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Weak solid–solid transitions in pharmaceutical crystalline solids detected via thermally stimulated current

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Abstract

Purpose: To demonstrate the ability of thermally stimulated current (TSC), normally used to study amorphous systems, in detecting weak solid–solid transitions in crystalline pharmaceutical compound. Methods: Polymorphs of a new chemical entity, LAU254, were generated and characterized using conventional and hot plate X-ray diffraction, DSC and TSC. Equilibration of 50:50 mixtures of the different polymorphs and solubility studies were conducted in aqueous and organic solvent at 25 and 50 °C and then analyzed by X-ray and DSC. Results: Four crystalline forms (A–D) were isolated. Form B showed one single endotherm at 180 °C while the other forms showed lower melting endotherms, a crystallization exotherm and eventually a final melting endotherm corresponding to that of form B (180 °C). The heat of fusion of form B was the highest. In contrast, solubility as well as mixture equilibration studies resulted in all forms converting to form A. TSC analysis revealed a well-defined reproducible peak with a maximum at ∼130 °C which was suspected to be a solid–solid transition. This was confirmed by hot plate X-ray diffraction where careful probing around 120–130 °C revealed three different forms; form A (the initial form), a second form that appears above 150 °C, melts, crystallizes and produces form B. Careful inspection of larger sample sizes in DSC showed a small endotherm at ∼130 °C. Conclusions: TSC, normally used to study amorphous systems, proved to be useful in detecting weak solid–solid transitions in crystalline pharmaceuticals, an application that has never been explored or reported previously. This resulted in identifying a form, obtainable only at temperatures above the transition temperature (related enantiotropically to the form that is most stable at ambient temperatures) and in reconciling the DSC and solubility data. TSC can be very useful in detecting and probing those transitions that occur in the solid state due to subtle dipolar motion and are not associated with large changes in global motion and heat capacity that is needed for detection by DSC and therefore can be complementary to DSC in obtaining a more complete assessment of the polymorphism behavior of crystalline solids.

Keywords: Thermally stimulated current; Solid transitions; Crystalline to crystalline transitions; Pharmaceutical solids

1. Introduction

Polymorphism screening is typically conducted early in drug development to identify the thermodynamically stable drug form. Development of the most stable form at the outset of development reduces the need for concern once a formulation and process have been established. Due to very small changes in enthalpy, the detection of solid–solid transitions by DSC may be difficult [1,2]. This can lead to misinterpretations, as has been previously reported [3,4]. Yet these transitions are important not only for a full characterization of the system but also in investigating the relative thermodynamic stability of the different polymorphs if the compound exists in more than one crystalline form.

Several indirect approaches have been used to determine this type of transition. For example, solubility studies at several temperatures can be conducted to demonstrate if the polymorphs in question are monotropically or enantiotropically related [5,6]. Additionally, a combination of thermal and spectroscopic techniques has also been useful in demonstrating this relationship [7]. Differences in heats of fusion

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[4] and/or heats of solution [8] between two forms where a solid–solid transition is suspected have also been utilized to indirectly detect the relationship. In all cases, both forms have to be isolated and prepared, which might not always be possible.

The objective of this work is to demonstrate the ability of thermally stimulated current (TSC), normally used to study amorphous systems, to detect weak solid–solid transitions in crystalline pharmaceutical compounds, an application of TSC that has never been explored or reported previously. TSC may be beneficial in complementing DSC and in directly providing a more complete picture of solid state polymorphism behavior.

2. Materials and methods

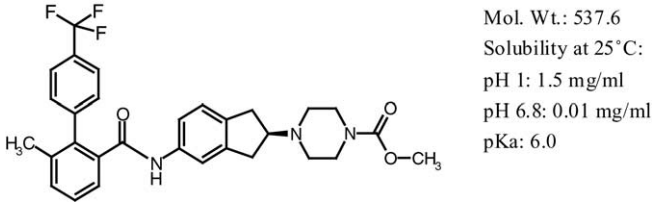
Drug substance: LAU254 (Scheme 1), form A, purity 99.8% by HPLC, was obtained from Novartis Pharmaceutical Corp.

Solvents: Ethyl alcohol 200 proof, USP grade was purchased from Pharmco Products Inc. (Brookfield, CT). Methanol 99.9% HPLC grade, ethyl acetate 99.8% HPLC grade, Heptane 99+% were all purchased from Aldrich Chemical Co. (Milwaukee, WI). Sterile water for injection, USP was purchased from Abbott Laboratories (North Chicago, IL).

2.1. Polymorphism screening

Solvents used in the isolation and crystallization steps of drug synthesis were selected to conduct polymorphism screening. These solvents were water, ethyl acetate, ethanol, methanol, methanol/water (2/1) and ethyl acetate/heptane (1/1.3). The first crystalline form isolated (form A) was used as the starting material and screening was conducted according to the following three procedures:

1. Phase equilibration: Excess solid (form A) was equilibrated in 10 ml of each of the solvents above at 25 and 50 °C for 72 h. Solid remaining after 72 h was collected by filtration and then analyzed by X-ray diffraction (XRD) and differential scanning calorimetry (DSC).
2. Slow evaporation: The supernatant obtained from the phase equilibration studies described above was collected and allowed to evaporate at room temperature for 12 h. This was done for all the above solvents excluding water. The solids remaining after the evaporation of solvents were analyzed using X-ray diffraction (XRD) and differential scanning calorimetry (DSC).
3. Precipitation of solid by the addition of a non-solvent: This was performed to simulate the recrystallization processes used to produce form A. Form A was dissolved in either methanol or ethyl acetate and heated to 50 °C for 2 h, followed by the addition of water (to methanol at 1/2 ratio) or the addition of heptane (to ethyl acetate at 1/2.6 ratio). The precipitated solid was isolated by filtration and was analyzed by XRD and DSC.



Scheme 1. Structure of LAU254.

2.2. Solubility and mixture equilibration

Solubility values of the forms obtained from screening, exhibiting different XRD and DSC patterns from the starting form A, were determined in water and in methanol/water (2/1) at 25 °C after equilibration for 3 days. Analysis was performed with HPLC (Waters 2695 separation module equipped with Waters photodiode array detector 2996, Waters Corp., Milford, MA). In addition, the excess solids remaining at the end of the solubility studies were analyzed using XRD and DSC.

Mixture equilibrium studies (4 days at 25 and 50 °C) were conducted with 50:50 mixtures of two of the forms obtained from the screening studies, prepared in a suspension form in 2/1 methanol/water. The excess solids remaining at the end of the suspension equilibration study were analyzed using XRD and DSC.

2.3. Differential scanning calorimetry (DSC)

A Mettler Toledo differential scanning calorimeter (DSC 30, Mettler-Toledo Inc., Colombus, OH) equipped with a computer analyzing system (STAR^e Program) was used for all the studies. Samples weighing 5–10 mg were placed in sealed aluminium DSC pans with a pinhole (to prevent pressure build-up) and heated in an atmosphere of nitrogen.

2.4. Thermo-gravimetric analysis (TGA)

A Mettler TG 50 thermal gravimetric analyzer equipped with a Mettler M3 microbalance (Mettler-Toledo Inc., Colombus, OH) and a computer analyzing system (STAR^e Program) was used to determine the water/volatile loss through the measurement of percentage weight change upon heating. Samples weighing 40–50 mg were placed in open aluminium oxide crucibles and heated in an atmosphere of nitrogen.

2.5. Powder X-ray diffraction (PXRD)

Hot stage PXRD measurements were performed with a ThermoARL (Scintag) XDS2000 powder diffraction system fitted with a copper X-ray tube and peltier detector. The tem-

perature was controlled using a Scintag model T-L-23 temperature attachment with a Micristar 828D controller. The samples were heated to the desired temperature and held isothermally during PXRD data collection. The PXRD data were collected at 3°/min using a continuous scan mode and step size of 0.02.

Conventional X-ray diffractograms were obtained using a Rigaku RINT 2200 equipped with a Cu target X-ray tube. Scans from 2° to 40° 2θ angle (x-axis) were used for all samples and data were reported as count/s (y-axis). Samples weighing approximately 50 mg were loaded onto a 0.2 mm aluminium holder.

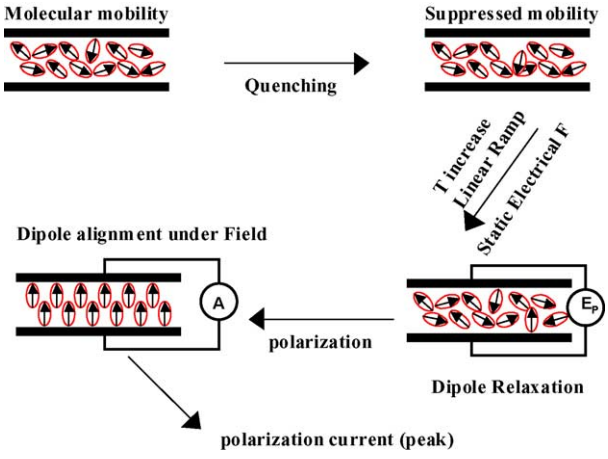
2.6. Thermally stimulated polarization current (TSPC)

Thermally stimulated current (TSC) is a general term that is applied to the measurement of current generated by the temperature activated relaxation of molecular scale dipoles in response to the application of a static electric field [9–11].

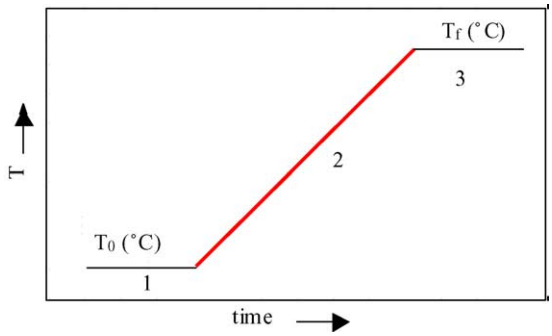
Thermally stimulated polarization current (TSPC) is one of the experimental procedures in TSC and is used in this study. A sample is cooled to temperature below the suspected transition(s) and held there for a short time. The sample is then subjected to an electric field and a current is observed as the dipolar structures orient in response to the application of the field while heating at a constant rate to a temperature above the transition. This is represented in Scheme 2. Experiments were conducted according to the TSPC procedure shown in Scheme 3.

The electric field was on only in step 2 at a voltage of 100 V mm^{-1} where the sample was heated at a constant rate (7 °C/min) during which polarization current was generated and measured giving rise to an asymmetrical peak.

In this work, TSPC experiments were carried out using a TSC/RMA 9000 instrument (TherMold Partners, Stamford, CT). Samples (5–7 mg) were weighed into aluminium DSC pans then covered with a small piece of Teflon. The samples were placed between the electrodes of a parallel plane capacitor and shielded by a Faraday cage that was evacuated to 10^{-4} mbar and flushed several times with 1.1 bar of high purity helium prior to experiments. Cooling was conducted using liquid nitrogen connected to the Faraday cage according to the Newtonian cooling mode, which allows the sample to reach initial temperature T_0, as fast as possible (≥20 °C/min).



Scheme 2. Principle of thermally stimulated polarization current (TSPC).



Scheme 3. Schematic representation of TSPC procedure, T_0 is the initial temperature (below the suspected transition) and T_f is the final temperature to which a sample is heated.

3. Results and discussion

Four LAU 254 polymorphs resulted from the screening studies. The PXRD patterns are shown in Fig. 1 (1) demonstrating clearly different patterns. These forms also exhibit different thermal behavior as shown by the different DSC thermograms shown in Fig. 2, top. The corresponding peak temperatures and enthalpies of the transitions are summarized in Table 1. The ΔH_{fusion} of the lower temperature melting endotherms for all forms has a lower value than that of the highest temperature melting endotherm. The Heat-of-Fusion Rule

Table 1
Summary of thermal parameters obtained from DSC for different LAU254 forms

Transition	Thermal parameter		
	T_{peak} (°C)	ΔH (J/g)	$\Sigma\Delta H$ (J/g)
Form A			
Endotherm	170.5	10.0	58.7
Exotherm	173.4	−3.2	
Endotherm	180.5	51.9	
Form B			
Endotherm	181.0	66.9	66.9
Form C			
Endotherm	155.3	22.1	64.6
Exotherm	159.6	−17.9	
Endotherm	180.9	60.5	
Form D[a]			
Endotherm	155.4	3.1	61.2
Exotherm	158.1	−3.1	
Endotherm	165.6	11.3	
Exotherm	169.6	−9.9	
Endotherm	180.9	59.8	

[a] Form D might be a mixture of forms C and A.

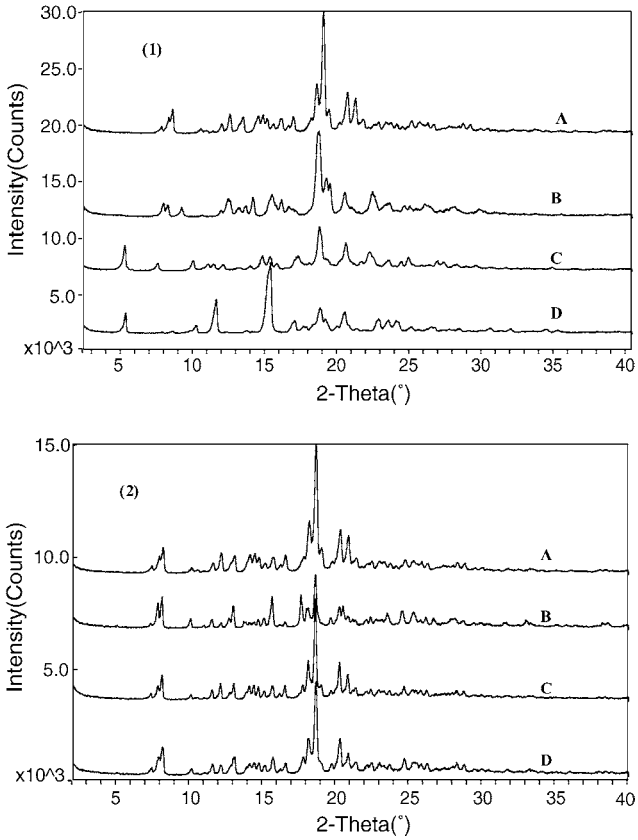


Fig. 1. X-ray diffraction (XRD) patterns of LAU254 forms: (1) results of polymorph screening; (2) polymorph transitions exhibited in the solid phase remaining at end of the solubility study in methanol:water (2:1).

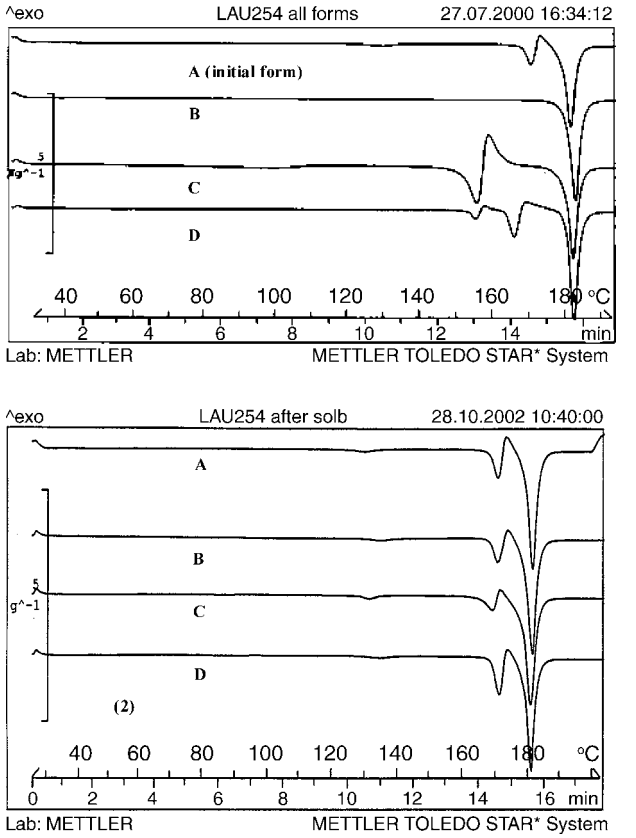


Fig. 2. Differential scanning calorimetry (DSC) thermograms of LAU 254 forms: (top) results of polymorph screening; (bottom) polymorph transitions exhibited in the solid phase remaining at end of the solubility study in methanol:water (2:1).

[1] states that if the higher melting form has the higher heat of fusion, the two forms are monotropically related, otherwise they are enantiotropic and that in the case of monotropy the higher melting form is always the thermodynamically stable form. Accordingly, form B appears to be the most thermodynamically stable form and is monotropically related to the other forms.

Thermo-gravimetric analysis (TGA) results (Fig. 3) were used to assess if the low temperature endotherms corresponded to solvates and/or hydrates. The results demonstrated that all forms obtained exhibited less than 1.2% weight loss, indicating the absence of stoichiometric hydrates/solvates.

The 25 °C water solubility values of LAU254 polymorphs are summarized in Table 2. Form B shows the highest solubility, indicating that it is the form with the highest free energy. This is contradictory to what was observed by DSC and to the Heat-of-Fusion Rule that suggested form B is the most stable form since it exhibited the highest temperature melting endotherm and the highest heat of fusion.

To further understand this result, the solid remaining at the end of the solubility study was collected and analyzed by DSC. The patterns indicated no change in form for any of the forms tested, likely due to the very low water solubility. The LAU254 polymorph solubility values were also determined in the crystallization solvent methanol:water (2:1), after

Table 2
Solubility of different LAU254 forms in water (25 °C, 3 days)

LAU254 form	Solubility (μg/ml)
A	1.0
B	5.2
C	2.51
D	2.0

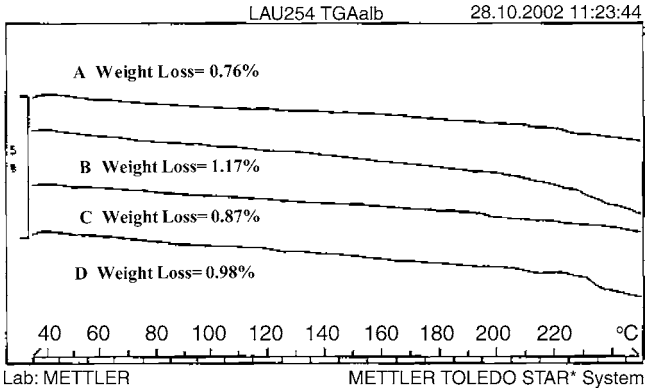


Fig. 3. Thermo-gravimetric analysis (TGA) thermograms of the different forms of LAU254.

Table 3
Solubility of different LAU254 forms in crystallization solvent (methanol:water (2:1))

LAU254 form	Solubility (mg/ml)
A	2.85
B	3.73
C	3.42
D	3.44

equilibration at 25 °C for 4 days. This solvent was chosen particularly because it was used for compound recrystallization. The results are summarized in Table 3. The solubility values clearly demonstrate form A having the lower value. The solid remaining was collected and analyzed with XRD and DSC. The patterns obtained (bottom) are compared to the initial patterns (top) and are shown in Fig. 1 (XRD) and Fig. 2 (DSC), respectively. Fig. 1 shows that all forms tested in this solubility study converted to form A instead of to form B. This was also confirmed by the DSC results as demonstrated in Fig. 2 where all forms converted to form A. These data confirm form A as the most stable form at 25 °C. One should note that the reported solubility values for forms B–D clearly indicate incomplete conversion to form A but the amount of these respective forms are undetectable by DSC and XRD.

To further confirm and understand the result obtained from the solubility studies, that form A is the most thermodynamically stable form, 50:50 mixtures of forms A:B were prepared in a suspension, in 2:1 methanol:water and were equilibrated at 25 and 50 °C for 4 days. Methanol:water (2:1) was chosen as a solvent in this study due to the poor solubility of the compound (different forms) in water, which would lead to very long conversion times. The solid remaining at the end of 4 days was collected and analyzed by XRD and DSC in comparison to 50:50 dry physical mixtures of forms A and B. The results for the samples generated at 25 °C are demonstrated in Fig. 4 (XRD) and Fig. 5 (DSC). Both XRD and DSC show that the suspension of A:B (50:50) did not convert to form B at 25 °C but rather converted to form A. This is in agreement with the result obtained from the solubility study (Figs. 1 and 2) indicating that form A, and not B, is the thermodynamically most stable form. The same was observed at 50 °C.

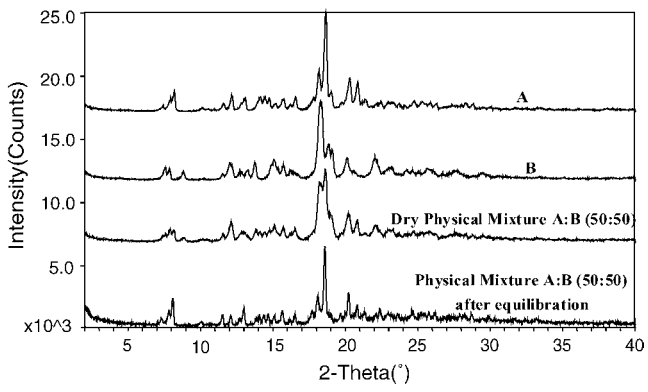


Fig. 4. XRD patterns of the solid remaining at the end of mixture equilibration studies (25 °C) compared to the dry physical mixtures.

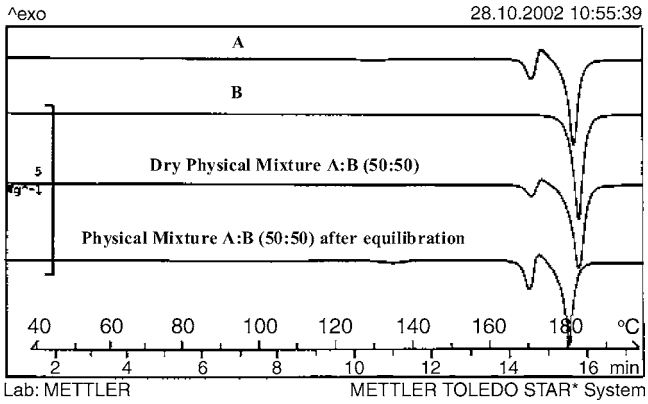


Fig. 5. DSC patterns of the solid remaining at the end of mixture equilibration studies (25 °C) compared to the dry physical mixtures.

Note that mixtures of forms A and B only were investigated and not combinations of the other forms because forms C and D converted to form A while form B had the highest melting point/heats of fusion, therefore, investigation of forms A and B only was necessary.

Thermally stimulated current (TSC), a technique that is relatively new to the pharmaceutical community, has been widely used in the polymer industry to study slow molecular transitions in amorphous systems [12,13]. In an attempt to understand the relationship between forms A, B and the amorphous form, crystalline form A was tested using the TSC polarization mode (TSPC). Form A was also used as a starting material to prepare the amorphous form in situ. Typically in TSPC the melting phenomenon of a crystalline material (or any fast transition) produces a noisy signal (negative and positive current) with strong intensity superimposed on the baseline with no net change in current. This is due to the fast and random nature of molecular motions characterizing the melting transition, which does not allow for a net change in molecular orientation to occur. This is in contrast to a well-structured peak that appears in TSPC as a result of a slow transition/molecular motion with a

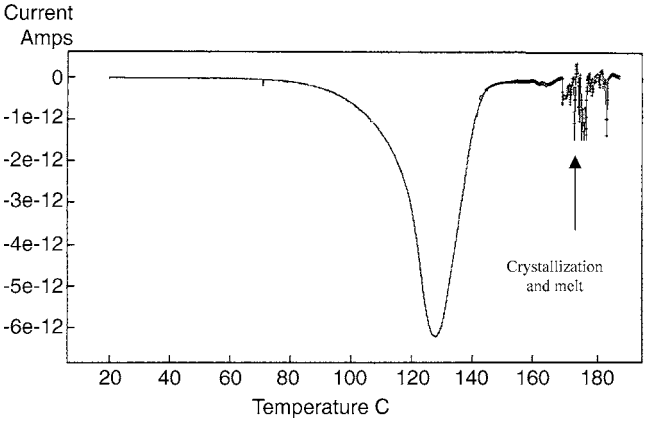


Fig. 6. TSPC thermogram of crystalline LAU254 form A.

net change in molecular orientation, as observed for the glass transition phenomenon of amorphous materials [14,15]. Interestingly, when form A was tested, in the crystalline form, a well-structured peak appeared with a maximum at

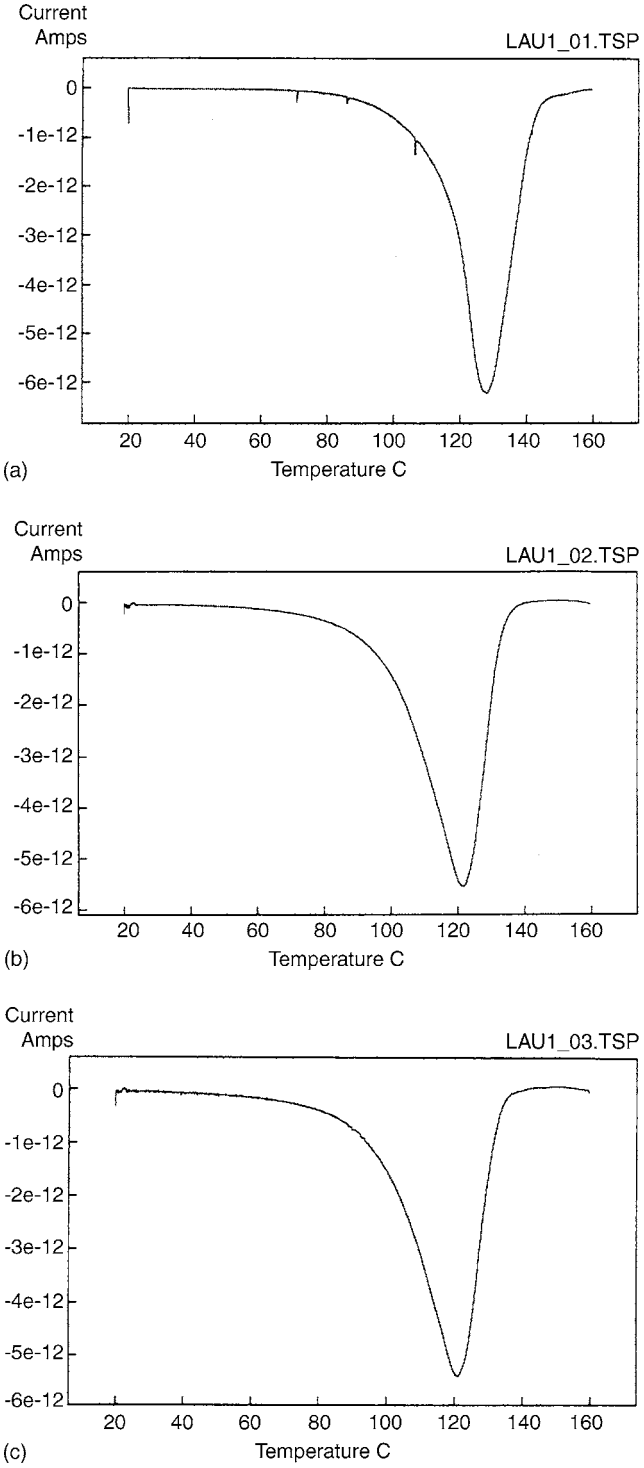


Fig. 7. TSPC thermogram of crystalline LAU254 form A after (a) first run (heated only to below melt) (b) second, and (c) third run. The second run was conducted after cooling down from 160 °C from the first run at 1 °C/min to 20 °C then re-heating to 160 °C at 7 °C/min. The same was repeated for the third run.

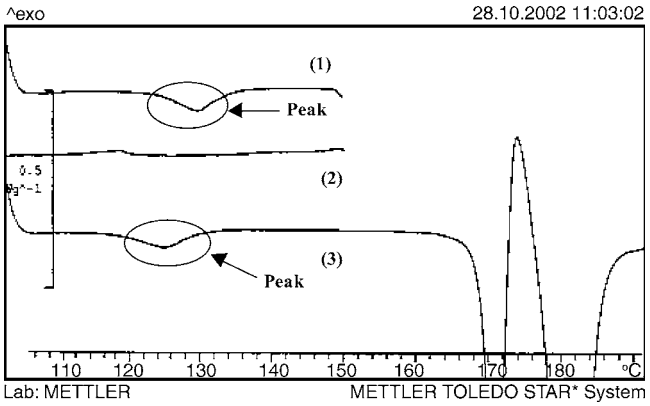


Fig. 8. Enlarged DSC thermogram of form A showing the small endotherm at 130 °C. Reversibility of this peak is demonstrated by: (1) heating from 100 to 150 °C at 10 °C/min and holding at 150 °C for 1 min; (2) cooling from 150 to 100 °C at 1 °C/min and holding at 100 °C for 1 min; (3) re-heating from 100 to 200 °C at 10 °C/min.

∼130 °C followed by a noisy signal (spike) starting at 170 °C corresponding to the crystallization and melt processes in agreement with DSC. This is depicted in Fig. 6. Another sample was tested to investigate the reproducibility of the peak obtained below the melting point for crystalline form A. Again, a well-structured peak appeared with a maximum at ∼130 °C as shown in Fig. 7. To investigate reversibility, a sample was heated only to 160 °C (below the melting point) then cooled down slowly at 1 °C/min to 20 °C then re-heated to 160 °C at 7 °C/min. The transition (well-structure peak) at ∼130 °C was again observed thus showing the reversibility of the event (Fig. 7). The presence of this peak in the crystalline form (versus the amorphous form) indicates a somewhat slow transition/rearrangement occurring in the solid state at around 120–130 °C.

Upon careful inspection of the DSC thermograms of form A in Figs. 2 and 7, a small endotherm at ∼130 °C (Fig. 8 (1)) is observed. This result is also obtained upon cooling and re-heating as described above in agreement with the TSPC results. For comparison, Fig. 9 (2) shows the thermogram of amorphous LAU254 obtained by quenching form A from

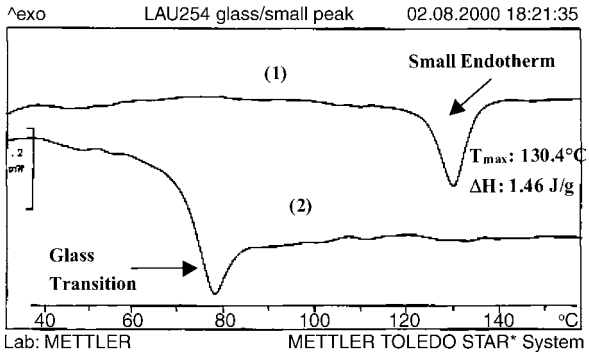


Fig. 9. Comparison between the DSC thermograms of the small endotherm of crystalline form A and that of the amorphous form of LAU254: (1) crystalline form A, (2) amorphous.

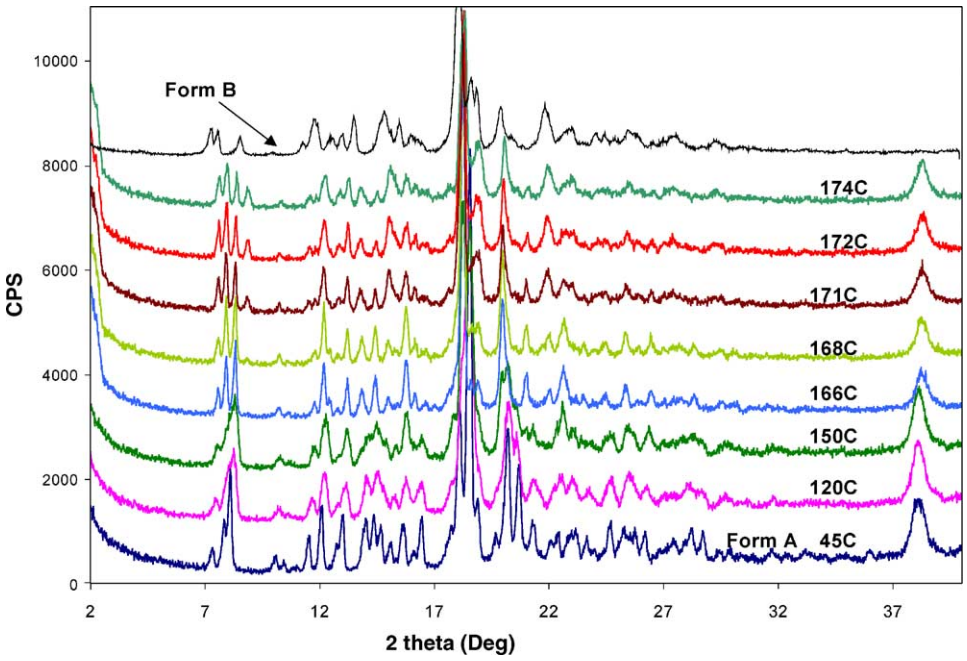


Fig. 10. Hot stage X-ray diffraction of form A. From bottom to top (for form A): 45, 120, 150, 166, 168, 171, 172 and 174 °C. The topmost diffraction pattern is that of form B obtained at RT, added for comparison.

the melt. This was investigated to discern that this small endotherm is not a glass transition event (with relaxation enthalpy) of what might be any residual amorphous substance in the crystalline form A.

The above may be explained if a solid–solid transition is assumed to take place, with a transition temperature (T_t) between 120 and 130 °C. What is postulated to be form A, the initial form, would be the thermodynamically stable form below the transition temperature. Heating above that temperature leads to an endothermic transformation, in the solid state, from form A to a second form (AI), which is the thermodynamically stable form above the transition temperature. The two forms would be related enantiotropically according to the Heat-of-Transition Rule [1,2].

XRD was performed at room temperature (below T_t), therefore the pattern obtained is for form A. In addition, since all the solubility studies and mixture equilibration were performed below the transition temperature (at 25 and 50 °C), the form obtained was indeed the most stable form at those temperatures. However, if a solid–solid transition occurs then form A does not melt (melting point is above transition temperature), but converts in the solid state to a second form (AI) and it is this form that melts at 170 °C, crystallizes out and produces form B which in turn melts at 180 °C. Form B in this case although has a higher melting temperature, is not the most stable form below the transition temperature.

To test the hypothesis above, it was necessary to perform hot stage X-ray diffraction studies on form A. The results are shown in Fig. 10, which confirms that a solid–solid transition takes place after the endothermic transition at 120–130 °C. Heating the initial form (shown in Fig. 10 at 45 °C) results in a gradual (but not complete) loss of crystallinity demonstrated by diffused, less sharp XRD patterns at temperatures above 45 °C and ranging from 120 to 150 °C. This corresponds to where the peak in TSC and the endothermic small transition in DSC were observed. The crystal lattice appears to be partially but not completely destroyed as the transition proceeds gradually through the lattice as a result of the molecules reorienting gradually to form a new crystal form. The sensitivity of the TSC to slow molecular motions would suggest that the solid–solid transition observed with LAU 254 results from a slow intermolecular rearrangement of molecules within the crystal lattice and not a simultaneous melt and recrystallization. This is confirmed by the PXRD patterns between 120 and 150 °C (Fig. 10) where the "halo" that normally accompanies amorphous systems was not exhibited during the course of the transition. After the endothermic transition, the crystalline form obtained evolves with heating into a form different from the initial form A and from the high melting form B. This can be shown by comparing the diffraction patterns at 166, 168, 171, 172, 174 °C to the initial form A at 45 °C and to form B. This form, called here (AI) is the more stable form at temperatures above the transition temperature compared to form A. Form AI melts and recrystallizes above 172 °C to form B. No attempts were made to determine the exact (equilibrium) value of the transition temperature (T_t) since it is at minimum above 50 °C (the highest temperature at which equilibration studies were performed) and therefore, irrelevant to drug substance/product manufacture and storage stability.

4. Conclusions

In this study, TSPC proved to be a useful technique in detecting weak solid–solid transitions in a crystalline compound. This resulted in identifying a form, obtainable only at temperatures above the transition temperature (related enantiotropically to the form that is most stable at ambient temperatures) and in reconciling the DSC and solubility data. TSC, being a sensitive probe of internal structure, is beneficial and complementary to DSC in obtaining a more complete assessment of the polymorphism behavior of crystalline solids. In particular, it can be very useful in detecting and probing those transitions that occur in the solid state due to subtle dipolar motion and are not associated with large changes in global motion and heat capacity that is needed for detection by DSC. Moreover, the current data illustrate that a solid–solid transition results from slow molecular reorientation of the molecules in a crystal lattice and not a melt–recrystallization process.

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