found that the ground α -form samples converted faster than the untreated α -form crystals. We attribute this to the existence of surface defects caused by grinding. As the surface areas of seed crystals increases as expected after grinding, the mass transfer increases, in turn, promoting the overall transformation.

Figure 4.17 Effect of seed size on the transformation from α -form to γ -form in 80 mg/mL NaCl aqueous solutions at 20 °C.

Figure 4.18 Effect of grinding seeds on the transformation from α -form to γ -form in 80 mg/mL NaCl aqueous solutions at 20 °C.

4.3.2.4 Effect of stirring rate on the transformation behavior

The effect of stirring rate on the transformation was investigated using seeds of 200–300 μm in aqueous solution with 80 mg/mL NaCl at temperature of 20 °C. In crystallization processes, mechanical stirring is often used to attain homogeneous temperature and concentration over the entire volume. As shown in Figure 4.19, the transformation rate was enhanced as the stirring rate was increased from 200 to 500 rpm. Generally, the stirring rate in a suspension system has an appreciable influence on the mass transfer coefficient and interfacial surface area (Liang et al., 2004). This effect results in an increase of the crystallization kinetics at higher stirring rate, thus increasing the formation rate of the stable form and hence the transformation rate.

Figure 4.19 Effect of stirring rate on the transformation from α -form to γ -form in 80 mg/mL NaCl aqueous solutions at 20 °C.

4.3.2.5 Effect of ultrasound on the transformation behavior

Ultrasound has been known to bring considerable benefits to several

crystallization processes (Li et al., 2003; Gracin et al., 2005). There is reliable evidence that applying ultrasound not only can induce primary nucleation, reduce metastable zone width and shorten induction time, but also can change crystal size distribution and crystal habit, inhibit agglomeration and modify polymorphism of products (Lyczko et al., 2002; Kurotani and Hirasawa, 2008). The effect of ultrasonic irradiation on the solution-mediated transformation was investigated in this work. The experiments were carried out using periodic ultrasonic irradiation (irradiation with 2 s pulse on and 10 s pulse off) with different intensity (30% and 60% of maximum amplitude, MA). Figure 4.20 presents the transformation behavior of the α -form in 80 mg/mL NaCl solutions with and without application of ultrasound. The results show that the transformation rate from form α to form γ was enhanced significantly with the presence of ultrasonic irradiation.

Figure 4.20 Effect of ultrasound on the transformation from α -form to γ -form in 80 mg/mL NaCl aqueous solutions at 20 °C.

The role of ultrasound in crystallization processes is complex. Previous investigations have revealed that the influence of ultrasound on the nucleation and crystal growth might be performed through the mechanisms of cavitation and acoustic streaming. The created mechanical disturbance is thought to accelerate the mass transfer so as to prompt the nucleation as well as the crystal growth. In addition, it was hypothesized that ultrasonic energy may change the nucleation kinetics of different polymorphs (Kurotani and Hirasawa, 2008). This implies that similar to using additives, impurities or solvents, the application of ultrasound can direct the nucleation of a specific polymorphic form.

4.4 Summary

The nucleation and transformation behavior of two common polymorphs of glycine (α -form and γ -form) in aqueous solutions with and without additives have been investigated. The nature of the polymorphic form that nucleates initially from glycine solutions with NaCl strongly depends on NaCl concentration and supersaturation. In aqueous solutions without additive or at low NaCl concentration, the α -form spontaneously nucleates, while the γ -form crystallizes preferentially at high NaCl concentration and low initial concentration of glycine. Based on the experimental results, it is proposed that the presence of electrolytes can modify the packing arrangements of glycine molecules resulting from the Coulombic interactions between the ions and glycine molecules. The growth units of the α -form may be disrupted due to the presence of NaCl, while the formation of the head-to-tail chains could benefit from its existing.

The solution-mediated transformation from α -form to γ -form was successfully monitored and analyzed using in situ Raman spectroscopy, ATR-FTIR and real-time

microscope. The effects of additives and process parameters including temperature, seed properties, stirring rate and ultrasound on the transformation were investigated. The combination of the measurements of solid-phase and liquid-phase properties using various techniques provides an effective tool to reveal the influence of various operating factors on the polymorphic transformation process. This methodology will be valuable in studying the crystallization and transformation mechanism of polymorphic systems and developing a robust crystallization process to obtain the desired polymorphic crystal products.

Part of the results in this Chapter have been reported on Journal of Crystal Growth, 2008, 310, 604-611 and Journal of Raman Spectroscopy, 2008, 39, 1433-1439.

Chapter 5 Metastable zone, nucleation kinetics of glycine and effects of seeding and cooling profile in cooling crystallization

5.1 Introduction

In pharmaceutical and fine chemical industry for separation and purification, crystallization is commonly preceded by cooling the solution from a higher initial temperature at which it is saturated or undersaturated. In cooling crystallization, changes of operating conditions, such as temperature, cooling rate, mixing and seeding, can result in a significant effect in product properties, such as crystal size distribution (CSD), crystal purity, desired morphology and polymorphism. These product properties may have a great impact on downstream unit operations such as filtration, washing, drying and formulation. To meet the desired product specifications, it is desirable to understand the nucleation and growth kinetics of the batch crystallization process. Furthermore, a detailed knowledge of the characteristics of the crystalline materials is required.

Metastable zone width (MSZW) is an extremely important parameter in the design and optimization of crystallization processes as it allows an insight into the nucleation behavior of systems (Mullin, 2001; Teja and Rousseau, 2004). The metastable zone is a region where the solution is supersaturated but primary nucleation does not occur. Figure 5.1 displays the schematic diagram of solubility curve and metastable zone. The difference in concentration or the difference in temperature between a saturated solution and a solution on supersolubility curve is called metastable zone width (MSZW) or maximal allowed temperature of supercooling. The solubility curve and metastable zone width provide graphical information for understanding both the thermodynamic and kinetic aspects of the crystallization processes. In cooling

crystallization, it is often preferred to operate within the metastable zone and close to the solubility curve. Therefore, the quantitative determination of MSZW under various operating conditions are important to achieve the required quality for the final product. The measurement of metastable zone width is mainly carried out by polythermal method. For the polythermal method (Nyvlt et al., 1970; Nyvlt et al., 1985; Mullin and Jancic, 1979; Mullin, 2001), a saturated solution is cooled from the overheating temperature until first crystals are detected visually or instrumentally. The MSZW is thus determined from the difference between the saturation and crystal detection temperature. The metastable zone width is affected considerably by temperature, solution concentration, cooling rate, presence of impurity, mechanical effects (i.e. stirring rate), etc (Young and Van Sicklen, 1913; Nyvlt et al., 1985).

Figure 5.1 Solubility and metastability curves.

Supersaturation is the driving force for both nucleation and crystal growth. The level of supersaturation significantly affects the rate of these two competing processes which in turn affects the product properties. Therefore, in order to obtain product

crystals with particular properties, it is necessary to control the supersaturation as desired. Proper cooling and seeding are known to be the two most important factors to keep the supersaturation at desired level throughout the crystallization process. Much attention has been paid to the optimization of these two aspects to improve product properties (Jones, 1974; Jones and Mullin, 1974; Jones and Chianese, 1987; Chung et al., 1999; Bohlin and Rasmuson, 1992). There are four cooling modes commonly employed in crystallization: natural cooling, linear cooling, optimal cooling and controlled cooling. In natural cooling, the solution is cooled by circulating constant temperature coolant. In linear cooling, the cooling rate is kept constant during the whole process. The optimal cooling was proposed by Mullin and Nyvlt (1971). This cooling mode exhibits a convex profile, in which the temperature decreases slowly in the early stage and then rapidly at the late stage of the batch. The controlled cooling is a cooling mode employed to keep the supersaturation at low level during the whole crystallization process. In this cooling mode, the cooling profile is adjusted at different operating conditions so as to optimize the product properties.

The addition of crystal seeds is another important method to lower the supersaturation and to control the product particle size distribution. Results of previous research show that seeding is a powerful technique in crystallization to produce crystals with a desired CSD (Jagadesh et al., 1999; Doki et al., 2001; Funakoshi et al., 2001). These work mainly targeted to the optimization of seed size and seed loading. According to previous studies reported by the research group of Kubota (Doki et al., 2001; Kutoba et al., 2001), the desired unimodal distribution of final products could be achieved when seed loading exceeded a critical value.

The purpose of this chapter is to measure the MSZW and determine nucleation kinetics parameters associated with batch cooling crystallization of glycine. The role of

cooling rate on the MSZW was demonstrated. Because only α -form glycine was produced by cooling crystallization from pure water, this chapter focuses on crystallization kinetics of glycine regardless of polymorph. Effects of cooling profiles and seed loadings on the crystal size distribution of final products were investigated. In situ FTIR was employed to monitor the solute concentration and supersaturation. The results would provide us a better understanding of the cooling crystallization of glycine.

5.2 Experimental

The α -form glycine was used in the present work. The seed crystals were prepared by sieving the ground α -form glycine. The particles in the size range of 50–150 μm were taken as seed crystals.

Cooling crystallizations was carried out in a Mettler Toledo LabMax Automatic Laboratory Reactor system. Experiments were performed in a 1 L jacketed glass crystallizer, which was equipped with an overhead mixer and a thermostat. All experiments were stirred at 350 rpm and temperature control was achieved by manipulating the heating/chiller. The MSZW was measured by using a Lasentec S400A FBRM. Solution concentration was determined via in situ ATR-FTIR. The spectra and quantitative calibration of FTIR measurements have been described in detail in Chapter 4. After crystallization, samples of the product suspension were withdrawn and filtered, and then the samples were dispersed in pure isopropanol for crystal size analysis using a Malvern Mastersizer 2000 laser diffraction at ambient temperature.

5.3 Results and discussion

5.3.1 Metastable Zone Width (MSZW) of glycine in water

Figure 5.2 presents the solubility curve and MSZW for different cooling rates measured by FBRM. Figure 5.3 shows the measured MSZW as a function of the initial equilibrium temperature. The present results show that the MSZW of glycine in aqueous solution increases with an increase of cooling rate for all the experimental conditions explored. The faster the cooling rate, the wider the MSZW becomes. This could be explained by the classic nucleation theory. As a faster cooling rate in principle leads to a higher supersaturation generation rate, a longer relaxation time is required to establish a quasi-steady-state distribution of molecular clusters (Ni and Liao, 2008). Hence, the faster cooling rate results in wider MSZW than the slower cooling rate. Furthermore, the results also show that the width of the MSZ increases with a decrease in the initial concentration of glycine. That is, increasing equilibrium temperature shortens the MSZW.

Figure 5.2 Experimental metastable zone width of α -form glycine in aqueous solutions under different cooling rates.

Figure 5.3 Metastable zone width for different initial equilibrium temperatures.

5.3.2 Nucleation order and nucleation rate constant

Nyvt et al. (Nyvt, 1968; Nyvt et al., 1970) mathematically investigated a kinetic model for nucleation rate from the relationship between MSZ and cooling rate. In this model, the primary nucleation rate J is expressed as

$$J = b \left(\frac{dc^*}{dT} \right) \quad (5.1)$$

where b is the cooling rate ($\Delta T/\Delta t$).

This model has been widely accepted and used to describe the kinetics behavior of a solution under constant cooling rate and constant solution concentration. According to the classical nucleation theory (Mullin, 2001), the nucleation rate can be written as a function of maximum supersaturation ($\Delta c_{\max}$)

$$J = k_n \Delta c_{\max}^n \quad (5.2)$$

$$\Delta c_{\max} = c - c^* \quad (5.3)$$

where k_n is the nucleation rate constant, $\Delta c_{\max}$ is the maximum supersaturation, c is the solution concentration, c^* is the equilibrium saturation concentration at a given temperature and n is the apparent nucleation order.

For the solution cooled at a constant rate, the relationship between the maximum supersaturation $\Delta c_{\max}$ and maximum supercooling $\Delta T_{\max}$, or metastable zone width (MSZW) is as follows

$$\frac{\Delta c_{\max}}{\Delta T_{\max}} = \left(\frac{dc^*}{dT} \right) \quad (5.4)$$

where $\frac{dc^*}{dT}$ is the slope of solubility curve.

Combining Equations (5.1)–(5.4), the relationship between the MSZW and the cooling rate b can be obtained

$$\log b = n \log \Delta T_{\max} + (n-1) \log \frac{dc^*}{dT} + \log k_n \tag{5.5}$$

Therefore, plotting the logarithm of b against the logarithm of $\Delta T_{\max}$ should result in a straight line with slope equal to the nucleation order, n . The nucleation rate constant k_n can be evaluated from the intercept.

Figure 5.4 Plots of the logarithm of cooling rate (b) versus the logarithm of $\Delta T_{\max}$ for glycine in water at various equilibrium temperatures.

The extracted MSZW data for different cooling rates and initial equilibrium temperatures are plotted in Figure 5.4. All data are well fitted by straight lines. The values of nucleation order and nucleation rate constant were calculated from the slopes and intercepts of these straight lines. The results are listed in Table 5.1 with the

root-mean square deviations. The nucleation order and nucleation rate constant appear to be dependent on the initial equilibrium temperature. Apparently, the nucleation order decreased slightly with the initial equilibrium temperature, which indicates that higher initial solution concentration makes the nucleation easier. On the other hand, the value of nucleation rate constant increased significantly with an increase in the initial equilibrium temperature, which means the solution with higher concentration crystallizes at higher nucleation rate.

Table 5.1 Nucleation kinetics parameters calculated from Equation 5.5.

T* (°C)	<i>n</i>	<i>k_n</i>	rmsd
14	8.648	1.849 × 10 ⁻¹¹	0.1548
25	6.534	6.982 × 10 ⁻⁷	0.09846
37	5.022	1.763 × 10 ⁻⁴	0.04215
50	3.589	0.0198	0.03879

From the metastable zone width data, we are able to obtain some insight into the nucleation mechanisms. There are different mechanisms for the formation of crystal nuclei. Homogeneous primary nucleation occurs when there are not any solid particle in the supersaturated solution. In the heterogeneous primary nucleation, the crystallization takes place in the presence of foreign particles. Secondary nucleation requires the presence of seeds or existing crystals (surface nucleation) and attrition (secondary nucleation induced by attrition) to initiate crystal formation. Mersmann et al. (Mersmann, 1998; Kim, 2001) proposed a method to determine the nucleation mechanism by measuring the metastable zone width and solubility at a constant cooling rate. This method provides a simple criterion for different nucleation

mechanisms through a diagram giving the relationship between dimensionless metastable supersaturation $\Delta C_{\max}/C_c$ and dimensionless solubility C^*/C_c . C_c is molar-based density of crystal. As shown in Figure 5.5, glycine-water system by cooling crystallization is dominated by primary heterogeneous nucleation.

Figure 5.5 Mersmann's nucleation criterion.

5.3.3 Cooling profile and seeding effects in batch cooling crystallization

Two different cooling profiles (linear and optimal cooling) were employed in this work, starting from 50 °C and decreasing to the final temperature of 25 °C in 2.5 h. The temperature in linear and optimal cooling is governed by following equation derived by Nyvlt (Nyvlt, 1973)

$$\frac{T(t)-T_i}{T_f-T_i} = \left(\frac{t}{t_f}\right)^n \tag{5.6}$$

where T_i and T_f are initial and final temperatures, and t_f is batch time. The linear

cooling profile is determined at parameter $n = 1$, while $n = 3$ leads to the optimal cooling mode.

The applied linear and optimal cooling profiles are given in Figure 5.6. The linear cooling profile shows a decrease in crystallization temperature at a constant cooling rate of 10 °C/h. In the optimal cooling profile, the temperature decreases very slowly at first and then the decreasing trend accelerates towards the end of the process.

Figure 5.6 Linear and optimal cooling profiles.

5.3.3.1 Cooling effect on the unseeded crystallization of glycine

The real-time variation of solute concentration during the unseeded crystallization with the two different cooling modes is shown in Figure 5.7. It can be seen that the solute concentration is consumed rapidly in linear cooling than that in optimal cooling. Normally, a faster decrease in solute concentration results in a quick increase of supersaturation. As a driving force for crystallization, the degree of supersaturation governs both nucleation and crystal growth. Generally, a high level of supersaturation

promotes nucleation, while low supersaturation favors crystal growth. As a result, the supersaturation of the solution has a significant effect on product characteristics such as crystal size distribution. Figure 5.8 displays the supersaturation profiles produced under the two cooling modes. In linear cooling, there is a rapid increase of supersaturation in the initial stage of the crystallization until nucleation occurs. For optimal cooling, the supersaturation increases gradually at the beginning and then drops to close to zero after nucleation.

Figure 5.7 Solute concentration profiles of two different cooling modes for the unseeded crystallization.

Figure 5.8 Supersaturation profiles of two different cooling modes for the unseeded crystallization.

Figure 5.9 presents the CSD of product crystals obtained for the two cooling modes. Both products exhibit unimodal distribution. As expected, the mean size of the crystals produced under the optimal cooling are larger than that obtained under the linear cooling. For linear cooling, the supersaturation increases rapidly with temperature in the early stage, resulting in high generation rate of nuclei. In this case, most of the supersaturation is consumed by the nucleation of new particles. This leads to a decrease of driving force for growth of the existing crystals. Comparing to the linear cooling, the initial supersaturation decreases slowly when the optimal cooling is applied. This implies that unwanted excessive nucleation is suppressed and the supersaturation is consumed through crystal growth.

Figure 5.9 Crystal size distribution of products obtained by unseeded crystallization from different cooling modes.

5.3.3.2 Seeding effect on the crystallization of glycine

Seeding has been demonstrated to be a critical factor in batch crystallization due to its important role in controlling the final particle size distribution. To study the effect of seed mass on the supersaturation and CSD of final product, the α -form glycine crystals with size range of 50–150 μm were used as initial seeds. Figure 5.10a and b and Figure 5.11a and b show the solute concentration and supersaturation profiles with different seed loadings under the linear and optimal cooling modes. The seed loading is ratio of the seed amount to the final mass of product. It can be found that with the increase of the seed loading, the supersaturation was significantly reduced for the two cooling modes. Figure 5.12 and Figure 5.13 compare the CSD of products obtained from the crystallization carried out under the two cooling modes with different seed loadings. For the two cooling modes, increasing seed loading results in a narrower

product CSD and a decrease in the mean particle size. This is probably because the low supersaturation does not provide sufficient driving force for crystals to grow.

Figure 5.10 Effect of seed loading on the solute concentration (a) and supersaturation (b) for the linear cooling mode.

Figure 5.11 Effect of seed loading on the solute concentration (a) and supersaturation (b) for the optimal cooling mode.

Figure 5.12 Effect of seed loading on the crystal size distribution for the linear cooling mode.

Figure 5.13 Effect of seed loading on the crystal size distribution for the optimal cooling mode.

5.4 Summary

The crystallization kinetics of glycine in aqueous solutions was investigated. Metastable zone widths for glycine in water were measured by using FBRM. The mathematical relationship between the MSZW ($\Delta T_{\max}$) for the solutions at various saturated temperatures and cooling rates (b) was analyzed using Nyvlt's approach. The nucleation order (n) and nucleation rate constant (k_n) were obtained from the linear regression of $\log(\Delta T_{\max})$ and $\log(b)$. These nucleation parameters are useful for the practical design and control of crystallization process. Crystallization experiments with varying cooling profiles and seed loadings were performed to investigate their effects on the crystal size distribution of final products. The solute concentration and supersaturation during the crystallization were monitored using in situ FTIR. For the crystallization of glycine from aqueous solutions, cooling mode and seed loading have great effects on the supersaturation and CSD of the final products. Comparing to linear cooling, the optimal cooling mode produces larger mean crystal size. Increasing seed loading decreases the supersaturation and results in narrower CSD and smaller mean crystal size.

Chapter 6 In situ monitoring polymorphic crystallization of *p*-aminobenzoic acid

6.1 Introduction

From thermodynamic consideration, only one polymorph is stable at a specific temperature and pressure, and a metastable polymorph will theoretically transform to a more stable one. Polymorphic transformation from a less stable form to a more stable form is predicted to be spontaneous and inevitable, and the transformation process may last for seconds, days or even millennia depending on the environmental conditions (temperature, pressure, solvent, etc.). However, it has been noted that transformation may not necessarily occur despite a polymorphic system within an environment which theoretically allow it happen because transformation between polymorphs involve both thermodynamic and kinetic control. Typically in solution-mediated transformation, seeding of stable form crystals is always necessary to induce the transformation.

Polymorphic transformation proceeds generally via solution-mediated transformation and solid-state transition. Solution-mediated transformation occurs in the presence of a solvent via dissolution of a less stable form and nucleation of a more stable form. The thermodynamic driving force for the polymorphic transformation is the difference of free energy, specifically the solubility difference between the two polymorphs (Mukuta et al., 2005; Jiang et al., 2008). Solid-state transition is believed to result from the molecular reorientation in the crystal lattice of metastable form (Singh et al., 1998; Petit and Coquerel, 1996; Kim et al., 2005). The solid-state transition takes place without passing through liquid or vapor media and its occurrence is much influenced by kinetic parameters.

The stability of biomolecules and pharmaceutical products is a critical issue in the

chemical and pharmaceutical industry. As for a compound having more than one polymorph, the transformation between different polymorphs can have significant effects on its physical and chemical properties, and eventually modify the pharmaceutical properties, such as solubility, dissolution and bioavailability. Therefore, monitoring and controlling polymorphic transformation of a pharmaceutical compound under various operating conditions are of increasing importance in chemical and pharmaceutical industries.

The conventional analytical techniques frequently used for characterizing transformation processes include PXRD (Sheridan and Anwar, 1996), DSC (Otsuka et al., 1993) and FTIR (Rustichelli et al., 2000; Yang et al., 2008a). These off-line techniques, however, usually require sample isolation and invasive preparation before the measurements, which may introduce error into the measured kinetic parameters. The development of in situ analytical techniques applied to provide real time information about transformation processes is crucial in understanding transformation mechanism as well as for process modeling, control and design (Mougin et al., 2002; Yu et al., 2004; Kim and Kim, 2008). Recently, in situ Raman spectroscopy has been proved to be a powerful process measurement technique for real-time monitoring polymorphic crystallization (Ono et al., 2004; Schöll et al., 2006; Yang et al., 2008b; O'Brien et al., 2004; Qu et al., 2006; Hu et al., 2005; O'Sullivan et al., 2003; Aaltonen et al., 2006). These studies show that Raman spectroscopy is a simple, rapid and reliable real-time technique for probing the polymorphic transformation.

In the present study, transformation between two polymorphs of a model compound, *p*-aminobenzoic acid (PABA) in aqueous solutions and in solid state as well as cooling crystallization of the two forms from water were investigated. The chemical structure of PABA is given in Figure 6.1. The crystal structure of the two

polymorphs of PABA (α -form and β -form) has been reported in literatures (Gracin and Rasmuson, 2004). The two forms exhibit different packing arrangements and show different behavior in solvents of varying polarity and hydrogen bonding capability.

Figure 6.1 Chemical structure of *p*-aminobenzoic acid.

6.2 Experimental

6.2.1 Materials

The α -form PABA (99%) was purchased from Sigma-Aldrich and used as received. The β -form was prepared by solution-mediated transformation of the α -form in aqueous solution. Various analytical techniques include PXRD, microscopy and Raman were used for the characterization and identification of the two polymorphs. The ultra pure deionized water was obtained through a Millipore ultrapure water system (Milli-Q Gradient A10 System).

6.2.2 Solution-mediated transformation

Solution-mediated transformation of PABA between the two polymorphs were carried out in a 50 mL jacketed crystallizer equipped with magnetic stirring bar (stirring rate of 350 rpm). The jacket temperature was controlled by a Julabo programmable circulator FP50-ME. The saturated solutions with respect to α -form or

β -form were prepared at the required temperature. When the concentration and temperature of the solution were steady, a certain amount of the corresponding crystals was added into the prepared solution. The composition changes of the two polymorphs during the transformation processes were monitored using in situ Raman (785 nm RamanRXN1 analyzer from KOSI). The calibration and measurement methods are similar to the approach established in Chapter 4.

6.2.3 Solid-state transition

The solid-state polymorphic transition of PABA from β -form to α -form was performed at various temperatures under ambient environment. The experiments were carried out on a Linkam hot and cold microscope stage (Linkam THMS 600, UK) accommodating a micro quartz cell (15 mm diameter x 2 mm depth). Approximately 80 mg of powder sample was compressed to form a 13 mm diameter disk in an evacuated die at 3.5 tons for 1 min. The disk was placed in the quartz cell mounted on the hot stage which had been preheated to the desired temperature. During the transition, Raman spectra of the sample were in situ collected via an immersion fiber-optic probe (305 mm length x 12.7 mm diameter) attached to the 785 nm RamanRXN1 analyzer from KOSI. The Raman spectra were collected at an interval of 0.1–5 min. The accumulation was 2 scans, and the exposure time was 10 sec. The obtained Raman spectra were processed using the KOSI's HoloGRAMS software package. In current work, "semi-quantitative analysis" was performed to determine the polymorphic transformation trend. In all transition experiments, pure β -form was used as initial phase and only these two polymorphs existed during the whole process. Raman spectra in a selected characteristic region were analyzed and calculated by HoloGRAMS software package to obtain the transformation trend.

6.3 Results and discussion

6.3.1 Solid state characterization

6.3.1.1 Powder X-ray Diffraction (PXRD)

The PXRD patterns of the two polymorphs of PABA over the range of $2\theta = 5$ to 40° are shown in Figure 6.2. The characteristic peak of the α -form is observed at 15.36° and the peak at 27.88° is characteristic of β -form.

Figure 6.2 Powder X-ray diffraction patterns of PABA polymorphs: (a) α -form and (b) β -form.

6.3.1.2 Polarized light microscopy

Figure 6.3 Polarized light microscopy images of the crystals of form α and form β .

The crystal morphology of the two PABA polymorphs were investigated using optical microscopy. As shown in Figure 6.3, the α -form appears as needle-shape crystals while form β displays a prismatic morphology.

6.3.1.3 Raman spectroscopy

Figure 6.4a shows the Raman spectra of the forms α and β in the range of $200\text{--}3100\text{ cm}^{-1}$. Some distinct differences can be discerned between the two forms. One of the major differences is the strong band at 1695 cm^{-1} corresponding to the C=O stretching mode of carboxylic acid groups for the β -form. The absence of C=O stretching mode of α -form in this wavenumber region may be interpreted in terms of the changes in hydrogen bonding. As it was previously reported (Gracin and Rasmuson, 2004), the α -form and β -form have different molecular packing arrangements in the crystal lattice. The crystal structure of α -form consists of hydrogen-bonded dimers by the association of the carboxylic acid groups. It is possible that the C=O bond is weakened as hydrogen-bonded dimer is formed. While in the crystal structure of β -form, there are no carboxylic acid dimers but four-membered rings of alternating

amino groups and carboxylic acid groups.

Figure 6.4 Raman spectra of solid-state α -form and β -form of PABA: (a) 200–3100 cm^{-1} ; (b) highlighted region of 1150–1350 cm^{-1} .

The comparative spectra differences of the two forms are highlighted in the region of 1150–1350 cm^{-1} , as shown in Figure 6.4b. In the α -form there are peaks at 1181, 1286, 1313, and 1434 cm^{-1} , while the peaks at 1167, 1253, 1274, and 1309 cm^{-1} are characteristic for the β -form. Therein, the bands associated with C–H in-plane bending at 1181 and 1167 cm^{-1} for α -form and β -form, respectively (inset of Figure 6.4b), were used to interpret events during the in situ monitoring of solid state polymorphic transition. Table 6.1 summarizes the main characteristic bands with corresponding assignments by reference to published literatures (Samsonowicz et al., 2005; Świsłocka et al., 2006; Sundaraganesan et al., 2007) for the α -form and β -form.

Table 6.1 Selected peak positions in the Raman spectra and assignments for the α -form and β -form of PABA.

Approximate assignments	Frequency (cm^{-1})	
	α -form	β -form
$\gamma(\text{C}-\text{C}-\text{C})$	505vw	512vw
$\gamma(\text{C}=\text{O})$	616vw	615vw
$\beta(\text{C}-\text{C}-\text{C})$	640w	639w
$\gamma(\text{C}-\text{H})$	846m	845m
$\beta(\text{C}-\text{H})$	1181m	1167m
$\nu(\text{C}-\text{OH})$	1286m	1274w
$\nu(\text{C}-\text{C})$	1313w	1309w
$\nu(\text{C}-\text{C})$	1434w	
$\nu(\text{C}-\text{C})$	1602vs	1602vs
$\nu(\text{C}=\text{O})$		1695s
$\nu(\text{C}-\text{H})$	3067w	3075w

ν , stretching; β , in-plane bending; γ , out-of-plane bending; vs, very strong; s, strong; m, medium; w, weak; vw, very weak.

6.3.1.4 Thermal analysis

The thermal analysis of α -form and β -form of PABA were conducted using TGA and DSC. In order to remove the effect of particle sizes on the thermograms (Gracin and Rasmuson, 2004), our experimental samples were prepared by grinding the α -form and β -form crystals and separating them using a L3P sonic sifter with 75 μ m diameter sieves. The sample was collected from sieve aperture between 20 μ m and 25 μ m. Figure 6.5 shows TGA–DSC thermograms for the α -form and β -form at a heating rate of 10 $^{\circ}$ C/min. For the α -form, there is no significant weight loss before 180 $^{\circ}$ C and a steady decrease in weight is observed up to 290 $^{\circ}$ C. The DSC trace exhibits a sharp melting endothermic peak with an onset of 190 $^{\circ}$ C, which eliminate the possibility of phase transition from α -form to other polymorphs. Next, another partially resolved endothermic peak is observed at about 245 $^{\circ}$ C due to the decomposition of the compound.

The DSC trace of form β is quite similar to that observed for the α -form except that there is a small endothermic peak at 95 $^{\circ}$ C. Two major endothermic peaks around 189 and 243 $^{\circ}$ C are observed attributed to melting and decomposition of the compound, respectively. No significant weight loss can be observed for the β -form until the melting point was reached. It has been reported that the β -form undergoes an endothermic solid-solid transition to the α -form below the melting point (Gracin and Rasmuson, 2004). In order to confirm that the DSC peak at 95 $^{\circ}$ C is due to the transition of form β to form α , the β -form crystals have been heated up to 100 $^{\circ}$ C at 10 $^{\circ}$ C/min and then cooled back to room temperature at 20 $^{\circ}$ C/min. The PXRD pattern of the sample after the heating-cooling circle was found to present the same feature of form α . The results of thermal analysis for forms α and β of PABA state that the two forms are related enantiotropically.

Figure 6.5 Combined TGA and DSC traces of PABA: (a) α -form and (b) β -form.

To conclude, these significant differences in XRD patterns, thermal behavior and Raman spectra make it very easy for the two PABA polymorphs to be identified and quantified.

6.3.2 Solubility

The solubility data of each form of PABA in water were measured in the range of 0 to 45 °C. The temperature dependence of solubility is described by the van't Hoff relationship (logarithmic solubility versus reciprocal of absolute temperature). As shown in Figure 6.6, the solubility lines of the two forms intersect at approximately 14.9 °C, which is assumed to be the transition point between the two polymorphs. Below this transition temperature, form β is the stable form (lower solubility), whereas form α is the stable one above the transition point.

Figure 6.6 Van't Hoff plots of logarithm of solubility for α -form (—) and β -form (---) as a function of the reciprocal of absolute temperature.

However, the solubility data and transition point shown for α -form and β -form obtained in the current study are different from the results reported by Gracin and Rasmuson (Gracin and Rasmuson, 2004). They estimated the transition temperature to be between 23 and 26 °C from solubility data. The considerable discrepancy reflects a

general problem associated with the solution-mediated transformation of metastable form to stable form during the solubility measurement experiments. A relatively fast transformation may lead to solubility values of metastable form unreliable, which could yield an inaccurate transition point estimate.

6.3.3 Solution-mediated transformation

Even though the solution-mediated transformation of metastable form would result in somewhat incorrect solubility data of the metastable form, it gives a chance to estimate the transition point from the kinetics of transformation as a function of temperature. Since the two polymorphs of PABA are related enantiotropically, the stability order is reversible with varying temperature. That is, the polymorphic transformation of PABA in aqueous slurry is reversed at temperatures above and below the transition temperature. By monitoring the transformation behavior at different temperatures, the transition point can be closely approximated via progressively narrowing the temperature range.

In a typical solution-mediated transformation experiment, a suspension of pure form α in water at temperature of 3 °C was stirred continuously. In situ Raman was used to monitor the changes of polymorph contents during the transformation. Figure 6.7 shows Raman waterfall plot in the range of 1150–1200 cm^{-1} over the course of transformation. The characteristic peaks of α -form at 1181 cm^{-1} gradually decrease, while those due to the β -form at 1167 cm^{-1} increase in intensity. The transformation trends based on the normalized peak areas are computed automatically by the HoloReact software. The solid fraction of the two forms were scaled from 0 to 100% to fulfill the mass balance. The transformation profile as a function of time for the selected spectral region is illustrated in Figure 6.8. As can be seen, the transformation

curve displays a typical sigmoidal shape. The induction time, which is the time required for the formation of β -form nuclei, was about 7 h while complete transformation required circa 25 h.

Figure 6.7 Real-time waterfall plot for the transformation of α -form at 3 °C in the range of 1150–1200 cm^{-1} .

Figure 6.8 Transformation behavior of the α -form PABA in water at 3 °C.

The transformation profiles from α -form to β -form measured at 3, 6, 9, and 14 °C are presented in Figure 6.9. Obviously, the transformation rate is highly dependent upon the operating temperature. The polymorphic transformation from a less stable form to a more stable form is predicted to be spontaneous. The solubility difference between the stable and metastable polymorphs is expected to be the major driving force for the solution-mediated transformation. As shown in Figure 6.6, the transition temperature of α -form and β -form determined from solubility experiments is about 14.9 °C. In these transformation experiments carried out at temperatures below the transition point, the lower the transformation temperature, the larger the solubility difference between α -form and β -form, resulting in the faster transformation rate from α -form to β -form. As a result, the induction time decreases and the transformation rate increases with reducing the operational temperature.

Figure 6.9 Transformation profiles of the α -form PABA in water at different temperatures: (x) 3 °C; (■) 6 °C; (o) 9 °C; (▲) 14 °C.

For the transformation experiments carried out at temperatures close to the transition point, i.e., 14 °C, a considerably long induction time was observed. This comparatively slow transformation kinetics could be attributed to the small solubility difference between the two polymorphs, where thermal energy is insufficient to overcome the required kinetic barrier for the nucleation and growth of stable form β . Therefore, small percentage of form β seeds was used to induce the transformation of α -form to β -form at temperature near to the transition point.

In order to kinetically investigate the transformation of α -form to β -form, the transformation rate was quantified from the experimental data in the range of 10–90 wt.% fraction of transformation. A Johnson-Mehl-Avrami-Erofeev-Kolmogorov (JMAEK) model (Weinberg et al., 1997; Woldt, 1992; Al-Zoubi et al., 2002) was used to describe the transformation kinetics. The JMAEK equation can be written as

$$[-\ln(1-\alpha)]^{1/n} = k_T t \quad (6.1)$$

where α represents the transformed fraction at time t , k_T is rate constant and n is a unitless exponent which is associated with the nucleation mechanism and the geometry of growth, i.e., $n = 2$ for two-dimensional growth, $n = 3$ for three-dimensional growth.

The experimental data measured at different temperatures were fitted to the JMAEK equations with various exponent values of n . The best fit (for which $R^2 > 0.99$ at each temperature) was obtained by using $n = 3$. Plots of $(-\ln(1-\alpha))^{1/3}$ vs time are shown in Figure 6.10. The transformation rate constant (k) is therefore determined from the slope.

Figure 6.10 Dependence of the function $f(\alpha)$ on time for the transformation of α -form at different temperatures: (×) 3 °C; (■) 6 °C; (○) 9 °C; (▲) 14 °C.

Transformation experiments were also performed at four temperatures above the transition point (17, 24, 30 and 35 °C) by using the β -form as initial material. Similar data analysis and curve-fitting method as described above were carried out. The transformation rate constants as a function of transformation temperature are plotted in Figure 6.11. These rate constants clearly depend on the transformation temperatures. It can be noted that the changes in temperature not only affect the transformation rate but also orient the transformation direction between the two polymorphs. With changing the temperature from below the transition point to above the transition point, the transformation direction is reversed. At temperatures above the transition point, increasing transformation temperature causes a greater solubility difference and a larger driving force for the transformation. Therefore, it can be observed that above the transition point the transformation rate from β -form to α -form sharply increased with an increase in temperature.

Figure 6.11 Transformation rate constants as a function of temperature. Positive rate constants represent the transformation from α -form to β -form, while negative rate constants represent the transformation from β -form to α -form.

Arrhenius plots were constructed for the transformation rate constants. Figure 6.12 shows the Arrhenius plot depicting variation of natural logarithm of the absolute value of the rate constants with corresponding reciprocal temperatures. Two lines can be linearly regressed from the experimental data and give a point of intersection at around 14.8 °C. It is known that at the transition point, α -form and β -form have equal solubility, Gibbs free energy and stability and therefore there is no driving force for transformation between the two forms. It seems plausible to hypothesize that zero overall transformation rate at the transition point is attributed to equal rates of the forward and reverse transformations. Thus, the intersection shown in Figure 6.12 can be accepted as the transition point between the two polymorphs. This transition point is in reasonable agreement with our previous determination by solubility measurement.

Since polymorphic transformations can only occur from the metastable form to the stable form, the solution-mediated transformation is especially useful for accurate

determination of relative stability and transition point for an enantiotropic system. As has been discussed above, the dissolution of the metastable form and the nucleation and subsequent growth of the stable form is driven by supersaturation with respect to the stable form, which is generated from the solubility difference between the two polymorphs. As the temperature is changed away from the transition point, the large solubility difference can contribute to a high supersaturation level and result in a fast transformation. With either increasing or decreasing temperature close to the transition point, the decreased solubility difference produces a low degree of supersaturation and consequently results in a slow transformation. Especially, at temperatures very close to the transition point, the driving force resulted from the solubility difference may be insufficient to overcome the free energy barrier for the nucleation of the stable form. This explains why the induction time for our experiment carried out at 14 °C is beyond the time frame in the absence of stable β -form seeds. And even when seeded with β -form crystals, the transformation rate of α -form into β -form at 14 °C is still rather slow.

Figure 6.12 Arrhenius plots for the transformation between α -form and β -form.

6.3.4 Solid-state transition

As revealed by DSC, the β -form of PABA undergoes a solid-solid transition to the α -form on heating before melting. To get a better understanding of the solid-state transition process between two polymorphs of PABA, the transition was monitored using in situ hot-stage microscopy. The sequence in Figure 6.13 shows the isothermal transition of the β -form to α -form at 120 °C. The prismatic β -form crystals were placed in the center of the preheated hot stage. With continuous heating, the particles gradually show rough surface morphology and needle-shape α -form began to appear. Finally, the α -form and β -form crystals condensed and sublimed.

Figure 6.13 Real-time microscope sequence of the solid-state transition of PABA at 120 °C.

In order to get more information about the solid state transition, kinetic study was performed under isothermal conditions using the experimental setup as described in Experimental section. The β -form crystals were present on the hot stage and held isothermally at different temperatures. The Raman spectra of the sample were collected in situ during phase transition experiments. A typical Raman waterfall plots during the transition in the region of 1150–1350 cm^{-1} are illustrated in Figure 6.14. It shows the time course of the transition from β -form to α -form at 96 °C. As the transition proceeds, the characteristic peaks of the β -form are decreasing in intensity while those of the α -form appear and increase in intensity.

Figure 6.14 Real-time waterfall plots for the solid-state transition from β -form to α -form at 96 °C in the region of 1150–1350 cm^{-1} .

Figure 6.15a presents the Raman spectra changes between selected region between 1150 and 1190 cm^{-1} . The appearance, disappearance and changes in intensity of the Raman characteristic peaks for each form can be clearly observed. The spectra changes in the region of 1155 and 1190 cm^{-1} were used to monitor the transition progress. The normalized spectra changes during the transition process were computed by the HoloReact software, which used the baseline-integrated areas to do calculations. The pure Raman spectra were normalized to a particular band of an internal standard. Figure 6.15b shows the transition trends of the two forms presented in arbitrary units. The grey and black curves represent the time profiles of the relative changes in the 2nd derivative peak areas of α -form and β -form, respectively. As revealed by Raman waterfall plot, only two species (α -form and β -form) exist in the course of the transition, and the initial and final materials are pure β -form and pure α -form. A semi-quantitative analysis could now be employed to convert the original Raman response to mass fraction transformed. The original data in Figure 6.15b were then

rescaled between 0 and 100% to reflect the corresponding mass fraction of the two forms.

Figure 6.15 The solid-state transition from β -form to α -form at 96 °C: (a) selected spectra and (b) corresponding transition trends presented in arbitrary units.

Figure 6.16 presents the rescaled transition profiles from isothermal experiments at six different temperatures: 78, 82, 86, 90, 96 and 100 °C. It can be seen all the transition curves exhibit a sigmoidal shape: (1) an initial period of induction, defined as the time required to detect a considerable increase in conversion, (2) an acceleratory period of rapid increase in conversion which is corresponding to the accelerated three-dimensional growth of crystals, and (3) a decay period indicating the completion of the crystallization process. The time required to complete the transition decreases with increasing isothermal temperature, being of circa 115, 56, 30, 18, 11 and 5 min at 78, 82, 86, 90, 96 and 100 °C, respectively. In order to kinetically determine the transition of β -form to α -form, various solid state reaction kinetic models (Avrami, 1939; Prout and Tompkins, 1944; Garner, 1955; Zhou et al., 2003; Urakami and Beezer, 2003; Amado et al., 2007) have been employed to derive the transition rate (Table 6.2). By fitting the experiment data in the range of 5–95 wt.% fraction transformed to the equations $f(\alpha)$, a linear relation is predicted between $f(\alpha)$ and transition time t , and the corresponding slope gives the transition rate constant (k) at different temperatures.

A correlation coefficient (R^2) analysis was performed for all the kinetic models and tabulated in Table 6.3. The results demonstrate that several models fairly fitted the data well, particularly for Avrami–Erofeev models (M1, M2 and M3) and Prout–Tompkins model (M8). The development of the Avrami-Erofeev relationship is based on the assumption that the solid phase transformation proceeds by a nucleation-and-growth mechanism (Zhu and Grant, 2001). The Prout–Tompkins model is based on branching nuclei with possible interference occurring between these nuclei (Gundusharma and Secco, 1990). This kinetic model has been commonly adapted to nucleation-rate-limited solid-state transformations. These analyses imply that the nucleation and growth of α -form within α -form and β -form phase boundaries may play

an important role in PABA polymorphic transition process.

Figure 6.16 Contents of the α -form against time at different temperatures: (◇) 78 °C; (▲) 82 °C; (○) 86 °C; (●) 90 °C; (×) 96 °C; (■) 100 °C.

Table 6.2 Kinetic equations and mechanisms of solid-state reactions.

Model	Equation, $f(\alpha) = kt$	Mechanism
M1	$[-\ln(1-\alpha)]^{1/2}$	Avrami-Erofeev, $n = 2$
M2	$[-\ln(1-\alpha)]^{1/3}$	Avrami-Erofeev, $n = 3$
M3	$[-\ln(1-\alpha)]^{1/4}$	Avrami-Erofeev, $n = 4$
M4	α^2	One-dimensional diffusion
M5	$(1-\alpha)\ln(1-\alpha)+\alpha$	Two-dimensional diffusion
M6	$[1-(1-\alpha)^{1/3}]^2$	Three-dimensional diffusion (Jander)
M7	$1-2\alpha/3-(1-\alpha)^{2/3}$	Three-dimensional diffusion (Ginstling-Brounshtein)
M8	$\ln[\alpha/(1-\alpha)]$	Random nucleation (Prout-Tompkins)
M9	$-\ln(1-\alpha)$	First-order reaction
M10	$1/(1-\alpha)-1$	Second-order reaction
M11	α	One-dimensional phase boundary reaction
M12	$1-(1-\alpha)^{1/2}$	Two-dimensional phase boundary reaction
M13	$1-(1-\alpha)^{1/3}$	Three-dimensional phase boundary reaction

α : Mass fraction of α -form, $\alpha = 0.05-0.95$.

Table 6.3 Correlation coefficients (R^2) for different kinetic models.

Model	Correlation coefficient, R^2					
	78 °C	82 °C	86 °C	90 °C	96 °C	100 °C
M1	0.968	0.978	0.994	0.989	0.996	0.999
M2	0.989	0.993	0.996	0.989	0.989	0.994
M3	0.994	0.997	0.9921	0.985	0.9812	0.987
M4	0.918	0.927	0.974	0.952	0.978	0.983
M5	0.871	0.883	0.935	0.940	0.970	0.959
M6	0.777	0.792	0.841	0.897	0.926	0.895
M7	0.843	0.856	0.908	0.930	0.959	0.945
M8	0.992	0.995	0.989	0.994	0.988	0.989
M9	0.874	0.891	0.923	0.951	0.970	0.960
M10	0.581	0.596	0.619	0.750	0.763	0.734
M11	0.979	0.982	0.992	0.962	0.965	0.984
M12	0.954	0.962	0.992	0.976	0.991	0.997
M13	0.934	0.945	0.978	0.974	0.990	0.991

The suitability of these models to describe the kinetic data was also evaluated by the Arrhenius equation

$$k = A \exp\left(-\frac{E_a}{RT}\right) \quad (6.2)$$

where E_a is activation energy, R is Boltzman constant, and T is absolute temperature. The k values determined from these kinetic models were fit to Arrhenius plots of the natural logarithm of rate constants ($\ln k$) against corresponding reciprocal temperatures, as shown in Figure 6.17. The transition rate constants clearly increase with the rise of temperature. The activation energy E_a can be determined from the slope of the fitted Arrhenius plot. The activation energy is related to the hindrance involved in the transition of form β to form α . Table 6.4 shows the activation energy and R^2 produced with models M1, M2, M3 and M8. All of these models provide similar correlation coefficient, R^2 , around 0.99. The corresponding standard deviation of slope, S_b , is also reported. The average activation energy over the four models is 136.08 kJ/mol.

In addition, it was found that the solid transition behavior was significantly affected by powder packing. In our experiments, the powder samples in compressed form generally show excellent repeatable results due to good thermal contact among solid particles. While for the powder sample with loose packing, heat transfer through the sample would be a key factor controlling the transition rate. To reduce heat transfer effects, the same amount of sample was compressed to disk with similar diameter and thickness before Raman analysis. It was found that no polymorphic transition of β -form crystals was observed during the compression.

Figure 6.17 Arrhenius plots for the β - α transition of PABA using rate constants determined from the four kinetic models: ($\diamond$) M1 model; ($\blacktriangle$) M2 model; ($\circ$) M3 model; ($\times$) M8 model.

Table 6.4 Calculated activation energies (E_a) for the transition using the four kinetic models.

Model	Activation energy (kJ/mol)	R^2
M1	137.7	0.991
M2	135.9	0.991
M3	134.7	0.990
M8	134.9	0.991

6.3.5 Cooling crystallization

In the selective crystallization of polymorphs, it is important to control supersaturation to suppress nucleation of undesired polymorph (Lin et al., 2007). Especially for an enantiotropic system, design and adaptation of an operating curve leading specifically to the desired polymorph are strong challenges. The main

objective of this work is to study the effects of cooling modes on the supersaturation and hence the product polymorph. The solubility of the two polymorphs of PABA and the metastable zone limit of form α are presented in Figure 6.18. At the cooling rate of 5 °C/h, the average MSZW values of the α -form from 10 °C to 30 °C is between 3 and 5 °C.

Figure 6.18 Solubility of the α -form and β -form and metastable zone width in aqueous solutions with cooling rate of 5 °C/h.

A series of experiments as illustrated in Figure 6.19 and Table 6.5 were designed to examine the effects of initial concentration and cooling mode on the crystallization process. The linear cooling rates applied were 0.35, 0.5, 1 and 5 °C/h. The optimal cooling mode as described in Chapter 5 was also conducted.

Table 6.5 Experiments for cooling crystallization.

Exp	Initial concentration (mg/mL)	Cooling rate (°C/h)
1	3.3	5
2	3.3	1
3	3.3	0.5
4	3.3	optimal cooling
5	2.6	0.5

Figure 6.19 Various cooling profiles for cooling crystallization.

Figure 6.20 Supersaturation profiles of cooling crystallization experiments under different conditions.

Crystallization pathways under different cooling conditions are presented in Figure 6.20. Solid composition profiles quantitatively obtained from in situ Raman spectroscopy are plotted in Figure 6.21. It can be seen that at initial solution concentration of 3.3 mg/mL, all initial crystals are α -form regardless of cooling mode. However, the final products show different polymorphs under different cooling modes. In aqueous solutions, the α -form has a faster nucleation rate than the β -form and the α -form therefore crystallizes firstly upon fast cooling. This result is in agreement with Ostwald's rule of stage, in which the metastable form initially appears and subsequently transforms to a more stable form. Slow cooling and optimal cooling are in favor of keeping the supersaturation at a low level throughout the crystallization processes, which in this case provide enough time for the β -form to nucleate. At initial concentration of 2.9 mg/mL and cooling rate of 0.5 °C/h, the β -form can nucleate solely from solution.

The above results show that in the cooling crystallization of PABA from aqueous solution, starting concentration and cooling mode have remarkable effects on the nucleation of polymorph. The selective crystallization of desired polymorph can be achieved through supersaturation control. Apart from cooling mode, seeding is another effective method used for preferential crystallization of polymorph (Courvoisier et al., 2003). The core of such strategies is the supersaturation control which ensures the crystallization process is operated following a specific procedure (Kee et al., 2009).

Figure 6.21 Solid composition during the cooling crystallization under different conditions: (a) 3.3 mg/mL and cooling rate of 5 °C/h, (b) 3.3 mg/mL and cooling rate of 1 °C/h, (c) 3.3 mg/mL and cooling rate of 0.5 °C/h, (d) 3.3 mg/mL and optimal cooling, (e) 2.9 mg/mL and cooling rate of 0.5 °C/h.

6.4 Summary

This chapter focused on in situ monitoring of polymorphic transformation and cooling crystallization of PABA. Two different polymorphic forms of PABA were prepared and characterized using various typical analytical techniques. Crystals of the two polymorphs have been obtained: α -form, the commercial one and β -form, which was prepared through solution-mediated transformation of α -form from aqueous solution. The relative thermodynamic stability of the two forms is evaluated from DSC and solubility measurements. Our results confirm the enantiotropic relationship between the two forms. The β -form is the more stable one at temperatures below the enantiotropic transition temperature. The transition point was quantified by means of solution-mediated transformation experiments. The transformation between the two forms at different temperatures was monitored using Raman spectroscopy. The in situ Raman data obtained during the transformation were modeled using JMAEK equation. Following the model, the transformation rate constants were calculated and the transition point was obtained from the Arrhenius plots. The solid-state transition from β -form to α -form has been probed using in situ microscopy and Raman spectroscopy. The transition kinetics was analyzed by fitting the experimental data to several solid-state reaction kinetic models. The best-fit model is chosen according to maximum correlation coefficient analysis. Cooling crystallization was performed under different conditions to selectively produce the two polymorphs. In situ Raman and FTIR spectroscopy were used to track the solid composition and solute concentration during the crystallization processes. Results show that the preferential crystallization of kinetically unfavored β -form can be achieved by adapting starting concentration and cooling rate.

Chapter 7 Conclusions and future work

7.1 Conclusions

The present work was motivated by the critical requirement in pharmaceutical industry to prepare crystalline materials with different polymorphic forms: a specific desired polymorph must be produced with a high level of reproducibility and reliability. We focus our study on polymorphic crystallization and transformation of two chosen model compounds, glycine and *p*-aminobenzoic acid, under various operating conditions. Several in situ techniques, such as Raman spectroscopy, ATR-FTIR, real time microscopy and FBRM were applied to investigate the crystallization and transformation mechanism.

In chapter 3, two polymorphs of glycine, namely α -form and γ -form, were prepared and identified by PXRD, DSC-TGA, FTIR, SEM and microscopy. Thermal analysis results indicate the enantiotropic relationship between the two polymorphs. Solubility of the two forms in various aqueous solutions was measured by using analytical gravimetric method. In aqueous solutions containing antisolvents (methanol, PEG and isopropanol), the solubility of both forms decrease with the increase of concentration of antisolvents. With the presence of electrolyte additives (NaCl, KCl, NaNO₃ and Na₂SO₄), the solubility of γ -form increase with additive concentrations. The solubility curve of γ -form in water at different pH values shows a U-shape with the minimum solubility at the isoelectric point (pH 5.9). The solubility characteristic of γ -form glycine is assumed to be a result of the interactions between glycine molecules and electrolytes.

Chapter 4 investigates the nucleation and transformation behavior of the two polymorphs in aqueous solutions with and without additives. The polymorphic nature

of glycine crystals was found to be dependent on supersaturations and NaCl concentrations. The results show that the α -form spontaneously nucleates from pure water or solutions with low NaCl concentrations, while the γ -form crystallizes preferentially from solutions with high NaCl concentrations. With the presence of NaCl, the α -form tends to crystallize from solutions at higher supersaturation level. The nucleation behavior of the two forms can be explained by the different packing arrangements. The growth unit of α -form, which is composed of hydrogen bonded dimers, may be disrupted with the presence of electrolyte ions. The head-to-tail chains, as growth unit of γ -form, could benefit from the Coulombic interactions between glycine molecules and electrolyte ions. The solution-mediated transformation from α -form to γ -form was carried out using different additives and under various process variables. In situ techniques utilized to monitor the transformation process include Raman spectroscopy, ATR-FTIR and real time microscopy. The results indicate that the solution-mediated transformation of glycine in aqueous solutions is controlled by the nucleation and crystal growth of the γ -form. The presence of NaCl accelerates the transformation as it shortens the induction time and promotes the nucleation of the γ -form. It was concluded that higher temperature, smaller seed size, higher stirring rate and ultrasonic irradiation can lead to an increase in the transformation rate.

The purpose of chapter 5 is to study the crystallization of α -form glycine in water. The dependence of MSZW on cooling rate was investigated and modeled using the empirical equation developed by Nyvlt. The nucleation order and nucleation rate constant at various equilibrium temperatures were calculated. In this chapter, we also studied the effects of cooling modes and seed loadings on the CSD of final products. ATR-FTIR provided the real time solute concentration and supersaturation during the crystallization processes. It was observed that optimal cooling mode produced larger

mean crystal size than linear cooling, which can be due to the slow decrease in supersaturation under optimal cooling conditions. Three different seed loadings were applied to study the role of seed amount in supersaturation during the crystallization process and CSD of final products. Higher loading of seed crystals resulted in narrower CSD and smaller mean crystal size. Results obtained could be beneficial for process design and control to improve the product properties.

Chapter 6 focuses on the polymorphic transformation of PABA in solution and in solid-state. DSC analysis and solubility data show that the α -form and β -form of PABA exhibit an enantiotropic relationship. The β -form is stable below and metastable above the transition temperature. The transition point in water was determined by analyzing the transformation kinetics at various temperatures. An important issue in this method is the role that the less stable polymorph will convert to a more stable one at a given temperature. The solution-mediated transformation between α -form and β -form in water was carried out at different temperatures away from the transition point. In situ Raman spectroscopy was employed for determination of transformation direction and for quantitative measurements of transformation rate. The solid-state transition from β -form to α -form was investigated by in situ microscopy and Raman spectroscopy. The transition kinetics was examined by in situ quantitative analysis using Raman spectroscopy. Various empirical kinetic models were evaluated to describe the transformation kinetics. Selection of the best-fit model was performed by maximum correlation analysis. Cooling crystallization at different cooling modes were carried out to selectively produce the two polymorphs. In situ Raman and FTIR provided the real time solid composition and solute concentration throughout the crystallization processes. For the studied PABA polymorphic system, fast cooling is beneficial for the formation of α -form while slow cooling mode at low initial solution concentration is

preferred to produce the β -form.

7.2 Suggestions for future work

The real time monitoring of polymorphic crystallization and transformation using in situ techniques, as discussed in chapters 4, 5 and 6, has been shown to have great advantages in understanding the crystallization and transformation mechanism. Our future work in this field may combine Raman spectroscopy and ATR-FTIR with other in situ techniques, such as FBRM, near-infrared spectroscopy (NIR) and particle vision measurement (PVM), to provide more real time information about crystallization processes.

An important issue for future studies is to establish the optimal operating conditions for crystallization of desired polymorph of model compounds. Future efforts will focus on design and optimization of cooling profiles, seeding and addition of antisolvent and additive.

Another area of future interest is to understand and predict the nucleation behavior of polymorphs using molecular modeling approaches. Molecular modeling studies can hopefully explore molecular interactions between host crystals and additives and predict the crystal morphology.

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