ELSEVIER

International Journal of Pharmaceutics 179 (1999) 179–207

international journal of pharmaceutics

The relevance of the amorphous state to pharmaceutical dosage forms: glassy drugs and freeze dried systems

Duncan Q.M. Craig *, Paul G. Royall, Vicky L. Kett, Michelle L. Hopton

Centre for Materials Science, School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK

Received 25 May 1998; accepted 6 August 1998

Abstract

Many pharmaceuticals, either by accident or design, may exist in a total or partially amorphous state. Consequently, it is essential to have an understanding of the physico-chemical principles underpinning the behaviour of such systems. In this discussion, the nature of the glassy state will be described, with particular emphasis on the molecular processes associated with glass transitional behaviour and the use of thermal methods for characterising the glass transition temperature, T_g. The practicalities of such measurements, the significance of the accompanying relaxation endotherm and plasticization effects are considered. The advantages and difficulties associated with the use of amorphous drugs will be outlined, with discussion given regarding the problems associated with physical and chemical stability. Finally, the principles of freeze drying will be described, including discussion of the relevance of glass transitional behaviour to product stability. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Amorphous; Crystallisation; Differential scanning calorimetry; Freeze drying; Glass transition; Lyophilisation

1. Introduction

The majority of solid drugs and dosage forms are prepared in the crystalline state, characterised by a regular ordered lattice structure. In practical terms, the physical structures of these systems are generally thermodynamically stable and are relatively simple to study using techniques such as differential scanning calorimetry and X-ray diffraction. However, it has been recognized for a considerable period of time that pharmaceutical materials may also be prepared in an amorphous form (Haleblian, 1975; Byrn, 1982), where there is no long range order. The classic example of this approach is that of novobiocin which was shown

* Corresponding author. Tel.: +44-171-753-5863; fax: +44-171-753-5863; e-mail: duncraig@ulsop.ac.uk.

0378-5173/99/$ - see front matter © 1999 Published by Elsevier Science B.V. All rights reserved.
PII: S0378-5173(98)00338-X

to exhibit favourable dissolution properties when prepared in an amorphous as opposed to crystalline state (Mullins and Macek, 1960). Furthermore, processes such as freeze and spray drying may lead to the generation of amorphous systems, while grinding or conventional drying may result in materials which are partially or wholly disordered.

The amorphous state may arise as a result of three sets of circumstances. Firstly, the drug, excipient or delivery system may be deliberately produced in an amorphous form in order to enhance product performance characteristics. Examples of this strategy include the preparation of glassy drugs for enhanced dissolution behaviour or freeze drying, both of which will be discussed in more detail in later sections. Secondly, the material may be intrinsically at least partially amorphous at room or body temperature, examples including D/L polylactic acid, polyvinylpyrrolidone or polyethylene glycol. Consequently, dosage forms prepared using these materials will inevitably be at least partially amorphous. Thirdly, the amorphous state may be generated accidentally, examples including milling, drying and compression. In many ways accidental production can prove to be the most problematic, as the levels of disordered material generated may be sufficiently large to cause changes in product performance but also too small to be easily detected.

While the preparation of amorphous systems may be desirable, there are a number of difficulties associated with their use. Amorphous materials are thermodynamically unstable and will tend to revert to the crystalline form on storage (devitrification); such behaviour has been well documented for a number of drugs (Fukuoka et al., 1986; Yoshioka et al., 1994; Hancock et al., 1995). It should be stressed at this point that the onset of the devitrification process may be so slow so as to be effectively irrelevant within the storage time of a product, although an understanding of the nature and characterisation of the glass transitional behaviour is nevertheless essential in order to enhance predictability of stability.

The mechanical properties and vapour sorption profiles of amorphous systems may be markedly different from the crystalline material (Hancock and Zografi, 1997), while the chemical reactivity of amorphous drugs may be greater (Pikal et al., 1978). In addition, the behaviour of the system below and above the glass transition temperature (T_g, at which the material changes on cooling from a liquid or rubbery state to a brittle state) will differ; the rate of crystallisation is much greater above T_g, while freeze dried products are less likely to physically collapse if stored below T_g. A further consideration which is particularly pertinent to commercial uses of amorphous materials is the lack of a 'comfort factor' associated with such systems. The physical structure of glassy materials is more difficult to characterise and quality control than is that of crystalline systems. Similarly, the knowledge base concerning the relationship between T_g and pharmaceutical product performance is not fully developed. Furthermore, considerable care must be taken with regard to the use of conventional accelerated stability studies in order to predict chemical or physical stability, as the behaviour above and below T_g is not directly comparable (Duddu and Dal Monte, 1997). In common with phenomena such as polymorphism, the characteristics of amorphous drugs must also be considered in the light of regulatory requirements (Byrn et al., 1995). These difficulties have led to a number of instances whereby the use of amorphous drug forms has been rejected as a formulation strategy by pharmaceutical companies, despite the fact that this route offers a potential means of considerably enhancing product performance.

The subject of glassy systems has received considerable attention in other fields such as the polymer and food sciences (Wunderlich, 1990; Slade and Levine, 1991, 1995) and the interested reader is referred to these and related texts for more detailed information. Similarly, the reviews by Hancock and Zografi (1997) and Kerč and Srčič (1995) are also recommended, the former containing a detailed discussion of the nature of the glassy state of pharmaceuticals and the latter placing emphasis on published examples of amorphous drug systems, with particular consideration of thermal analysis. Given the breadth that the topic of amorphous pharmaceutical systems has

now assumed, it is not possible to cover all aspects of the subject in a single review. In this discussion, therefore, the basic principles underlining the behaviour of amorphous materials will be described, with particular emphasis on characterisation of these systems using thermal analysis. In terms of applications, the discussion will be confined to glassy drugs and freeze dried materials, as these are two of the most important systems in which the amorphous state may be encountered.

2. The amorphous state and glass transitions

2.1. The generation of amorphous materials by cooling from the melt

While partially or wholly amorphous solids may be generated via a number of routes, most texts describing glassy systems work on the basis that the material has been formed by rapid cooling from the melt. For simplicity, this will also be assumed in the forthcoming description of the theoretical basis of glass formation, although in practice processes such as solvent precipitation or milling may also lead to the generation of amorphous systems. A number of texts are available for more information on the nature of the glass transition (Elliott, 1983; Wunderlich, 1990; Angell, 1995a; Hancock and Zografi, 1997).

The essential differences between the formation of amorphous and crystalline systems may be illustrated with respect to Fig. 1. For crystalline systems, decreasing the temperature from the liquid state to the melting point (T_m) results in a transition to the crystalline form (assuming no supercooling), which below T_m is the thermodynamically stable state with respect to non-crystalline forms. The exothermic crystallisation process leads to a sudden contraction of the system due to a decrease in free volume (defined as the difference between the total volume and the actual volume displaced by the constituent molecules). Consequently, both the enthalpy (H) and specific volume (V) decrease at T_m. Further less marked decreases in the above may be seen as the temperature is lowered as a result of heat capacity and thermal contraction effects. It should be noted, however, that in the case of water, crystallisation leads to expansion rather than contraction.

In the case of a glass-forming material, the cooling process is too fast for the crystallisation process to take place, either due to the use of a rapid rate of cooling or the crystallisation process being unfavoured because of molecular size and shape (as is the case with the majority of proteins). No discontinuity in enthalpy or volume is seen on cooling the material below T_m and the system forms a supercooled liquid. As the material is cooled further, a point is reached at which the material becomes 'frozen' into the glassy state. At this temperature, the bonding between molecules remains essentially the same as that of the liquid but the translational and rotational motions of those molecules are dramatically reduced, with principally vibrational motions taking place below T_g. The glass transition will thus be characterised by a step change in heat capacity C_p which is the derivative of enthalpy with respect to temperature ($(\partial H/\partial T)_p$), hence the transition is dependent on molecular mobility with no associated heat transfer for the process. The transition

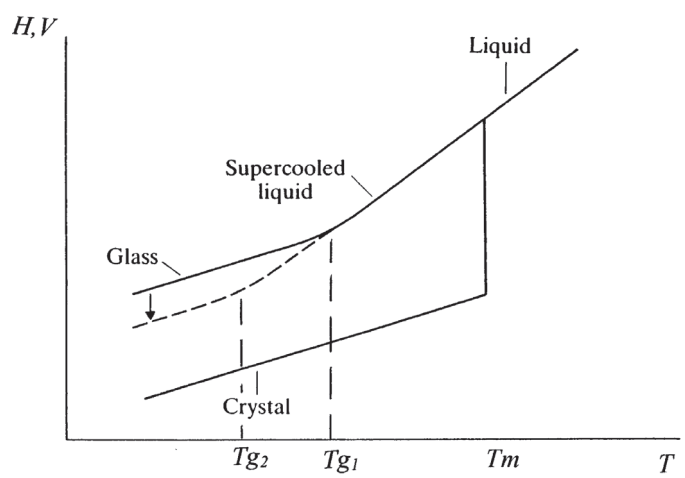

Fig. 1. Schematic illustration of the change in volume or enthalpy with temperature for a material undergoing crystallisation or a glass transition. The first-order crystallisation at T_m is shown in conjunction with the discontinuity in volume/enthalpy at T_g. The short-dashed line indicates the behaviour of a system cooled at a slower rate than that corresponding to the solid line. The long-dashed line shows the T_g values for the fast cooled (T_{g_1}) and slow cooled (T_{g_2}) systems [adapted from Elliott (1983)]

is rate dependent, with slower cooling rates resulting in lower values for T_g, as indicated in Fig. 1. In practical terms, the material is considered to be in the liquid (or 'rubbery' in the case of some polymers) and glassy states above and below T_g, respectively. In the case of many polymers the mechanical properties of the system change from those of a pliable to a brittle material as the system is cooled through the glass transition, these changes arising as a result of the decrease in molecular motions.

There are a number of theories associated with the nature of the glass transition but, as yet, there is no universally accepted single explanation for the phenomenon. The observation that T_g is represented by a change in the derivative of extensive thermodynamic parameters such as volume, enthalpy and entropy suggests that the glass transition is a second order thermodynamic phase transition, where the term second order refers to the order of the lowest derivative of the Gibbs free energy which shows a discontinuity at the transition point (processes such as crystallisation being first order). However, there are a number of difficulties associated with this explanation, not least of which is the common observation that the value of T_g is dependent on cooling rate. As a second order thermodynamic transition is by definition rate independent, the glass transition is not ideally second order.

It is also helpful to consider the entropy of the system, which at equilibrium is related to the heat capacity via $C_p = T\,(\partial S/\partial T)_p$ The heat capacities of the glassy ($< T_g$) and crystalline states for a given material are essentially the same and arise principally from vibrational contributions, while the higher values of C_p observed at temperatures $> T_g$ are caused by the material possessing additional configurational degrees of freedom when in the rubbery state. Consequently, the T_g may be considered to occur at a given value of excess entropy (Elliott, 1983). Kauzmann (1948) addressed the question of the temperature dependence of T_g, in particular whether there is a lower limit to the value of the glass transition at infinitely long cooling times. It was suggested that such a lower limit does indeed exist and is governed by the fact that if the T_g represents a loss of excess entropy, that value may not exceed the entropy associated with the change from the liquid to the crystalline form (i.e. the entropy of fusion). Otherwise, the entropy of the glass would be lower than that of the crystal which would violate the third law of thermodynamics. This situation is known as the Kauzmann paradox and is resolved by there being a lower limit to T_g for any system (termed either the calorimetric ideal glass transition temperature T_{oc} or the Kauzmann temperature T_k). In practice, the experimental T_g may occur 20 K or more above T_k. It may therefore be postulated that, over and above the possibility of a second order phase transition taking place, the heat capacity will decrease with temperature in any case as a result of equilibrium thermodynamics. On this basis, the glassy behaviour of materials may be considered to be a function of their configurational entropy, hence it should theoretically be possible to relate the T_g value to the 'stiffness' of the molecule in question. This has been discussed in detail for linear polymers (Gibbs and Di Marzio, 1958; Gibbs, 1960; Gee, 1970).

The glass transition may also be considered in terms of the relaxation processes that occur as the liquid is cooled. These relaxation times will be temperature dependent, with longer times being observed as the liquid is cooled; for hydrogen-bonded fluids, these relaxation processes will be governed largely by reorganisation of those hydrogen bonds. If the 'structural' relaxation time of the material (τ_r) is short with respect to the time of observation t_0 (which will be the case at temperatures above T_g) then the material will appear 'liquid-like' as the sample will be able to respond to changes in temperature within the timescale of the cooling process. Consequently, above T_g the sample is in equilibrium with the cooling programme. Below T_g, however, t_0 will be $< \tau_r$, hence the material will assume solid-like characteristics due to the relaxation process being slow with respect to the timescale of the cooling programme, leading to reduced molecular mobility. T_g may therefore be considered to occur when $\tau_r \approx t_0$. A related approach to interpreting the glass transition is to consider the free volume of the system. This model was developed for fluids

(Cohen and Turnbull, 1959; Turnbull and Cohen, 1961, 1970) and considers the liquid to be composed of a volume occupied by the constituent molecules (V_{occ}) and a volume in which the molecules are free to move (the free volume V_f). The free volume is composed of voids of varying sizes which are continuously redistributed through the system via random movements of the constituent molecules. It is further assumed that the molecules may diffuse through the system only when the free volume is above a critical value. As the temperature is lowered, both volumes contract. For a glassy system, the free volume reaches a lower limit at Tg and is thereafter temperature independent, hence in essence the glass transition occurs when V_f falls below a critical value (Fox and Flory, 1950, 1951, 1954).

The relaxation behaviour of glassy systems may be associated with molecular mobility and macroscopic fluidity, which in turn determine many of the important properties of the amorphous materials. These properties will therefore be outlined separately in a subsequent section. However, the above discussion illustrates the difficulty in establishing an exact definition of the glass transition. The process has been described as a second order thermodynamic phase change (although, as previously stated, the rate dependence of the process weakens this interpretation considerably), as an equilibrium process involving excess entropy or as a kinetic relaxation process. In practice, it is usual to work on the basis of the process being essentially kinetic, hence the T_g is considered to be a function of the relaxation behaviour of the material. It is important to stress, however, that this is not the only interpretation of this event.

2.2. The viscosity of glasses

At the Tg, the viscosity of the liquid attains a value in the region of 10^{12}–10^{14} Pa.s, while above the glass transition the material may show considerable temperature dependence in the viscosity value, as shown in Fig. 2. The extent and nature of this temperature dependence itself varies between materials. 'Strong' glass formers such as SiO_2 which form networks in the liquid state obey the Arrhenius relationship over a wide temperature range, while 'fragile' glass formers such as organic glasses (e.g. *O*-terphenyl and i-butyl bromide) show strong deviation from such behaviour (Wong and Angell, 1976). In the region immediately above T_g, such materials may follow the Vogel–Tammann–Fulcher (VTF) relationship which may be expressed in terms of the relaxation time τ via:

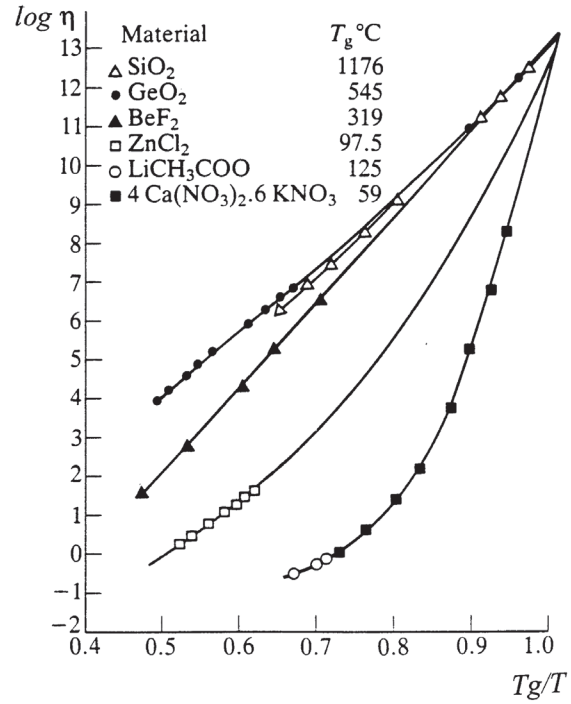

Fig. 2. Viscosities of a variety of glass-forming systems as a function of reduced reciprocal temperature. The curves have been normalised such that the glass-transition temperature is defined to be when $\eta = 10^{13}$ Poise (Reprinted from Glass: structure by spectroscopy, J. Wong and C.A. Angell, Marcel Dekker, New York, Copyright (1976), with permission from Elsevier Science).

$$\tau = \tau_0 \exp\left(DT_0/T - T_0\right) \quad (1)$$

where T is the temperature and τ_0, D and T_0 are constants. As can be seen from Fig. 2, systems obeying this relationship show a temperature-dependent activation energy as opposed to the constant value predicted by the Arrhenius relationship. A related empirical expression is the Doolittle equation (Doolittle, 1951) which relates the viscosity to the free volume of the sample via:

$$\eta = A \exp\left(BV_{occ}/V_f\right) \quad (2)$$

where A and B are constants, if the thermal expansion of the system is linear with temperature then Eq. (1) may be obtained from the Doolittle equation. Alternatively, the Williams–Landel–Ferry (WLF) equation (Williams et al., 1955), a special case of the VTF equation, has been shown to be applicable to a wide range of glass forming systems whereby:

$$\eta = \eta_g \exp\left[C_1(T - T_g)/(C_2 + (T - T_g))\right] \quad (3)$$

where η_g is the mean viscosity at T_g and C_1 and C_2 are constants. This equation allows calculation of the relaxation times of the system over a range of temperatures and therefore allows prediction to be made with regard to temperature-dependent behaviour. As will be demonstrated later, these relationships may be highly important in understanding the behaviour of pharmaceutical products.

Strong glass-formers exhibit minimal molecular mobility changes at T_g, hence the shift in heat capacity tends to be small. Conversely, fragile glass-forming systems exhibit marked changes in mobility through the T_g due to their non-directional, non-covalent interactions, hence the heat capacity alterations at T_g are larger (Angell, 1995a) and easier to detect. A 'rule-of-thumb' with regard to predicting the fragility has been proposed (Slade and Levine, 1995; Angell, 1995a,b) whereby if T_m/T_g is less or greater than 1.5 the glass can be considered fragile or strong, respectively. Unfortunately, native globular proteins are generally strong glass forming systems, hence the heat capacity changes associated with T_g tend to be small which renders their measurement using thermal methods more difficult. The glassy behaviour of proteins has been the subject of considerable discussion (Doster et al., 1989; Frauenfelder et al., 1991; Green et al., 1994) and, given the preponderance of proteinaceous drug molecules under development, this area may represent a subject of considerable future interest within the pharmaceutical sciences.

2.3. Available methods for the measurement of T_g

As T_g is associated with a change in molecular mobility, the transition will consequently affect many of the material's physical characteristics. It is therefore possible to detect glass transitional behaviour by changes in volume/density, heat capacity, viscoelastic moduli, electrical permittivity and refractive index. There are a number of available methods by which the glass transition may be studied and the interested reader is referred to the text by Hancock and Zografi (1997) for a more detailed discussion of these approaches along with other methods such as NMR. In the present text the use of thermal methods will be outlined. The simplest method with which to measure the change in heat capacity associated with the glass transition is differential scanning calorimetry (DSC). This technique lends itself to the measurement of T_g values for small quantities of powdered material, which is the most likely state in which an amorphous pharmaceutical system will be presented to the analyst. Other thermal approaches include thermomechanical analysis (TMA), whereby the dimensional changes of a sample are measured as a function of temperature. This method is usually employed for the measurement of polymeric films, although in practice cast films of a range of amorphous materials may be analysed (Hancock et al., 1995).

The change in the viscoelastic properties of an amorphous material with temperature can be measured by dynamic mechanical analysis (DMA, Craig and Johnson, 1995; Haines, 1995). An oscillatory stress is applied to the sample and both the magnitude and phase relationship of the stress and strain values are measured as a function of either frequency or temperature. When the sample is heated through the glass transition the shear modulus decreases, often by several orders of magnitude. The ratio of the loss and storage modulus is equal to tan δ, and the glass transition is seen as a peak in tan δ when plotted against temperature. Andronis and Zografi (1997) have described the use of DMA for the determination of the glass transitional behaviour for amorphous indomethacin. Dielectric analysis (DEA) is another oscillatory technique, but in this case a sinusoidal oscillatory electric field is applied to the sample. The complex dielectric permittivity is measured as a function of temperature and may change abruptly through the

glass transition, thereby allowing identification of the T_g (McCrum et al., 1967).

2.3.1. The measurement of T_g by differential scanning calorimetry

DSC is the most frequently used technique for the measurement of glass transitional behaviour. The method involves the heating or cooling of a sample and reference and the measurement of the differential heat flow (power) between them with respect to temperature (or, less usually, time). There are two principal approaches to such measurements. Heat flux DSC involves the measurement of the temperature differential between the sample and reference and the subsequent calculation of the equivalent heat flow, as given by:

$$\Delta Q = (T_s - T_r)/R_T \quad (4)$$

where Q is heat, R_T is the thermal resistance of the cell and T_s and T_r are the sample and reference temperatures, hence at a given scanning rate the heat flow (power, P) is obtained and displayed against temperature.

Power compensation DSC involves the application and measurement of a compensatory power input (P) to one or other pan in order to maintain both at the same programme temperature, hence:

$$P = I^2 R \quad (5)$$

where I is the current supplied to the heater of resistance R in order to maintain equality of temperature.

As power is energy per unit time and the sample is being heated or cooled at a predetermined rate, in effect the instrument is measuring the difference in energy required to raise the temperature of either sample or reference by a unit amount, i.e.

$$P = \mathrm{d}Q/\mathrm{d}t = C_p \cdot \mathrm{d}T/\mathrm{d}t + f(t,\ T) \quad (6)$$

where $f(t,\ T)$ is a function of temperature and time and reflects kinetically controlled events such as melting and crystallisation. In essence, therefore, DSC is measuring the difference in apparent heat capacity between the two pans. Consequently, the glass transition is seen as a step in the baseline which allows identification of both the temperature and, with suitable calibration, quantification of the ΔC_p value, although the latter has not been extensively employed in the study of pharmaceutical systems. It should also be pointed out that, due to the scaling direction on the ordinate, power compensation DSC shows endotherms and exotherms pointing upwards and downwards respectively, while heat flux instruments show the opposite.

Fig. 3 shows an idealised sketch of a typical glass transitional response measured using DSC, the response being expressed in terms of the change in heat capacity. T_g is preferably specified as the temperature of half vitrification on cooling, i.e. the temperature at which the heat capacity is midway between the liquid and glassy state (Wunderlich, 1990). It is determined by the extrapolation of the C_p (or power) plots for the glass and the liquid/rubber state, with T_g given as the midpoint between the two lines. In addition the glass transition range and onset can be used to characterise this region. The beginning of the transition is given by T_b, whereas the extrapolated onset temperature is T_1. Similarly, the extrapolated endpoint is T_2 and the transition end is given by T_e. However, in practice the use of parameters such as T_b and T_e may be limited by the difficulties

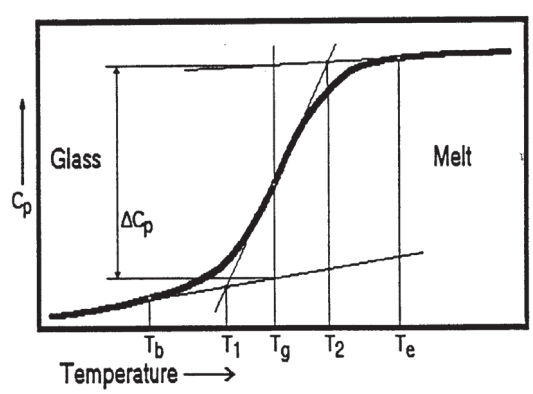

Fig. 3. Schematic representation of the change in heat capacity through the glass transition, indicating the various parameters that may be used to express the glass transitional behaviour (Reprinted from Thermal Analysis, B. Wunderlich, Academic Press, London, Copyright (1990), with permission from Elsevier Science).

 Sun Ex. 1025

associated with distinguishing the beginning and end of the transition from the baseline.

It is important to note that the value of the glass transition depends on the heating and cooling rate. As illustrated in Fig. 1, a fast cooling rate produces a higher value for T_g than does the use of slower rates (Richardson and Savill, 1975). This relationship may be described in terms of the relaxation behaviour of the system. The time scale for the relaxation processes is higher at lower temperatures, i.e. the relaxation will be slower (Moynihan et al., 1974). Consequently, at slower rates the temperature at which the relaxation process becomes comparable with the time scale of the experiment will be lower, hence the measured T_g will be lower. This issue is a source of considerable confusion when constructing quality control tests, as unlike melting which is independent of heating rate, the glass transition is a response to a heating or cooling signal and hence will vary depending on the method of measurement.

The difficulty of scanning rate dependence may be overcome by use of the fictive temperature T_f, which represents the temperature at which the extrapolated enthalpies above and below to the glass transition are equal. As described above, the glass transition is a kinetic event and therefore highly dependent on the heating or cooling rate of the temperature programme. However, the true value for the glass transition temperature is only dependent on the conditions of formation, e.g. on the cooling rate of the sample in the case of quenching. The heating rate dependence is seen because DSC is a dynamic technique, leading to possible differences in the experimental and molecular time scales. Consequently, analysis of the transition on heating will not give the 'true' T_g value, but a dynamic glass transition temperature dependent on the underlying heating rate. To remove this problem, Richardson and Savill (1975) have suggested measurement of the fictive temperature which is independent of heating rate. This method involves raising the temperature from a steady value below the glass transition region, T_1, to a steady value above T_g, T_2, and, using suitable calibration, recording the specific heat as a function of temperature, i.e.

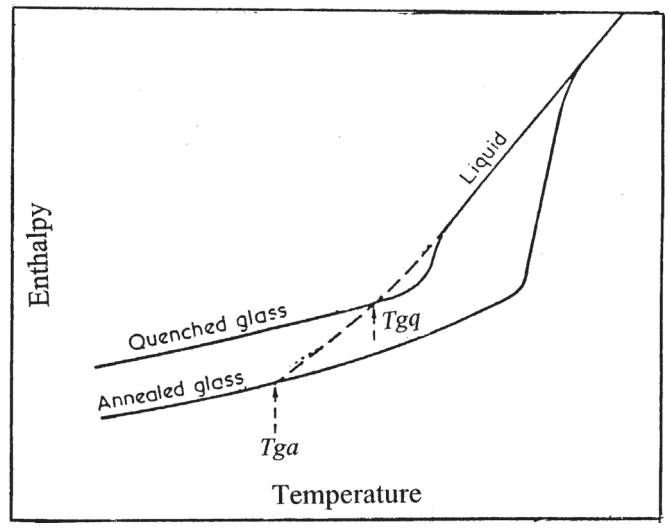

Fig. 4. Schematic representation of the fictive temperature, showing the extrapolation of the enthalpy curves below and above T_g for a quenched (T_{gq}) and annealed (T_{ga}) glass. The solid lines depict typical experimental paths, while the dashed line shows how the fictive T_g is defined [adapted from Richardson and Savill (1975)].

$$C_{pg} = a + bt, \qquad C_{pl} = A + BT \tag{7}$$

where C_{pg} and C_{pl} are the specific heat capacities of the glassy and liquid states and a, b, A and B are constants determined by linear regression. Integration of these equations gives:

$$H_g(T) = aT + 1/2\, bT^2 + P,$$
$$H_l(T) = AT + 1/2\, BT^2 + Q \tag{8}$$

where P and Q are constants. The fictive temperature is defined as the intersection of the extrapolated enthalpy curves and is obtained by solving for the quadratic for T when $H_g(T) = H_l(T)$. is shown schematically in Fig. 4. The value may also be determined via calculation of the areas under the experimental heat capacity curves; interested readers are referred to the work by Moynihan et al. (1976) and Moynihan (1994) for more details. Use of the fictive temperature could have several advantages as far as pharmaceutical systems are concerned, particularly in terms of establishing reliable quality control protocols.

A further, often considerable problem associated with the measurement of T_g using DSC is the presence of a relaxation endotherm; this feature is seen on heating as an endothermic response superimposed on the baseline shift which may ren-

der identification and quantification of the T_g extremely difficult (an example of an amorphous drug showing this behaviour is given in Fig. 5). Indeed, for complex or multicomponent samples such as freeze dried formulations it may be extremely difficult to differentiate between a glass transition and a melting response, although differences in rate dependence of the relaxation and melting endotherms may allow distinction between the two. The relaxation endotherm may arise as a result of one of two processes. Firstly, it may reflect a mismatch in the rate of cooling and subsequent heating of the sample. This may be explained with reference to Fig. 6. When cooling is slow the glass that forms has long relaxation times 'frozen in'. Subsequently, when this material is heated quickly, with a faster rate through the glass transition than was the case on cooling, the relaxation times are slow with reference to the heating rate, thereby producing an overshoot in the enthalpy curve. In other words, the molecules within the glass cannot achieve the motion required for the glass transition within the time scale of the heating rate and the glass briefly superheats (Wunderlich, 1990). Once the relaxation times lower to the order of the heating rate the superheated glass reverts quickly towards the liquid line in the enthalpy curve. This overshoot and subsequent recovery in the enthalpy curve produces the characteristic endothermic peak in the ΔC_p (or power) curve. The second reason for the appearance of the endothermic relaxation is that because glasses are not in an equilibrium state, they can relax over time, thereby decreasing the enthalpy and volume of the material and increasing the structural relaxation time. Consequently, such annealing also produces a relaxation endotherm at the glass transition for the same reasons as above, the magnitude of which may be used to calculate relaxation times for the sample (Montserrat, 1989; Hancock et al., 1995), this is described in more detail in Section 3.2.

There are a number of difficulties associated with the presence of relaxation endotherms. Over and above problems in identifying glass transitions, the quantification of both the T_g value and the relaxation endotherm may be difficult, as illustrated in Fig. 5. It is not clear, for example, exactly where under the peak the T_g lies (although the fictive temperature may be of use in this

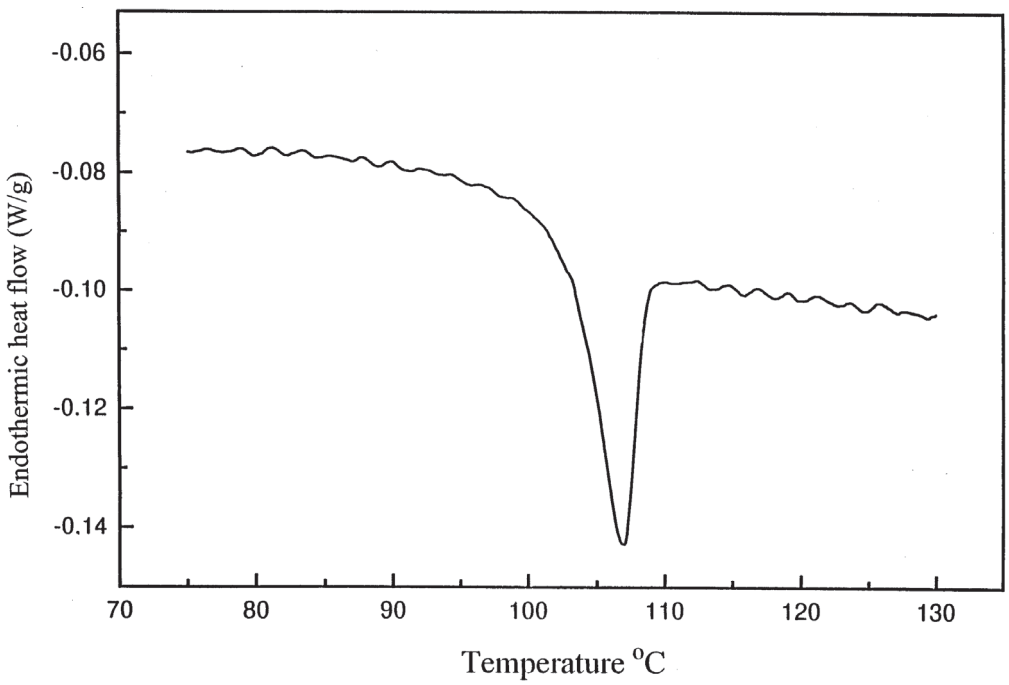

Fig. 5. Glass transition of saquinavir, showing the relaxation endotherm associated with T_g (Reprinted from Pharm. Res., 15, P.G. Royall, D.Q.M. Craig and C. Doherty, Characterisation of the glass transition of an amorphous drug using modulated DSC, pp. 1117–1121, Copyright (1988), with permission from Elsevier Science).

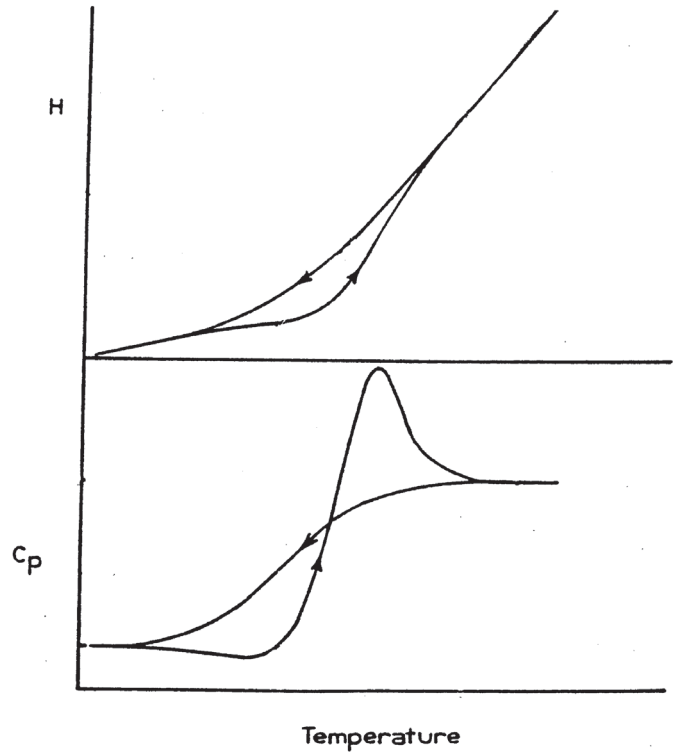

Fig. 6. Schematic representation of the origin of the relaxation endotherm caused by a mismatch in cooling and heating rates. The figure shows the enthalpy and corresponding heat capacity changes through the T_g region on slow cooling and fast heating.

respect), hence it is also difficult to calculate the value of the relaxation enthalpy. This may be overcome by controlling the thermal history of the sample, particularly by heat/cooling which should remove annealing effects. However, this may not be appropriate for pharmaceutical samples which are often multicomponent and hence may not be reversibly temperature cycled. An alternative approach involves the use of modulated temperature DSC, which allows separation of the relaxation endotherm from the glass transition, although Royall et al. (1988) have identified certain provisos associated with such measurements. This technique is discussed in more detail below.

In addition to the above, there are also a number of practical considerations associated with the measurement of glass transitions using DSC. The baseline shifts may be small, particularly for strong glasses, rendering differentiation from baseline noise a non-trivial problem. However, a true glass transition will be reproducible and should be seen on both heating and cooling (given the provisos listed above). Furthermore, it is essential that adequate baseline calibration is performed to ensure as flat a baseline as possible; if extensive bowing is present then quantitative identification of the T_g will be extremely difficult. Baseline calibration is achieved by subtraction of the response from two empty DSC pans in the reference and sample positions. It is also possible to use higher scanning rates to aid visualisation of the T_g. Inspection of Eq. (6), for example, indicates that at higher values of dT/dt, the heat flow will be greater, hence the sensitivity of the measurement may be improved (although there will inevitably be a concomitant loss of resolution); the influence of the heating rate on the glass transition value should, however, be noted. Great care is required if the ΔC_p value at T_g is to be measured quantitatively, as it is essential to perform adequate reference calibration using a standard such as sapphire in order to obtain meaningful data.

Sample preparation conditions must be carefully controlled (and stated), particularly in terms of the choice of pans and level of residual moisture or other solvents, as otherwise changes to the sample either during or prior to analysis may have a profound influence on the measured T_g. A recent investigation (Hill et al., 1998) indicated that the measured T_g of spray dried lactose may vary by $\approx 35°C$ depending on pan type. This is due to the retention of water in hermetically sealed pans which acts as a plasticizer (discussed below), thereby reducing T_g compared to non-hermetically sealed or open pans from which water is lost during the heating run.

T_g measurements are, ideally, performed in cooling rather than heating cycles, as in the former the sample starts from the equilibrium liquid state before entering the nonequilibrium glass, which is a reproducible route compared to starting from the glass in a heating cycle (Wunderlich, 1990). However, as mentioned above, many pharmaceutical systems such as drugs and freeze dried systems are heat sensitive and may not withstand cycling. Similarly, other systems are multicomponent, hence heating through the T_g of one compo-

nent may result in irreversible changes in the structure of the system as a whole. Consequently, most pharmaceutical studies have involved measuring T_g upon heating.

The development of MTDSC has removed or lessened some of the difficulties associated with characterising glass transitions. This technique represents a change in the software of the conventional DSC and, in the model marketed by TA Instruments (modulated DSC, MDSC) involves the application of a sinusoidal heating signal superimposed on the linear programme, hence information may be derived from both the sine wave response and the Fourier transformed total heat flow output (equivalent to conventional DSC). The advantage of the technique is that changes in heat capacity (i.e. glass transitions) may be seen in isolation from other events, particularly relaxation endotherms, with a considerably enhanced signal-to-noise ratio. An example of this is shown in Fig. 7 for the amorphous drug saquinavir. More details of this technique are available elsewhere (Royall et al., 1988; Reading et al., 1993; Coleman et al., 1996; Hill et al., 1998) and the method is expected to make a major contribution to the assessment of glassy pharmaceuticals.

2.4. Plasticization of amorphous materials

A highly important consideration with regard to the glass transition of amorphous materials is the effect of the presence of additional materials, particularly water, on the value of T_g. The study of mixed systems has been of particular importance in the polymer science field, whereby materials may be multicomponent, with the degree of mixing being estimated by observing the glass transitions of the product in relation to those of the individual components. Mixing of amorphous pharmaceuticals is an approach that may be used to raise the glass transition of a product in order to improve stability (Fukuoka et al., 1989).

The work on which amorphous mixing theory is based is that of Gordon and Taylor (1952), which was originally used to describe the behaviour of polymer blends. The Gordon Taylor equation is based on free volume theory and gives the glass transition, $T_{g_{mix}}$, of a binary mixture,

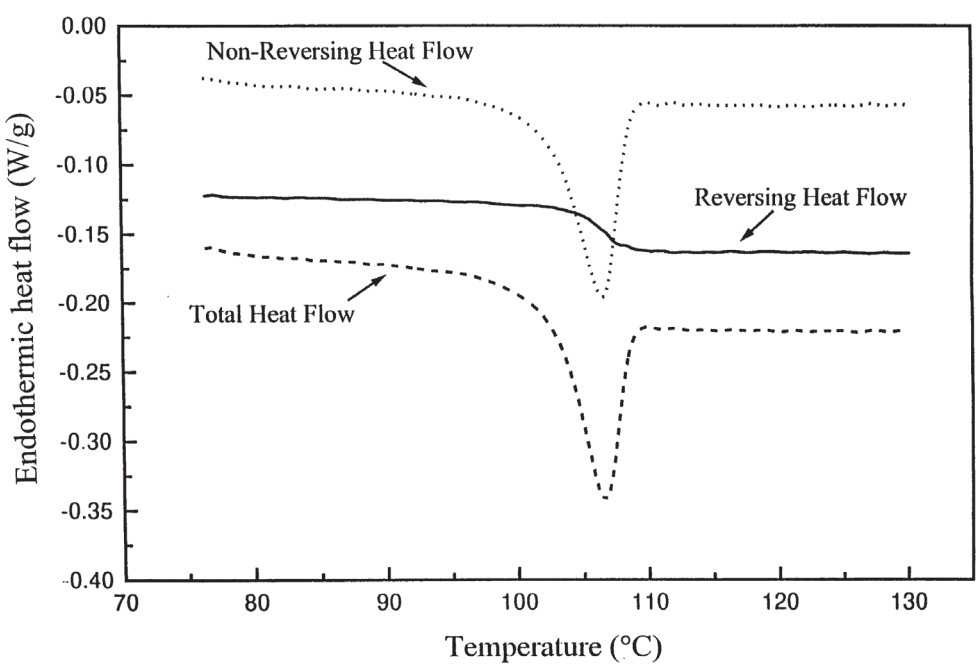

Fig. 7. Separation of the DSC response of amorphous saquinavir into the reversing and non-reversing signals, in which the glass transition and relaxation endotherm may be seen in isolation (Reprinted from Pharm. Res., 15, P.G. Royall, D.Q.M. Craig and C. Doherty, Characterisation of the glass transition of an amorphous drug using modulated DSC, pp. 1117–1121, Copyright (1988), with permission from Elsevier Science).

assuming no specific interaction between the two components, via:

$$T_{g_{mix}} = \varphi_1 T_{g_1} + \varphi_2 T_{g_2} \quad (9)$$

where φ is the volume fraction and the subscripts represent the two components. The volume fraction can be described in terms of the weight fraction of the components w, as $\varphi = (w\Delta\alpha)/\rho$, where $\Delta\alpha$ is the change in thermal expansivity at T_g and ρ is the density of the material. Redefining Eq. (9) in terms of weight fraction gives:

$$T_{g_{mix}} = \frac{(w_1 T_{g_1}) + (K w_2 T_{g_2})}{w_1 + (K w_2)} \quad (10)$$

where:

$$K = \frac{(\rho_1 \Delta\alpha_2)}{(\rho_2 \Delta\alpha_1)} \quad (11)$$

which can be simplified by application of the Simha–Boyer rule to:

$$K = \rho_1 T_{g_1} / \rho_2 T_{g_2} \quad (12)$$

K can be considered the ratio of the free volumes of the two components. The goodness of fit of experimental data to the Gordon–Taylor equation gives an idea of the ideality of mixing of two components, as well as providing a predictive tool for assessing the effects of different levels of a second material on T_g. In terms of pharmaceutical systems, the area in which this approach has proved to be particularly important is in the study of the effects of water on the glass transition. It is well recognised that residual water levels may be an important factor in determining chemical stability and mechanical properties. However, water will also have a profound effect on the glass transition of amorphous pharmaceuticals, acting as a plasticizer by increasing the free volume of the material, hence leading to a decrease in T_g. Exactly the same principle applies to the inclusion of plasticizers in polymeric film coats, although in the light of the systems discussed in the next section the question of water sorption will be emphasised here.

In the amorphous state considerably more water may be taken up relative to the crystalline form (Hancock and Zografi, 1997), effectively due to absorption of water into the solid, hence in contrast to crystalline systems the uptake process tends to be dependent on sample mass rather than surface area. A typical profile of T_g against water uptake is shown in Fig. 8 (Hancock and Zografi, 1994). As the concentration of water in the solid increases, the T_g is seen to decrease according to the Gordon–Taylor equation (or an approximation of this relationship if the system is non-ideal). At any particular temperature, therefore, the system may change from the glassy to the rubbery state if water uptake takes place, with concomitant implications for chemical degradation or devitrification (discussed below). Ahlneck and Zografi (1990) have emphasised that at higher storage temperatures, a lower amount of water is required to lower the T_g to that particular temperature (Fig. 9). As a general principal, this has profound implications for accelerated stability testing of amorphous pharmaceuticals. Hancock and Zografi (1994) have reported that water is a potent plasticizer for a wide range of pharmaceutical materials including PVP, lactose, starch, sucrose and others.

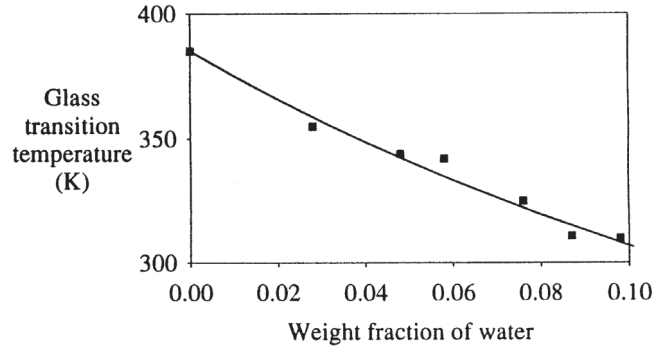

Fig. 8. Variation in the glass transition of freeze dried lactose with water content. Solid line is the predicted behaviour from Eq. (8)(Reprinted from Pharm. Res., 11, B.C. Hancock and G. Zagrafi, The relationship between the glass transition temperature and the water content of amorphous pharmaceutical solids, pp. 1166–1173, Copyright (1994), with permission from Elsevier Science).

3. Relevance of the glassy state to amorphous drugs

There are numerous ways in which the amorphous state is of relevance pharmaceutically and a full discussion of all such considerations would be

prohibitively lengthy. Instead, this and the following section will focus on two of the most important aspects of glassy pharmaceuticals, namely amorphous drugs and freeze dried systems.

3.1. Amorphous drugs and dissolution behaviour

It is well recognised that, for many drugs, dissolution within the gastrointestinal tract may be the rate limiting step to absorption, hence improvement in the dissolution rate may enhance the bioavailability of that drug. One approach to such improvement is to prepare the drug in an amorphous form, the higher molecular mobility of this form compared to the equivalent crystalline material may lead to enhanced dissolution rate and bioavailability, although there are important disadvantages to the approach which will be discussed below.

From the discussion given above, it is clear that the basic physico-chemical parameter that may be used to characterise amorphous drugs is the glass transition temperature. Kerč and Srčič (1995) have prepared a highly useful compilation of T_g and melting point (T_m) data for a number of drugs which is reproduced in Table 1. It should be born in mind that the conditions under which the various samples were prepared and measured are highly unlikely to be uniform, hence absolute comparisons between data points may not be applicable. Furthermore, it is extremely difficult in practice to remove all of the water associated with such samples, hence plasticization may have taken place in some cases. Nevertheless, a number of useful trends may be extracted from the data. In particular, the authors have discussed the significance of the ratio between the glass transitional and melting parameters, as T_g/T_m tends to be ≈ 0.5 for symmetrical polymers and 0.7 for asymmetrical (Gujrati and Goldstein, 1980), this refers back to the concept of fragile and strong glasses outlined in Section 2.2, although here the ratio is inverted. Kerč and Srčič (1995) report values between ≈ 0.6 and 0.8 for low molecular weight pharmaceuticals, which are slightly higher than those found for polymeric systems. According to the 'rule of thumb' of T_m/T_g ratios outlined earlier, this indicates that low molecular weight pharmaceutical systems are more fragile glass-forming systems than polymers. The knowledge of this ratio is of use as it does allow the formulator to make a rough estimate of where a glass transition is likely to be found for a drug with a known melting point.

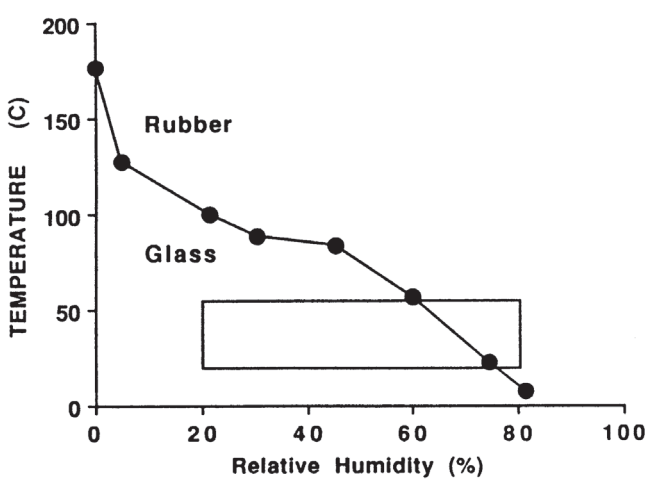

Fig. 9. Effect of relative humidity in the glass transition temperature of PVP K30. The box illustrates conditions typically used in accelerated storage testing (Reprinted from Int J. Pharm., 62, C. Ahlneck and G. Zografi, The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state, pp. 87–95, Copyright (1990), with permission from Elsevier Science, data compiled from Oksanen (1992)).

A number of examples of enhanced dissolution for amorphous drugs are available in the literature. For example, 9,3″-diacetylmidecamycin (MOM), a 16-membered macrolide antibiotic, was prepared in the crystalline and amorphous forms and the dissolution behaviour of both at a range of temperatures compared (Sato et al., 1981). Figs. 10 and 11 show the corresponding dissolution profiles for the two forms; the amorphous form clearly shows higher dissolution rates, although at longer dissolution times the concentration in water decreases due to the formation of crystalline MOM from the supersaturated solution. Similarly, the dissolution rate of amorphous indomethacin has been reported to be greater than for the crystalline material (Fukuoka et al., 1986) as shown in Fig. 12 for water–ethanol and water systems. Interestingly, these authors also studied the relaxation behaviour of the glassy system on storage below T_g, showing that while quench cooled indomethacin was stable over a 2 year period at room temperature, pulverised

Table 1
Glass transition (T_g) and melting point (T_m) data for a range of pharmaceuticals [reproduced from Kerč and Srčič (1995)]

Pharmaceutical	T_g/K	T_m/K	T_g/T_m
Glycerol	180	291	0.62
Aspirin	243	408	0.59
Dibucaine	246	336	0.73
Mephenesin	247	340	0.73
Antipyrine	256	380	0.67
Ribose	263	360	0.73
Sorbitol I	270	384	0.70
Methyltestosterone	270	421	0.64
Sorbitol II	271	367	0.74
Phenylbutazone	277	377	0.73
Quinine ethylcarbonate	278	362	0.77
Progesterone	279	399	0.70
Pentobarbital	279	408	0.69
Atropine	281	379	0.74
Ethacrynic acid	282	398	0.71
Citric acid	283	432	0.72
Xylose	283	426	0.66
Tolbutamide	284	403	0.70
Hexobarbital	286	423	0.68
Amobarbital	286	432	0.66
Fructose	286	373	0.77
Tolnaphtate	287	384	0.75
Nimodipine	288	389	0.74
Tartaric acid	289	430	0.67
Flufenamic acid	290	406	0.71
santonin	290	434	0.67
Ergocalciferol	290	376	0.77
Proxyphylline	295	403	0.73
Acetaminophen	295	447	0.66
Cholecalciferol	296	352	0.84
Paracetamol	297	347	0.86
Eserine	297	378	0.79
Nialamide	297	427	0.70
Chlorotrianisene	298	393	0.76
Chlorimphenicol I	301	349	0.86
Acetaminophen	302	441	0.69
Glucose	303	419	0.72
Nitrendipine	303	429	0.71
Sulphisoxazole	306	460	0.67
Chloramphenicol II	306	414	0.74
Stilbestrol	308	439	0.70
Estradiol-17β-cypionate	309	425	0.73
Dextrose	310	432	0.72
Diphylline	315	438	0.72
Phenobarbital	315	452	0.70
Maltose	316	375	0.84
Felodipine	316	407	0.76
Phenobarbital	321	443	0.72
Norethynodrel	324	453	0.72
Quinidine	326	445	0.73
Sucrose	329	453	0.73
Spironolactone	331	478	0.69
Salicin	333	466	0.71
Sulphathiazole	334	471	0.71
Chlormadinone acetate	334	483	0.69
β-Estradiol-3-benzoate	336	472	0.71
Amlodipine besylate	337	467	0.72
Sulphadimethoxine	339	465	0.73
Glibenclamide	344	447	0.77
Dehydrocholic acid	348	502	0.69
Cellobiose	350	498	0.70
Trehalose	350	476	0.74
17β-Estradiol	354	445	0.80
Nicardipin hydrochloride	358	440	0.81
Griseofulvin I	362	422	0.86
Brucine	365	451	0.81
Griseofulvin II	370	497	0.74
Chenodeoxycholic acid	371	436	0.85
Deoxycholic acid	377	447	0.84
Ursodeoxycholic acid	378	477	0.79
Cholic acid	393	473	0.83

amorphous drug crystallised over a period of months.

Amorphous drugs may be prepared in a number of ways such as rapidly cooling from the melt or precipitation from suitable solvent systems. Drying or grinding may deliberately or accidentally induce amorphous characteristics. A ground mixture of griseofulvin and microcrystalline cellulose significantly improved both the dissolution rate and bioavailability of the drug compared to a micronised griseofulvin powder preparation (Yamamoto et al., 1974). This was ascribed to an increase in amorphous content of the drug as a result of the grinding process. Grinding of phenytoin with microcrystalline cellulose was also found to enhance drug dissolution rate, this again being attributed to the formation of an amorphous form of the drug (Yamamoto et al., 1976).

Spray drying may also be used to prepare amorphous systems. A review of the physical structure of spray dried products, with special reference to thermal analysis, has been given by Corrigan (1995). The formation of amorphous pharmaceuticals via this process has been demonstrated for a number of drugs such as digitoxin (Nurnburg, 1976) and a range of thiazide diuretics

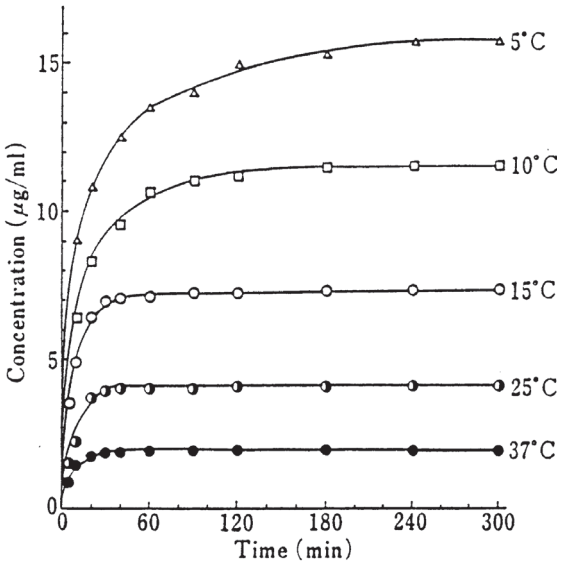

Fig. 10. Concentration-time curves for crystalline MOM in distilled water at various temperatures [reproduced from Sato et al. (1981)].

(Corrigan et al., 1984). In addition, Hill et al. (1998) have recently studied the glass transitional behaviour of spray dried lactose using modulated DSC. Interestingly, Corrigan (1995) has described how changing the spray drying conditions may result in the formation of materials with differing T_g values and physical properties. For example, Matsuda et al. (1992) prepared amorphous frusemide using two spray drying protocols and reported that the resultant materials had differing T_g values (44.2 and 54.4°C). These changes may be a result of differences in moisture content but the study clearly demonstrates the dependence of T_g on spray drying protocol. Similarly, dichloromethane solutions of 4″-*O*-(4-methoxyphenyl)acetyltylosin

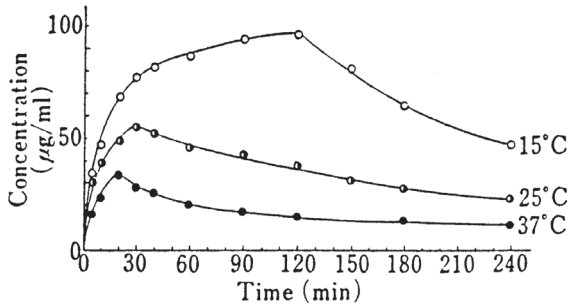

Fig. 11. Concentration-time curves for amorphous MOM in distilled water at various temperatures [reproduced from Sato et al. (1981)].

(MAT) were spray dried using a range of inlet temperatures (Yamaguchi et al., 1992). The authors performed storage studies on the samples at temperatures $< T_g$, finding that materials prepared using inlet temperature between T_g and T_m were more stable than those prepared at lower or higher temperatures, even though the storage conditions were identical for the various samples.

These studies touch on one of the questions which, to date, does not appear to have been fully answered, namely to what extent can the amorphous 'form' of a drug vary depending on manufacturing or processing conditions? It is predictable from basic theory that changing the preparation conditions such as cooling rate from the melt or the presence of trace plasticisers (particularly water) may alter the T_g (and hence 'structure') of the material. For example, Kerč et al. (1991) have reported (comparatively small) changes in the T_g value of felodipine on altering the cooling rate from the melt. However, marked changes in the dissolution rate were observed depending on cooling conditions (Fig. 13), even though the glass transition values for systems cooled at 1°C/min and those cooled at the maximum set rate of the instrument showed differences in T_g of < 2°C. It is conceivable that other factors such as the macroscopic integrity of the sample may have contributed to this effect. However, the change in dissolution profile is clearly of potential practical significance. Angell (1995a) has discussed the concept of 'polyamorphism', whereby materials may exist in distinct disordered forms, as has been reported for water (Mishima et al., 1984, 1985, 1991). The generation of such forms appears to take place under fairly extreme conditions and hence the direct relevance to pharmaceutical materials is far from certain. However, the suggestion that polyamorphism may exist at all is of interest.

3.2. The physical stability of amorphous drugs

Given the potential advantages of preparing drugs in an amorphous form, the question arises as to why this approach is not used more often. The single most important reason is undoubtedly the problems associated with stability, both physical and chemical. The amorphous state is, by defini-

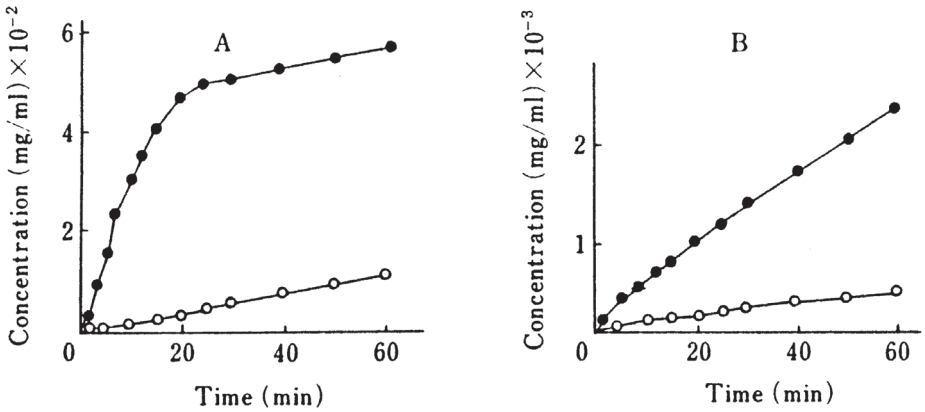

Fig. 12. Dissolution of crystalline and amorphous indomethacin in water-ethanol (A) and water (B) ● glass ○ crystalline material [reproduced from Fukuoka et al. (1986)].

tion, metastable with regard to the crystalline material, hence amorphous drugs will tend to revert to the crystalline form over a period of time. Prediction of the timescales involved is clearly critical and yet may be difficult to achieve. However, with some knowledge of the fundamental principles of the crystallisation process and the physico-chemical properties of the drug in question it is possible to make some assessment of the likelihood of devitrification and to recommend storage conditions which will minimise the risk of the process occurring.

Crystallisation from the amorphous state is governed largely by the same factors that determine crystallisation from the melt and has been discussed in more detail by Hancock and Zografi (1997). In the case of amorphous materials, there are two conflicting phenomena associated with the crystallisation process. As the temperature is lowered, the rate of nucleation may be expected to increase. However, the molecular mobility decreases as the temperature decreases, particularly below T_g, thereby slowing the molecular diffusion and reducing the rate of crystallisation. This is summarised in Fig. 14 which shows that the maximum rate of crystallisation will take place between T_m and T_g. If a sample is stored below T_g the risk of devitrification is considerably reduced due to the molecules having lower mobility with which to undergo crystal growth; studies on the crystallisation behaviour of sucrose and lactose appear to confirm the importance of T_g in this respect (Makower and Dye, 1956; Saleki-Gerhardt and Zografi, 1994). However, it should be stressed that storage below T_g is far from a guarantee of physical stability. Hancock et al. (1995) have shown that the molecular mobility below T_g may be sufficient to result in devitrification over the typical storage periods encountered for pharmaceutical products. The relationship between T_g and the stability of glassy pharmaceuticals is therefore complex; studies comparing the stability of glassy indomethacin and phenobarbital, both of which have similar T_g values, showed very considerable variation in stability profiles even though both were stored below their T_g values

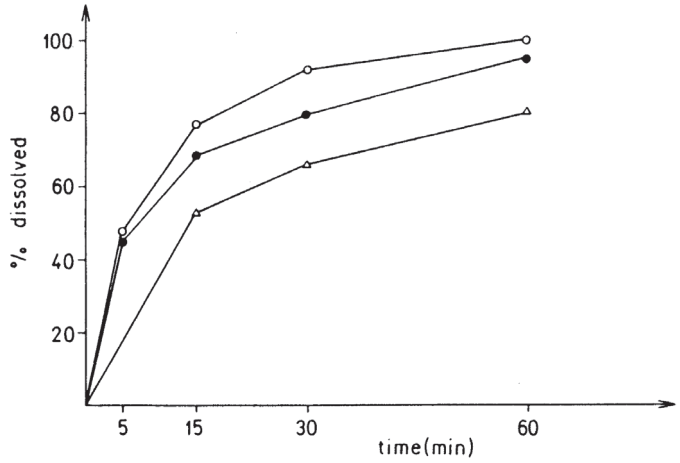

Fig. 13. Dissolution of felodipine in water-ethanol. △ crystalline felodipine ● glassy felodipine prepared by cooling under ambient conditions o glassy felodipine prepared by quenching in liquid nitrogen (Reprinted from Int. J. Pharm., 68, J. Kerč, S. Srčič, M. Mohar and Smid-Korbar, Some physiochemical properties of glassy felodipine, pp. 25–33, Copyright (1991), with permission from Elsevier Science).

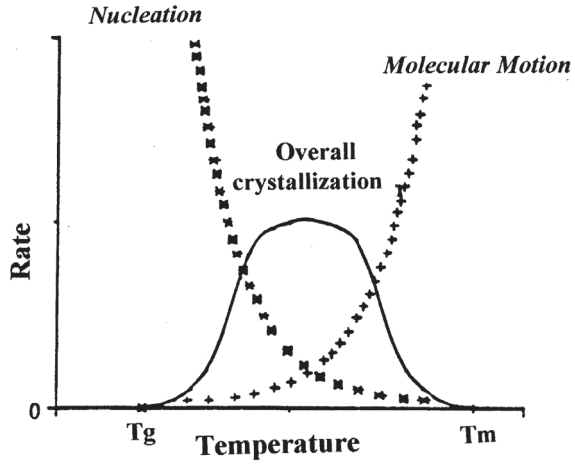

Fig. 14. Schematic representation of the temperature dependence of the crystallisation process for an amorphous system [Hancock and Zografi, 1997, after Jolley (1970)].

(Fukuoka et al., 1989). Glassy indomethacin was reported to be stable over a 2 year period at room temperature, while phenobarbital devitrified within 1 week. These differences were ascribed to variations in steric structure and hydrogen bonding within the two materials. It is also important to emphasise that even if one is storing the material below the T_g of the dry materials, the presence of trace levels of water may plasticize the sample sufficiently to bring the T_g value to that of the storage temperature. Consequently, it is recommended that if possible the material should be kept as dry as possible. It has been shown, for example, that the crystallisation temperature of salbuterol sulphate may be reduced by the presence of sorbed water (Ward and Schultz, 1995). The converse also applies; inclusion of materials such as PVP, which has a high T_g value ($\approx$ 180°C), may improve the physical stability of glassy drugs by raising the T_g value of the binary system (Fukuoka et al., 1986; Yoshioka et al., 1995).

Hancock et al. (1995) have argued that the key issue is that of the temperature in relation to the T_g at which a glassy drug should be stored in order to provide a pharmaceutically acceptable stability profile. From the above discussion it is clear that storage below T_g is advantageous but is not a guarantee of stability due to the molecular mobility which is present below the glass transition temperature. The authors have argued that a reasonable guide would be that, if possible, the sample should be stored at least 50°C below the T_g value. Clearly, this may not always be practically possible and, in addition, the stability will inevitably be system dependent. However, the estimate affords the operator some prediction as to the likelihood of there being a physical stability problem, which may be of considerable benefit when at the early stages of planning a formulation strategy. The authors describe the measurement of the magnitude of the relaxation endotherm after storage at different temperatures as a means of calculating the relaxation time of the sample. More specifically, the maximum enthalpy recovery (ΔH_∞) is calculated via:

$$\Delta H_\infty = (T_g - T)\cdot\Delta C_p \tag{13}$$

where T_g and T are the glass and experimental temperatures and ΔC_p is the change in heat capacity at T_g. The extent of relaxation ϕ_t is then calculated at any time (t) and temperature (T) conditions via:

$$\phi_t = 1 - (\Delta H_t/\Delta H_\infty) \tag{14}$$

where ΔH_t is the measured enthalpy of recovery under those conditions. The relaxation time may then be calculated via the Williams–Watts equation (Williams and Watts, 1970):

$$\phi_t = \exp(-t/\tau)^\beta \tag{15}$$

where τ is the mean relaxation time constant and β is an empirical relaxation time distribution parameter ($0 \le \beta \le 1$). By non-linear regression it is possible to calculate the relaxation times over a range of conditions, thereby allowing an insight into the relationship between molecular mobility and temperature, with clear implications for storage stability. There are a number of difficulties associated with this approach, including the necessity to accurately calibrate for ΔC_p and the difficulty in reliably measuring the magnitude of the relaxation endotherm; given the associated shift in baseline, modulated temperature DSC may in the future prove to be highly useful in both respects. In addition, it is interesting to note that in the dielectrics field, from which the Williams–Watts equation was obtained, there is growing emphasis on non-empirical analysis of

non-Debye relaxation behaviour, particularly in terms of the Dissado–Hill theory (Dissado and Hill, 1979) which describes the relaxation behaviour in terms of many-body interactions rather than a spread of relaxation times which is in itself a controversial concept. As yet there has been no extensive cross-referencing of this theory with regard to glass transition measurements; such an approach could prove to be highly useful.

The recrystallisation behaviour of the sample on heating above T_g may also be characterised. This is commonly seen as an exotherm above the glass transition, as shown in Fig. 15 for spray dried lactose. This approach has been studied by Kerč et al. (1991), who describe two main methods for obtaining kinetic data from the exotherm. The heat evolution method (Caroll and Manche, 1972; Torfs et al., 1984) involves the measurement of the rate constant k at any temperature during the crystallisation process via:

$$k = \mathrm{d}H/\mathrm{d}t \cdot [(\Delta H_{tot}(\Delta H_{rem}/\Delta H_{tot}))^n]^{-1} \quad (16)$$

where $\mathrm{d}H/\mathrm{d}t$ is the heat flow, ΔH_{tot} is the total enthalpy, ΔH_{rem} is the enthalpy corresponding to the non-crystallised fraction at temperature T and n is the order of the reaction. An alternative approach is the variable heating rate method (Ozawa, 1975) whereby the exothermic peak maximum temperature is measured as a function of heating rate. These approaches are of use in estimating the kinetics of the crystallisation above T_g, although they are not applicable to the prediction of the behaviour at temperatures below the glass transition.

3.3. The chemical stability of amorphous drugs

Numerous reports have shown that rates of drug degradation may be enhanced in the amorphous state compared to the crystalline material. A series of papers in the 1970s explored the drug degradation of amorphous and crystalline forms of drugs. For example, the temperature dependent degradation of cefoxitin sodium was found to be markedly enhanced when the drug was prepared in an amorphous form (Fig. 16, Oberholtzer and Brenner, 1979), while Pikal et al. (1977) showed that the degradation rate of a range of β-lactam

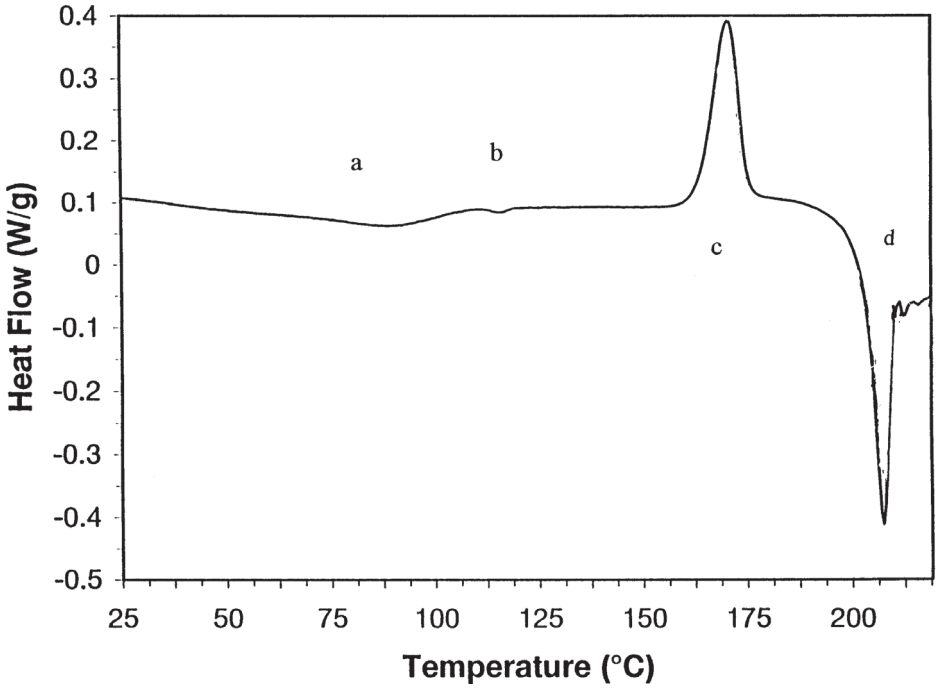

Fig. 15. DSC response for spray dried lactose, showing a water loss endotherm (a), a glass transition with accompanying relaxation endotherm (b), recrystallisation (c) and melting with decomposition (d) (Reprinted from Int. J. Pharm., 161, V.L. Hill, D.Q.M. Craig and L.C. Feely, Characterisation of spray-dried lactose using modulated differential scanning calorimetry, pp. 95–107, Copyright (1998), with permission from Elsevier Science).

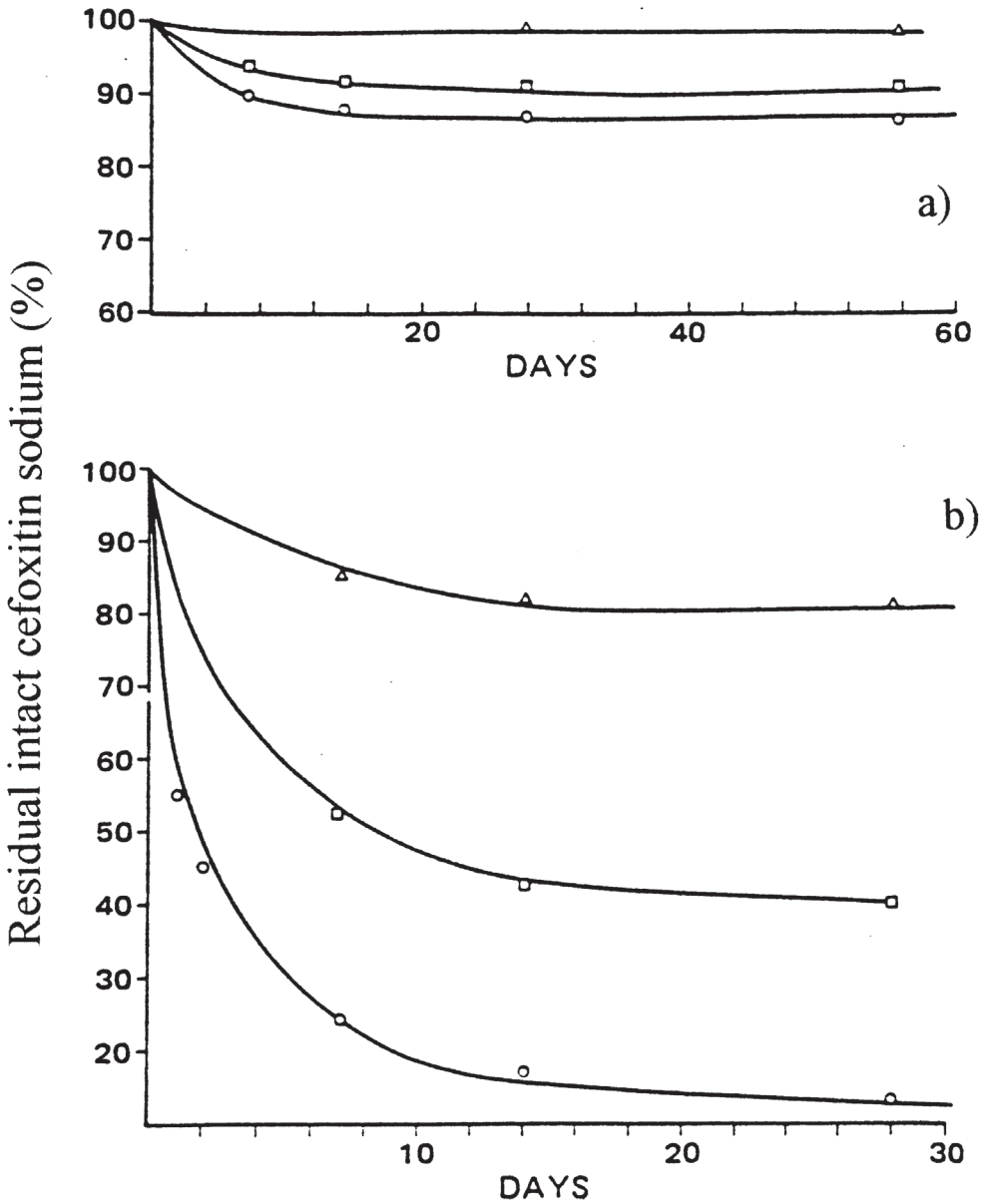

Fig. 16. Typical plots of decomposition of (a) crystalline and (b) amorphous cefoxitin sodium at various temperatures △ 40°C, □ 60°C and ○ 80°C [reproduced from Oberholtzer and Brenner (1979)].

antibiotics was enhanced by approximately an order of magnitude for amorphous as opposed to crystalline drugs, even when sorbed water levels were very low. Interestingly, these authors also reported a discoloration of amorphous cephalothin sodium prior to the development of a measurable potency loss.

The mechanism and kinetics of such degradation reactions have been the subject of some speculation. As one might expect, the presence of sorbed water has been reported to increase the degradation of a range of drugs including insulin (Strickley and Anderson, 1996) and the aforementioned β-lactam antibiotics (Pikal et al., 1977). In the latter study, the authors discussed the kinetics of the degradation process, finding that in general the antibiotics showed what appeared to be first order kinetics as opposed to the sigmoidal curve expected for solid state reactions (although this was not the case for all the drugs under study). However, the authors also reported that the temperature dependence of the decomposition rate indicated that the activation energy for the process was decreasing as the temperature was raised.

Pikal et al. (1977) suggested that this may be a function of the rate limiting step of the decomposition being associated with molecular reorientation processes, whereby certain steric conditions need to be satisfied in order for the reaction to occur. In other words, the degradation may be a function of molecular mobility and relaxation behaviour. The concept of orientation-specific degradation has been discussed by several authors. Sukenik et al. (1975, 1977) suggested that the solid state reactivity of aminobenzoate derivatives may be associated with their orientation within the crystal, while Hageman et al. (1992) have suggested that protein intermolecular reactivity may be higher in the solid state due to a higher 'effective concentration' compared to the solution. Similarly, Strickley and Anderson (1996) demonstrated that the formation of the [desamido$_{A21}$–Gly$_{A1}$] dimer was enhanced in glassy insulin compared to the aqueous solution. Pikal et al. (1977) suggested that as non-Arrhenius behaviour (involving the activation energy decreasing with temperature) is characteristic of glassy behaviour in the vicinity of T_g then the kinetics of degradation may be associated with relaxation behaviour of the amorphous solid. This is also consistent with the observation that the presence of water enhanced the decomposition rate, as over and above the presence of a chemical reactant, the water may be plasticizing the amorphous material and decreasing the relaxation time at the temperature of storage, several other studies have demonstrated a correlation between the chemical reactivity of an amorphous material and the glassy behaviour (Roy et al., 1992; Levine and Slade, 1993).

4. Freeze dried systems

4.1. Introduction

Freeze-drying has been used as a pharmaceutical unit operation for a number of years for the low temperature drying of injectable systems. However, the approach has recently become more prominent due to the necessity of preparing novel peptide and proteinaceous drugs in a dry, stable form which may be easily reconstituted prior to parenteral administration. The challenges associated with preparing such formulations have necessitated re-examination of the physico-chemical principles underlying the freeze drying process, hence research is ongoing in order to enhance predictability of the chemical and physical stability of such products. One aspect of this technology which has been the subject of considerable study is the glass transitional behaviour of both the frozen system prior to drying and of the finished product. A number of excellent texts are available on the subject of freeze drying to which the interested reader is referred to for more details (Holdsworth, 1987; Pikal, 1990a,b; Nail and Gatlin, 1993; Pikal, 1993; Franks, 1994). In the interests of brevity, the description of the freeze drying process given here is intended as an outline of the amorphous nature of these systems and is not meant as a comprehensive discussion of all aspects of the process.

Freeze drying involves the desiccation of a substance by crystallisation of ice, followed by sublimation of water vapour from the solid state at reduced pressure. Drying at low temperatures theoretically avoids extensive chemical degradation and the resulting solid tends to have an open porous structure that facilitates the rehydration process, this may be essential in life threatening conditions whereby rapid reconstitution may be critical. The process has found considerable application in the processing of pharmaceutical products of biological origin such as serums, vaccines, peptide drugs and liposomes, as the stability of freeze-dried solids is usually much higher than the equivalent aqueous solution or suspension. In addition, there has also been considerable interest in the use of freeze drying as a means of producing rapidly dissolving oral dosage forms (Corveleyn and Remon, 1997). However, there may be substantial difficulties associated with both the chemical and physical stability of freeze dried products, hence there is considerable interest in establishing the nature of the interplay between the formulation, the process used and the stability of freeze dried systems.

4.2. The freeze drying process

4.2.1. Initial Freezing

Freezing occurs upon cooling aqueous solutions to temperatures below 0°C and takes place via ice nucleation (Körber, 1988). This process involves the generation of water aggregates with sufficiently long lifetimes so as to allow interactions with surrounding water molecules. As the temperature is lowered, molecular mobility decreases and the lifetimes of the aggregates increase, leading to nucleation and subsequent crystal growth. It is important to note that, depending on factors such as the cooling rate, water may undergo considerable supercooling. Ice formation may therefore take place at temperatures down to −20°C using rates which are practical in most freeze dryers (for homogeneous nucleation, undercooling to −40°C is possible in microlitre volumes, although in practice heterogeneous nucleation will inevitably place at higher temperatures during the freeze drying process). The degree of supercooling in turn determines the size distribution and morphology of the ice crystals formed, with faster freezing rates giving rise to smaller crystals which permit more rapid sublimation in the primary drying step (although secondary drying may be slower). The resulting product will have a fine pore structure that facilitates rehydration.

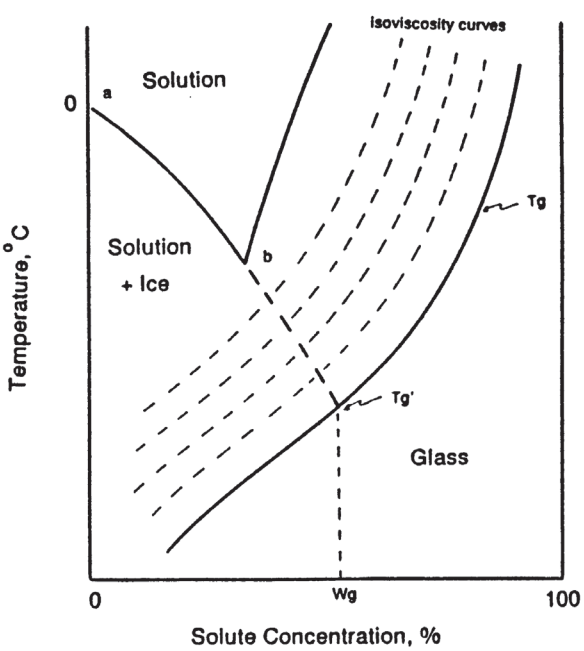

Fig. 17. Schematic representation of the phase diagram of the NaCl–water eutectic system (Reprinted from In: Avis, K.E., Lieberman, H.A., Lachman, L. (Eds.), Pharmaceutical Dosage Forms: Parenteral Medications. Marcel Dekker, New York, S.L. Nail and L.A. Gatlin, pp. 163–233, Copyright (1993), with permission from Elsevier Science).

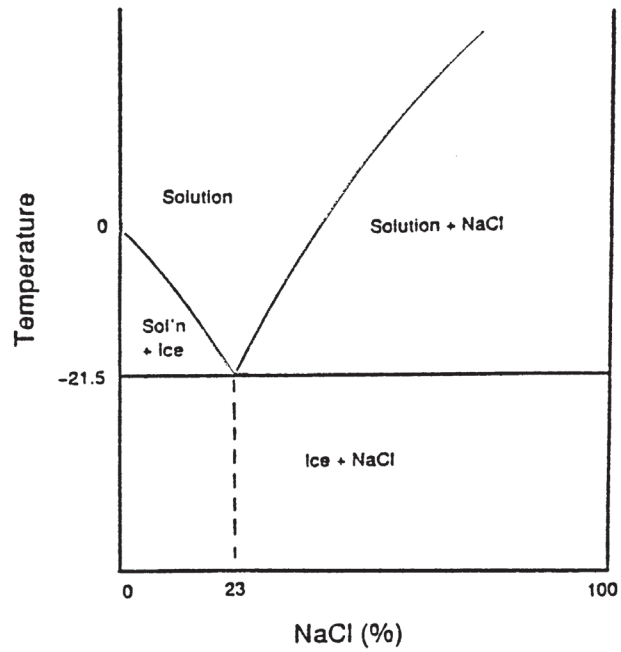

Fig. 18. Schematic representation of the state diagram of a glass forming binary system (Reprinted from In: Avis, K.E., Lieberman, H.A., Lachman, L. (Eds.), Pharmaceutical Dosage Forms: Parenteral Medications. Marcel Dekker, New York, S.L. Nail and L.A. Gatlin, pp. 163–233, Copyright (1993), with permission from Elsevier Science).

The removal of water to form ice has the effect of increasing the solute concentration, which when coupled with cooling causes an increase in viscosity of the solute phase. This concentration effect is responsible for many of the deleterious effects of freeze drying. Nucleation of the solute(s) may occur leading to crystallisation and the formation of a eutectic system (Fig. 17). For example, sodium chloride is expected to form a eutectic with water at −21°C, although the rate of crystal growth is slow and complete crystallisation is not generally observed. If nucleation does not occur within the timescale of the cooling process then eventually the remaining 'solution' will form a glass with a viscosity sufficiently high (typically $\approx 10^{13}$ Pa.s) to inhibit further ice formation. The characteristics of this glass may be of considerable importance in determining the freeze drying pro-

tocol and hence will be discussed in more detail with reference to Fig. 18.

Taking the simplest scenario, the solution is cooled and ice crystals form, hence the remaining solution becomes increasingly concentrated until a saturation value is reached and no further increase in concentration is possible (C'_g), the system is said to be in the maximally freeze concentrated state (Franks, 1990). The system undergoes a glass transition and the temperature at which this occurs is denoted T'_g. However, rapidly cooled systems containing very low solute concentrations may exhibit a transition at temperatures lower than T'_g because the glass formed will include more water than the ideal case; this problem is prevented by the use of lower cooling rates. It should be noted that T'_g is a very low energy transition which may be obscured by the ice melting peak and by the accompanying relaxation endotherm (if present). Clearly, such a measurement is prone to considerable variation, which is reflected by the relatively poor consistency of data for T'_g and C'_g available in the literature, with a range of values reported for materials such as sucrose. The corresponding values for this material are now generally accepted to be -32°C and 83% sucrose (Slade and Levine, 1988; Franks, 1990).

Identification of T'_g may be further complicated by the presence of a second transition at slightly higher temperatures. One system which has been widely studied in this respect is sucrose, which is important not only as a pharmaceutical excipient but in frozen foods. DSC studies of frozen carbohydrate solutions (Ablett et al., 1992; te Booy et al., 1992) have revealed two transitions below the onset of melting of ice. DSC studies have shown that annealing at temperatures immediately below T'_g yielded a characteristic double transition below T_m of water, while annealing at the estimated T'_g gave only one; it was concluded that the two transitions had coalesced at this temperature (Ablett et al., 1992). Current thinking is that the lower transition is the true glass transition while the second is caused by the irreversible collapse of the glass and is denoted T_s (softening point, Shalaev and Franks, 1995).

4.2.2. Primary drying

During this stage the water separated from the solute phase in the form of ice during freezing is removed by sublimation under vacuum. The product temperature remains relatively constant and drying follows a pseudo-steady-state rate with heat removal by sublimation at the same rate as that of the heat input supplied by the shelves. This can be expressed by (Pikal, 1993):

$$\Delta H_s \cdot \frac{dm}{dt} = \frac{dQ}{dt} \tag{17}$$

where ΔH_s is the heat of sublimation, dm/dt is the sublimation rate and dQ/dt is the rate of heat input. The rate of sublimation can be expressed as:

$$\frac{dm}{dt} = \frac{(P_0 - P_c)}{(R_p + R_s)} \tag{18}$$

where $(P_o - P_c)$ is the thermodynamic driving-force for sublimation where P_0 is the vapour pressure of ice in the frozen sample and P_c is the total pressure in the chamber. $(R_p + R_s)$ is the total resistance to sublimation, where R_p is the product resistance and R_s is the resistance of the stopper in the vial. The rate of heat input can be expressed as:

$$dQ/dt = A_v \cdot K_v\,(T_s - T_p) \tag{19}$$

where A_v is the cross-sectional area of the vial, K_v is the vial heat transfer coefficient and $(T_s - T_p)$ is the heat difference between the shelf and the product, T_s being the shelf temperature and T_p the product temperature. P_0, the vapour pressure of the ice in the product, increases exponentially with temperature, so that an increase in product temperature will cause a large increase in rate of sublimation. This also implies that the chamber pressure should not be set to below that of the standard vapour pressure of water over ice at the product temperature since this will decrease the rate of sublimation. In addition, the dimensions of the cake influence the effectiveness of drying since the product resistance will increase as the thickness of dried cake increases, hindering diffusion of water to the solute/vapour interface. This has the effect of reducing the rate of sublimation which will give rise to a temperature gradient in

the product. At the end of primary drying, secondary drying will begin spontaneously in some regions of the product where all the ice has sublimed. It is generally assumed that primary drying has ended when the product temperature is at the shelf temperature.

Knowledge of the glass transitional behaviour of the frozen system is extremely useful for choosing the most appropriate processing parameters. If during primary drying the product temperature is raised above T'_g then ice will melt back into the product, causing softening which may lead to collapse (discussed below), hence identification of T'_g and C'_g allows greater predictability of the effects of drying temperature on the physical integrity of the sample, allowing drying to occur at the highest possible temperature without compromising the physical integrity of the sample. Given that a temperature increase of 1°C may lead to a reduction in primary drying time of 13% (Pikal, 1985), the economic importance of choosing the drying temperature on a rational basis is clear. Product collapse during drying has been discussed in detail by Bellows and King (1972), Pikal and Shah (1990) and more recently Sun (1997) and is associated with viscous flow of the amorphous material; this phenomenon leads to an inelegant product but may also increase reconstitution times and residual water levels. It is therefore essential to dry the material below the collapse temperature (T_c) which, for most practical purposes, is ≈ 20°C above T'_g (Sun, 1997). A range of T'_g and C'_g for commonly used freeze drying excipients has been given by Franks (1990).

4.2.3. Secondary drying

This is the term given to the process during which 'unfrozen' water is removed from the freeze concentrate. Secondary drying is usually aided by increasing the shelf temperature, usually to 25–60°C. For this reason it is very important that all ice has been removed by sublimation before the temperature is raised in order to prevent melt-back. In the case of amorphous products, the water remaining after primary drying is trapped in the glassy phase. Initially the rate of water loss (the first few hours) will be great but a plateau level is reached beyond which further water removal (below $\approx 2\%$) is very slow. The rate of water removal is not proportional to the concentration of water in the freeze concentrate, but is controlled by the rate of diffusion to the solute/vapour interface and the subsequent evaporation. As the solid dries, the motion in the concentrate becomes much slower and consequently diffusion becomes more difficult. Diffusion is a function of the porosity of the concentrate; small pores caused by extreme supercooling before ice formation during the initial freeze hinder the diffusion of water to the solid/vapour interface, although chamber pressure (P_c) and dried cake thickness have little effect on the rate of secondary drying. The removal of plasticizing water during secondary drying has the effect of raising the glass transition and hence the collapse temperature, therefore an ideal secondary drying protocol would follow the T_g increase of the sample.

4.2.4. Product stability during storage

The glass transition of the freeze dried product must be well above the subsequent storage temperature in order to prevent collapse, hence determination of T_g for the freeze dried product has important implications for storage stability (Nail and Gatlin, 1993). Since collapse occurs when the sample is unable to hold its own weight, its apparent value will depend on sample mass and period of observation (and applied pressure if mechanical methods of detection are used).

As yet a limited number of reports have discussed the significance of product collapse and implications for stability of pharmaceutical products, although numerous studies may be found linking the glass transition and stability in the food science literature, describing phenomena such as volumetric shrinkage at collapse (Levi and Karel, 1995), caking and stickiness (Chuy and Labuza, 1994) and increased rates of nonenzymic browning (Buera and Karel, 1995). Early studies have indicated that modulated DSC may be particularly useful for measuring transitions of the dried product in terms of identifying and quantifying the value of T_g in complex systems (Kett et al., 1988; Van Winden et al., 1998).

 Sun Ex. 1025

4.3. Freeze drying of biological products

4.3.1. The effects of water on stability

The principal challenge associated with freeze drying proteins and other biological products is loss of activity during freezing, drying or storage; for example, lactate dehydrogenase may show complete loss of enzymatic activity after freeze drying unless protective measures are taken (Pikal, 1993). A number of texts (Franks, 1985; Baffi and Garnick, 1991; Constantino et al., 1995; Anchordoquy and Carpenter, 1996; Chang et al., 1996a,b; Carpenter et al., 1997; Colaco et al., 1997) are available which outline the current thinking in this area.

One highly important issue associated with the stability of freeze dried proteins is the optimal residual water of the dried product. Aggregation of excipient free human growth hormone (hGH) has been attributed to overdrying (Pikal et al., 1991a), while excipient free tissue type plasminogen activator (tPA) was found to aggregate at an enhanced rate if the residual water level was low (Hsu et al., 1991a). However, these observations are not the norm, as in general lower water contents improve storage stability. For example, the rate of haemoglobin oxidation at room temperature is doubled if the residual water content is increased from 1 to 4% (Pristoupil et al., 1985), while the rate of degradation of human growth hormone at elevated temperatures is increased tenfold if the water content is increased from ≈ 1 to 2.5% (Pikal et al., 1991b). The detrimental effect of water is usually attributed to the increased mobility and hence reactivity of solute species. It has been postulated (Hsu et al., 1991b) that above monolayer levels of water the protein has increased conformational mobility and that additional water can mobilise reactant species in the amorphous phase. It should be emphasised that the distribution of the water throughout the product is also important. A low overall %w/w water content may be misleading due to the possibility of localised regions containing higher water concentrations.

4.3.2. Cryoprotectants

Cryoprotectants are materials which are commonly added during the freeze drying process in order to afford protection of the drug from degradation. The term literally refers to protection during freezing or freeze-thawing rather than drying and hence the term lyoprotectant is preferred if the additive is capable of preventing degradation during the lyophilisation process as a whole. This distinction may be important as the freeze-drying process is much harsher than freeze-thawing due to the additional sublimation stage. For example, the enzyme L-aspariginase can be freeze-thawed without any loss of activity, but retains only 20% activity after being exposed to freeze-drying (Hellman et al., 1983). Indeed, many cryoprotectants that are useful additives during freeze-thawing have little effect during freeze-drying, examples of such additives being proline and trimethylamine *N*-oxide (Carpenter et al., 1991).

The most commonly used cryoprotectants are sugars, although polymers and amino acids may also be used. The use of sugars as a means of protection against freezing or dehydration is well known in nature. For example, certain organisms possess the ability to survive complete dehydration and exist in a state of anhydrobiosis for tens of decades, without loss of activity upon rehydration. An example of such an organism is brewers' yeast, which is fully active only minutes after rehydration. This is possible because the organism accumulates large amounts of saccharides, usually either sucrose or trehalose (Crowe et al., 1984), and their ability to survive dehydration can be correlated with the concentration of sugar present. Both of these sugars have been investigated as additives to freeze dried protein formulations (Carpenter and Crowe, 1989; Hall et al., 1995) and liposomes (Van Winden et al., 1998).

It has been postulated that carbohydrates and some amino acids stabilise proteins during freezing by their thermodynamically favourable exclusion from the protein surface (Arakawa et al., 1991). This situation arises when it is more favourable for the stabilizer molecules to interact with each other than with the protein. In this case the native state of the protein is favoured because unfolding (or breaking up of multi-unit proteins to sub-units) would give greater opportunity for such stabilizer–protein interactions to occur, and so raise the Gibbs free energy of unfolding (Arakawa and

Timasheff, 1982). In contrast, it has been suggested that polymers such as polyvinylpyrrolidone (PVP) and maltodextrins are believed to exert their stabilising influence by raising the average molecular weight, and so increasing the viscosity of the formulation (Blond, 1994; Anchordoquy and Carpenter, 1996).

There are two main theories that have been put forward to account for the stabilising effect of cryoprotectants during drying and storage, namely the 'water substitute' and the 'vitrification' theories. The water substitute theory is thermodynamically based (Franks et al., 1991; Slade and Levine, 1991) and assumes that the stabiliser interacts with the protein in the same way as does water, i.e. that the native state is stabilised because water lost during the drying process is replaced by the stabiliser molecules (Prestrelski et al., 1993a). Studies using FTIR spectroscopy (Prestrelski et al., 1993b,c) have indicated that the conformation of proteins that have bonded sugars are similar to those in aqueous solution.

The vitrification hypothesis states that the activity of the stabilisers stems from their glass-forming properties. It is assumed that in the glassy state molecular motion is effectively removed, hence degradation is slowed. Evidence supporting this theory includes the observation that mobility and reactivity are both often decreased as the product temperature is reduced below the glass transition temperature region (Roozen et al., 1991). This hypothesis has also been used to explain the cryoprotective ability of high molecular weight polymers in some instances, which are considered to raise the glass transition temperature of a formulation. However, a clear link between the T_g of the formulation and the stability of the product has not yet been established.

5. Conclusions

This discussion has attempted to tie together some of the concepts and challenges facing the formulation scientist with respect to the understanding and handling of amorphous materials. In particular, the principles underpinning the glassy state have been described, along with a consideration of thermal analysis as a means of characterising such materials. Two applications have been discussed, namely the behaviour of amorphous drugs and freeze dried systems. In all cases, the need to understand the glass transitional and relaxation behaviour of the systems under study has been stressed.

Amorphous systems are clearly an integral component of the development of effective dosage forms and the importance of these materials is likely to increase both due to the need to develop oral dosage forms which show favourable bioavailability profiles and the growing emphasis on freeze drying for the preparation of proteinaceous drugs. By understanding the relationship between the glassy behaviour and the product performance characteristics of these materials it is therefore possible to approach formulation strategies on a more rational basis.

Acknowledgements

We wish to thank and GlaxoWellcome and Rhone-Poulenc Rorer for financial support of VK and MH, respectively.

References

Ablett, S., Izzard, M.J., Lillford, P.J., 1992. Differential scanning calorimetry study of frozen sucrose and glycerol solutions. J. Chem. Soc. Farad. Trans. 88, 789–794.

Ahlneck, C., Zografi, G., 1990. The molecular basis of moisture effects on the physical and chemical stability of drugs in the solid state. Int. J. Pharm. 62, 87–95.

Anchordoquy, T.J., Carpenter, J.F., 1996. Polymers protect lactate dehydrogenase during freeze drying by inhibiting dissociation in the frozen state. Arch. Biochem. Biophys. 332, 231–238.

Andronis, V., Zografi, G., 1997. Molecular mobility of supercooled amorphous indomethacin, determined by dynamic mechanical analysis. Pharm. Res. 14, 410–414.

Angell, C.A., 1995. The old problems of glass and the glass transition and the many new twists. Proc. Nat. Acad. Sci. USA 92, 6675–6682.

Angell, C.A., 1995. Formation of glasses from liquids and biopolymers. Science 267, 1924–1935.

Arakawa, T., Timasheff, S.N., 1982. Stabilization of protein structure by sugars. Biochemistry 21, 6536–6544.

Arakawa, T., Kita, Y., Carpenter, J.F., 1991. Protein solvent interactions in pharmaceutical formulations. Pharm. Res. 8, 285–291.

Baffi, R.A., Garnick, R.L., 1991. Quality control issues in the analysis of lyophilized proteins. Dev. Biol. Stand. 74, 181–184.

Bellows, R.J., King, C.J., 1972. Freeze drying of aqueous solutions: maximum allowable operating temperature. Cryobiology 9, 559–561.

Blond, G., 1994. Mechanical properties of frozen model solutions. J. Food Eng. 22, 253–269.

Byrn, S.R., 1982. Solid-State Chemistry of Drugs. Academic Press, London.

Byrn, S.R., Pfeiffer, R., Ganey, M., Hoilberg, C., Poochikian, G., 1995. Pharmaceutical solids: a strategic approach to regulatory considerations. Pharm. Res. 12, 945–954.

Buera, M.P., Karel, M., 1995. Effect of physical changes on the rates of nonenzymic browning and related reactions. Food Chem. 52, 167–173.

Carpenter, J.F., Crowe, J.H., 1989. An infrared spectroscopy study of the interactions of carbohydrates with dried proteins. Biochemistry 28, 3916–3922.

Carpenter, J.F., Arakawa, T., Crowe, J.H., 1991. Interactions of stabilizing additives with proteins during freeze-thawing and freeze drying. Dev. Biol. Stand. 74, 225–239.

Carpenter, J.F., Pikal, M.J., Chang, B.S., Randolph, T.W., 1997. Rational design of stable lyophilized protein formulations: some practical advice. Pharm. Res. 14, 969–975.

Caroll, B., Manche, E.P., 1972. Kinetic analysis of chemical reactions for non-isothermal processes. Thermochim. Acta 3, 449–459.

Chang, B.S., Beauvais, R.M., Dong, A., Carpenter, J.F., 1996. Physical factors affecting the storage stability of freeze dried interleukin-1 antagonist: glass transition and protein conformation. Arch. Biochem. Biophys. 331, 249–258.

Chang, B.S., Kendrick, B.S., Carpenter, J.F., 1996. Surface-induced denaturation of proteins during freezing and its inhibition by surfactants. J. Pharm. Sci. 85, 1325–1330.

Chuy, L.E., Labuza, T.P., 1994. Caking and stickiness of dairy-based food powders as related to glass transitions. J. Food Sci. 59, 43–46.

Cohen, M.H., Turnbull, D., 1959. Molecular transport in liquids and glasses. J. Chem. Phys. 31, 1164–1169.

Colaco, C.A.L.S., Smith, C.J.S., Sen, S., Roser, D.H., Newman, Y., Ring, S., Rosers, B.J., 1997. In: Cleland, J.L., Langer, R. (Eds.), Formulation and Delivery of Proteins and Peptides. Pp. 222–240

Coleman, N.J., Craig, D.Q.M., 1996. Modulated temperature differential scanning calorimetry: a novel approach to pharmaceutical thermal analysis. Int. J. Pharm. 135, 13–29.

Constantino, H.R., Griebenow, K., Mishra, P., Langer, R., Klibanov, A.M., 1995. Fourier-transform infrared spectroscopic investigation of protein stability in the lyophilized form. Biochim. Biophys. Acta 1253, 69–74.

Corrigan, O.I., Holohan, E.M., Sabra, K., 1984. Amorphous forms of thiazide diuretics prepared by spray-drying. Int. J. Pharm. 18, 195–200.

Corrigan, O.I., 1995. Thermal analysis of spray dried products. Thermochim. Acta 248, 245–258.

Corveleyn, S., Remon, J.P., 1997. Formulation and production of rapidly disintegrating tablets by lyophilisation using hydrochlorothiazide as a model drug. Int. J. Pharm. 152, 215–225.

Craig, D.Q.M., Johnson, F.A., 1995. Pharmaceutical applications of dynamic mechanical analysis. Thermochimica Acta 248, 97–115.

Crowe, J.H., Crowe, L.M., Chapman, D., 1984. Infrared spectroscopic studies on interaction of water and carbohydrates with a biological membrane. Arch. Biochem. Biophys. 232, 400–407.

Dissado, L.A., Hill, R.M., 1979. Non-exponential decay in dielectrics and dynamics of correlated systems. Nature 79, 685–689.

Doolittle, A.K., 1951. Studies in newtonian flow, 2: the dependence of the viscosity of liquids on 3-space. J. Appl. Phys. 22, 1471–1475.

Doster, W., Cusack, S., Petry, W., 1989. Dynamical transition of myoglobin revealed by inelastic neutron scattering. Nature 337, 754–756.

Duddu, S.P., Dal Monte, P.R., 1997. Effect of glass transitional temperature on the stability of lyophilized formulations containing a chimeric therapeutic monoclonal antibody. Pharm. Res. 14, 591–595.

Elliott, S.R., 1983. Physics of Amorphous Materials. Longman, London.

Fox, T.G., Flory, P.J., 1950. Second order transition temperatures and related properties of polystyrene, 1: influence of molecular weight. J. Appl. Phys. 21, 581–591.

Fox, T.G., Flory, P.J., 1951. Further studies on the melt viscosity of polyisobutylene. J. Phys. Chem. 55, 221–234.

Fox, T.G., Flory, P.J., 1954. The glass temperature and related properties of polystyrene—influence of molecular weight. J. Polym. Sci. 14, 315–319.

Franks, F., 1985. Biophysics and Biochemistry at low temperatures, 1st ed. Cambridge University press, Cambridge.

Franks, F., 1990. Freeze drying: from empiricism to predictability. Cryo-Letters 11, 93–110.

Franks, F., 1994. Effective freeze drying: a combination of physics, chemistry, engineering and economics. Proc. Inst. Refrig. 91, 32–39.

Franks, F., Hatley, R.H.M., Mathias, S.F., 1991. Materials science and the production of shelf stable biologicals. Biopharmacy 4, 38.

Frauenfelder, H., Sligar, S.G., Wolynes, P.G., 1991. The energy landscapes and motions of proteins. Science 254, 1598–1603.

Fukuoka, E., Makita, M., Yamamura, S., 1986. Some physicochemical properties of glassy indomethacin. Chem. Pharm. Bull. 34, 4314–4321.

Fukuoka, E., Makita, M., Yamamura, S., 1989. Glassy state of pharmaceuticals 11: thermal properties and stability of glassy pharmaceuticals and their binary glass systems. Chem. Pharm. Bull. 37, 1047–1050.

Gee, G., 1970. Glassy state in polymers. Contemp. Phys. 11, 313–334.

Gibbs, J.H., 1960. In: Mackenzie, J.D. (Ed.), Modern Aspects of the Vitreous State. Butterworth, chapter 7.

Gibbs, J.H., Di Marzio, E.A., 1958. Nature of the glass transition and the glassy state. J. Chem. Phys. 28, 373–383.

Gordon, M., Taylor, J.S., 1952. Ideal copolymers and the second-order transitions of synthetic rubbers, 1: non-crystalline copolymers. J. Appl. Chem. 2, 493–498.

Green, J.L., Han, J., Angell, C.A., 1994. The protein–glass analogy: some insights from homopeptide comparisons. J. Phys. Chem. 98, 13780–13790.

Gujrati, P.D., Goldstein, M., 1980. Viscous liquids and the glass transition, 9: nonconfigurational contribution to the excess entropy of disordered phases. J. Phys. Chem. 84, 859–863.

Hageman, M.J., Bauer, J.M., Possert, P.L., Darrington, R.T., 1992. Preformulation studies orientated towards sustained delivery of recombinant somatotropins. J. Agric. Food Chem. 40, 348–355.

Haines, P.J., 1995. Thermal Methods of Analysis. Blackie Academic and Professional, Glasgow.

Haleblian, J.K., 1975. Characterisation of habits and crystalline modifications of solids and their pharmaceutical applications. J. Pharm. Sci. 64, 1269–1288.

Hall, D.R., Jacobson, M.P., Winzor, D.J., 1995. Stabilizing effect of sucrose against irreversible denaturation of rabbit muscle lactate dehydrogenase. Biophys. Chem. 57, 47–54.

Hancock, B.C., Zografi, G., 1994. The relationship between the glass transition temperature and the water content of amorphous pharmaceutical solids. Pharm. Res. 11, 1166–1173.

Hancock, B.C., Shamblin, S.L., Zografi, G., 1995. Molecular mobility of amorphous pharmaceutical solids below their glass transition temperatures. Pharm. Res. 12, 799–806.

Hancock, B.C., Zografi, G., 1997. Characterisation and significance of the amorphous state in pharmaceutical systems. J. Pharm. Sci. 86, 1–12.

Hellman, K., Miller, D., Cammack, K., 1983. The effect of freeze drying on the quaternary structure of L-asparaginase from erwinia-carotovora. Biochim. Biophys. Acta 749, 133–142.

Hill, V.L., Craig, D.Q.M., Feely, L.C., 1998. Characterisation of spray-dried lactose using modulated differential scanning calorimetry. Int. J. Pharm. 161, 95–107.

Holdsworth, S.D., 1987. In: Thorne, S. (Ed.), Developments in Food Preservation—4. Elsevier Applied Science, New York, pp. 153–204.

Hsu, C.C., Ward, C., Pearlman, R., Ngiyen, H., Yeung, D., Curley, J., 1991. Hemoglobin lyophilized with sucrose—the effect of residual moisture on storage. Dev. Biol. Stand. 74, 255–271.

Hsu, C.C., Ward, C.A., Pearlman, R., Nguyen, H.M., Yeung, D.A., Curley, J.G., 1991. Detwermining the optimal residual moisture in lyophilised protein pharmaceuticals. Devel. Biol. Stand. 74, 255–271.

Jolley, J., 1970. The microstructure of photographic gelatine binders. Photogr. Sci. Eng. 14, 169–177.

Kauzmann, W., 1948. The nature of the glassy state and the behaviour of liquids at low temperatures. Chem. Rev. 43, 219–256.

Kerč, J., Srčič, S., Mohar, M., Smid-Korbar, 1991. Some physicochemical properties of glassy felodipine. Int. J. Pharm. 68, 25–33.

Kerč, J., Srčič, S., 1995. Thermal analysis of glassy pharmaceuticals. Thermochim. Acta 248, 81–95.

Kett, V., Craig, D.Q.M., Taylor, K.M.G., Deutsch, D., 1988. The use of modulated differential scanning calorimetry in the study of freeze dried systems. In: Proceedings of The Seventeenth Pharmacy Tech. Conference, Dublin, pp. 104–108.

Körber, C., 1988. Phenomena at the advancing ice-liquid interface, solutes particles and biological cells. Q. Rev. Biophys. 21, 229–298.

Levi, G., Karel, M., 1995. Volumetric shrinkage (collapse) in freeze dried carbohydrates above their glass transition temperature. Food Res. Int. 28, 145–151.

Levine, H., Slade, L., 1993. In: Blanchard, J.M.Y., Hillford, P.J. (Eds.), The Glassy State in Foods. Nottingham Press, Nottingham, pp. 35.

Makower, B., Dye, W.B., 1956. Equilibrium moisture content and crystallisation of amorphous sucrose and glucose. J. Agr. Food Chem. 4, 72–77.

Matsuda, Y., Otsuka, M., Onoe, M., Tatsumi, E., 1992. Amorphism and physicochemical stability of spray dried frusemide. J. Pharm. Pharmacol. 44, 627–633.

McCrum, N.G., Read, B.E., Williams, G., 1967. Anelastic and dielectric effects in polymeric solids. John Wiley and Sons, London.

Mishima, O., Calvert, L.D., Whalley, E., 1984. Melting ice 1 at 77 K and 10 kbar: a new method of making amorphous solids. Nature Lond. 310, 393–395.

Mishima, O., Calvert, L.D., Whalley, E., 1985. An apparently first-order transition between two amorphous phases of ice induced by pressure. Nature Lond. 314, 76–78.

Mishima, O., Takemare, K., Aoki, K., 1991. Visual observations of the amorphous–amorphous transition in H_2O under pressure. Science 254, 406–408.

Montserrat, S., 1989. Physical ageing studies in epoxy resins: 1, kinetics of the enthalpy relaxation process in a fully cured epoxy resin. J. Polym. Sci. Polym. Phys. Edn. 32, 509–522.

Moynihan, C.T., Easteal, A.J., Wilder, J., 1974. Dependence of the glass transition temperature on heating and cooling. J. Phys. Chem. 78, 2673–2677.

Moynihan, C.T., Easteal, A.J., DeBolt, M.A., 1976. Dependence of the fictive temperature of glass on cooling. J. Am. Ceram. Soc. 59, 12–21.

Moynihan, C.T., 1994. In: Segler, R.J. (Ed.), Phenomenology of the structural relaxation process and the glass transition: assignment of the glass transition ASTM STP 1249. American Society for Testing and Materials, Philadelphia, pp. 32–49.

Mullins, J., Macek, T., 1960. Some pharmaceutical properties of novobiocin. J. Am. Pharm. Assoc. Sci. Ed. 49, 245–248.

Nail, S.L., Gatlin, L.A., 1993. In: Avis, K.E., Lieberman, H.A., Lachman, L. (Eds.), Pharmaceutical Dosage Forms: Parenteral Medications. Marcel Dekker, New York, pp. 163–233.

Nurnburg, E., 1976. Kolloids Verteilungszuständs in der pharmazeutischen Technologie: herstellung und Eigenschaften pharmazeutischer Präparate durch Sprühtrocknung. Prog. Colloid Polym. Sci. 59, 55–69.

Oberholtzer, E.R., Brenner, G.S., 1979. Cefoxitin sodium: solution and solid state chemical stability. J. Pharm. Sci. 68, 863–866.

Oksanen, C.A., 1992. Molecular mobility in mixtures of adsorbed water and poly(vinyl pyrrolidone), PhD Thesis. University of Wisconsin-Madison.

Ozawa, T.J., 1975. Critical investigation of methods for kinetic analysis of thermoanalytical data. J. Therm. Anal. 7, 601–617.

Pikal, M.J., Lukes, A.L., Lang, J.E., 1977. Thermal decomposition of amorphous β-lactam antibacterials. J. Pharm. Sci. 66, 1312–1316.

Pikal, M.J., Lukes, A.L., Lang, J.E., Gaines, K., 1978. Quantitative crystallinity determinations for β-lactam antibiotics by solution calorimetry: correlation with stability. J. Pharm. Sci. 67, 767–772.

Pikal, M.J., 1985. Use of laboratory data in freeze drying process design: heat and mass transfer coefficients and the computer simulation of freeze drying. J. Parenteral Sci. Technol. 39, 115–138.

Pikal, M.J., 1990a. Freeze drying of proteins. part 1: process design. Biopharmacy (September issue) 18–20.

Pikal, M.J., 1990b. Freeze drying of proteins. part 2: Formulation selection. Biopharmacy (September issue) 26–30.

Pikal, M.J., Shah, S., 1990. The collapse temperature in freeze drying: dependence on measurement methodology and rate of water removal from the glassy phase. Int. J. Pharm. 62, 165–186.

Pikal, J., Dellerman, K., Roy, M.L., 1991. effects of moisture and oxygen on the stability of freeze dried formulations of human growth hormone. Dev. Biol. Stand. 74, 21–38.

Pikal, M.J., Dellerman, K.M., Roy, M.L., Riggin, R.M., 1991. The effect of formulation variables on the stability of freeze-dried human growth hormone. Pharm. Res. 8, 427–436.

Pikal, M.J., 1993. Freeze-drying of proteins. In: Cleland, J.L., Langer, R. (Eds.), Formulation and Delivery of Proteins and Peptides, Chapter 8. ACS Symposium series 567, American Chemical Society, Washington.

Prestrelski, S.J., Arakawa, T., Carpenter, J.F., 1993. Separation of freezing and drying induced denaturation of lyophilized proteins using stress specific stabilization. Arch. Biochem. Biophys. 303, 465–473.

Prestrelski, S.J., Tedeschi, N., Arakawa, T., Carpenter, J.F., 1993. Dehydration induced conformational transitions in proteins and their inhibition by stabilizers. Biophys. J. 65, 661–671.

Prestrelski, S.J., Arakawa, T., Carpenter, J.F., 1993c. Structure of proteins in lyophilized formulations using Fourier Transform infrared spectroscopy. In: Cleland, J.L., Langer, R. (Eds.), Formulation and Delivery of Proteins and Peptides. ACS Symposium Series 187, American Chemical Society, Washington.

Pristoupil, T.I., Kramlova, M., Fortova, H., Ulrych, S., 1985. Haemoglobin lyophilised with sucrose: the effect of residual moisture on storage. Haematologia 18, 45–52.

Reading, M., Elliott, D., Hill, V.L., 1993. MDSC, a new approach to the calorimetric investigation of physical and chemical transitions. J. Therm. Anal. 40, 949–955.

Richardson, M.J., Savill, N.G., 1975. Derivation of accurate glass transition temperatures by differential scanning calorimetry. Polymer 16, 753–757.

Roozen, M., Hemminga, M., Walstra, P., 1991. Molecular motion in glassy water maltooligosaccharide (maltodextrin) mixtures as studied by conventional and saturation spin probe ESR spectroscopy. Carbohydr. Res. 215, 229–237.

Roy, M.L., Pikal, M., Richard, E.C., Maloney, M., 1992. The effects of formulation and moisture on the stability of a freeze dried monoclonal antibody–vinca conjugate: a test of the WLF theory. Dev. Biol. Stand. 74, 323–340.

Royall, P.G., Craig, D.Q.M., Doherty, C., 1988. Characterisation of the glass transition of an amorphous drug using modulated DSC. Pharm. Res. 15, 1117–1121.

Saleki-Gerhardt, A., Zografi, G., 1994. Non-isothermal and isothermal crystallisation of sucrose from the amorphous state. Pharm. Res. 11, 1166–1173.

Sato, T., Okada, A., Sdekiguchi, K., Tsuda, Y., 1981. Differences in physico-pharmaceutical properties between crystalline and noncrystalline 9,3′-diacetylmidecamycin. Chem. Pharm. Bull. 29, 2675–2682.

Shalaev, E.Y., Franks, F., 1995. Structural glass transitions in aqueous carbohydrate solutions and their relevance to frozen food stability. Thermochim. Acta 280/281, 449–464.

Slade, L., Levine, H., 1988. Thermomechanical properties of small carbohydrate-water glasses and rubbers—kinetically metastable systems at subzero temperatures. J. Chem. Soc. Farad. Trans. 84, 2619.

Slade, L., Levine, H., 1991. Beyond water activity: recent advances based on an alternative approach to the assessment of food quality and safety. Crit. Rev. Food Sci. 30, 115–360.

Slade, L., Levine, H., 1995. Glass transitions and water–food structure interactions. Adv. Food Nutr. Res. 38, 103–269.

Strickley, R.G., Anderson, B.D., 1996. Solid-state stability of human insulin 1: mechanisms and the effects of water on the kinetics of degredation in lyophiles from pH 2–5 solutions. Pharm. Res. 13, 1142–1153.

Sukenik, C.N., Bonapace, J.A.P., Mandel, N.S., Bergman, R.G., Lau, P.-Y., Wood, G., 1975. Enhancement of a chemical reaction rate by proper orientation of reacting molecules in the solid state. J. Am. Chem. Soc. 97, 5290–5291.

Sukenik, C.N., Bonapace, J.A.P., Mandel, N.S., Lau, P.-Y., Wood, G., Bergman, R.G., 1977. A kinetic and X-ray diffraction study of solid state rearrangement of methyl

P-dimethylaminobenzene sulfonate: reaction rate enhancement due to proper orientation in a crystal. J. Am. Chem. Soc. 99, 851–852.

Sun, W.Q., 1997. Temperature and viscosity for structural collapse and crystallisation of amorphous carbohydrate solutions. Cryo-Letters 18, 99–106.

te Booy, M.P.W.M., de Ruiter, R.A., de Meere, A.L.J., 1992. Evaluation of the physical stability of freeze-dried sucrose-containing formulations by differential scanning calorimetry. Pharm. Res. 9, 109–114.

Torfs, J.C.M., Deij, L., Dorrepaal, A.J., Heijens, J.C., 1984. Determination of Arrhenius constants by differential scanning calorimetry. Anal. Chem. 56, 2863.

Turnbull, D., Cohen, M.H., 1961. Free-volume model of amorphous phase glass transitions. J. Chem. Phys. 34, 120–125.

Turnbull, D., Cohen, M.H., 1970. On free-volume model of liquid–glass transitions. J. Chem. Phys. 52, 3038–3041.

Van Winden, E.C.A., Talsma, H., Crommelin, D.J.A., 1998. Thermal analysis of freeze-dried liposome-carbohydrate mixtures with modulated differential scanning calorimetry. J. Pharm. Sci. 87, 231–237.

Ward, G.H., Schultz, R.K., 1995. Process-induced crystallinity changes in albuterol sulphate and its effect on powder physical stability. Pharm. Res. 12, 773–779.

Williams, M.L., Landel, R.F., Ferry, D.J., 1955. The temperature dependence of relaxation mechanisms in amorphous polymers and other glass-forming systems. J. Am. Chem. Soc. 77, 3701–3707.

Williams, G., Watts, D.C., 1970. Non-symmetrical dielectric relaxation behaviour arising from a simple empirical decay function. Trans. Farad. Soc. 66, 80–85.

Wong, J., Angell, C.A., 1976. Glass: structure by spectroscopy. Marcel Dekker, New York.

Wunderlich, B., 1990. Thermal Analysis. Academic Press, London.

Yamaguchi, T., Nishimura, M., Okamoto, R., Takeuchi, T., Yamamoto, K., 1992. Glass formation of 4″-*O*-(4-methoxyphenyl) acetyltylosin and physicochemical stability of the amorphous solid. Int. J. Pharm. 85, 87–96.

Yamamoto, K., Nakano, M., Arita, T., Nakai, Y., 1974. Dissolution rate and bioavailability of griseofulvin from a ground mixture with microcrystalline cellulose. J. Pharmacokinet. Biopharm. 2, 487.

Yamamoto, K., Nakano, M., Arita, T, Takayama, Y., Nakai, Y., 1976. Dissolution behaviour of phenytoin from a ground mixture with microcrystalline cellulose. J. Pharm. Sci. 65, 1484–1488.

Yoshioka, M., Hancock, B.C., Zografi, G., 1994. Crystallisation of indomethacin from the amorphous state below and above its glass transition temperature. J. Pharm. Sci. 83, 1700–1705.

Yoshioka, M., Hancock, B.C., Zografi, G., 1995. Inhibition of indomethacin crystallisation in poly(vinylpyrrolidone) coprecipitates. J. Pharm. Sci. 84, 983–986.