Solubility of Glycine Polymorphs and Recrystallization of β-Glycine

Andréanne Bouchard,* Gerard W. Hofland, and Geert-Jan Witkamp

Process & Energy, Delft University of Technology, Leeghwaterstraat 44, 2628 CA, Delft, The Netherlands

The solubilities of α-, β-, and γ-glycine in aqueous solutions containing methanol, ethanol, 2-propanol, or acetone were measured at 310 K. The solubility of all the polymorphs dropped rapidly as a function of the concentration of antisolvent. The solubility of the glycine polymorphs in water−antisolvent mixtures was, in decreasing order: methanol > ethanol > 2-propanol > acetone. The solubility of α-glycine was slightly higher than that of γ-glycine, but the solubility of β-glycine was significantly higher by up to 17 %. The induction time for the recrystallization of β- to α-glycine in those water−antisolvent mixtures was, in decreasing order: methanol > ethanol > acetone > 2-propanol. This signifies that the selection of an antisolvent for preparation of β-glycine can have an important effect on the product.

Introduction

Glycine is the simplest amino acid and is often used as a model compound as it has at least three polymorphs and is a zwitterion. However, solubility data available in the literature normally do not mention the polymorphic forms.[1,2] The solubility of α- and γ-glycine in water as a function of temperature is available.[3] The solubility data in aqueous mixtures of methanol,[2] ethanol,[1] and 2-propanol[1] are assumed to be of α-glycine. Measuring the solubility of β-glycine poses the problem of a fast recrystallization into the α form, even at high organic antisolvent concentration.[4]

In this work, solubility measurements were performed on α-, β-, and γ-glycine in aqueous solutions containing methanol, ethanol, 2-propanol, or acetone at 310 K. Furthermore, the induction time for recrystallization of β-glycine was investigated with these same aqueous−organic mixtures.

Materials and Methods

Materials. The following materials were used as received: L-glycine (EP, Fluka, Steinheim, Germany), ethanol, methanol, 2-propanol, and acetone (all 100 %, technical grade, Chemproha, Dordrecht, The Netherlands).

Preparation of Polymorphs. β-Glycine. β-Glycine was prepared by antisolvent precipitation, starting from a saturated solution of glycine in a water−acetic acid mixture (5:1 volume ratio). The filtered solution (up to 100 mL) was mixed with ethanol in a volume ratio of 1:1. The precipitate was recovered by filtration as soon as the solution became turbid and rinsed twice with the same volume of ethanol. The crystals were then dried under vacuum for 2 h and placed in a dedicated oven set at 65 °C until use.[5] The polymorphic composition of the powder was verified by X-ray diffraction.[6]

γ-Glycine. γ-Glycine was prepared by seeding of α-glycine slurry with γ-glycine crystals. The slurry was agitated with a magnetic stirrer for at least 3 days at room temperature, and the completion of the conversion was verified by Raman spectroscopy. The slurry was filtered, and the crystals were dried in an oven at 65 °C.

* Corresponding author. E-mail: a.m.j.l.bouchard@tudelft.nl. Phone: (+31) 15 278 5561. Fax: (+31) 15 278 6975.

γ-Glycine seeds were prepared by quench freezing glycine solution (5 % w/v) using liquid nitrogen. The 1 mL freeze-drying vials were then put on freeze-dryer shelves precooled to −45 °C and kept at that temperature for 4 h. The temperature was then increased to −5 °C for 5 h for the annealing step and finally decreased back to −45 °C at a rate of 0.1 °C·min^{-1}.[7,8] The pressure was maintained at 0.013 mbar during the entire drying cycle, and vials were stoppered under vacuum. The polymorphic composition of the powders prepared by conversion and quench freezing was verified by X-ray diffraction.[6]

Raman Spectroscopy. Raman spectra of glycine were recorded using a Hololab Series 5000 Raman Spectrometer (Kaiser Optical System, Inc.) with a near infrared excitation radiation of 785 nm. For analytical purposes, different glycine polymorphs were prepared by standard techniques and probed by X-ray powder diffraction[7] before being used as a reference in the Raman identification. The regions (300 to 400) cm^{-1} and (1425 to 1465) cm^{-1} were used for characterization of the polymorphs (Figure 1).

X-Ray Powder Diffraction (XRPD). X-ray scattering measurements were performed using a Bruker D8 Discover diffractometer possessing a two-dimensional (1024 × 1024 channels) detector. The incident Cu radiation of 1.54 Å was used, and the sample-to-detector distance was set at 6 cm. An incident beam with a cross section of 0.5 mm was used, and dried sample powders were prepared in a 0.5 mm thick holder. The background scattering (air + sample holder) was subtracted from each measurement. Data were integrated along the χ angle, giving rise to an intensity as a function of the scattering angle (2θ). For verification purposes, XRPD diffractograms of reference polymorphs were compared to the literature.

Measurement of the Solubility. The water−organic mixtures were prepared by mass. For α- and γ-glycine, the saturated solutions (slurries) were placed in a thermostated (310.0 ± 0.1 K) shaking bath (Julabo SW22) for one week before the solutions were rapidly filtered (Glass fiber prefilters AP20, Millipore). The glycine concentration was simply determined by gravimetry (Balance AE 100 Mettler Toledo, ± 0.0001 g). The estimated uncertainty on the solubility is better than 0.002 g·g^{-1} of solution. The polymorphic form of the α and γ crystals

10.1021/je700014k CCC: $37.00
Published on Web 08/24/2007

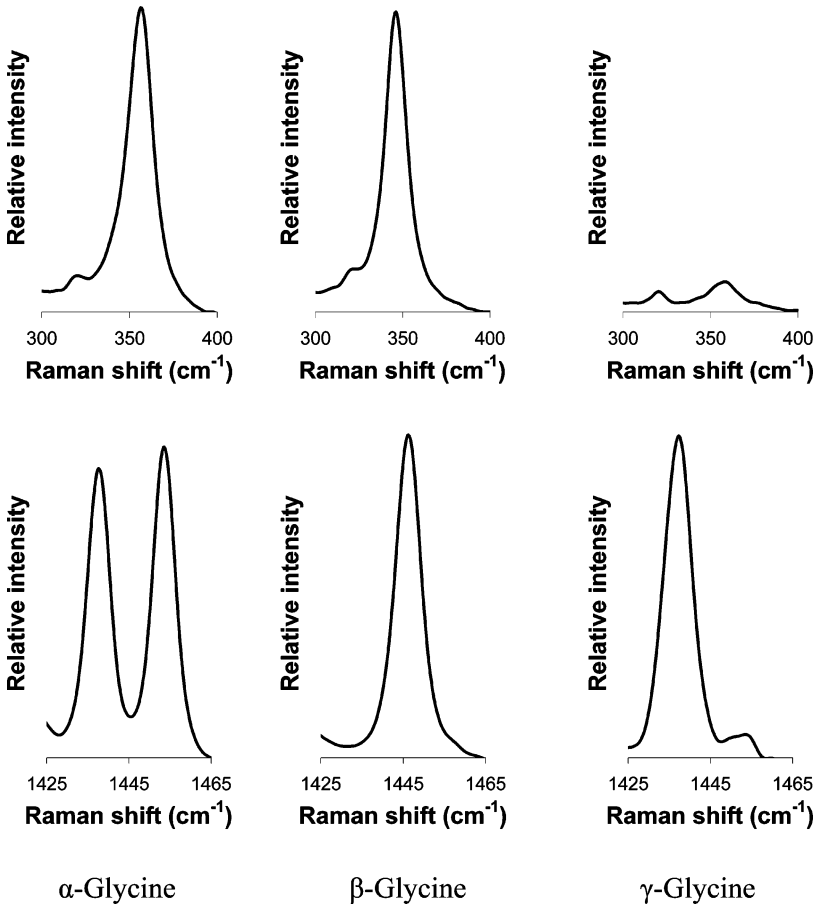

Figure 1. Section Raman spectra of α-, *β*-, and *γ*-glycine used for characterization.

Table 1. Solubility of α- and *γ*-Glycine in Aqueous Mixtures of Methanol, Ethanol, 2-Propanol, or Acetone Measured at 310 K

solvent composition (w/w)		solubility of α-glycine ($g \cdot g^{-1}$ solution)				solvent composition (w/w)		solubility of *γ*-glycine ($g \cdot g^{-1}$ solution)			
% water	% antisolvent	methanol	ethanol	2-propanol	acetone	% water	% antisolvent	methanol	ethanol	2-propanol	acetone
100	0	0.234	0.234	0.234	0.234	100	0	0.228	0.228	0.228	0.228
90	10	0.173	0.167	0.166	0.179	90	10	0.164	0.164	0.161	0.162
80	20	0.114	0.109	0.112	0.110	80	20	0.110	0.106	0.105	0.108
70	30	0.070	0.067	0.077	0.065	70	30	0.067	0.064	0.071	0.063
60	40	0.042	0.041	0.047	0.036	60	40	0.040	0.040	0.045	0.034
50	50	0.024	0.022	0.027	0.017	50	50	0.023	0.020	0.026	0.010
30	70	0.007	0.005	0.005	0.002	30	70	0.007	0.004	0.005	0.002
0	100	0.002	0.000	0.000	0.001	0	100	0.001	0.000	0.000	0.004

was checked afterward to verify that it remained unchanged through the solubility measurements.

In the case of *β*-glycine, saturated solutions were prepared in 60 mL round flasks in a thermostated bath (Lauda C6/CS, 310.0 ± 0.1 K) fitted with an impeller. Aliquots of 5 mL of slurry were collected at regular intervals and immediately filtered. The uncertainty on the solubility of *β*-glycine is estimated to be better than 0.005 $g \cdot g^{-1}$ of solution. The crystals from each sample were rinsed in a large volume of the organic solvent that was in the water−organic mixture. After filtration, the cake was dried under vacuum for at least 30 min and then put in an oven at 65 °C until analysis by Raman spectroscopy.

Results and Discussion

Solubility of* α- *and* *γ*-*Glycine. The solubilities of α- and *γ*-glycine in various aqueous mixtures of methanol, ethanol, 2-propanol, or acetone were measured at 310 K, and the values are listed in Table 1 and plotted in Figure 2.

The solubility of α- and *γ*-glycine decreased with an increase of organic fraction and an increase of carbon atoms in alcohols, meaning that the solubility of glycine in alcohols increases with increasing polarity of the solvent. We obtained results for the solubility of α-glycine similar to those available in the literature data[1] (313 K) (Figure 2A). The solubility of α- and *γ*-glycine was possibly lower in acetone than in alcohols because of differences in solvent−solute interactions and polarity. The difference in solubility between α- and *γ*-glycine was minor, which is in line with what has been previously observed by Park et al.[3] in a study of the solubility of α- and *γ*-glycine in water as a function of the temperature.

Solubility of* *β*-*Glycine. The rapid recrystallization of *β*- to α-glycine complicates the solubility measurement of *β*-glycine. The crystal form of the solids needs to be assessed simultaneously with the solubility over time. The maximum solubility measured at various molar fractions of antisolvent is plotted in Figure 3, and the values are listed in Table 2. In some instances, at high antisolvent concentration (70 % (w/w)), we observed a higher solubility when only the *β*-glycine form was detected by Raman (Figure 4A), but at lower antisolvent concentration, the higher solubility was measured for a product containing

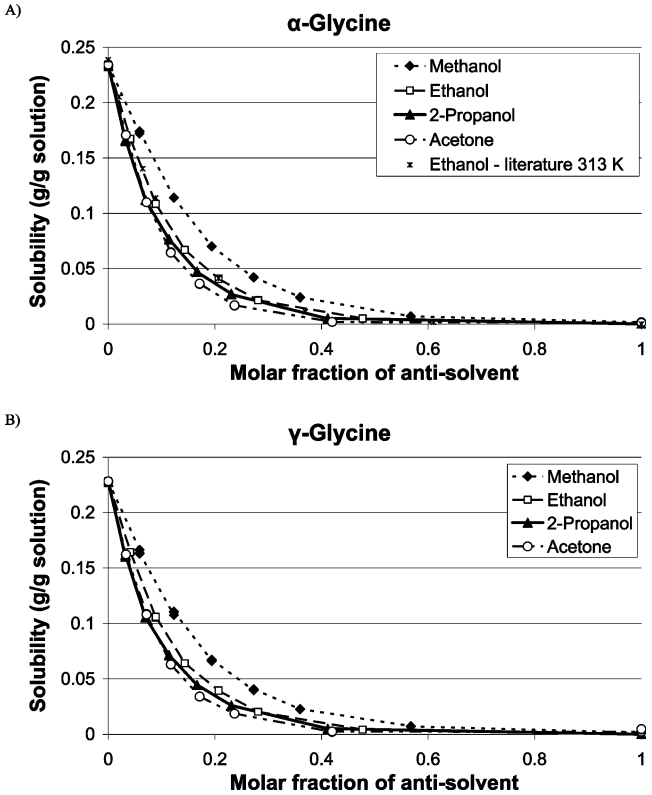

Figure 2. Solubility of (A) α-glycine and (B) γ-glycine in aqueous mixtures of methanol, ethanol, 2-propanol, or acetone measured at 310 K.

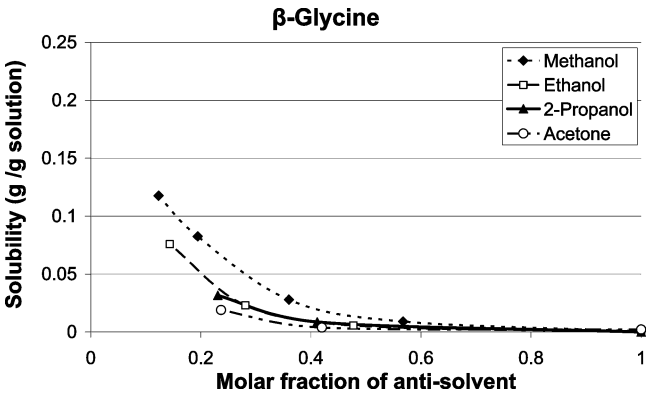

Figure 3. Maximum measured solubility of β-glycine in aqueous mixtures of methanol, ethanol, 2-propanol, or acetone at 310 K.

Table 2. Maximum Solubility of β-Glycine in Aqueous Mixtures of Methanol, Ethanol, 2-Propanol, or Acetone Measured at 310 K

% water	% antisolvent	solubility of β-glycine ($g \cdot g^{-1}$ solution)			
(w/w)	(w/w)	methanol	ethanol	2-propanol	acetone
80	20	0.119	n/a	n/a	n/a
70	30	0.083	0.076	n/a	n/a
50	50	0.028	0.023	0.019	0.032
30	70	0.009	0.006	0.004	0.009
0	100	0.001	0.000	0.002	0.000

α-glycine (Figure 4B). It should then be noted that the concentrations plotted in Figure 3 were often from α−β mixtures as it corresponded to the highest solubility measured. The real solubility of the β-glycine could then be slightly higher (estimated to ∼20 % relative) than that measured. The solubility of β-glycine in the various antisolvent mixtures follows the trend

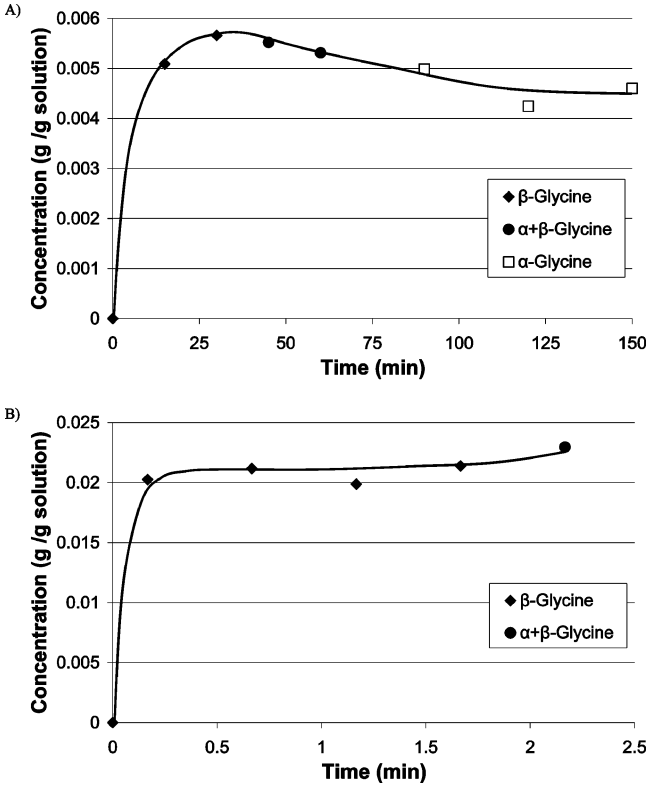

Figure 4. Solubility of β-glycine in aqueous mixtures over time for mixtures containing (A) 70 % and (B) 50 % ethanol at 310 K.

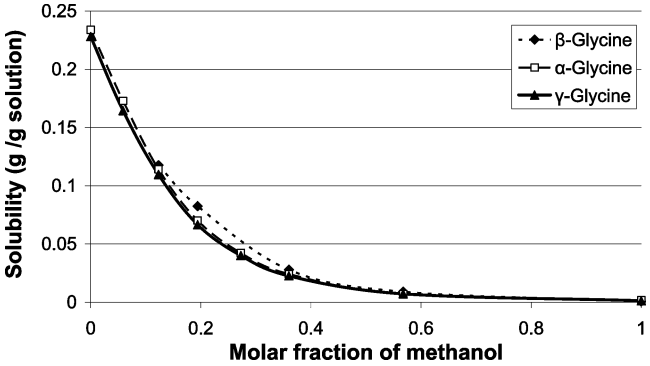

Figure 5. Solubility of α-, β-, and γ-glycine in aqueous mixtures of methanol at 310 K.

observed with α- and γ-glycine: methanol > ethanol > 2-propanol > acetone.

Figure 5 shows the difference in solubility of the three polymorphs in water−methanol mixtures. The solubility of α-glycine is only slightly higher than that of γ-glycine. However, the solubility of β-glycine could reach 17 % more than that of α-glycine. This is in line with the stability of these three polymorphs at 310 K (γ > α > β).

Recrystallization of β-Glycine. In the presence of water, β-glycine recrystallizes rapidly into α-glycine. The induction time to recrystallization was defined as the time required for the powder fraction of α-glycine in the powder to reach ∼5 %, which is slightly above the detection limit of Raman spectroscopy. The induction time is plotted (Figure 6) for the various antisolvents and molar fraction and is in decreasing order: methanol > ethanol > acetone > 2-propanol. The difference in interaction between glycine and different organic solvents (e.g., solubility) is expected to influence the recrystallization rate.

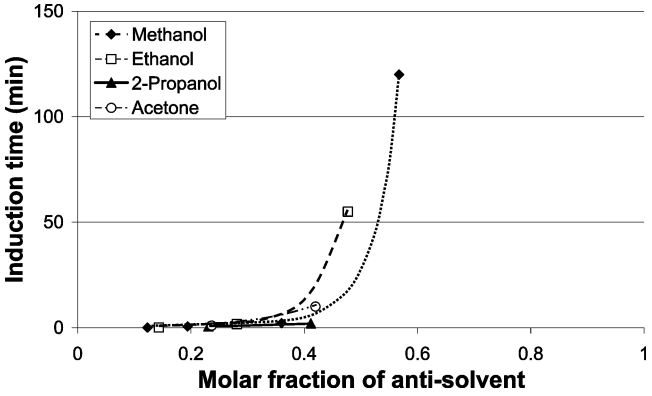

Figure 6. Induction time for recrystallization from β- to α-glycine in slurries of water and various antisolvents at 310 K.

On the basis of our results on the solubility (methanol > ethanol > 2-propanol > acetone) and on the induction time for recrystallization of β-glycine (methanol > ethanol > acetone > 2-propanol), we can suggest that the preparation of β-glycine should preferably be done with methanol > ethanol > 2-propanol $\approx$ acetone. Even though the use of methanol appears more appropriate for the precipitation of β-glycine, significant attention to the fast reduction of the residual water content is required to circumvent the recrystallization.[4,5]

Acknowledgment

The authors thank Hélène Brunel for her crucial help with the solubility measurements and Jesper Knijnenburg for the preparation of β-glycine.

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Received for review January 9, 2007. Accepted July 21, 2007. This research was supported by the Technology Foundation, STW, the Applied Science Division of NWO, and the Technology Program of the Dutch Ministry of Economic Affairs.

JE700014K