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Estimation of the transition temperature for an enantiotropic polymorphic system from the transformation kinetics monitored using Raman spectroscopy

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Abstract

Polymorphism is a frequently encountered phenomenon in organic materials and is particularly important for pharmaceuticals. For enantiotropically related polymorphs, one important property of the polymorphic pair is the thermodynamic transition temperature. The transition temperature is sometimes difficult to determine experimentally due to the rapid transformation between the two polymorphic forms in solution. Due to its relatively rapid spectral acquisition rate, as well as the possibility of in-line monitoring, Raman spectroscopy is ideally suited to monitoring the kinetics of transformation between different solid-state forms. In this study, it was demonstrated that the transition temperature could be estimated from polymorphic transformation profiles obtained from real-time *in situ* Raman data. Using this method, the estimated transition temperature for flufenamic acid was in good agreement with the previously published value. These results suggest that Raman spectroscopy may be a useful method to determine transition temperatures in systems not amenable to other methods.

Keywords: Polymorphism; Transformation; Raman spectroscopy; Transition temperature

1. Introduction

Polymorphism is a common phenomenon in organic compounds and is of particular importance for pharmaceuticals. Polymorphism results from the different packing arrangement of molecules in the crystal lattice leading to forms possessing different Gibbs free energy. Polymorphs can either be monotropic where one form has a lower Gibbs free energy than the other form at all temperatures below the melting point or enantiotropic, whereby at a certain temperature, called the transition temperature, the Gibbs free energy of the two solids is equal [1].

For enantiotropic polymorphs, the transition temperature is of great importance since it defines the temperature at which the stability relationship between the two forms becomes inverted. There are several methods currently available to experimentally determine or estimate this value. The most commonly used method is the van't Hoff plot [2–6]. The logarithmic solubility of each polymorphic form at different temperatures is plotted against the reciprocal of absolute temperature. The transition temperature is the temperature at which the two forms have the same solubility. Although this method works well for some systems, it is often difficult to determine meaningful solubility values for metastable forms due to rapid transformation to a more stable solid. In addition, if the temperature range used to determine the solubility is too wide, the linearity of the plot can be compromised, as discussed by Grant et al. [7]. Intrinsic dissolution rate measurements can also be used in some instances to determine solubility ratios as a function of temperature [8,9]. Additionally, combining solubility and heat of solution measurements have been used to determine the transition temperature of different polymorphic systems [10,11]. Finally, Yu described how the transition temperature could be determined from differential scanning calorimetry data [12].

Slurry transformation is a potentially practical way to determine the transition temperature. In this method, the two

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polymorphic forms are mixed and held at a range of temperatures in a suitable solvent system. The metastable form will convert with time to the stable form via a solvent-mediated transformation. By monitoring the solids composition using a suitable analytical technique, and gradually narrowing the temperature range, the transition temperature can be estimated.

Raman spectroscopy is a potentially useful technique for estimation of the transition temperature by monitoring of the solids composition as a function of time. As a result of advances in instrumentation, Raman spectroscopy has been widely applied within the pharmaceutical industry to provide information about the chemistry and solid-state composition of various systems, as well as for monitoring crystallization and solvent-mediated phase transformations [13–16]. Recently, Hu et al. reported the use of Raman spectroscopy for the real-time monitoring of crystallization and polymorphic transformation of a model compound, flufenamic acid (FFA) [17]. FFA can exist as two polymorphic forms (Forms I and III) at room temperature. The two forms are enantiotropically related with a reported transition temperature of 42 °C [18] and Form III is the stable form below this temperature. Although Forms I and III can both exist in the solid-state at room temperature, they cannot co-exist in a slurry for prolonged periods of time; this is especially true at high temperatures. For example, at approximately 53 °C, the transformation from Form III to Form I take approximately 15 min when seeds of Form I are present [17]. Although the relatively fast transformation in slurries makes solubility determination challenging, it provides an excellent opportunity to monitor transformation kinetics. This study illustrates how the transition temperature can be determined from the kinetics of transformation as a function of temperature, using Raman spectroscopy to monitor the transformation kinetics.

2. Materials and methods

2.1. Materials

FFA was purchased from Aldrich, Inc. (Milwaukee, WI). Pure FFA Form III was obtained by dissolving an appropriate amount of FFA (as received) in toluene at about 80 °C and quench cooling to 0 °C with vigorous stirring. FFA Form I crystals were obtained by the same method, the only difference was that Form I seeds (about 50 mg) were added to the clear solution before Form III crystals were formed during the cooling stage. Sodium dodecyl sulfate (SDS 95%) was purchased from Mallinckrodt Chemical, Inc. (St. Louis, MO). Double deionised water was used for all experiments. Ethanol (200 proof) was purchased from Aaper Alcohol and Chemical Company (Shelbyville, KY).

To control particle size while avoiding changes in morphology, particles were sieve separated into different particle size fractions by gentle agitation. To facilitate the separation and diminish friction between the particles, wet sieving was performed using a 0.25% aqueous solution of SDS (FFA has a very low aqueous solubility). The particles in the size range of 75–106 μm were collected and dried under ambient condition in a light protected environment.

2.2. Solubility determination

The solubility of Forms I and III in pure water was determined at three different temperatures by measuring the concentration of FFA at λ_{max} = 288 nm [19], with a UV–vis spectrophotometer (DU 7400, Beckman, Irvine, CA). Two analysis temperatures were chosen, one above and one below the transition temperature reported in literature [18]. Since the metastable form can rapidly convert to the stable form in the presence of seeds, rigorous steps were taken to eliminate seeds. These included an extra purification step for each polymorph and the use of closed containers for the solubility experiments. An excess amount of solid was added to a 50 ml glass bottle with approximately 25 ml of water. The sealed container was immersed in water held at the temperature of interest and agitated for 3 days. Triplicate samples were measured for each polymorphic form at each temperature. The samples were filtered using preheated 0.45 μm filters (Alltech, Deerfield, IL). Aliquots of the filtrate were diluted in 50:50 (v/v) ethanol/water with the dilution factor being determined gravimetrically. UV spectroscopy was then used to determine the concentration of the diluted samples.

2.3. Preparation of samples for slurry transformation experiments

Different amounts of Forms I and III were accurately weighed and geometrically mixed to produce samples of different weight ratios. The purpose of mixing the two forms was to bypass the nucleation stage of the solvent-mediated transformation. By varying the weight ratio of each form, the kinetics of transformation could be manipulated based on how far the system was from the transition temperature. An appropriate amount of the solid mixture was added to 80 ml of 70:30 EtOH/water (v/v) saturated with respect to FFA, held at the temperature of interest. Immediately after solids addition, the suspension was agitated using an overhead stirrer and spectral acquisition was initiated. The overhead stirrer speed was set at 450 rpm, and the Raman probe tip was submerged approximately 5 mm under the surface of the solution. These conditions were selected to provide minimal variation in signal intensity.

2.4. Raman spectroscopy

Raman spectra were collected using Raman Rxn1-785 spectrometer (Kaiser Optical Systems, Inc., Ann Arbor, MI), equipped with a 1/4 inch diameter MultiRxn stainless steel immersion optic with a flat sapphire window attached to a MR Probe. A 400 mW diode laser at 784.8 nm was used for excitation, and the power at the window was measured to be around 100 mW using a Coherent LaserCheck power meter (Auburn, CA). Spectral data between −100 and 3450 cm^{-1} were collected using a 5 s integration time with 10 accumulations summed for each spectrum. A spectral collection rate of one spectrum per

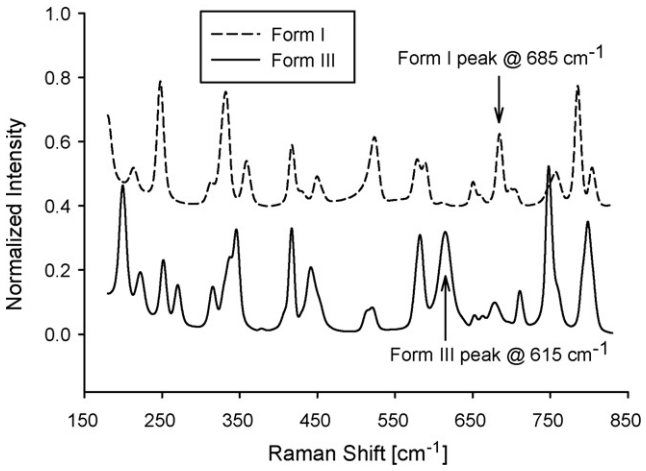

Fig. 1. Raman spectra of the Forms I and III polymorphs of flufenamic acid.

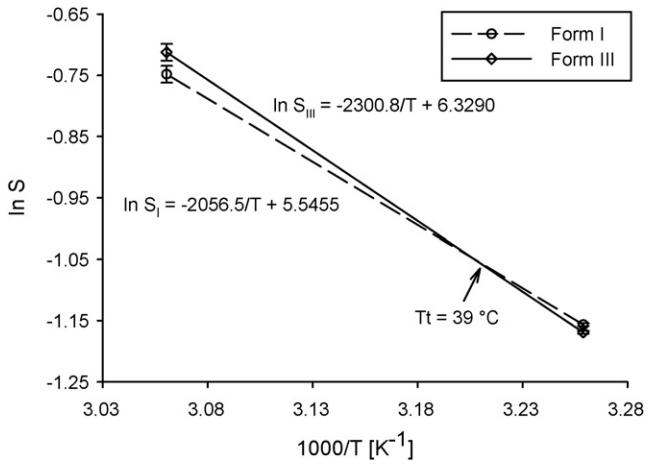

Fig. 2. van't Hoff plot of flufenamic acid Forms I and III.

minute was used for each experiment. The Raman spectra of the two polymorphic forms are shown in Fig. 1.

2.5. Data analysis

Univariate analysis was used to assess polymorphic composition as described in detail previously [17]. This analysis enabled the percentage of FFA Form I in the solid phase to be determined. Hence, the variation in polymorphic composition could be monitored as a function of time. Using the percentage of FFA Form I as a function of time, polymorphic form transformation profiles were constructed. The transformation profiles were then fitted to a sigmoidal equation (Eq. (1)).

$$X = X_0 + \frac{A}{1 + e^{-(t-t_m)/b}} \quad (1)$$

where X is the percentage of Form III formed, X_0 the initial amount of Form III, A the difference between the maximum possible amount of Form III subtracted by the initial amount, t_m the time to 50% transformed, and t is the time. The rate of the transformation (b) can be empirically related to the transformation rate constant (k) of a first-order reaction according to the following equation:

$$k = \frac{\ln 2}{b} \quad (2)$$

The calculated transformation rate constants were then used to find the transition temperature of this polymorphic pair.

2.6. Software

HoloGRAM software (version 4.0, Kaiser Optical Systems, Inc., Ann Arbor, MI) was used to control the Raman spectrometer. Origin (version 7, OriginLab Co., Northampton, MA) was used for fitting the Raman peaks. Sigma Plot (version 8.02, SSPS, Inc., Chicago, IL, USA) was used for curve fitting and graph plotting.

3. Results and discussion

3.1. van't Hoff plot of solubility data

The solubility data collected for each polymorph at two temperatures are plotted as a van't Hoff type plot in Fig. 2. This experiment was carried out to confirm the approximate temperature of the transition point, and to select the appropriate experimental conditions for the transformation rate experiments, hence only very limited solubility measurements were made. A common problem with this approach for determining the transition temperature is that the metastable form may transform during the experiment. For that reason, the remaining solids at the end of the experiment were examined using Raman spectroscopy to verify that no transformation had occurred throughout the experiment. It can be seen from the plot that the solubilities of the two forms intersect at approximately 39 °C, close to the literature value of 42 °C reported for the transition temperature of flufenamic acid [18].

3.2. Deducing kinetic and thermodynamic information

Fig. 3 shows how the spectra change as a function of time as the relative proportion of each polymorphic form varies. It can be seen from Fig. 3a that, at 25 °C, the peaks characteristic of Form I are decreasing in intensity, while those arising from Form III become more prominent. At 49 °C, shown in Fig. 3b, the converse is seen, and with time, the spectra become dominated by Form I peaks. These results are consistent with the Form I polymorph being the metastable form at 25 °C and hence converting to Form III, while at 49 °C, Form III is metastable and converts to Form I.

Following application of the calibration curve to find the percentage of Form I in the total solid mass as a function of time, a transformation profile showing conversion from the metastable to the stable form at a particular temperature can be generated as shown in Fig. 4. The initial ratios of the stable form were different for different temperatures to compensate for the differences in transformation rates. The transformation profiles provide a

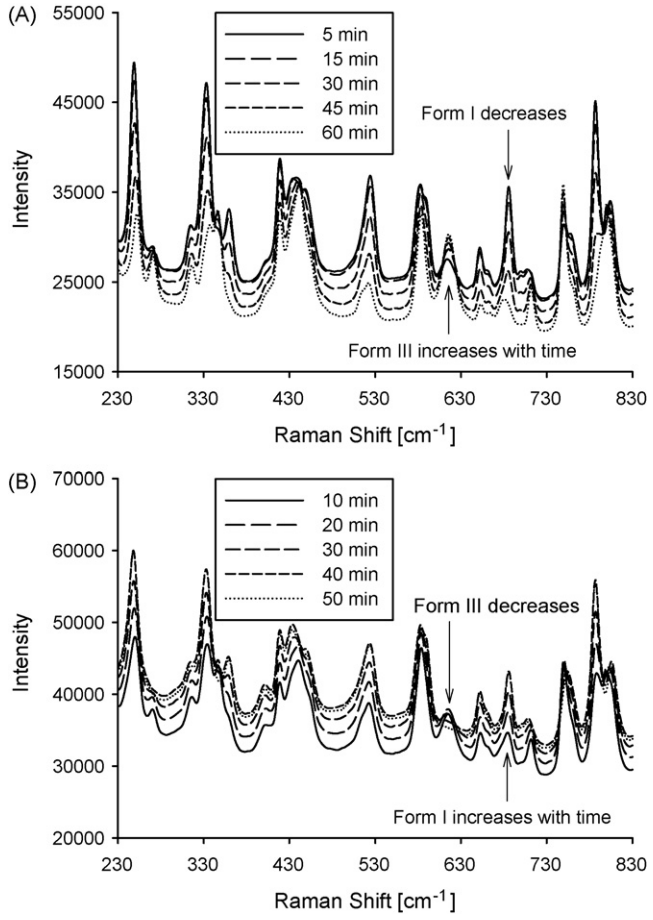

Fig. 3. Raman spectra of showing the solvent-mediated polymorphic transformation of flufenamic acid during slurry experiments. (A) Transformation at 24.7 °C showing the transformation of the Form I polymorph to the Form III polymorph. (B) Transformation at 49.1 °C showing the transformation of the Form III polymorph to the Form I polymorph.

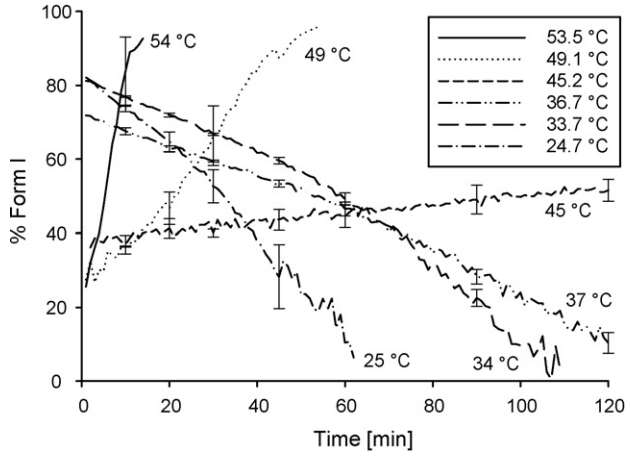

Fig. 4. Transformation profiles obtained at different temperatures showing the conversion of Form III to Form I (percentage of Form I increases towards 100%) above the transition temperature and the transformation of Form I to Form III (percentage of Form I decreases towards 0%) below the transformation temperature.

Table 1
The transformation rate of Form I to Form III at different temperatures ($n = 3$)

Temperature (°C)	$1000/T$ (K^{-1})	First-order rate constant (k) (%/min)[a]		
		Average	S.D.	$\ln(\lvert k \rvert)$
24.7	3.357	0.0541	0.0061	−2.925
33.7	3.259	0.0315	0.0008	−3.458
36.7	3.227	0.0221	0.0011	−3.812
45.2	3.141	−0.0037	0.0023	−5.924
49.1	3.103	−0.0922	0.0215	−2.420
53.5	3.061	−0.3061	0.0568	−1.210

[a] The first-order order rate constant is shown as negative when the transformation is from Form III to Form I, i.e. when the transformation occurs above the transition temperature.

clear indication of which form is the stable form at a given temperature, since the amount of the stable form will increase with time, with a concurrent decrease in the quantity of the metastable form. Hence, below the transition temperature (experiments performed at 25, 34 and 37 °C), the amount of Form I, which is the metastable form, decreased towards zero with time, while at higher temperatures (experiments performed at 45, 49 and 54 °C) the quantity of Form I increased towards 100% (Fig. 4). In addition, it can be qualitatively noted that the transformation rates of the experiments run at 25, 34 and 37 °C decrease with an increase in temperature, while the transformation rates of the experiments run at 45, 48 and 54 °C increase with temperature. Based on these observations, the spectroscopic data indicate that the transition temperature for the transformation between Forms I and III of FFA must fall within the range from 37 to 45 °C, in excellent agreement with the literature value and the results obtained from the van't Hoff experiments.

In order to extract quantitative information about the transformation kinetics as a function of temperature from the experimental data, the transformation profiles were fitted to a sigmoidal equation (Eq. (1)) in order to determine the first-order rate constant k (Eq. (2)). The rate constants are shown in Table 1 and are plotted as a function of temperature in Fig. 5. As can be seen from Table 1, the transformation rate above the

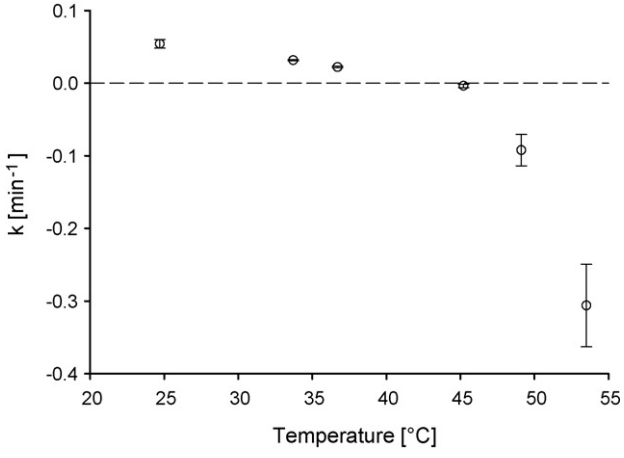

Fig. 5. Transformation rates of Form I to Form III as a function of temperature. Positive rate constants indicate that Form I is transforming to Form III, while negative rate constants indicate the Form III is transforming to Form I.

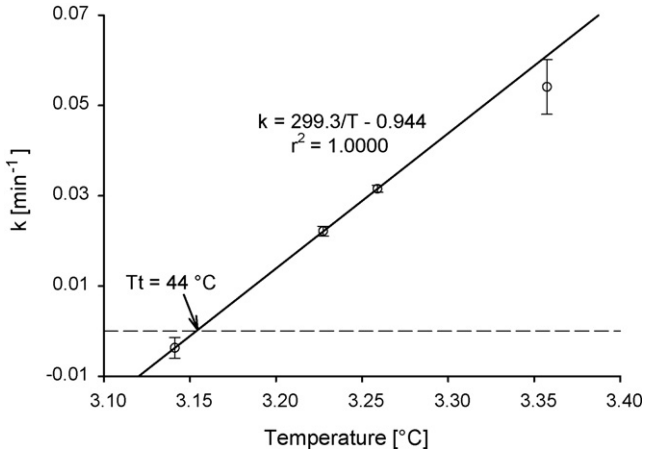

Fig. 6. The transition temperature could be estimated using the transformation rate of Form I to Form III at the temperature region below the transition temperature. It also demonstrated that when close to the transition temperature, in the temperature region above the transition temperature, the transformation rate still followed the same linear relationship.

transition temperature, from polymorphic Form III to Form I, increased steeply with temperature. For the temperature range from 34 to 45 °C the rate constant displayed a linear relationship with respect to temperature changes, as shown in Fig. 6. At the transition temperature, the two polymorphs have the same free energy and thus there is no thermodynamic driving force for conversion between the two forms. Hence, at the transition temperature, a rate constant of zero is theoretically predicted. A linear regression of the data points plotted in Fig. 6 showed that the polymorphic transformation rate constant would be zero at 44.0 ± 0.6 °C, which infers that at this temperature, the two polymorphic forms are in equilibrium. Four individual experiments were carried out at 45.2 °C, and in each of these experiments, any Form III present transformed completely to Form I, confirming that the transition temperature is below 45 °C. This result is close to the reported transition temperature of 42.0 °C [18]. The difference between the value derived from the spectroscopic data and the literature value may be related to the slight deviation from linearity over the 25–45 °C temperature range and a more accurate value may be obtained if more experiments closer to the transition temperature were carried out.

3.3. Relationship between polymorphic transformation rate and temperature

As shown in Fig. 5, the rate of the solvent-mediated transformation passes through zero at the transition temperature and increases with either an increase or decrease in temperature. A rate constant of zero at the transition temperature is consistent with each polymorphic form having the same free energy and therefore existing in equilibrium Accordingly, changing the temperature above or below the transition temperature, should result in the conversion of one polymorph to the other since one polymorph is now metastable and it would thus be expected that the rate of the transformation would no longer be zero, as is observed experimentally. It can be observed from Fig. 5 that the rate increases very steeply with an increase in temperature, whereas decreasing the temperature below the transition temperature results in a more moderate increase in the transformation rate. The relationship between transformation rate and temperature has been discussed theoretically by Giron [20]. In their review, the author highlighted that once the temperature increases or decreases from the transition temperature, the Gibbs free energies for the two forms begin to differ, and hence there is a tendency for the metastable form to convert to the stable form. The energy difference increases as the temperature differential between the experimental temperature and the transition temperature widens. However, while the difference in Gibbs free energy increases as the temperature is lowered, the solubility of the two forms decreases, which can negatively impact the transformation process. This may explain the decreasing change in the rate constant as the temperature is lowered, which is evident in Fig. 6.

4. Conclusions

The utility of Raman spectroscopy for in-line monitoring of a solvent-mediated polymorphic transformation has been demonstrated. By monitoring the polymorphic transformation in solution at different temperatures, the transformation profile as a function of temperature was obtained and from these profiles, rate constant information could be extracted. The transition temperature, estimated from the rate constant data as a function of temperature, was found to be in good agreement both with the value obtained using the conventional van't Hoff approach and the value reported in literature [18]. Spectroscopic monitoring of solvent-mediated polymorphic conversions may provide a convenient method to assess the thermodynamic transition temperature, in particular for rapidly converting systems where it is not possible to measure equilibrium solubility of the metastable form.

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