Detection of Low Levels of the Amorphous Phase in Crystalline Pharmaceutical Materials by Thermally Stimulated Current Spectrometry[1]

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Purpose. To demonstrate the applicability of thermally stimulated current (TSC) spectrometry for the detection of low levels of the amorphous phase in crystalline pharmaceutical materials.

Methods. A crystalline drug substance was melt quenched to produce an amorphous material. Blends of the crystalline and amorphous phases in different ratios (from 75:25 to 99:01) were prepared by serial dilution. TSC studies were performed by applying an electric field at a temperature above the glass transition temperature (T_g) to orient the dipoles, rapidly cooling to 0°C, short circuiting for 1 min, and scanning at 7°C/min to measure the depolarization current. The temperature of the peak in the spectrum corresponds to the T_g of the amorphous phase. Modulated differential scanning calorimtery (DSC) studies were performed using three different test protocols (varying linear heating rate, modulation amplitude, and time period). Powder X-ray diffraction (XRD) studies were performed using a Siemens D500 diffractometer.

Results. The ability to detect the amorphous phase by powder XRD is beset with problems due to indirect inference, orientation effects, and instrument-related intensity variations. Even using a consistent sampling procedure and an internal standard, the XRD could quantify the amorphous phase at a level of 5%. In the conventional or modulated DSC, the amorphous phase manifests itself as a shift in the baseline. Using modulated DSC it was possible to detect the amorphous phase at a level of 5% when tested at a heating rate of 2°C/min and an amplitude of ±1.0°C with a period of 30 s. The moisture sorption method appears to have a similar detection capability. In TSC scans, the glass transition event due to molecular/segmental mobility in the amorphous phase was manifested as a peak/shoulder on the low-temperature side of the depolarization peak of the crystalline phase. The amorphous phase was unambiguously detected at 2% with a lower detection limit of ~1%.

Conclusions. On the basis of the results of this preliminary investigation, TSC appears to be capable of detecting the amorphous phase at as low as ~1% in crystalline pharmaceuticals, thus offering a much needed capability in discerning factors.

KEY WORDS: amorphous phase; crystalline pharmaceuticals; TSC; detection.

INTRODUCTION

Pharmaceutical processes, such as recrystallization from solvent systems, milling (size reduction for improving dissolution, processability, or bioavailability), granulation, drying, and compaction, may result in disorder in the form of crystal defects or an amorphous phase (1–6). The amorphous phase, which is of a higher energy state than the crystalline phase, may be formed throughout the particle, in parts of it, or at its surface. Although the amorphous fraction is low, these "high energetic" regions may result in a significant enhancement in dissolution rate, decreased chemical stability, solid-state transition, and moisture-induced recrystallization during storage. Hence, it is important to measure low levels of the amorphous phase in crystalline materials in order to ensure the development of robust, commercializable formulations.

The thermal stimulated current (TSC) technique is widely used in characterizing the amorphous phase of polymers (7,8) by monitoring molecular motions (relaxations) which are affected by the degree of curing, orientation, internal stress, ageing, and phase separation in copolymers and thermoplastic resins. Relaxations in materials take place as a result of internal motions, which are induced by either thermal, mechanical, or electrical energy disturbances. The method consists of applying an electric field (direct current) to a material at an appropriate temperature in order to orient dipoles within the molecular chains, which are sensitive to the electric field. The temperature is then lowered (with the electric field maintained) in order to reduce internal motions, trapping the polarized dipoles within the material. During reheating of the sample, the thermal energy helps relax the trapped molecular segments. As this occurs, a small current is observed, corresponding to one or more relaxations (9). Recent TSC studies on pharmaceutical materials (10), though limited in scope, have shown TSC technique to be particularly useful in characterizing the amorphous phase in crystalline pharmaceutical materials. Amorphous and crystalline phases in pharmaceutical materials have also been detected, quantified, and/or characterized by a variety of techniques, such as thermomechanical analysis (11), dielectric analysis (12), solid-state nuclear magnetic resonance (13), FT-Raman (14), FT-IR (15), enthalpic relaxation by differential scanning calorimetry (DSC) (11), moisture sorption, isothermal calorimetry (13), powder X-ray diffraction (XRD) (15,16), and modulated DSC (17,18).

The main objective of this study was to demonstrate the applicability of TSC in the detection of low levels of the amorphous phase in crystalline pharmaceutical materials by a comparative evaluation of modulated DSC, moisture sorption/desorption, powder XRD, and TSC methods.

MATERIALS AND METHODS

Materials

Carvedilol, a novel antihypertensive drug with a chemical structure as shown below, was used as a model pharmaceutical material for this investigation. Seven lots of crystalline drug substance (one lot A contained some amorphous phase while 6 lots, lots B–G, were ~100% crystalline) were tested as received.

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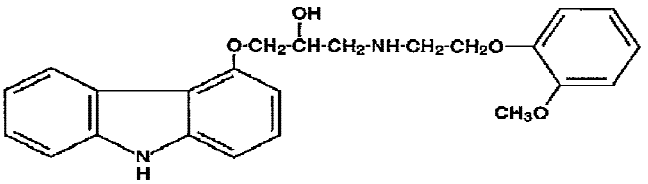

Methods

Preparation of Crystalline/Amorphous Blends

Drug substance, lot C, was heated to about 135°C, about 20°C above its melting temperature and held at that temperature in a vacuum oven until the drug substance was completely in the molten state. The sample was quickly removed and allowed to cool to ambient temperature in a desiccator. The cooled drug substance was triturated and passed through a #60 mesh sieve. Two grams of this sample was blended with 6.0 g of crystalline sample in a Turbula blender to produce a 75:25 blend. Other blends, 90:10 to 99:01, were produced using this blend and crystalline material by serial dilution.

Thermogravimetric Analysis (TGA) and Modulated DSC

TGA was performed on Perkin-Elmer 7 series Thermal Analysis System. The samples were heated at a rate of 10°C/min. Modulated DSC thermograms were generated using a TA Instruments 2920 Modulated DSC in unsealed aluminum pans under a nitrogen purge. The following modulated DSC testing protocols were used in order to determine the optimum conditions to quantify low levels of the amorphous phase in crystalline/amorphous blends: (a) underlying heating rate, 5°C/min with a modulation amplitude of ±1.0°C and a period of 60 s; (b) heating rate, 2°C/min with a modulation amplitude of ±1.0°C and a period of 30 s; and (c) heating rate, 2°C/min with a modulation amplitude of ±0.16°C and a period of 30 s. From their complex specific heat versus temperature relationships, glass transition temperature previously defined, T_g, was calculated.

Moisture Sorption Behavior

The moisture sorption behavior of the drug substance was obtained using the VTI microbalance, VTI MB-300W. The sample (50–100 mg) was placed on the microbalance pan, surrounded by a thermal jacket used for controlled thermal scanning. The sample was first dried at 60°C using an equilibrium weight change criterion of "no more than 5 μg weight change in a 5 min sampling interval" over three consecutive measurements. The apparatus was programmed for moisture sorption from 0% to 90% relative humidity (RH) and desorption from 90% to 10% RH in 5% RH steps at 25° C with the same equilibration criterion as used in the drying cycle.

Powder XRD

Qualitative XRD. Powder XRD studies were performed by filling samples of the drug substance into a specimen holder by a side drift method and exposing the sample to CuK_α radiation (45 kV × 30 mA) in a wide-angle X-ray diffractometer (model D500, Siemens). The instrument was operated in the step scan mode in increments of 0.05° 2Θ. The angular range was 5–40° 2Θ, and counts were accumulated for 1 s at each step.

Amorphous Content by XRD. For a quantitative XRD run, samples of the drug substance (crystalline, amorphous, or crystalline/amorphous blend) and LiF (internal standard) were prepared by mixing 240 mg drug substance (crystalline, amorphous, or crystalline/amorphous blend) and 60 mg LiF using the geometric mixing method. The mixtures were loaded into the aluminum XRD holders by the side-drift method and exposed to CuK_α radiation (45 kV × 30 mA) in a wide-angle X-ray diffractometer (model D500, Siemens). The instrument was operated in the step scan mode in increments of 0.05° 2Θ in the angular range of 5–40° 2Θ. From these XRDs, two crystalline peaks of the drug substance at 5.2 and 14.9° 2Θ and two peaks of LiF at 38.8 and 45.0° 2Θ were chosen for the quantitative determination of low levels of the amorphous phase in otherwise crystalline samples. Four angular ranges were scanned in the step scan mode in increments of 0.01°: 5.0–6.8° 2Θ (drug substance peak, hereafter referred to as peak A1), 14.1–16.2° 2Θ (drug peak referred to as peak A2), 37.5–39.4° 2Θ (LiF peak referred to as peak A3), and 44.1–45.7° 2Θ (LiF peak referred to as peak A4). The counts were accumulated for 1 s at each step. The data collection and analysis program used was JADE 3.0. The integrated intensities of the peaks in the four angular regions were calculated after appropriate background subtraction. The ratios of A1:A3, A1:A4, A2:A3, and A2:A4 were calculated for each XRD run. Two to four replicate tests were run for each sample.

Thermally Stimulated Current Spectrometry

TSC studies were performed on powder samples in the form of ~1 mm thick hand-pressed disk placed between the electrodes of Thermal Stimulated Current 9000 Spectrometer (TherMold Partners, Stamford, CT). An insulating film of polyimide (Kapton® of E. I. Dupont de Nemours, Wilmington, DE) was inserted between the sample and the electrode, which would block ohmic current. Consequently, only the current generated due to segmental molecular motions in the amorphous phase was observed. All tests were performed using the following test protocol: polarization at 70°C for 5 min by applying a DC electric field at 100 V/mm to orient molecular dipoles; rapidly cooling the sample to 0°C while maintaining the electric field to trap the polarized dipoles; short circuiting the electrodes for 1 min; and scanning the sample at 7°C/min up to 110°C while monitoring the current generated due to the relaxation of polarized dipoles.

RESULTS AND DISCUSSION

Amorphous Material

From the powder XRD pattern of the milled amorphous material presented in Fig. 1, it was obvious that the melt-quenched material was largely amorphous. The thermal data were also consistent with this conclusion. The total degradation levels determined using a validated stability-indicating HPLC method were about 0.2% for both the starting crystalline and the melt-quenched samples. The chemical stability of the amorphous material stored in a tightly closed bottle for over 6 months was established by HPLC, while its physical

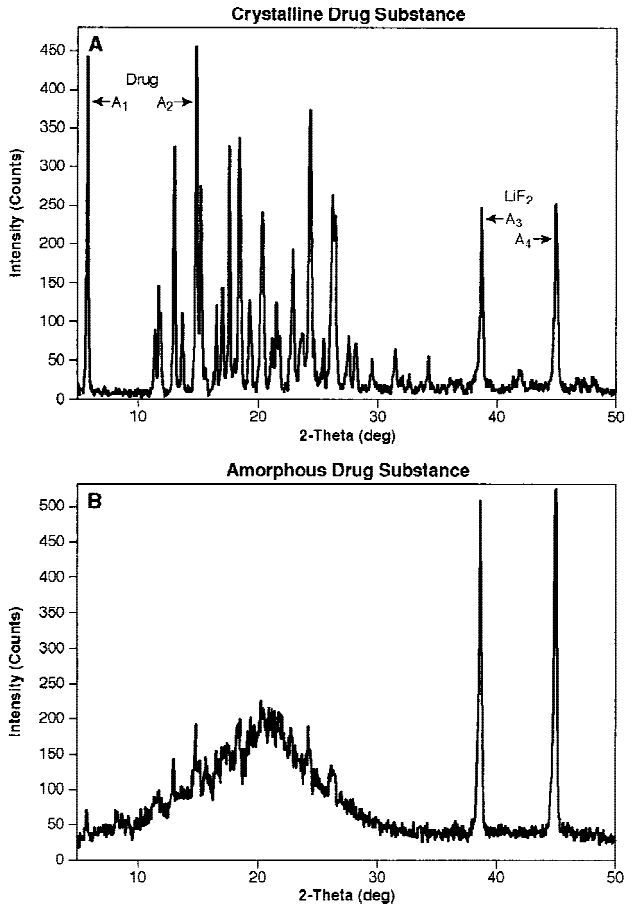

Fig. 1. Powder XRD of carvedilol drug substance: (A) ~100% crystalline material and (B) ~100% amorphous material.

stability, i.e., the absence of significant recrystallization, was established by modulated DSC.

Crystalline Samples

Thermal Analysis (DSC and TGA)

The six lots of the drug substance exhibited similar DSC profiles. The modulated DSC showed only one event at about 116°C ascribed to the melting of the drug substance. The endotherms of all the samples showed similar onset temperatures and also, similar values of melting enthalpies. The TGA results of all lots showed a small weight loss (<0.1%) due to desorption of surface moisture between 25 and 150°C. However, the moisture sorption data indicated that the drug substance lot A behaved differently, i.e., it picked up more moisture (about 0.3%) in comparison to that of the other lots (~0.1%). This suggested that this drug substance lot had some amorphous phase, which was in agreement with the XRD and TSC observations (to be discussed in later sections). The discrepancy between the TGA data and those of XRD, TSC spectrometry, and moisture sorption measurements could be attributed to the lack of sensitivity of the TGA balance while monitoring fractional changes in sample weights of the order of 4 mg.

Powder XRD

All XRD patterns for crystalline samples appeared to be identical except for lot A, which showed the presence of some amorphous phase. Although XRD is widely used for quantification of crystalline fractions in pharmaceutical materials, its ability to detect the amorphous phase is beset with problems due to indirect inference, orientation effects, and instrument-related intensity variations. Recently, the use of a pattern subtraction approach enabled identification of the crystalline drug substance even when it constituted only 5% w/w of the formulation (16). However, in the present study, large variations in the integrated peak intensity ratios of A2:A3 and A2:A4 were observed in almost 100% crystalline samples. For example, the peak intensity ratio A2:A3 varied from 1.835 to 2.30, while the ratio A2:A4 varied from 1.44 to 2.02 for almost 100% crystalline samples. These variations were not likely due to the crystal orientation effect alone. The values of the median particle size for all the lots were in the range of 7.92–14.3 μm. The internal reference standard, LiF, also had a median particle size of approximately 5 μm. Furthermore, the side drift filling procedure has been shown to minimize orientation effects. One other factor that could also partly account for the observed variability was the spatial separation of the sample and reference XRD peaks, which were measured in the region of 5–16° and 37–46° 2Θ, respectively.

Crystalline/Amorphous Blends

Quantitative XRD

The standard curves were generated for 100% crystalline sample (lot C), 100% amorphous material, and 75:25, 90:10, 95:05, 98:02, and 99:01 samples. On the basis of the calculated correlation coefficients, the ratio of A2:A3 was found to be most reproducible. The next was A2:A4, while the ratios A1:A3 and A1:A4 were least reproducible. The XRD peak area ratios, A2:A3 and A2:A4 are presented in Fig. 2 as a function of the amorphous content. The inclusion of the 100% crystalline lot C and the 99/01 blend significantly affected the correlation coefficients. The linear relationship for 2–25% w/w amorphous materials (correlation coefficient: 0.96) used to determine the amount of amorphous phase in a blinded sample (sample labeled as *xx:xx*) yielded a value between 1.5 and 3.1%, which agreed with the actual value of 2.0%. However, the amorphous contents predicted for the crystalline samples varied from 3.9 to 22.9%. No evidence could be found from any other technique including TSC to confirm these predictions. Hence, the conclusion was that the current quantitative XRD approach was not successful in properly quantifying the crystallinity of almost 100% crystalline samples, although the most logical reference peaks were chosen for estimation.

Modulated DSC

The quantification of the amorphous phase by T_g measurement in a conventional DSC which records the total heat flow is generally complicated by the presence of an enthalpic relaxation endotherm positioned directly over the T_g event. In contrast, the modulated DSC has the ability to separate reversing (such as glass transition event, T_g) and nonreversing (such as melting and crystallization events) heat signals; hence, it has the capability of providing a clearer visualization of T_g. However, a proper choice of the experimental parameters, sinusoidal (modulated) heating signal (amplitude), its

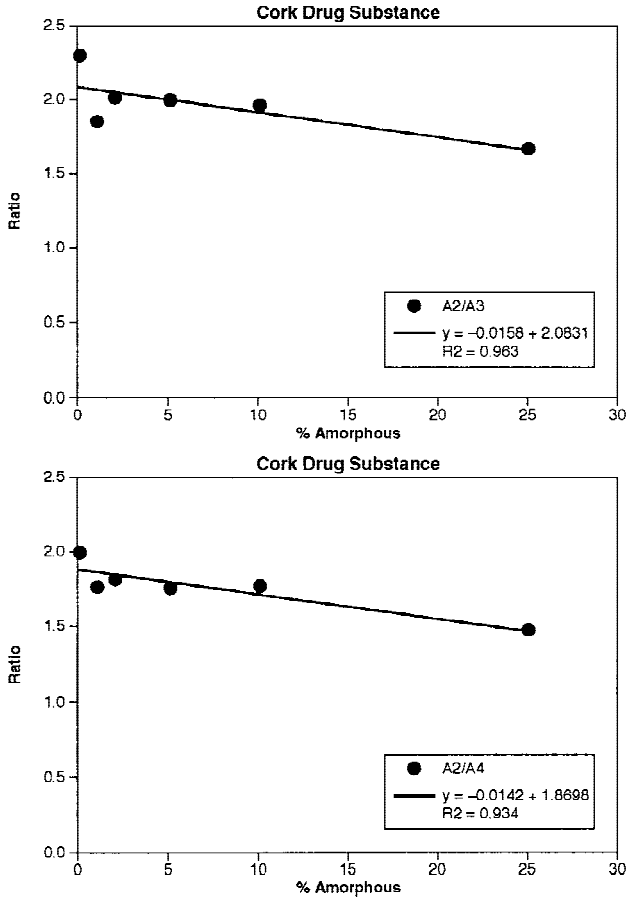

Fig. 2. X-ray diffraction peak area ratios for A2:A3 and A2:A4.

duration/period, and underlying linear heating rate, must be made in order to allow reliable deconvolution of the data (17,18). Figure 3 shows the complex heat capacity signals obtained for the 75:25 crystalline/amorphous blend at the three experimental protocols used. Identical glass transition events were obtained only at the heating of 2°C/min with an amplitude of ±1.0°C and a period of 30 s. Similar results were obtained for the 90:10 crystalline/amorphous blend. Figure 4 shows the complex heat capacity data obtained at the above-

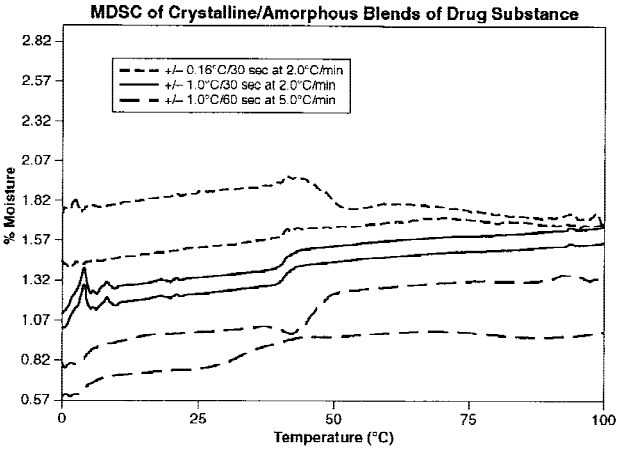

Fig. 3. Complex specific heat plots for 75:25 crystalline/amorphous blend.

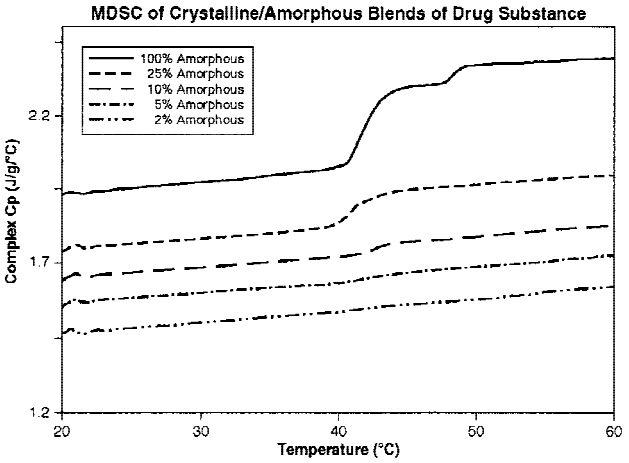

Fig. 4. Complex specific heat plots for crystalline/amorphous blends.

mentioned experimental protocol for different crystalline/amorphous blends. The shift in the heat capacity signal at the glass transition temperature for 95:05 crystalline/amorphous blend was hardly visible. This observation suggested a limit of detection of the amorphous content at ~5%.

Moisture Sorption/Desorption

Figure 5 shows the moisture sorption/desorption behavior exhibited by the crystalline/amorphous blends. With increasing amorphous content, both moisture pickup during sorption and retention during desorption increased. Linear relationships, each with a high degree of correlation, obtained for the moisture sorbed at 90% RH and retained at 60% RH with increasing amorphous content are presented in Fig. 6. The moisture sorption/desorption behavior appeared to be a sensitive parameter for the detection/quantification of the amorphous phase in crystalline materials. The estimated lowest level of detection was ~5%.

Thermally Stimulated Current Spectrometry

Figure 7 shows the TSC spectra of two lots of the drug substance, which were ~100% crystalline. Each lot of the drug substance exhibited a depolarization peak centered at 77–

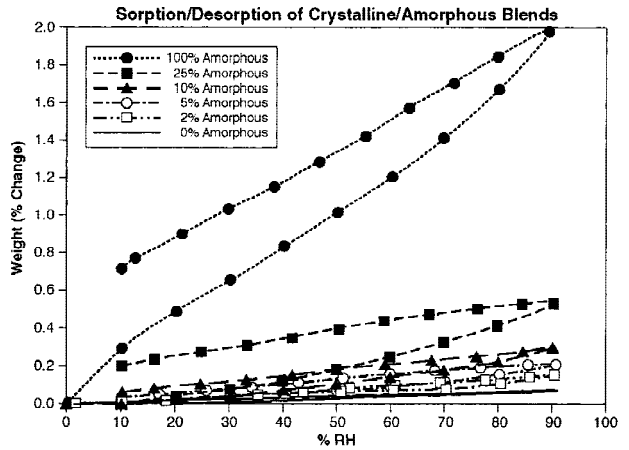

Fig. 5. Moisture sorption/desorption behavior for crystalline/amorphous blends.

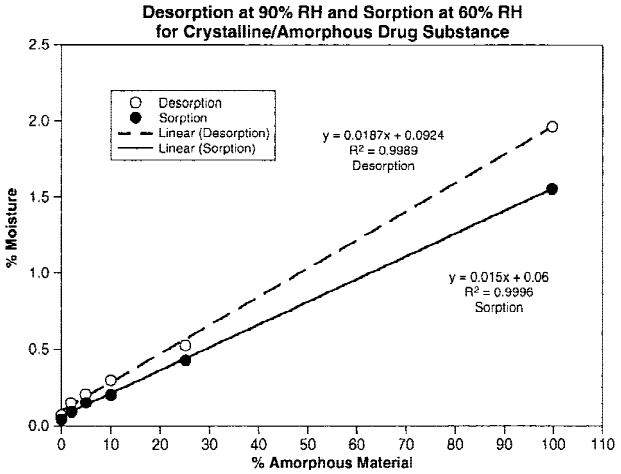

Fig. 6. Moisture sorption at 90% RH and desorption at 60% RH for crystalline/amorphous blends.

81°C. Figure 8 shows the TSC spectra for 75:25, 90:10, and 98:02 crystalline blends. Each spectrum is composed of two events; one is the T_g event while the other is the polarization peak. At higher amorphous contents (≥5%), the glass transition event appeared as a peak on the low temperature side of the polarization peak. At lower amorphous contents (≤5%), the T_g appeared as a shoulder. It appeared possible to achieve a limit of detection of ~1.0%. The estimated amorphous content for the blinded sample was between 2 and 3%,

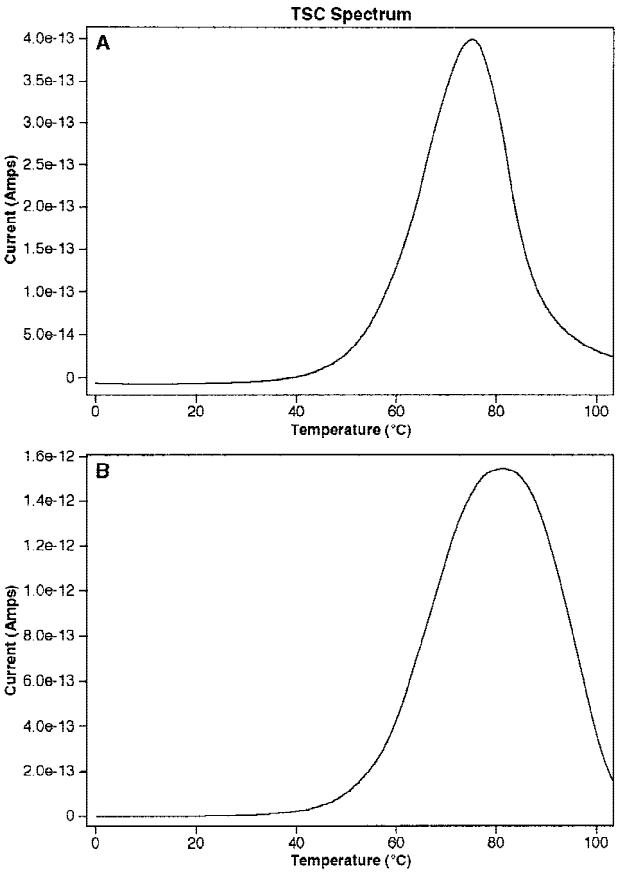

Fig. 7. TSC spectra of ~100% crystalline samples: (A) lot C and (B) lot E.

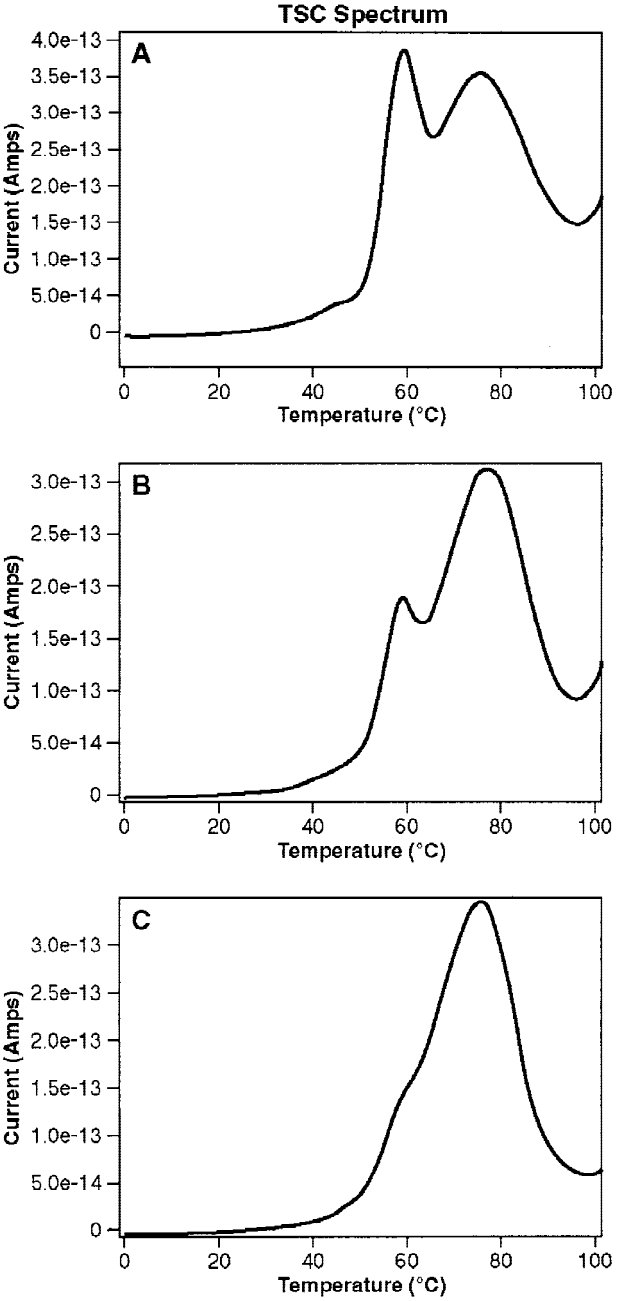

Fig. 8. TSC spectra of crystalline/amorphous blends: (A) 75:25, (B) 90:10, and (C) 98:02.

while the actual value was 2%. The TSC measurements performed by polarizing at 90°C for all the blend samples resulted in similar observations. However, the TSC measurement performed by polarizing at 60°C for the 98:02 blend resulted in a similar but less pronounced T_g event; furthermore, the glass transition event was observed at a lower temperature. Galop and Collins (19) developed a procedure to calculate the normalized contribution of the glass transition relaxation by subtracting from the TSC curve a fitted polynomial curve (degree higher than 6) using the data outside the 45–65°C window. A linear relationship between the area under the glass transition curve vs. the percent amorphous content with a high degree of correlation was established for these carvedilol samples (19).

Comparison of TSC vs. Other Techniques

The moisture sorption and quantitative XRD measurements have shown the capability of detecting the amorphous contents at or above 5% w/w. Glass transition events measured by TSC are similar to those measured by conventional or modulated DSC. However, TSC offers not only the flexibility of quantitatively determining low levels of the amorphous content by polynomial curve fitting, but also enhancing the response by increasing the stimulus, i.e., by applying increasing electric fields. In the present study, the TSC technique consistently detected or estimated the presence of the amorphous content at 2%.

CONCLUSIONS

The usefulness of analytical techniques, such as modulated DSC, powder XRD, moisture adsorption, and TSC spectrometry, was evaluated for a qualitative/ quantitative estimation of low levels of the amorphous phase in predominantly crystalline pharmaceutical materials. The TSC measurement appears to have the capability of providing a clearer visualization of the presence of low levels of the amorphous material, which manifests as a glass transition event, T_g.

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