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Full Length Research Paper

Characterization and pseudopolymorphism of L-phenylalanine anhydrous and monohydrate forms

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In this work, the preformulation study on the solid state characterization for the anhydrous form and the monohydrate form of L-phenylalanine has been initially conducted by the use of powder X-ray diffraction (PXRD), Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and microscopy. Meanwhile, the solubility of both forms and the transformation kinetics from anhydrous form to monohydrate form in different water/acetone mixtures were studied as well. The transformation rate can be prohibited by increasing temperature and reducing water content in the solvent mixtures. These results will contribute to a better understanding of drugs' pseudopolymorphism for pharmaceutical industry.

Key words: Crystallization, L-phenylalanine, polymorphism, identification, transformation.

INTRODUCTION

Pseudopolymorphism refers to the crystalline forms of a compound in which solvent molecules are included as an integral part of the structure (Pedireddi and PrakashaReddy, 2003). Pseudopolymorphs, also termed solvates or hydrates, generally show different solubility, dissolution rate, mechanical behavior, stability and bioavailability from their unsolvated counterparts (Bechtloff et al., 2001).

The propensity of a compound to form pseudo-polymorphs is deemed to be relevant to molecular structures, hydrogen bonding ability and crystal packing (Khankari and Grant, 1995; Bingham et al., 2001; Infantes and Motherwell, 2002; Gillon et al., 2003). Multi-point recognition with strong and weak hydrogen bonds between solvent and solute molecules can facilitate the retention of organic solvents in crystals (Nangia and Desiraju, 1999). During a crystallization process, strong solute-solvent interactions shall result in the nucleation of solvated crystals (Rodríguez-Spong et al., 2004). In particular, the water molecule, because of its small size, activity and ability to act as both a hydrogen bond donor and acceptor, is found to be more capable of linking to drug molecules to form new crystal structures than any other solvent. To date, it is found that approximately one-third of active pharmaceutical ingredients (APIs) can form crystalline hydrates (Stahl, 1980; Giron et al., 2002).

In the pharmaceutical industry, identification of pseudopolymorphs of APIs during their early stage development is critical (Byrn et al., 1995; Starbuck et al., 2002; Dahiya and Gautam, 2011; Fan and Luo, 2011; Ding et al., 2011). In addition, the study on the solvent-mediated transformation between pseudopolymorphs is highly important as well. The transformation may happen during both primary manufacturing of the product (e.g. crystallization) and secondary manufacturing (e.g. wet granulation). The knowledge of the solvent-mediated transformation rates from anhydrate to hydrate or vice versa, and the mechanism of the phase transformation is a prerequisite for controlling the solid phase during the manufacturing of drug products (Qu et al., 2006). L-Phenylalanine, an essential amino acid for human nutrition and widely used in pharmaceutical industry (Jia et al., 2008), has been found to have two solid forms, anhydrous form and monohydrate form. In this work, a group of analytical techniques were first employed to

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characterize the two forms, and then the solvent-mediated transformation between anhydrous form and monohydrate form in pure water and acetone/water mixtures at different temperatures was studied quantitatively. Data processing and statistical analysis were conducted using Microsoft Excel. Experimental results will be useful in the preparation of pure solid forms of L-phenylalanine.

MATERIALS AND METHODS

L-Phenylalanine anhydrate and acetone were purchased from Sinopharm Chemical Reagent Company, and used without further purification. The monohydrate form of L-phenylalanine was obtained by recrystallization method described as follows: A saturated solution of anhydrate was prepared at 70°C and then quenched in a thermostat maintained at 5°C. After 24 h, crystals of the monohydrate were formed. After filtration, the monohydrate products were dried for 24 h at 30°C under vacuum, and then stored in a desiccator at room temperature. The pure deionized water was obtained with a Direct-Q Millipore system (Millipore, Billerica, MA).

Characterization

The solubility of the two forms of L-phenylalanine in water at different temperatures was measured experimentally by a gravimetric technique (Lu et al., 2010). Powder X-ray diffraction (PXRD) was conducted by a Bruker AXS Advance diffractometer (Bruker, Germany) at 40 kV and 30 mA with a Ni-filtered Cu KR radiation source (γ = 1.54 Å). The samples were scanned from 3 to 40° (2θ) at a step size of 0.05° and at a scanning rate of 5° min^{-1}.

Thermal analysis methods used in this study included differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC was performed using a Mettler-Toledo DSC-822^e differential scanning calorimeter (Mettler-Toledo, Columbus, OH). Indium was used for calibration. Accurately weighed samples (5 to 8 mg) were placed in hermetically sealed aluminum pans and scanned at 10°C min^{-1} under a nitrogen purge. TGA was conducted with a Mettler-Toledo TGA/DSC 1/1100 SF instrument (Mettler-Toledo, Columbus, OH) that was also calibrated with indium prior to analysis. The sample weight was approximately 5.5 mg and a heating rate of 10°C min^{-1} under nitrogen purge was used. The powders of both forms were heated at 10°C min^{-1} to 350°C.

The morphology of each crystalline form was observed by an optical microscope (C3230B, Shanghai Precision Instruments Company) and recorded with a camera (CX-1, Shanghai Precision Instruments Company). Fourier transform infrared spectroscopy (FTIR) spectra were recorded from KBr disks using a FTLA2000-104 spectrophotometer (ABB Bomem, Canada). Ground KBr powder was used as the background in the measurements. The number of scans was 32 and the resolution was 4 cm^{-1}. The measured wave number range was from 4000 to 400 cm^{-1}. To construct the calibration curve for quantitative analysis, we selected the characteristic peaks at 1002 and 1197 cm^{-1} for pure anhydrous and monohydrate forms, respectively. The reference peak at 1410 cm^{-1}, common to both forms, was selected as an internal standard (Skrdla et al., 2001). And the following equation was used for calibration:

$$Y = \frac{a'bc'}{ab'c + a'bc'} \quad (1)$$

where a and a' are the intensities of the reference peak at 1410 cm^{-1} of pure anhydrous and monohydrate forms, respectively, b and b' are the intensities of the peak at 1002 cm^{-1} of pure anhydrous form and the sample, respectively, and c and c' are the intensities of the peak at 1197 cm^{-1} for pure monohydrate form and the sample. Y is the calculation factor. The calibration curve was thus obtained from plotting the calculation factor Y against the concentration of monohydrate form in standard samples (Lu and Rohani, 2009a). The standard samples were prepared as the mixtures of two forms, in various mass fractions: 0.00, 21.54, 28.57, 40.0, 59.75, 78.57 and 100 wt% monohydrate form in the mixture. The mixing was done by hand for more than 10 min.

Solvent-mediated transformation

Solvent-mediated polymorphic transformation from anhydrous form to monohydrate form in various solvents was performed isothermally under ambient pressure at different temperatures (Lu and Rohani, 2009b).

RESULTS AND DISCUSSION

Solid characterization of pure forms

Figure 1 shows typical PXRD patterns for the two forms of L-phenylalanine. For example, the monohydrate form and the anhydrous form have characteristic diffraction peaks at 6.62, 10.82, 14.94, 17.66, 19.68, 21.56, 33.12 and 5.64, 16.98, 22.70, 28.50, 34.36°, respectively (Kee et al., 2009).

The IR spectra of two forms are shown in Figure 2. For instance, the anhydrous and monohydrate forms have characteristic absorption bands at 1002 and 1197 cm^{-1} (Figure 3), respectively. The thermogravimetric (TG) thermograms in Figure 4 shows the existence of a dehydration step for the monohydrate form during heating, giving a result of 8.53 wt% water in the monohydrate sample as the hydrated water, which is approximately equal to the theoretical value, 9.82 wt%.

Figure 5 shows DSC thermograms of two L-phenylalanine crystal forms. The anhydrous form shows three endothermic peaks at 262.1, 276.8 and 292.4°C which correspond to the simultaneous processes of melting and decomposition, common to most amino acids. As to the monohydrate form, in addition to melting and decomposition, there exists an endothermic dehydration (peak maximum at 88.8°C and heat of dehydration = 34.75 J/g).

Figure 6 presents the morphologies of two forms of L-phenylalanine crystals. The anhydrous form appears as rhombic platelets, while the monohydrate form exhibits needle-like.

Solubility of pure forms

Figure 7 presents experimental results over the solubility of the two forms of L-phenylalanine in water at different

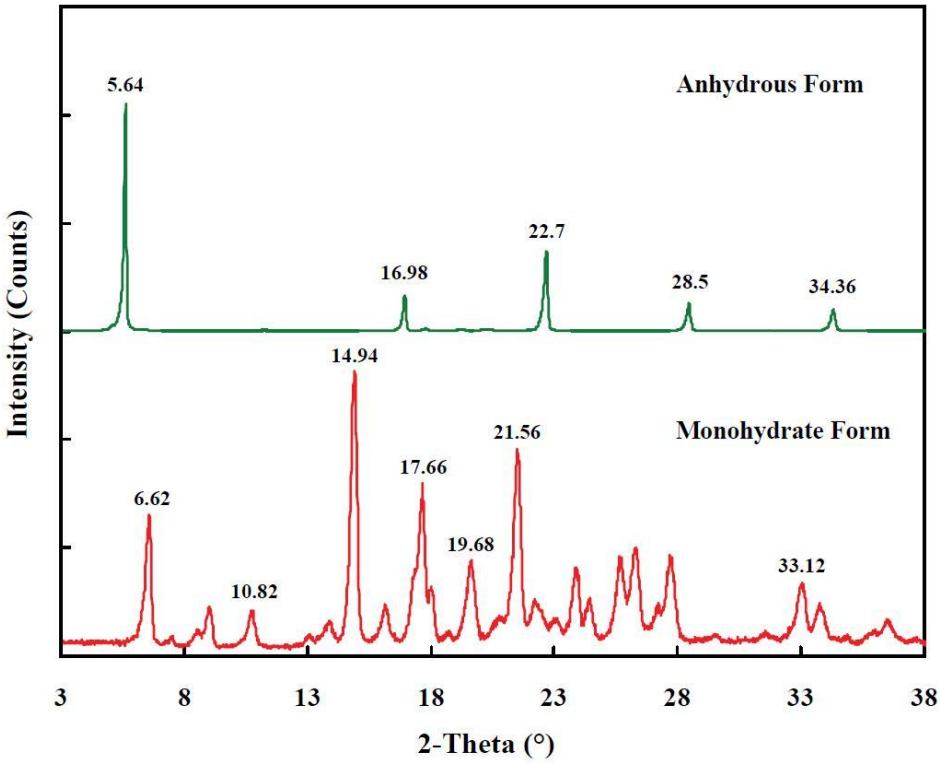

Figure 1. Powder X-ray diffraction patterns for anhydrous and monohydrate forms of L-phenylalanine.

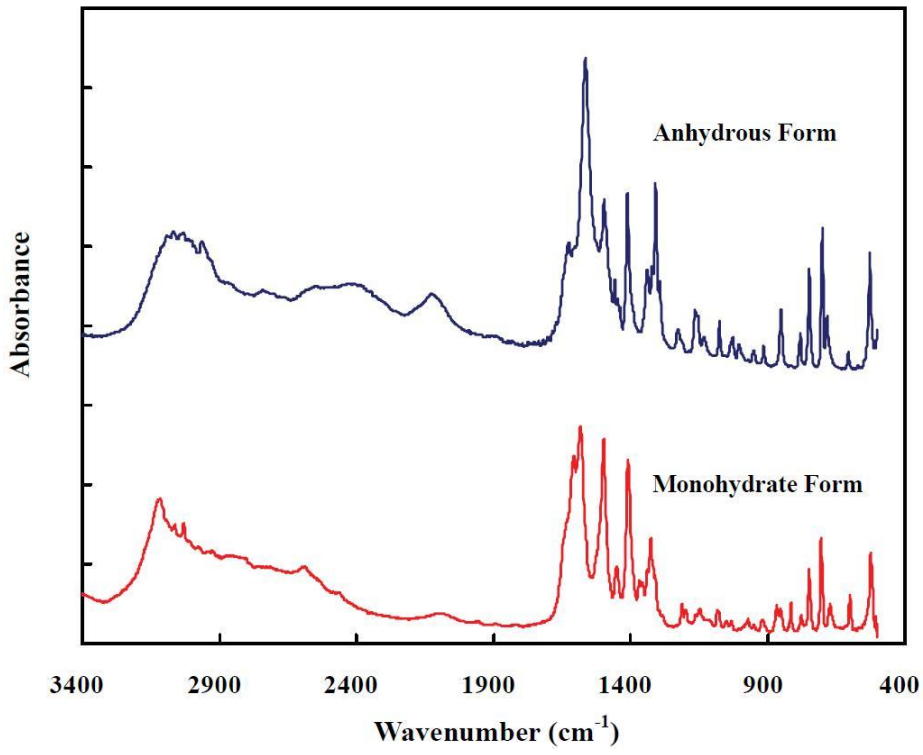

Figure 2. FTIR spectra of anhydrous and monohydrate forms of L-phenylalanine crystals.

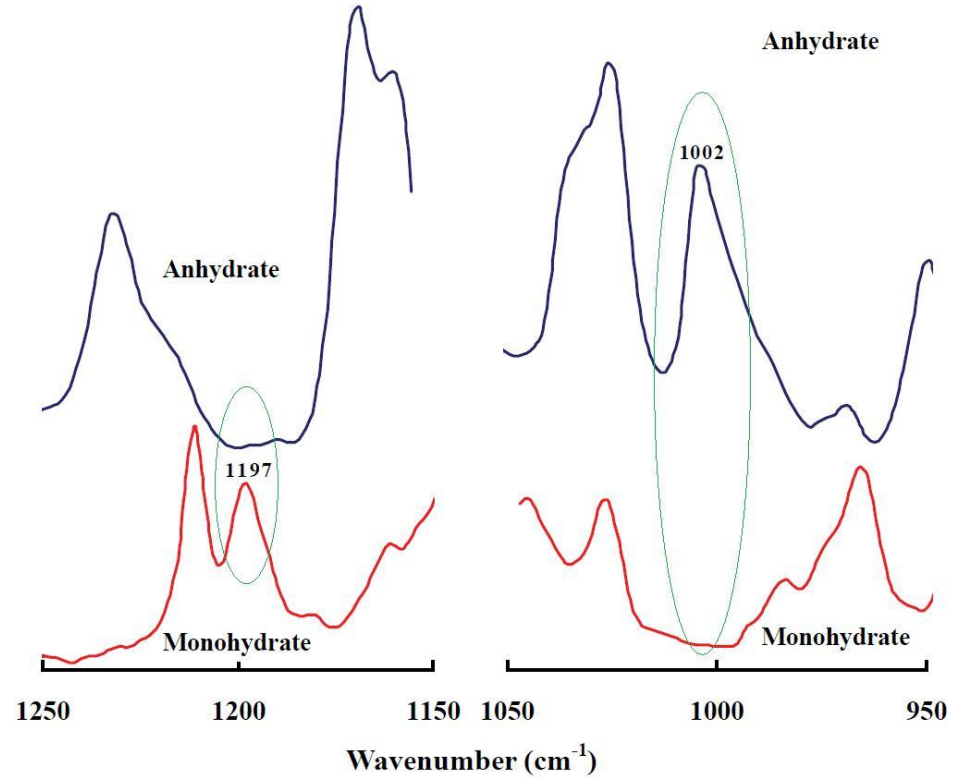

Figure 3. The characteristic absorption bands of two forms of L-phenylalanine crystals.

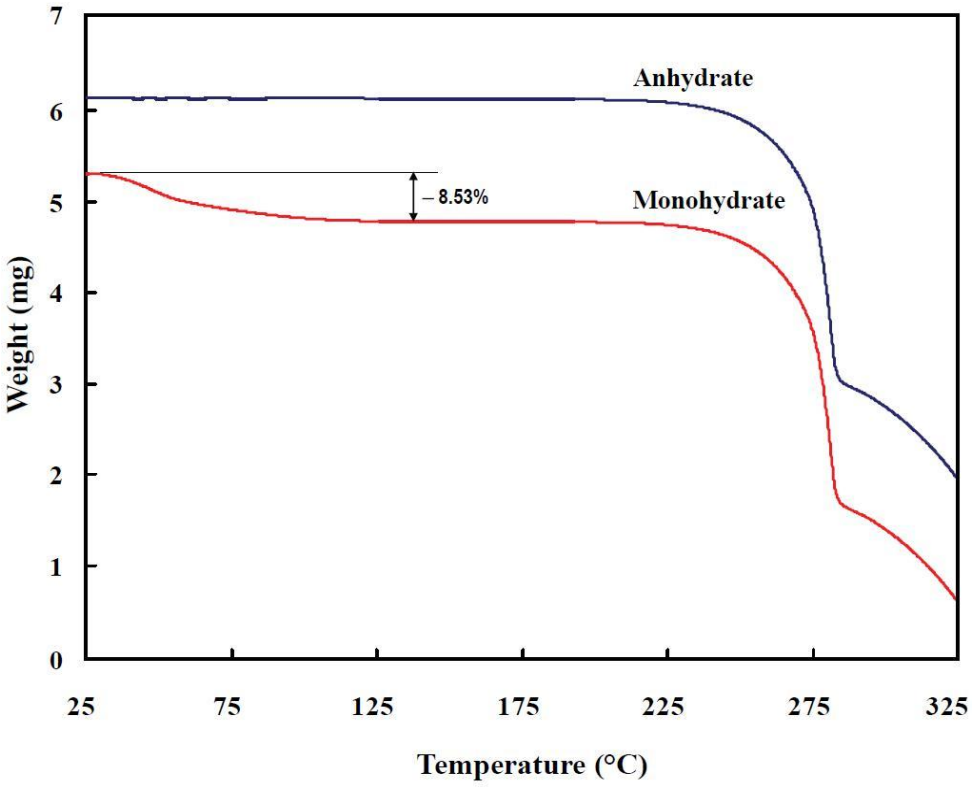

Figure 4. The TG curves of anhydrous and monohydrate forms of L-phenylalanine crystals.

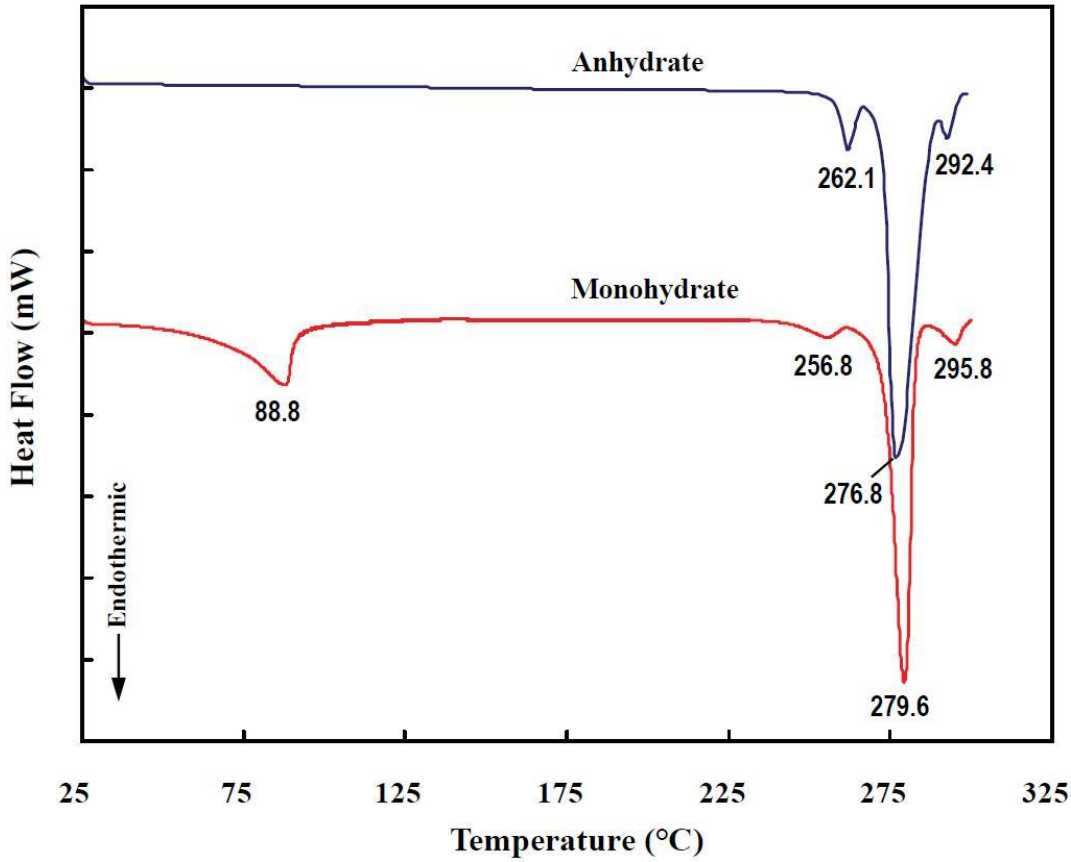

Figure 5. The DSC curves of anhydrous and monohydrate forms of L-phenylalanine crystals.

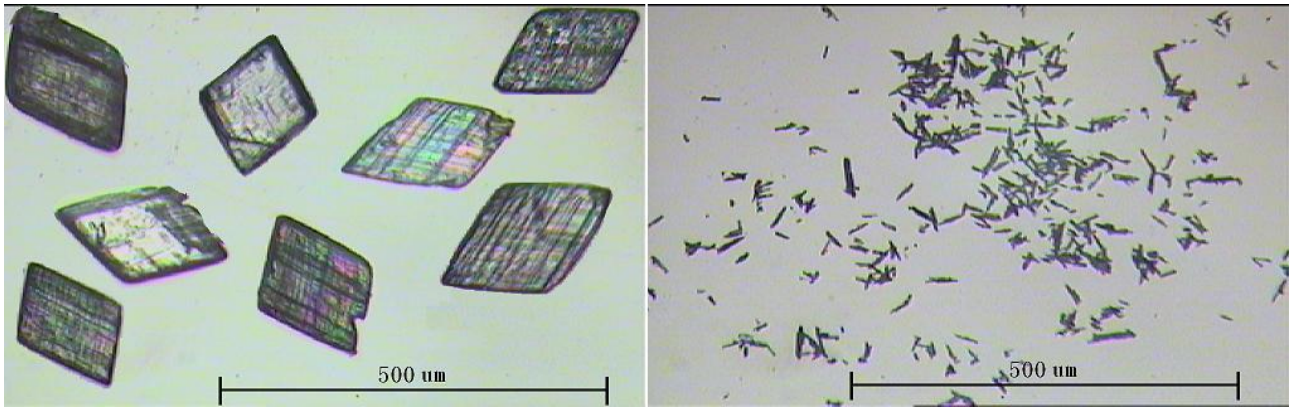

Figure 6. Micrographs of anhydrous (left) and monohydrate (right) forms of L-phenylalanine crystals.

temperatures. The solubility curves expose an enantiotropic nature of the two forms of L-phenylalanine and the transition point between them is about 35.4°C, which is in accordance with the results of Mohan et al. (2001), in which the anhydrous form is found to be the thermodynamically favored form (stable form) above 37°C, whereas the monohydrate is the kinetically favored form (metastable form) of L-phenylalanine.

The solubility of both forms increases with the temperature. Using the van't Hoff relation, $\ln S = -(\Delta H^{diss}/RT) + (\Delta S^{diss}/R)$ for the solubility curves of Figure 7, we obtain the dissolution enthalpy and dissolution

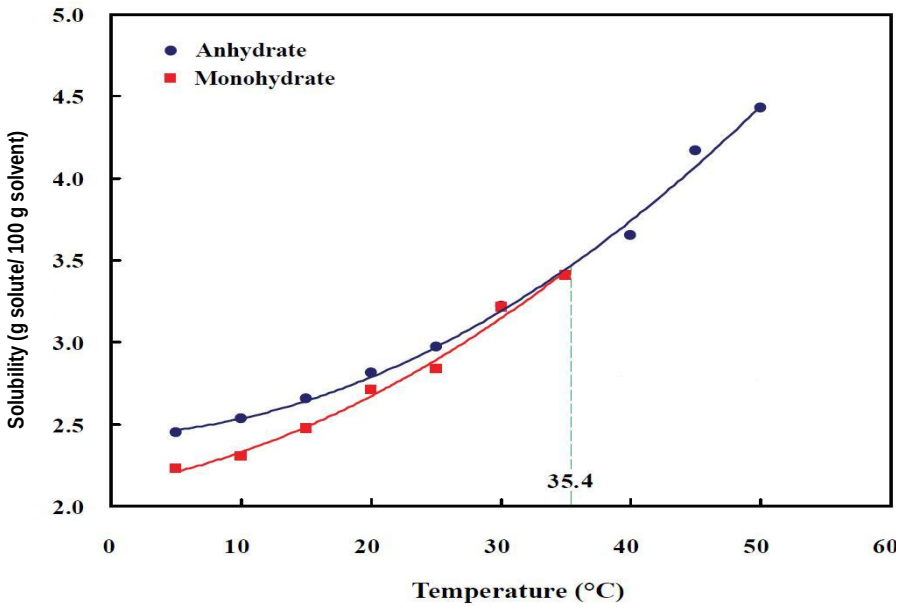

Figure 7. Solubility of the two forms of L-phenylalanine, anhydrate and monohydrate.

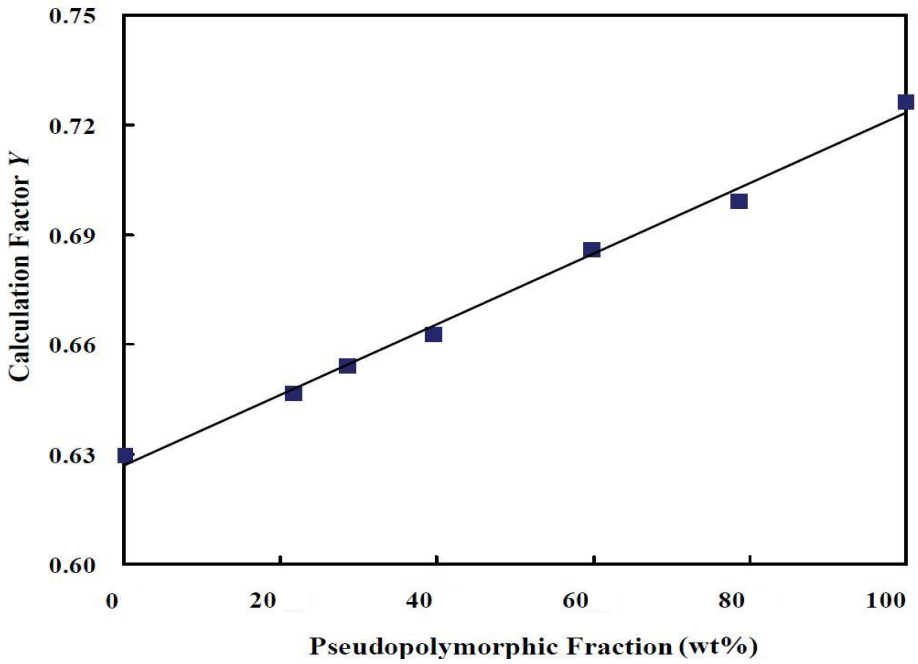

Figure 8. Calibration curve established by plotting the calculation factor *Y* versus the pseudopolymorphic fractions of monohydrate form in the samples.

entropy of anhydrate are 9.69 kJ/mol and 41.77 J/mol, respectively, while those of monohydrate are 10.54 kJ/mol and 44.28 J/mol, respectively. The enthalpy and entropy of dissolution of the monohydrate form are higher than that of the anhydrous form.

Solvent-mediated transformation

The calibration curve plotted in Figure 8 exhibits a good linearity over nearly the entire concentration range studied, which suggests that the simple approach applied

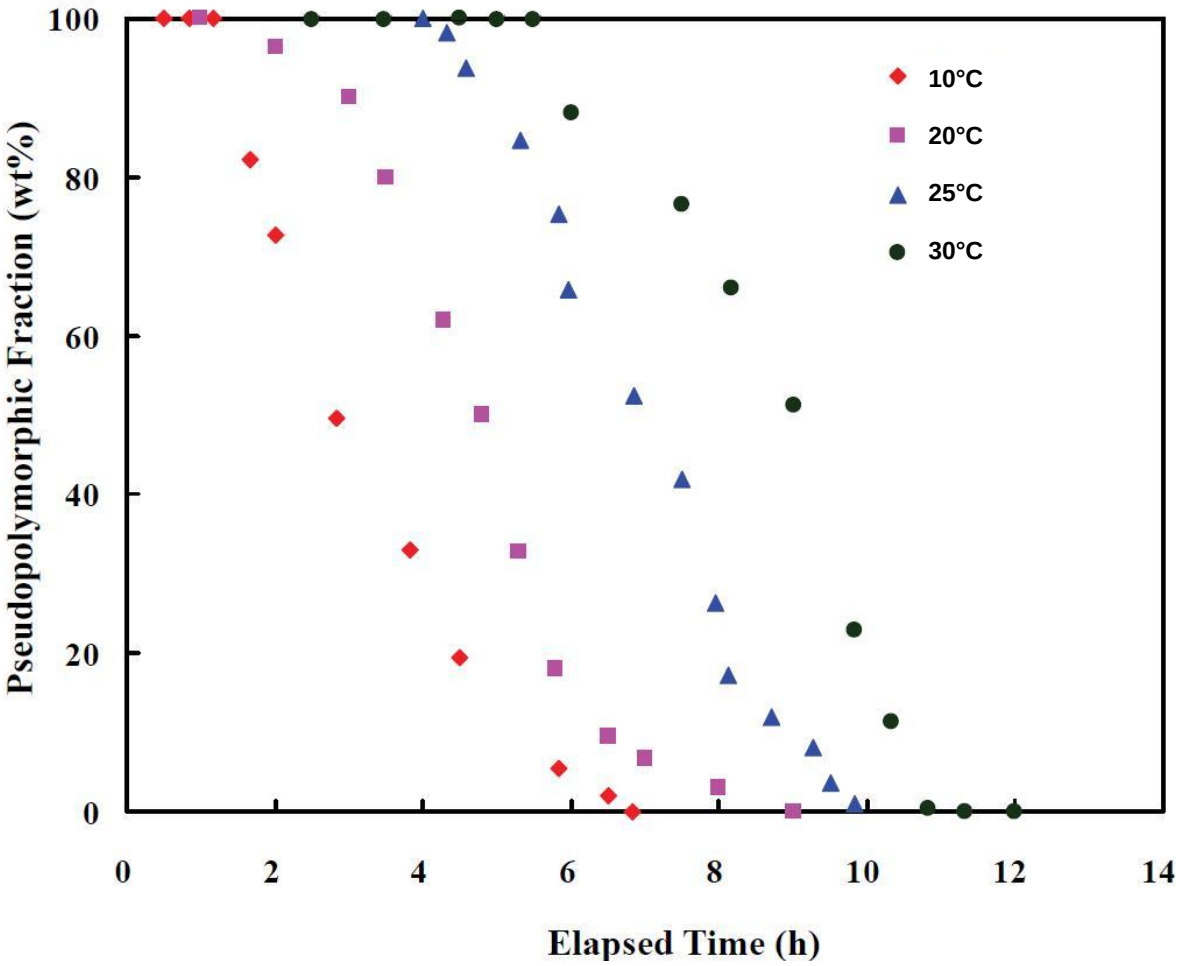

Figure 9. Pseudopolymorphic fractions of anhydrous form of L-phenylalanine slurry as a function of time, at temperatures of 10, 20, 25 and 30°C.

in this work for the quantification of the pseudo-polymorphic mixture of L-phenylalanine via FTIR is practical.

The solvent-mediated transformation rates from anhydrate to monohydrate in water were measured at temperatures of 10, 20, 25 and 30°C, as shown in Figure 9. It is clearly shown that the transformation rates measured are highly sensitive to the temperature: Transformation rate decreases with an increase in temperature. This result can be explained by the fact that the solvent-mediated transformation is controlled by differences in solubility of stable and metastable forms, where a metastable form possesses higher solubility (Sonoda et al., 2006). Solvent-mediated transformation proceeds through two sequential processes, the dissolution of the metastable form and the recrystal-lization of the stable form. When the metastable phase is within the metastable zone width of the stable phase, there will be an induction time of nucleation for the stable phase. If the metastable phase is outside the metastable zone width, the stable form will be produced without an induction time (Nývlt, 1997). That is, if the starting point lies within the metastable zone of the stable phase, the transformation starts only after elapse of the corresponding induction period. In the case when the starting point is located outside the metastable zone of the stable phase, the transformation proceeds without any delay. In this work, decreasing temperature will bring down the nucleation rate constant, but will more remarkably increase the difference in solubility of two forms of L-phenylalanine (Figure 7, that is, the driving force for transformation), thus comprehensively results in enhanced transformation rates.

Figure 10 shows the transformation rates from anhydrate to monohydrate at 25°C in pure water, and water/acetone mixed solvents with different volumetric ratios. It is interesting to note that higher water content in the solvents leads to shorter transformation time and ultimately the direct crystallization of monohydrate form. With the content of acetone in the solvent mixture increasing, more water molecules are surrounded by acetone molecules, which results in less water molecules

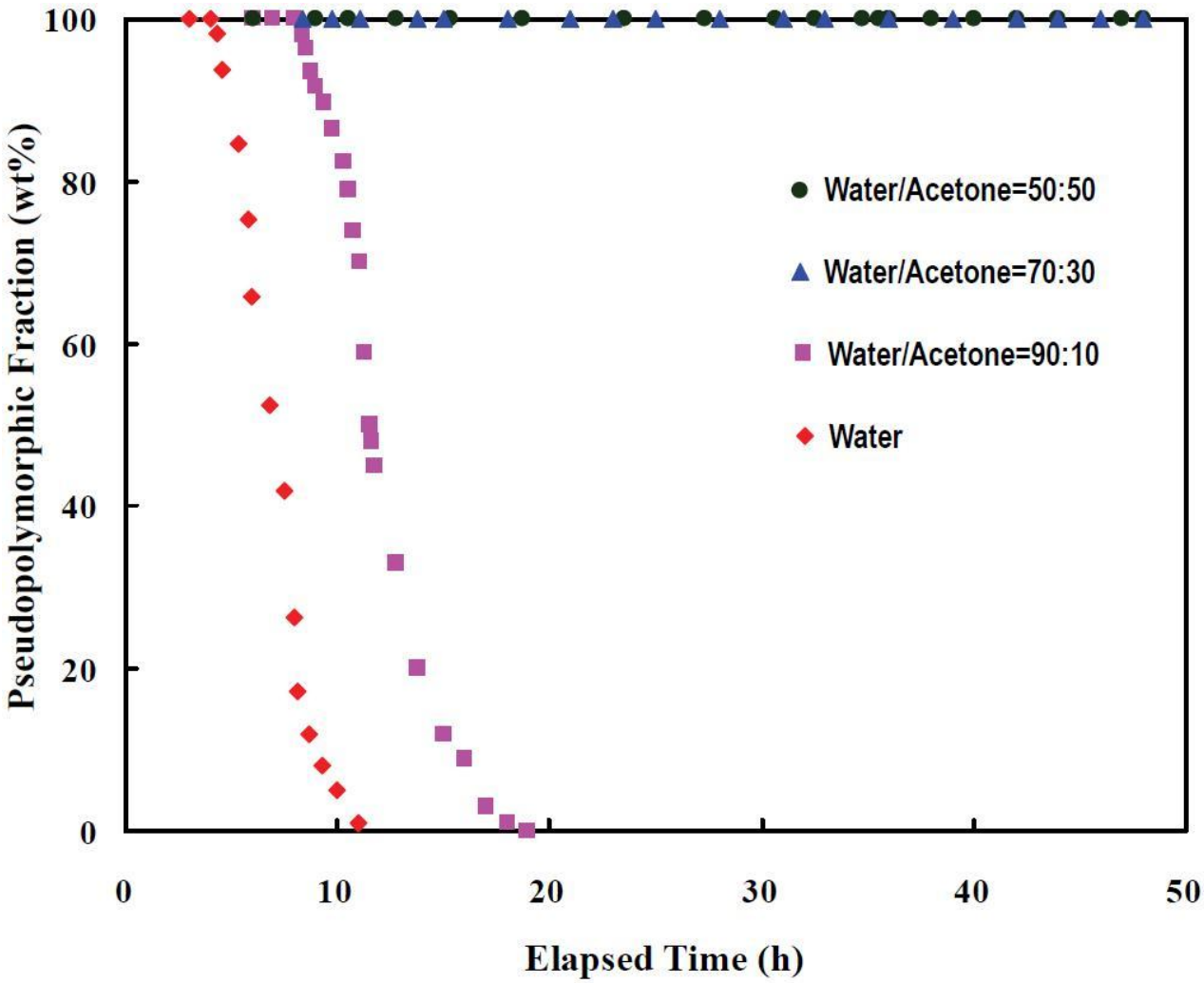

Figure 10. Pseudopolymorphic fractions of anhydrous form of L-phenylalanine slurry as a function of time in pure water and water/acetone mixture solvents (90:10, 70:30 and 50:50 (v/v)), at 25°C.

to participate in the transformation from L-phenylalanine molecules to their hydrated ones (Davey et al., 2002).

Conclusions

We have presented experimental results on the solid state characterization and the solubility of two forms of L-phenylalanine crystals (anhydrous form and monohydrate form). Besides, the transformation rates from the anhydrous form to the monohydrate form in various aqueous solutions and at different temperatures have been investigated as well, and the transformation is found to be remarkably dependent upon the temperature and the acetone content in the solutions. All these results shall provide a better understanding about this enantiotropic pseudopolymorphic system for pharmaceutical industry.

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