A Mechanistic Investigation of an Amorphous Pharmaceutical and Its Solid Dispersions, Part I: A Comparative Analysis by Thermally Stimulated Depolarization Current and Differential Scanning Calorimetry

Rama A. Shmeis,[1,2,3] Zeren Wang,[1,2] and Steven L. Krill[1,2]

Received April 13, 2004; accepted July 15, 2004

Purpose. To explore using thermally stimulated depolarization current (TSDC), in comparison to differential scanning calorimetry (DSC), for the characterization of molecular mobility of an amorphous pharmaceutical new chemical entity (LAB687), an amorphous polymer (PVPK-30), and their combination as solid dispersions at different % drug loadings.

Methods. Amorphous drug was prepared by quenching from the melt. Solid dispersions containing 10–60% of drug in polymer were prepared by solvent evaporation method. Glass transition temperatures (T_g) were determined by DSC and TSDC.

Results. In comparison to a single T_g obtained from DSC for the drug substance, TSDC shows two overlapping relaxations. Both peaks correspond to α-relaxations that are associated with the glass transition, with the second peak corresponding to the rigid fraction that is difficult to be detected by DSC because it is associated with only small changes in heat capacity. Two overlapping relaxations were also observed for the polymer vs. one T_g by DSC. The lower temperature relaxation is believed to be a β-relaxation, whereas the higher temperature transition corresponds to an α-relaxation. For the solid dispersions, one single peak was obtained for each of the 20% and 30% dispersions in excellent agreement with the DSC results. However, at the 40% drug load, a small shoulder was observed by TSDC at the low temperature of the main peak. This shoulder becomes more pronounced and overlaps with the main peak as the drug load increases to 50% and 60%. Agreement between the T_g values calculated by the Gordon-Taylor equation and the DSC and TSDC experimental data, especially for the 20% and 30% drug loading, indicate ideal miscibility. At higher drug loads, only by TSDC was it possible to detect the saturation level of the drug in the polymer.

Conclusions. TSDC proved to be very sensitive in detecting small reorientational motions in solids and in separating overlapping events with only slight differences in molecular motion exhibited as broad events in DSC. This allowed for detection of the rigid fraction of the amorphous drug, the sub-glass transition β- relaxation in the polymer, and the limit of miscibility between the drug and the polymer in the solid dispersions.

KEY WORDS: amorphous systems; differential scanning calorimetry (DSC); glass transition; solid dispersions; thermally stimulated depolarization current (TSDC).

[1] Pharmaceutical and Analytical Development, Novartis Pharmaceutical Corporation, East Hanover New Jersey 07936, USA.

[2] Present address: Pharmaceutics, Boehringer-Ingelheim Pharmaceuticals, Inc., Ridgefield Connecticut 06877, USA.

[3] To whom correspondence should be addressed. (e-mail: rshmeis@rdg.boehringer-ingelheim.com)

INTRODUCTION

Preparation of pharmaceutical materials in an amorphous form has been recognized as an approach to enhance product performance such as dissolution behavior and bioavailability (1). However, the amorphous state is a nonequilibrium state (thermodynamically unstable) and will tend to revert to the crystalline form on storage (1,2). This could result in poor product performance over time.

Several investigators have reported that the origin of instabilities might be attributed to the molecular motions that can still exist below the glass transition temperature (T_g) (2,3). Long time-scales of molecular motions and the heterogeneous nature of glassy systems makes direct experimental measurements of relaxation times (indicators of molecular mobility) below T_g difficult to obtain.

Differential scanning calorimetry (DSC) is a thermal analytical technique that is routinely used not only for the characterization of active drug substance properties but also for excipients and their combinations with the active as formulations and finished products. Due to the complexities of systems constituted of more than one or two components and due to sensitivity limits dictated by the principle of measurement, there are limitations to the information provided by this technique especially in the area of amorphous systems.

Thermally stimulated depolarization current (TSDC) is a dielectric thermal technique which was first used to investigate ionic motion in crystals (1964) and since 1967 has been used widely to study the dynamics and molecular motions in semicrystalline and amorphous polymers (4). Important features of this technique are its low equivalent frequency and high resolving power [probes a time window between 25 and 3000 s which corresponds to a frequency window between 5×10^{-5} and 6×10^{-3} Hz (5) and detects currents as small as 5×10^{-15} Amps (6)]. This reveals low-frequency molecular motions, enhances the resolution of complex and overlapping processes, and may provide better sensitivity to glass transition and sub-glass transition relaxations, making TSDC particularly suited to investigations of slow re-orientational molecular motions and mobility in amorphous solids composed of one or more components.

In the pharmaceuticals arena, this technique is relatively new. Our interest in exploring and applying TSDC to pharmaceutical systems is timely with the continuous emergence of new chemical entities that are increasingly poorly water soluble. One of the formulation approaches mostly researched to meet the challenge of developing such compounds is to explore using amorphous drug alone or incorporated into a polymer as a solid dispersion to enhance solubility and dissolution. This makes the need to use highly sensitive techniques capable of investigating the glass transition and sub-glass transition mobility more urgent than ever. A more thorough understanding of amorphous system characteristics will ultimately lead to successful predictions of physical stability and optimal design of amorphous formulations. Currently this ability is not available and thus limits the utilization of these drug delivery systems.

Accordingly, the main purpose of this work is to explore applying TSDC, in combination with DSC, to investigate pharmaceutically relevant problems in the area of molecular motions and stability of amorphous systems. Part I, the sub-

ject of this paper, is a comparative analysis between DSC and TSDC in characterizing molecular mobility of an amorphous pharmaceutical new chemical entity, a polymer frequently used in the pharmaceutical industry, and the impact of combining the drug with the polymer as solid dispersions at different % drug loading.

Part II analyzes and discusses in depth molecular mobility, relaxation times, and activation thermodynamic parameters of the same systems and is presented in the second part of this work (7).

MATERIALS AND METHODS

Materials

The drug substance LAB687 (Scheme 1), form D, purity 99.9% by HPLC, was provided by Novartis Pharmaceutical Corp. (East Hanover, NJ, USA). The polymer Kollidon 30 powder (polyvinylpyrrolidone PVPK-30; Scheme 2) was purchased from BASF (Mt. Olive, NJ, USA). The solvent ethyl alcohol (200 proof, USP grade) was purchased from Pharmco Products Inc. (Brookfield, CT, USA), and the solvent dichloromethane (99.8% HPLC grade) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA).

METHODS

Preparation of the Amorphous Form of LAB687

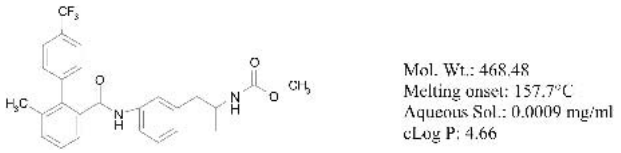
Amorphous LAB687 was prepared by melting the crystalline form (melting point 157.7°C) in a thermally resistant container and quenching with liquid nitrogen. The samples were stored in a desiccator containing anhydrous CaSO4. HPLC analysis (Waters 2695 separation module equipped with Waters photodiode array detector 2996, Waters Corp., Milford, MA, USA) revealed no evidence of any thermal degradation.

Preparation of Solid Dispersions

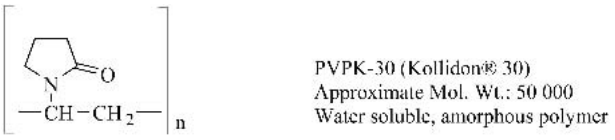
Solid dispersions containing 10–60% of LAB687 in PVPK-30 were prepared by the solvent evaporation method. LAB687 in amounts varying between 100 mg and 600 mg and various amounts of PVPK-30 (between 900 mg and 400 mg) were dissolved in 20 ml of dichloromethane:ethyl alcohol (23:77) at room temperature. The solvents were removed using a rotary evaporator (Büchi R-200, Büchi Labortechnik AG, Flawil, Switzerland) at 50°C. The remaining solid was dried in a vacuum oven at 40°C for 12 h and then ground slightly in a mortar, passed through 60 mesh sieve, and stored under refrigeration (5°C) until time of use (1–4 days).

Differential Scanning Calorimetry

A Mettler Toledo differential scanning calorimeter (DSC-30, Mettler-Toledo, Inc., Columbus, OH, USA) equipped with a computer analyzing system (STAR^e Program) was used to determine glass transition temperatures.



Scheme 1. Structure of LAB687.



Scheme 2. Structure of polyvinylpyrrolidone, PVPK-30.

Samples weighing 5–10 mg were placed in sealed aluminum DSC pans with a pinhole to prevent pressure buildup and heated in a flowing atmosphere of nitrogen (50 ml/min.).

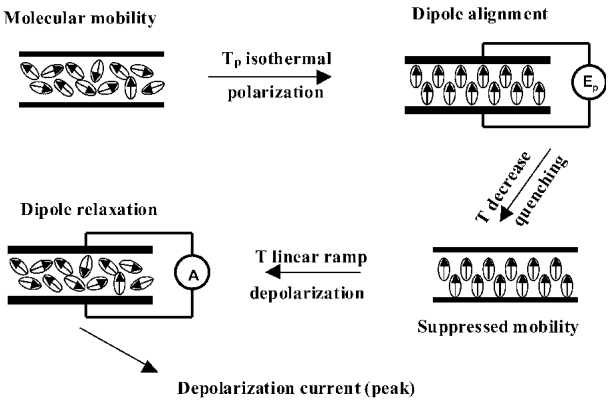
All samples were given similar thermal histories by linearly heating to 190°C at 7°C/min and cooling at 50°C/min to −10°C prior to measurement. Samples were then heated again to 190°C at 7°C/min.

Thermally Stimulated Depolarization Current

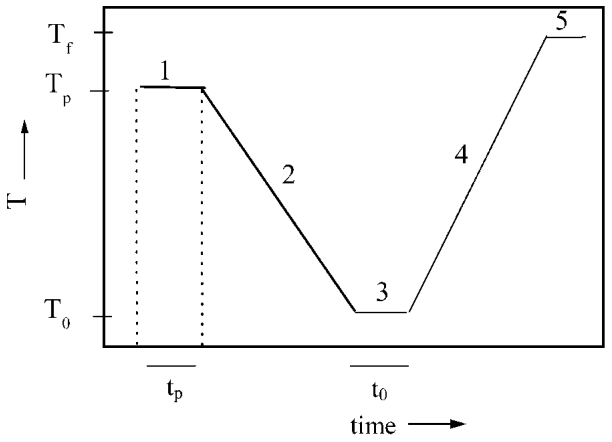
The principle of TSDC is to orient polar molecules or pendant polar groups by polarizing a sample placed between the electrodes of a parallel plane capacitor and applying an electric field at a given temperature for a given time. The orientation is then "frozen-in" by quenching the material to a much lower temperature where molecular motion ceases. Subsequent heating (with the polarizing field removed) causes the oriented dipoles to relax and this relaxation motion generates a depolarization current (peak) that can be related directly to molecular mobility. This is demonstrated in Scheme 3. The output of a TSDC experiment is a peak corresponding to the depolarization current intensity as a function of temperature.

TSDC experiments were conducted according to the procedure shown in Scheme 4.

Step 1 is the polarization step during which the sample is held for a certain amount of time (t_p) at a given temperature (T_p) under the effect of an electric field. This step orients the dipoles within the molecular structure. Because molecular mobility increases as the temperature increases, the nature and the amount of polarization created by the field will depend on the polarization temperature. Step 2 is the cooling step during which the sample is cooled to a given temperature (T_0), in the presence of the electric field. The purpose of this step is to freeze-in the dipolar orientation, that is, to retain (at least partially) the polarization created by the electric field at



Scheme 3. Principle of thermally stimulated depolarization current (courtesy of TherMold Partners, personal communication).



Scheme 4. Representation of TSDC global experiment procedure where T_P is polarization temperature, t_P is polarization time, T_0 is freezing temperature, and T_f is final temperature to which a sample is heated.

the polarization temperature. In step 3, the polarizing electric field is removed and the sample is held for a certain amount of time with no field (t_0). Some of the polarization will dissipate and some will be retained. Relaxation time of the molecular motions in general is temperature dependent in such a way that it increases with decreasing temperature. Thus, the retained polarization corresponds to dipolar motions that were activated by the electric field at the polarization temperature and whose characteristic time [relaxation time $\tau(T)$] is sufficiently temperature dependent to give rise to a "freezing-in" of the polarization. That is, the retained polarization contains the contribution of the molecular motions that are relatively fast at T_p but that become slower than the time scale of the experiment at T_0. The state of the sample at the end of the freezing-in step of the TSDC experiment is thus a nonequilibrium state, where the depolarization (that is due to molecular motion) is prevented for kinetic reasons. During the cooling phase (step 2), the temperature interval $\Delta T = T_p - T_0$, where T_0 is the temperature to which a sample is cooled before starting the linear heating ramp, is wide. The polarization created in the sample by this step will encompass a wide variety of dipole motions or a wide distribution of relaxations. These experiments are called global experiments. The global experiments are used in order to detect and localize the different relaxations in the TSDC spectrum, whereas in another procedure called thermal windowing [discussed in part II (7)], the experiments are performed in order to study the detail of each complex relaxation (distribution of enthalpies and entropies of activation).

Finally, the last step (step 4) is a constant rate linear heating step where the relaxation time of the molecular motions decreases allowing the return of the sample to the equilibrium state (depolarization step). This gives rise to a small intensity electric current (I), which is measured as a function of temperature (peaks) and constitutes the experimental output of a TSDC experiment.

In this work, TSDC experiments were carried out using a TSC/RMA 9000 instrument (TherMold Partners, Stamford, CT, USA) equipped with a computer analyzing system (TSC 9000 Analysis). Samples (3–5 mg) were weighed into aluminum DSC pans, covered with a small piece of Teflon, and placed between the electrodes of a parallel plane capacitor that was then shielded by a Faraday cage and evacuated to 10^{-4} mbar and flushed several times with 1.1 bar of high-purity helium prior to experiments. All samples were given similar thermal histories prior to measurement as was done for the DSC experiments. In all experiments, polarization time (t_p) = 2 min, polarizing field intensity (E)= 300 $V \cdot mm^{-1}$, freezing temperature (T_0) = −10°C, holding time at the freezing temperature (t_0) = 1 min., heating rate (r) = 4°C/min, and the final temperature to which a sample was heated (T_f) = 200°C. Cooling was conducted using liquid nitrogen connected to the Faraday cage according to the Newtonian cooling mode, which allows the sample to reach the freezing temperature T_0, as fast as possible (≥20°C/min). Values of the experimental parameter that varied between experiments, that is, T_P, the polarization temperature, will be presented in the "Results and Discussion" section under the respective figures. Polarization temperature for each material was selected such that it was 15–30°C higher than the glass transition temperature obtained by DSC to ensure that all molecular dipolar motions below, at or above at the glass transition detected by DSC if any were activated.

RESULTS AND DISCUSSION

DSC results exhibit single glass transition temperatures (T_g) obtained for the amorphous drug substance LAB687 (71.0°C) as well as for the polymer PVPK-30 (168.0°C) as shown in Fig. 1. A single T_g was also obtained for all the solid dispersions tested (10–60% loading), which shifted systematically toward that of the drug as the ratio of drug/polymer increased (Fig. 1). This behavior is indicative of miscibility at the molecular level, that is, the formation of solid solutions.

The global spectrum of amorphous LAB687 obtained by TSDC is demonstrated in Fig. 2. In comparison to a single T_g obtained from DSC at 71.0°C (7°C/min heating rate), TSDC global spectrum shows two overlapping relaxations at 77.1°C and 92.0°C in the temperature range probed (–10 to 190°C at 4°C/min heating rate). The first peak corresponds to an α-relaxation that is associated with the glass transition event and in agreement to what was obtained by DSC. The second apparent T_g, is possibly also an α-relaxation that corresponds to the rigid fraction of the amorphous drug. Due to rigidity, this transition is expected to be associated with only small changes in the global motion, leading to small changes in heat capacity that are difficult to be detected by DSC. Similar behavior was reported for other molecules that are rigid in the glassy state

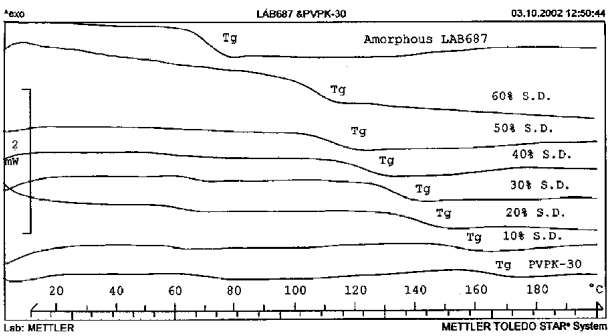


Fig. 1. DSC thermograms of amorphous LAB687, PVPK-30, and their solid dispersions at drug loads of 10–60% (open pan, 7°C/min).

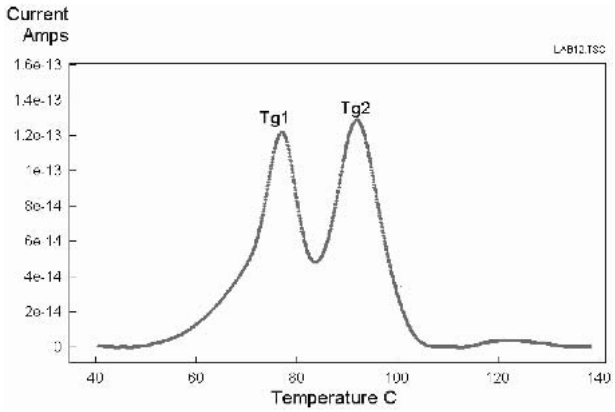


Fig. 2. TSDC thermogram of amorphous LAB687 obtained from global experiment. The polarization temperature was $T_P = 100°C$ with polarization time $t_P = 2$ min at an intensity of the polarizing field E = 300 V/mm. The freezing temperature to which the sample was cooled (quenched) was $T_0 = -10°C$ with a holding time at this temperature (t_0) = 1 min, after which the sample was heated to a final temperature $T_f = 200°C$ at a heating rate r = 4°C/min.

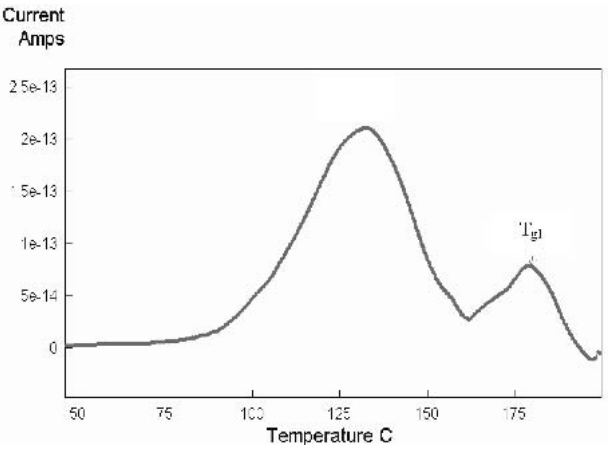


Fig. 3. TSDC thermogram of PVPK-30 obtained from global experiment. The polarization temperature used was $T_P = 180°C$ for a polarization time $t_P = 4$ min at an intensity of the polarizing field E = 300 V/mm. The freezing temperature to which the sample was cooled down (quenched) $T_0 = -10°C$ with a holding time at this temperature (t_0) = 1 min, after which the sample was heated to a final temperature $T_f = 200°C$ at a heating rate r = 4°C/min.

(strong glasses) that did not show a glass transition when tested by DSC [β-cyclodextrin and HP-β cyclodextrin (8)]. Because TSDC is capable of measuring currents as low as 10^{-15} A (6), it is very sensitive in detecting small reorientational motions in solids and therefore can provide more detail about the heterogeneity of these amorphous systems, and hence, two peaks were detected for the relaxation of the amorphous drug substance. Both peaks are somewhat sharp and asymmetric, showing a gradual increase followed by a sharp decrease in current intensity. This pattern corresponds to an α-transition as described by Correia *et al.* (5). In addition, calculation of the kinetic parameters (activation enthalpy and entropy) using the thermal windowing procedure (TW) suggests that the second transition is indeed an α-relaxation as presented in part II of this work (7). Two peaks for α-transition indicates a spatial non-uniformity, that is, relaxations occurring in a variety of microenvironments for the rigid and less rigid components.

The PVPK-30 TSDC global spectrum (Fig. 3) is similar to the drug substance with two overlapping relaxations, at 132.3°C and 178.9°C, whereas only one T_g was observed by DSC at 168.0°C. As opposed to being an α-relaxation as is the case for the drug alone, the lower temperature relaxation is possibly a β-relaxation, whereas the higher temperature transition corresponds to the glass transition (α-relaxation). In the TSDC spectrum, β-relaxation usually appears as a broad relaxation that is observed over a large temperature interval compared with the α-relaxation (5), which is narrower. Furthermore, thermal windowing (TW), (7) experiments allows each peak to be more fully characterized and supports this interpretation.

As for the solid dispersions of LAB687 and PVPK-30, the global TSDC spectra (Fig. 4) show one single peak obtained for each of the 20% and 30% dispersions, with a maximum intensity at 145.2°C and 137.2°C, respectively. This is in agreement with the results obtained from DSC (T_g = 143.4°C and 134.5°C, respectively). At the 40% drug load, a single peak is also observed (134.3°C) in addition to a small shoulder at the low temperature end (123°C). This shoulder becomes more pronounced and overlaps with the main peak as the drug load increases to 50% and 60% (Fig. 4). Glass transition values for the drug, polymer and their solid dispersions obtained from DSC and TSDC are summarized in Table I.

The Gordon-Taylor equation, which is frequently used to evaluate the glass transition of miscible mixed amorphous systems, was used to analyze both the DSC and TSDC data summarized in Table I. This relationship assumes that the two components are ideally miscible and that the free volumes of the components are additive:

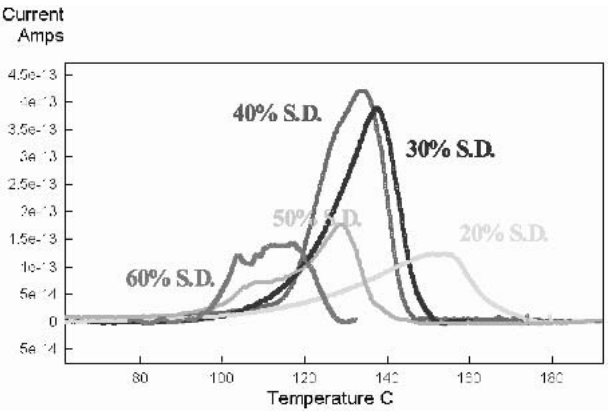


Fig. 4. TSDC thermograms of solid dispersions of LAB687/PVPK-30 obtained from global experiments. The polarization temperatures used were $T_P = 160°C$ for the 20% S.D., 150°C for the 30% S.D., 145°C for both the 40% and 50% S.D.s, and 140°C for the 60% S.D. The other experimental parameters were the same for all solid dispersions: polarization time $t_P = 2$ min at an intensity of the polarizing field E = 300 V/mm. The freezing temperature to which the samples were cooled down (quenched) $T_0 = -10°C$ with a holding time at this temperature (t_0) = 1 min, after which samples were heated to a final temperature $T_f = 200°C$ at a heating rate r = 4°C/min.

Table I. Comparison Between Glass Transition Temperatures Obtained from DSC and TSDC

Material	Glass transition temperature (°C)	
	DSC	TSC
PVPK-30	168	132.3, 178.9
20% S.D.	143.4	145.2
30% S.D.	134.5	137.2
40% S.D.	125	123 (shoulder), 134.3
50% S.D.	114.5	126.7, 108.8
60% S.D.	107.3	115, 103.73
LAB687	71.0	77.1, 92.0

$$T_{g12} = \frac{w_1 T_{g1} + K w_2 T_{g2}}{w_1 + K w_2} \quad (1)$$

$$K \approx \frac{T_{g1}\rho_1}{T_{g2}\rho_2} \quad \text{or} \quad \frac{\Delta C_{p2}}{\Delta C_{p1}} \quad (2)$$

where T_{g12} is the glass transition of the mixture, w_1 and w_2 are the mass fractions of components 1 (drug) and 2 (polymer), ρ_1 and ρ_2 are the true densities of components 1 and 2, and ΔC_{P1} and ΔC_{P2} are the changes in heat capacity at T_{g1} and T_{g2} of components 1 and 2, respectively. The K value in this work was calculated from the changes in the heat capacities at the glass transition temperatures of the polymer and the drug and was found to be 0.86.

The DSC data agrees well with the values calculated by the Gordon-Taylor equation, indicating ideal miscibility between the drug and the polymer at the drug loads tested (10–60%) as demonstrated in Fig. 5. In comparison, Fig. 6 shows the TSDC experimental data and the values calculated by the Gordon-Taylor equation compared to the DSC results. There is very good agreement between the calculated values and the DSC and TSDC data, especially for the 20% and 30% drug loading (only one peak was observed), given that the heating rates are slightly different (7°C/min vs. 4°C/min for the DSC and the TSDC experiments, respectively). At higher loading (≥40%), a second peak was detected only by TSDC, indicating that the polymer has approached saturation levels

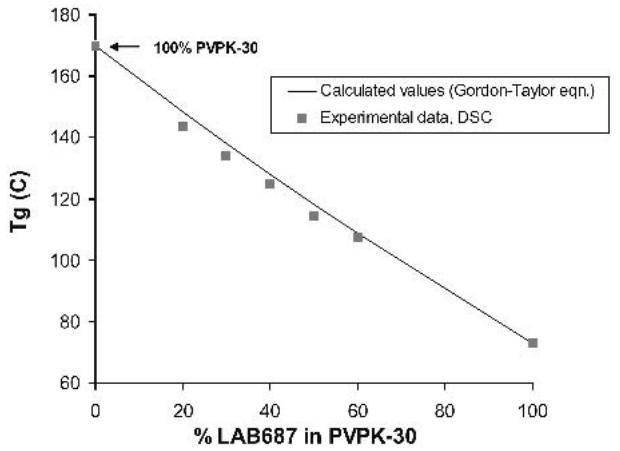


Fig. 5. Gordon-Taylor equation predictions (calculated values) to the glass transition temperatures of amorphous LAB687, PVPK-30, and their solid dispersions (10–60% drug load) measured by DSC.

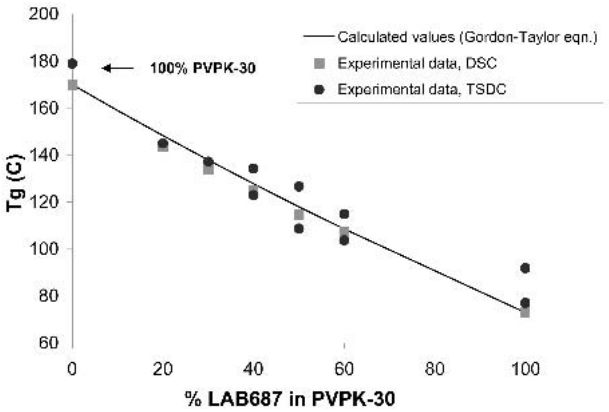


Fig. 6. Gordon-Taylor equation predictions (calculated values) to the glass transition temperatures of amorphous LAB687, PVPK-30, and their solid dispersions (10–60% drug load) measured by TSDC (global experiments) as compared to DSC.

with drug. Due to its high sensitivity, TSDC was able to separate a single DSC event (where a broad glass transition is observed) into two overlapping events having slight differences in microenvironmental molecular motion. Using TSDC, it was possible to detect saturation of the drug in the polymer that was not seen by DSC. It is advantageous to detect the supersaturation of the drug in the polymer at an early stage (i.e., freshly quenched glasses as is the case in this work) because with storage and upon aging, the supersaturated drug will separate from the polymer and due to its higher mobility compared to the molecularly dispersed drug will revert to the crystalline state faster at the same storage temperature. Therefore, having this information early on in development helps in designing the most appropriate storage conditions/temperatures which might not be the case if the storage conditions were chosen based on the higher single T_g value for the solid solution obtained from DSC alone.

The ideal miscibility between the drug and the polymer demonstrated by the linearity of the Gordon-Taylor relationship, especially at the lower drug loading was independently confirmed as exhibited by the values of the activation thermodynamic parameters (activation enthalpy and entropy) as presented in part II of this work (7).

CONCLUSIONS

In this work, for the first time, the molecular mobility of an amorphous pharmaceutical new chemical entity, a polymer frequently used in the pharmaceutical industry and their formulations in solid dispersions, was directly probed as a function of drug loading using TSDC. This technique being capable of measuring currents as low as 10^{-15} A proved to be very sensitive in detecting small reorientational motions in solids and therefore provided more detail about the heterogeneity of both the amorphous drug (LAB687) and the polymer (PVPK-30). Amorphous drug by itself exhibited two overlapping events in the region of glass transition as compared to only one obtained by DSC, Two overlapping relaxations were also observed for the polymer (PVPK-30), where only one transition was observed by DSC. In the later case, TSDC was able to detect the sub-glass transition β-relaxation as well as the α-relaxation.

This was applied not only to single components (drug or polymer) but also to their formulations in solid dispersions at different drug loads where the saturation level of the drug in the polymer at higher drug loads was only detected by TSDC. Overlapping events with only slight differences in molecular motion were separated whereas these were exhibited by a broad event in DSC. Superior sensitivity permitted the limit of miscibility and heterogeneity of the glassy state to be detected.

Part II (7) focuses on characterizing the motional processes in these systems at and below glass transition and on determining the distribution of temperature dependent relaxation times using thermal windowing vs. a single average value, which allows for relevant kinetic parameters to be obtained and used in mechanistically delineating the effects on molecular mobility of incorporating the drug in a polymer. This will ultimately lead to appropriate choices to be made regarding drug loading and storage temperature that would realistically correlate to the physical stability during storage.

ACKNOWLEDGMENTS

George Collins, Ph.D. (NJIT) is gratefully acknowledged for useful and informative discussions about TSDC. Yatindra Joshi, Ph.D., and Weiqin Tong, Ph.D., from Novartis are gratefully acknowledged for continuous support of this work.

REFERENCES

1. B. C. Hancock and G. Zografi. Characteristics and significance of the amorphous state in pharmaceutical systems. J. *Pharm. Sci.* **86**:1–12 (1997).
2. V. Andronis and G. Zografi. Crystal nucleation and growth of indomethacin polymorphs from the amorphous state. *J. Non-Cryst. Solids.* **270**:236–248 (2000).
3. S. L. Shamblin, X. Tang, L. Chang, B. C. Hancock, and M. J. Pikal. Characterization of the time scales of molecular motion in pharmaceutically important glasses. *J. Phys. Chem. B* **103**:4113–4121 (1999).
4. M. Zielinski and M. Kryszewski. Thermal sampling technique for the thermally stimulated discharge in polymers. *Phys. Stat. Sol.* **42**:305–314 (1977).
5. N. T. Correia, C. Alvarez, J. J. Moura Ramos, and M. Descamps. The β-α- branching in d-sorbitol as studied by thermally stimulated depolarization currents (TSDS). *J. Phys. Chem. B* **105**:5663–5669 (2001).
6. *TSC/RMA 9000 Instrument Manual*. TherMold Partners, Stamford, CT.
7. R. A. Shmeis, Z. Wang, and S. L. Krill. A mechanistic investigation of an amorphous pharmaceutical and its solid dispersions, part II: molecular mobility and activation thermodynamic parameters. *Pharm. Res*. **21**:2031–2039 (2004).
8. J. Li, Y. Guo, and G. Zografi. The solid-state stability of amorphous Quinapril in the presence of β-cyclodextrins. *J. Pharm. Sci.* **91**(1):229–243 (2002).