

## **Application of Phase Equilibria for the Precipitation from Aqueous Solution**

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## **Abstract**

Supercritical CO<sub>2</sub> can be used as anti-solvent for the precipitation of pharmaceuticals from organic solution. Processing of aqueous solutions can cause considerable difficulties, because water is immiscible with CO<sub>2</sub> in contrast to the usually employed organic solvents. A water miscible organic co-solvent has to be added to successfully precipitate an active from aqueous solutions. Depending on the choice of solvent the resulting ternary phase equilibrium is affecting the precipitation behavior. Morphology, particle size and other solid-state properties can also be influenced.

For this study, the amino acid glycine was chosen as test substance and its precipitation from an aqueous solution with different organic co-solvents was investigated with the Nektar Supercritical Fluid Technology™ process. Temperature, pressure, concentration and flow rates were varied to measure the influence of these process parameters on the particles generated. Pressure was varied between 10-30 MPa and temperature ranged between 298-353 K. Further, the choice of organic co-solvent on the particle properties of the precipitated powders was studied. Additionally, glycine exhibits three polymorphs and the occurrence of the different forms with varying processing conditions in the Nektar Supercritical Fluid Technology™ process was investigated. Determination of the polymorphs was conducted by XRPD. The morphology of the particles was studied by SEM.

## **1. Introduction**

Precipitations from aqueous solution using supercritical fluids are rarely performed due to the complexities of the underlying ternary phase equilibria. In the literature, it has been shown that the orientation of the tie-lines in the ternary liquid-vapor equilibrium

can have a profound effect on the crystallization from aqueous solution. When the tie-lines exhibit a negative slope (i.e. critical point at CO<sub>2</sub> rich compositions) then precipitation will occur rapidly. Conversely, if the tie-lines have a positive slope (i.e. critical point at H<sub>2</sub>O rich compositions) precipitation will occur significantly slower. These differences in precipitation behavior should allow for possible polymorph separation with supercritical CO<sub>2</sub> using an aqueous feed.

In this study, separation of the three polymorphs of glycine was attempted, with efforts to show that particle size can be modified depending which operating conditions and solvent system used. The amino acid glycine is highly soluble in water and virtually insoluble in organic solvents. It is therefore a good model compound to carry out a systematic investigation for crystallising water soluble compound using the Nektar Supercritical Fluid Technology™ process. Glycine is known to exhibit three polymorphs  $\alpha$ ,  $\beta$  and  $\gamma$ . The unstable  $\beta$  form is monotropic to the other two and the  $\alpha$ - and  $\gamma$ -forms are enantiotropic to each other [1]. The objectives of the present work were to evaluate the effect of various process parameters on the crystallization process, polymorph separation and their influence on the physicochemical properties of the crystallized glycine.

## **2. Experimental**

### *2.1. Chemicals*

Glycine (aminoacetic acid) had a purity of 99.7% and was supplied by BDH laboratory supplies (Poole, UK). Acetone was 99.99% pure and was supplied by Fisher Chemicals (Loughborough, UK). Methanol was 99.99% pure and was supplied by Fisher Chemicals (Loughborough, UK). Acetic acid was more than 99.8% pure and was

supplied by BDH laboratory supplies (Poole, UK). Pure water for HPLC application was supplied by Fisher Chemicals (Loughborough, UK). CO<sub>2</sub> was 99.99% and supplied by BOC (Manchester, UK). All chemical were used without further purification.

## *2.2. Equipment*

Crystallization experiments and solubility measurements were carried out in a Nektar Supercritical Fluid Technology™ apparatus. The Nektar Supercritical Fluid Technology™ equipment shown in Fig. 1 is used to produce particulate materials. In this process, a solution of the material of interest is fed with a supercritical fluid, (e.g. CO<sub>2</sub>) and a third flow of organic solvent through a specially designed nozzle into the particle formation vessel under stable conditions of temperature and pressure. The supercritical fluid disperses, mixes and rapidly extracts the solvent leading to the formation of particles, which are retained in the vessel. By manipulating the operating conditions of pressure, temperature, solution concentration and flow rates in the nozzle, it is possible to control the size, shape and morphology of the products. HPLC pumps were used for solvent delivery (JASCO model PU-980). CO<sub>2</sub> was supplied from a gas cylinder and chilled to 258 K before entering the pump. A fan oven (Applied Separations, model Spe-ed SFE) and a back-pressure regulator BPR (JASCO, model 880-81) were used to control system temperature and pressure. The CO<sub>2</sub>, solution and solvent stream passed through heat exchangers before entering any crystallization or solubility vessel to ensure that all streams had reach the operating temperature. Additionally, the CO<sub>2</sub> passed through a pulse dampener to provide a smooth flow. A ball flow meter monitored the CO<sub>2</sub> effluent flow rate in standard litres per minute

(Applied Separations, USA). A more detailed description of the equipment and its operating procedure has been given elsewhere [2-5].

### *2.3. Procedures*

#### *2.3.1. Solubility measurements*

For the solubility measurements, two types of experiments have been carried out. Beaker test at ambient conditions and solubility tests under operating conditions. These experiments were performed to predict the minimum amount of solvent required to precipitate glycine in aqueous mixtures. Solubility experiments under pressure were also performed to check the influence of pressure on solubility.

##### *2.3.1.1. Solubility at ambient conditions*

An exact amount of glycine (between 0.1 and 0.5 g), initially finely ground in a mortar, was added into a beaker. The beaker was placed onto a magnetic stirrer and the solvent or solvent mixture was added in steps of 200  $\mu\text{L}$  until disappearance of the last crystal. Along with the total volume of solution added and the exact mass of glycine, the solubility was calculated (Fig. 2).

##### *2.3.1.2. Solubility under pressure*

Glycine was mixed with glass beads (220-310  $\mu\text{m}$ ) in a ratio 4:1 of beads:solute. The mixture was homogenised and packed in a 10 mL high pressure stainless steel vessel (Keystone, USA). The vessel was mounted in the oven at 313 K. Solvent flow rate was set at 0.2  $\text{mL}\cdot\text{min}^{-1}$  for 60 min. The operating pressure was set to 20 MPa. The vessel was filled with solvent under pressure and left 30 min for equilibration. The effluent

solution was collected in a beaker. The glycine in solution was precipitated by adding an excess of acetone which was partially evaporated using a rotary evaporator. The remaining mixture was then filtered off. The collected powder was left to dry overnight in an oven at 343 K. The mass of the dried glycine was recorded and the solubility calculated.

### *2.3.2. Crystallizations using the Nektar Supercritical Fluid Technology™ process*

For the crystallization experiments, 5 or 15% w/v aqueous solutions of glycine were pumped together with CO<sub>2</sub> into the crystallization vessel. Solvent flow rates varied from 0.5 to 5 mL·min<sup>-1</sup> for acetone and acetic acid and from 0.5 to 10 mL·min<sup>-1</sup> for methanol per 10 mL·min<sup>-1</sup> CO<sub>2</sub>. After all solute solution had been fed into the vessel the apparatus was flushed with pure CO<sub>2</sub> for 15 min to replace the CO<sub>2</sub>/solvent medium in the vessel with pure CO<sub>2</sub>. The experiments have been carried under the following operating conditions: Acetone: 298, 313 and 353 K and 20 MPa, methanol: 313 K and 20 MPa and acetic acid: 313 K and 15 MPa. For acetic acid, the operating pressure had to be lowered to 15 MPa to avoid solidification of the acid in the HPLC pump.

## *2.4. Analytical methods*

### *2.4.1. Scanning electron microscopy (SEM)*

Scanning electron microscopy (SEM) was performed using an FEI XL30 TMP instrument. Dry powder samples were fixed to aluminium SEM stubs using self-adhesive carbon disks, and were subsequently sputter-coated with a conducting gold layer (Emitech K550 Sputter Coater). The SEM stubs were inserted into the SEM, and the sample was viewed and photographed under a range of magnifications. This

technique was used to provide visual information regarding the morphology, size, size distribution and degree of agglomeration of the particles within a test sample.

#### 2.4.2. X-Ray Powder Diffractometer (XRPD)

Confirmation of chemical and polymorphic identity, and assessment of crystallinity, were carried out using the Bruker AXS (Karlsruhe, Germany) model D8 Advance X-Ray diffractometer. Intensity data were collected between  $10^\circ$  and  $50^\circ$   $\theta$ - $\theta$  with Cu  $K\alpha_1$  radiation ( $\lambda=1.5406 \text{ \AA}$ ). Calculations of d-spacings and intensity values were made using the integrated instrument control software EVA, and compared to literature values for sample identification [6,7].

### 3. Results

#### 3.1 Solubility in methanol/water mixtures

The solubility of glycine in methanol/water mixtures was determined at  $293^\circ\text{C}$ , 0.1 MPa. The results are shown in Fig. 2. The solubility of glycine varies from 16% w/v in pure water to an extrapolated  $<3 \cdot 10^{-4}$  % w/v in pure methanol. The measurements were performed with mixtures up to 60 mol % methanol (x: mole fraction of solvent). Data beyond this mixture were extrapolated using a second order polynomial with:

$$\text{Solubility} = (\text{x solvent})^2 \times b_2 + \text{x solvent} \times b_1 + b_0$$

#### 3.2 Solubility in acetone/water mixtures

The solubility of glycine in acetone/water mixture at  $293^\circ\text{C}$ , 0.1 MPa was also determined. From Fig. 2, the solubility of glycine in solvent/water mixtures was

extrapolated for all compositions. Glycine is less soluble in acetone than in methanol ( $3 \cdot 10^{-3}$  % w/v) with a solubility lower than  $1 \cdot 10^{-4}$  % w/v.

### *3.3 Solubility under pressure*

Additionally the solubility of glycine in acetone/water mixture was investigated under pressure at operating conditions 313 K, 20 MPa. The solubility of glycine measured at 313 K and 20 MPa is higher than at 293 K and 0.1 MPa. Solubility usually increases with temperature and decreases with pressure. With glycine it appears that both effects are almost cancelling each other at the operating conditions of 313 K and 20 MPa. Only a slight increase in solubility was observed when compared to the values measured at 293 K.

### *3.4. Crystallization experiments*

With the information gained from the solubility experiments different regions of the ternary phase equilibria were targeted to crystallize glycine using the Nektar Supercritical Fluid Technology™ process. The ternary phase diagrams available in the literature for (CO<sub>2</sub> + water) and methanol, acetone and acetic acid are given at 15, 10 and 10.4 MPa respectively (Fig. 3, 4 and 5). The literature values differ slightly from our chosen operating conditions, but it was assumed that changes to the diagrams would be negligible. Experimental conditions are summarized in Table 1. All precipitated material were analysed by XRPD and their spectra compared to literature traces (Fig. 6). As expected from earlier literature [8] results the orientation of the tie-line in the liquid-vapor equilibrium should have an effect on the precipitation. Increasing the solvent flow rate in the CO<sub>2</sub> + acetone + water system did not have a strong effect on the particle size

of the precipitate. Only minor increase in growth was detected due to the large increase in overall flow rate. Precipitation is occurring from the liquid phase, which according to the phase diagram does not significantly change when increasing the acetone amount in the system. The relatively slow precipitation led to large particles (Fig. 7), which were solely comprised of the  $\alpha$ -polymorph of glycine. Varying the operating temperature to 298 or 353 K also did not alter the polymorph of the precipitate. The  $\alpha$ -form of glycine is not the energetically most stable form of glycine and it was assumed that an excess of water in the system paired with the slow precipitation might lead to the formation of the most stable  $\gamma$ -form. Experiments with equilibrium compositions within the vapor-liquid envelope were performed, but again yielded the metastable  $\alpha$ -form. The energy difference between the metastable  $\alpha$ -form and the stable  $\gamma$ -form is very small and although precipitation in the  $\text{CO}_2 + \text{acetone} + \text{water}$  system is slow, it is still too fast to allow crystallization of the energetically most stable form.

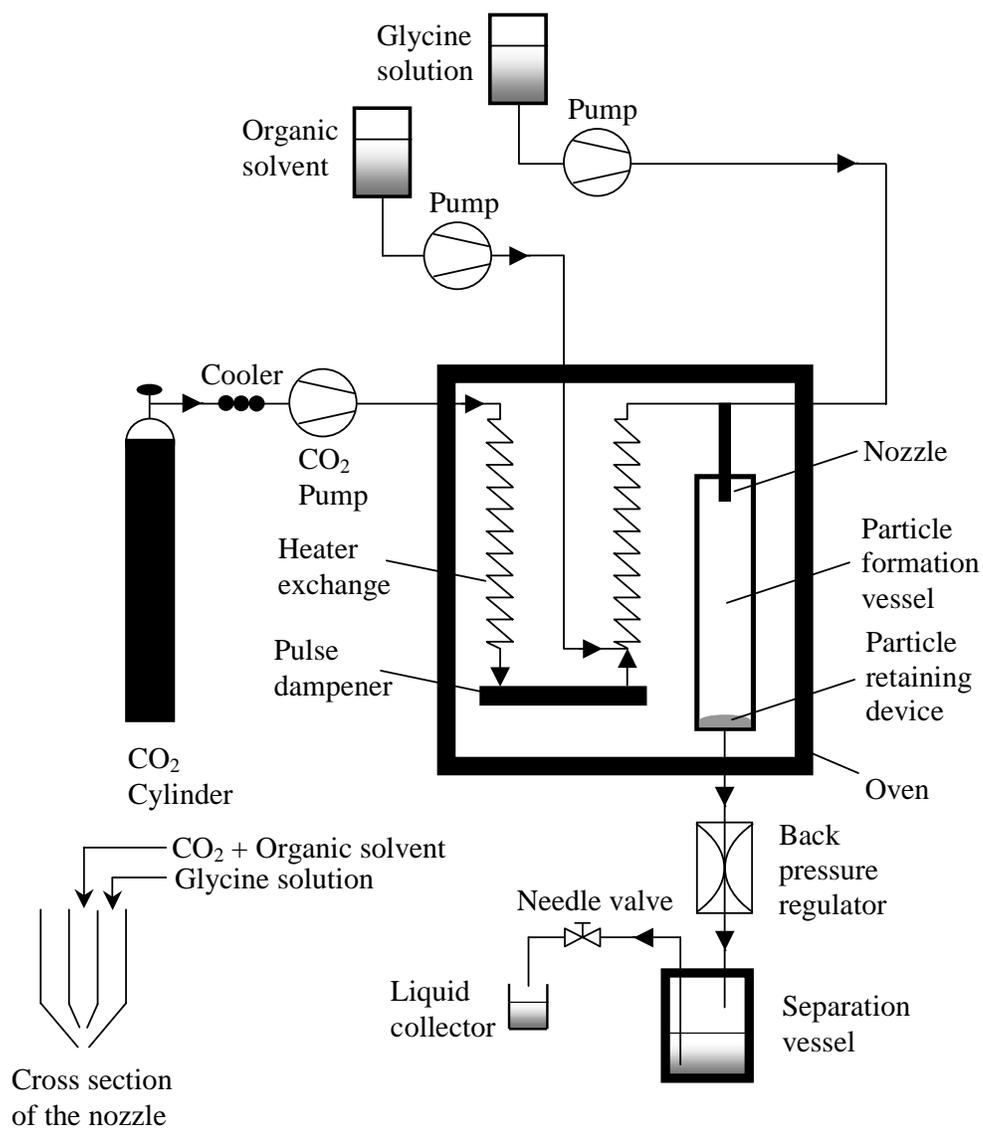
In contrast, precipitations from the  $\text{CO}_2 + \text{methanol} + \text{water}$  system should show an influence of the solvent flow rate on particle size and speed of crystallization. The equilibrium composition of the tie-lines changes drastically with slight increases in methanol flow rate and shows a decrease in particle size. Additionally, with the highest methanol flow rate a mixture of  $\alpha$ -glycine and  $\beta$ -glycine could be formed (Fig. 8).

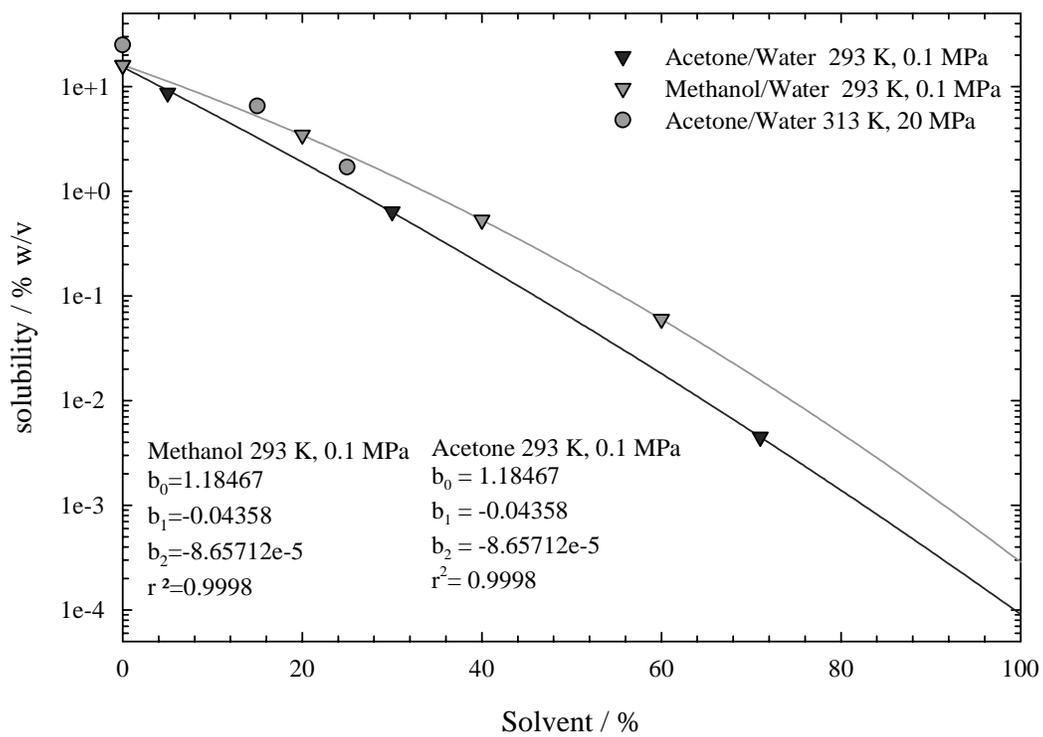
Literature results suggested [9] that the stable form might be obtained from acidified solution. Acetic acid has been used for this purpose in classical beaker experiments. Acetic acid is a powerful antisolvent for glycine and can be used in a similar way to other organic solvents. Some rudimentary phase equilibrium information for the  $\text{CO}_2 + \text{acetic acid} + \text{water}$  system [10] is available and exhibits a similar behavior to the methanol system (Fig. 5). With low flow rates of acetic acid the  $\alpha$ -form of glycine was

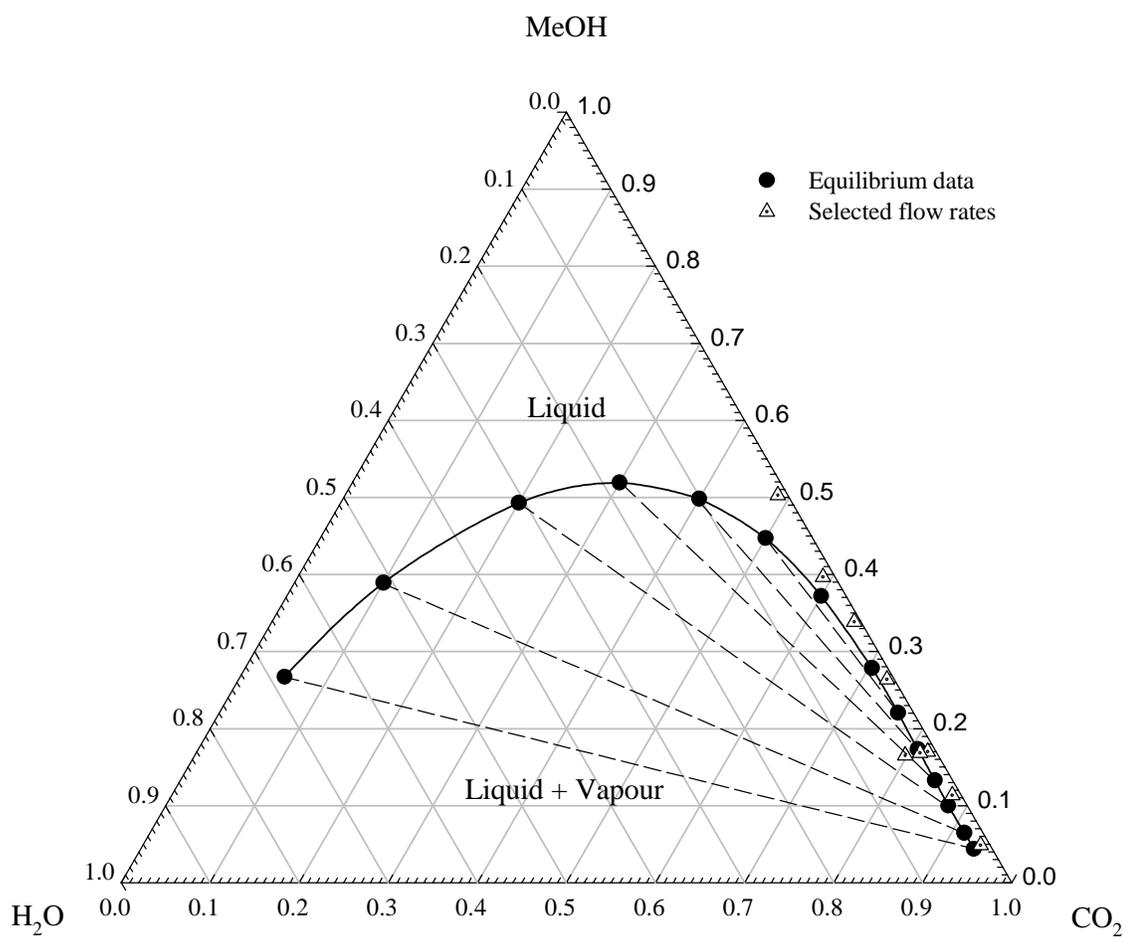
obtained and with slightly higher acid flow rates the unstable  $\beta$ -form was precipitated (Fig. 9). As with the acetone containing system, the stable  $\gamma$ -form was not observed. Despite the increased ionic strength of the solution, the rate of precipitation is still too fast to allow the formation of the stable form.

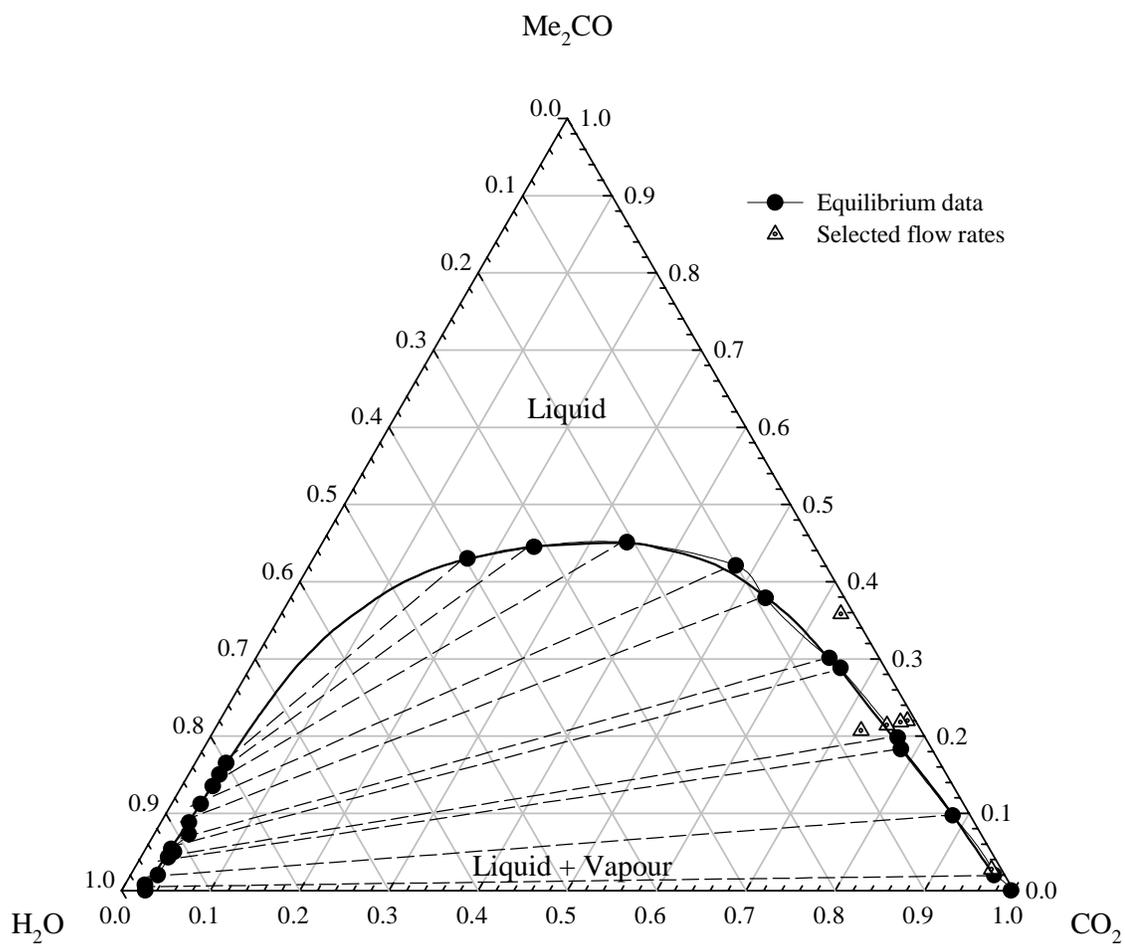
#### **4. Conclusions**

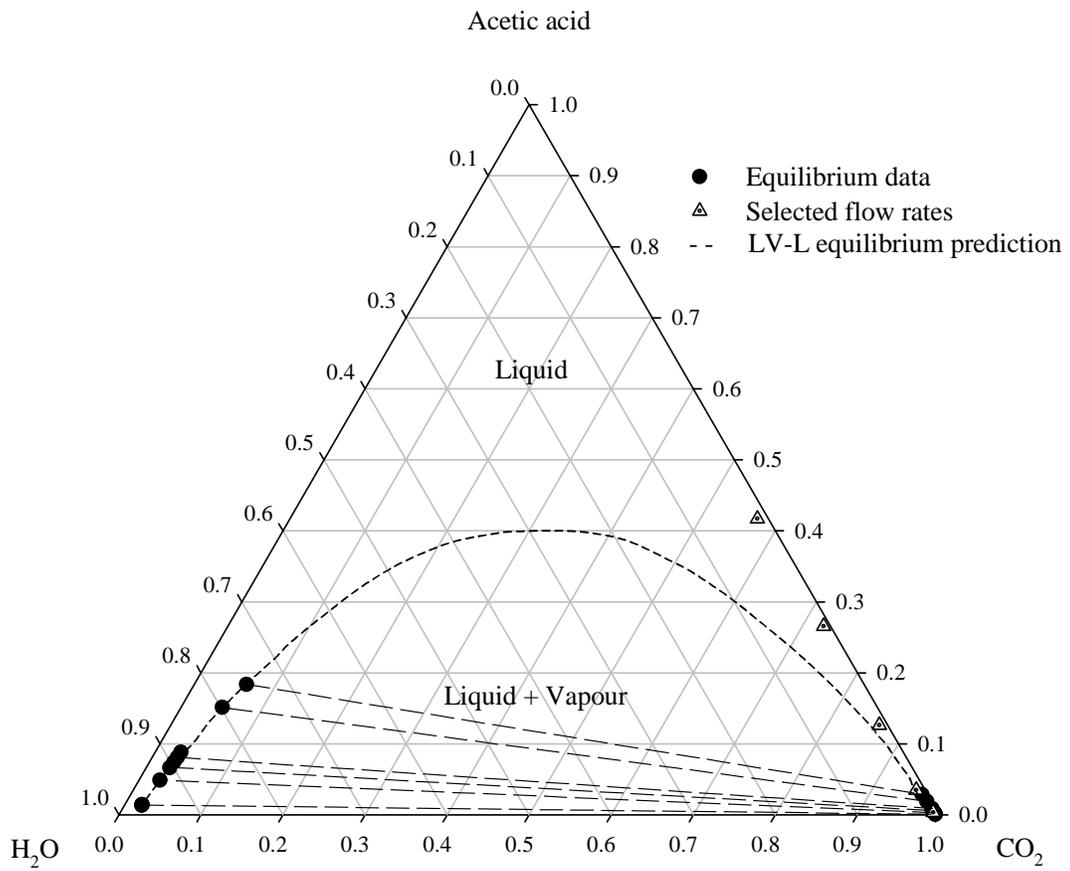
Glycine is a good model substance for a water soluble compound in aqueous environment. It was shown that the ternary phase equilibrium influences the precipitation of glycine. The polymorph formation appears to be governed by the phase equilibrium of the system (orientation of the tie-lines). It was possible to perform polymorph separation of glycine using the Nektar Supercritical Fluid Technology™ process. This was illustrated with the acetic acid + water + CO<sub>2</sub> and the methanol + water + CO<sub>2</sub> systems where the  $\alpha$ -form of glycine is formed with a low organic solvent flow rate and the  $\beta$ -polymorph at higher flow rate. Using acetone as anti-solvent, particle growth was governed by the acetone flow rate whilst larger particles were precipitated for lower acetone flow rate, no major size change was observed for the methanol + water + CO<sub>2</sub> system. For the systems studied, the stable  $\gamma$ -polymorph was not precipitated.



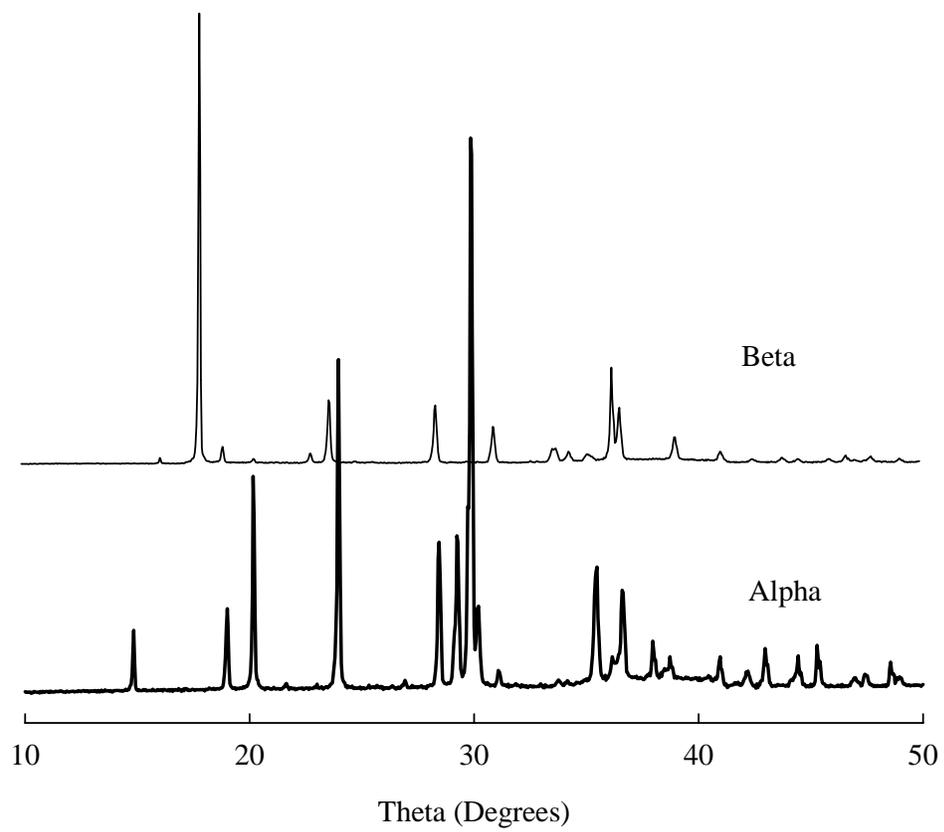


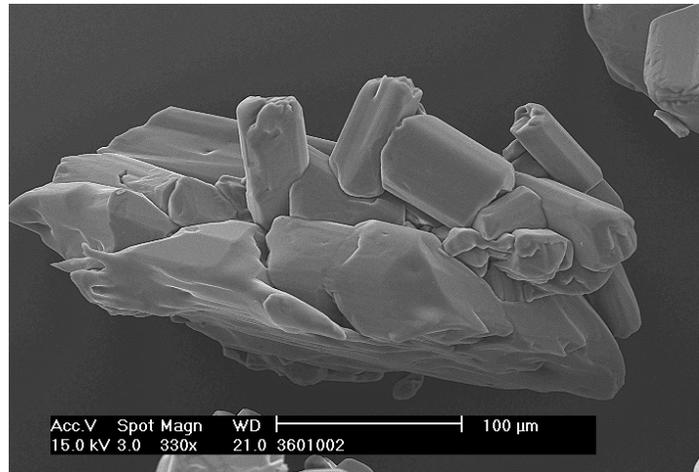




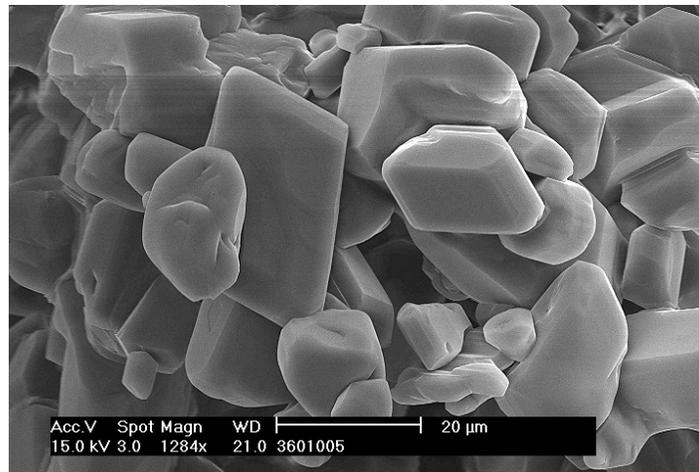


CO <sub>2</sub> flow (mL·min <sup>-1</sup> )	Glycine solution flow (mL·min <sup>-1</sup> )	Solvent flow (mL·min <sup>-1</sup> )	Glycine conc. (% w/v)	Form crystallized
Methanol 313 K, 20 MPa				
10	0.05	0.5	15	$\alpha$
10	0.05	1.25	15	$\alpha^*$
10	0.05	2	15	$\alpha$
10	0.1	2	15	$\alpha$
10	0.2	2	15	$\alpha$
10	0.05	3.5	15	$\alpha$
10	0.05	5	15	$\alpha$
5	0.05	3.25	15	$\alpha^*$
5	0.05	5	15	$\alpha+\beta^*$
Acetone 313 K, 20 MPa				
10	0.05	0.5	15	$\alpha^*$
10	0.05	5	15	$\alpha^*$
10	0.1	5	15	$\alpha$
10	0.2	5	15	$\alpha$
10	0.4	5	15	$\alpha$
5	0.05	5	15	$\alpha^*$
Acetone 298 K, 20 MPa				
5	0.05	5	15	$\alpha$
Acetone 353 K, 20 MPa				
10	0.05	5	15	$\alpha$
Acetic Acid 313 K, 15 MPa				
10	0.05	0.5	15	$\alpha$
20	0.05	0.1	15	$\alpha^*$
10	0.05	2	15	$\beta$
10	0.05	5	15	$\beta$
5	0.05	5	15	$\beta^*$

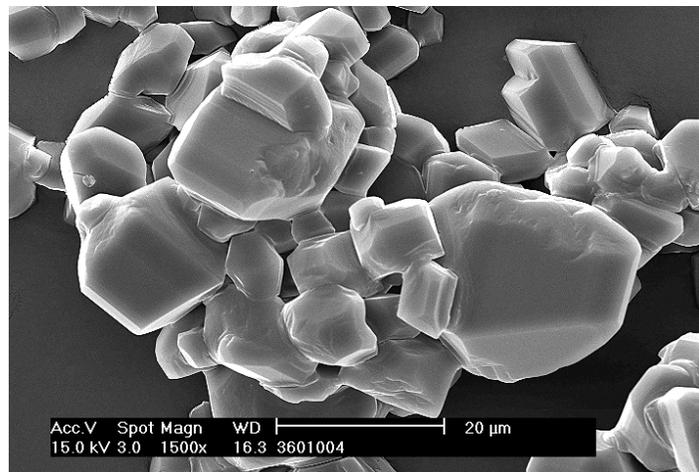




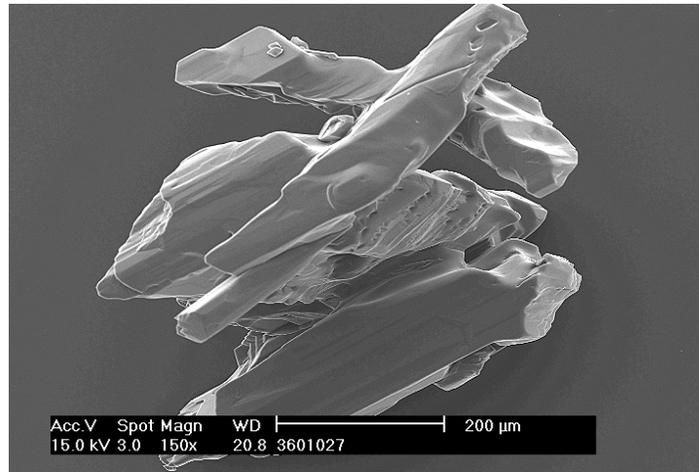
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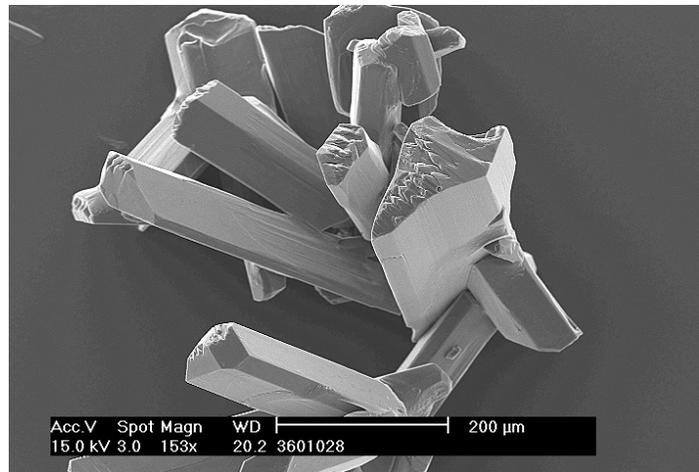
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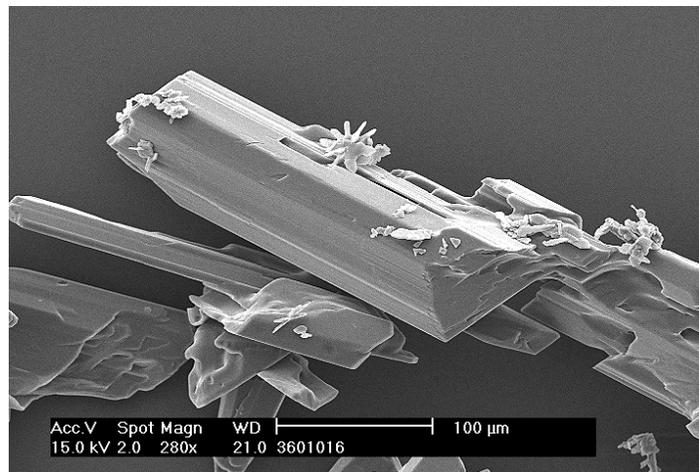
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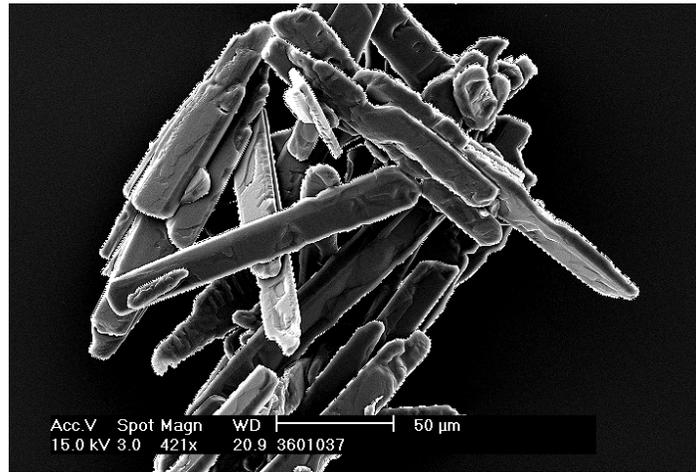
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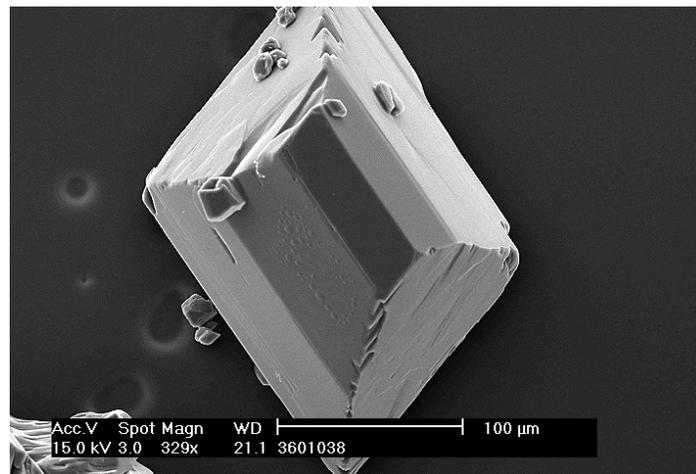
**b**



**c**



**a**



**b**

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## List of captions

Fig. 1. Schematic representation of the Nektar Supercritical Fluid Technology™ apparatus

Fig. 2. Isothermal solubility of glycine in solvent/water mixtures at 293 °C, 0.1 MPa and at 313 K, 20 MPa

Fig. 3. Ternary phase diagram of the MeOH + H<sub>2</sub>O + CO<sub>2</sub> system at 323 K, 15 MPa [11]

Fig. 4. Ternary phase diagram of the Acetone + H<sub>2</sub>O + CO<sub>2</sub> system at 313 K, 10 MPa [12]

Fig. 5. Ternary phase diagram of the acetic acid + water + CO<sub>2</sub> system at 323 K, 10.4 MPa [10]

Table 1. Experimental conditions for the crystallization of glycine using the Nektar Supercritical Fluid Technology™ process. (\*) SEM pictures available for these conditions

Fig. 6. XRPD patterns of Nektar Supercritical Fluid Technology™ processed α- and β glycine

Fig. 7. Size and morphology of glycine particles crystallized at 313 K, 20 MPa. Feed solution 15% w/v glycine in water and CO<sub>2</sub>, solution and acetone flow rates in mL·min<sup>-1</sup>: (a) 10/0.05/0.5; (b) 10/0.05/5; (c) 5/0.05/5

Fig. 8. Size and morphology of glycine particles crystallized at 313 K, 20 MPa. Feed solution 15% w/v glycine in water and CO<sub>2</sub>, solution and methanol flow rates in mL·min<sup>-1</sup>: (a) 10/0.05/1.25; (b) 5/0.05/3.25; (c) 5/0.05/5

Fig. 9. Size and morphology of glycine particles crystallized at 313 K, 15 MPa. Feed solution 15% w/v glycine in water and CO<sub>2</sub>, solution and acetic acid flow rates in mL·min<sup>-1</sup>: (a) 5/0.05/5; (b) 20/0.05/0.1