Determination of Glass Transition Temperature and *in Situ* Study of the Plasticizing Effect of Water by Inverse Gas Chromatography

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Purpose. To use an inverse gas chromatographic (IGC) method to determine the glass transition temperature (Tg) of some amorphous pharmaceuticals and to extend this technique for the *in situ* study of the plasticizing effect of water on these materials.

Methods. Amorphous sucrose and colyophilized sucrose-PVP mixtures were the model compounds. Both IGC and differential scanning calorimetry (DSC) were used to determine their Tg. By controlling the water vapor pressure in the IGC sample column, it was possible to determine the Tg of plasticized amorphous phases. Under identical temperatures and vapor pressures, the water uptake was independently quantified in an automated water sorption apparatus.

Results. The Tg of the dry phases, determined by IGC and by DSC, were in very good agreement. With an increase in the environmental relative humidity (RH), there was a progressive decrease in Tg as a result of the plasticizing effect of water. Because the water uptake was independently quantified, it was possible to use the Gordon-Taylor equation to predict the Tg values of the plasticized materials. The predicted values were in very good agreement with those determined experimentally using IGC. A unique advantage of this technique is that it provides complete control over the sample environment and is thus ideally suited for the characterization of highly reactive amorphous phases.

Conclusions. An IGC method was used (a) to determine the glass transition temperature of amorphous pharmaceuticals and (b) to quantify the plasticizing effect of water on multicomponent systems.

KEY WORDS: inverse gas chromatography (IGC); glass transition; amorphous; sucrose; plasticizer.

INTRODUCTION

Inverse gas chromatography (IGC) is a variation of conventional gas chromatography wherein the functions of stationary and mobile phases are reversed. A stationary phase of the solid material of interest (i.e., analyte) is prepared in a column, and a volatile probe of known characteristics is passed through it. The probe residence time and shape of the chromatogram indicate the characteristics of the stationary phase and its interaction with the probe (1).

The technique has found widespread application in the characterization of surface properties (surface free energy, heat of adsorption, and acid-base properties of the surface) of polymeric materials (1) and recently pharmaceuticals (2–5). IGC has also been used to determine the solubility parameters, solid–solid interaction parameters, phase transition temperatures, and degree of crystallinity (1). Current work in the field of pharmaceutical sciences has dealt with the use of IGC to study the effects of processing conditions on pharmaceutically relevant properties (6–8).

Because of their desirable pharmaceutical properties and also widespread occurrence, amorphous pharmaceuticals are of great interest and have been the subject of several recent reviews (9–11). The major factor limiting the use of amorphous substances in dosage forms is their potential instability. This is because of their higher molecular mobility and consequently higher reactivity compared to their crystalline counterparts. Amorphous systems are characterized by the glass transition temperature (Tg), which is the transition point between a highly viscous brittle structure called glass and a less viscous, more mobile, rubbery state. The rubbery state (above the Tg), represents a liquid-like structure with high molecular mobility and is thus more prone to physical and chemical changes than the glassy state.

Several amorphous pharmaceuticals are in the glassy state under ambient conditions (12). However, the sorption of water is known to cause plasticization, resulting in a concentration-dependent lowering of Tg (13,14). Water sorption can occur both during the manufacture of the dosage form (for example, aqueous wet granulation) or during storage, potentially resulting in profound changes in the physicochemical properties of the amorphous phase. Unless this factor is recognized, one might draw erroneous conclusions about their properties. For example, the reported Tg of amorphous sucrose ranges from 60 to 75°C (15,16). This could be attributed, at least partially, to the water content, which could vary significantly from one sample to another. Similarly, depending on water content, the Tg of amorphous lactose varied by ~35°C (17).

Differential scanning calorimetry (DSC) is the method of choice for the determination of Tg of amorphous pharmaceuticals. The technique permits rapid data collection with minimal sample preparation and has wide applicability. However, the technique has some drawbacks. The major limitation is the difficulty in controlling the environment during sample preparation and analysis. Thus, with highly hydrophilic materials (for example, sugars), it is very difficult to prepare completely dry samples for DSC analysis. A consequence of residual water is that the observed Tg is lower than that of the completely dry sample. Although the sample chamber can be maintained "dry" by purging an inert gas, it is not possible to control the water vapor pressure during the analysis.

IGC enables us to circumvent these problems by providing complete control over the sample environment. Once the sample is packed into the column and dried, exposure to the ambient environment can be completely avoided. The *in situ* drying at the desired temperature, using a dry carrier gas, ensures complete removal of the sorbed water and other volatile impurities. Thus, the sample history is completely controlled. An added advantage is that the water (or organic solvent) vapor pressure in the sample chamber can be con-

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trolled over a wide temperature range, enabling quantification of its plasticizing effect. This feature is not available in the other techniques currently used for Tg determination.

IGC has been extensively used to study amorphous polymers, but it has found limited application in the characterization of pharmaceuticals (18). Recently, Newell *et al.* used IGC to study the effect of processing on the surface properties of lactose (6), and Thielman and Williams determined the Tg of amorphous maltose under a variety of conditions (19).

The objective of this work was to use an IGC method for determining the Tg of some amorphous pharmaceuticals. By determining the Tg at controlled water vapor pressures, this technique was extended to quantify the plasticizing effect of water. As mentioned earlier, the Tg determination was accomplished with complete *in situ* control of the sample environment, in both the presence and absence of water vapor. The model systems were amorphous sucrose and colyophilized sucrose–poly(vinylpyrrolidone) mixtures. From here on, poly(vinylpyrrolidone) will be referred to as PVP throughout this document.

MATERIALS

Sucrose (99+% pure, reagent grade, J.T. Baker Chemical Co.) and PVP (Plasdone® K-90, average molecular weight of 1,000,000, ISP Technologies, Inc.) were used as received. The hydrocarbon probes used, *n*-nonane, *n*-decane, and *n*-dodecane (all 99+% pure), were obtained from BDH Chemicals, UK. Methane (1% v/v in helium) was obtained from Scott Specialty Gases.

METHODS

Preparation of the Amorphous Phases

Amorphous sucrose and sucrose-PVP mixtures (25 and 50% w/w PVP) were prepared by freeze-drying. Aqueous solutions (10% w/v of solute) were frozen to –45°C and subjected to high vacuum for 48 h in a tray freeze-drier (Model UNITOP 400L,Virtis). Over the next 24 h, the temperature was gradually increased to 50°C. The secondary drying was carried out at 50°C for 24 h, after which the temperature was raised to 60°C, and the drying continued for 24 more hours. The lyophile was removed and stored in a desiccator at room temperature over anhydrous calcium sulfate (RH ~0%).

Inverse Gas Chromatography

A commercially available inverse gas chromatograph (Surface Measurement Systems, London, UK) was used. Nonane, decane, and dodecane were the probes. Methane was used as the noninteracting marker for the determination of null volume, and helium was the carrier gas.

About 200–400 mg of the sample was packed, in dry nitrogen environment, in a cylindrical glass column (30 cm long and 2 mm inner diameter) that was pretreated with dimethyldichlorosilane. The sample was initially conditioned under dry helium (flow rate of 10 ml/min) at room temperature for 2 h and further conditioned for 2 h at 60°C. This treatment ensured removal of sorbed water or volatile impurities from the system. The sample thermal history was removed by heating it to ~10°C above Tg. It was then cooled back to room temperature. The sample was then subjected to a temperature program from room temperature to 20–30°C above Tg. The probe and the marker were injected together at various temperatures in this range. The output from the column was analyzed using a flame ionization detector (FID). At each temperature of analysis, the system was equilibrated for 40 min before the injections were made. The desired water vapor pressures were achieved using humidified helium. The experimental setup allowed independent control of the temperature and water vapor pressure (relative humidity). The initial experiments were carried out over a wide range of temperature and relative humidity values. Once the temperature and RH of interest were identified, the final experiments were performed over a narrow range in an effort to accurately determine the Tg.

Karl Fischer Titrimetry

The water content was determined using a Karl Fischer titrimeter (Model CA-05 Moisture Meter, Mitsubishi). A small amount of dry sample was directly added to the Karl Fischer cell, and the water content was determined.

Thermal Analysis

A differential scanning calorimeter (MDSC, Model 2920, TA Instruments, New Castle, DE) with a refrigerated cooling accessory was used. The instrument was calibrated with pure samples of tin and indium at the heating rates of interest. About 4–8 mg of the sample was weighed into an open aluminum pan and dried isothermally at temperatures ~10–15°C below its Tg under a stream of dry nitrogen. The sample history was removed by heating the sample to a temperature about 15–20°C above its Tg and cooling back to 25°C. Finally, the Tg of this pretreated sample was determined at heating rates ranging from 0.5°C/min to 20°C/min.

X-Ray Powder Diffractometry

About 200 mg of sample was filled in an aluminum holder by the side-drift method and exposed to CuKα radiation (45 kV × 40 mA) in a wide-angle X-ray diffractometer (Model D5005, Siemens). The instrument was operated in the step-scan mode, in increments of 0.05°2θ. The angular range was 5 to 40°2θ, and counts were accumulated for 1 s at each step. The data collection and analyses were performed with commercially available software (JADE, version 5.1, Materials Data, Inc.).

Density

The density determination was performed by helium pycnometery (AccuPyc 1330, Micromeritics).

Water Vapor Sorption

About 10–12 mg of the sample was placed in the sample pan of an automated moisture balance (DVS-1000, Surface Measurements Systems, London, UK). Before equilibration at the desired temperature and water vapor pressure, the sample was dried under dry nitrogen flow (~0% RH) at 40°C for 24 h. The water uptake was quantified over a range of temperatures and RH values. The microbalance was calibrated using a 100-mg standard weight. The relative humidity

sensor was calibrated at 5.0, 11.3, 32.8, 52.8, 75.3, and 84.3% RH (25°C), using saturated salt solutions.

Surface Area Determination

Specific surface area was determined by the multipoint (5 points) BET method using a surface area analyzer (Gemini, Micromeritics, Norcross, GA). Accurately weighed samples were degassed under vacuum at room temperature for at least 12 h, and measurements were made using nitrogen as an adsorbate and helium as a carrier gas.

Determination of Tg by IGC: Theory

IGC, at infinite dilution, can be used for the characterization of phase transitions in solids. At infinite dilution, the surface coverage is well below monomolecular layer, and the net retention of a probe is independent of the quantity of probe injected (20). The basis of IGC at infinite dilution is the equilibrium partitioning of probe molecules between the vapor and stationary phases. The net retention volume, V_n, describes the equilibrium state of partitioning. It is defined as the volume of carrier gas required to elute the probe from the column and can be calculated from the net retention time (t_n), flow rate (F), and a correction factor, J, for pressure drop across the column [Eq. (1)]. The net retention time t_n is the difference between the retention time for the probe (t_r) and the noninteracting marker (t_o).

$$V_n = J\,F(t_r - t_0) = J\,F t_n \quad (1)$$

In the infinite dilution region, V_n is independent of the probe concentration and depends on its interaction energy with the stationary phase (20,21). The retention volume can be normalized both for temperature and the sample amount by using the specific retention volume, V_g, which is defined as:

$$V_g = V_n \frac{273.16}{T\,w} \quad (2)$$

where T is the temperature of the system and w is the sample weight (22). Though this normalization method is questioned in the chromatographic literature (23), it is a widely accepted procedure for reporting the retention volume data.

When studying adsorption on solid surfaces using gas–solid chromatography, a plot of the natural log of the retention volume vs. the reciprocal of the absolute temperature is expected to be linear. The heat of adsorption can be determined from the slope using Henry's law. When amorphous materials are heated across the glass transition region, usually a z-shaped plot (Fig. 1) is obtained because of change in the retention mechanism across Tg (24). In region I (T < Tg), surface adsorption is the proposed probe retention mechanism. The first deviation from linearity is attributed to the onset of glass transition. The glassy and rubbery states coexist in the transition region (region II), where the retention mechanism is a combination of surface adsorption and bulk sorption. The retention volume increases, possibly as a result of bulk sorption in the rubbery state. This continues until the end of region II, where the conversion to the rubbery state is complete. Region III is characterized by surface and bulk sorption, wherein the retention volume decreases with increasing temperature.

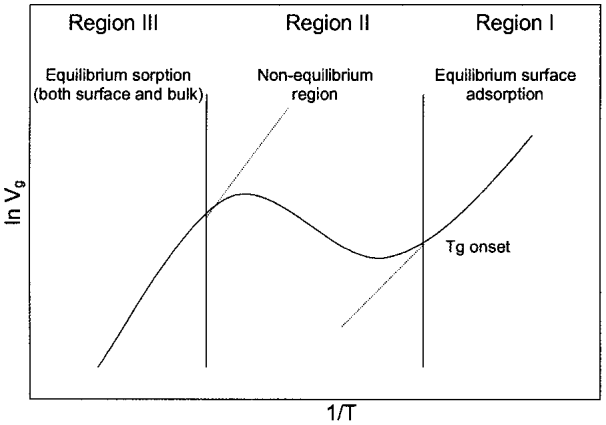

Fig. 1. Schematic of the retention of a probe through the glass transition region (24). V_g is the probe retention volume, and T is the temperature.

RESULTS AND DISCUSSION

Baseline Characterization

The freeze-dried sucrose as well as the sucrose–PVP mixtures were X-ray amorphous. The other relevant physicochemical properties are summarized in Table I. The experimentally determined Tg values were in good agreement with the reported values (25).

Determination of Tg

Tg in the Absence of Plasticizer

The retention behavior of the probes across the glass transition region of sucrose and sucrose–PVP mixtures was

Table I. Baseline Characterization of Amorphous Samples

Material	Water content (% w/w)	Tg (°C)		Width of Tg (°C)‡	Density (g/cm^3)§	Surface area (m^2/g)§
		Experimental*	Reported†			
Sucrose	<0.3	73	75	5	1.49 ± 0.01	0.13
Sucrose-PVP (75:25 w/w)	<0.2	81	~84	8	1.45 ± 0.01	0.58 ± 0.14
Sucrose-PVP (50:50 w/w)	<0.2	96	~98	16	1.42 ± 0.02	0.87 ± 0.04

* The onset temperature determined by DSC at a heating rate of 10°C/min. The standard deviation in the measurement was <1°C.
† Determined by DSC at a heating rate of 20°C/min (25). The Tg values for colyophilized mixtures were obtained by interpolation of graphical data points, and are thus approximate.
‡ Determined by DSC at a heating rate of 10°C/min.
Standard deviations are shown when n ≥ 3.

studied (Fig. 2; Table II). At temperatures substantially below the calorimetric Tg, a plot of specific retention volume as a function of 1/T was linear (Fig. 2). There was deviation from linearity as the Tg was approached. Invariably, the temperature of this deviation was lower than the Tg onset temperature in the DSC (at 10°C/min). As the temperature was increased further, the profile became linear again, indicating complete conversion to the rubbery phase. As discussed later, the nature of the profile in the transition region was influenced by the stationary phase as well as the probe used.

In all cases, the first deviation from linearity (the discontinuous vertical lines in Fig. 2) was considered as the onset of Tg. In an effort to obtain Tg values objectively, two approaches were attempted. The first method was based on visual inspection of the data points for deviation from linearity. The second approach was based on the observation that the slope values were approximately constant at temperatures substantially above and below the Tg. Thus, starting at the lowest temperature of measurement, three data points were taken at a time and subjected to linear regression. Tg onset was that temperature at which there was a pronounced change in the slope value. The Tg values obtained by the two methods were in good agreement (Table II).

Comparison of Tg Values Obtained by IGC and DSC

The Tg values obtained by IGC and DSC are compared in Table II. As expected, the Tg increased with an increase in the PVP content of the system (antiplasticizing effect). As the PVP content increased, the transition region (region II in Fig. 1) became wider. The width was 8°C for sucrose and 11 and 18°C, respectively, for sucrose–PVP mixtures with 25 and 50% w/w PVP. The wide molecular weight distribution of PVP is responsible for the wide temperature range of the glass transition. This can explain the wider transition temperature as the PVP content increases.

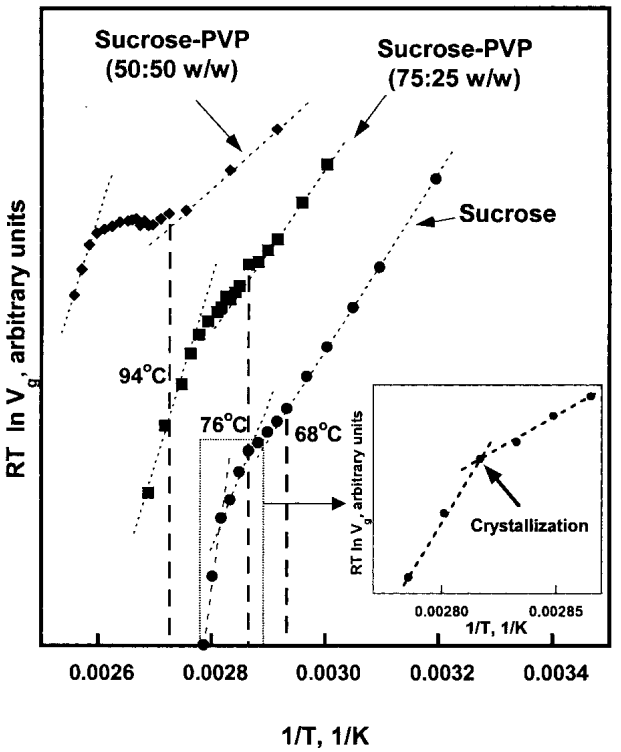

Fig. 2. Retention behavior of decane on sucrose and sucrose–PVP mixtures as a function of 1/T (at 0% RH). The discontinuous lines indicate the lowest temperature at which there was a deviation from linearity. The inset shows crystallization of amorphous sucrose.

DSC experiments are routinely carried out at a heating rate of 10°C/min. The Tg values determined by DSC, at this heating rate, were invariably higher than those obtained by IGC (Table II). The experimental conditions may be responsible for these differences. A decrease in the heating rate usually lowers the observed Tg, as is evident from Table II. In the glass transition region, each 2°C increase in the IGC column temperature was followed by an isothermal hold for 60 min, resulting in a low overall heating rate of 2°C/hour. DSC experiments at such low heating rates did not result in a discernible Tg. Therefore, it was not practically possible to compare the Tg values obtained by the two methods under the same heating rate. Moynihan *et al.* have shown that a plot of heating rate (logarithmic scale) vs. 1/Tg is linear provided the heating and cooling rates are identical (26). Such a plot, over a range of 0.5 to 20°C/min, yielded linear profiles (Fig. 3). Assuming linearity at slower heating rates, we extrapolated these profiles to a heating rate of 1°C/h (Table II). The extrapolated Tg values were much lower than those determined by IGC.

There are some fundamental differences between the IGC and DSC experimental methodology, which warrant consideration. Although the overall heating rate in the IGC is very low, at each heating step, the sample experiences an initial rapid heating rate (for example, 2°C increase in temperature in 2–3 min) followed by an isothermal hold for ~1 h. Thus, the initial instantaneous heating rate is much higher than the overall heating rate. One other consequence of the isothermal hold at temperatures close to Tg is the enthalpic relaxation of the glassy material, resulting in an increase in the observed Tg. Because of the influence of these effects on the observed Tg values, there may not be a perfect agreement between the IGC and DSC results.

In the retention diagram of sucrose, the second change in slope at ~82°C (Fig. 2; inset) is attributed to initiation of crystallization (27). In order to confirm sucrose crystallization, when this sample was heated to ~90°C, cooled to room temperature, and reheated, there was no evidence of glass transition. As is evident from Fig. 2, crystallization caused a pronounced decrease in the net retention volume that was possibly a consequence of a number of factors including decrease in surface area and change in retention mechanism, both due to crystallization.

The retention profiles in Fig. 2 are different from the idealized schematic in Fig. 1. According to Fig. 1, since there is a change in the retention mechanism in the transition region, there will be an increase in retention volume with an increase in temperature. This should be followed by a decrease in the retention volume in the rubbery region. When decane was the probe, there was no substantial increase in the retention volume for sucrose and sucrose–PVP mixtures in the transition region (Fig. 2). Although the glass-to-rubber transition in sucrose occurred over a narrow temperature range (~6–8°C), the presence of PVP caused a significant increase in the width of the transition region (Table II).

The nature of the retention profiles can be influenced by the probe used, width of the glass transition, and the relative

Table II. Comparison of Tg Values Obtained by DSC and by IGC

Material	Tg (°C) determined by					
	DSC*				IGC	
	10°C/min	5°C/min	0.5°C/min	1°C/h‖	Tg (°C)‡	Tg (°C)§
Sucrose	73	71	69	62	68[a], 68[b]	70[b]
Sucrose-PVP (75:25 w/w)	81	79	77	70	76[b]	76[b]
Sucrose-PVP (50:50 w/w)	96	93	89	83	94[b], 94[c]	96[b]

† [(a)] Nonane, [(b)] decane, and [(c)] dodecane.
‡ Based on visual observation of the first deviation from linearity.
§ Based on linear regression of three data points at a time (details in the text).
‖ Extrapolated.
* Standard deviation in the DSC measurements was <1°C.

strengths of interactions involved in surface adsorption and bulk sorption. If the differences in retention volumes between the glassy and rubbery states are not large, the transition region may not be discernible. The size and type of probe molecule might also influence the retention profile (28). Unlike the retention diagram obtained with decane (Fig. 2), the use of dodecane as the probe resulted in a profile closely matching the "ideal" shown in Fig. 1. In Fig. 4, the retention behavior of decane and dodecane have been compared. Although the transition temperatures using the two probes were identical (94°C), the retention profiles were different. Dodecane appeared to be an ideal probe, but the long retention times at low temperatures yielded broad, shallow peaks, thus precluding its use for low-Tg materials.

Effect of Water on the Tg

During pharmaceutical processing as well as storage, amorphous samples are likely to come in contact with water or other solvents. Sorption of these small molecules results in plasticization of the amorphous phase, lowers the Tg, and can facilitate physical and chemical transformations. It is therefore very useful to simulate the manufacturing or storage conditions and determine the Tg of the plasticized amorphous phase. However, in order for these results to be meaningful, the amount of sorbed plasticizer must be known so the Tg can be correlated with composition. Our next objective, therefore, was to study, *in situ*, the plasticizing effect of water. The Tg was determined at several RH values ranging from 5 to 20%. In each case, the RH was maintained constant over the entire temperature range of Tg determination. The plasticizing effect of water was evident from the progressive decrease in Tg with increasing RH (Table III).

In an independent series of experiments, the water content of amorphous samples was determined using the automated water sorption balance. The water uptake as a function of RH was determined at several temperatures ranging from 25 to 70°C. In these experiments, the RH was progressively increased while the temperature was kept constant. The results from the sucrose–PVP mixture are shown (Fig. 5), whereas those of sucrose alone are not shown. These profiles enabled us to obtain the water content as a function of temperature at a constant RH (the continuous lines in Fig. 5). This information is relevant to the IGC experiments, wherein the temperature is varied while the RH remains constant. By combining the two techniques, it was possible to determine the water content of these plasticized systems at their Tg values. In DSC, when the sample is heated, it is not possible to control the water vapor pressure. Moreover, when this

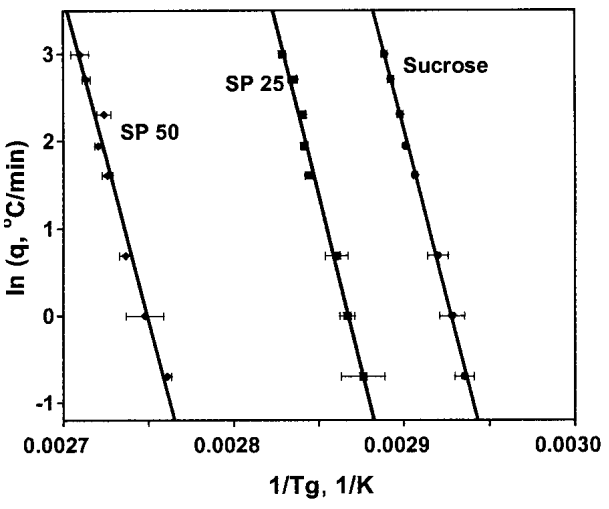

Fig. 3. Plot of natural log of heating rate as a function of inverse of Tg for sucrose and sucrose–PVP mixtures (n ≥ 3). SP25 = Sucrose-PVP (75:25 w/w); SP50 = Sucrose-PVP (50:50 w/w).

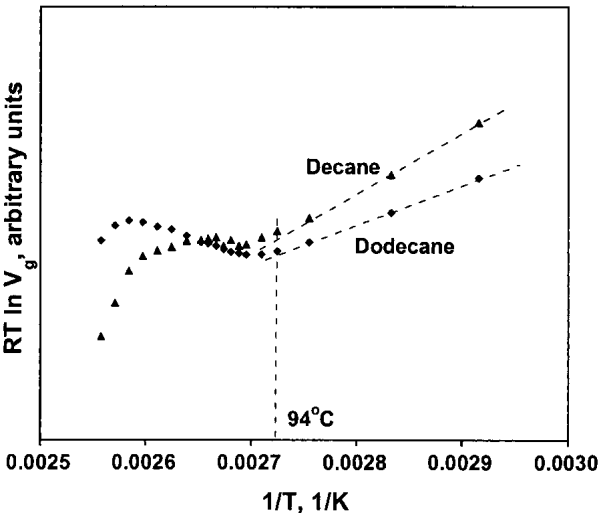

Fig. 4. Retention behavior of decane and dodecane on sucrose–PVP (50:50 w/w) mixture. The discontinuous vertical line indicates the Tg determined in both cases.

Table III. Plasticizing Effect of Water on Amorphous Sucrose and Sucrose–PVP (75:25 w/w) Mixture

RH (%)	Tg by IGC (°C)‡	Water content (% w/w)*	Predicted Tg† (°C)
Sucrose			
0	68[n], 68[d]	ND	—
5	60[n], 60[d]	0.79	62
10	55[d]	1.48	56
15	50[n], 51[d]	2.45	50
20	45[n], 46[d]	3.53	43
Sucrose–PVP (75:25 w/w)			
0	76[d]	ND	—
5	69[n], 70[d]	0.75	69
10	63[n], 61[d]	1.51	64
15	57[n], 55[d]	2.49	58
20	51[n], 50[d]	3.55	51

The water content was calculated at the mean value of Tg at each condition.
* Determined at Tg using a water sorption apparatus.
† Using the Gordon-Taylor equation.
‡ n, nonane; d, decane.
ND, not detected.

technique is used to determine the Tg of plasticized systems, the water content of the sample at Tg is not known. This is particularly important in light of the fact that water content of the amorphous sample changes as a function of temperature. From Fig. 5, it is clearly evident that at a fixed RH, the water content decreases slightly with increase in temperature. The saturated water vapor pressure has a pronounced temperature dependence. With a rise in temperature, a fixed RH can be maintained only with a pronounced increase in water vapor pressure. In spite of this increase in water vapor pressure, the water content is largely unaffected.

The water content determined under different conditions (Fig. 5) enabled us to independently predict the glass transition temperatures of the plasticized phases. These profiles provided a highly reliable estimate of the water content at Tg,

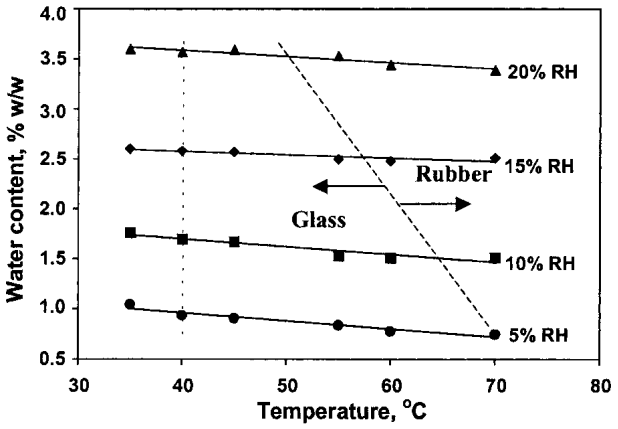

Fig. 5. Water content in sucrose–PVP (75:25 w/w) mixtures determined using a water sorption apparatus, over a range of temperatures and relative humidities. At a selected temperature, the water content was determined at progressively higher RH values. The vertical discontinuous line shows the results of one such experiment. From several such isothermal experiments, the profiles of the water uptake as a function of temperature (at a fixed RH) were drawn. The glassy and rubbery regions are approximately separated.

which formed the basis for calculating the Tg of the plasticized phase using the Gordon-Taylor equation. The Tg values used for dry sucrose (68°C) and sucrose–PVP (75:25) mixture (76°C) were those determined by IGC, whereas the literature value of 135 K for water was used for these calculations. The predicted glass transition temperatures were compared with the experimentally determined values (Table III), and there was an excellent agreement between the two.

It was necessary to determine the effect, if any, of the water sorbed by the stationary phase on the retention behavior of the probes. With decane used as a representative example, its retention on amorphous sucrose was evaluated at a constant temperature of 40°C, at RH values ranging from 0 to 20%. At this temperature, in the RH range selected, no transitions are expected. The mean retention volume was 127 (± 1) ml and ranged between 125 and 128 ml. Although there seemed to be no systematic effect of RH on the retention volume, the limited amount of data available was insufficient to conclusively prove this. In this context, it is important to recognize that for the determination of Tg, while the sample was subjected to a controlled temperature program, the RH was maintained constant. As mentioned previously, it is evident from Fig. 5 that so long as the RH was fixed, the amount of water sorbed did not change significantly with temperature. Thus, the role of sorbed water is expected to be constant throughout the experiment.

Determination of Tg: DSC vs. IGC

DSC is the technique of choice for determining the Tg of amorphous materials. This simple technique enables rapid determination of Tg using a very small amount (milligrams) of sample, and the results are usually reproducible. In comparison, IGC is much more labor intensive, time consuming, and requires a larger amount of sample. Moreover, it is not possible to measure enthalpic relaxation by this technique. The major advantage of IGC is that it permits complete control of the sample environment during the entire experiment. This feature enables the unambiguous Tg determination of

plasticized materials. This issue is discussed in the following paragraphs.

Water is the most common plasticizer of amorphous pharmaceuticals. The method of preparation and storage conditions determine the water content in amorphous materials. Even when the solid is completely dried, exposure to ambient atmosphere for a short time—for example, during sample preparation—can cause considerable water sorption, resulting in an unreliable Tg value. In contrast, the IGC experimental setup enables conditioning of the sample under dry helium purge, a procedure facilitating complete removal of sorbed water and eliminating the uncertainty in Tg determination.

Let us finally consider the Tg determination of amorphous samples containing known amounts of sorbed water. DSC experiments are usually carried out in non–hermetically crimped pans and under nitrogen purge. When the sample is heated, desorption is expected to occur, and the amount of sorbed water would decrease as a function of temperature. The non-hermetic crimping will allow the desorbed water to leave the pan. In other words, the composition of the system will be changing continuously. Although it may not be possible to prevent water desorption, the loss of desorbed water can be prevented by the use of hermetically sealed pans. However, the water vapor pressure inside the pan will be uncontrolled and will be a function of sample size, sample packing and the sample holder geometry. The major disadvantage, with both hermetic and non–hermetically sealed pans, is that the water vapor pressure (or the RH) in the sample chamber is unknown and uncontrolled as the sample is heated.

In contrast, IGC offers complete control of the temperature as well as the water vapor pressure. The latter is controlled using humidified helium as the purge gas. When there is a programmed increase in temperature (but RH is fixed), there is a corresponding increase in the vapor pressure so that the RH is maintained constant. In other words, it is possible to independently vary the temperature and relative humidity, a feature unavailable in commercial differential scanning calorimeters.

Although the chromatographic technique of measuring the Tg is indirect, and morphologic changes in the amorphous phase cannot be directly observed during the experiment, simultaneous determination of not only the Tg but also the amount of plasticizer in the system is possible. Recently, Edwards *et al*. combined the data for CO_2 solubility in poly(methyl methacrylate), obtained by tracer pulse chromatography, with Tg measurement using IGC to correlate the chromatographically observed Tg depression and composition (29). This approach involved modeling of the data with some extrapolations. A rather different and more direct approach was used in our work, as shown in Fig. 5.

DSC has not been found to be very useful in the determination of Tg of some amorphous proteins because of the wide transition range and the small heat capacity change at Tg. This could be attributed to the internal heterogeneity of these molecules and the broad distribution of relaxation times (30). IGC methodology for the determination of Tg was developed for polymeric materials, which are usually similar to amorphous proteins in terms of the structural heterogeneity and distribution of relaxation times. It is hypothesized that the changes in bulk structure across the Tg would produce measurable alterations in the retention behavior. In such cases, IGC is expected to be a much more sensitive technique than differential scanning calorimetry. This issue deserves detailed investigation.

CONCLUSIONS

An IGC method was used for determining the glass transition temperature (Tg) of amorphous sucrose and colyophilized sucrose–PVP mixtures. The Tg values determined by IGC were, in general, in very good agreement with those obtained by DSC. A unique feature of the IGC methodology is that it enables complete control over the sample environment during the *entire* measurement.

The amorphous samples were plasticized *in situ* by controlling the temperature and relative humidity (RH) in the column. This enabled Tg determination over a range of relative humidities. The water uptake at the same temperatures and relative humidities was determined in an automated vapor sorption balance, which permitted us to calculate the Tg of these plasticized systems. The experimental values were in very good agreement with the calculated values.

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