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Polymorphic Crystallization and Transformation of the Anti-Viral/HIV Drug Stavudine

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Abstract:

The effect of supersaturation, nucleation temperature, cooling rate and solvent on the polymorphic crystallization of stavudine has been studied. Supersaturation is found to be the predominant controlling factor for the occurrence of polymorphs. When stavudine crystallizes from methanol or 2-propanol, form I nucleates preferentially at a low supersaturation level, whereas form II can be obtained at a high supersaturation level. When 1-butanol is used as solvent, apart from forms I and II, a newly found metastable form IV can crystallize out at a moderate supersaturation level. The relative stability of the above three polymorphs in decreasing order is: I > IV > II. In addition, the polymorphic transformation of stavudine in 2-propanol from form II to form I has been investigated by in situ Raman spectroscopy. The transformation rate is found to be accelerated significantly by increasing temperature and seeding with form I.

1. Introduction

No matter whether as pure drug substances or in formulated products, active pharmaceutical ingredients (APIs) can exist in various solid forms, such as polymorphs, pseudopolymorphs (solvates and hydrates), salts, cocrystals and amorphous solids.[1,2] Thereamong, polymorphism refers to that an API has two or more crystalline forms in which the molecules have different arrangements (packing polymorphism) and/or conformations (conformational polymorphism) in the crystal lattice.[3,4] As a matter of fact, polymorphism is a widespread phenomenon observed for more than half of all active pharmaceutical ingredients.[5] Polymorphs generally have different physical and chemical properties including solubility and melting point resulting in different stability and bioavailability of drug products.[6] Thus, in both theoretical and practical contexts, understanding and controlling the polymorphic outcome of pharmaceutical crystallization is very important.

During the past decades, various methods have been developed to generate and control different polymorphs of an organic compound, such as cooling or quenching of melts,[7] desublimation,[8] solvent drop grinding,[9] spray drying,[10] supercritical fluid crystallization,[11] solution crystallization from single or mixed solvents,[12,13] seeding strategy,[14,15] introduction of conformational mimicry[16] or additives,[17] capillary crystallization,[18,19] polymer-induced heteronucleation,[20,21] nucleation confined in nanopores,[22] heteronucleation on substrates[23] or templates,[24] laser-induced nucleation,[25,26] etc. Recently, high-throughput crystallization[27] and crystal structure prediction[28−30] have been developed to facilitate the polymorph discovery for

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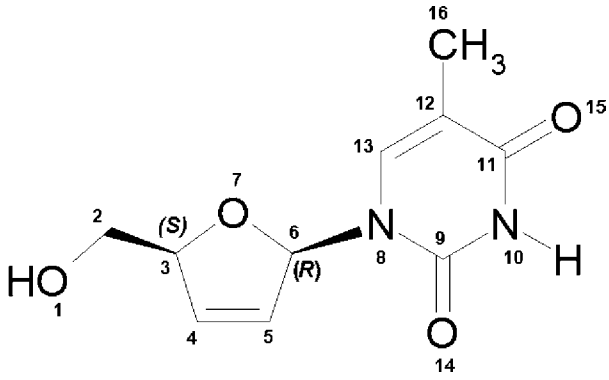
Scheme 1. **Structural formula of stavudine**



pharmaceutical active ingredients. Despite decades of great efforts, the fundamental mechanisms and molecular properties that drive molecular crystal form diversity are not well understood.[31,32] As a result, the prediction of the polymorphic behaviors of pharmaceutical compounds remains a great challenge.[33,34]

Stavudine (d_4T: 2′,3′-Didehydro-3′-deoxythymidine, Scheme 1). Has been applied to the treatment of AIDS because of its inhibitory activity against reverse transcriptase of the human immunodeficiency virus.[35] Stavudine has three known solid forms designated as anhydrous polymorphs I and II, and a hydrated pseudopolymorph III [$(d_4T)_3 \cdot H_2O$]. Polymorph I of stavudine is demonstrated as the thermodynamically most stable form and is the marketed form. The single-crystal X-ray structures of forms I and II have been reported by Gurskaya et al.[36] and Harte et al.,[37] respectively. Moreover, Gandhi et al.[38] characterized the three solid-state forms of stavudine and studied their hygroscopicity and dissolution in aqueous and nonaqueous solvents. Mirmehrabi and Rohani[39,40] have found such impurities as thymine and thymidine show significant changes in the crystal habit but have no influence on the polymorphic outcome.

In the present work, the influence of crystallization process parameters on the polymorphism of stavudine has been investigated in depth, and meanwhile the polymorphic transformation process has been investigated by in situ Raman spectroscopy. The operational parameters studied include the solvent, cooling rate, supersaturation and nucleation temperature.

2. Experimental Section

Materials. Raw stavudine was provided by Apotex PharmaChem Inc. (Brantford, ON). Such solvents as methanol, 2-propanol and 1-butanol, purchased from Sigma-Aldrich (Milwaukee, WI), were of the highest grade available and were used without further purification. Pure form I was slowly recrystallized from 2-propanol solution, whereas pure form II was obtained by quenching a concentrated 2-propanol solution into an ice-bath.

Powder X-ray Diffraction. PXRD was conducted by a MiniFlex II benchtop X-ray diffractometer at 30 kV and 15 mA with a Ni-filtered Cu Kα radiation source ($\lambda = 1.54$ Å) (Rigaku, The Woodlands, TX). The samples were scanned from 3° to 43° (2θ) at a scanning rate of 0.5 deg per min. The diffractograms were processed using JADE 7.0 software (Materials Data, Livermore, CA).

Raman Spectroscopy. Raman spectra were collected using a RamanRXN System (Kaiser Optical Systems, Ann Arbor, MI) equipped with a diode laser (784.8 nm) and a fiber optic probe. Calibration was performed using a silicon standard.

Thermal Analysis. Thermal analysis methods employed in this work included differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and hot-stage microscopy (HSM). DSC was conducted by use of a Mettler-Toledo DSC-822^e differential scanning calorimeter (Mettler-Toledo, Columbus, OH). Indium was used for calibration. Accurately weighed samples (5−10 mg) were placed in hermetically sealed aluminum pans and scanned from 25 to 250 °C at 10 °C/min under nitrogen purge. TGA was performed on a Mettler-Toledo TGA/SDTA 851^e instrument. Approximately 7−9 mg sample was heated from 25 to 300 °C at 5 °C/min under nitrogen purge. HSM analysis was carried out with a Linkam THMS 600 hot-stage (Linkam, Tadworth, UK) and an Axioskop 40 microscope (Carl Zeiss, Oberkochen, Germany) with an attached CCD video camera (Qimaging, Surrey, BC). The powders of pure polymorphs I and II were heated to 200 °C, at 2 °C/min, respectively.

Moisture Sorption and Desorption. Dynamic gravimetric vapor sorption (DVS) is a well-established method for the measurements of vapor sorption and dehumidification. The DVS Advantage instrument (Surface Measurement Systems, London, UK) was employed to investigate the hygroscopicity of stavudine polymorphs by measuring the uptake and loss of vapor gravimetrically using a Cahn D-200 ultrasensitive recording microbalance (Thermo Fisher, Waltham, MA) with a mass resolution of ±0.1 μg. The required relative humidity (RH) levels were generated by mixing dry and saturated vapor gas flows in the correct proportions using 1179A flow controllers (MKS Instruments, Cheshire, UK). The temperature was maintained constant at 25 ± 0.1 °C. About 100 mg of pure polymorphs were loaded onto a quartz DVS round-bottom sample pan and pre-equilibrated at 0% RH (±0.4%) in a continuous flow of dry nitrogen. The relative humidity was then increased to 90% in a 10% increment per step. The equilibration criterion dm/dt (change in mass as a function of time) was set at 0.0002%/min for all steps. After each experiment, the data were exported to Microsoft Excel using a DVS Macro.

Solubility Measurements. The solubility of the two polymorphs of stavudine was measured in methanol, 2-propanol and 1-butanol at various temperatures. Saturated solutions were prepared in a 100-mL jacketed glass crystallizer by adding

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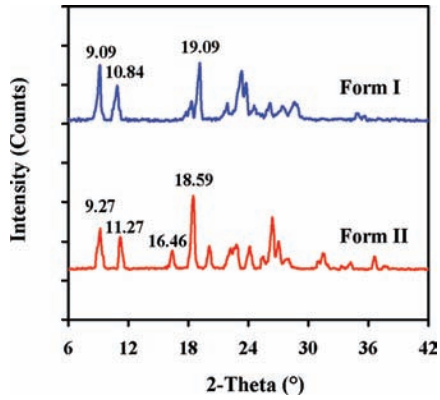

Figure 1. **Powder X-ray diffraction patterns for the polymorphs I and II of stavudine crystals.**

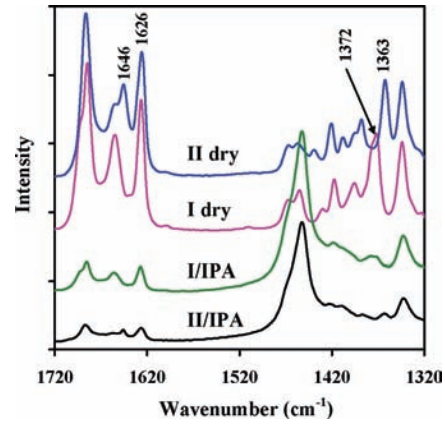

Figure 2. **Raman spectra of the forms I and II of stavudine in dry state and suspended in 2-propanol.**

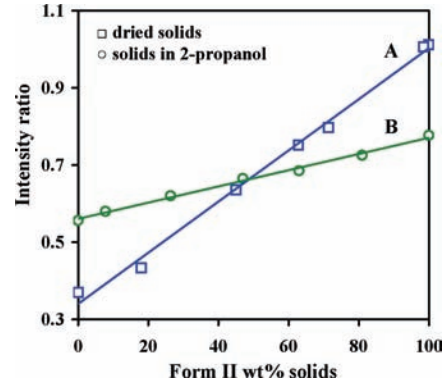

Figure 3. **Raman spectroscopy calibration curves for quantitative analysis.**

excess powders of pure polymorph. The Teflon-coated magnetic stirring bar ensured proper mixing in the crystallizer. The temperature of the crystallizer was controlled by a RTE-740 Digital Plus refrigerated bath (Thermo Neslab, Newington, NH), and the solution was stirred gently for about 8 h at each temperature. After the agitation was stopped, the suspensions were allowed to settle. The supernatant in equilibrium with a macroscopically observable solid phase was then filtered and diluted for concentration analysis. The solid residues were also filtered and dried for the analysis of their polymorphic content by PXRD. The concentration of diluted supernatant was determined spectroscopically by measuring absorbance at 266 nm with a Cary 100 Bio UV−visible spectrophotometer with WinUV Bio software (Varian, Palo Alto, CA). The extinction coefficient obtained through calibration experiments was 0.51 mL/(mg cm). The calibration curve was determined in deionized water. Solubility of each sample was measured in triplicate.

Cooling Crystallization Experiments. The experiments were performed in a 50-mL jacketed glass crystallizer, the temperature of which was controlled by a Julabo FP 50 programmable circulator (Julabo Labortechnik, Seelbach, Germany). The cooling rates conducted were 0.1, 0.2, 0.8, 1.6 °C/min. The crystals that first appeared were filtered off and analyzed by PXRD or Raman for their polymorphic compositions, and meanwhile the temperatures at which the nucleation took place were recorded. For all cooling crystallization experiments, the stirring was employed by a Corning PC-353 magnetic stirrer (Corning, NY). In addition to cooling rate, the studied variable crystallization parameters included initial solute concentration and the type of the solvent.

Nucleation Experiments. Two series of nucleation experiments were performed: In the first set, nucleation took place at various initial concentrations at constant temperatures; in the second set, nucleation occurred at various temperatures at constant initial concentrations. Thus the occurrence domain of each respective polymorph with respect to nucleation temperature and initial concentration were obtained. During the nucleation experiments, a suspension of the desired amount of the polymorph I of stavudine was heated to 3 °C above the equilibrium temperature to dissolve all powders in the 200-mL vessel. It was then filtered and added to the 50-mL crystallizer in which temperature was kept at the desired value. Agitation was provided by the magnetic stirrer. When crystals appeared, the time was recorded, and the crystals were filtered off immediately and analyzed by PXRD or Raman for their polymorphic content. Experiments under each set of conditions, defined by initial supersaturation ratio S_0 and nucleation temperature T_0, were replicated. The initial supersaturation ratio S_0 was calculated as $S_0 = c_0/c_{eq}$, where c_0 and c_{eq} are the initial concentration of stavudine and the equilibrium solubility at the nucleation temperature T_0 of the form I, respectively.

Solvent-Mediated Polymorphic Transformation. First, suspensions of the excess amount of pure polymorph I or II in 2-propanol were stirred in a 200-mL jacketed glass crystallizer at 25 and 50 °C. The polymorphic content of the solids in suspensions were monitored in real time using the RamanRXN analyzer. Raman spectra were collected every 20 min. The intensity ratio of the characteristic peak of form II at 1646 cm^{-1} and the reference peak common to both forms at 1626 cm^{-1}, respectively, was used to estimate the percent of form II in the solids in suspensions.[40] After 16 h, no interconversion between two polymorphs from their pure samples was observed. Second, the suspensions of polymorphic mixtures were employed. Saturated solutions of form II in 2-propanol were prepared in the 200-mL jacketed glass crystallizer at 25 and 50 °C, respectively. The mixtures of both forms (about 1% form I in the mixtures) were then added into the saturated solutions. In all experiments, the suspension density of the slurries was about 15 mg/mL, and vigorous agitation was employed to prevent the adhesion of solids onto the window of the RamanRXN analyzer. Apart from the in situ observation by the RamanRXN analyzer, a portion of each suspension was withdrawn and

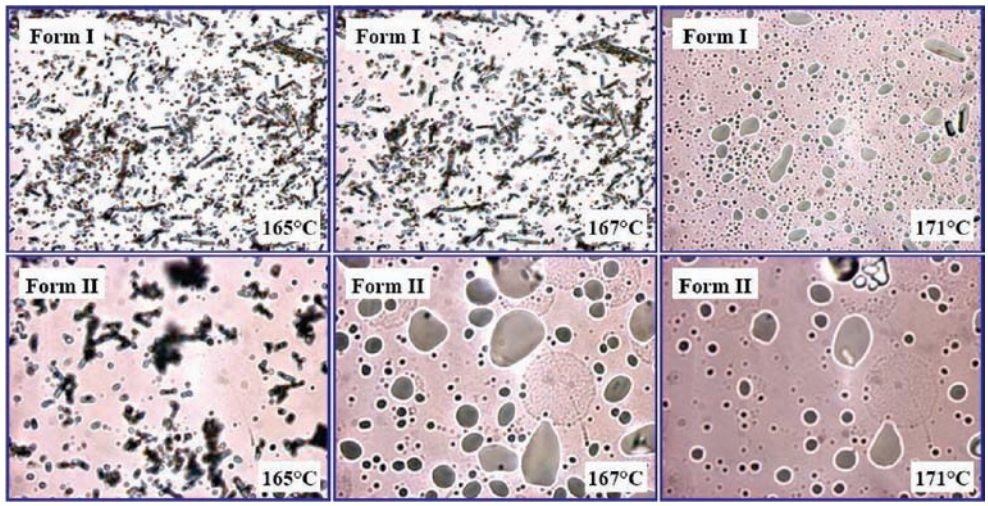

Figure 4. **Melting behaviors of polymorphs I and II examined under HSM.**

filtered at designated times for double-checking the polymorphic content of the solids in suspensions.

3. Results and Discussion

Solid-State Characterization of Pure Polymorphs. Figure 1 shows typical PXRD patterns for the two polymorphs of stavudine. The form I has characteristic diffraction peaks at 9.09°, 10.84°, 19.09°, etc., whereas the form II has the characteristic peaks at 9.27°, 11.27°, 16.46°, 18.59°, etc.

Raman spectra in the range of 1320−1720 cm^{-1} of forms I and II in dry state and suspended in 2-propanol, respectively, are presented in Figure 2. No matter whether in dry state or suspended in 2-propanol, form I shows unique peaks at 1372 cm^{-1}, whereas form II has characteristic peaks at 1646 and 1363 cm^{-1} which corresponds to the torsion motions of C9−N8−C6−O7. The differences in Raman spectra of the two forms of stavudine in the above range can be attributed to the differences in their spatial structures, i.e. their different conformations.

As shown in Figure 2, the Raman spectra of solids in dry state and in 2-propanol are different. For quantitative analysis of the polymorphic content of solids in dry state and in suspensions, two calibration curves were constructed respectively. The reference peak at 1626 cm^{-1}, common to both forms, was selected as an internal standard. The characteristic peak of form II at 1646 cm^{-1} was chosen for quantitative analysis. First the standard dry samples were prepared as the mixtures of the two polymorphs in various mass fractions of form II in the mixture. The calibration curve A for dry samples was thus plotted. Then the calibration curve B for the solids in suspensions was obtained through analyzing various suspensions by the calibration curve A. Both curves in Figure 3 exhibit good linearity over nearly the entire concentration range studied.

The measured onset and peak maximum of the melting endotherm of polymorph I are 168.1 and 170.1 °C, respectively, while those of polymorph II are 165.5 and 166.6 °C, respectively. In addition, the hot-stage microscopy experiments confirm that the form II has a lower melting point, as shown in Figure 4.

Figures 5 and 6 display the typical moisture sorption and desorption results for the polymorphs of stavudine crystals at 25 °C, respectively. With respect to both polymorphs, the

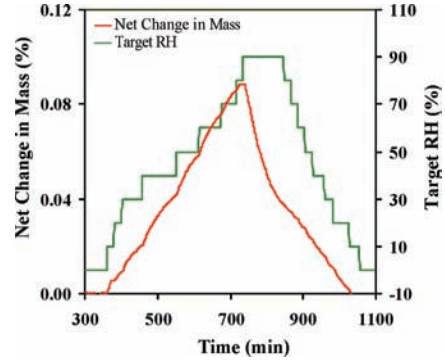

Figure 5. **Moisture sorption and desorption kinetics for the polymorph I at 25 °C.**

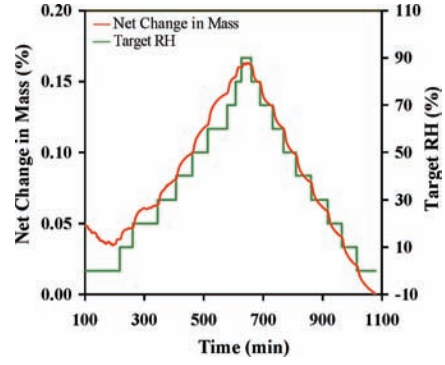

Figure 6. **Moisture sorption and desorption kinetics for the polymorph II at 25 °C.**

sample mass slightly increases or decreases with each corresponding increase or decrease in humidity, which suggests that both polymorphs have low hygroscopicity. Figure 5 displays an indication of a hydrae change, as the sample loses weight at the maximum humidity after gaining it.

Solubility of Polymorphs. Figure 7 presents the solubility of the two polymorphs of stavudine in methanol, 2-propanol and 1-butanol at different temperatures. The solubility of form II is higher than that of form I, and the solubility of both forms increases with the temperature. In addition, the solubility in the 3 alkyl alcohols is in the order methanol > 2-propanol > 1-butanol; that is, the solubility decreases with the increase in the alkyl chain length of alcohol.

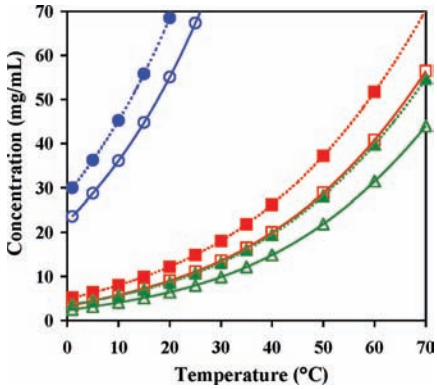

Figure 7. **Solubility of the forms I and II of stavudine in different solvents: Solid blue circle - form II in methanol. Open blue circle - form I in methanol. Solid red square - form II in 2-propanol. Open red square - form I in 2-propanol. Solid green triangle - form II in 1-butanol. Open green triangle - form I in 1-butanol.**

Table 1. **Effect of cooling rate on the crystallization of stavudine from 2-propanol solutions at various initial concentrations**

cooling rate (°C/min)	initial concentration (mg/mL)	product form
0.1	66.66	I
	54.10	I
	40.40	I
	33.33	I
	25.51	I
0.2	66.66	I
	33.33	I
0.8	66.66	I
	33.33	I+II
1.6	66.66	I
	33.33	I+II

Form I has a higher melting point (170.1 °C), and its solubility is always lower than form II that has a lower melting point (166.6 °C). According to the Solubility Rule,[41] the nature of the relationship between the forms I and II of stavudine is monotropic, and the form I is the thermodynamically stable form, whereas the form II is a metastable form.

Metastable Zone Width. At a cooling rate of 0.1 °C/min, the average metastable zone widths of polymorph I in methanol and 2-propanol are 6.9 and 7.5 °C, respectively. That suggests that the metastable zone width is wider in the solvent with a lower solubility.

Effect of Cooling Rate. Usually, it is expected that slow crystallization from dilute solution will produce the stable form, whereas rapid crystallization from concentrated solution in which the kinetics dominate, will generate metastable forms. Our experiments (Table 1) show that, when 2-propanol is used as solvent, cooling rate can affect the polymorphic outcome only at low concentrations and polymorph I, instead of polymorph II, preferentially nucleates from concentrated solutions at the high cooling rates of 0.8 and 1.6 °C/min. That is because, when initial concentration is low, the number of collisions between the solute molecules is greatly reduced, the metastable limit of form II can be exceeded when fast cooling rate is employed.

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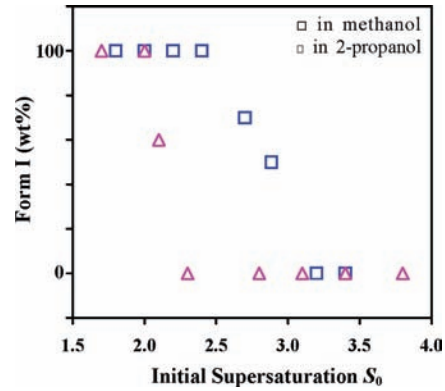

Figure 8. **Effect of supersaturation on the form of products crystallized from methanol and 2-propanol at 20 °C.**

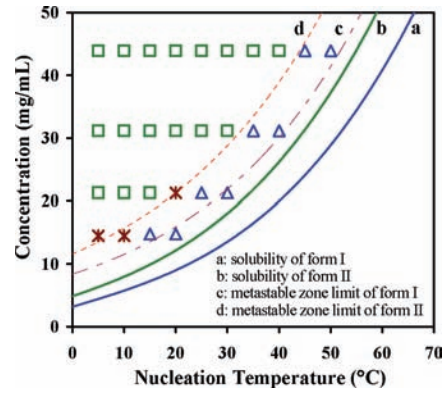

Figure 9. **Occurrence domains of stavudine polymorphs crystallized from 2-propanol: Open blue triangle - form I. Open green square - form II. * - mixture of forms I and II.**

Effect of Supersaturation. Figure 8 shows the polymorphic outcome of crystallization from 2-propanol and methanol solutions at various initial concentrations at a constant nucleation temperature 20 °C. The supersaturation for the nucleation of form II from 2-propanol is found to be lower than that from methanol. This means that the nucleation barrier of form II crystallized from methanol is larger than that from 2-propanol.

Occurrence Domain. From the nucleation experiment, the occurrence domain[42] of each respective polymorph with respect to nucleation temperature and initial concentration, can be determined. To control a crystallization process to produce the desired polymorph, the occurrence domain that is unique for that particular polymorph needs to be delineated. The occurrence domain for the polymorphic crystallization of stavudine from 2-propanol solution is described in Figure 9. From the nucleation experiments, the metastable zone limit of form II can be outlined.

As for the effect of initial concentration, several types of behaviour have been recognized: (a) the more stable form crystallizes preferentially at all concentrations, (b) the less stable form will crystallize preferentially only at high concentrations, (c) the less stable form crystallizes preferentially only at intermediate concentrations, (d) the less stable form nucleates at all concentrations. As shown in Figure 9, during the crystallization of stavudine, both form I and form II can crystallize from either concentrated or diluted solutions, depend-

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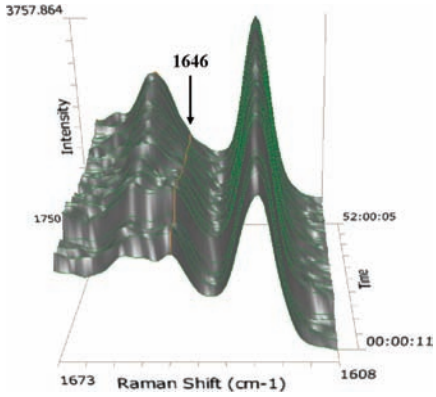

Figure 10. **In situ Raman analysis of the transformation from form II to form I of stavudine in 2-propanol at 25 °C (1% form I added as seeds).**

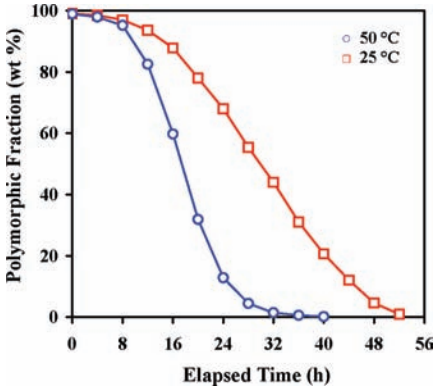

Figure 11. **The polymorphic fractions of form II of stavudine in slurry as a function of time, at the temperatures of 25 and 50 °C, seeded with form I.**

ing upon the level of supersaturation provided. On the other hand, Figure 9 also implies that nucleation temperature presents an insignificant effect on the polymorphic crystallization of stavudine, i.e. no matter whether high or low nucleation temperature is employed, the form I always nucleates at lower supersaturation levels, whereas the form II always occurs at higher supersaturation levels. In other words, the level of supersaturation can be regarded as a predominant controlling factor of the polymorphic crystallization of stavudine.[43]

Solvent-Mediated Transformation. As shown in Figure 10, in the presence of form I as seeds, the intensity of the peak at 1646 decreased with the time, indicating that form II can transform into the form I. When form I was added as seeds, the primary nucleation step was bypassed; thus, the transformation rate shall correspond to the crystal growth rate of form I and the dissolution rate of form II.

On the other hand, as shown in Figure 11, the transformation rate remarkably increases with the temperature, which can be attributed to that the crystal growth rate of form I and the dissolution rate of form II increased with the temperature.[44] In fact, apart from temperature and seeding, other factors such as

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(44) Bernstein, J.; Davey, R. J.; Henck, J. O. *Angew. Chem., Int. Ed.* **1999**, *38*, 3440–3461.

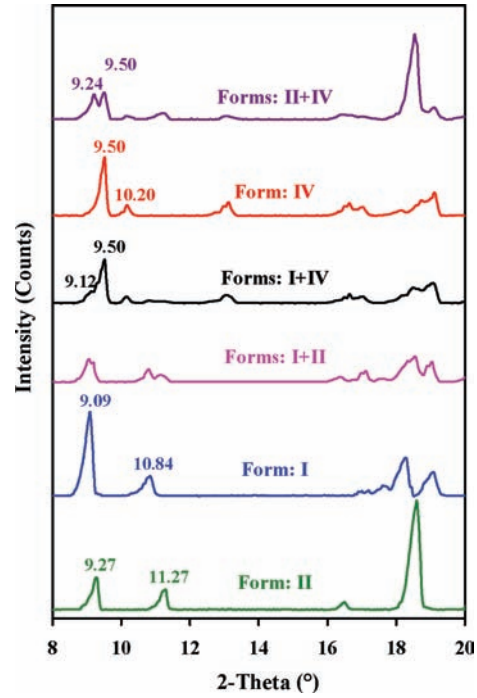

Figure 12. **Comparison of the PXRD patterns of various solid forms of stavudine.**

Table 2. **Product form of stavudine crystallized from 1-butanol at a constant nucleating temperature 20 °C**

initial supersaturation S_0	product form
1.8	I+IV
2.1	I+IV
2.4	IV
2.7	II+IV

solvent, agitation, slurry density, etc. have been found to greatly influence the polymorphic transformation processes.[45]

Effect of Solvent. A new crystalline form of stavudine was found in this work and designated as form IV, when crystallized from 1-butanol. The polymorphic outcome of the crystallization of stavudine from 1-butanol at various initial supersaturation levels at the nucleation temperature 20 °C is summarized in Table 2. The form IV is characterized by PXRD, TGA and DSC. As shown in Figure 12, the form IV has characteristic peaks at 9.50°, 10.20°, etc. The TG and DSC analysis shows that the form IV is anhydrous polymorph, and has a peak maximum of the melting endotherm at 169.5 °C. From the Table 2, form IV is suggested as a metastable form, its thermodynamic stability is between those of polymorph I and polymorph II.

4. Conclusions

This work has studied the effect of supersaturation, solvent, cooling rate, initial concentration and nucleation temperature on the polymorphism of stavudine. When methanol and 2-propanol are employed as solvents, polymorph I crystallizes preferentially at a low supersaturation level, whereas polymorph II can be obtained at a high supersaturation level. When 1-butanol is employed as solvent, a newly found metastable

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polymorph, designated as polymorph IV, can be obtained at a moderate supersaturation. The thermodynamical relative stability of the form IV is suggested to be between the form I and the form II. In addition, *in situ* Raman spectroscopy has been successfully applied to monitor the polymorphic transformation of form II to form I in 2-propanol, and the transformation rate is found to be significantly accelerated by increasing temperature and seeding with form I.

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