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FREEZE-DRYING OF PROTEIN PHARMACEUTICALS - THE APPLICATION OF THERMAL ANALYSIS

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Summary

This review describes the range of thermal analysis techniques that may be used to investigate the key thermal properties of formulations to be freeze dried and the resultant products. The use of these allows development of cost-effective processing while maintaining optimum product quality.

Keywords: thermal analysis, freeze-drying, glass transition, protectant, protein, lyophilisation

FREEZE DRYING AS AN INDUSTRIAL PROCESS

A number of excellent reviews are available which describe the freeze-drying process (lyophilisation) in great detail. Franks (1-3), Mackenzie (4-7) Pikal [1-7] and Rey [8-16] have been especially prolific in this field and more recently Wang[17] has added to this body of work. However, a brief outline of the process is warranted before discussing the thermal techniques that can be applied to study of the process. In brief, it is the desiccation of a substance by sublimation of vapour from the solid state. This review describes the key temperatures associated with the process and some of the thermal analysis techniques with which these parameters are commonly determined.

Freeze-drying is commonly used to stabilise a range of products such as pharmaceuticals (biotechnological drugs, antibiotics, vaccines) that are reconstituted prior to parenteral injection [3, 18, 19]) as well as diagnostic kits used in clinical settings. It is also used to preserve various materials including proteins [20], liposomes [21], drug-loaded nanoparticles [22], red blood cells [23], spermatozoa [24], microbes [25], tissue for transplant [26], gene delivery systems [27] and bone [28, 29] as well as in the preparation of bone scaffolds based on poly(ether ester) segmented block copolymers [30] in a stable form. In the food industry it is used for products including meats [31], vegetables [32], fruit products [33-35], coffee [36] and bacterial cultures [37]. The process was first used on an industrial scale during the Second World War when it became necessary to preserve large quantities of human blood serum and plasma as well as the antibiotics that had just been developed [38].

OVERVIEW OF THE FREEZE DRYING PROCESS

The process essentially consists of three steps. These are the initial freezing of the sample, sublimation of the resulting ice (primary drying) and the subsequent removal of any
unfrozen water remaining within the solute phase (secondary drying). However, these steps are not necessarily discrete. For example primary drying may not be complete in one area of a product before secondary drying has begun elsewhere. Low temperature drying minimises adverse effects such as chemical degradation, bacterial activity or skin formation. The final product tends to have an open porous structure with a high surface area, in pharmaceutical products or diagnostics that require reconstitution prior to use this facilitates rehydration.

**STABILITY OF FREEZE DRIED PRODUCTS**

In the case of pharmaceutical products the ideal formulation for parenteral delivery would be a stable solution. However, biological material such as peptides and proteins are prone to many degradative reactions in solution, for example hydrolysis, aggregation or oxidation [39, 40] making this format impractical. Freeze drying is a preferred method of formulating these types of product because it is generally thought that drying at lower temperatures is less harmful than comparable processes that require the input of heat such as spray-drying [41].

However, the process places both freezing and drying stresses on sensitive molecules. Protectants are commonly added to minimise these, of which there are two types; cryoprotectants act during freezing; lyoprotectants act throughout drying and storage. Much research has been conducted in this area, with the groups of Carpenter [18, 42-55], J and L Crowe [56-58], and Randolph [51, 55, 59-70] being especially prolific. A recent review by Skrabanja et al. has also discussed the nature of the interactions between drugs and excipients in freeze dried pharmaceutical products [71].

**KEY PARAMETERS IN THE FREEZE-DRYING PROCESS**

Any material that is going to be freeze-dried will have a characteristic “critical temperature”, often termed the collapse temperature, Tc [6, 72-75] above which it will suffer processing defects during freeze-drying. Therefore, it is important to maintain the primary drying temperature below this. However, maintaining the product too far below this temperature will lead to the drying process becoming unacceptably slow, since the kinetics of solvent removal are temperature-dependent. Therefore, it is essential to know the Tc of a material prior to freeze-drying so that appropriate conditions of temperature, time and pressure can be employed for its successful drying in a reasonable time.

For a crystalline (eutectic) system, the critical temperature will be that at which its eutectic melting temperature (Te) [10, 76-78]. Exceeding this temperature will cause the material to melt during processing. However, most formulations remain in an amorphous state, in which case their critical temperature is termed the ‘collapse temperature’ (Tc), which is the temperature at which the material softens to the point of not being able to support its own structure. This phenomenon is linked to undesirable product appearance, incomplete drying, reduced stability and slow reconstitution, Tc is closely related to the glass transition temperature (Te’ , Figure 1)[74, 79-82].

* Strictly Tg’ refers to the glass transition for the maximally freeze concentrated amorphous phase, in freeze-drying this concentration may not be reached. However, Tg’ is still commonly used to refer to the formulation glass transition in order to distinguish it from that of the product, denoted Tg.
Amorphous materials exhibit a glass transition, denoted \( T_g \), which may be observed as a discontinuity in the plots of heat capacity, \( C_p \), and expansivity, \( \alpha_p \), against temperature. These materials are thermodynamically unstable but their viscosity is so high that any motion is in the order of mm/year making reversion to the more stable crystalline state unlikely as long as the storage temperature does not exceed the \( T_g \). A \( T_g \) may be detected by thermal analysis because of the increase in heat capacity that occurs upon warming through this temperature which occurs because the material suddenly becomes more mobile.

An amorphous (glassy) phase is generally formed upon freezing because of ice formation and temperature lowering that together conspire to increase the viscosity of the solute-rich phase. At this point the formulation corresponds to the “ice and rubber” region shown in Figure 1. If nucleation of the solute(s) occurs then the resultant crystallisation leads to the formation of a eutectic system, corresponding to the point labelled \( T_e \) (the eutectic temperature). If nucleation does not occur then eventually a temperature will be reached at which the solute phase becomes so viscous that it effectively solidifies preventing further ice formation. The temperature at which this transition occurs is \( T_g' \) and \( C_g' \) is the maximal concentration of solute in the glassy phase that is obtained at this temperature [85].

The amorphous phase now has the structure of a liquid but, because of its high viscosity, is effectively solidified. This region corresponds to the area labelled “ice and glass” in the phase diagram for sucrose depicted in Figure 1. The ice crystals formed during freezing will be distributed throughout this phase. On a phase diagram \( T_g' \) is the point that intersects the \( T_g \) (also termed glass or isoviscosity) and the \( T_m \) (also known as fluid or liquidus curve that describes the solubility of ice in the solute phase) curves. Phase diagrams describe the kinetically-constrained behaviour of the system, but do not show the thermodynamic equilibrium conditions. The \( T_g' \) point can be determined indirectly by extrapolation of the \( T_g \) and \( T_m \) lines or directly by differential scanning calorimetry (DSC).

Previously, the terms glass transition, collapse and eutectic have often been used interchangeably, in the belief that they were the same. Therefore many workers determined \( T_g' \) and assumed that it was the same as the collapse temperature. While this is safe for processing, since collapse occurs only at temperatures higher than \( T_g' \) this may lead to a reduction in processing efficiency.
THERMAL ANALYSIS TECHNIQUES

A range of thermal analysis techniques are available with which to determine the various key parameters associated with the freeze drying process. These can be broadly divided according to the properties that they measure; calorimetric [86-91]; mechanical [92-96]; optical [73, 75, 97] and electrical/dielectric [93, 98-105]. A number of reviews have evaluated the use of these techniques particularly with reference to low-moisture or frozen foods, which have much in common with freeze dried systems at various stages of the process [32, 74, 83, 106-127].

CALORIMETRIC METHODS

DTA essentially involves heating or cooling a sample and a reference (generally water for the study of frozen material) under identical conditions [125, 128-130]. The differential temperature is then recorded as a function of time or temperature. If a process leads to the ingress or loss of heat from the sample relative to the reference then it can be detected. The technique was first introduced for the investigation of alloys in 1899 [131] but did not become popular until the 1950s when significant improvements in both the measurement and the collection of the experimental data were made [132]. DTA was used for the first systematic investigation of the thermal behaviour of frozen aqueous solutions [133]. A series of transitions were reported prior to melting and in particular one such transition was noted which was called “antemelting” and characterised by an apparent heat capacity increase just below the main melting endotherm. Reid et al. [134] have described some of the concerns that need to be addressed when using DTA (and DSC, described later) for the analysis of frozen systems. One of the main disadvantages of DTA is concerned with the arrangement of the thermocouples within the sample holder. This can cause problems because the sample acts as a heat source (upon heating) and as a thermal resistance that impedes the flow of heat to and from the thermocouple.

The difference between DSC and DTA is that a differential scanning calorimeter measures the power output of a sample relative to an inert reference rather than measuring the temperature difference between the two. There are two designs of DSC available, heat flux or power compensation. Both measure the response of a sample to an applied thermal stimulus, but they differ in the way that the stimulus is applied and the response measured. In this review the description will be restricted to that of heat flux DSC only, MTDSC, which is a software modification of DSC will be described later.

In a heat flux DSC heat is transferred to and from the sample and reference pans by a constantan (copper nickel alloy) disc. The differential heat flow between the sample and reference is measured by thermocouples that cover the underside of the platforms supporting the pans.

The heat flow, \( \frac{dQ}{dt} \) or \( P \) is described in terms of the applied heating rate as follows;

\[
P = \frac{dQ}{dt} = Cp \cdot \frac{dT}{dt} + f(t, T)
\]

Cp is the heat capacity, \( \frac{dT}{dt} \) is the applied heating rate and \( f(t, T) \) is the heat flow from kinetic processes. This equation shows that the heat flow is a function of the change in temperature and of the absolute temperature. Data are presented in the form of a thermal analysis curve, depicting change in heat flow against temperature. Traditionally power compensation DSC analyses show endotherms as peaks in the baseline whilst heat flux DSC shows exotherms as peaks. The integral of enthalpic peaks is directly proportional to the
quantity of heat evolved or taken up and the mass of sample under analysis, and is given by the following equation;

Equation 2  \( A = K \cdot m \cdot (-\Delta H) \)

where A is the peak area, K is the calorimetric sensitivity, m is the mass of sample, -\( \Delta H \) is the heat change.

Tg values are ideally specified as the temperature of half vitrification on cooling, which is the temperature at which the heat capacity is halfway between that of the liquid and glassy states [135]. It is determined by extrapolation of the baselines. The Tg is then calculated as the midpoint between the lines. However, other parameters may also be quoted in the literature. These include \( T_b \), the beginning of the transition; the extrapolated onset, \( T_1 \); the end is \( T_e \) and the extrapolated end is \( T_2 \). However, the small changes in heat capacity at the Tg are often hidden by an accompanying enthalpic relaxation, which can result in Tg being mistaken for a melting transition. The measured Tg is dependent on the scanning rate [136] so it is therefore important to note this when recording a Tg measurement or comparing data. This issues of rate-dependence may be overcome by determining the fictive temperature, \( T_f \), which is independent of heating rate[137] [138]. For frozen systems the determination of Tg’ can be difficult because rapidly cooled systems containing low solute concentrations may form less ice than the ideal case giving rise to a more dilute glass which will exhibit a glass transition at temperatures < Tg’. Tg’ is a very low energy transition and may be obscured by the onset of melting or by an accompanying endotherm, which has led to considerable variation of published data. A typical output for a frozen amorphous system is shown in Figure 2.

![Figure 2 DSC Data Showing the Tg’ of a 10% PVP Solution [99]](image)

As outlined above, the Tg is often obscured upon heating by an accompanying relaxation endotherm. These peaks occur because of two processes. The first is because of differences in cooling & heating rates, the second is because of aging effects (also termed annealing). If a sample is cooled slowly long relaxation times will be “frozen” into the system. If the sample is then heated at a higher rate these relaxations will be slower than the heating rate and the sample will superheat, as it cannot rearrange quickly enough to form the liquid at Tg. This phenomenon gives rise to an overshoot in the enthalpy curve and derived heat capacity curve. With higher heating rate, the peak is shifted to higher temperature with increasing enthalpy, but the peak height reaches a maximum. Once the relaxation times are in the same timescale
as the heating rate, the enthalpy curve follows the predicted liquid base line. These effects may cause errors in the calculated Tg temperature and accompanying ΔCp value.

An endotherm may also be caused by ageing, which occurs if an amorphous material is left to come towards equilibrium leading to structural relaxation. These ageing effects are affected by the length of the annealing period and the annealing temperature. For example, the endotherm seen if a product is stored at room temperature will be much larger than that seen if the same material is stored in a refrigerator prior to thermal analysis. The magnitude of an endothermic relaxation can be used to determine relaxation times of a sample, that in turn give information about the molecular mobility in the glassy phase [139], [140]. This effect has been investigated for annealing of freeze dried lactose below its Tg [141] and will be discussed in more detail in the MTDSC section.

MTDSC is a modification of DSC, in which a modulation is superimposed onto the linear heat ramp [142, 143]. Deconvolution is applied to the data to obtain the underlying linear and modulated responses. This added information makes it possible to measure heat capacity data as a direct output from the experiment. The modulated temperature programme is given by

Equation 3
\[ T = T_0 + \beta t + B \sin \omega t \]

where \( T_0 \) is the start temperature, \( \beta \) is the heating rate, \( B \) is the amplitude of the modulation and \( \omega \) is the angular frequency of modulation. The heat flow in a modulated temperature differential scanning calorimeter is given by the following equation, which is essentially the same as that for conventional DSC;

Equation 4
\[ \frac{dQ}{dt} = Cp \cdot (b + B \cos \omega t) \cdot f(t, T) + C \cdot \sin \omega t \]

The heating rate (d\( T \)/d\( t \) in the conventional DSC heat flow equation) is denoted by \((b + B \omega \cos \omega t)\), \( b \) is the underlying heating rate; \( f(t, T) \) is the underlying kinetic function once the effect of the sine modulation has been subtracted; \( C \) is the amplitude of the kinetic response to the sine wave modulation.

From inspection of Equation 4 it can be seen that the heat flow signal will contain a component corresponding to the values of \( B \), \( \omega \) and \( C \). The sine component only contributes to the experimental output when an enthalpic event occurs. However, the cosine function is always present. When this is the case it can be assumed that \( C \) is zero and a Fourier transform deconvolution procedure can be applied that removes the underlying sine wave to give the traditional DSC output. In order for this procedure to be valid, it is assumed that the temperature excursions from the underlying heating rate are small so that the response of the rate of the process being analysed is approximately linear. In order to prevent artefacts arising in the data produced at least six modulations are required to take place during the course of one thermal event. This requires low heating rates that increase the length of experiments. Caution must also be exercised if very accurate Tg measurements are required because of its dependence on both the heating rate and modulation frequency, \( \omega \) [144]. The same procedure as outlined above is applied to the heat input and the temperature signals to give the underlying heating rate and temperature at any time.

Comparison of the modulated component of the heat flow and the heating rate allows the heat capacity, \( C_p \), to be determined using Equation 5 below, where \( A_{HF} \) refers to the amplitude of the modulation in the heatflow and \( A_{HR} \) refers to the amplitude of the modulation in the applied modulation.

Equation 5
\[ C_p = \frac{A_{HF}}{A_{HR}} \]

When heating through a Tg the resultant increase in \( C_p \) causes an increase in amplitude of the modulated heat flow output. Multiplication of the underlying heating rate by \(-C_p\) allows
the reversing component of the heat flow to be determined. Transitions that appear in the reversing signal are thermodynamically reversible at the time and temperature at which they are detected. All other events will appear in the non-reversing signal, which is calculated by subtraction of the reversing signal from the total heat flow.

MTDSC is especially useful advantageous for the measurement of small Tg or Tg’ because the deconvolution procedure removes the accompanying endothermic relaxation (which appears in the non-reversing signal) to give the heat capacity change only in the reversing signal. Figure 3 shows the data produced from analysis of a sample of 30%w/w aqueous sucrose solution.

![Figure 3 Tg' Region Of A 30%W/V Sucrose Solution As Measured By MTDSC](image)

**MECHANICAL METHODS**

Thermomechanical Analysis (TMA) is used to investigate the effect of temperature change on sample length or volume while it is subjected to a constant mechanical stress [145]. For example upon heating through a melt or Tg a material will suddenly deform. For the analysis of frozen samples a constant load is applied by resting a flat-ended probe onto it. The temperature around the sample is controlled by placing it inside a furnace through which cold nitrogen is circulated. The technique has been used to investigate the mechanical properties of frozen solutions of various protectants in the Tg’ region and at the beginning of melting [79, 96, 123]. Similar studies have also investigated mixed systems, for example the effects of different concentrations of sucrose on the viscoelastic properties in the Tg’ region when various polymers had been added [115].

Dynamic Mechanical Analysis (DMA) is used to measure how the mechanical modulus or stiffness is affected by change in temperature, in the classical experiment the sample is heated while being subjected to a load oscillating at constant frequency. The resulting deformation is resolved into the in-phase (storage modulus, E’) and out-of-phase (loss modulus, E’”) signals and the ratio E’”/E’ gives the damping factor tan δ. The instrument is similar to TMA, and generally TMA experiments can also be performed in a dynamic mechanical analyser. In order to apply the oscillating load different geometries may be used, while the analysis may also be designed such that frequency sweeps are performed at set temperature increments, which may be particularly useful when investigating Tg phenomena. DMA is often used for the investigation of Tg and morphological properties of polymeric
materials and composites and may be advantageous in the measurement of low energy transitions that may be missed by DSC.

DMA has been used in the determination of the Tg’ region of sucrose solutions [125] and compared with DSC and dielectric analysis in the study of the Tg’ region of frozen wheat doughs [146].

**OPTICAL METHODS**

Cold stage microscopy (CSM) is often used to directly observe the processes such as crystallisation in frozen solutions. The use of polarised light and differential interference contrast can also aid in the characterisation of the types of sample under observation. In the modern microscope the temperature control is generally provided by connection to a gas flow controller using liquid nitrogen while the temperature of the block itself is automatically calibrated each time the instrument is switched on by reference of the resistance in the sample heating block to electrical resistors in the heating unit. Temperature ramps or annealing steps may be programmed in order to mimic those used in DSC studies or indeed in the freeze-drying process. Some temperature stages even allow the connection of a vacuum pump in order to control the pressure in the system too. CSM has been used to corroborate calorimetric observations of frozen solutions [147] and has even been coupled to DSC in order to investigate the crystallization of ice in aqueous solutions of glycerol and dimethyl sulfoxide [148].

Freeze Drying Microscopy allows Tc itself to be directly observed. This is useful because the temperature interval between a measured Tg’ and the onset of collapse will vary between formulations. Freeze-drying microscopy (FDM) allows the direct microscopic observation of a thin section of material as it freezes and dries, enabling the structure of the drying material to be assessed under a range of conditions. The first freeze-drying microscopes were developed in the middle of the last century[149], but it was only in recent times that such apparatus became commercially available. An image captured from a FDM experiment where a dextran solution has been heated to above its Tc is shown in Figure 4.

![Figure 4 FDM Image Showing Collapse of Dextran Solution at -9.1°C](image)

The technique enables collapse temperatures to be detected in situ as well as the characterisation of skin or crust formation. The use of compound microscopes with these systems also enables the use of polarised light and differential interference contrast (DIC) in order to gain more detailed information about the behaviour of different regions within a formulation. The combination of FDM with other thermoanalytical methods can provide a
comprehensive means of formulation characterisation [75, 150, 151], for example in the determination of effective diffusion coefficient ($D_{eff}$) values for the diffusion of water vapour through freeze dried cakes formed by the sublimation of water from a frozen mass of buffer solution [75]. The values determined were shown to be in reasonable agreement with those estimated from theory.

**DIELECTRIC AND ELECTRICAL METHODS**

There are several techniques that measure the change in electrical properties of a material with respect to temperature usually by measuring the response of a material to an applied current [102, 152]. Electrical resistance analysis (ERA) is also known as freezing resistance analysis (FRA) when used to measure the subambient behaviour of a sample, and involves measuring the resistance of a system to an alternating current as it is cooled and rewarmed [88]. This can provide crucial information as to the state of the interstitial (non-solvent) phase at a range of temperatures, which becomes critical at the point where ice is removed during freeze-drying.

Her et al [153] have shown that addition of low levels (about 0.1%) of electrolyte to frozen solutions was necessary to obtain the sharpest Tg values using electrical thermal analysis (ETA). For example when compared with DSC data of solutes with and without electrolyte (0.1%) it was shown that the electrolyte decreased Tg' by ~0.5-1.0°C. They also reported that the Tg' values obtained by ETA were higher than those measured by DSC and that the difference between the two methods increases as the Tg' value decreased.

Smith & Pearson have reviewed the potential application of dielectric analysis to frozen and freeze-dried materials [154]. In essence, dielectric spectroscopy measures the response of a sample to an applied stress in the form of an alternating current [102]. From this response it is possible to obtain information concerning the structure and behaviour of the sample. Frequency domain measurement can be used to obtain information regarding the electrical properties of a sample that can be related to changes in structure or morphology, for example in the cases of crystals or gels. It can therefore be used to fingerprint materials or to compare similar samples. The ability to measure the behaviour of water is ideal for the determination of the structure of frozen systems. It is particularly suited to the analysis of glassy systems where it is important to determine how much water is present in the glassy phase and whether the remainder of the water in the system is ice or free water. It has been used to study the low frequency dielectric response of aqueous solutions containing low concentrations of polyvinylpyrrolidone (PVP) [99]. It was concluded that the low frequency dielectric response in these systems is primarily a reflection of the relaxation behaviour of the water molecules in the nonfrozen fraction.

A combined approach is often useful when examining behaviour of frozen solutions. For example cold stage microscopy (CSM) DSC, MTDSC and temperature controlled XRD have been used in combination used to examine the effects of cooling rate and annealing on the subambient behaviour of mannitol solutions [155]. CSM showed the formation of a separate phase upon cooling, MTDSC was used to determine the Tg (Figure 5) while DSC and XRD were used to examine the crystallisation behaviour. MTDSC indicated that the transitions observed upon reheating corresponded to a Tg immediately followed by crystallisation, XRD data showed that crystallisation was into the β form. The combination of these techniques also allowed the effect of annealing to be investigated.
FREEZE-DRIED PRODUCTS

A successful freeze-dried product must survive processing and also storage over the claimed shelf life without significant loss of activity, accumulation of decomposition products or chance in texture/appearance. For example stability of freeze-dried pharmaceuticals is usually much higher than the equivalent aqueous solution, but inactivation may occur either during the drying process or storage. Some products that exhibit desirable characteristics immediately after drying may exhibit poor long-term stability that may be attributed to their generally (at least partially) amorphous nature. Unlike crystalline materials, amorphous freeze-dried products are not thermodynamically stable, and with time they will tend to revert to the more stable crystalline state. While the recrystallisation process is hindered by the very low mobility that exists in amorphous systems [7, 139, 156], freeze-dried products also tend to be extremely hygroscopic, readily picking up water which can cause products to collapse into sticky gums, once water content has increased then the rates of degradation reactions will also increase [157-160]. Therefore product stability is generally a function of the formulation and the residual water content [111, 112, 161, 162].

ANALYSIS OF FREEZE-DRIED PRODUCTS

EFFECT OF WATER ON Tg

Water lowers the Tg of amorphous products. It does this by increasing the molecular mobility lowering the average molecular weight of the products. Water is termed a plasticizer, the traditional definition of which comes from polymer chemistry and is “a material incorporated into a polymer to increase the polymers workability, flexibility and extensibility”[163]. When concerned with glasses the term is used to mean a molecule that when added to a pure compound reduces the Tg by lowering the viscosity. Water is the most common plasticizer, a 1% increase in the water content of a formulation may decrease the Tg by up to 10K. For example the Tg of anhydrous saquinavir, a protease inhibitor drug molecule, is 105°C [164], but the Tg of the same material with 5% water content is 55°C.

The effect of water content on the Tg of a range of common pharmaceutical excipients including polymers and sugars was investigated in relation to the Gordon-Taylor equation [165] (Equation 1), which is used to describe the Tg of compatible polymer blends [166] and can be a useful aid in predicting Tg changes. This equation assumes no interaction between the components and that volume additivity occurs. Tg_{mix} is the Tg of the formulation
containing components 1 and 2, \( w_1 \) and \( w_2 \) refer to the weight fractions of components 1 and 2 respectively, \( T_{g1} \) and \( T_{g2} \) refer to \( T_g \) values of components 1 and 2 respectively and \( K \) is a constant that is a ratio of the free volumes of the two components under any given conditions.

Equation 1

\[
T_{g(mix)} = \frac{w_1 * T_{g1} + w_2 * T_{g2}}{w_1 + (K * w_2)}
\]

The study showed that if two amorphous solids had the same \( T_g \) the one with the highest density mass would be plasticized the most by a given amount of water, whereas for materials with the same density the one with the highest \( T_g \) was plasticized the most. \( T_g \) and density are closely related so that the Gordon-Taylor constant, \( K \), is usually approximately equal to 0.25. Plots of \( T_g \) against water content for the model compounds used gave the predicted results for most of the materials studied.

Thermogravimetric analysis (TGA) is routinely used in the determination of water content for amorphous materials. It involves heating material at a defined rate in a controlled environment and measuring mass changes. TGA is routinely used to characterise materials that exhibit mass gain or loss caused by decomposition, oxidation or dehydration. It is particularly useful in the measurement of water contents [88], especially where only small amounts of sample are available or if it is not compatible with the Karl-Fischer medium [167, 168]. The disadvantage of TGA is that it determines mass changes but it cannot identify the nature of the evolved species. For this it is necessary to connect the outlet to a mass-spectrometer. If this is not possible then TGA may be validated by comparison with Karl-Fischer analyses for standard samples.

**STORAGE STABILITY**

It has already been stated that since freeze dried products are generally amorphous there may be issues associated with storage stability. This will be especially true if any temperature or relative humidity changes are likely to be incurred during shipping or storage. For this reason it may be desirable to investigate the effect of water on the \( T_g \) of the formulation and also to investigate the effect of temperature on mobility in the product.

**CALCULATION OF RELAXATION TIMES FROM ENTHALPY RELAXATION AT THE Tg**

DSC and, more recently, MTDSC have been used to accurately measure the endothermic relaxation accompanying the \( T_g \) that is caused by structural rearrangement of the glass [139, 141]. The relaxation enthalpy is a gauge of the mobility present in the glassy state. If an amorphous material is stored below its \( T_g \), the size of the endothermic relaxation obtained upon reheating can be related to the storage temperature. With increased annealing time the endothermic relaxation increases as more of the material has relaxed to a more stable state. If the heat capacity change at the \( T_g \) of the material is determined (e.g. from a MTDSC experiment) the maximum endothermic relaxation at a given temperature can be calculated from

Equation 2

\[
\Delta H_\infty = \left( T_g - T \right) \Delta C_p
\]

Where \( \Delta H_\infty \) is the maximum enthalpic relaxation, \( T \) is the storage temperature and \( \Delta C_p \) is the change in heat capacity at the \( T_g \). The extent to which the material has relaxed is then calculated from the following equation
Equation 3 \( \phi_t = 1 - \left( \frac{\Delta H_t}{\Delta H_w} \right) \)

where \( \phi_t \) is the extent to which the material has relaxed and \( \Delta H_t \) is the measured enthalpy at time \( t \). If a series of annealing experiments are performed at different annealing temperatures and times the degree of stability below the Tg can be calculated. For example Figure 6 shows the effect of storage temperature below the Tg on the relaxation constant, \( \tau \), of freeze dried lactose. The molecular time constant is calculated from the Kohlrausch-Williams-Watts (KWW) equation and is a measure of molecular mobility in a sample. \( \beta \) is the exponential power that represents the distribution of molecular relaxation times, which varies between 0 and 1.

Equation 4 \( \phi_t = \exp\left[ \left( -\frac{t}{\tau} \right)^\beta \right] \)

Figure 6 The Effect of Storage Temperature on \( \tau \) of Freeze Dried Lactose [141]

**ISOTHERMAL MICROCALORIMETRY**

Isothermal microcalorimetry, sometimes referred to as heat conduction or heat flow calorimetry is used to observe the heat flow associated with processes occurring at constant temperature [169-171]. The instrument records power output as a function of time with extreme sensitivity, detecting enthalpy changes of the magnitude of tens of nW. In theory any sample that can be contained within a sample ampoule can be observed using the technique. However, larger quantities are required when compared with DSC and since samples are not heated times of observation may be considerably longer. Microcalorimetry has been used to directly measure the rate of heat change during the relaxation processes in freeze dried formulations of saccharides [172], where the structural relaxation time was obtained from a fit of the power data to the derivative version of the KWW equation.

Microcalorimetry can also be used to investigate the effect of solvent vapours on recrystallisation process in amorphous materials if a perfusion accessory is attached to the apparatus. This allows the effect of relative humidity in the space above the sample to be accurately controlled by mixing defined ratios of an inert carrier gas (such as dry nitrogen) with solvent vapour (usually water) [170]. In a similar manner a hydrostat containing a saturated salt solution can be included in the sealed sample cell to generate a defined vapour.
pressure of water. The recrystallisation of the amorphous material can then be observed with time. However, a drawback associated with the use of hydrostats is that for very fast recrystallisation processes the event under scrutiny may either be obscured by the heat changes generated by loading the sample into the calorimeter or may indeed even take place before the sample has been loaded.

DSC IN COMBINATION WITH MICROSCOPY

Mention has already been made of use the value of combined techniques for the analysis of frozen systems and it may also be useful for the observation of the collapse process in products. Mazzobre et al. [150] have used the combined approach of DSC and microscopy to measure crystallisation kinetics, induction times and time for complete sugar crystallisation at a range of storage temperatures. Videoing the microscopy images allowed the direct real time observation of individual crystal growth and morphological aspects at resolution not detected by DSC. They also showed that the data obtained from the microscopy data relating to the temperature dependence of crystallisation rate and time to complete lactose crystallisation were comparable to those obtained by DSC.

SUMMARY

A range of thermal techniques are now available to aid the formulation scientist and process engineer in the design of the optimum freeze-dried formulation using the most efficient freeze drying process. Thermal analysis can assist in the determination of the key parameters at all stages of the freeze drying process right through from the initial solution to the final product.

REFERENCES


