

EFFECTS OF MOISTURE SORPTION AND TEMPERATURE ON AMORPHOUS SPRAY DRIED LACTOSE

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ABSTRACT

An isothermal microcalorimetry technique based on the ability of amorphous spray dried lactose to absorb large amounts of water was developed in order to follow the transition of the metastable amorphous form to the more stable crystalline form. A sharp response was measured, indicating a rapid process with the whole sample crystallising simultaneously. Crystallisation was monitored by weight changes due to water uptake and subsequent loss after crystallisation. DSC and TGA data revealed that the crystallised form contained a mixture of β -, α -anhydrous and α monohydrate forms. Physical mixtures of amorphous and crystalline lactose were used to investigate the lower detection limit of these two techniques for amorphous material. These were 0.3% for microcalorimetry and ca 0.1% for the gravimetric method.

The water sorption behaviour of the amorphous lactose and water mobility below and close to the glass transition temperature (T_g) was investigated. Exposure of the spray dried lactose to 50%RH for varying lengths of time resulted in structural collapse of the amorphous sample to different degrees. The collapsed material did not crystallise instantaneously and water desorption from this structure was shown to be slow. DSC and TGA studies revealed that during heating the collapsed lactose crystallised at around 70°C compared to the uncollapsed material, which crystallised at around 180°C. Drying at 0%RH and 25°C also resulted in eventual crystallisation of the collapsed material. DSC studies on compressed samples revealed a reduced crystallisation temperature of around 120°C. With increased temperature crystallisation occurred more rapidly and required a lower water content.

Moisture uptake and crystallisation in bulk samples (up to 100g) of two partially amorphous lactose materials, Zeparox and Pharmatose, was also investigated. These samples crystallised gradually, on exposure to 75%RH, as water was passed down through the powder bed. A net negative weight change indicated that the spray dried lactose did not form a complete hydrate on crystallising.

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*this thesis is dedicated to
my dear mother and in loving memory of my late father
with deepest love, respect and gratitude*

“Everything that happens, happens as it should,
and if you observe carefully, you will find this to be so.”

Marcus Aurelius Antoninus (121 – 180)
Meditations IV, 10

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ABBREVIATIONS

%RH	% relative humidity
d.i.	Disruption index
dm/dt	Changes in mass with time (% / min.)
D	Diffusion coefficient
C_p	Heat capacity
g	Grams
G	Gibbs free energy
H	Enthalpy
J	Joules
P	Pressure
Q	Heat
sccm	Standard cubic centimetres
s	Seconds
S	Entropy
T	Temperature
T_c	Crystallisation temperature
T_g	Glass transition temperature
T_m	Melting temperature
U	Internal energy
V	Volume
w	Weight fraction
W	Work
α	Thermal expansion coefficient
η	Viscosity
η_g	Viscosity at T _g
ρ	Density
ΔH_c	Enthalpy of crystallisation
ΔH^f	Enthalpy of fusion
ΔS^f	Entropy of fusion
ΔS^m	Entropy of mixing
ΔS^p	Entropy of processing
ΔS^s	Entropy of solution

CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Particles used in the pharmaceutical industry are often recognised as crystalline and thus considered to be in a stable physical state. However, in a lot of cases, crystals aren't totally perfect and have defects or imperfections in their lattice that cause disorder in the particles. Regions of molecular disorder can therefore exist in crystalline materials, and particles that contain randomly positioned molecules without any repetition in structure are said to be in the amorphous state as there is an absence of long range order. Huttenrauch et al (1985) differentiated two categories of disorder and instability resulting from mechanical activation of pharmaceutical systems, one of which concerns short-living states (10^{-7} to 10^{-3} s), which decay as soon as they are generated, and secondly, "freezed, metastable states" with a duration ranging from 10^{-3} to 10^6 s (1670h or 70days).

The existence of disorder in a pharmaceutical material may be because conventional crystallisation techniques cannot produce the material in a crystalline form, or processing (e.g. milling and micronising) might introduce a certain level of amorphous structure to an otherwise highly crystalline material. It is also possible to have a partially amorphous system as in the case of spray dried lactose used for direct compression tableting, which is usually around 15% amorphous. The amorphous state may also be introduced deliberately to enhance the performance of a product, for example, the use of the amorphous form of drugs and excipients to improve properties such as rate of solution has been well documented (Florence and Salole, 1976, Chiou and Kyle, 1979 and Mosharraf and Nystrom, 1996).

Drying methods such as freeze drying and spray drying, commonly used in the pharmaceutical and food industries can result in the conversion of many carbohydrate and sugar systems into the amorphous form. Since a variety of these high molecular weight carbohydrate materials such as proteins, starches and celluloses and water soluble systems are often used in pharmaceutical dosage forms it is clear that the amorphous state is a critical issue in both the pharmaceutical and food industries. The amorphous state is critical in determining the solid state physical and chemical properties and stabilities of many pharmaceutical dosage forms and on the properties and quality of many food products. Hence, knowledge regarding the effect of factors such as moisture, temperature and time on these systems is of utmost importance.

1.2 BASIC THERMODYNAMIC CONCEPTS IN RELATION TO THE CRYSTALLINE AND AMORPHOUS STATES.

Thermodynamics, which studies the transformation of energy, can be used to follow the release of energy stored by molecules, due to the production of heat. The thermodynamic state of a system is described by specifying its properties, which include temperature (T), pressure (P), volume (V), internal energy (U), enthalpy (H), Gibbs free energy (G) and entropy (S). These properties are related by the following fundamental equations:-

$$H = U + PV \quad \text{.....Equation 1.1}$$

$$G = H - TS \quad \text{..... Equation 1.2}$$

According to the 1st Law of Thermodynamics which states that energy can neither be created or destroyed, the energy change for a system can be defined as:

$$\Delta U = Q - W \quad \text{.....Equation 1.3}$$

where Q is absorption of heat and W is work done by the system. Since the internal energy (U) of a system is related to the enthalpy (H), the enthalpy change (ΔH) is the heat which is absorbed or evolved when a system changes its thermodynamic state under constant pressure.

When heat flows into a substance the motion of the atoms and molecules in the system increases. As a result of their increased motion, the atoms or molecules take up more space and the substance expands. Their motion gives every object its internal energy, which depends on how rapidly the atoms or molecules move. Temperature is an indication of an object's internal energy level, while heat on the other hand is the passage of energy from one object to another. As heat raises the internal energy of an object; that objects atoms and molecules move around more and become more disorderly. The term entropy is used to describe the amount of disorder in an object.

The entropy of a substance increases whenever the energy it possesses to do work decreases. A system that has a high degree of unpredictability has high entropy. Heat,

therefore, flowing into an object will always increase the internal energy and disorder in that object and increases the temperature. Consequently, heat disrupts the orderly arrangement of the atoms or molecules of an object.

The second Law of Thermodynamics, which describes the degree of disorder of a system, says that a spontaneous change in an isolated system proceeds in the direction of increased entropy. This law deals with heat efficiency and is also related to the definition of energy as the stored ability to do work. The internal energy of a system is stored in the movement of the molecules, but this law states that a temperature difference is necessary before work can be done. According to this law therefore, a consequence of a spontaneous process is that there is an increase in the disorder or decrease in ΔG . It involves the entropy change ΔS , which is related to the enthalpy and free energy according to equation 1.2. A decrease is not the preferred direction for entropy changes, consequently there must be a favourable change in the enthalpy which more than compensates for the loss of entropy. In the context of the present discussion of powdered materials, the processing of such materials may involve the use of high energy procedures such as comminution, which consumes a lot of energy but only a very small amount of the energy is used to fracture the material. Energy is also released as heat, vibration and sound. Similarly, the efficiency with which the applied force is used is very low in grinding processes since many particles are deformed by the applied force, but are not broken, so that the energy expended on squeezing and releasing them is turned into heat. Laws governing the energy requirements for grinding processes predict that the energy requirement must increase as the particle size decreases.

1.3 THE CRYSTALLINE STATE

1.3.1 DEFINITION AND DESCRIPTION

The crystalline state involves a 3-dimensional ordered array of molecules in which there is periodicity and symmetry, with atoms and molecules in a regular and repeating arrangement. All molecules are equivalent with respect to their binding energy, which results in the breakdown of the crystal occurring at a fixed, unique energy level (i.e. temperature).

Single crystal X-ray characterisation is a powerful tool in studying crystals, and thermal methods such as DSC are also commonly used in investigating crystalline materials. Drug substances are usually purified by recrystallisation or precipitation. Many different crystal systems exist e.g. the cubic system, orthorhombic and monoclinic systems. Inorganic compounds usually crystallise in a specific system e.g. sodium and potassium chloride crystallise in the cubic system. Cubic crystals usually form if crystal growth is of equal rate in all directions or a plate-like structure if the growth is inhibited in one direction. If, on the other hand, it is inhibited in 2 directions then a needle will be the result. Such shape differences do not imply different crystal systems but are called crystal habits. They are important since a cubic-like shape for instance will flow better in a powder hopper, which of course is important in tableting and encapsulation. On the other hand the less cubic a crystal becomes the larger the specific surface area so dissolution rates are favourably affected.

Organic compounds, however, can crystallise to form different crystal systems depending on the recrystallisation or precipitation conditions and this phenomenon where the same substance crystallises in different forms is referred to as polymorphism. Polymorphism refers to different crystals of the same substance with different unit cells and packing arrangements e.g. graphite and diamond are different polymorphs of carbon. Solvates exist when the same substance crystallises in different unit cells that contain solvent of crystallisation. If water is incorporated the solvates are called hydrates e.g. α lactose monohydrate which contains one molecule of water for each lactose molecule. This hydrate is only removed when the sample is heated to around 150°C, as shown by Berlin et al (1971) from TGA studies, resulting in the formation of the hydrate form. Desolvated solvates exist when the solvent is removed leaving the same crystal structure. The polymorphic form with the lowest energy content is the most stable one, and forms with higher energy content often have lower melting points, higher dissolution rates and often, lower chemical stability, (Nyqvist, 1993). It can be expected that a crystal form with a high energy content should transform more or less rapidly into a stable form. However, as will be seen later on in the case of amorphous systems, addition of activation energy is often necessary to induce such a transformation, and in this case the high energy form is called the metastable form.

Polymorphism is remarkably common, particularly within certain structural groups; 63% of barbiturates, 67% of steroids and 40% of sulphonamides exhibit polymorphism. Traces levels of solvents (either organic or water) can remain in crystallised species after precipitation, and can become molecular additions to the crystal and change its habit. This is termed pseudopolymorphism, and one way in which pseudopolymorphs can be differentiated from true polymorphs is by observing the melting behaviour. Pseudopolymorphs evolve a gas whereas true polymorphs merely melt. As mentioned earlier crystals are never totally perfect and have defects or imperfections in the crystal lattice which cause disorder in the crystals.

1.4 THE AMORPHOUS STATE

1.4.1 DEFINITION AND DESCRIPTION.

A majority of drug compounds and excipients are solids at room temperature, mainly in the form of powders. However, a solid compound in the form of a powder does not necessarily mean that it is crystalline. Occasionally, addition of activation energy is necessary in order to obtain a certain crystal form. This means that if the activation energy is high a spontaneous transformation e.g. at room temperature, is not possible. Consequently, when a substance doesn't crystallise, the amorphous form exists.

The 3-dimensional long-range order that normally exist in a crystalline material does not exist in the amorphous state, and the position of molecules relative to one another is more random as in the liquid state (Hancock and Zografi, 1997). Typically, amorphous solids exhibit short-range order over a few molecular dimensions and have physical properties quite different from those of their corresponding crystalline states. Thus, the ideal amorphous state can be recognised as randomly positioned molecules without any repetition in structure.

1.4.2 PRODUCTION OF THE AMORPHOUS STATE IN SOLIDS.

There are a number of ways in which amorphous character may be induced in a solid and the four most common ways are:

- (1) condensation from the vapour state
- (2) supercooling of the melt
- (3) mechanical activation of a crystalline material (e.g. grinding, milling and micronising) and
- (4) rapid precipitation from solution (e.g. freeze drying or spray drying).

Sometimes, amorphism is compared to 'freezing' of a liquid structure, i.e. there is an absence of long range order. This concept is best understood by considering a scene where a molten substance is cooled slowly and without disturbing it, whence it is possible to super-cool it (i.e. cool it below its melting point, without solidification) (Carstensen, 1993). If the liquid at this point is not very viscous then it usually will not take much of a disturbance to make it precipitate out. An example of this is water if cooled carefully, temperatures as low as -20°C can be easily attained. However, the slightest stir will cause the ice to freeze out. Crystallisation rates are inversely proportional to viscosity and if the substance is very viscous at its melting point the super-cooling becomes much easier. The solid thus formed is actually a liquid with (infinitely) high viscosity, but even glass (which is mostly amorphous) is 'liquid'. This is the reason why old church windows are thicker at the bottom than at the top showing the flow with time. The best way of visualising this idea of an amorphous system can be seen in Figure 1.1, where a schematic representation of the enthalpy or volume - temperature relation is illustrated for a typical amorphous solid i.e. specific volume dependence on temperature is shown.

1.4.3 ASSOCIATED ENERGY AND MOLECULAR MOBILITY OF AN AMORPHOUS SYSTEM.

For a crystalline material, at very low temperatures a small increase in enthalpy (H) and volume (V) with respect to temperature occurs, indicating a heat capacity (C_p) and thermal expansion coefficient (α) (Hancock and Zografi, 1997). There is then a sharp break at the melting point T_m (characterised by a large change in H/V) for the crystalline compound (Figure 1.1), which represents the first-order phase transition to the liquid state. As already mentioned rapid cooling of this melted material may result

in H and V following the equilibrium line for the liquid beyond the melting temperature into a super-cooled region. The enthalpy and volume changes immediately below T_m exhibit no discontinuity with those observed above T_m , so this amorphous state is considered to be an equilibrium super-cooled liquid.

This amorphous state is also called the ‘rubbery’ state because of the macroscopic properties of amorphous solids in this region. This state is further characterised by considering its rate and extent of molecular motions, both of which are strongly temperature dependent. Cooling the super-cooled liquid even further appears to reduce the molecular mobility of the material to a point at which the material is kinetically unable to attain equilibrium in the time-scale of the measurement as it loses thermal energy.

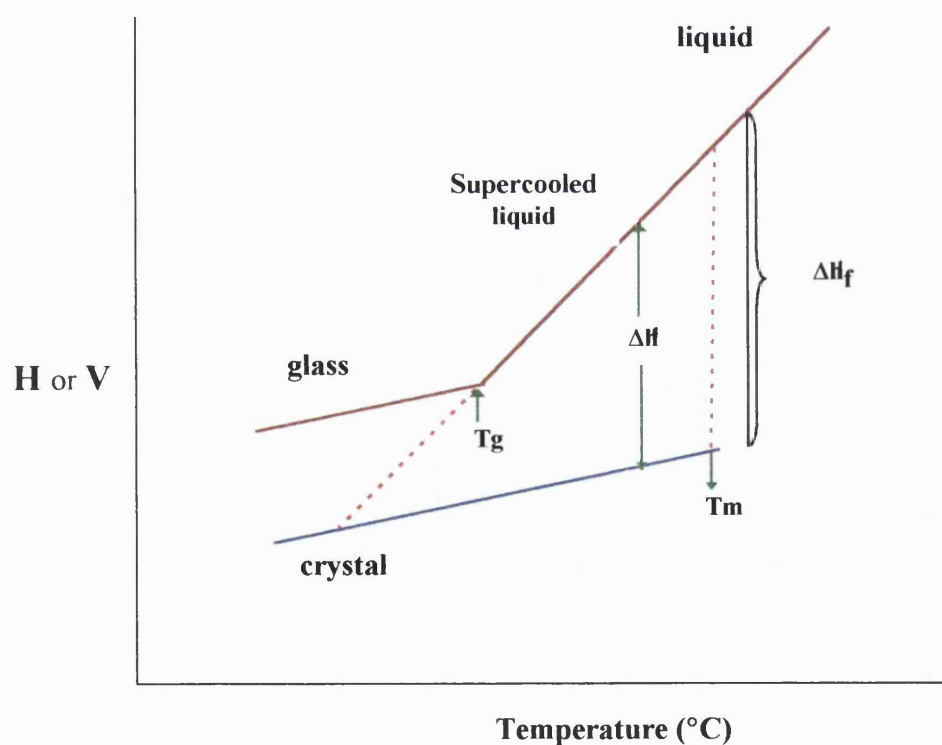


Figure 1.1 Schematic representation of the enthalpy(H) or volume(V) and temperature relationship for a typical amorphous solid. (adapted from Hoffman, J.D., *J. Chem. Phys.*, **29** (1958) 1192 - 1193).

Hence, for crystal forming materials the basic factor that will determine whether the eventual solid is crystalline or amorphous will be the extent to which the molecules are able to attain equilibrium configuration. Low molecular mobility due to high sample viscosity, as already mentioned, will result in the inability to attain equilibrium configurations and consequently in amorphous formation (Flink, 1983).

On production of the amorphous material, a system is created which is unstable (in a metastable state), and which has a higher free energy (G) than the corresponding crystalline state, as can be seen from Figure 1.1. The internal energy (U) and the entropy (S) are also larger than the corresponding values for the pure crystal (York and Grant, 1985). Salvetti et al (1996) used heats of solution measurements to indicate the excess energy of amorphous/glassy forms of sucrose, glucose and glucose monohydrate over the crystalline forms. They stated that the difference in energy is caused by differences in Van der Waals interaction energy, the extent of the total energy associated with H-bonding in the two solids and their vibrational frequencies. They also postulated that the enthalpies of solution of a glass will vary depending on its thermal history, since one of the characteristics of a glass is that its enthalpy decreases on spontaneous structural relaxation during its physical ageing. The irregular arrangement of molecules in the amorphous state usually causes them to be spaced further apart than in a crystal. Hence, the specific volume is greater and the density lower for an amorphous solid than that of the crystal and it is said that there is greater free volume (Hancock and Zografi, 1997). It has been postulated (Flink, 1983) that thermodynamically speaking, the presence of this metastable state indicates that there are free energy barriers, which hinder the attainment of the stable crystalline state, which is illustrated in Figure 1.2. The amorphous state existing as a supercooled liquid can be said to exist in a non-equilibrium state as if it were at equilibrium. The barrier that can prevent a system achieving its equilibrium state is termed an activation step. This is shown in Figure 1.2 where a system may need to undergo an energetically unfavourable transition in order for it to attain an equilibrium state. Hence, it may be necessary to impart sufficient energy to the system in order for it to overcome this activation energy barrier and reach equilibrium.

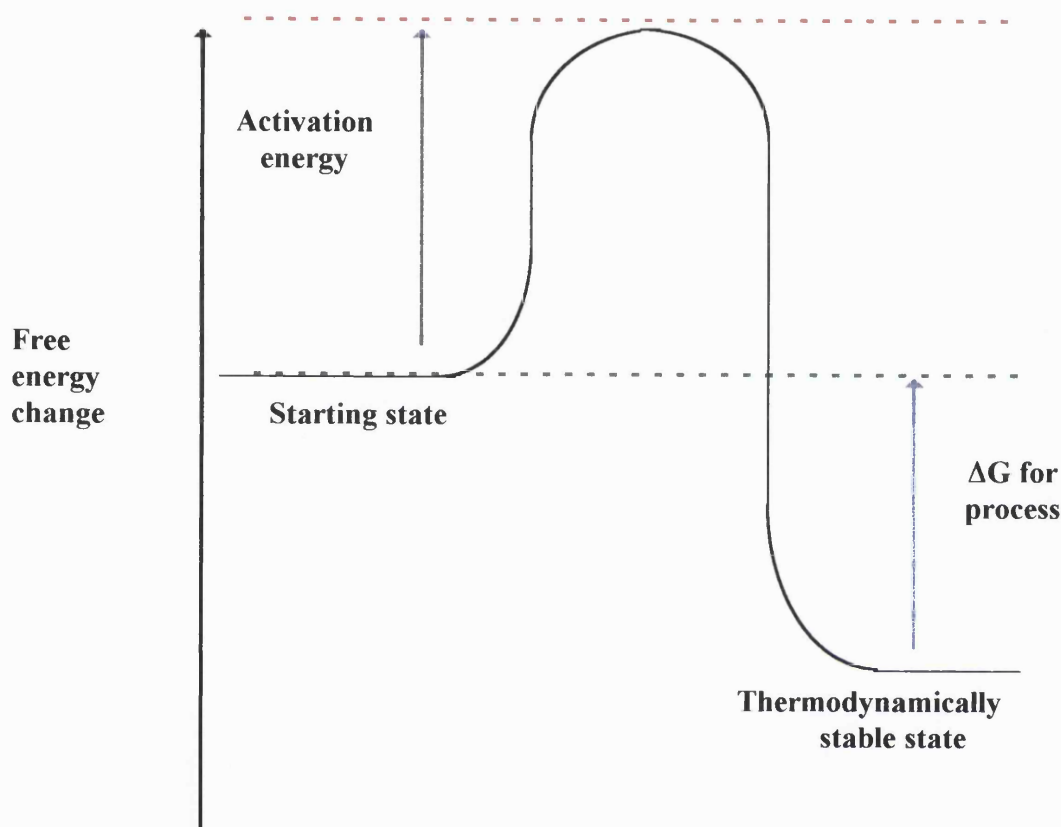


Figure 1.2 Energy diagram showing an activation barrier that must be overcome before a process can move to the thermodynamically stable state (adapted from Buckton, G., *Interfacial phenomena in drug delivery and targeting*, 1995).

1.4.4 THE GLASS TRANSITION TEMPERATURE (T_g).

At temperatures just below the crystalline melting point (T_m), molecular motions in the amorphous material are very rapid and the material has liquid-like properties. However, on cooling these molecular motions can be slowed down and eventually a temperature is reached at which the system viscosity is said to become too high for the volume relaxation to follow the temperature lowering and non-equilibrium conditions are established (Hancock et al, 1995).

The temperature at which the phase change takes place is called **the glass transition temperature (T_g)** and this is defined as the narrow temperature range over which an amorphous material changes from a 'glassy' state with low free volume to a 'rubbery' state with greater free volume (Zografi and Hancock, 1993).

This temperature is characterised by the change in slope at this point in Figure 1.1 where the properties of the glassy material deviate from those of the equilibrium super-cooled liquid to give a non-equilibrium state having even higher H and V than the super-cooled liquid.

It must be noted however that below T_g translational molecular motions in the amorphous material still occur but are relatively slower and occur over much longer periods of time (Hancock *et al*, 1995). Amorphous solids therefore undergo increasing translational and rotational motion as temperature increases with the most significant changes occurring above T_g in the rubbery state. Above T_g both the energy and entropy adjust to changing temperatures so that volume is at a minimum and the system is at equilibrium, as shown in Figure 1.1. The molecular movements (rotational, translational and vibrational) are such that they overlap and thus free volume exists throughout the system. Free volume is the volume unoccupied by the 'solid matter' of the molecules and represents the volume available for free movement of the molecules (Flink, 1983). On cooling at temperatures above T_g , liquids contract and as the molecules' kinetic energy decreases, the free volume decreases also. As long as free volume extends throughout the system formation of the crystalline form is possible. However, at the point when free volume no longer exists throughout the system, the glassy state is formed.

On re-warming, the molecules take up some thermal energy and movements increase. When the range of movements begin to overlap and extend throughout the system, then the system takes on liquid-like properties again and the T_g has been passed. **The glass transition temperature is therefore a reversible second order change in phase between the solid glassy phase and the liquid like rubbery phase.** It is characterised by a heat capacity change, which is usually indicated by an endothermic baseline shift in the DSC. Above this temperature the amorphous material retains the properties of a liquid (eg. Plastic deformation) and is denoted 'rubbery' and below this temperature it has more of the properties of a crystalline solid and is denoted glassy.

Chemical stability of rubbery amorphates is like the stability of the liquids and is generally worse than that of the crystalline material. Experimental studies of T_g are complicated by the existence of many different modes of molecular motion in most systems and changes in the scale and type of motions with temperature (Hancock and Zografi, 1997).

1.5 EFFECTS OF TEMPERATURE ON AMORPHOUS SOLIDS.

Amorphous materials can therefore undergo increasing translational and rotational motion as temperature increases with the most significant changes occurring above T_g in the rubbery state. Such molecular motion or molecular mobility increases significantly as the difference between T the operating temperature and T_g becomes greater (Zografi and Hancock, 1997). Williams et al (1955) showed that the mathematical analyses used for glass transition phenomena in polymers can also be used for low molecular weight organic glasses, such as sucrose. They demonstrated the existence of a unique equation that relates various properties of a material near T_g to the difference in temperature from T_g . Thus, the effect of $T - T_g$ on dynamic properties related to molecular mobility can be quantified using the Williams, Landel, Ferry (WLF) equation derived from free volume theory (Williams et al, 1955). Equation 1.4 is a form of the WLF equation describing the effect of $T - T_g$ on viscosity,

$$\ln \eta = \ln \eta_g - \frac{40.2 (T - T_g)}{51.6 + (T - T_g)} \quad \text{.....equation 1.4}$$

where η and η_g are viscosities at T and T_g , respectively and the constants are characteristic of amorphous solids in the range of T_g to $T_g + 100^\circ$. Viscosity is in units Pa s (1 Pa s equals 10 poise) and temperature is in Kelvin. At T_g , typical values of the shear viscosity of amorphous solids (e.g. sugars) are in the region of 10^{12} Pa s (Parker and Ring, 1995). This equation shows how significantly the viscosity drops and hence the mobility of the molecules increases as one raises the temperature above T_g . This was illustrated by Zografi and Hancock (1993), where it was reported that the viscosity of an amorphous solid changed from about 10^{13} Pa at T_g (i.e. $T - T_g = 0$) to 10^8 Pa when going just 20° above T_g (i.e. $T - T_g = 20$). This indicates that the

behaviour of all glasses is based on the same principle; that is, that as the glass transition is approached the relative free volume decreases sharply and that this is the primary cause for increase in viscosity. This indicates the importance of $T - T_g$ on the solid state molecular mobility of drugs and excipients which would in turn affect chemical degradation, solid state phase transformations and mechanical strength, all properties controlled by the level of molecular mobility possible in the sample (Ahlneck and Zografi, 1990).

Since it appears that the viscosity of an amorphous solid is significantly changed when going from the glassy to the rubbery state it is easy to see that only small changes in temperature near T_g can greatly change the reactivity of a drug. The Arrhenius equation is not valid under these circumstances as shown in Figure 1.3, where the validity of this equation for different states of a material is illustrated. Zografi (1988) stated that for amorphous solids as the temperature is increased the molar volume increases to a small extent until T_g is reached and the 'rubbery' state forms. Eventually this 'rubbery' amorphous solid will change into a liquid where the changes in molar volume of the substance will change with temperature according to the Arrhenius equation. However, in the rubbery state this change will be exponential with temperature.

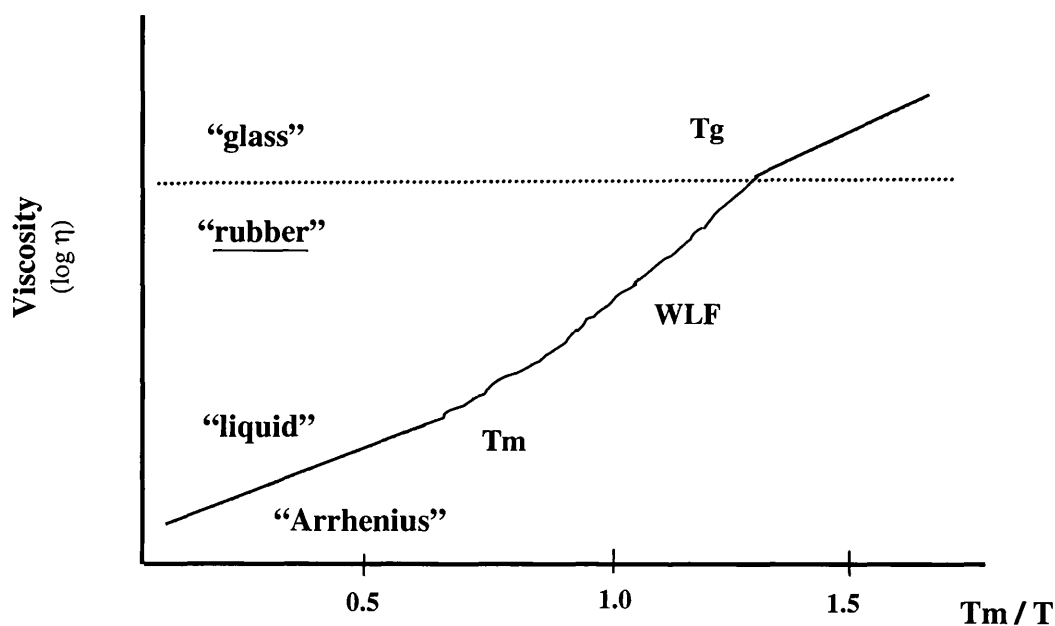


Figure 1.3 The validity of the Arrhenius relationship for different amorphous states (adapted from Ahlneck, C., *Industrial Aspects of Pharmacy* 1993).

Instead the WLF equation is required in the rubbery state above T_g , and is based on the temperature dependence of free volume. It defines the kinetics of molecular-level relaxation processes. It is therefore important to note that the Arrhenius equation is not always valid and can vary depending on the state of the amorphous solid. The temperature dependence of molecular motions directly determines many important properties of amorphous materials, including the location of the glass transition temperature and the ease of glass formation.

Hancock et al (1995) while investigating the molecular mobility of amorphous pharmaceutical solids below their T_g 's, agreed with earlier findings that amorphous materials could be classified as either 'strong glass formers' or 'fragile glass formers'. 'Strong glass formers' typically exhibit relatively small changes in heat capacity (ΔC_p) at their T_g while 'fragile glass formers' conversely exhibit large ΔC_p values. Proteins are examples of 'strong glass formers' with their change in ΔC_p at T_g often being extremely small, and exhibit Arrhenius-like changes in their molecular mobility with temperature.

Hancock et al (1995) postulated that the reason for these different types of amorphous behaviour, lies in the way in which molecular structures of different systems respond to changes in temperature. The important factor in investigating the molecular mobilities of these systems was molecular relaxation times that represent the average time taken for a single molecular motion of a particular type to occur and is calculated from enthalpy relaxation data. They further predicted both sucrose and indomethacin to be 'fragile glass formers' due to their relatively large ΔC_p values at T_g with sucrose being slightly more fragile. They also stated that such was the dependence of molecular mobility on T_g for the system, that the experimental temperature had to be lowered to at least 50K below the experimental T_g before the molecular motions detected by DSC could be considered negligible over the lifetime of a typical pharmaceutical product.

The T_g usually becomes a critical parameter when it is approached or exceeded by the temperature encountered by the product during processing (eg. drying temperature and storage temperatures). Hence the need for an adequate understanding of processing conditions and material performance characteristics.

1.6 WATER AND AMORPHOUS SOLIDS

Amorphous solids take up considerably more water than the crystalline form of the same chemical entity (Pikal *et al*, 1978). The reason for this is because of the disordered state of the amorphous solid it is possible for water to dissolve in the solid. This can be seen in Figure 1.4, which shows a schematic view of the effect of water on a disordered structure.

Therefore, in contrast to crystalline materials where water uptake is referred to as adsorption, where the amount of water taken up depends on the available surface area, uptake by amorphous solids is predominantly determined by the total mass of the amorphous solid. Adsorbed water can be adsorbed or desorbed reproducibly depending on the temperature and relative humidity of the environment and this type of water normally means 1 - 3 molecular layers which would have little consequence for any effects on the properties of the material (Konty *et al*, 1987). It is also possible for water to exist in other states or locations (apart from surface adsorption) in crystalline materials. Capillary condensation (which can occur with any solid possessing a highly porous structure with very small pores) and deliquescence (which occurs with water-soluble materials when the relative humidity of the atmosphere exceeds the relative humidity of a saturated salt solution of the solid) are processes that can produce liquid water in a solid. Adsorbed water on a crystalline particle will not affect the solid to any great extent before condensed water is present at the particle surface or deliquescence occurs.

Water interactions with amorphous solids of pharmaceutical interest such as starches, celluloses and proteins, however, are characterised by significant uptake into the solid structure far in excess of what would be predicted for adsorption by a crystalline material. This large water uptake is termed absorption or 'sorption' to differentiate it from adsorption (Zografi, 1988). In such cases there is usually a significant hysteresis between sorption and desorption. As mentioned, water is assumed to be taken up, by dissolving into the solid or by a simultaneous filling up of 'micropores' in the glassy amorphous pores of the solid. Absorption of water into amorphous regions of a solid therefore is predominantly determined by the total mass of the solid material instead of the specific surface area.

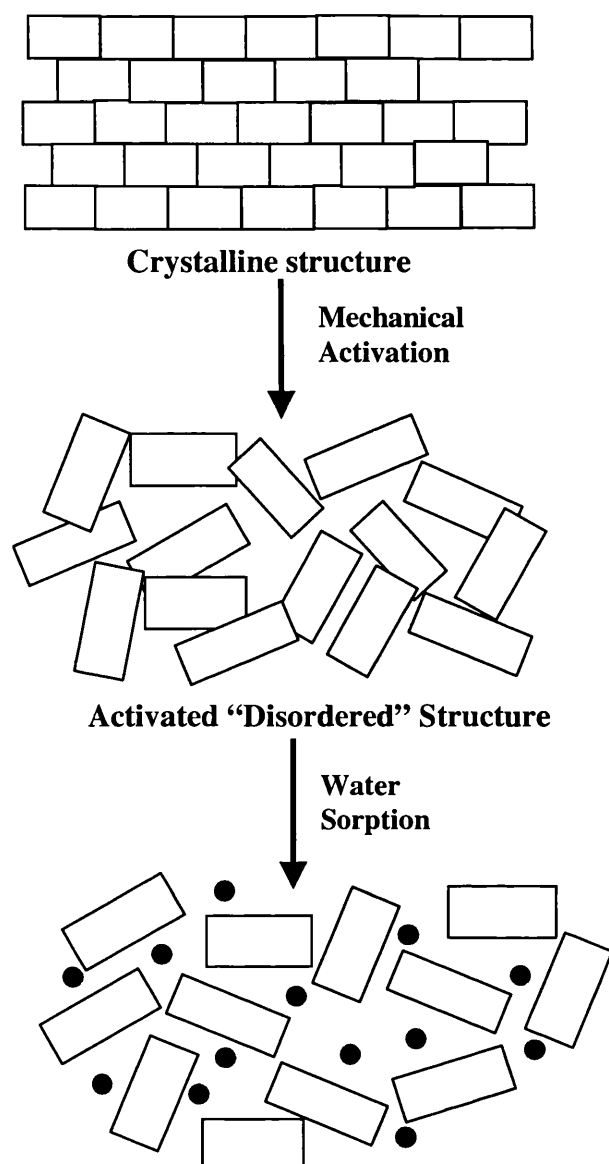


Figure 1.4 Schematic representation of the effect of moisture sorption on an amorphous solid (reproduced from Ahlneck, A., *Industrial Aspects of Pharmacy* 1993).

When water molecules are dissolved in an amorphous solid, these polar molecules increase the free volume of the solid by rupture or breakage of hydrogen bonds between solid molecules. It has been well established that the addition of one or more amorphous materials to an amorphous solid can cause a change in the T_g of that solid by its ability to dissolve in the solid and thereby affect the overall free volume. This in turn changes the value of $T - T_g$ for any system at constant T and alters molecular mobility.

1.6.1 WATER AS A PLASTICISER

When a substance lowers the glass transition temperature of an amorphous solid it is referred to as a plasticiser (Hancock and Zografi, 1993). Water molecules dissolved in an amorphous solid can act as a plasticiser thereby lowering T_g. This is illustrated in Figure 1.5 which shows a schematic representation of this change in T_g for a typical amorphous solid having very high water solubility and a high T_g in the dry state. Thus, water with its very low T_g (-134°C), its H-bonding ability and its small molecular size increasingly and continually reduces the T_g of the solid system as its concentration in the solid increases (Hancock and Zografi, 1993). Therefore, the solid state of an amorphous material can change from the glassy state to a rubbery state due to temperature increase above T_g or to changes in the water content depressing the T_g below the operating temperature of the solid. An equation has been developed using a mixing rule, which can be used to estimate the effect of adding a given amount of one substance to another is a modified Gordon Taylor equation (Gordon and Taylor, 1952) which is based on free volume theories.

For a 2 component mixture the equation used is as follows:

$$T_{g_{mix}} = (w_1 T_{g1} + k w_2 T_{g2}) / (w_1 + k w_2) \quad \text{.....equation 1.5}$$

where T_{g_{mix}} for a system containing water and an amorphous solid, is the glass transition temperature of the mixture and T_{g1} and T_{g2} are the glass transition temperatures of the water and an amorphous solid material and w₁ and w₂ are the weight fractions of water and the solid respectively. In addition, *k* is a constant defined as:

$$k = \rho_1 T_{g1} / \rho_2 T_{g2} \quad \text{.....equation 1.6}$$

In equation 1.6 ρ_1 and ρ_2 are the densities of water and the amorphous solid, respectively. *k* is a measure of the relative free volume contributed to the mixture by each of the components at any temperature and its presence in equation 1.6 reflects the importance that free volume and therefore molecular mobility have on T_g.

Thus, the mixture of the 2 amorphous components if kept below the T_g line in Figure 1.5, will remain as an extremely viscous immobilised glassy solution where water molecules in this highly immobilised state behave as if they were in a tightly bound state. If however, the temperature of the system is allowed to go above this line, a significantly less viscous rubbery state will be formed with greatly enhanced molecular mobility of both the solid and water. Hancock and Zografi (1994) and Oksanen and Zografi (1990) both carried out studies on the relationship between T_g and water content (vapour sorption) by amorphous pharmaceuticals.

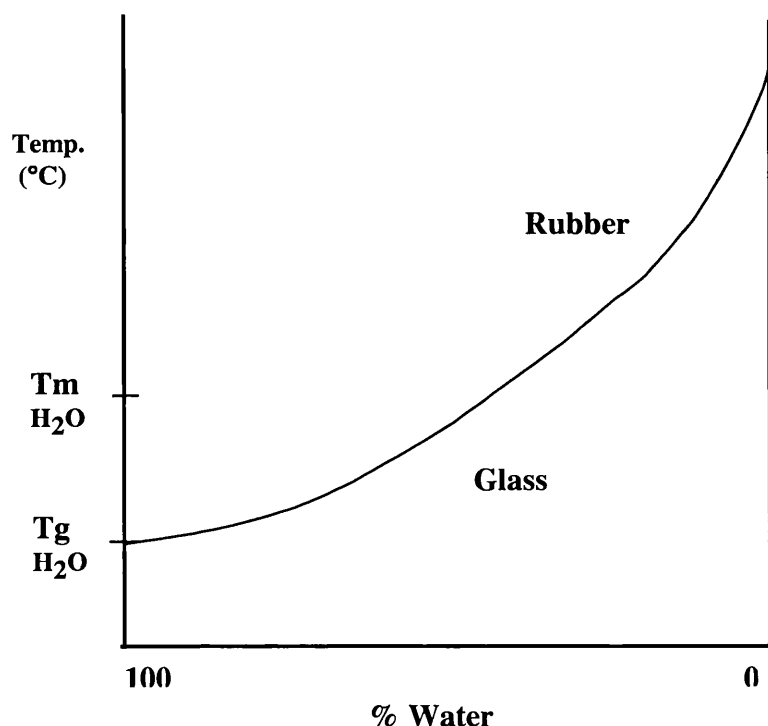


Figure 1.5 Solute-water state diagram that illustrates the effect of water plasticisation and its effect on T_g (adapted from Ahlneck and Zografi, *Int.J.Pharm.*, 62 (1990)).

Oksanen and Zografi (1990) observed that a critical water content existed at which a glassy macromolecular material is sufficiently plasticised by a low molecular weight penetrant, e.g. water, that it transforms to a rubbery amorphous solid under ambient conditions. They further observed and stated that rubbery amorphous materials usually 'sorb' considerably greater quantities of vapour than their respective glasses due to differences in morphology, and that this phenomenon can be used to identify the state

of an amorphous material. This concept and its importance in terms of product stability is illustrated in Figure 1.6 where a plot of T_g versus relative humidity exposed to the solid is given for the amorphous polymer PVP (reproduced from Oksanen and Zografi (1990)).

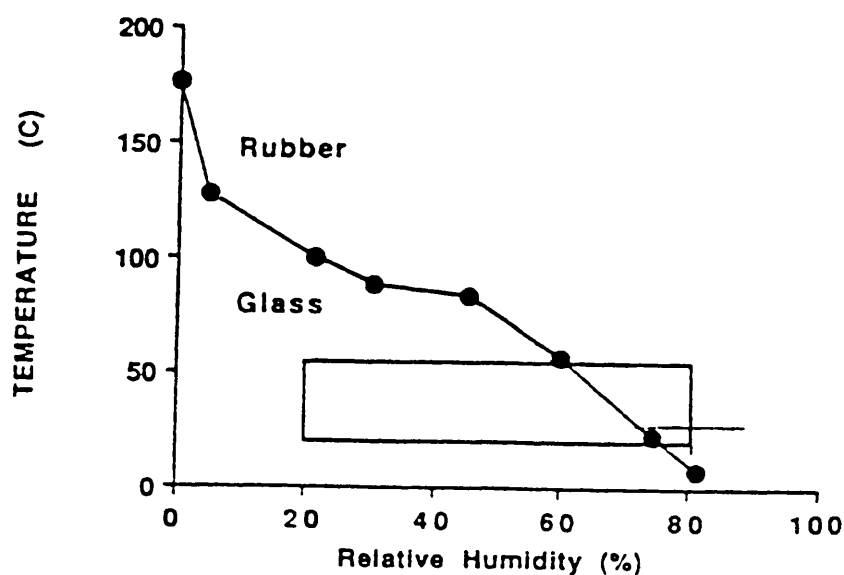


Figure 1.6 Effect of relative humidity on the glass transition temperature of PVP-K30 (reproduced from Oksanen, C.A. and Zografi, G., *Pharm. Res.*, 7 (1990)).

This graph shows that a sample of PVP stored at 25°C and 80%RH would have its T_g decreased to approximately 10°C and hence would have been converted from a glassy to a rubbery state. At 40°C such a conversion would only require storage at about 65%RH. This indicates the importance of knowing the state of the ingredients, both drug and excipients, in a formulation. Along with T_g of the drug being very important,

so also is the presence of water in an excipient as the resulting molecular mobility may be increased if the rubbery state is reached. Therefore, the excipient is potentially a source of water thus affecting the stability of the drug substance. This concept is of particular interest to us since the way in which held and water mobility in the amorphous structure of lactose is a main objective of this project. As mentioned earlier, the amount of water required to lower T_g to T can be calculated using the Gordon-Taylor equation, therefore, it is possible at any T to identify this critical water content that is required to convert an amorphous solid from the glassy to the rubbery state. It is also possible from this equation to predict what $T_{g_{mix}}$ will be, at any particular water content, thereby giving a good indication of the stability of the system.

Therefore, each point on a sorption isotherm (which depicts the relation between %RH and equilibrium moisture content for a substance), reflects the physical state of both the water and the solid and how each have influenced one another under a given set of conditions. Also, although the operating temperature (T) of the experiment might be constant, the difference between this temperature and the T_g ($T - T_g$) will be different for different amounts of absorption and consideration must be given to contributions of the solid to water activity in such a system. Hence along with thermodynamic considerations the kinetic effects of the solid on water are contributing to the behaviour of the system, with the molecular mobility of the system and hence the diffusion of water molecules changing accordingly.

Roos (1993) carried out a study investigating melting and T_g of low molecular weight carbohydrates of varying water content using DSC. The Gordon-Taylor equation was used to predict T_g from the varying water contents thus enabling an evaluation of the physical state of the materials studied. Bell (1995) investigated if it was possible to determine T_g from moisture sorption isotherms, using a hydrophilic polymeric system (PVP) of different molecular weights and various additives. As expected, decreasing molecular weights or addition of plasticisers reduced T_g of the system. However, these studies suggested that a more extensive knowledge of the systems properties was needed in order to extract T_g from these isotherms.

1.7 COLLAPSE OF THE AMORPHOUS STRUCTURE

1.7.1 LOSS OF POROSITY

The idea of an amorphous material changing from the glassy to the rubbery state at a characteristic temperature (T_g), based on certain properties and characteristics of the material e.g. moisture content and experimental temperature at any time, is now well established. It is also accepted that the molecular mobility and viscosity change greatly at this temperature. Furthermore, due to numerous studies and observations of changes and defects in amorphous glasses especially carbohydrates such as proteins and sugars, these structural changes were stated to be the result of the same basic phenomena (time, temperature and moisture dependent viscous flow) (Tsourouflis et al, 1976, White and Cakebread, 1966). In the food industry especially, these structural changes (loss in structure) in amorphous products have been referred to as collapse.

The concept of collapse on the premise of the above changes has been described as the result of structural relaxation in a material. Furthermore, Levine and Slade (1986) stated that this structural relaxation represents the “manifestations of an underlying molecular transformation from kinetically metastable amorphous solid to unstable amorphous liquid, which occurs at T_g ”. The critical effect of plasticisation by water on T_g , and a subsequent knowledge of $T-T_g$, is a central element to the concept and the mechanisms which have been proposed to explain it. White and Cakebread (1966) in discussing the glassy state in boiled sweets containing sucrose and glucose mixes, stated that collapse was caused by 3 main factors:

- (1) a low content of solute in a freeze-dried mixture
- (2) high residual moisture or
- (3) high storage temperature.

It was stated that these factors cause collapse by decreasing the viscosity, in the first 2 cases due to T_g being lowered below T and in the third one due to T being raised above T_g . Roy et al (1991) stated that the tendency of a freeze-dried amorphous antibody system to collapse could be minimised by the presence of low concentrations

of salts and excipients such as mannitol, presumably due to their ability to absorb moisture.

Collapse, therefore, could be described as the macroscopic manifestations of a glass transition in an amorphous material (e.g. a freeze-dried sample) where the subsequent viscous flow occurs over a time frame so that it is visible. The collapsed sample resembles a highly viscous glassy material compared to the pre-collapsed appearance which is that of a porous solid, and collapse therefore represents a loss of porosity as the amorphous material is unable to support its own weight under gravity. This can be seen from Figure 1.7 where the particle can be seen to shrink and lose its porous appearance with increasing temperature. Flink (1983) stated that although it is obvious that collapse and T_g are closely related, however, an important difference exists between these two phenomena in that T_g is reversible while collapse is not.

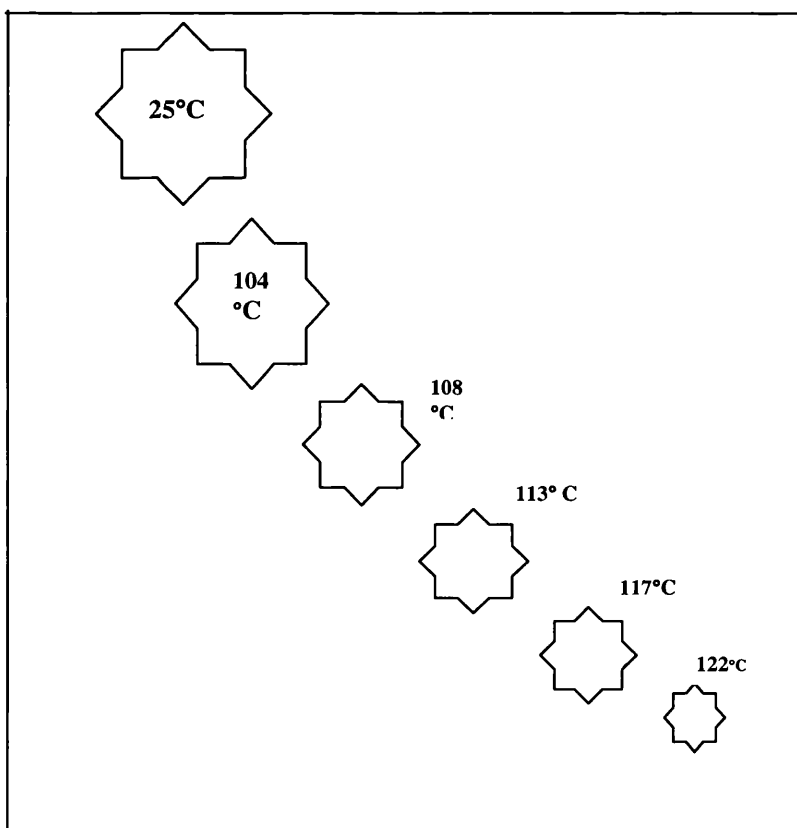


Figure 1.7 Area of an amorphous freeze dried maltose particle at various stages of collapse, on increasing temperature (reproduced from To, E.C. and Flink, J.M., *J. Fd. Technol.*, **13** (1978)).

1.7.2 A GENERAL PHYSIOCHEMICAL MECHANISM.

Levine and Slade (1986) proposed a generalised mechanism for collapse based on the occurrence of a critical structural relaxation at T_g , followed by viscous flow in the rubbery liquid state. The mechanism is derived from the WLF free volume theory mentioned earlier for amorphous polymers and leads to the fundamental equivalence of T_g and collapse temperature. This states that as T rises above T_g or as T_g falls below T due to water plasticisation of an amorphous material, polymer free volume increases. Due to decreased viscosity the glass to rubber transition occurs, permitting viscous flow. In this rubbery state translational diffusion can occur in practical time frames and diffusion-controlled relaxations occur, with time and %moisture being the influencing variables. The % water content is the critical determinant of collapse and the resulting changes occurring, through the effect of water on T_g . Franks (1982) stated that whenever T_g and the resultant collapse phenomena share a common time frame, T_g equals the minimum onset temperature for all collapse-related phenomena e.g. caking, stickiness.

White and Cakebread (1966) further observed that in many cases crystallisation of the collapsed amorphous substances occurred. To and Flink (1978b) found that in the absence of deliberate rehumidification, crystallisation of some freeze-dried carbohydrates was found to occur by heating past collapse and holding at these elevated temperatures. To and Flink (1978b) also stated that there may also be moisture content requirements that must be met if collapsed amorphous carbohydrates are to undergo crystallisation. Thus, heating of a carbohydrate that forms the monohydrate crystal in the absence of water can cause collapse without subsequent crystallisation. Hence, any further changes in moisture content and / or temperature will almost inevitably result in crystallisation of the collapsed structure. It can be seen that the amorphous solid carbohydrate is a metastable structure and that through the action of temperature and moisture this metastable state will ^{convert} to the stable crystalline form as was observed by To and Flink (1978b).

1.8 CRYSTALLISATION: THE AMORPHOUS TO CRYSTALLINE TRANSITION.

Levine and Slade (1986) stated that collapse could be prevented by keeping $T < T_g$, and formulation to increase T_g to a temperature above processing or storage temperature. However, once either of these criteria is met i.e. if proper precautions aren't taken during handling and storage subsequent crystallisation can occur due to increased molecular mobility. Makeover and Dye (1956) observed that during crystallisation water which was present in the amorphous regions is expelled from the solid. This water loss occurred at a specific relative humidity, again indicating that once a critical water content has been achieved crystallisation will occur. Once the metastable amorphous material converts to the stable crystalline state the surface energy is lowered, therefore crystallisation is considered to be a deactivation process.

Furthermore, such crystallisation can be responsible for post compression hardness of tablets (Sebhatu et al 1994, 1997) and loss of volatile components (Flink 1975). Puri et al (1996) reported also on the influence of moisture on the physical and chemical stability of spray-dried protein formulations containing trehalose. After storage at a range of relative humidities from 0 – 75% at 50°C for 10 days, the formation of soluble and insoluble aggregates were observed with the degree of aggregation higher at high RHs. Ward and Schultz (1995) and Leung and Schultz (1996) stated that crystallisation of micronised salbutamol sulphate and terbutaline sulphate can cause an increase in particle size due to particle aggregation, which in turn affects their performance in dry powder inhalers. Consequently, knowledge of the conditions favourable for crystallisation from the amorphous state, is of the utmost importance in ensuring and maintaining both chemical and physical stability of such systems.

1.8.1 NUCLEATION AND CRYSTAL GROWTH

Hancock and Zografi (1997) stated that crystallisation from the amorphous state is primarily governed by the same factors that determine crystallisation from the melt, i.e. nucleation and crystal growth. Makeover and Dye (1956) studied the crystallisation of spray-dried sucrose and glucose occurring during storage at various relative humidities and noted that at high humidities the absorbed water initiated

crystallisation of the sugars with subsequent release of moisture to yield essentially anhydrous materials. The course and rate of crystallisation was evaluated by Makeover and Dye (1956), from changes in moisture content and was found to follow an exponential law with respect to time, after an initial induction period. This period was interpreted to be the time for a build up of sufficient nuclei to initiate an appreciable rate of crystallisation.

Saleki-Gerhardt and Zografi (1994) stated that crystallisation from the amorphous state is primarily controlled by the rate of nucleation. Of particular interest in their work was the effect of sorbed water on T_g and their aim was to be able to propose a model for nucleation-controlled crystallisation from the amorphous state which would ultimately predict under what conditions (water content and temperature) crystallisation would occur. Nucleation, they stated, is affected by 2 opposing factors i.e. as temperature is reduced below the melting temperature, T_m , and hence supercooling is increased the nucleation rate would be expected to increase significantly. However, with such a decrease in temperature a significant increase in viscosity would be expected and therefore a marked reduction in diffusion and molecular motion in general. This is illustrated in Figure 1.8, which shows how the affect of changing the nucleation rate due to supercooling below T_m is balanced by the loss in molecular motion as temperature is brought closer to T_g . This is the point where such motion is assumed to approach very small values to give a crystallisation temperature (T_c) and maximum crystallisation rate which is between T_g and T_m .

1.8.2 THE EFFECT OF ADDITIVES ON NUCLEATION AND CRYSTAL GROWTH.

Islesias and Chirife (1978) studied aspects of sucrose crystallisation in humified freeze dried systems when other food components (e.g. carboxymethylcellulose and microcrystalline cellulose) were present. The study showed that the presence of these components greatly reduced the apparent rate of crystallisation of sucrose. This was attributed to interactions with the other components, as well as to the increased viscosity of the medium, which reduced the mobility of the sucrose molecules leading to its transformation to the crystalline state.

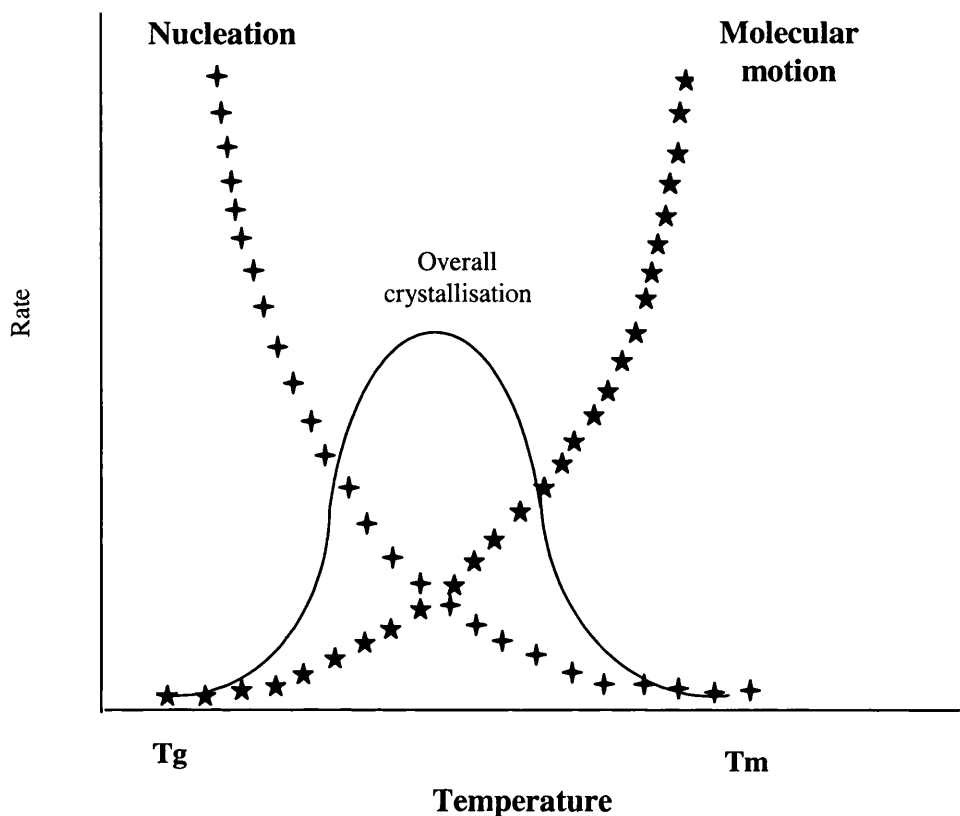


Figure 1.8 Schematic representation of the rates of parameters controlling crystallisation from the amorphous state above T_g and below T_m (reproduced from Saleki-Gerhardt, A. and Zografi, G., *Pharm. Res.*, **11** (1994)).

Van Scoik and Carstensen (1990) and Saleki-Gerhardt and Zografi (1994) also carried out studies on crystallisation of amorphous sucrose systems. Van Scoik and Carstensen paid particular attention to nucleation phenomena and investigated the effects of additives, temperatures and relative humidity on the inhibition or acceleration of nucleation with the view that if nucleation could be inhibited then the stability of an amorphous system could be increased. In the system that was studied it was found that once nucleation occurred crystallisation was rapid, and the effect of the presence of additives such as lactose was to double the time delay before nucleation occurred. One of the mechanisms thought responsible for inhibition of nucleation was a mass transfer step, whereby hydrophilic materials such as raffinose having a strong tendency to H-bond with any available water, would thereby impede the movement

and collisions of sucrose molecules necessary to build up the critical nucleus. Another inhibiting mechanism was suggested to be the ability of some substances to prevent the molecules of the crystalline material from approaching the growing nucleus or crystal lattice in the proper orientation.

Saleki-Gerhardt and Zografi (1994) also stated that crystal growth can be inhibited by the presence of a second component due to mass transfer rates or competitive adsorption at the nucleating or crystal growth sites. Any additive causing an increase in T_g would be expected to produce a corresponding increase in viscosity and reduction in molecular motion. The induction time i.e. the time required for crystallisation to commence which in the present discussion would be due to plasticising effects of water, which is assumed to be mostly affected by rates of nucleation is therefore greatly affected by the presence of additives. The general conclusion from these studies is that crystallisation can be prevented by keeping T (operating temperature) below T_g and by reducing water content or by raising T_g of the system using additives with high T_g values.

1.8.3 'SEEDING' EFFECTS ON CRYSTALLISATION

Important also in the discussion of nucleation and crystal growth is the idea that some initial defects are necessary for growth to occur and that these defects are formed early during nucleation. Furthermore, it is accepted that just as in the case of crystallisation from solution surfaces with defects grow faster than surfaces without defects. Ahlneck (1993) stated different crystal forms can be grown by the introduction of additives that show different pharmaceutical properties due to differences at the crystal faces. The idea of seeding during crystallisation involves adding another species (e.g. crystals of the desired crystalline product) in order to stimulate nucleation and crystal growth to produce a particular form of the material being studied.

Van Scoik and Carstensen (1990) noted that insoluble additives have been used as seeds to promote nucleation in various crystallisation operations. The solid surface, they postulated, is thought to act as a heterogeneity, which decreases the free energy necessary for formation of critical nuclei. Yoshioka et al (1994) studied the effect of isothermal crystallisation of 2 different amorphous forms of indomethacin in the

presence of 2 different seed crystals, at different temperatures. Considerable differences were observed in the crystal products obtained i.e. proportions of the forms varied depending on the temperature and seed present, with the crystallisation of one form being inhibited at the higher temperature by the presence of the other seed crystal. Thus, it appears that amorphous material may be responsive to some type of surface nucleation and growth that selectively favours one crystal form over another.

1.9 PROCESSING OF PHARMACEUTICAL SOLIDS LEADING TO MOLECULAR DISORDER IN CRYSTALLINE MATERIALS.

Formulation of a drug in the solid state is a procedure where the drug substance is subjected to a variety of different processes to ensure maximum efficacy, performance and stability of the product. These include comminution to provide a larger surface area in order to increase the rate of dissolution and hence the bioavailability of the drug, or to reduce the particle size to ensure dosage uniformity as in the case of aerosol inhalation systems. There are many other processes including heat drying, spray drying and lyophilization, which have been reported to cause disruption of the crystalline structure leading to various degrees of disorder. Powder particles therefore may have a totally disordered structure i.e. are totally amorphous, or the disorder may be located on the surface of the particle. Processing of powders therefore will often alter the form of the surface to some extent. The significance of the change will relate to the amount of disruption caused and the rate at which the material recovers from the disruption. The greater the degree of disruption then the greater will be the change in the physical properties of the material. Consequently, detecting and quantifying the degree of surface disorder in crystalline materials is a very important issue in the chemical and physical stability of pharmaceutical materials.

1.9.1 PHARMACEUTICAL PROCESSES LEADING TO CRYSTAL DISRUPTIONS AND MODIFICATIONS.

There has been considerable interest and attention paid to changes in crystallinity and its effects on certain properties and the chemical stability of many drugs and excipients. Numerous studies have indicated modifications to crystalline drugs on physical manipulation, including digoxin (Florence and Salole, 1976; Black and

Lovering, 1971; Chiou and Kyle, 1979), digitoxin (Chiou and Kyle, 1979), cephalothin sodium (Otsuka and Kaneniwa, 1990), griseofulvin (Elamin et al, 1994; 1995), ampicillin trihydrate (Takahesi et al, 1984), albuterol sulfate (Ward and Schultz, 1995) and aspirin (Buckton et al, 1988). Changes in the crystalline nature of excipients such as lactose (Lerk et al, 1984; Huttenrauch and Keiner, 1979) and sucrose (Saleki-Gerhardt, 1994) have also been widely reported. A material can therefore have disorder throughout the entire particle i.e. a totally amorphous particle, or the disorder may only be present at and affect the particle surface regions, whereby the mobility of molecules at the particle surface is enhanced. This of course will have a profound effect on the reactivity of the substance since many particle characteristics depend on the surface structure and state e.g. adsorption, adhesion, flow, dissolution, compressibility, compactibility etc.

1.9.1.1 Comminution

Mechanical activation such as comminution results in a high degree of activation of pharmaceutical materials due to either endothermic (formation and deformation) or exothermic (bonding which is a heat releasing event) processes occurring. During size reduction the total surface area will increase, which is often the object with a comminution process, to improve the bioavailability of the substance. Particles undergoing size reduction are subjected to stress and are thereby strained and deformed (Ahlneck, 1993). When the application of a force leads to deformation cracks in particles can arise. These cracks will arise locally at specific points because the particle surface is normally irregular. After completion of the comminution process e.g. milling, cracks and defects propagated during the size reduction will remain. Thus local points or 'hot spots' at the surface of the particles storing energy are created. These changes in the crystalline material during or after processing have manifested themselves significantly in product performance and characteristics such as solubility and dissolution, chemical stability and tablet compression.

Numerous studies have shown the effect of comminution i.e. grinding, milling and micronising, in affecting crystalline structures. These include Lerk et al (1984), Waltersson and Lundgren (1985), Otsuka and Kaneniwa (1990) Mosharraf and Nystrom (1996) and Forni et al (1988). While some of these studies reported on the transformation from one polymorphic form to another, e.g. Forni et al found that

grinding of 2 polymorphic forms A and B resulted in turning form A into form B, most of these studies have reported the formation of amorphous material after processing.

Lerk et al (1984) found that α lactose monohydrate lost its water of crystallisation and like β lactose changes into an amorphous state on intensive grinding. Otsuka and Kaneniwa (1990) found that the crystallinity of cephalothin sodium fell to as low as 30% after 2 hours milling and then recovered slightly to plateau in the region of 50% amorphous after longer milling times. Takahashi et al (1984) reported that % crystallinity of ampicillin trihydrate decreased from 100 – 77% after 3 hours grinding as measured by X-ray diffraction. Saleki-Gerhardt et al (1994) was able to produce sucrose samples with different degrees of disorder by varying milling times. It is clearly evident that particle size reduction may lead to fundamental changes in the properties of a solid material.

However, it has proved extremely difficult to define the degree of disruption induced during processing or to accurately quantify it. Buckton et al (1988) investigated different comminution techniques on the surface energy of a model drug aspirin. The interaction of water vapour with drug samples which had been exposed to different milling processes was investigated. It was found that milling altered surface energetics (different enthalpies of adsorption of water vapour on solids were observed) according to how energetic the milling process was. Furthermore, powders milled by 2 consecutive processes were found to retain the surface energetics imparted by the first process. It was suggested that the wetting process was controlled by the degree of disorder at the powder surface which was due to the milling process. As stated earlier, comminution is a high-energy technique, consequently, after milling cracks and defects propagated during the size reduction will remain.

1.9.1.2 Heating, Lyophilization (Freeze-drying) And Mixing.

The production of disorder in crystalline materials on heating has been reported by Huttenrauch and Keiner (1979) who found that progressive drying (under vacuum at 125 – 126 °C) of α lactose monohydrate, resulted in a continuous decrease in the degree of order. It was concluded that heat-drying operations can therefore induce crystal defects. The production of amorphous material from spray-drying has been

widely reported such as in the production of partially amorphous lactose materials for use in direct compression tableting. Pikal et al (1978) showed how lyophilization of β lactam antibiotics produced disorder in the crystalline material. Mixing has also been reported to cause disruption of the crystalline structure as shown by a study carried out by Konno (1990), who used reduced pressure mixing of an organic crystalline drug with an adsorbant to readily produce an amorphous system. The temperature, pressure and rotation speed of the mixer all affected the degree of crystallinity of the product.

It is apparent therefore that due to various processing and storage conditions, pharmaceutical solids can undergo significant phase transformations from a crystalline form to a disordered form (activation), whereby the extent of activation will depend on the energy input during processing.

1.9.1.3 Disruption Index.

Changes in crystal properties, such as habit, density, energy, entropy are affected by changes in the concentration or density of crystal defects and these may be induced by 'impurities' present during crystallisation. The disruptive influence of an additive or impurity may be quantified by the entropy, and York and Grant (1985, 1986) introduced the concept of entropy of processing as a means of comparing the levels of solid state disorder of pharmaceutical materials. These authors proposed a dimensionless disruption index (d.i.) for quantifying the disruptive influence of an additive or impurity when present in solid solution in the crystal lattice of a host substance. They defined the entropy of processing (ΔS^P) as the difference between the entropy of the solid substance under investigation and the entropy of the same amount of a reference sample.

Consequently

$$\Delta S^P_{\text{solid}} = -\Delta S^f_{\text{solid}} + \Delta S^f_D \quad \text{equation.....1.7}$$

where $\Delta S^P_{\text{solid}}$ is the entropy of processing of the solid under study. ΔS^f_D is the entropy of fusion of the sample and ΔS^f_D is the entropy of fusion of the reference substance D.

The entropies of fusion may be obtained from DSC and DTA using the relationship

$$\Delta S^f = \Delta H^f / T_m \quad \text{.....equation 1.8}$$

and $\Delta S^p_{\text{solid}}$ may be determined from solution calorimetry. The d.i. was then given by

$$\text{d.i.} = -\delta(\Delta S^f) / \delta(\Delta S^m_{\text{ideal}}) \quad \text{.....equation 1.9}$$

or

$$\text{d.i.} = -\delta(\Delta S^s) / \delta(\Delta S^m_{\text{ideal}}) \quad \text{.....equation 1.10}$$

where ΔS^f is the entropy of fusion and ΔS^s is the entropy of solution and $\Delta S^m_{\text{ideal}}$ is the ideal entropy of mixing. Arbitrarily choosing as reference sample a highly crystalline sample of the pure drug D these authors analysed a range of published data and estimated entropies of processing. Among the data examined were solution calorimetry results which had been determined using spray dried and freeze dried antibiotics (Pikal et al, 1978). Values for the spray dried antibiotics (in terms of the enthalpy $\Delta S^p_{\text{solid}} / T$) ranged from 173 to 1.1 ($\text{J K}^{-1} \text{mol}^{-1}$), while higher values were obtained in all cases when freeze drying was the drying process employed (185-78) ($\text{J K}^{-1} \text{mol}^{-1}$). It is evident from the results that the method could quantify differences between samples prepared by two drying methods and also different spray dried samples of the same material (cephalothin sodium).

It was also suggested from these studies that dehydration (i.e. removal of hydrate water from a crystalline material) can be considered to be equivalent to the formation of a solid solution of vacancy point defects, each resulting from the loss of a water molecule. Since each vacancy is equivalent to a single anhydrate molecule such a system was said to be equivalent to doping the hydrate form with anhydrate molecules. Loss of moisture therefore from crystal structures was said to produce small amounts of disorder in the system. It was postulated that d.i. may be useful in accounting for and predicting certain solid batch-to-batch variations since virtually all the pharmaceutically significant properties of a solid drug depend to some extent on the lattice disorder, crystallinity and the concentration of crystal defects.

1.9.2 ADVANTAGEOUS PROPERTIES OF DISORDERED PHARMACEUTICALS.

The formation of disordered regions in a solid produces areas that are in a higher energy state than that of the crystal. Although in some cases processing induced disorder and crystal modifications are manifest only at surfaces and localized 'hot spots' nevertheless these surface changes have been shown to have far reaching formulation, performance and processing effects. They are known to affect the manufacture of dosage forms and the bioavailability of drugs. Increased surface energy and / or formation of amorphous states on milling with resulting higher dissolution rates have been widely reported (Chiou and Kyle, 1979, Burt and Mitchell, 1981, Mosharraf and Nystrom, 1996, Hendriksen, 1990 and Florence and Salole, 1976). Florence and Salole found that milled samples of digoxin had a dissolution rate 2.6 times that of the unmilled sample and concluded that these results were influenced by such parameters as particle size, agglomeration and polymorphism.

Burt and Mitchell investigated the relationship between crystal defects and dissolution and stated that dislocations are thermodynamically unstable, having localized energy, which results in an increase in the free energy and a reduction in the activation energy for dissolution at points where they emerge on the crystal face. They speculated that a crystal with a higher dislocation density should have a higher thermodynamic activity which may result in a greater overall dissolution rate. They also stated that crystal defects determine the strength of cohesive forces between atoms and molecules and hence will also affect dissolution rates. Burt and Mitchell (1981) found in their study a positive correlation between dissolution rate and content of dislocations in crystals. Elamin et al (1994) reported that milling of griseofulvin (a hydrophobic drug) particles produced an increased metastable solubility, which was thought to correspond to the solubility of an activated state. Material bulk properties as characterised by DSC, and individual primary particle size or surface area values did not appear to have been affected by the milling process. However, an increase in the free energy along with a decrease in the enthalpy of solution of the milled griseofulvin was observed, which suggested that the increase in solubility was due to disordering of the solid structure. Mosharraf et al (1996), on the other hand, investigated the importance of disordered structure in determining the solubility and dissolution rate of a number of hydrophilic drugs of low solubility, where overcoming the strong

intermolecular forces seemed to be the main dissolution barrier. The solubility was reported to increase dramatically as a function of the amount of disordered structure present.

Another advantageous property of disordered material is the increased tablet strength, which has been reported by Vromans et al (1986) and Sebhatu et al (1994; 1997) for partially amorphous systems. This increased tablet strength during storage has been reported for systems containing partially amorphous lactose (15%) at low /medium relative humidities. Sebhatu et al (1997) suggested that the increase in tablet strength of sucrose and sodium chloride tablets could be ascribed to a rearrangement of solid material at the particle surface by the action of limited amounts of sorbed water. Increased tablet strength was attributed to crystallisation of the disordered surfaces of the mainly crystalline material, whereby solid bridges were formed. A reduced tablet porosity and an increased tablet fracture resistance with increased moisture content of the particles was also observed in this study. Elamin et al (1994) similarly reported from studies on mechanically activated sugars the transformation of the disordered surfaces to the crystalline state, resulting in significant changes in material properties such as powder surface areas, agglomeration tendencies, compactability and would potentially affect powder flow, die filling and particle size and distribution.

1.9.3 UNDESIRABLE PROPERTIES OF DISORDERED PHARMACEUTICALS.

However, undesirable properties such as greater chemical instability of various amorphous and disordered drug entities has been widely reported e.g. Pikal et al (1978), Otsuka and Kaneniwa (1990), Roy et al (1991) and Waltersson and Lundgren (1985). Furthermore, there is a tendency of disordered regions to transform from the amorphous to the crystalline state, as already described for totally amorphous systems. Consequently, numerous reports of decreased stability on storage at high temperatures and relative humidities have been reported for materials containing crystal modifications and regions of disorder. Waltersson and Ludgren (1985) reported an increasing rate of material degradation with increasing milling time at moderately elevated temperature and relative humidity. Roy et al (1991) reported that substantial degradation occurred in a freeze-dried antibody system with increasing water content, when it was stored at 25°C and 40°C. The effect of excipients on improving the

stability of the system was also investigated, however these also crystallised on exposure to moisture which released water and resulted in lowering the T_g of the antibody which resulted in it crystallising. Konno (1990) also reported decreases in dissolution behaviour when amorphous mixtures were stored under humid conditions. Elamin et al (1994) reported similar findings from solubility studies on milled griseofulvin, where a decrease from initially high metastable solubility levels to a stable low equilibrium solubility was observed. This was assumed to be due to the transformation of the disordered material to a more ordered (crystalline) form, which was suggested to be a slow process where the rate-limiting step appeared to be a solid state rearrangement process. Similarly, Mosharraf et al (1996) reported from a similar study of some hydrophilic sparingly- soluble drugs containing disordered structure, that while the solubility of the disordered fraction was the rate determining factor during the first phase, the solubility of the crystalline state was the rate determining factor during the second phase. This again suggests the solid-state transformation of the metastable disordered structures to the more stable crystalline forms.

Of major importance therefore, in relation to decreased chemical stability and the tendency to crystallise, is the ability of the amorphous material to take up large amounts of water. Consequently, deactivation from the amorphous to the crystalline state can occur where the extent of deactivation depends on whether the molecules of the solid exhibit sufficient translational and rotational molecular mobility to allow molecular rearrangements.

1.10 EFFECTS OF MOISTURE ON PARTIALLY DISORDERED SYSTEMS.

The amorphous state or defects in the crystal structure have been shown to be able to act as 'active centres' for the removal or addition of atoms to a crystal as well as sites for adsorption of gases and sorption of water vapour. Crystals are more reactive at these points and thereby able to retain a higher state of energy than a perfect crystal state.

The molecules located in such regions of local disorder have been shown to exhibit greater chemical reactivity and solubility as reported by Waltersson and Lundgren (1985), which has been attributed to their 'activated state' due to a combination of greater molecular mobility and the exposure of more reactive chemical groups. As

discussed already for totally amorphous solids these regions of greater local disorder and reactivity should exhibit an ability to take up more water than would ordinarily be adsorbed on the surface of the crystalline parts of the material. Therefore relatively small amounts of water absorbed into these 'hot spots' can produce significant increases in the molecular mobility at low temperatures due to the plasticising effect of the water. It would appear therefore that if the amount of water taken up was sufficient to plasticise the local region to a point where molecular mobility would be greatly enhanced and hence the physical stability of the system would be greatly affected. This is an important consideration in relation to processing induced disorder since this type of disorder is normally at the surface of the particle e.g. after grinding or milling.

1.10.1 AMPLIFICATION THEORY OF WATER SORPTION

Ahlneck and Zografi (1990) showed and explained this concept of water uptake in the disordered amorphous regions of a processed material on a quantitative basis. They considered the case of a totally crystalline sample which would be expected to take up (adsorption) in the region of 0.1% water and this was then compared to a sucrose sample with various percentages of amorphous structure between 0.5 and 5% of the total solid. The amount of water absorbed into the disordered amorphous regions for 0.1 and 0.5% total moisture contents was then considered assuming that essentially all of the water is preferentially taken up in the amorphous regions. Depending on the amount of amorphous material present, a considerable concentration of water would be present, especially as the amount of amorphous material becomes quite small. The effect of these 'apparently' small quantities of water on T_g of sucrose (Levine and Slade, 1988) can be seen in Table 1.1, and it could be seen that only 0.1% total moisture, if concentrated in the 1% of the mass of the solid that was amorphous, the T_g was lowered to a value approaching room temperature and higher moisture contents lowering T_g much further resulting in regions of very high molecular mobility at room temperature. As already discussed the amorphous regions will crystallise once T_g has been lowered below T and a certain level of molecular mobility has been reached. Consequently, the effects of water on the solid state stability of a partially disordered structure are extremely important. This selective tendency, therefore for water to absorb into amorphous regions of a crystalline solid is

important because it means that the 'local' water concentration is much higher than would be predicted on the basis of total weight of the sample (Alneck and Zografi, 1990).

Ward and Schultz (1995) investigated the effect of moisture uptake on salbutamol sulphate after micronizing (to obtain optimum particle size) and its effect on powder physical stability. This effect of moisture on a processed pharmaceutical solid is shown schematically in Figure 1.9, which shows how the surfaces of 2 crystalline particles have become disordered following milling. Subsequent exposure of the system to water vapour (or heat) can then lead to enhanced molecular mobility whereby free volume increases and the glass transition temperature is lowered. Once T_g is lowered below T crystallisation can occur with the metastable disordered regions rearranging into a stable crystalline structure (expelling the sorbed water) forming a bridge between the particles. Interparticle bridging or fusion can occur therefore if the particles containing surface disorder are in contact during crystallisation. Significant agglomeration of particles can in the worse case effectively cancel out particle size reduction efforts, thereby dramatically reducing the effectiveness of powder inhalation products.

Amount of moisture (%)	Amount of Amorphous material (%)	Moisture content in amorphous material (mg H ₂ O/ 100mg solid)	Glass transition Temperature (°C)
0.1	0.5	20	9
	1.0	10	27
	2.5	4	45
	5.0	2	49

Table 1.1 Moisture content in the amorphous portion of sucrose and the glass transition temperature if a total of 0.1% moisture is taken up. (Compiled from Slade and Levine (1988), assuming that the glass transition temperature of purely amorphous sucrose is 52°C).

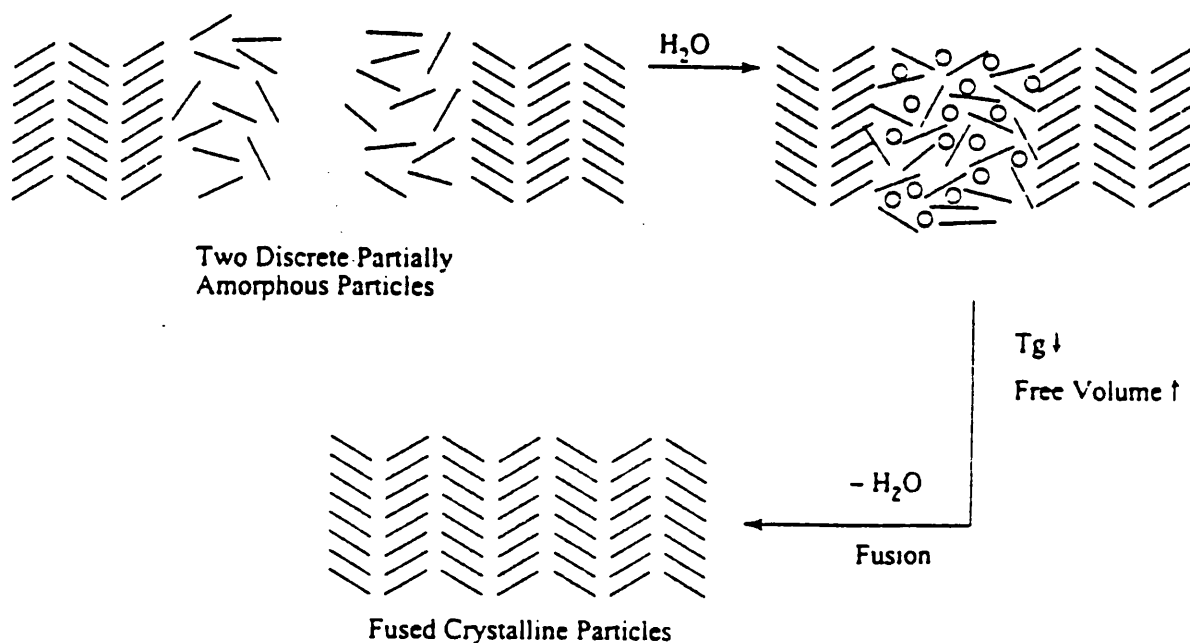


Figure 1.9 Schematic representation of the crystalline surfaces of two discrete particles after milling and the effect of water sorption which leads to crystallisation (reproduced from Ward G.H., and Schultz, R.K., *Pharm. Res.*, 12 (1995)).

It would appear therefore, that if small amounts of amorphous character are introduced into a crystalline material during processing, whether desirable or not, raising the operating temperature above T_g or exposing the sample to elevated humidity, will result in some samples changing back to the more stable crystalline form. The problem with samples containing small regions of disorder due to processing is that this type of amorphous character usually exists at the surface (as shown in Figure 1.9) at levels not easily detected. However, it has the potential to produce instability and unwanted changes in the physical and chemical properties of a system. Hence, the need for adequate methods to detect and quantify such disorder.

1.11 LACTOSE: A MODEL MATERIAL TO INVESTIGATE AMORPHOUS BEHAVIOUR.

1.11.1 APPLICATIONS IN PHARMACEUTICAL FORMULATION AND TECHNOLOGY.

Lactose has become an important excipient in the pharmaceutical industry where it is widely used as a filler or diluent in tablets and capsules and to a more limited extent in lyophilized products and infant feed formulas. Spray dried lactose was first developed over 50 years ago for use in solid dosage forms. Many other lactose grades and forms exist and are commercially available including anhydrous α lactose, α lactose monohydrate and anhydrous β lactose. Direct compression grades of lactose (which can be used in tablets without granulating) are more fluid and more compressible than crystalline or powdered lactose and are generally composed of spray-dried lactoses which contain specially prepared pure α lactose monohydrate along with a quantity of amorphous lactose. The use of direct compression grades of lactose results in tablets of higher breaking strength than standard lactose. Lactose is also used as a carrier or diluent for inhalation products and in lyophilized products, where lactose is added to freeze-dried solutions to increase plug size and aid in caking. Lactose is also used in combination with sucrose to prepare sugar coating solutions.

1.11.2 DESCRIPTION AND CHARACTERISTICS.

Lactose is a whitish odourless slightly sweet tasting powder also known as milk sugar. It occurs in mammals milk at levels between 1 and 7% and is the disaccharide of glucose and galactose as shown from its structural formula in Figure 1.10. It exists naturally in 2 isomeric forms α and β lactose which differ in the orientation of a $-H$ and $-OH$, shown in Figure 1.10. Its empirical formula is $C_{12}H_{22}O_{11}$ (anhydrous lactose) which has a molecular weight of 342.3 and $C_{12}H_{22}O_{11} \cdot H_2O$ for the monohydrate form which has a molecular weight of 360.31. Commercially, lactose is produced from the whey of cow's milk, whey being the residual liquid of the milk following cheese and casein production.

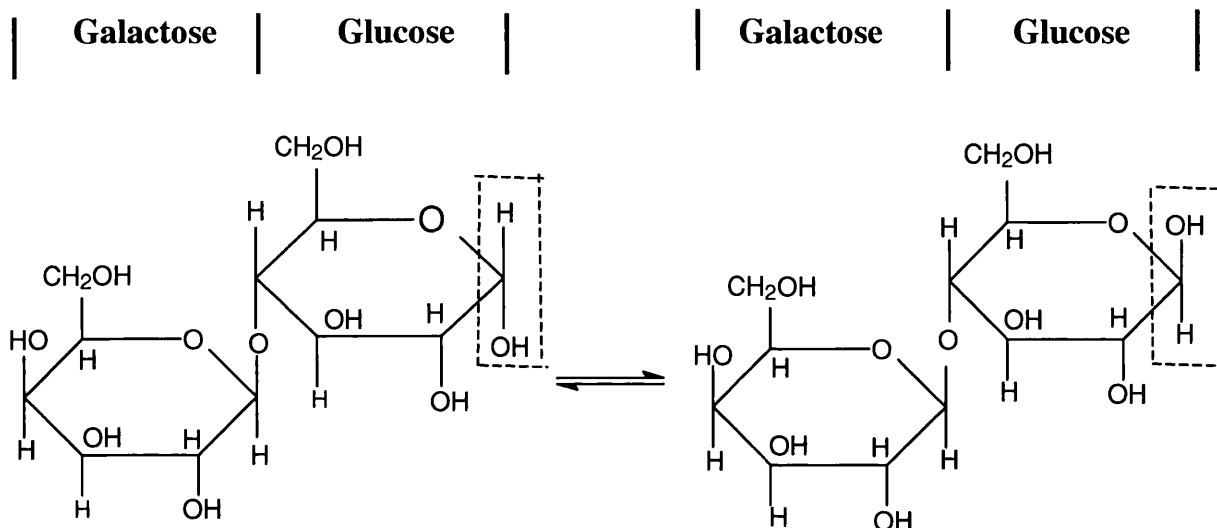
α lactose β lactose

Figure 1.10 Lactose, the disaccharide of glucose and galactose, showing the 2 isomeric forms α and β lactose.

The manufacturing procedure involves crystallisation from the concentrated whey as outlined in Figure 1.11. The α lactose monohydrate is obtained by crystallisation from a supersaturated solution below 93 °C, whereby the unstable α anhydrate absorbs one molecule of water per lactose molecule. α lactose is available primarily as the monohydrate but 2 anhydrous forms exist, stable and unstable anhydrous α lactose. A very hygroscopic product generally called unstable α lactose is formed when α lactose monohydrate crystals are heated mostly under vacuum at temperatures of 100 – 130 °C. Production of the stable form of anhydrous α lactose is more difficult but has been achieved by thermal treatment in a moist atmosphere at temperatures over at least 110 °C, or desiccation with liquids such as dry methanol where the non-hygroscopic form is produced.

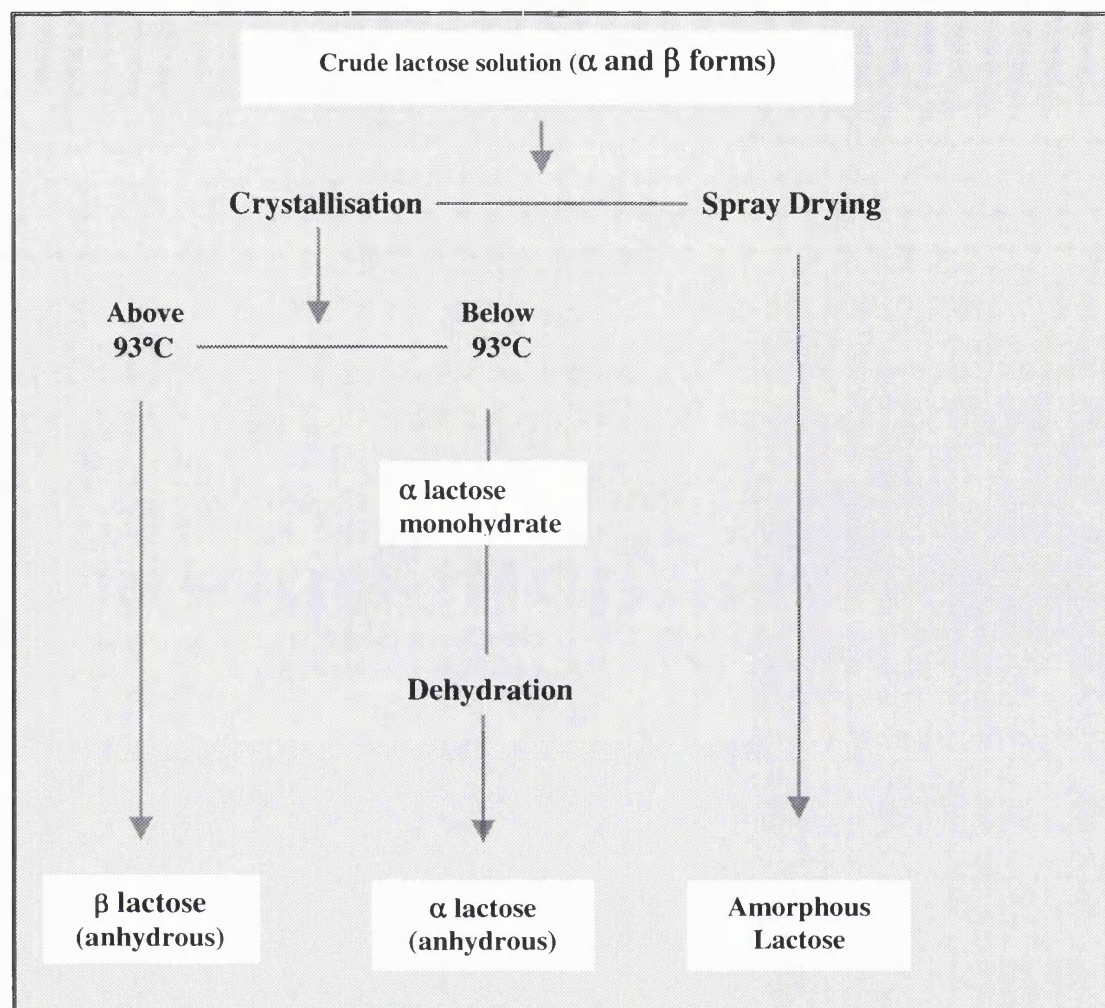


Figure 1.11 An illustration showing how the various forms of lactose are obtained by treatment.

β lactose, on the other hand, is prepared from solutions above 93 °C. Solubility of α and β lactose in water is influenced greatly by temperature, below 93 °C the β form is the more soluble isomer hence crystallisation of the α form from a concentrated supersaturated solution. Above 93 °C the β form becomes less soluble than α and hence β is the stable form precipitated from lactose solutions. During crystallisation of β lactose no water is incorporated in the crystal lattice, hence the β form only exists in the anhydrous state. The crystals of β lactose normally exist in a non-hygroscopic anhydrous form which is stable at low relative humidities, but is hygroscopic at high relative humidities, in contrast to the α form, which occurs both as the monohydrate

and as anhydrous α lactose. The commercially available β lactose typically contains 70% of the β form and 30% of the α form and is prepared by roller drying.

Amorphous lactose is obtained commercially either by spray drying from an aqueous lactose solution or by lyophilization (freeze-drying). During spray drying the suspension is pumped via an atomiser, either spinning disc or pressurised nozzle into a stream of hot air in a drying chamber. The lactose obtained consists of spherical particles made up from α lactose monohydrate crystals glued together by amorphous lactose. The amorphous material is produced by rapid drying of the lactose solution, and gives the spray dried lactose its good binding properties. The spherical particle is mainly responsible for the good flow characteristics. The manufacturing process can therefore be seen to alter the physical characteristics of the lactose, making each product more or less suitable for a particular task. Generally, in pharmaceutical formulation the grade of lactose chosen is dependent on the type of dosage form being delivered.

1.11.3 PROPERTIES AND APPLICATIONS OF DIFFERENT FORMS OF LACTOSE.

Bolhuis et al (1985) carried out an evaluation of anhydrous lactose as a potential new excipient in direct compression tableting. It was found in some formulations to be a very useful filler / binder producing tablets with a low weight variation, sufficient strength, a low friability, a fast disintegration and a high drug release. Spray dried lactose, as mentioned, is normally used in direct compression tableting which is widely seen as a more favourable than wet granulation methods, where active drugs and other excipients may be sensitive to moisture and/or heat. Sebhatu et al (1994) while investigating the effect of moisture sorption on tablets containing spray dried lactose (15% amorphous) found that if the spray dried lactose was exposed to 57%RH for a short period before compaction, the initial tablet strength increased markedly. This was due to moisture uptake, which resulted in a higher molecular mobility of the amorphous spray dried lactose and to an increase in plastic flow leading to the formation of solid bridges between amorphous particles.

1.11.4 THERMAL ANALYSIS AND MUTAROTATION.

Lactose has been studied extensively due to the number of different forms and grades available which all exhibit varying physical and chemical behaviour and characteristics. Thermal analysis has been the main analytical method used in this study, however, difficulties exist since some forms of lactose may exhibit complex thermoanalytical transitions during heating in the DSC. Olano et al (1983) carried out studies on β lactose crystallisation and he presented a brief review of some physical properties of the different crystalline forms of lactose. The variability in measurements and the difficulty in characterising the material was apparent from the range of different melting points listed from various literature sources for the different forms. Lerk et al (1984) investigated the alterations of α lactose during heating in the DSC and concluded that thermal treatment of α lactoses involves changes in β content and in crystal structure.

Angberg and co workers (1991a, 1991b, 1992, 1995) carried out numerous studies investigating the use of microcalorimetry in the characterisation of various changes in the α and β forms of lactose on exposure to moisture. This work focused mainly on investigating changes in crystalline forms of anhydrous lactose at elevated relative humidity i.e. the mutarotation process and subsequent incorporation of water. It was possible using microcalorimetry to monitor the incorporation of water into anhydrous lactose (31% α and 69% β) after storing for 1 day at 58%RH, and after 12 days at 94%RH microcalorimetry was still detecting a process although conventional DSC could not detect a change in dehydration heats. Angberg's studies showed that anhydrous α and β lactose both transform to α lactose monohydrate, when exposed to water vapour, but she stated that β lactose must first mutarotate to α lactose, which was found to be a rate limiting process.

A further complication in the mutarotation issue was highlighted by Olano et al (1983) who stated that mutarotation of α hydrate proceeds several tenths of degrees below the melting and that the unstable anhydrous α lactose has been suggested as a possible intermediate in the transformation. Olano et al (1983) found from their studies that in the absence of water vapour lactose could be heated to 170 °C under dynamic conditions (4 °C/min) without changing the initial isomeric composition. The presence of water vapour, however, caused mutarotation to all forms of α lactose and

to the β form. The amount of β formed was dependent on the %RH, with maximum transformation being obtained under 100%RH. The rate of mutarotation increased with temperature and was also dependent on the crystalline form of α lactose, with α hydrate and unstable anhydrous α lactose showing similar behaviour whereas stable anhydrous α lactose mutarotated at the slowest rate.

1.12 AIM OF THE THESIS

The aim of this thesis is to investigate transitions occurring in a model amorphous solid (spray dried lactose) as a result of moisture uptake and exposure to increased temperature. The uptake, movement and nature of water in the amorphous structure will be examined. The role of this water (and in particular its effect on T_g) in facilitating the transformation of the metastable amorphous lactose through various structural changes to attain the most stable form, through eventual crystallisation, will be followed. The temperature dependence of these structural changes during exposure to moisture will also be examined. Detection of small amounts of disorder in predominantly crystalline samples is also an objective. An investigation into water uptake and movement and the crystallisation process in bulk samples (20-100g) of two partially amorphous spray dried lactose materials will also be carried out in order to compare this to small samples (20-100mg) of spray dried lactose.

CHAPTER 2

METHODS AND MATERIALS.

2.1 MICROCALORIMETRY

2.1.1 INTRODUCTION

Isothermal heat-conduction microcalorimetry measures any heat change occurring in a sample. The system can observe and quantify both exothermic (heat producing) and endothermic (heat absorbing) processes, and therefore has the capacity, in principle, to be able to monitor all physical and chemical processes occurring in a sample. Information can be obtained concerning the rate and extent of basic chemical reactions, changes of phase, changes of structure and the metabolism of living systems. The technique is non-invasive and non-destructive towards the sample and more importantly, it is not dependent upon the sample form, which can be solid, liquid or gaseous. However, a possible disadvantage to the technique is that since it measures all processes occurring with a related heat change, hence there is the possibility of confusion in identifying the actual process taking place. The applications and use of microcalorimetry (and in particular isothermal microcalorimetry) in the fields of physical pharmacy and pharmaceuticals has been reviewed by Buckton and Beezer (1991), Buckton (1995) and Ladso (1997), where the different types of experimental set-ups have been considered.

2.1.2 INSTRUMENTATION

The instrument used in these studies was an LKB Thermal Activity Monitor (TAM) Thermometric AB, Sweden. Temperature differences as low as 10^{-6} °C are detectable using TAM, which utilizes the heat-flow principle. This is where heat produced in a thermally defined vessel flows away in an effort to establish thermal equilibrium, as shown in Figure 2.1. The instrument consists of a 25 litre water bath which surrounds the reaction measuring vessels of which there are four, as shown in Figure 2.2. The water bath acts as a heat sink and is maintained at $\pm 2 \times 10^{-4}$ °C within the working range of 5 - 80 °C. An external water circulator bath helps to maintain accurate temperature control. Temperature control is therefore extremely accurate making this a highly sensitive technique. The measuring vessels, which are maintained at constant temperature in the water thermostat, are surrounded by thermopiles through which any heat changes occurring are channelled to the heat sink. Each measuring vessel

contains a sample side A and a reference side B. A range of different reaction vessels are available e.g. titration, perfusion, flow system and glass and steel ampoules enabling a wide range of experiments to be carried out.

The heat energy is converted into a voltage signal proportional to the heat flow. Results are then presented as a measure of the thermal energy produced by a sample per unit time (dq/dt) and hence the technique is capable of analysis to produce both thermodynamic and kinetic data. An in-built interface is provided for control of the system by an external computer. The software version used here was 'Digitam 3'.

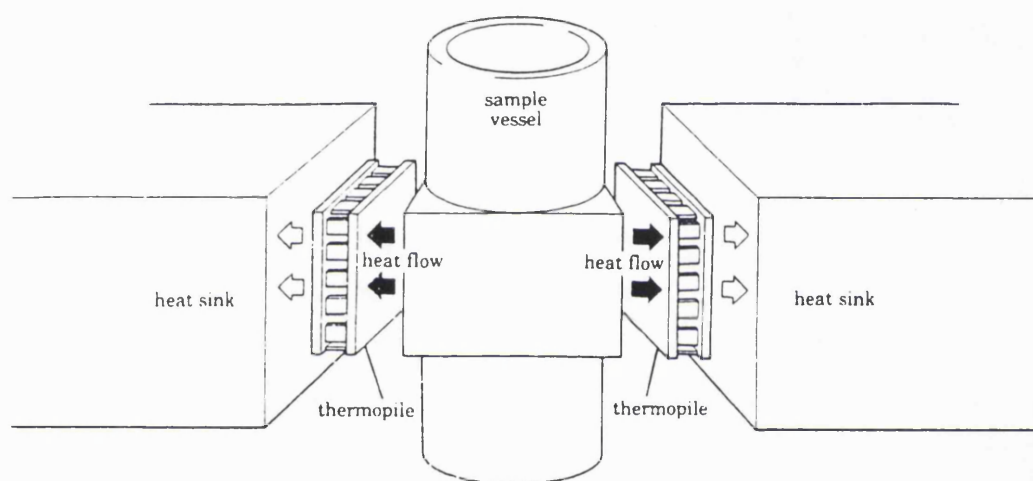


Figure 2.1 Diagram of the heat-flow (conduction) principle (reproduced from LBK 2277 Thermal Activity Monitor Instruction Manual).

2.1.3 EXPERIMENTAL

All experiments carried out in this work involved using glass ampoules. The sample in a sealed glass ampoule is introduced to the instrument along with a reference. The sample and reference are then pre-equilibrated at the half-way mark, before being introduced to the lower measuring position. The instrument was operated in a temperature controlled room, which was kept at $20\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$.

2.1.4 CALIBRATION

Calibration was performed at regular time intervals or whenever experimental conditions were changed (e.g. amplifier range or bath temperature), using two sealed empty glass ampoules. A range of amplifier settings (3, 10, 30, 100, 300, 1000 and 3000 μ W) is available. The calibration is performed by initially zeroing the instrument once the ampoules have been temperature equilibrated in the measuring position. Calibration is then initiated whereby a known current is passed through the appropriate channel heater resistor and a specific thermal power is dissipated. Both static and dynamic calibrations can be made and the resultant signal can be adjusted to the correct value.

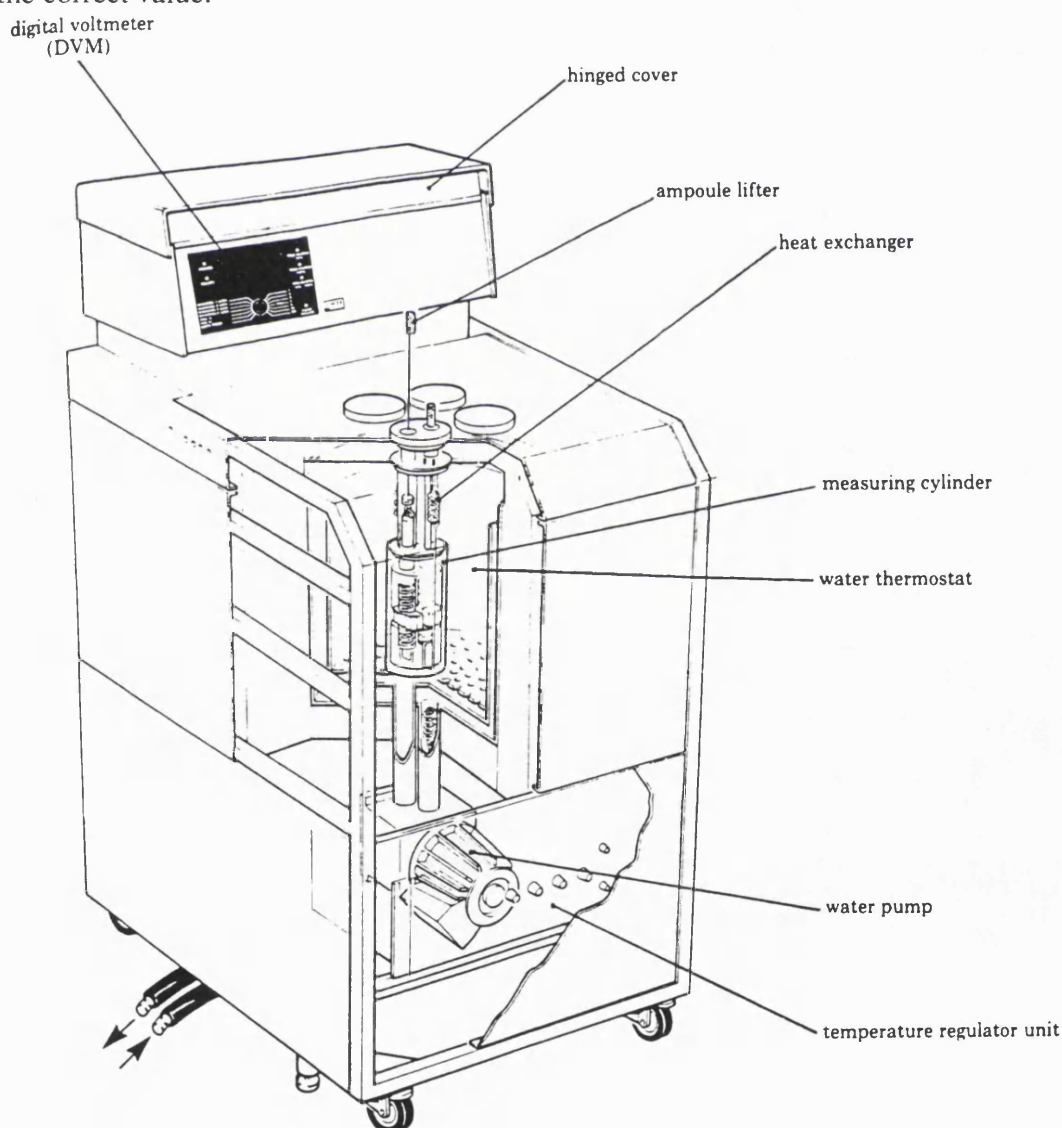


Figure 2.2 Diagram of an Isothermal Microcalorimeter (reproduced from LBK 2277 Thermal Activity Monitor Instruction Manual).

2.2 DYNAMIC VAPOUR SORPTION (DVS)

2.2.1 INTRODUCTION AND INSTRUMENTATION.

The gravimetric studies were undertaken in a humidity controlled microbalance system, Dynamic Vapour Sorption (DVS-1, Surface Measurement Systems, UK) as shown in Figure 2.3. This provided a fast, accurate and fully automated method for the moisture studies carried out in this work. The sample to be investigated is placed on a Cahn microbalance, which is housed in an incubator (to ensure accurate temperature control), and it is exposed to a continuous flow of air with a predetermined and constant relative humidity. The humidity is controlled by the flow of nitrogen through switching valves, which determine the total flow to pass through a humidification stage. The humidified air passed over both the sample and the reference sides of the balance, where the reference contains an empty sample pan. The mass change of a sample due to water sorption/desorption between 0%RH and 98%RH, across a temperature range of 25 °C to 80 °C can therefore be measured. Since the instrument is computer controlled it was possible to choose a number of experimental cycles and sorption/desorption steps, the relative humidity for each step and the length of time that the humidity is to be maintained. Sample weights in the range of 10 - 100mg were normally used.

Since the balance is extremely accurate it was necessary to ensure thorough cleaning of the sample pan after each experiment. This was performed by washing the pan firstly in distilled water (hot water was used if the sample was difficult to remove), after which it was rinsed thoroughly with either alcohol or acetone. The pan was then replaced on the balance and allowed to dry at 0%RH by evaporation. In order to remove any static which may have been present the relative humidity was set at 90%RH for around 5minutes, after which time it was returned to 0%RH, and allowed to stabilise at 0mg before commencing an experiment.

2.2.2 CALIBRATION

(a) Weight calibration.

Weight calibrations were performed on a monthly basis or whenever the operating temperature was changed using a 100mg weight which was placed on the sample pan and the weight was adjusted if different to the actual expected value.

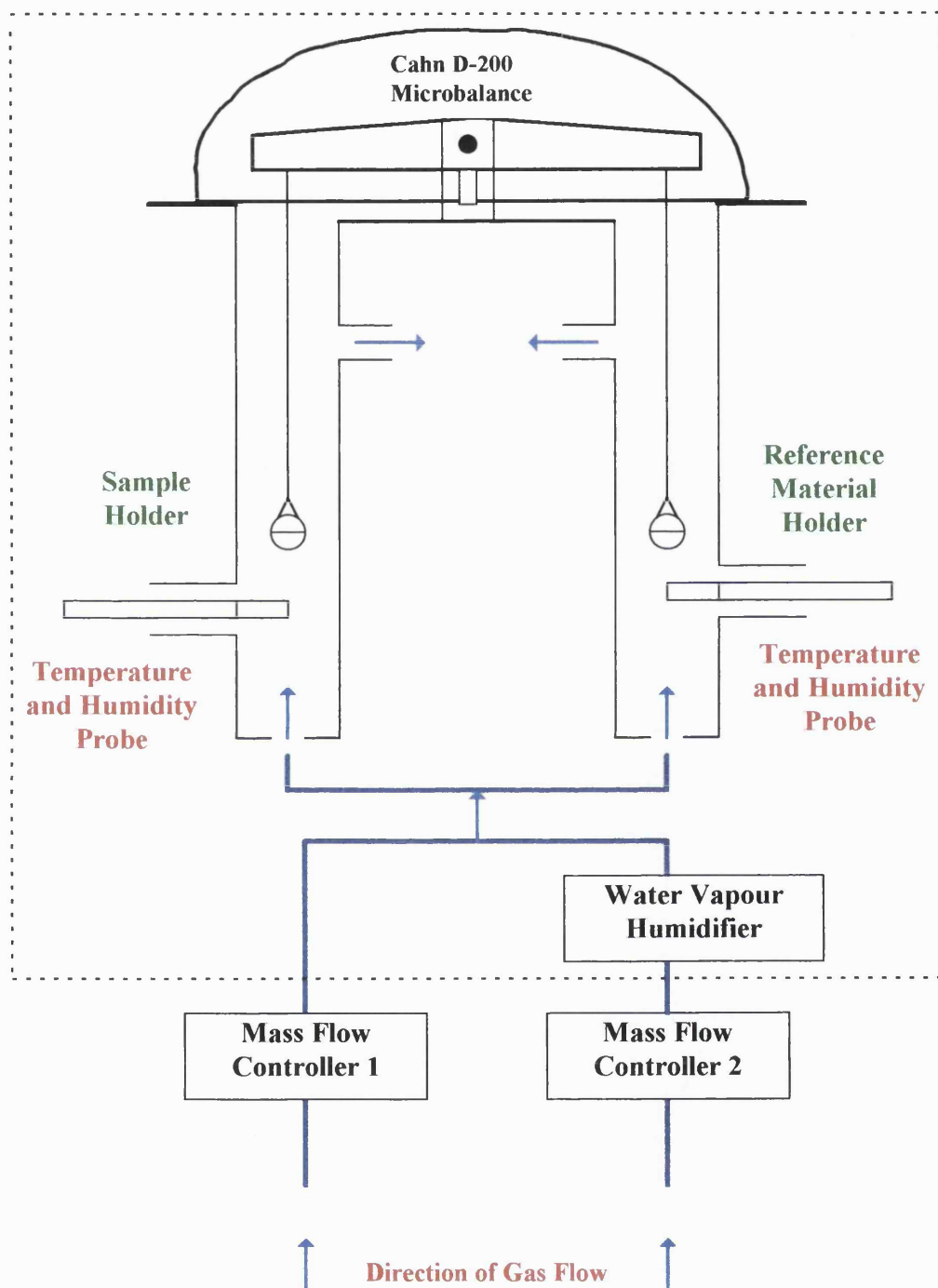


Figure 2.3 Schematic layout of the DVS-1 system (reproduced from DVS-1 Operating Manual).

(b) Relative humidity (RH) validation.

The calibration method used relies on the principle that the vapour pressure of water above a saturated salt solution in equilibrium with its surroundings is constant at a particular temperature. It involved placing a known mass of a particular salt on the sample pan (it is possible to calibrate in this way using either the pure dry salt or a saturated solution of the salt) which gave a specified %RH. The experimental set-up was such that a relative humidity ramp of 1%/hour over a 10% range around the literature value for the critical relative humidity of each salt (Nyqvist, 1983) was used. The salts which were normally used were sodium chloride (75.5%RH) and lithium chloride (12.0%RH). By plotting the change in mass with time (dm/dt) against the RH over the sample, the critical RH of the salt could be calculated, thus allowing the RH of the system to be validated.

2.3 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

2.3.1 Introduction

Differential scanning calorimetry involves heating a sample and reference pan separately, with the power supply to the sample heater variable so that the temperature difference can be maintained at zero even when a thermal transition (a chemical or physical change that results in the emission or absorption of heat) occurs in the sample. The difference in power supplied to the two heaters is exactly equivalent in magnitude to the energy absorbed or evolved in the transition and therefore yields a direct calorimetric measurement (ΔH) of the transition energy. The types of transitions and reactions which can be measured using this technique include fusion, sublimation and vapourisation (exothermic heat changes), crystallisation (endothermic heat change) and glass transitions (baseline shifts).

2.3.2 INSTRUMENTATION

The DSC 7 differential scanning calorimeter employed in these studies operates using Perkin Elmer's power compensation design, with the UNIX operating system. The power compensation design uses individual sample and reference micro-furnaces, Figure 2.4, and so operates as a 'thermal-null' system. The micro-furnace lends itself to very fast heating and cooling rates, rapid temperature equilibrium and fast sample

turn around time. Platinum resistance thermometers (PRT's) are used to make the temperature and energy measurements. The micro-furnaces, being made of a platinum-iridium alloy, are very inert in order to resist chemical attack. In the base of the furnace are two identical platinum resistance elements. One of these elements is used to provide power (heat) to the furnace. The other is used to detect the smallest change in the temperature of that furnace. The platinum windings are distributed over the full area of the furnace base and so provide truly distributed heating and temperature monitoring at all points in the furnace. Both sample and reference furnaces are mounted in cavities of a large aluminium block or 'heat-sink'. The heat sink is kept at a constant temperature which is well below the temperature range of the experiment to ensure that heat can be lost from the micro-furnace to the large heat sink very quickly.

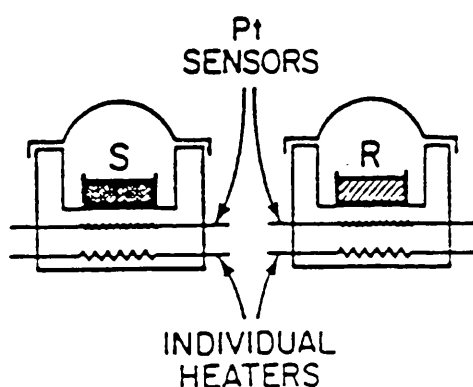


Figure 2.4 The design of a power compensated DSC (reproduced from 7 Series/UNIX DSC 7 Differential Scanning Calorimeter Users Manual).

2.3.2.1 The 'Null Balance' principle.

Two separate control loops are used to precisely control the temperature of the sample and reference furnaces as follows:

Average Temperature Control: This loop provides power to the sample and reference furnace in accordance with the program temperature selected and therefore ensures that heat is supplied at the selected rate over the chosen temperature range and that the two furnaces are always at the same temperature.

Differential Temperature Control (DCT): This loop is the actual measuring circuit that drives the instruments output signal. The system measures precisely any differences in the temperature that occurs between the sample and reference furnaces. If the material in the sample furnace gives out or takes up energy, the temperature of the sample furnace will change slightly from that of the reference furnace. This temperature change is accurately sensed by the platinum resistance thermometer in the base of the furnace and the DCT loop will adjust the power to bring the two furnaces back to the same temperature, thus the system is always kept at a thermal null. The amount of energy that must be provided or removed from the system to maintain the thermal null by the DCT loop is directly proportional to the energy change of the system so no heat flux equations are necessary. Direct measurements of energy and therefore ΔH can be made with a power compensation DSC.

2.3.3 EXPERIMENTAL

To achieve maximum peak sharpness and resolution, proper sample preparation is needed to maximise the contact surface between the pan and sample in order to reduce the resistance of the sample to heat flow through the DSC temperature sensors. It is therefore best with powder samples to spread a thin layer on the pan bottom for optimum performance. The pans used here were Perkin Elmer aluminum pans (cat no. 0219-0041) which were not hermetically sealed. The sample was encapsulated in the pan which ensures good thermal contact between the sample and furnace. Sample mass used in these studies was normally in the region of 4mg and samples were weighed using an AD-4 Perkin Elmer autobalance.

The purge gas was nitrogen and a flow rate of 20 - 30 psi was used. The temperature range over which the samples were scanned was normally 25 °C - 250 °C at a scan rate of 10 °C/minute unless otherwise stated. An empty sealed aluminium pan was used as a reference in these studies.

Generally, slow scanning rates improve the peak resolution while faster scanning rates improve the sensitivity. If a sample is melted in the DSC at increasing heating rates, then the size of the fusion endotherm will increase accordingly, but the area under the peak in J/g will remain the same because the same amount of heat will be needed by the sample to go from solid to liquid. Consequently, sample ΔH measurements can be

made at any heating rate. As the heating rate of the experiment increases so will the observed transition temperatures.

2.3.4 CALIBRATION

Thermal analysers need to be calibrated in order to ensure that any transition recorded is in all respects accurate. Both temperature and energy ranges must be calibrated and this is achieved by running high purity standard materials with known temperature and energy transitions. The standard materials used to calibrate the DSC 7 were Indium which is a stable metal of high purity (expected temperature onset = 156.60 °C and expected peak area (heat of fusion) = 28.45 J/g) and Zinc (expected temperature onset = 419.47 °C). The instrument is adjusted once the calibration check has been carried out if the measured values are not correct. Normally, calibration checks were carried out on a weekly basis or if any of the operating conditions were changed e.g. scan rate or use of different temperature ranges.

2.5.5 DATA ANALYSIS AND MANIPULATION (CONVERTING DSC DATA FILES TO *microsoft* EXCEL FORM).

In order to analyse DSC data in excel form the steps below must be followed using the UNIX software once an experiment is complete:

- (1) use **optimize data(F6)** to change the curve to the desired form eg. slope and axis adjustments.
- (2) save this as a snapshot by carrying out the following steps:-
 - **F4 recall data**
 - **F9 snapshot 1st**
 - entering a **filename**
 - **F9 save**
- (3) then go to **file utilities** and copy this snapshot to DOS after setting the DOS destination to a:\

It is then possible to open and analyse the data file in excel.

2.4 THERMOGRAVIMETRIC ANALYSIS (TGA)

2.4.1 INTRODUCTION.

Thermogravimetric analysis (TGA) is a thermal analysis technique for measuring the amount and rate of change in sample mass as a function of temperature and time. It is used to characterise any material that exhibits weight loss or phase changes as a result of decomposition, dehydration and oxidation (due to chemical and physical bonds forming and breaking at elevated temperatures). TGA data is therefore useful in characterising materials as well as investigating the thermodynamics and kinetics of the reactions and transitions from the application of heat to a material. Two modes are commonly used for investigating thermal stability behaviour in controlled atmospheres: (1) dynamic, in which the temperature is increased at a linear rate, and (2) isothermal, in which the temperature is kept constant.

2.4.2 INSTRUMENTATION

The TGA employed in these studies was a TGA 2950 Thermogravimetric Analyser (TA Instruments) as shown in Figure 2.5, and uses a null position balance. The balance arm is kept horizontal by an optically controlled meter movement. A small flag on the top of the balance arm blocks an equal amount of light (emitted from an LED) from reaching two photodiodes. If there is any weight change in the sample pan (which is connected to the balance arm via a kapton loop and hang-down wire, as shown in figure 2.6) the position of the balance arm and flag will move. This will cause an unequal amount of light to reach the two photodiodes. This unbalanced signal is then nulled by the control circuitry which causes the meter movement to bring the balance arm to the null (horizontal) position. The change in current needed to do this is thus directly proportional to the change in mass of the sample and is recorded by the software as a weight signal. The null position principle ensures that the sample maintains a constant position in the TGA furnace.

A tare pan hangs on the opposite side of the balance to the sample pan. The instrument has an accuracy of $< \pm 0.1\%$ and a resolution of $0.1\mu\text{g}$. The furnace can be heated to $1000\text{ }^{\circ}\text{C}$, and a purge gas is used to prevent contamination of the furnace and balance assemblies. The purge gas used here was nitrogen and the gas line is split before it enters the module and two flow meters are used to set the flow rates to

60cc/min through the purge inlet and 40cc/minute through the balance inlet. The furnace is cooled between runs by a compressed air supply, and a thermocouple positioned just above the sample pan is used to monitor the environment temperature.

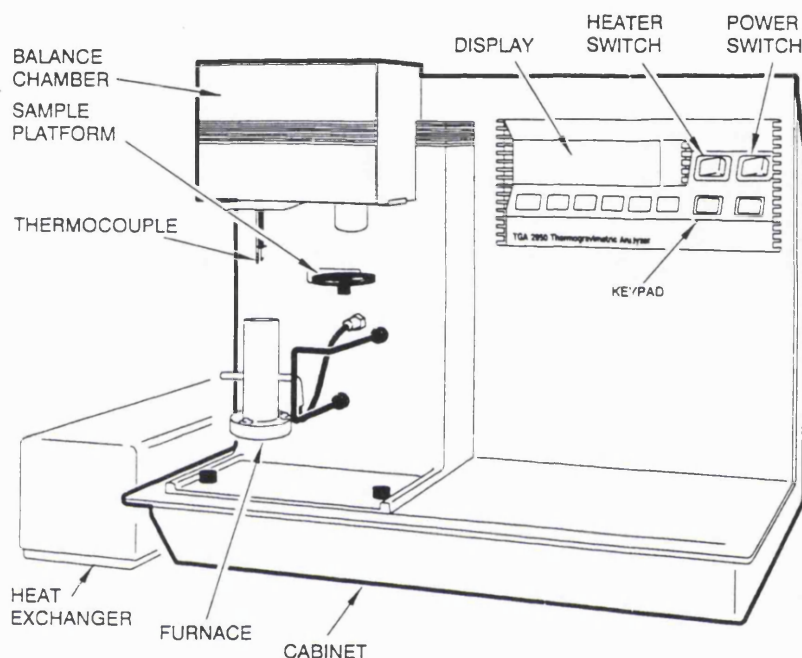


Figure 2.5 Diagram of the TGA 2950 Module. (reproduced from TGA 2950 Thermogravimetric Analyzer Operator's Manual).

2.4.3 EXPERIMENTAL

The sample pans used were Perkin Elmer aluminium pans (cat. No. 0219-0041). Open pans were used and the sample was weighed directly onto the tared pan on the TGA loading platform. Sample weights were in the region of 4mg and samples were normally scanned from 25 °C - 250 °C at a scan rate of 10 °C/minute unless otherwise stated. Data analysis was carried out using the TA software and was presented as a combination plot of weight (%) and derivative weight (%/min) as a function of temperature (°C).

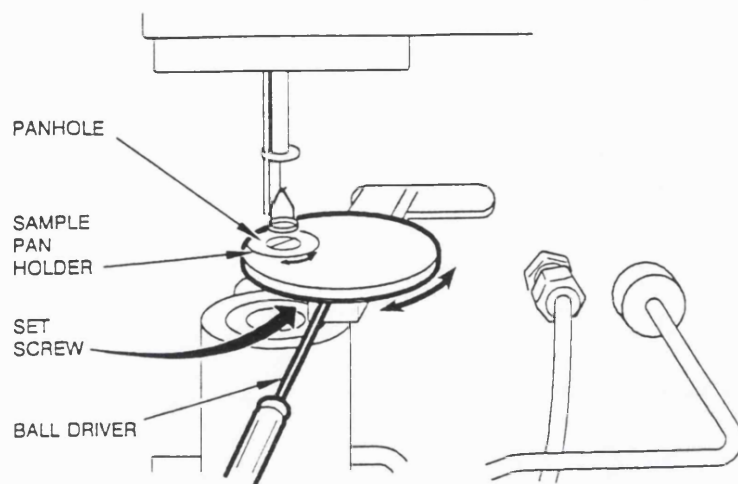


Figure 2.6 Diagram of the TGA sample platform assembly. (reproduced from *TGA 2950 Thermogravimetric Analyzer Operator's Manual*).

2.4.4 CALIBRATION

There are two calibration procedures i.e. weight and temperature calibrations. The weight one uses 100mg and 1g standards to calibrate the weight signal and is normally done once a month. Temperature calibration should be carried out if heating rate or purge gas flow-rate are changed or if the thermocouple position is changed.

2.5 SPRAY DRYING

2.5.1 INTRODUCTION

Spray drying is by definition the transformation of feed from a fluid state into a dried particulate form by spraying the feed into a hot drying medium. It is a one-step continuous particle-processing operation involving drying. The feed can either be a solution, suspension or a paste. The resulting product conforms to powders, granular agglomerates, the form of which depends upon the physical and chemical properties of the feed and the dryer design and operation. It has a wide range of applications especially in the pharmaceutical and food industries (Masters, 1976). Spray drying of milk products e.g. skim milk and other foods such as fruits and carbohydrates, is

advantageous since these spray dried materials retain nutritive content and are easy to use because they are readily reconstituted. The importance of spray drying methods in the manufacture of pharmaceutical dosage forms stems from its ability to alter and control such properties as particle shape, particle size and size distribution, bulk density, porosity, moisture content, flowability, stability, dispersability and friability (Corrigan, 1995).

The physiochemical properties of spray dried materials which may be affected by this process range from melting point, solubility and dissolution rate to crystal habit, cohesiveness and compressibility (Grant and York, 1986). Spray drying can therefore alter both physical and chemical characteristics of a material, the latter being reflected in enthalpy as well as entropy changes. This is evident from data reported by Pikal et al (1978) who carried out crystallinity determinations from solution calorimetry data on spray dried and freeze dried antibiotics. Increased enthalpy and entropy values were reported for the spray dried and freeze dried materials compared to the unprocessed sample. Frequently spray drying results in the formation of a glassy or amorphous phase i.e. a high energy forms of a material (Corrigan, 1995), which was the requirement of this technique as used in this study. The higher thermodynamic activity of the amorphous state of a material relative to the crystalline state has particular pharmaceutical significance in that the resulting increased solubility (Florence and Salole, 1976) can result in improved biological activity and product efficacy.

Newton (1966) discussed how spray dried product characteristics such as particle size and distribution, particle density and porosity, moisture content and bulk density are affected by the process. The hollow nature of the spray dried particle was said to give a low bulk density and was also thought to influence the rapid rate of solution of these materials. It was stated by Newton also that because of the spherical form and relatively narrow size distribution range spray dried powders are usually free flowing and hence their importance in tableting.

Fell and Newton (1970, 1971) investigated the production and properties of spray dried lactose and it was reported that by varying factors such as feed concentration, temperature and feed rate resulted in varying proportions of anhydrous α and β lactose and the α monohydrate form. This is in agreement with recent studies by Chidavaenzi et al (1997) who also noted variations in the lactose form obtained after

spray drying when feed concentration was altered. A decrease in the % lactose in solution due to an increase in feed concentration resulted in a decrease in the % amorphous content in the product. The different relative solubilities of the α and β forms, with β being more soluble in solution (Berlin et al, 1970) was considered to be a critical influencing factor in the spray dried product obtained. The use of spray dried lactose in direct compression tableting, which eliminates the need for a wet granulation step is also another important application of the process. The proportions of the amorphous and crystalline forms present in lactose have been reported to be important in determining the compression properties of the material (Vromans et al, 1987; Sebhatu et al, 1994, 1997).

Corrigan (1995) also mentions that spray drying is now frequently used to produce a range of encapsulated products, and biodegradable microparticles are increasingly being explored as components for entrapping therapeutic agents in drug delivery systems. The use of spray drying methods are also being investigated for the production of dry powder aerosol formulations (Venthoey, 1997) since spray-dried powder form is ideal for rapid assimilation into the body organs.

2.5.2 INSTRUMENTATION

Spray drying involves atomization of feed (normally aqueous) into a spray, and contact between spray and a drying medium (normally air) resulting in moisture evaporation. The drying of the spray proceeds until the desired moisture content in the dried particles is obtained and the product is then recovered from the air. These four stages are illustrated in Figure 2.7, where a diagrammatic representation of a spray dryer is shown. Atomization refers to the process whereby the liquid bulk is broken up into millions of individual droplets forming a spray for optimum evaporation conditions. During spray-air contact, droplets meet hot air and moisture evaporation takes place from the droplet surfaces. As soon as droplets of the spray come into contact with the drying air, evaporation takes place from the saturated vapour film which is quickly established at the droplet surface. Evaporation is rapid, due to the vast surface area of droplets in the spray. During evaporation, the atomized spray distribution undergoes change. Different products exhibit different evaporation characteristics. Some tend to expand, others collapse, fracture or disintegrate, leading to porous, irregularly shaped particles. The extent of any change in particle shape, and

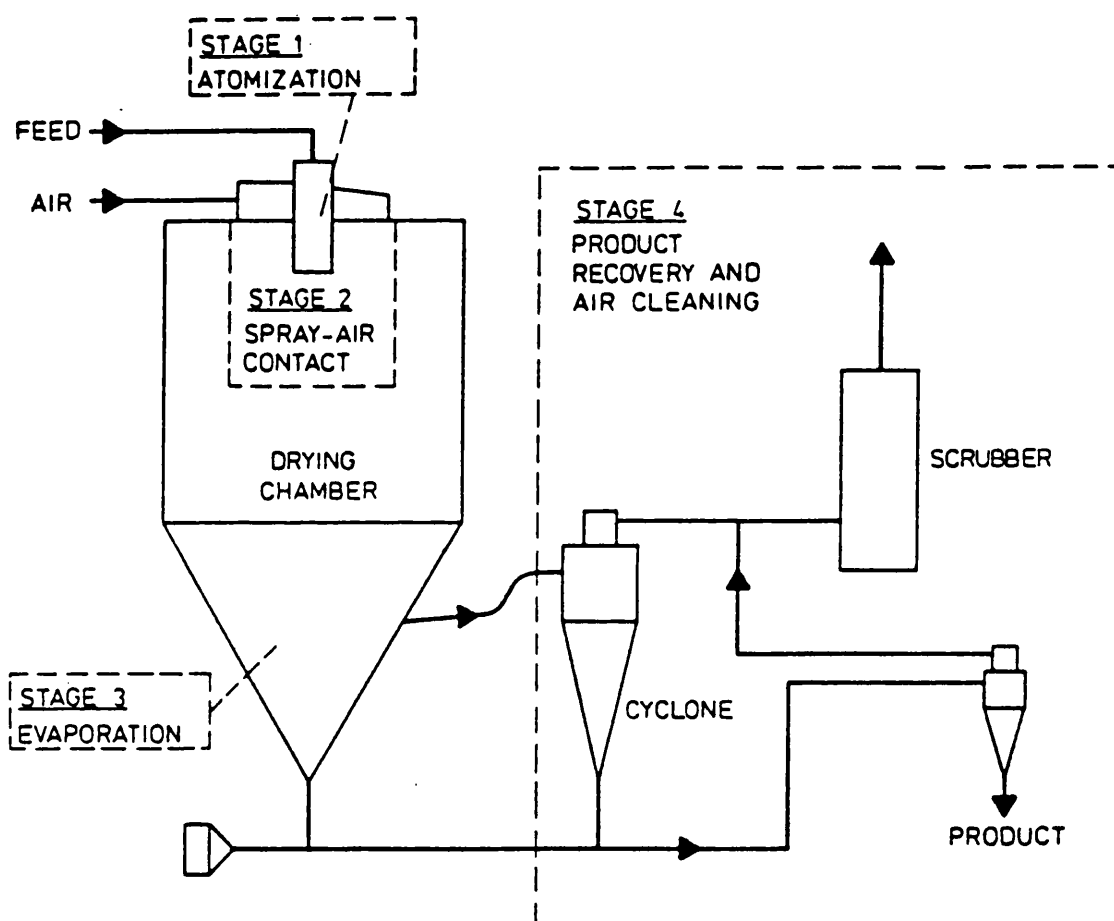


Figure 2.7 Diagrammatic representation of a spray dryer, showing the four stages of the process (reproduced from Spray drying. An introduction to Principles, Operational practice and Applications. Masters, K.).

hence the dried-powder characteristics are closely connected to the drying rate. Spray dried products are easily recognisable by being uniform in appearance and, if examined under the microscope the particles have a characteristic shape, in the form of hollow spheres with a small hole. This arises from the drying process since the droplet enters the hot air stream and dries on the outside to form an outer crust with the liquid still in the centre. This liquid then vapourises, the vapour escaping by blowing a hole in the sphere. This characteristic particle form gives the product a high bulk density, and in turn ready solubility.

Product separation from the drying air follows completion of the drying stage, when the dried product remains suspended in the air, and this can influence powder properties by virtue of the mechanical handling involved with excessive handling producing powders having a high percentage of fines.

2.5.3 EXPERIMENTAL

(1) Preparation of the amorphous lactose

The lactose solution to be spray dried was prepared by adding 15g of crystalline α lactose monohydrate (DMV, Netherlands) to 100ml of distilled water, after which it was placed in a sonicator until all the lactose was dissolved and the solution was clear in appearance. The spray dryer used was a Buchi 190 and it was set up under the operating parameters listed in Table 2.1. Care was taken to ensure the inlet temperature didn't go above 120 °C, to prevent spoilage of the lactose sample. The lactose was removed from the collecting vessel as quickly and as efficiently as possible in order to reduce handling time and exposure of the material to the atmosphere. It was then dried overnight in a vacuum oven at 40 °C. The material was stored in a dessicator over silica gel at 25 °C.

(b) Characterisation of the spray dried product

A TGA scan of the spray dried material after it had been dried overnight under vacuum at 40 °C revealed it to contain 2.7% residual moisture. DSC analysis resulted in the sample recrystallising at approximately 190 °C, with a large melting endotherm at 218 °C and a smaller one at 238 °C, which are the α and β lactose forms, respectively. These scans for the amorphous lactose are shown and discussed further in Chapter 3 (section 3.5).

Operating Parameter	Setting
Inlet temperature	120 °C
Outlet temperature	75 °C
Feed rate	2ml/min
Pressure	3 bar
Solution concentration	15% w/v
Pump	
■ aspirator	10
■ speed	3
■ heat control	3.5

Table 2.1 Spray drying parameters for the aqueous lactose solution.

2.6 PREPARATION OF SATURATED SALT SOLUTIONS FOR MAINTAINING SPECIFIED RELATIVE HUMIDITIES.

2.6.1 INTRODUCTION

Air at a given temperature is capable of taking up water vapour until it is saturated (at 100%RH). If the temperature is raised then the air will be able to take up more moisture and the relative humidity falls. The %RH may be defined as:

$$\frac{\text{vapour pressure of water vapour in the air}}{\text{vapour pressure of water vapour in air saturated at the same temperature}} \times 100$$

Saturated salt solutions can be used to maintain specified relative humidities in closed chambers (Nyqvist, 1983).

2.6.2 EXPERIMENTAL

These solutions were prepared by adding the particular salt, as listed in Table 2.2, to distilled water (about 20ml, depending on how much salt solution was needed), until an excess of salt remained at the bottom of the container. The solutions were normally prepared at room temperature, however, for high temperature studies i.e. 35, 45, 50 and 60 °C the solutions were prepared and stored at the experimental temperature.

The range of saturated salt solutions which were prepared and their corresponding relative humidities over a range of temperatures (Nyqvist, 1983) are listed in Table 2.2.

Salt	Temp (°C)						
	20	25	30	35	45	50	60
Potassium sulphate, K_2SO_4	97.6	97.3	97.0	96.7	96.2	95.8	95.8
Ammonium-dihydrogen orthophosphate, $NH_4H_2PO_4$	93.0	93.0					
Zinc sulphate, $ZnSO_4$	90.0						
Potassium chloride, KCl	85.1	84.3	83.6	82.9	81.7	81.3	80.0
Ammonium-sulphate, $(NH_4)_2SO_4$	81.0	80.0	80.0	80.0	79.0	79.0	78.0
Sodium chloride, $NaCl$	75.5	75.3	75.1	74.8	74.7	74.7	74.7
Sodium nitrite, $NaNO_2$	66.0	65.0	63.0	62.0		59.0	59.0
Magnesium nitrate, $(MgNO_3)_2$	54.5	52.8	51.5	50.0	47.1	45.5	45.5
Sodium dichromate, $Na_2Cr_2O_7$	54.8	54.4	54.0	53.5	51.8	51.4	
Potassium thiocyanate, $KSCN$	47.0						
Potassium carbonate, K_2CO_3	44.0	43.0	43.0	43.0	42.0		
Magnesium chloride, $MgCl_2$	33.1	32.8	32.4	32.0	31.1	30.5	30.0
Potassium acetate, $KC_2H_3O_2$	23.0	22.0	22.0	21.0	20.0		
Lithium chloride, $LiCl$	11.3	11.3	11.3	11.2	11.2	11.1	11.1

Table 2.2 Saturated salt solutions and their relative humidities over a range of temperatures (reproduced from *Pharmaceutical Handbook*, Wade (Ed.) and Nyqvist, 1983).

(The blank spaces at certain temperatures for some saturated salt solutions are where %RH values were not available from the literature.)

CHAPTER 3

INVESTIGATING THE CRYSTALLISATION OF AMORPHOUS SPRAY DRIED LACTOSE ON EXPOSURE TO WATER VAPOUR.

3.1 ISOTHERMAL MICROCALORIMETRY STUDIES.

3.1.1 INTRODUCTION.

It has been known for some time that amorphous sugars such as lactose, sucrose and glucose, when exposed to high humidity, absorb large amounts of water and eventually crystallise (Makower and Dye, 1956) releasing the sorbed water. This change from the amorphous to the crystalline form will be accompanied by a heat exchange and since microcalorimetry will measure any heat changes occurring in a sample, it would appear that this would be a useful method to monitor this process. Numerous recent studies have shown that the crystallisation process in both hydrophilic and hydrophobic amorphous materials can be followed by isothermal microcalorimetry. The sample is sealed in a glass ampoule in the presence of a saturated salt solution which gives a specific %RH or an organic solvent (in the case of hydrophobic materials), as described by Angberg (1992). Sebhathu et al (1994) showed that the crystallisation of lactose samples, rendered amorphous by spray drying, could be detected and measured by exposing the samples to elevated humidity in a sealed glass ampoule in the microcalorimeter. Buckton et al (1995) and Venthoye (1997) followed the crystallisation of amorphous spray dried salbutamol sulphate in a similar manner, by exposing the material to different saturated salt solution. Aso et al (1995) also monitored the crystallisation of an amorphous drug, nifedipine, using this same approach in the microcalorimeter.

Ahmed et al (1996) have shown that it is also possible to follow the crystallisation of a hydrophobic amorphous material, using organic solvent vapours e.g. ethanol and chloroform, to saturate the sample and cause a lowering of T_g in order to induce crystallisation of the amorphous material.

The aims of this study therefore were as follows:

- (1) To determine if it was possible to observe the crystallisation of spray dried lactose samples on exposure to water vapour in the microcalorimeter.
- (2) To subsequently determine the optimum conditions at which to carry this investigation i.e. %RH and sample weight necessary for crystallisation to occur.

- (3) To investigate the influence of additives on the crystallisation of the amorphous spray dried lactose in the microcalorimeter, with a view to finding a better understanding of water movement and transport in these samples.
- (4) to carry out water sorption studies on amorphous spray dried lactose using an automated vapour sorption microbalance
- (5) to attempt physical characterisation of spray dried lactose before and after crystallisation in the microcalorimetry.

3.1.2 MICROCALORIMETRY METHOD

The sample to be investigated was weighed accurately, using a 4-figure analytical balance into a 3ml glass ampoule. A Durham tube containing a saturated salt solution giving the %RH of choice was then placed in the glass ampoule and the ampoule was sealed as shown in Figure 3.1. The glass ampoule, Durham tube and saturated salt solution had all been previously stored overnight at the experimental temperature (25°C here) in order to minimise disruptions due to temperature variations in the measuring channel of the microcalorimeter. The sealed ampoule was then quickly placed in the equilibration position in the microcalorimeter, for exactly 30 minutes, after which time it was lowered very slowly (to avoid inducing any unwanted responses due to friction) into the measuring position and the experiment was started.

3.1.3 MICROCALORIMETRY CRYSTALLISATION RESPONSE FOR SPRAY DRIED LACTOSE.

The microcalorimetry response shown in Figure 3.2 is for a 27.5mg sample of spray dried lactose which was exposed to 75%RH (sodium chloride saturated salt solution).

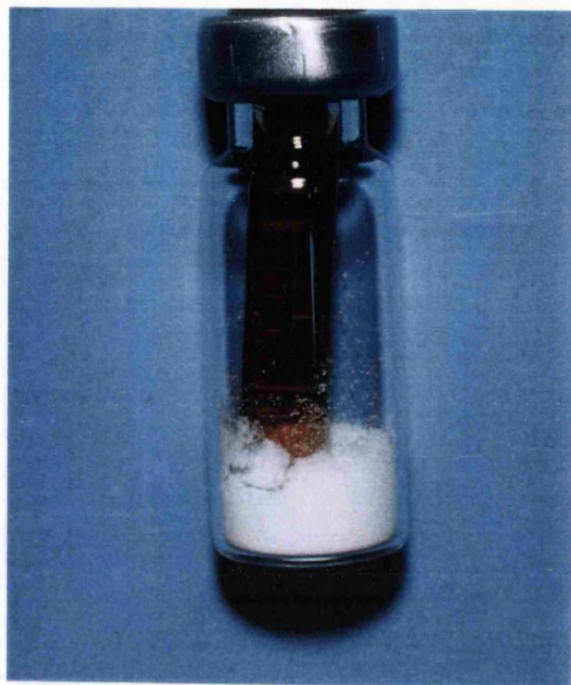


Figure 3.1 Glass ampoule with powder and Durham tube containing saturated salt solution.

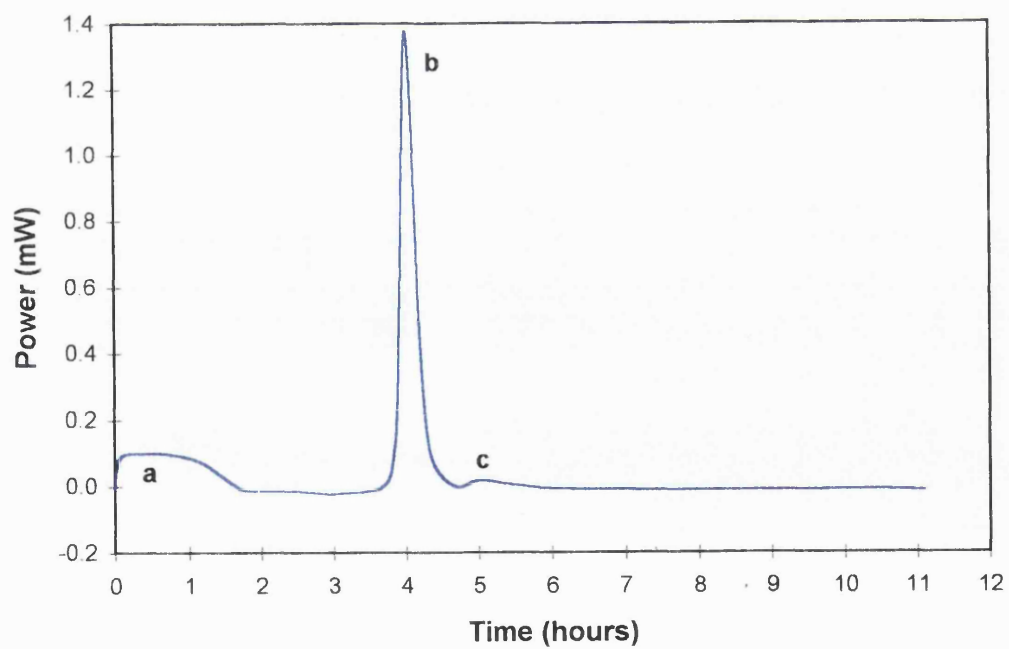


Figure 3.2 Typical microcalorimetry response for the crystallisation of 100% amorphous lactose on exposure to 75%RH.

3.1.3.1 Initial peak.

The response shows an initial small broad peak running for about 1.5 hours. This is thought to be mainly a wetting response due to water being absorbed into the amorphous regions of the powder (Sebhatu et al,1994; Ahmed et al 1995; Buckton et al, 1995c). However, there may also be a possible contribution due to the collapse of the amorphous material, which will be discussed further in Chapter 5. Since any process occurring in the cell which is accompanied by a heat change will be measured, this initial response will have a contribution due to the evaporation of the salt solution and a contribution due to the absorption of water into the amorphous material. The evaporation will be an endothermic response and the absorption will be exothermic response. If the water vapour was generated from an external source outside the measuring cell, as shown by Sheridan et al 1994, this wetting response would be much larger as there would be no endothermic effect from the evaporation of the salt solution.

3.1.3.2 Main peak.

After the initial peak there is a lag time of about 2 hours where only a flat baseline response is obtained. This is due to the endothermic vapourisation of the salt solution and the exothermic vapour sorption by the sample being equal and opposite and therefore canceling each other out. A large sharp exothermic peak is then observed at around 4 hours as shown in Figure 3.2. The shape of this peak indicates a very rapid process to be occurring in the cell. The sample was analysed by X-Ray powder diffraction on removal from the microcalorimeter after this peak and it was found to be crystalline (see Fig. 3.18). Another sample was removed here, dried and weighed into a new glass ampoule with the same salt solution and was again placed in the microcalorimeter. The experiment was then carried out as before, whereby no response was observed, also indicating the material to be already crystalline. The sample, if viewed on removal from the microcalorimeter after this sharp peak, was seen to have changed from a sticky particulate material to a hard fused solid mass.

The above observations indicate that crystallisation of the amorphous material has taken place, with the whole sample crystallising simultaneously. This sharp peak must therefore be due to the transformation of the sample from the amorphous to the crystalline state as observed also by Sebhatu et al (1994).

3.1.3.3 Effect of water uptake on the amorphous material.

As the amorphous sample is exposed to the high humidity environment in the glass ampoule it will begin to absorb large amounts of moisture due to it being in an activated state (Ahlneck and Zografi, 1990). It is probable that water vapour is taken up at the powder surface and then transported into the powder bed. Only when the whole sample is fully saturated will crystallisation occur with the whole sample crystallising simultaneously, as evidenced by the sharp peak observed. Sebhatu et al (1994) observed and described a similar response for lactose samples rendered amorphous by milling. The effect of water uptake on the sample is that it acts as a plasticising agent lowering the glass transition temperature (T_g) of the amorphous lactose. This leads to an increase in the free volume of the sample and when there is sufficient molecular movement (when the T_g equals the temperature (T) of the experiment which is 25°C in this case, crystallisation will occur (Zografi, 1988). As the sample begins to crystallise water will be desorbed, some of which will form the monohydrate while the remainder will be absorbed by the neighbouring amorphous material, where it will then cause it to crystallise. This continuous effect will give rise to the sharp crystallisation response that is seen here in the microcalorimeter. When the whole amorphous sample has crystallised the absorbed water will be released (Makower and Dye, 1956), which is shown and discussed in section 3.4 for the gravimetric experiments carried out on spray dried lactose samples. The crystallisation response will be exothermic and the desorption response will be endothermic.

3.1.3.4 Smaller broad peak.

An enlargement of the y-axis in Figure 3.2 shows a third peak directly after the main crystallisation peak as shown in Figure 3.3. This is much smaller than the main crystallisation peak and is attached to the main peak. It is thought that this peak is due to either the mutarotation of β lactose to α -form at high humidity or the incorporation of water into anhydrous lactose (Angberg et al, 1991a, 1991b, 1992).

However, there may also be a contribution due to condensation of water back into the saturated salt solution, following crystallisation of the sample, which would be an exothermic event. The reason for this observation is that if we

compare the responses obtained for a range of salt solutions, it appears that this peak decreases in magnitude as the %RH of the salt solution increases as shown in Figure 3.4. The heat flow from the desorption of sorbed water after crystallisation and the condensation back into the tube should cancel each other out, however, the difference between the humidity in the cell and the desorbed water, will be larger where the humidity in the ampoule is lower.

At high humidities a much broader response is obtained, see Figure 3.4, which seems to be occurring much slower which would indicate that this is possibly the mutarotation response. Angberg et al (1991) found that it was only at the highest humidities investigated that the mutarotation became an extensive process, although it did occur at lower humidities.

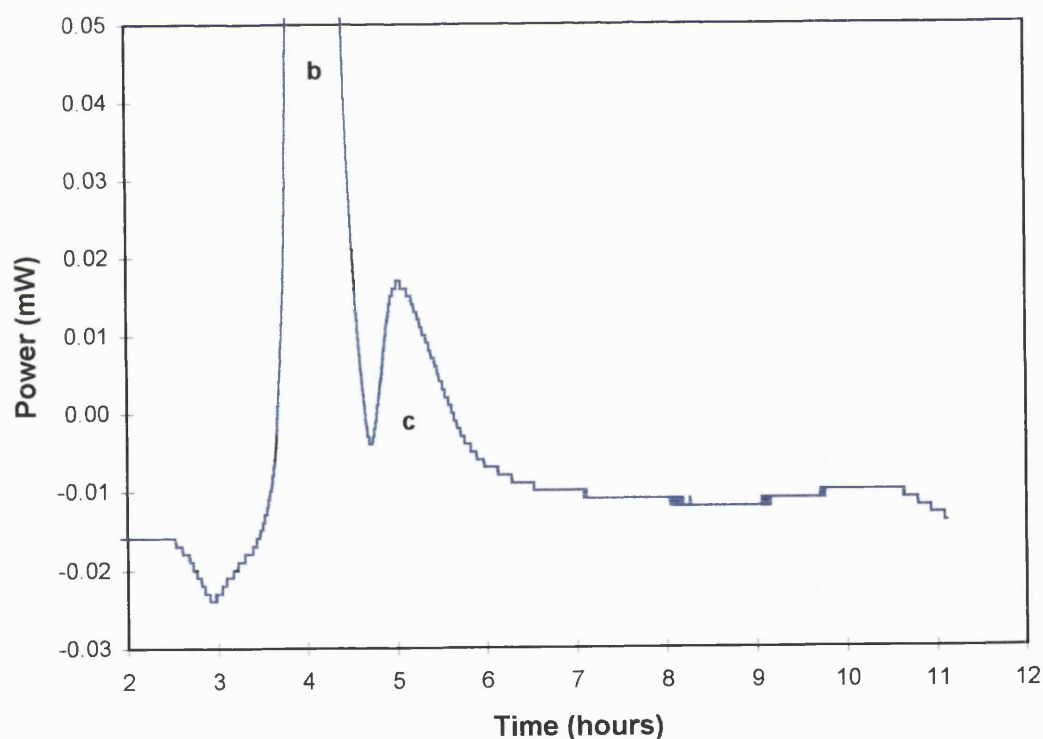


Figure 3.3 Enlargement of the microcalorimetry response for spray dried lactose.

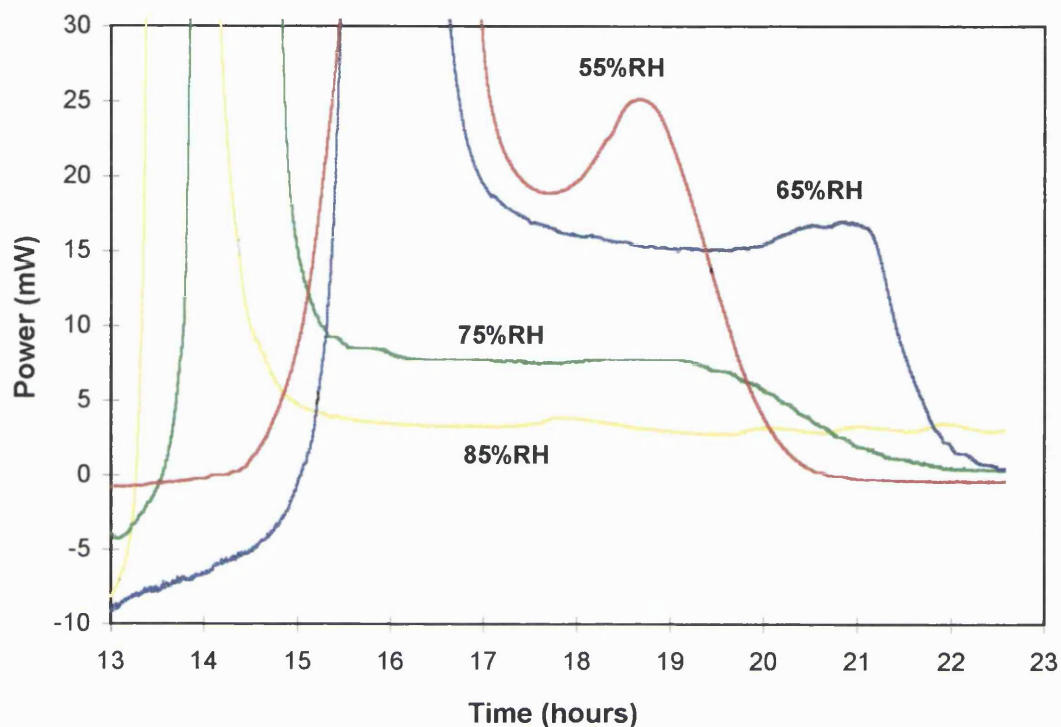


Figure 3.4 The effect of varying %RH on the magnitude of peak c.

3.1.3.5 Measuring the magnitude of the crystallisation response.

If the area under the curve is measured for stages b and c combined, it is found to be 48.0 J/g ($n=5$, ± 1.65), indicating this to be a very reproducible technique. This is equivalent to the total heat output for the process, i.e. the heat of crystallisation. This is probably also measuring a combination of other processes such as water desorption following crystallisation (endothermic response) and condensation back into the salt solution (exothermic response). Sebhatu et al (1994) reported a value of 32.0 J/g for stage 2 of this response. All peak area calculations performed here and from here onwards are on sections b and c combined.

3.2 FACTORS AFFECTING THE ISOTHERMAL MICROCALORIMETRY CRYSTALLISATION RESPONSE.

3.2.1 EFFECT OF SAMPLE SIZE ON THE CRYSTALLISATION RESPONSE

3.2.1.1 Introduction.

Since it was possible to follow the crystallisation of the amorphous lactose as it was occurring in the glass ampoule, it was decided to optimise the operating conditions for the experiment, by investigating such experimental variables as sample load.

3.2.1.2 Method.

The effect of sample size on the crystallisation response was investigated by carrying out experiments using 10, 20, 30, 40, 50 and 60mg samples of spray dried lactose. The experiments were set up and run exactly as described earlier using a potassium chloride (KCl) saturated salt solution which gave 85%RH at 25°C.

3.2.1.3 Results and Discussion.

The microcalorimetry crystallisation responses for the 10, 20, 30 and 40mg samples are shown in Figure 3.5. It can be seen from this figure that the effect of increasing sample size is to increase the delay time before crystallisation occurs. The time for crystallisation to occur, increased from just over 1 hour for the 10mg sample to approximately 5 hours for the 40mg sample, under the conditions used here. The relationship between sample size and crystallisation time is shown in Figure 3.6, where it can be seen that crystallisation time is proportional to sample size. It was not possible to measure a sample larger than 60mg using the present experimental conditions i.e. 85%RH and using the lowest sensitivity of 3000 μ W, since the resulting crystallisation peak was off-scale.

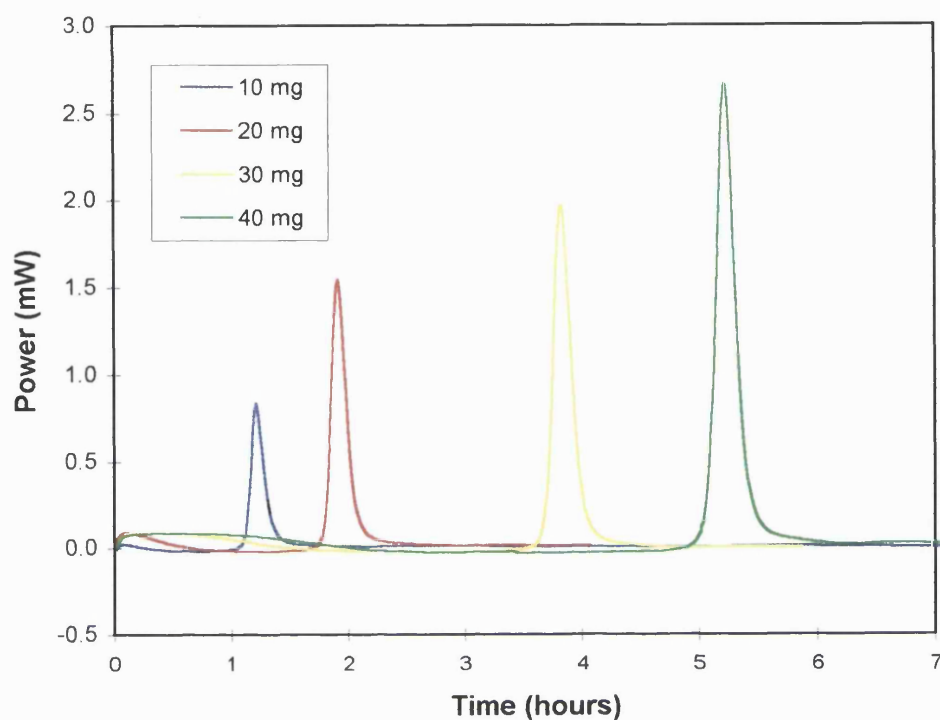


Figure 3.5. Microcalorimetry crystallisation responses for 10, 20, 30 and 40mg spray dried lactose at 85%RH.

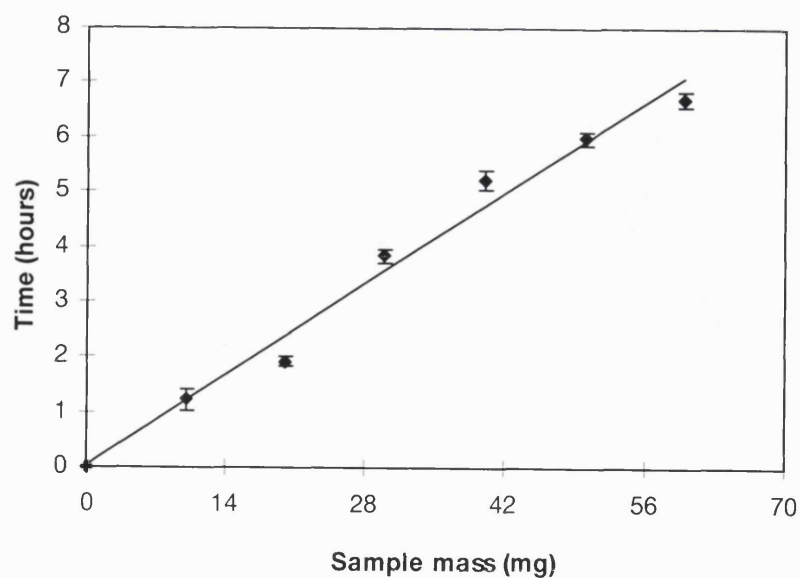


Figure 3.6 Graph showing the relationship between sample size and crystallisation time.

The longer time required for crystallisation to occur for larger sample sizes agrees with the idea that the sample must be fully saturated before crystallisation occurs. The larger sample masses will take longer to become fully saturated thereby delaying the onset of crystallisation.

If the area under the curves (as measured above for stages b and c) are calculated for each sample then they are found to be 44.9 J/g ($n=3, \pm 2.35$), 46.9 J/g ($n=3, \pm 1.33$), 47.8 J/g ($n=3, \pm 1.65$) and 47.3 J/g ($n=3, \pm 2.05$) for the 10, 20, 30 and 40mg samples respectively. The values obtained for the larger sample weights are probably more accurate since a larger peak was obtained and it was sufficiently separated from the initial wetting peak, and there will be less error due to weighing smaller quantities.

3.2.1.4 Conclusion

From the above results it would seem appropriate to use a sample mass of around 25mg, at this %RH. This would be appropriate for a number of reasons including weighing of the sample, where errors due to weighing too small samples will be reduced. The main crystallisation peak will also be sufficiently separated from the initial wetting peak, and the time for crystallisation to occur would be around 3 hours.

3.2.2 EFFECT OF VARYING %RH ON THE CRYSTALLISATION RESPONSE.

3.2.2.1 Introduction.

It appeared from the experiments carried out in section 3.2.1 that the delay in crystallisation for larger samples is due to the delay in saturating the powder bed. It therefore seems probable that this delay period is related to both the humidity of the air and the rate of supply of the water vapour to the air, to replace that which has been absorbed by the amorphous material. It was therefore decided to investigate this further by looking at the effect of changing the %RH in the measuring ampoule and to find an optimum %RH at which to carry out further experiments.

3.2.2.2 Method.

Experiments were carried out using a range of different saturated salt solutions to give a range of humidities (Nygqvist, 1983) as shown in Table 3.1. The sample weight was kept constant, at 20mg, for all the experiments, and the equilibrium time in the microcalorimeter, before measurement, was 30mins. as mentioned earlier.

SALT	%RH (25°C)
Potassium Sulphate	97
Ammonium Dihydrogen Orthophosphate	93
Zinc Sulphate	90
Potassium Chloride	85
Sodium Chloride	75
Sodium Nitrite	65
Sodium Dichromate	54
Potassium Thiocyanate	47

Table 3.1 Saturated salt solutions and their corresponding %RH at 25 °C

3.2.2.3 Observations and discussion.

Typical crystallisation responses for spray dried lactose samples exposed to 54%, 65%, 75% and 85%RH are shown in Figure 3.7. It can be seen that the effect of changing the %RH is to either increase or decrease the time for crystallisation to occur, with the crystallisation time increasing as %RH is decreased. The 20mg sample crystallised after about 2 hours at 85%RH, and this delay time before crystallisation increased to 3 hours at 75%RH, 5 hours at 65%RH and 7 hours at 54%RH. No crystallisation response was observed for the sample exposed to 47%RH within the time scale of the experiment which was 99 hours. The longer time required for crystallisation to occur at lower humidities is probably due to the sample taking longer to become fully saturated, due to a slower supply of water vapour at these lower humidities. Figure 3.8 shows the relationship

between %RH and crystallisation time and it can be seen that crystallisation time is inversely proportional to %RH.

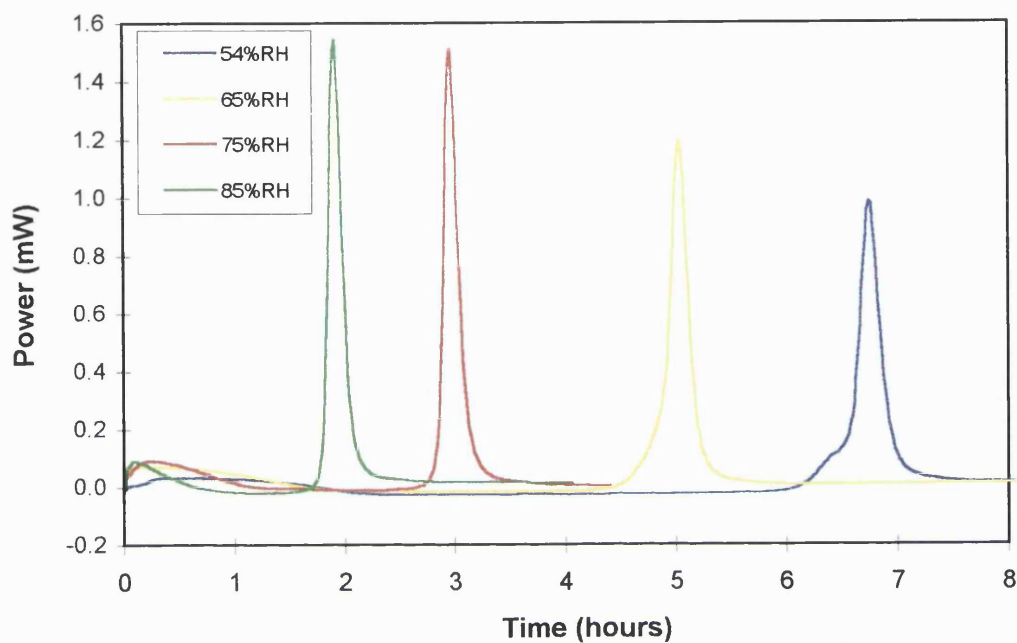


Figure 3.7. Crystallisation responses for spray dried lactose at 54%, 65%, 75% and 85%RH.

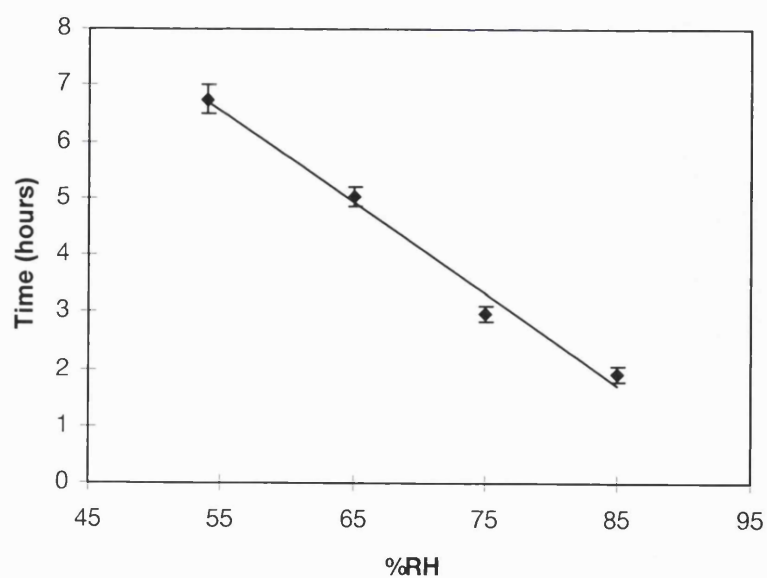


Figure 3.8 Graph showing the relationship between %RH and crystallisation time

The peaks are still sharp as observed previously indicating that the same process is taking place. At the lower %RH where the supply of water vapour to the samples will be much slower, the peak shape has changed slightly with a progressively broader peak being observed at the lower humidities.

The areas under the curves were measured and were found to be 48.0 J/g, exactly the same as those obtained for the experiments carried out using different weights of material. This indicates that the crystallisation process is again identical in each case.

3.2.2.4 Conclusion

From the above results it would seem appropriate to use a salt solution which gave a relative humidity of around 75%, for the sample weight used here which was 20mg. The main crystallisation peak will be sufficiently separated from the initial wetting peak, and the time for crystallisation to occur would be around 3 hours.

3.3 THE INFLUENCE OF ADDITIVES ON THE CRYSTALLISATION PROCESS.

3.3.1 INTRODUCTION

Since microcalorimetry can be used to detect and measure crystallisation of the amorphous material as it is occurring, it was decided to investigate the process more closely, with a view to a better understanding of water movement and transport in these samples. The results obtained and described so far indicate that all the amorphous material in a sample crystallises at the same time, rather than being a gradual process. This indicates that the water is transferred throughout the powder mass rather than remaining in one region to cause localised saturation and that the whole sample then crystallises simultaneously. The purpose of the following experiments was to investigate this cooperative crystallisation process, especially with respect to the influence of additives.

Van Scoik and Carstensen (1990) showed that small amounts of various organic substances, including gelatin, raffinose and lactose can have a significant effect on the nucleation rate of amorphous sucrose. Saleki-Gerhardt and Zografi,

(1994) and Hancock and Zografi (1997) both stated that the crystallisation of an amorphous material depends on two steps. Nucleation must first occur, then the nuclei must grow, therefore it follows that if nucleation can be inhibited by the addition of small amounts of additives then the stability of the amorphous material may be increased. Van Scoik found that the onset time for moisture loss due to crystallisation of the amorphous sucrose was increased considerably depending on the gelatin content of the sample, due to the latter having a strong tendency to hydrogen bond with any available water, thus impeding the movement and collision of sucrose molecules necessary for nucleation.

Saleki-Gerhardt and Zografi (1994) also observed that the onset of crystallisation of sucrose was delayed by colyophilization with low levels (1-10% w/w) of additives such as lactose, trehalose and raffinose. These additives all have T_g values greater than that of sucrose and hence their potential to inhibit crystallisation through antiplasticisation effects.

The additives chosen to be investigated in this study included both a hydrophobic and a hydrophilic material and an inert control. The experimental set-up involved placing a layer of the material being investigated in between two layers of spray dried lactose, as shown in Figure 3.9 and then allowing this sample to crystallise in the microcalorimeter as before. Physical mixes of the spray dried lactose and the additives were also investigated.

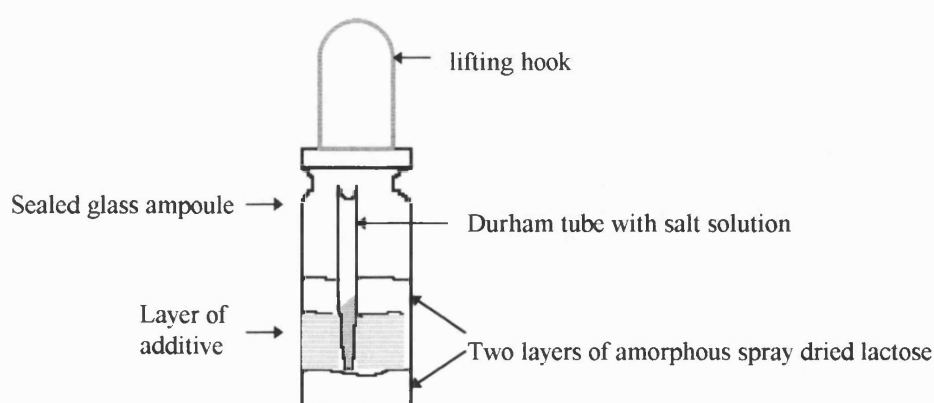


Figure 3.9 Diagrammatic representation of sample set-up for spray dried lactose and additives with Durham tube containing saturated salt solution.

3.3.2 THE EFFECT OF AN INERT MATERIAL

3.3.2.1 Material

The inert material which was chosen to act as a control was glass beads (Ballotini, Jencons no. 18, median diameter 66 μ m).

3.3.2.2 Method

The samples were prepared by first weighing approximately 14mg of spray dried lactose into a glass ampoule. A layer of glass beads (1.145g and depth 5mm) was then placed on top of the lactose and on top of this was placed another 14mg of lactose. These samples were then sealed with Durham tubes of NaCl saturated salt solution to give 75%RH, in the glass ampoules, and were equilibrated in the microcalorimeter as before. The experiment was then allowed to run overnight.

3.3.2.3 Results and discussion

The crystallisation responses for spray dried lactose and glass beads are shown in Figure 3.10, along with the response for spray dried lactose alone. The normal crystallisation response as seen previously for the amorphous lactose is a sharp response, occurring after the whole powder bed has become fully saturated with the water vapour. The response obtained for this sample containing lactose and glass beads is also a single sharp peak, which is only different to the response for lactose alone in that there is a slightly longer time delay before crystallisation occurs. A slightly broader peak with a lower peak height was obtained, indicating a possible change in kinetics for the crystallisation process. However the area under the curve was found to be 48J/g, which is the same as was obtained for the lactose alone.

It is clear therefore that the presence of an inert material between the two layers of lactose does not change the cooperativity of the process, with the entire lactose sample crystallising at the same time. However, it is possible from this data to speculate further on the exact nature of the crystallisation process. The rate of crystallisation will be influenced by the rate of supply of water vapour, water sorption by the amorphous lactose sample and transport of water within the sample. From Figure 3.10 it can be concluded that the rate of transport of water

from the upper layer to the lower one is faster than the rate of supply of water to the top one to replace that which has been transported away from the surface. Consequently, as the bottom layer approaches saturation the top layer will begin to crystallise releasing sorbed water which will rapidly complete the saturation of the bottom layer allowing the whole sample to crystallise together. A probable explanation for the slightly longer delay before the onset of crystallisation here may be that there is a delay in the entire powder bed becoming fully saturated because of the layer of glass beads.

3.3.3 THE EFFECT OF A HYDROPHOBIC MATERIAL.

3.3.3.1 Material

The hydrophobic material chosen was magnesium stearate, which is a fine white powder that is widely used in pharmaceutical formulations, primarily as a lubricant in capsule and tablet manufacture at concentrations between 0.25-1.0%. It's water sorption profile is shown in Figure 3.11. It's hydrophobic nature is confirmed here with it taking up around 1% water at 70%RH and reaching a maximum water uptake of around 3.5% at 90%RH, as can be seen from Figure 3.11.

3.3.3.2 Method

The samples investigated were prepared as for the control described earlier, with a layer of magnesium stearate being placed in between two layers of spray dried lactose. The weights of lactose and magnesium stearate used are listed in Table 3.2.

Wt. Mag. Stearate (mg)	wt.lactose (top layer) (mg)	wt.lactose (bottom layer) (mg)	wt.lactose (mix) (mg)	Cryst. time (hours)
0.0	-	-	35.0	4.8
93.1	17.0	17.0	-	6.1
122.3	17.0	17.0	-	7.1

Table 3.2 Weights and crystallisation responses for lactose and magnesium stearate samples.

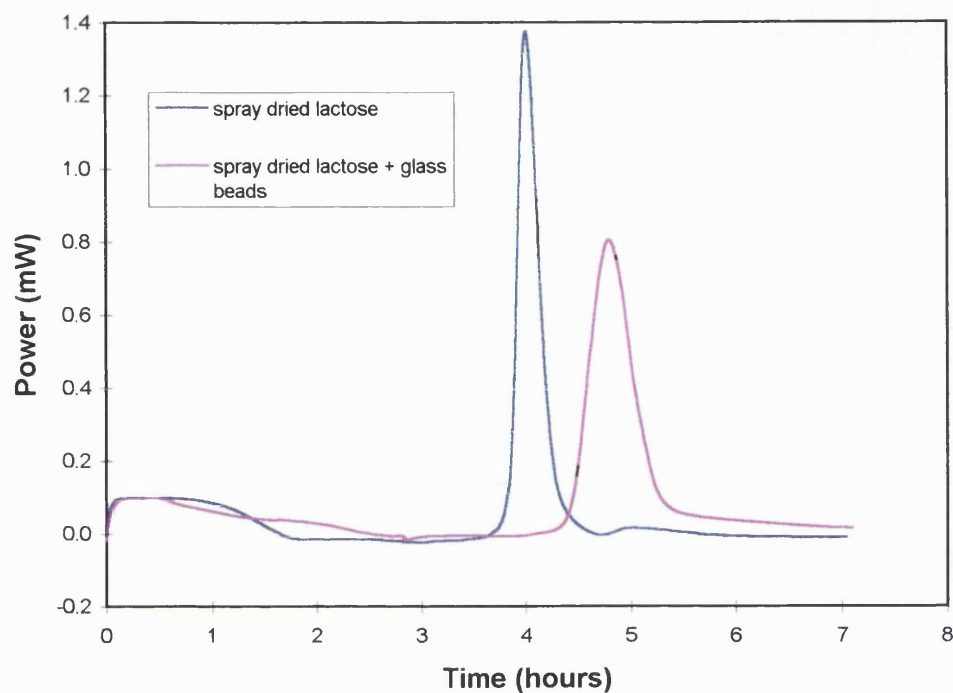


Figure 3.10 Microcalorimetry crystallisation response for spray dried lactose and glass beads.

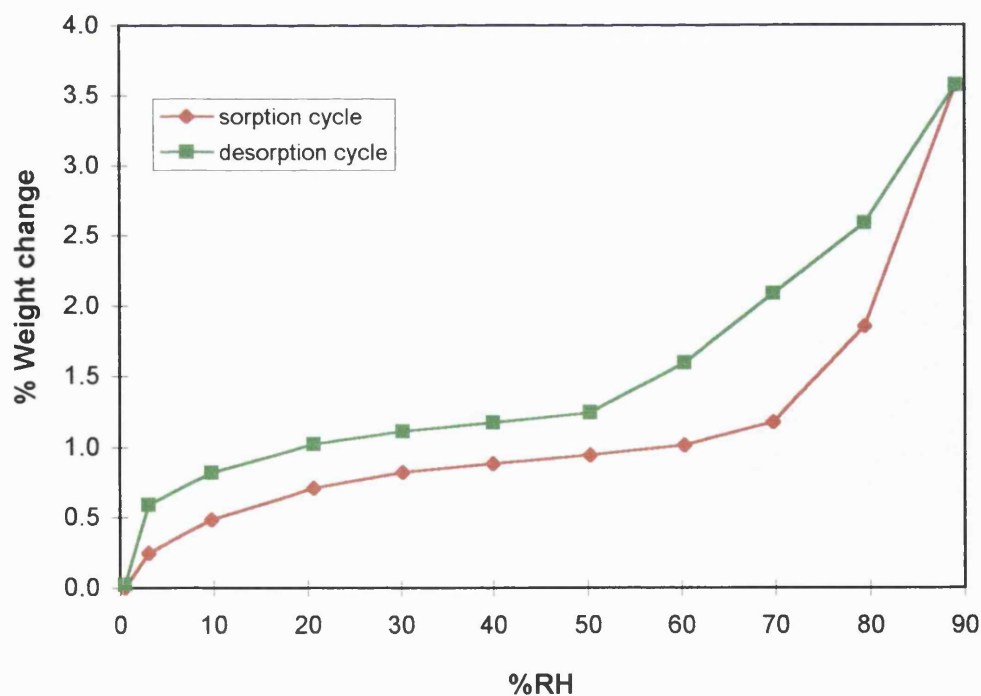


Figure 3.11 Water sorption and desorption isotherms for magnesium stearate.

3.3.3.3 Results.

The responses obtained for the spray dried lactose samples separated by a layer of magnesium stearate are shown in Figure 3.12, along with the response for lactose alone. It can be seen that a single crystallisation response was observed for all the samples investigated. However, the effect of having a layer of this hydrophobic material in between the 2 layers of amorphous lactose is to delay the onset of crystallisation. The sample which contained 93mg of magnesium stearate crystallised at around 6 hours (2 hours longer than the lactose alone under these conditions), and the sample containing 122mg crystallised at approximately 7 hours, a delay of 3 hours on the normal time for lactose to crystallise alone. Therefore the greater the amount of magnesium stearate present, the longer the delay before crystallisation. From these results it is possible to say that the magnesium stearate is not affecting the instantaneous rapid crystallisation process, since a single peak is obtained. The same heat change accompanied the crystallisation process here, with the peak areas still measuring 48 J/g.

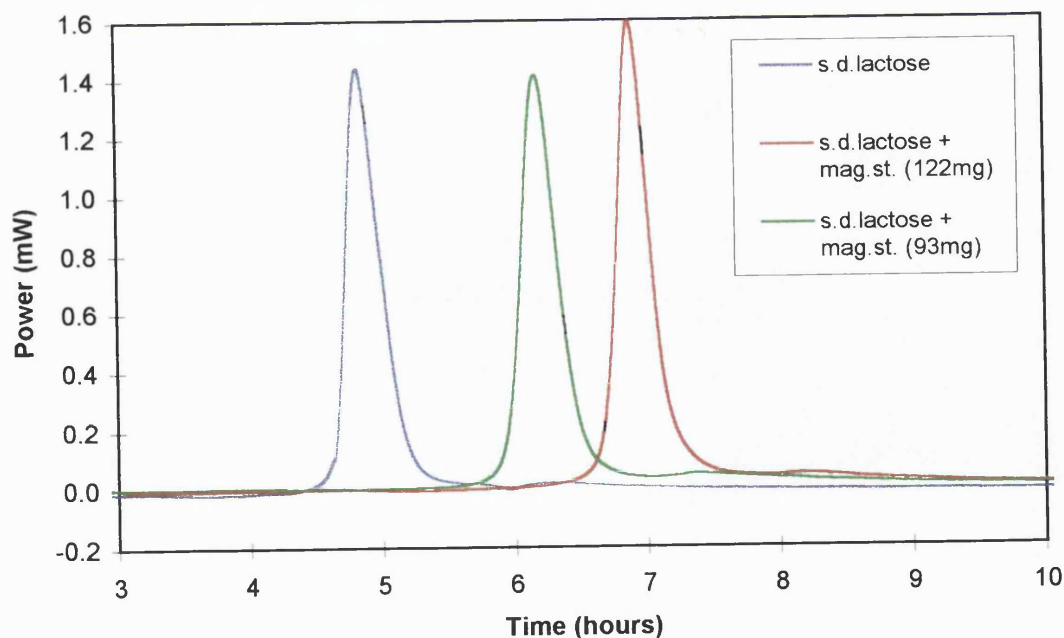


Figure 3.12 Microcalorimetry crystallisation responses for spray dried lactose and magnesium stearate

3.3.3.4 Discussion

Magnesium stearate, as mentioned previously, is a hydrophobic material which only took up 1% water at relative humidities up to 70%RH, as can be from Figure 3.11. However, its effect here is to slow down the crystallisation process. This indicates that the onset of crystallisation will be influenced by the rate of supply of water vapour to the sample, since the delay in crystallisation here is probably due to a delay in the bottom layer of lactose becoming saturated, as discussed in section 3.3.2.3. Crystallisation will therefore be influenced by the rate of absorption and movement of the water vapour through the powder bed. It is unlikely that the two layers are absorbing moisture simultaneously i.e. they have equal access to the water, since the presence of the magnesium stearate has an obvious effect on the kinetics of the process due to the longer delay time before crystallisation than for lactose alone. Therefore, it is probable that the rate of loss of water from the top layer is faster than the rate of uptake of water by the bottom layer, with crystallisation occurring simultaneously throughout the entire sample when the bottom layer is also saturated.

3.3.4 THE EFFECT OF A HYDROPHILIC MATERIAL.

3.3.4.1 Material

The hydrophilic material chosen was microcrystalline cellulose, and the type used here was ©Avicel PH101. This is a partially depolymerised white crystalline powder, composed of porous particles, widely used in the pharmaceutical industry as a diluent in oral tablet and capsule formulations, where it is used in both wet granulation and direct compression processes. It also has some lubricant and disintegration properties. Its water sorption profile is shown in Figure 3.13. Its very hydrophilic nature can be seen here, with it reaching a maximum water uptake of around 13% at 90%RH.

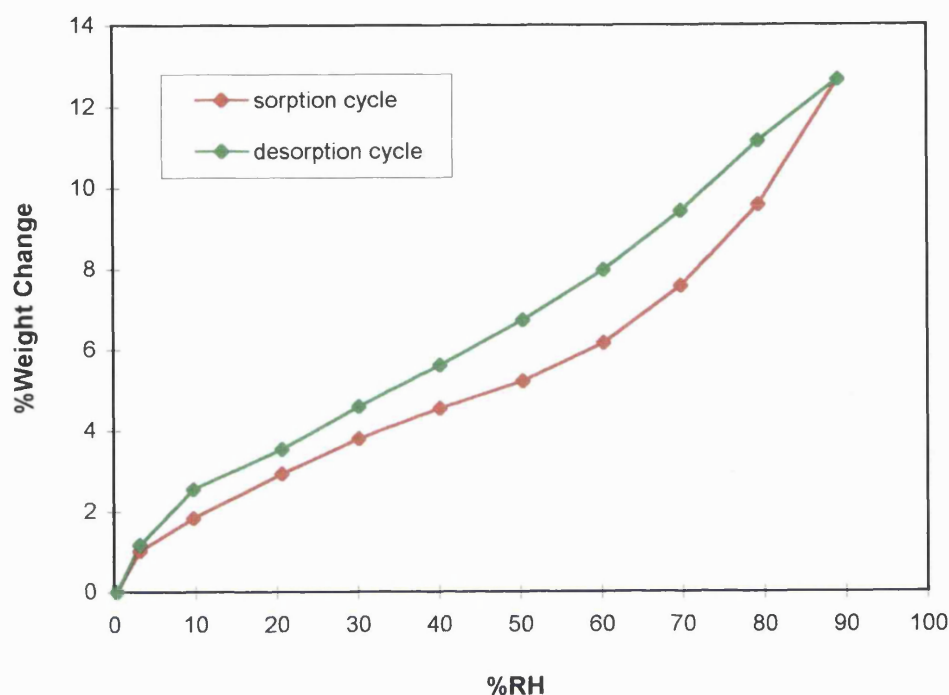


Figure 3.13 Water sorption and desorption isotherms for ©Avicel PH101.

3.3.4.2 Method

The samples here were prepared as for the control described earlier, with a layer of ©Avicel PH101 being placed in between two layers of spray dried lactose. The weights of lactose and ©Avicel PH101 used are listed in Table 3.3. Samples were also prepared here where the amount of lactose in the top and bottom layers was varied. Homogenous mixes of lactose and ©Avicel PH101 were also prepared in order to compare the crystallisation response for these with that of samples where two distinct layers of lactose were present.

3.3.4.3 Results.

3.3.4.3.1 Effect on crystallisation onset.

The responses obtained for samples containing two identical layers of spray dried lactose separated by a varying layers of ©Avicel PH101 are shown in Figure 3.14. The responses for a physical mixture of ©Avicel PH101 and lactose and lactose alone are also shown.

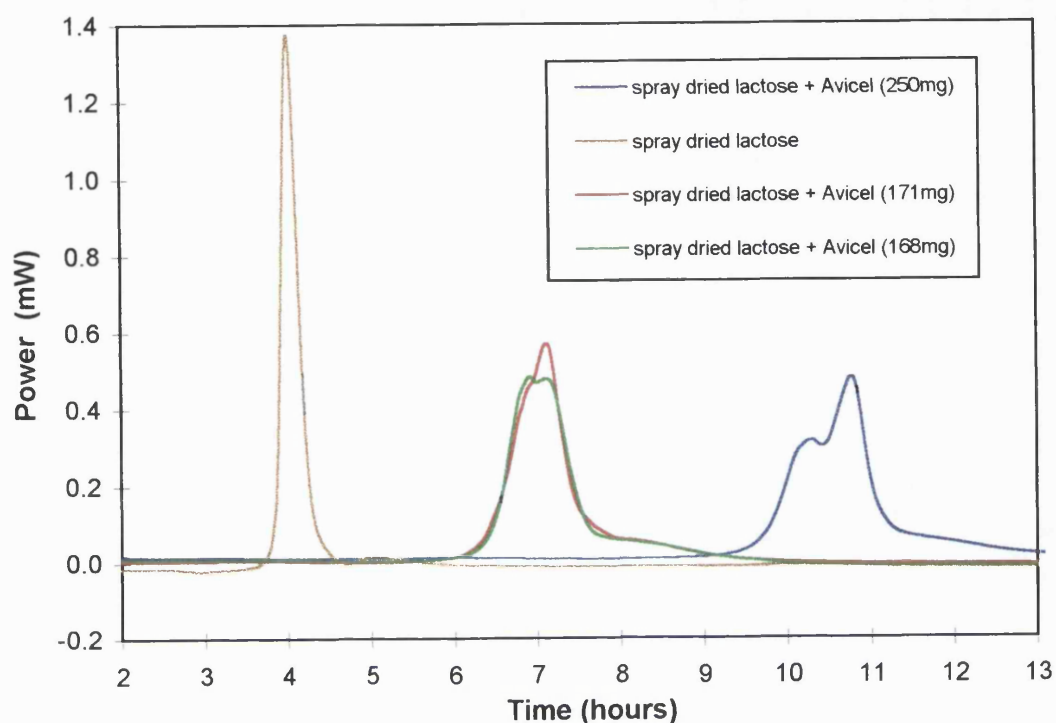


Figure 3.14 Microcalorimetry crystallisation responses for spray dried lactose and microcrystalline cellulose (©Avicel PH101) samples.

wt. ©Avicel (mg)	wt.lactose (top layer) (mg)	wt.lactose (bottom layer) (mg)	wt.lactose (mix) (mg)	Cryst. Time (hours)
0.0	-	-	27.5	4.5
120.0	-	-	27.5	6.5
168.0	13.7	13.7	-	7.0
171.0	13.7	13.7	-	7.0
250.0	13.7	13.7	-	10.0-11.0

Table 3.3 Weights and crystallisation responses for lactose and microcrystalline cellulose samples.

It can be seen that the effect of the layer of microcrystalline cellulose is to substantially increase the lag time before the onset of crystallisation, with increasing amounts of ©Avicel resulting in an increased delay time before crystallisation. The sample which contained the physical mix of spray dried lactose and ©Avicel PH101 crystallised approximately 3 hours later than the lactose alone. The samples which contained a layer of approximately 170mg of ©Avicel took slightly longer than the physical mixture to crystallise, and this delay time increased to around 7 hours for the sample which contained 250mg ©Avicel with crystallisation taking 11 hours to occur.

The long delay before crystallisation for these samples which contain ©Avicel is due to the hydrophilic nature of the ©Avicel, which results in it sorbing large amounts of moisture (which can be seen from it's sorption isotherm in Figure 3.13). This results in a delay in the amorphous lactose becoming saturated because of the competition for the water vapour whose supply is limited by the rate of evaporation from the saturated salt solution, hence the delay in the actual crystallisation process.

3.3.4.3.2 Effect on spontaneous crystallisation process.

It can also be seen from the data shown in Figure 3.14 that the samples containing ©Avicel appear to have 2 peaks present for the actual crystallisation response, whereas normally a very sharp peak is obtained. This may indicate that the 2 layers of amorphous lactose may be crystallising at slightly different times. It was decided to investigate this double peak phenomenon further by varying the amounts of lactose present in the top and bottom layers of the sample. The weights of ©Avicel and spray dried lactose used in these experiments are listed in Table 3.4, along with crystallisation times and peak areas for these samples. The expanded crystallisation responses are shown in Figure 3.15 for the samples which contained a homogenous mixture of the lactose and ©Avicel (250mg), and the samples which contained either a small top layer and larger bottom (or vice versa) of lactose separated by 250mg ©Avicel.

wt. ©Avicel (mg)	wt.lactose (top layer) (mg)	wt.lactose (bottom layer) (mg)	wt.lactose (mix) (mg)	cryst. time (hours)
0.0	-	-	27.5	4.5
250.0	-	-	27.5	11.0
250.0	8.3	21.4	-	10.0 - 12.0
250.0	13.7	13.7	-	10.0 - 11.0
250.0	20.0	7.1	-	16.0 - 18.0
250.0	21.0	21.7	-	48.0 - 50.0

Table 3.4 Microcalorimetry crystallisation responses for varying combinations of spray dried lactose separated and ©Avicel.

The first sample to crystallise was the one which contained 250mg of ©Avicel separating equal layers of spray dried lactose, which is followed quite closely by the sample with the smaller top layer of lactose. The homogenous mixture also crystallised at a similar time to these two. The sample containing the largest top layer took longest to crystallise i.e. around 16 hours. The response for the homogenous mixture is clearly only a single sharp peak, followed by the much smaller mutarotation / condensation peak described earlier. The 3 layered samples all show definite signs of 2 peaks being present as mentioned above and this can be seen more clearly in Figure 3.15, where the time axis has been expanded. It seems for the samples with 2 peaks present that the top layer may be crystallising first and this is being followed very soon afterwards by the bottom layer. This is evidenced by the fact that for the sample with the smaller top layer there is a smaller peak followed by a larger peak, which is probably due to a slight delay in the larger bottom layer crystallising. This phenomenon is then reversed for the sample containing a larger top layer where a larger first peak is observed, followed by a smaller peak (due to the smaller bottom layer crystallising). It would appear therefore from this data that the crystallisation of the two layers is occurring at slightly different times, with the top layer crystallising just before the bottom layer.

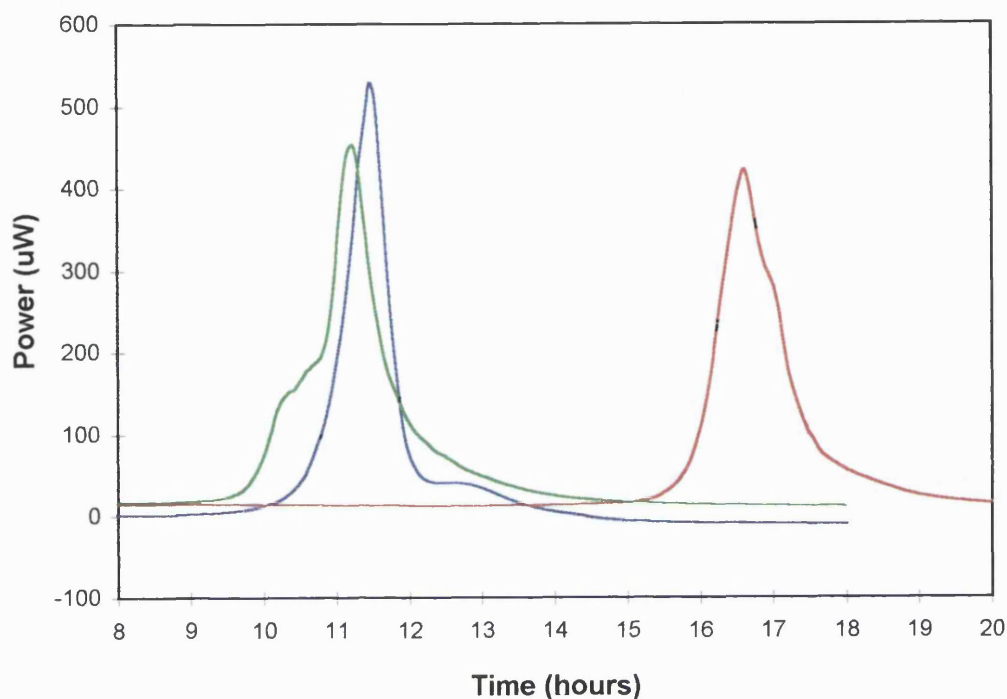


Figure 3.15 Microcalorimetry crystallisation responses for spray dried lactose (varying top and bottom layers) and ©Avicel PH101 samples.

- A** - Spray dried lactose (8.3mg **top**) and (21.4mg **bottom**) + Avicel (250mg)
- B** - Spray dried lactose (27.4mg) + Avicel (250mg) **mixture**
- C** - Spray dried lactose (20.0mg **top**) and (7.1mg **bottom**) + Avicel (250mg)

The fact that there is a much increased delay time before crystallisation for the sample that contained the larger top layer, indicates that water distribution and subsequent crystallisation in these samples depends on where the water is initially taken up and how it is transported through the powder bed. The kinetics of water distribution may well change depending on the size of the top layer i.e. where the water is first taken up. The sample containing the larger top layer is probably taking a longer time to crystallise due to this larger layer taking longer to saturate, since water will also be absorbing into the ©Avicel and the bottom layer of lactose. Once the top layer crystallises and water is expelled, this will

give a rapid supply of water to the ©Avicel and the smaller bottom layer. This bottom layer will probably become saturated quite quickly and then crystallise very soon after the top layer. This effect is further supported by the sample which contained 20mg of lactose in both the top and bottom layers, which didn't crystallise until 48 hours (see Table 3.4). The crystallisation peak for this sample also showed some indication of 2 peaks being present but it appeared that the crystallisation of the two layers was still occurring at almost identical times. Again, the much increased delay time before crystallisation is probably due to a delay in the similarly large bottom layer becoming saturated (once the top layer is saturated), due to competition with the ©Avicel for water vapour, whose supply will be controlled by the rate of evaporation from the saturated salt solution.

3.3.4.3.3 Water uptake and transport theory.

It appears therefore from the data presented in this investigation that water concentration (once the sample begins absorbing moisture) is greatest at the surface of these amorphous samples, probably resulting in a gradient throughout the sample. The top layer doesn't saturate immediately due to the faster rate of transport of water into the powder bed than supply from the salt solution, as indicated by the fact that single crystallisation responses were always seen for samples containing lactose layers which were physically separated, indicating that the layers were crystallising at virtually identical times. The bottom layer may not be fully saturated when the spontaneous crystallisation process begins, but will quickly become saturated due to the increased availability of water vapour which is expelled from the top layer as it crystallises. Crystallisation onset will therefore be controlled by the rate of saturation of the bottom layer. Once this layer begins to reach saturation the top layer will begin to crystallise thus releasing sorbed water which will lead to total saturation and consequently total crystallisation.

3.4 MOISTURE SORPTION STUDIES ON SPRAY DRIED LACTOSE USING DYNAMIC VAPOUR SORPTION (DVS)

3.4.1 INTRODUCTION

Vapour sorption studies employing commercially available computer-controlled moisture balances are now being used by an increasing number of laboratories to investigate and follow water uptake and subsequent crystallisation of amorphous materials, by observing the weight loss due to desorption of water on the crystallisation of the amorphous material. Ward and Schultz (1995) carried out water vapour sorption studies using an automated microbalance system, to investigate the moisture content necessary to bring about the amorphous to crystalline conversion of salbutamol sulphate. Schmitt et al (1996) used a similar set-up to obtain moisture sorption isotherms of a freeze-dried and spray-dried drug substance, and were able to observe the crystallisation of these materials by observing the weight loss due to water desorption from the amorphous regions on crystallisation.

3.4.2 METHOD

The gravimetric studies were undertaken using the dynamic vapour sorption apparatus (DVS) as described in Chapter 2. The spray dried lactose samples were weighed directly onto the sample pan. Weights in the region of 20mg were normally used and the exact weight was recorded by the computer, before commencing the experiment. The experimental method was set up such that the samples were initially held at 0%RH for 1hour. The %RH was then increased in 10%RH steps up to 90%RH and then decreased back down to 0%RH. A dm/dt value of 0.001%/min. (requesting the mass change to be below 0.001% for a minimum time of 10mins.) was used at each %RH step, thus ensuring that equilibrium was reached at each %RH before the experiment proceeded to the next step. All measurements were carried out at 25°C.

3.4.3 RESULTS AND OBSERVATIONS

3.4.3.1 Water vapour sorption profile for spray dried lactose

A plot showing a typical sorption/desorption experiment for spray dried lactose sample is shown in Figure 3.16. The plot shows change in mass (mg) and %RH as a function of time (hours). There is an initial weight loss at 0%RH since the sample contains a small amount of absorbed water, also observed from TGA data as indicated in section 3.5.3. On increasing the %RH in 10% steps up to 30%RH there is a steady weight increase. At 30%RH there is a larger and gradual uptake of water taking place. At 40%RH there is a much larger biphasic type uptake of water indicating a possible morphological change beginning to occur in the sample. The large water uptake is characteristic of amorphous materials which are known to absorb large amounts of water into the amorphous regions, due to their higher energy state (Zografi, 1988).

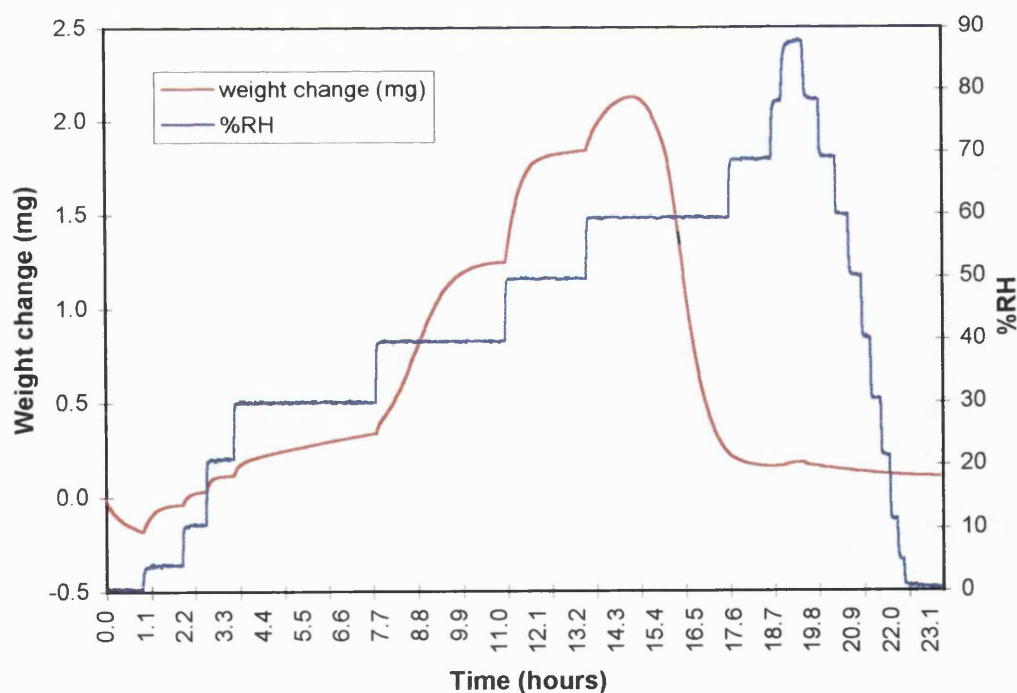


Figure 3.16 Moisture sorption and desorption data for spray dried lactose.

The weight increase continues up to 60%RH where there is a continued increase, followed by a large drop-off in weight. This dramatic weight loss suggests a physical change in the sample such as crystallisation of the amorphous material. It is well known that water is readily absorbed by amorphous materials whereby it acts as a plasticising agent lowering the T_g . The free volume of the sample is increased leading to greater molecular mobility, and when T_g falls below T of the experiment crystallisation will occur. This would correspond with the microcalorimetry observations in section 3.2 above where the amorphous lactose crystallised on exposure to relative humidities at and above 54%.

The dramatic weight loss seen in Figure 3.16 is due to water which was present in the amorphous regions being expelled from the sample on crystallisation. This occurs very quickly indicating that a certain water uptake must be achieved after which crystallisation will occur very rapidly. As the %RH is further increased up to 90%RH there is only very minimal water uptake, as expected for crystalline lactose (Berlin et al, 1970; Sheridan et al, 1994). There is also very minimal water loss on the desorption cycle i.e. as the %RH is decreased from 90% down to 0%RH. The sample when removed from the sample pan after the desorption cycle showed no response in the microcalorimeter when exposed to humidities above 54%, indicating it to be totally crystallised.

3.4.3.2 Water sorption/desorption isotherms for amorphous lactose.

In order to check if the sample was completely crystallised, 2 repeat sorption/desorption cycles were performed on the same sample before it was removed from the microbalance. The resulting isotherms for the 3 consecutive sorption/desorption cycles are shown in Figure 3.17, and the equilibrium % water content at each %RH for the three repeat cycles is presented in Table 3.5 along with hysteresis values for the sorption/desorption data at each %RH. The plot in Figure 3.17 shows %weight change as a function of %RH for the 3 repeat cycles. It can be seen that there is a difference between the first cycle and the other cycles with all cycles after the first showing superimposable responses. The higher weight increases for the first sorption cycles are a consequence of water being absorbed into the amorphous regions of the sample as well as being adsorbed onto the surface of the crystalline material as discussed earlier.

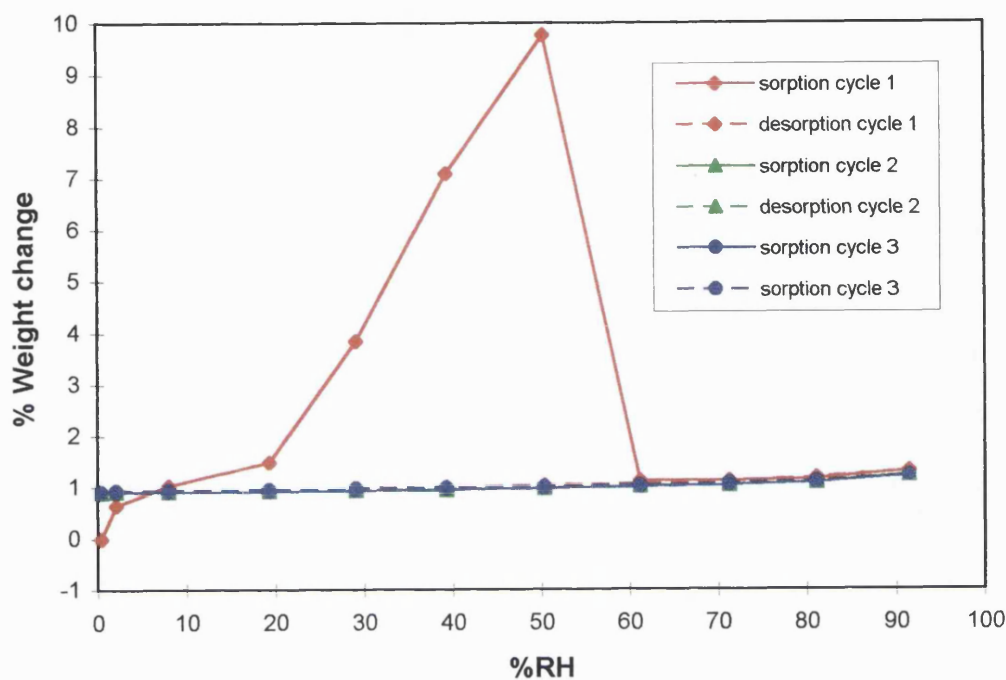


Figure 3.17 Moisture sorption and desorption isotherms for three repeat cycles of a spray dried lactose sample.

No dramatic weight loss was observed at or around 60%RH, as with the first sorption cycle. The second and third cycles show only minimal water uptake i.e. approximately 1% at 90%RH compared to a maximum water uptake of approximately 10% for the first cycle. This low water uptake for the second and third cycles is characteristic of surface adsorption by a crystalline species. The equilibrium moisture content of sample at the end of the first desorption cycle of around 1% is due to incorporation of hydrate water in the crystallised 100% amorphous lactose. This value of 1% is lower than would be expected if the sample crystallised to form a complete hydrate. Since α lactose monohydrate contains 5% water of hydration, then the expected weight increase over dry weight would be 5.26% if the fully hydrated form was obtained. Furthermore, repeat cycling of relative humidity up to 90%RH and desorption to 0%RH did not appear to result in formation of more hydrate. It must be concluded therefore

that the amorphous lactose does not crystallise to form a perfect monohydrate form. This is not expected to be due to an inadequate amount of water being present, since following crystallisation at 60%RH the RH is further increased to 90%RH, hence a plentiful supply of water for total hydration. Rather, it may be that incorporation of water into the crystallised form may occur over long periods of time, as observed by Angberg et al (1991). Although, as stated earlier, there is a plentiful supply of water, the construction of the experiment is such that the sample is only exposed to high RH's (>60%RH) for relatively short periods of time. This is due to the crystallised lactose adsorbing very small amounts of water, therefore equilibrium weight is reached quite rapidly.

%RH	Cycle 1			Cycle 2		
	sorption	desorption	hysteresis	Sorption	desorption	Hysteresis
0.4	0.000	0.902	0.902	0.902	0.902	0.000
2.1	0.647	0.929	0.282	0.904	0.927	0.023
8.0	1.032	0.944	-0.088	0.910	0.939	0.029
19.3	1.481	0.9615	-0.052	0.918	0.959	0.041
29.1	3.840	0.985	-2.856	0.935	0.975	0.040
39.3	7.090	1.008	-6.082	0.951	0.995	0.044
50.4	9.766	1.038	-8.728	0.981	1.018	0.037
61.2	1.120	1.076	-0.044	1.016	1.042	0.026
71.3	1.130	1.120	-0.010	1.044	1.075	0.031
81.1	1.166	1.188	0.022	1.091	1.128	0.037
91.5	1.314	1.314	0.000	1.233	1.233	0.000

Table 3.5 Equilibrium water content values for amorphous spray dried lactose for 2 repeat cycles from 0 – 90%RH.

3.4.3.3 Critical %RH and water content necessary to induce crystallisation in 100% amorphous lactose.

It has been shown from the earlier microcalorimetry investigations and is apparent from the gravimetric experiments carried out here that a region of critical relative humidity exists between 50 and 60%RH. The amorphous lactose didn't crystallise during microcalorimetry experiments when exposed to 47%RH, and the lowest RH salt solution available which resulted in crystallisation was the 54%RH one. It is apparent therefore the critical RH for the crystallisation process to occur at 25°C, is somewhere between these two points. It must be assumed that below this critical %RH the water content is not sufficient to plasticise the sample such that T_g reaches T and that above this point the sample has been sufficiently plasticised to allow T_g to drop below T , resulting in crystallisation of the amorphous sample. From the data presented in Table 3.5 it can be seen that the sample reaches a maximum water uptake of approximately 9.8% (at 50 - 60%RH) in the first sorption cycle before spontaneous crystallisation occurs. This amount of water uptake is equivalent to a 1.91mg weight increase for the 19.5mg sample investigated here, therefore the final sample weight is 21.41mg of which water is 8.9%. Since the amorphous lactose crystallises after this water uptake has been reached it is apparent therefore that this is the amount of water necessary to plasticise the sample such that crystallisation occurs. However, the sample being investigated is crystallising very rapidly once the critical water uptake has been reached, as shown by the dramatic weight loss at 60%RH, which indicates that it isn't actually at equilibrium and therefore contains more than sufficient water to plasticise the sample, and the T_g will be well below T . This is due to the experiment set-up and the constant supply of water vapour to replace that which has been sorbed, unlike in the microcalorimetry experiments where the water sorption by the sample is dependent on rate of evaporation from the salt solution which would be much slower. This phenomenon will be discussed further in Chapter 5 where water movement at and around the T_g and collapse of the amorphous structure prior to crystallisation is discussed.

3.5 PHYSICAL CHARACTERISATION OF SPRAY DRIED LACTOSE BEFORE AND AFTER CRYSTALLISATION.

3.5.1 X-RAY POWDER DIFFRACTION.

The spray dried lactose sample was analysed using a Scintag XDS200 X-ray diffractometer and the resulting trace is shown in Figure 3.18 (a). The broad response obtained which had no obvious peaks present is characteristic of amorphous materials and therefore indicates the spray dried lactose to be totally amorphous. A spray dried lactose sample which was removed from the microcalorimeter after the crystallisation peak was also analysed by X-Ray diffraction. This was shown to be crystalline, see Figure 3.18 (b), as evidenced by the sharp peaks which are characteristic of crystalline materials, thus confirming that the lactose has indeed crystallised on exposure to the saturated salt solution in the microcalorimeter. Figure 3.18 also shows XRPD patterns for α lactose monohydrate (c) and β lactose (d) standards and it can be seen that the crystallised lactose sample contains a mixture of both α and β lactose.

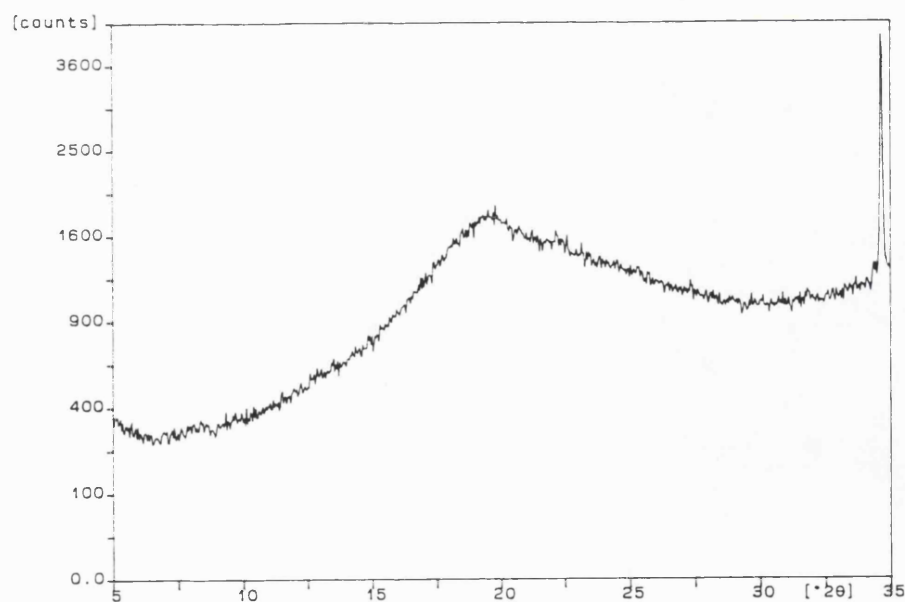


Figure 3.18 (a) X-Ray diffraction traces for amorphous spray-dried lactose

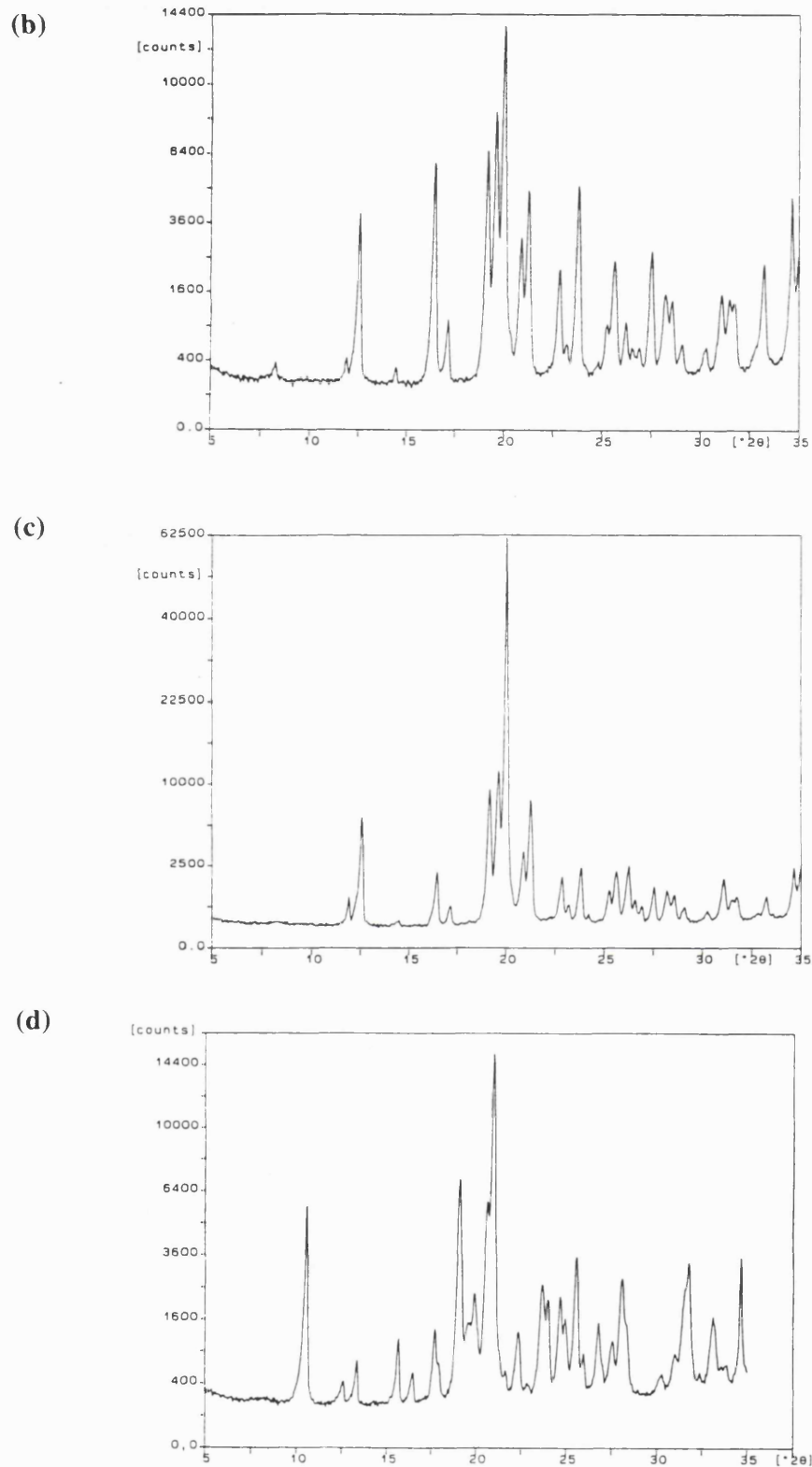


Figure 3.18 (b) – (d) X-Ray diffraction traces for (b) spray dried lactose after crystallisation in the microcalorimeter, (c) α lactose monohydrate and (d) β lactose.

3.5.2 DIFFERENTIAL SCANNING CALORIMETRY (DSC)

Spray dried lactose samples (before and after exposure to 75%RH in the microcalorimeter) were scanned in the DSC from 30°C to 250°C using 5mg of sample in non-hermetically sealed pans, at a scan rate of 10°/minute. The results of these scans can be seen in Figure 3.19, along with DSC traces for α and β lactose standards. The α lactose monohydrate standard has an exotherm at 150°C which is due to loss of the monohydrate water, and a melting exotherm at 218°C, which is due to α lactose melting. The β lactose standard has only one exotherm, at 238°C, which is the melting exotherm. These results are in agreement with the findings of Berlin et al (1971).

(a) original amorphous spray dried lactose

A broad endotherm is just visible from approximately 30°C to 100°C, which is due to loss of absorbed water from the sample. This is followed by an exotherm at approximately 187°C which is due to the amorphous lactose crystallising. Two subsequent endotherms are then observed at around 218°C and 238°C, which are due to melting of α and β lactose respectively, as seen from comparison to the α and β lactose standards. The peak at 218°C is considerably larger than that at 238°C indicating that the sample contains mostly α lactose.

(b) spray dried lactose after exposure to 75%RH in the microcalorimeter.

An endotherm is present at 150°C which is where α lactose monohydrate normally loses its hydrate water. Two endotherms are present again at around 218°C and 238°C as with the starting material, which are again due to melting of α - and β lactose respectively. However, the proportions of the α - and β - forms have changed considerably from those observed for the amorphous starting material. This sample contains a mixture of α - lactose monohydrate and β lactose. This indicates that the sample produced on crystallisation after exposure to 75%RH in the microcalorimeter contains some α lactose monohydrate and a substantial amount of the β - form. This is in agreement with recent reportings by Schmitt et al (1997) from their study on the crystallisation of dry and hydrated forms of amorphous lactose. Their studies indicated that crystallisation of spray dried lactose at RH's up to 57.5% primarily yielded the β anhydrous form of lactose.

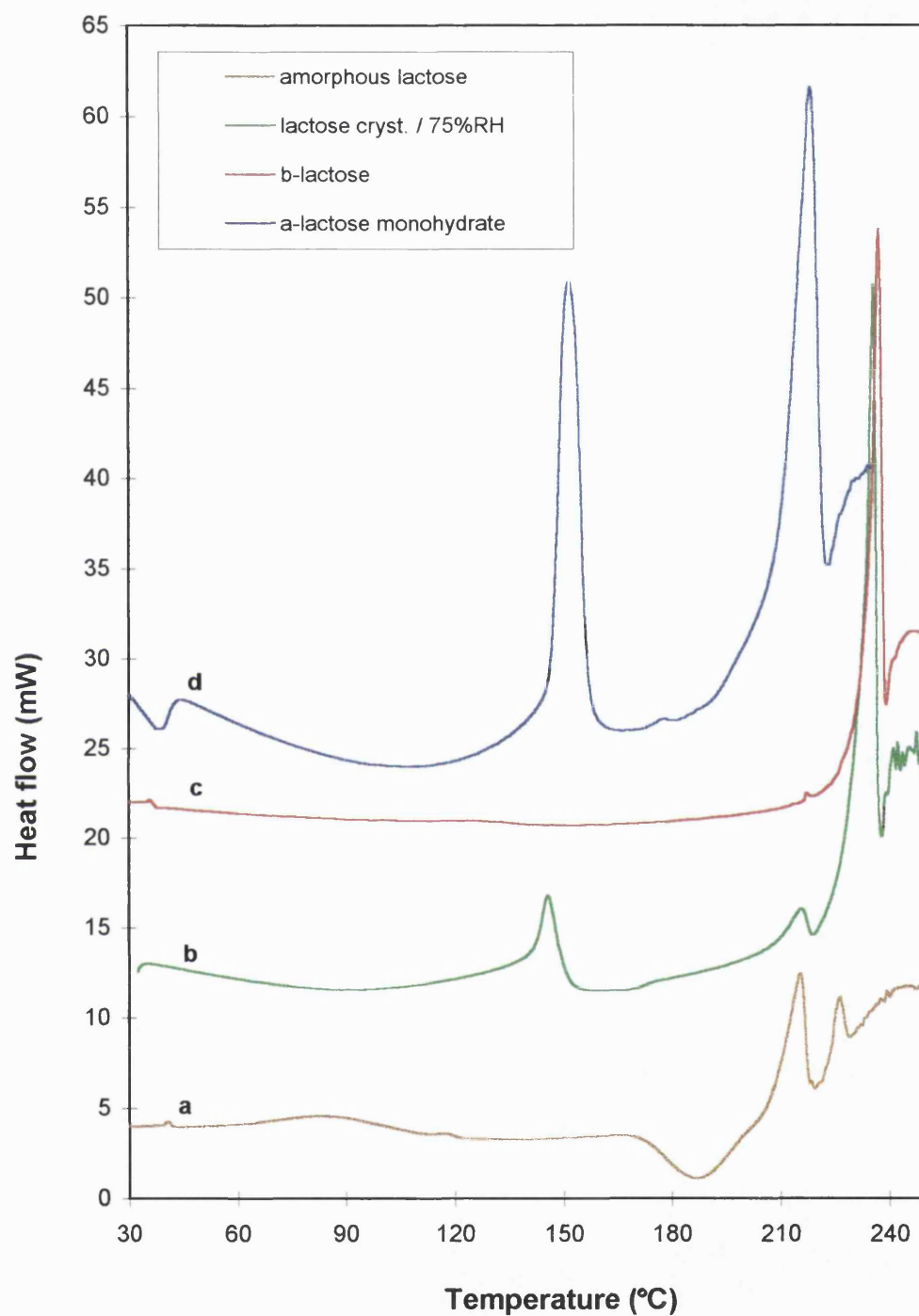


Figure 3.19 DSC scans for (a) spray-dried lactose, (b) spray dried lactose on removal from the microcalorimeter (after exposure to 75%RH), (c) α lactose monohydrate and (d) β lactose.

3.5.3 THERMOGRAVIMETRIC ANALYSIS (TGA)

Spray dried lactose samples (before and after exposure to 75%RH in the microcalorimeter) were scanned in the TGA from 30°C to 250°C using 5mg of sample in an open aluminium pan, at a scan rate of 10°/minute. The results of these scans can be seen in Figure 3.20, along with TGA results for α - and β lactose standards. The α lactose monohydrate standard shows a derivative weight loss of 5% at 150°C, which is loss of monohydrate water, which corresponds with the results reported by Berlin et al (1971). The β lactose standard doesn't show any weight loss.

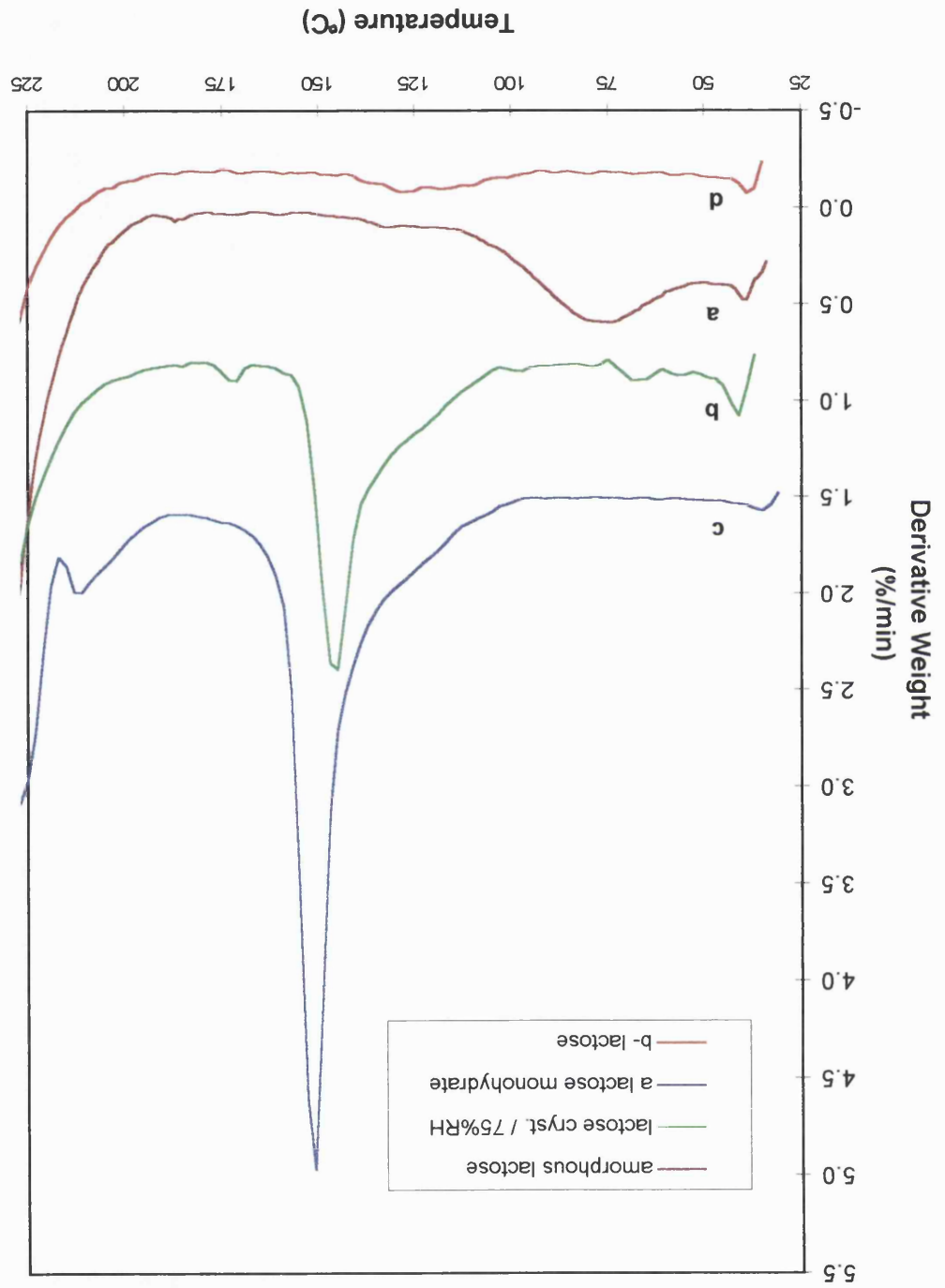
(a) original spray dried lactose

A large broad weight loss was observed from 50°C to 100°C which is due to loss of absorbed water from the amorphous lactose. This amounted to around 3% water loss, which is slightly higher (due to heating effects) than that observed during drying at 0%RH in the DVS studies.

(b) spray dried lactose after exposure to 75%RH in the microcalorimeter.

A derivative peak is present here at around 150°C which corresponds to that observed in the DSC and is due to loss of the hydrate water from the α lactose monohydrate sample. This again indicates that the crystallised product contains α lactose monohydrate. The magnitude of this peak was 3.1%, indicating that the sample wasn't fully hydrated, since α lactose monohydrate contains 5% water when fully hydrated i.e. the molecular weight of lactose monohydrate is 360g of which 5% is water of crystallisation. This observation that the sample is not fully hydrated is in agreement with the DSC data for this sample which showed that although there was some α - present, there was a substantial amount of the β - form present also. The β - form is known to exist only in the anhydrous form, but will mutarotate to α at very high relative humidities with subsequent incorporation of water (Berlin et al, 1971; Angberg et al, 1991).

Figure 3.20 TGA scans for (a) spray-dried lactose, (b) spray dried lactose on removal from the microcalorimeter (after exposure to 75%RH), (c) α lactose monohydrate and (d) β lactose.



3.6 CONCLUSIONS

The work carried out here has shown that amorphous spray dried lactose can be detected and studied by isothermal microcalorimetry and vapour sorption studies using an automated humidity controlled microbalance, by making use of its ability to absorb large amounts of water and convert to the crystalline form.

The following conclusions can be made from the experiments carried out:

- Isothermal microcalorimetry can be used to follow the 'real time' crystallisation of spray dried lactose on exposure to saturated salt solutions of specific relative humidity. A sharp instantaneous response was obtained and the accompanying heat change for the process was 48 J/g.
- Increasing sample weight increased the delay time before crystallisation due to the delay in the whole powder bed becoming saturated.
- Decreasing the $\%RH_A$ also increased the delay time before crystallisation due to the slower supply of water at lower RH's, and a critical relative humidity limit for the process was identified between 47% and 54%RH.
- The crystallisation process was seen to be influenced by the presence of additives where the onset of crystallisation was delayed depending on the hygroscopicity of the additive. Furthermore, a better understanding of the transmission kinetics of moisture through these powder beds and the mechanism for the cooperative crystallisation process was obtained.
- Water vapour sorption studies revealed important information on water uptake at different relative humidities and also indicated a critical %RH around 50 – 60%RH at which the amorphous to crystalline transition occurred. The water uptake necessary to plasticise the amorphous lactose prior to crystallisation was in the region of 9%.
- Thermal analysis, DSC and XRPD of the crystallised sample showed that a mixture of α lactose monohydrate and the β form were produced after crystallisation of the spray dried lactose, with approximately 3% hydrate present.

CHAPTER 4

DETECTION OF SMALL AMOUNTS OF DISORDER IN PREDOMINANTLY CRYSTALLINE SAMPLES.

4.1 INTRODUCTION

The introduction of disorder into pharmaceutical powders as a result of processing, either purposely or unintentionally means that reliable methods for detecting the degree of disorder is crucial. The formation of disorder in a solid produces regions that are in a higher energy state than that of the crystalline form, which can lead to better pharmaceutical properties such as improved dissolution rate (Chiou and Kyle, 1979) as well as undesirable properties such as increased chemical instabilities (Pikal et al, 1978) and a potential to convert to the more stable crystalline form on storage (Makower and Dye, 1956). Hence the importance of being able to quantitatively detect disorder in crystalline solids down to very low levels.

Until recently, conventional analytical techniques used to determine the amorphous content of pharmaceutical powders, have only been able to detect down to about 10% disorder in a sample. The most widely used technique in the past was X-ray powder diffraction where the characteristic peak intensities or integrated peak intensities are measured as the crystalline sample is mixed with varying proportions of an amorphous sample (Black and Lovering, 1977) to provide a calibration plot. Other techniques used in this way to assess the percent disorder in crystalline samples include: density (Duncan-Hewitt and Grant, 1986), heat of solution (Pikal et al, 1978), and infrared spectroscopy (Black and Lovering, 1977). These techniques measure the average degree of disorder throughout the sample and are therefore of limited use when measuring small amounts of amorphous content, especially if that amorphous content is at the surface.

Differential scanning calorimetry has been used to measure the heat evolved during the transformation of an amorphous material to its crystalline state, however the usefulness of this technique is limited to samples with high amounts of disorder i.e. >10% (Saleki-Gerhardt et al, 1994).

The aim of this study therefore was to investigate new methods to detect small amounts of disorder in lactose samples, containing physical mixes of amorphous spray dried lactose and crystalline α lactose monohydrate by:

- employing the microcalorimetry glass ampoule technique developed in Chapter 3 to measure the crystallisation response in physical mixes containing small amounts of amorphous and crystalline lactose
- carrying out gravimetric studies, using an automated microbalance, to monitor weight changes due to water uptake and subsequent crystallisation occurring in similar mixes, containing varying amounts of amorphous and crystalline lactose.

4.2 MICROCALORIMETRY STUDIES.

Microcalorimetry, being an extremely sensitive thermal analytical technique, and having already been shown to be able to detect and follow the crystallisation of amorphous lactose by exposure to elevated humidities in sealed glass ampoules, was chosen to investigate varying amounts of disorder in spray dried lactose samples. Sebhatu et al (1994) showed that it was possible to detect down to 1% disorder in lactose samples containing varying amounts of amorphous character (induced by milling), by exposing them to a saturated salt solution of defined relative humidity in the microcalorimeter (the mini humidity chamber technique) as described in Chapter 3. Aso et al (1995) also used isothermal microcalorimetry to study the physical stability of amorphous nifedipine. Ahmed et al (1996) showed that microcalorimetry is a suitable approach for crystallinity studies on hydrophobic powders (using organic vapours to bring about the amorphous to crystalline transition), giving a lower limit of detection for amorphous content in the order of 1%. Most recently Lusting et al (1997) investigated the degree of disorder in lactose using solid-state NMR and isothermal microcalorimetry. Mixtures of amorphous and crystalline lactose alone were studied, and a three component system containing acet ysalicylic acid also was investigated. The two methods used were reported to be able to detect as low as 0.5% amorphous lactose in the mixtures. It was suggested that the advantage of using NMR is that it can provide structural information about a sample containing several different components.

Thompson et al (1994) found that the most quantitative correlations with degree of crystallinity of a beta-lactam antibiotic were obtained by measurements of thermal activity and heats of solution determined by isothermal microcalorimetry and solution calorimetry, respectively.

4.2.1 PREPARATION OF SAMPLES.

The samples prepared and analysed in this set of experiments contained mixtures of the same amorphous spray dried lactose used in Chapter 3 and crystalline α lactose monohydrate, which had been pre-stored at 85%RH in a desiccator to ensure the material was totally crystalline, and this sample showed no response in the microcalorimeter when it was exposed to 75%RH.

Samples containing 1.25%, 0.625% and 0.31% amorphous lactose were prepared. The samples were prepared as follows:

- (1) the 1.25% sample was prepared by adding 10mg of spray dried lactose to 790mg of crystalline α lactose monohydrate.
- (2) the 0.625% sample was prepared by adding 5mg of spray dried lactose to 795mg of crystalline α lactose monohydrate.
- (3) the 0.31% sample was prepared by mixing 400mg of the 0.625% sample with 400mg of crystalline α lactose monohydrate.

Since the amount of amorphous spray dried lactose present was very small, hence the reason for preparing larger sample loads here than was investigated previously. All the samples were first mixed thoroughly using a spatula and then shaken in a turbula mixer for 20minutes.

4.2.2 MICROCALORIMETRY METHOD AND MEASUREMENTS

The samples were weighed (800mg, as prepared above) into glass ampoules as before and a durham tube containing NaCl saturated salt solution, which gave 75%RH was added. The 1.25% sample was equilibrated for 30 minutes as for previous experiments, however the 0.625% and the 0.31% samples were equilibrated for 10minutes, since a very rapid microcalorimetric response was expected. The glass ampoules, durham tubes and saturated salt solutions were pre-equilibrated in an oven at 25°C as done previously.

4.2.3 RESULTS

4.2.3.1 Response for 1.25% amorphous sample.

The microcalorimetric responses for two repeat mixes containing 1.25% amorphous lactose are shown in Figure 4.1. It can be seen for this mix that the overall response is the same as that obtained and described previously for 100% amorphous lactose in Chapter 3. An initial wetting peak is observed followed by the main peak at 2 hours indicating that the same spontaneous crystallisation process is occurring here. This indicates that a sample containing this small amount of amorphous material can be easily detected and measured using this experimental set-up. The areas under the curves were measured and a mean value of 58.9 J/g (± 1.20 , $n=4$) amorphous material was obtained. (This response is measured per mg of amorphous material rather than per mg of total sample weight).

This mean measured value is higher than that reported earlier for the 100% amorphous sample which was 48 J/g. There are probably a number of reasons why the peak areas are slightly higher than expected here. There will probably be a considerable contribution due to errors in weighing and mixing of such very small quantities of amorphous material. There may also be an error due to the main crystallisation peak not being completely separated from the initial wetting peak because of the rapid crystallisation process, thereby resulting in a higher than expected peak area.

4.2.3.2 Response for 0.625% amorphous sample.

The microcalorimetric responses for four repeat mixes containing 0.625% amorphous lactose are shown in Figure 4.2. These are less reproducible than those obtained for the 1.25% mixes but even at this low level it is still quite easy to observe and measure the crystallisation response. The overall response here is again similar to that obtained for the 100% amorphous sample, with an initial wetting peak followed by the main crystallisation peak at 1 hour. This graph shows the responses obtained for 4 replicate experiments, but it is possible to see here the first signs of deviation from reproducibility, since the shape of the main crystallisation peak has altered slightly for at least one of the repeats.

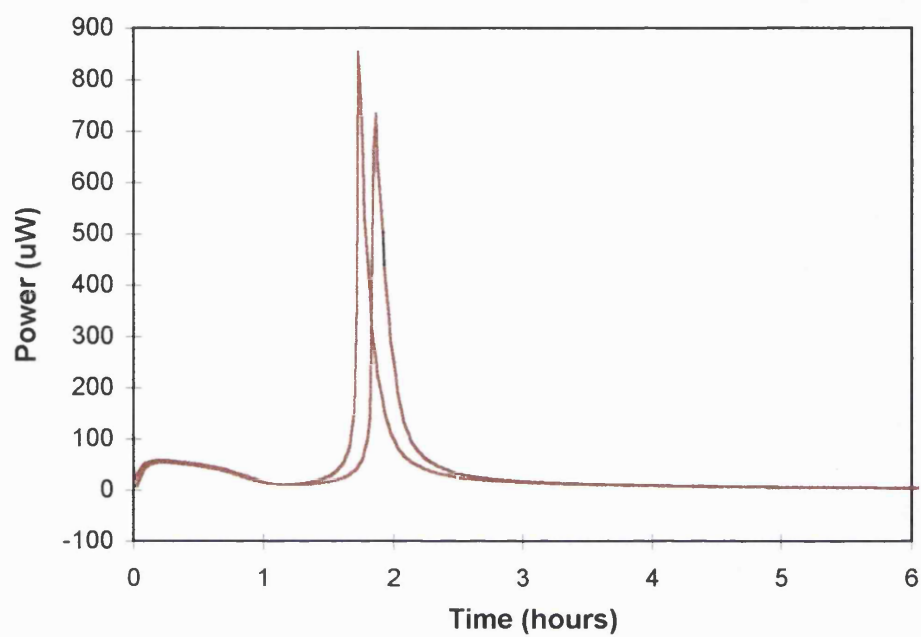


Figure 4.1 Crystallisation responses for mixes containing 1.25% amorphous lactose.

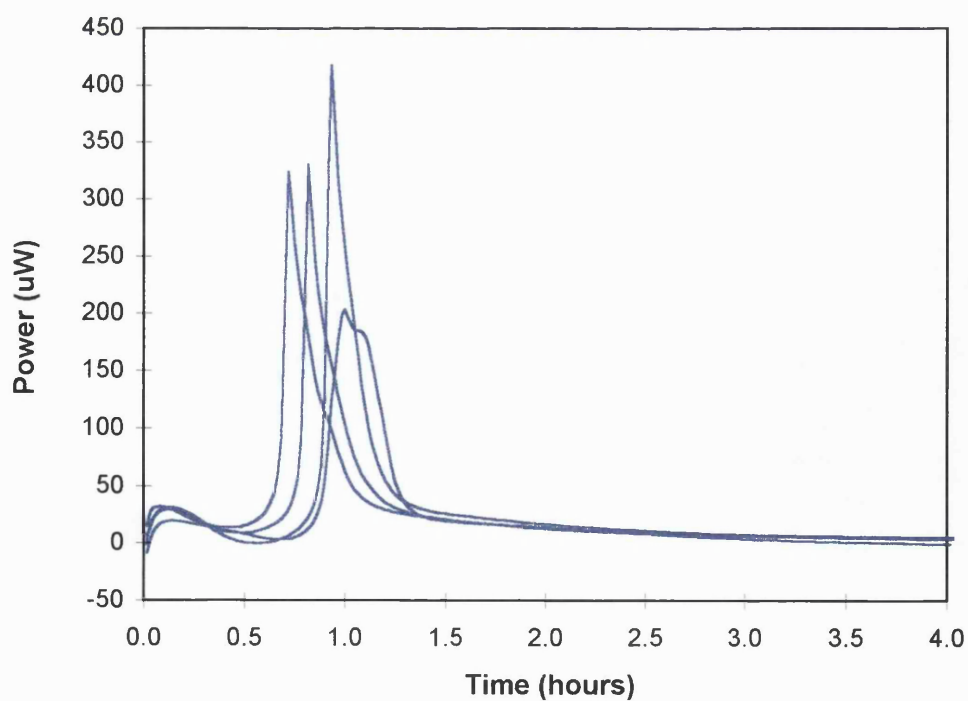


Figure 4.2 Crystallisation responses for mixes containing 0.625% amorphous lactose

The peak areas have a mean value of 53.9 J/g (± 8.50 , $n=4$) which again is higher than that obtained for the 100% amorphous sample. As explained earlier this is probably due to errors in sample weighing and mixing. Since the crystallisation peak occurs quite rapidly for the 0.625% mix, this peak isn't completely separated from the initial peak, which will result in a higher peak area measurement for the main peak.

4.2.3.3 Response for 0.31% amorphous sample.

The microcalorimetric responses for two repeat mixes containing 0.313% amorphous lactose are shown in Figure 4.3. The crystallisation response is still clearly present, however multiple peaks were obtained. The average heat of crystallisation for these two repeats was found to be 80.9 J/g (± 10.4 , $n=2$). This is much higher than expected, but is probably due to the initial wetting peak being superimposed on the crystallisation peak, which would also explain the presence of the multiple peaks.

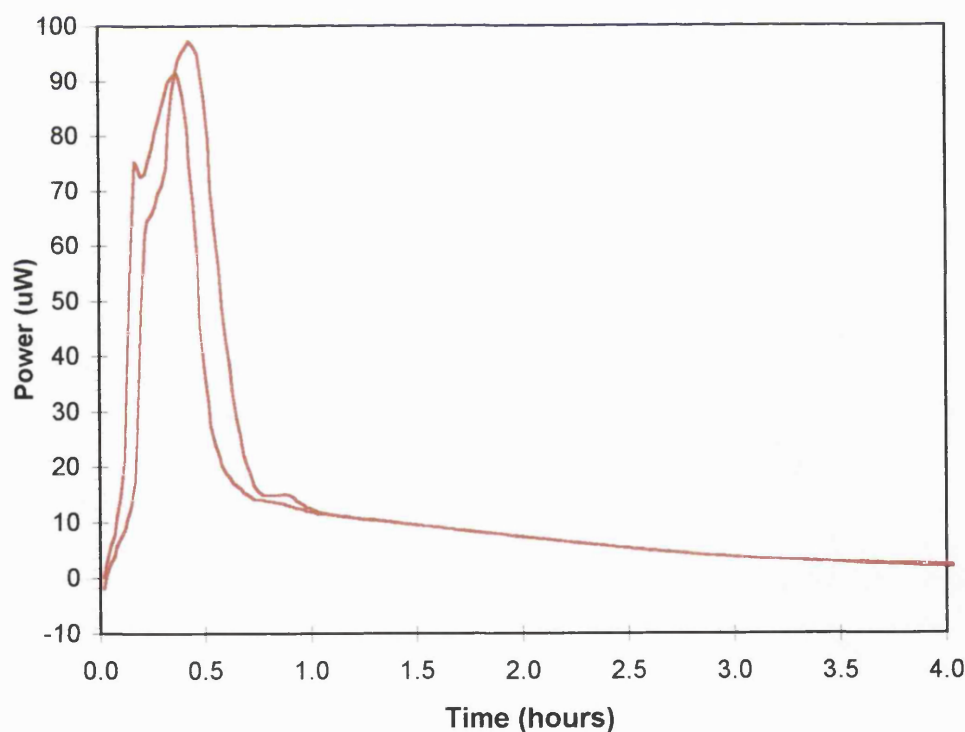


Figure 4.3. Crystallisation responses for mixes containing 0.31% amorphous lactose.

4.2.3.4 Multiple peaks

It is unclear as to why for those samples containing 0.31% and some of those containing 0.625% amorphous lactose that multiple peaks should be obtained. It may be that the crystallisation process is occurring at a slower rate. The crystallisation process is initiated by the water plasticising the amorphous material and allowing sufficient molecular movement for the crystallisation to occur. Upon crystallisation, the water will be desorbed from the sample. Therefore, there are a number of condensation and evaporation processes occurring with the sample, the saturated salt solution and the surrounding atmosphere. The sorption of water into the sample will be an exothermic event and the evaporation of the salt solution will be an endothermic event. On crystallisation, some of the water will be absorbed by the surrounding material while some will be desorbed (endothermic response) and this will then condense back into the saturated salt solution (exothermic response), in order to restore the humidity in the cell to that of the salt solution present. It may be that because we are seeing quite a small response here that these events are magnified and the response appears as multiple peaks. The mixes containing greater amounts of amorphous material show a larger crystallisation response, thus any underlying responses are probably hidden here. There may also be discrepancies due to poor mixing, since the system contains a physical mix of the amorphous and crystalline materials and sample uniformity will be an influencing factor in the ability of this technique to probe smaller increments of amorphous character in these samples. It is possible therefore that there is a slight delay in different parts of the amorphous material crystallising, as was found in chapter 3 where samples containing amorphous lactose in two separate layers showed signs of crystallising at different times giving rise to a multiple crystallisation response also.

4.2.3.5 Crystallisation responses for partially amorphous lactose samples.

It has been suggested (Saleki-Gerhardt et al, 1994) that the use of a physical mixture of amorphous and crystalline particles represents a 'two-state' model, where each particle is almost entirely crystalline or amorphous whereas in processed samples, it is likely that all or most of the solid particles are only partially crystalline or amorphous, in what is termed a 'one-state' model (Huttenrauch et al, 1985). It is accepted therefore that this approach may yield different results for systems containing samples

which have been rendered amorphous due to processing, since the uptake and transport of water in these systems will probably behave differently. However, the crystallisation responses for two commercially available partially amorphous lactose materials Pharmatose and Zeparox, were investigated and are shown in Figures 4.4 and 4.5. These materials are specified by the manufacturers to contain approximately 15% amorphous character, and as expected they show sharp crystallisation responses which are easily detected by the microcalorimetry approach being used here.

The interesting thing about these samples here is that they show the same spontaneous crystallisation response as was observed earlier for the totally amorphous sample and for the samples which contained physical mixes of the amorphous and crystalline materials. This indicates that the same cooperative crystallisation process is taking place in these samples, with the whole sample crystallising simultaneously once the whole powder bed is saturated.

The amorphous content for these unknown samples was calculated using their measured crystallisation responses and the heat of crystallisation for the 100% amorphous standard i.e. 48 J/g. The Pharmatose and Zeparox samples were found to contain 12% and 11% amorphous content, respectively. Although these samples contain a much higher amorphous content than was detected using the physical mixes, the onset time and magnitude of these peaks indicate that similar materials containing much smaller amounts of disorder could be measured. The uptake and transport of water in larger weights of these samples is investigated and discussed in more detail in Chapter 7.

4.2.4 CONCLUSION.

Isothermal microcalorimetry was able to detect down to 0.3% amorphous lactose in samples containing physical mixes of spray dried amorphous lactose and crystalline α lactose monohydrate. However, at this low level it was not possible to accurately quantify the amorphous content since the crystallisation response showed a multiple peak effect due to a very rapid crystallisation response, which resulted in a higher than expected heat of crystallisation being measured. The amorphous content for unknown samples was estimated by measuring the crystallisation responses for these samples and calculating %amorphous content using the heat of crystallisation for 100% amorphous lactose.

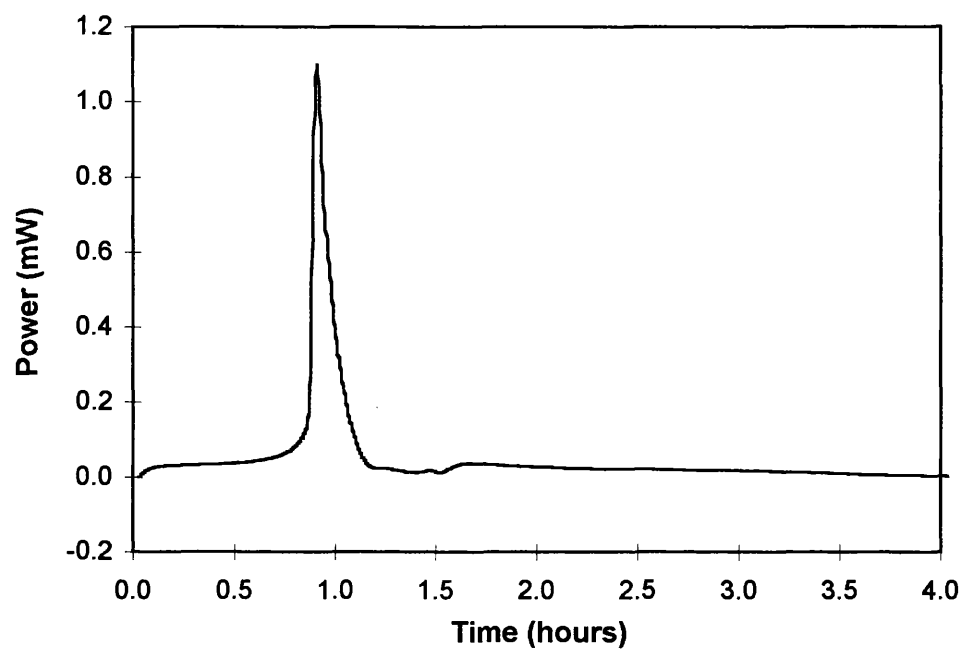


Figure 4.4 Typical microcalorimetry crystallisation response for Pharmatose.

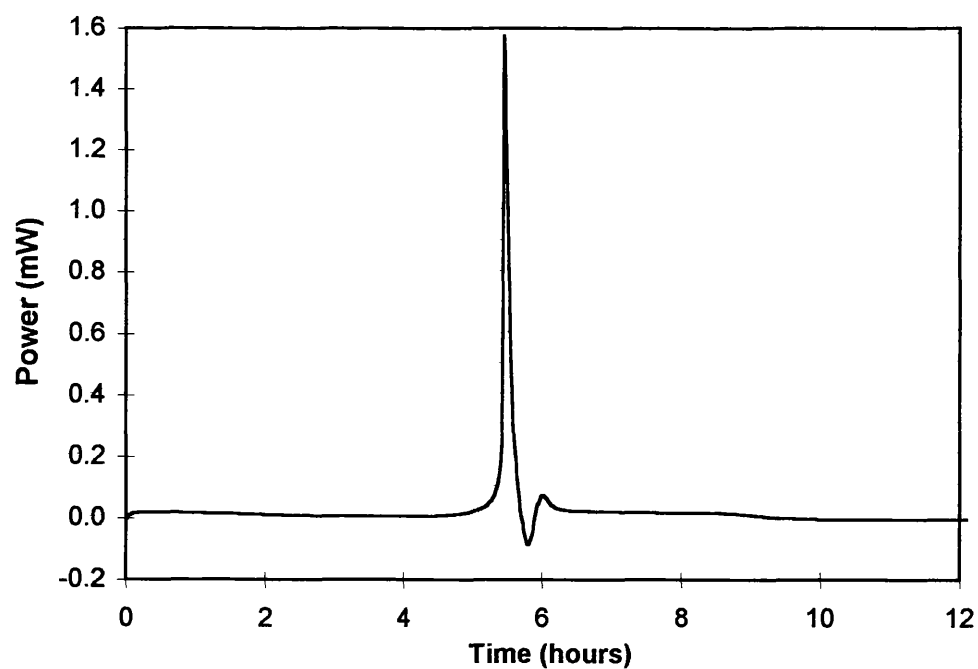


Figure 4.5 Typical microcalorimetry crystallisation response for Zeparox.

4.3 DYNAMIC VAPOUR SORPTION (DVS) STUDIES.

4.3.1 INTRODUCTION

Vapour sorption studies (employing commercially available computer-controlled moisture balances) are now being used by an increasing number of laboratories to investigate batch-to-batch variability in materials, which in a lot of instances is due to the presence of small amounts of disorder being present in these materials. It was shown in Chapter 3 that the crystallisation of spray dried lactose could be easily observed and measured in a dynamic vapour sorption microbalance system, by observing the weight loss due to desorption of water on crystallisation of the amorphous material. Ward and Schultz (1995) and Schmitt et al (1996) have both shown how crystallisation of amorphous materials can be followed using these automated vapour sorption techniques.

Schmitt et al (1996) were able to use the normalized weight loss during recrystallisation to detect as low as 2% amorphous content in physical mixes of the amorphous and crystalline form of a drug substance. Saleki-Gerhardt et al (1994) used the equilibrium moisture content of amorphous sucrose at a relative humidity before crystallisation occurred to quantitate amorphous content as low as 1% with an accuracy of $\pm 0.5\%$, compared to a lower detection limit of 10% using X-ray powder diffraction, density and heats of crystallisation.

4.3.2 SAMPLE PREPARATION.

The samples prepared and analysed in this set of experiments contained mixtures of the same amorphous spray dried lactose used in the microcalorimetry experiments described in 4.2 and crystalline α lactose monohydrate, which had been initially stored at 85%RH in a desiccator to ensure that it was totally crystalline. The water sorption / desorption isotherms for a sample of crystalline α lactose monohydrate is shown in Figure 4.6. There is very little water uptake by the sample below 80%RH, as expected for a crystalline lactose sample (Berlin et al, 1970 and 1971; Sheridan et al, 1994) and the sample reaches an equilibrium water uptake at 90%RH of 0.1%. The water sorption and desorption profile for spray dried lactose is shown in Chapter 3 section 3.4.

The mixes were prepared by weighing and mixing the spray dried and crystalline lactose samples as shown in Table 4.1. All the samples were first mixed thoroughly using a spatula and then shaken in a turbula mixer for 20 minutes.

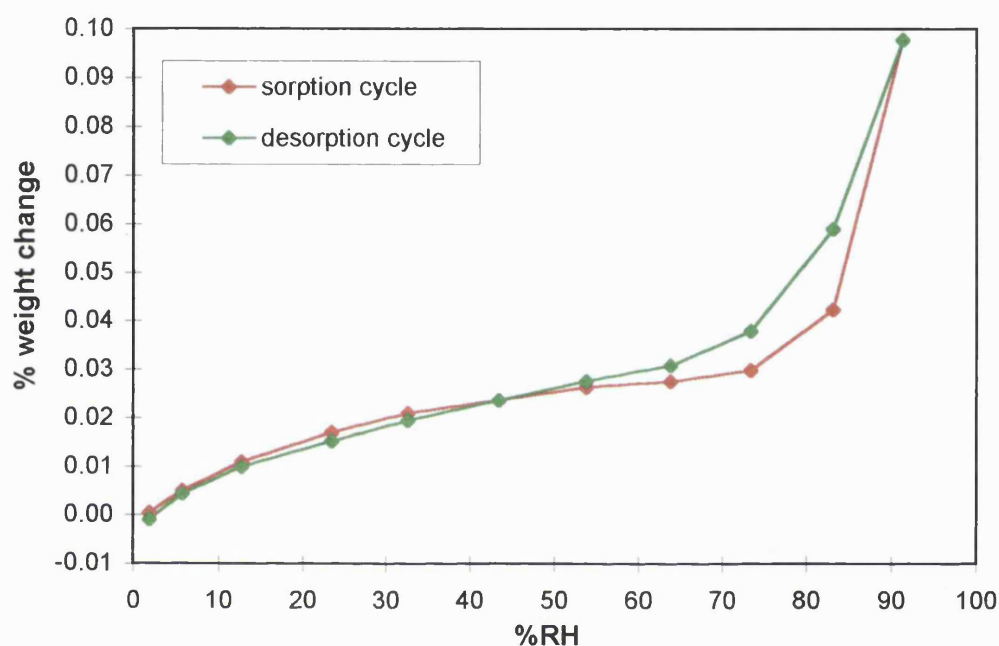


Figure 4.6 Sorption and desorption isotherms for crystalline α lactose monohydrate

4.3.2.2 Sample measurement

The samples investigated here were measured using exactly the same procedure as described for the 100% amorphous sample investigated in Chapter 3 (section 3.4). Weights in the region of 50mg were used and the experimental method was set up such that the samples were initially held at 0%RH for 1 hour. The %RH was then increased in 10%RH steps up to 90%RH and then decreased back down to 0%RH. A dm/dt value of 0.001 %/min (requesting the mass change to be below 0.001% for a minimum time of 10minutes) was used at each %RH step, thus ensuring that equilibrium was reached at each %RH before the experiment commenced to the next step. Four repeat cycles were performed on each sample and a minimum of three repeat samples were investigated. All measurements were carried out at 25°C.

% Amorphous content	Weight of material (mg)	Mix Number
10	10mg s.d.lactose + 90mg cryst.lactose	1
5	5mg s.d.lactose + 95mg cryst.lactose	2
1	5mg MIX 1 + 45mg cryst.lactose	3
0.5	5mg MIX 2 + 45mg cryst.lactose	4
0.25	5mg MIX 2 + 95mg cryst.lactose	5
0.125	5mg MIX 2 + 195mg cryst.lactose	6

Table 4.1 *Weights of spray dried and crystalline lactose used to prepare mixes.*

4.3.3 RESULTS

4.3.3.1 Water sorption and desorption profile for amorphous / crystalline mixes.

Typical sorption and desorption data for the first cycle of the mix containing 1.0% amorphous lactose are shown in Figure 4.7, while sorption and desorption isotherms for the first cycle of all the mixes investigated are shown in Figures 4.8 - 4.12. The overall water profile for each of the mixes is similar to that observed and discussed in chapter 3 for the 100% amorphous sample. There is considerable water uptake up to 60%RH, as seen in Figure 4.7, where there is a dramatic weight loss, corresponding to water being expelled from the amorphous regions of the sample on crystallisation. There is only minimal water uptake as the %RH is increased up to 90%RH, as observed for the 100% amorphous sample. This again is characteristic of crystalline material where there is adsorption onto the surface of the powder. As the %RH is decreased from 90%RH down to 0%RH very little weight loss is observed. Three consecutive repeat cycles for each sample showed very little water uptake and loss for all the samples.

This pattern of weight increase up to 60%RH followed by a sharp weight loss due to crystallisation of the amorphous material is clearly evident in the Figures 4.8 - 4.12 for the mixes containing 1.0%, 0.5%, 0.25% and 0.125% amorphous lactose. Therefore, for these samples it is clearly possible to easily detect down to 0.1% amorphous content.

The sample which contained 0.05% amorphous lactose, showed a slight weight loss at 60%RH indicating that this is the limit of detection for this system. It is possible at this low level that errors due to weighing and mixing of samples will occur.

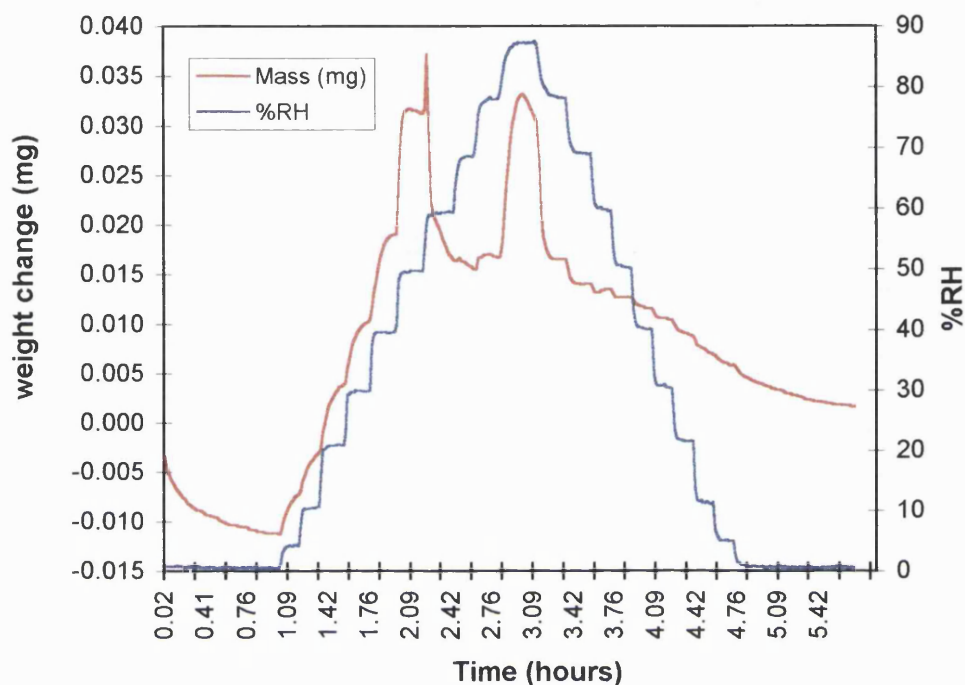


Figure 4.7 Water sorption / desorption data for mix containing 1.0% amorphous lactose.

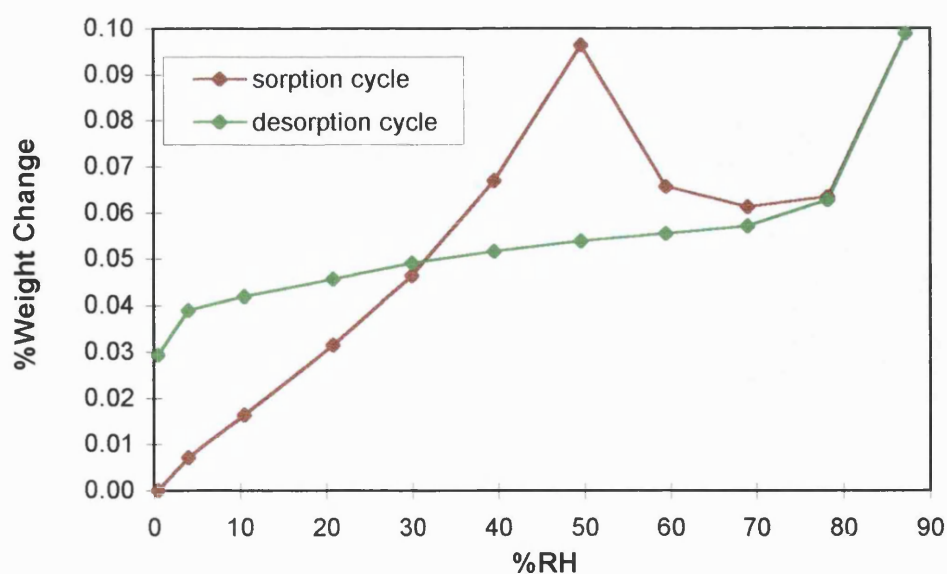


Figure 4.8 Water sorption / desorption isotherms for mix containing 1.0% amorphous lactose.

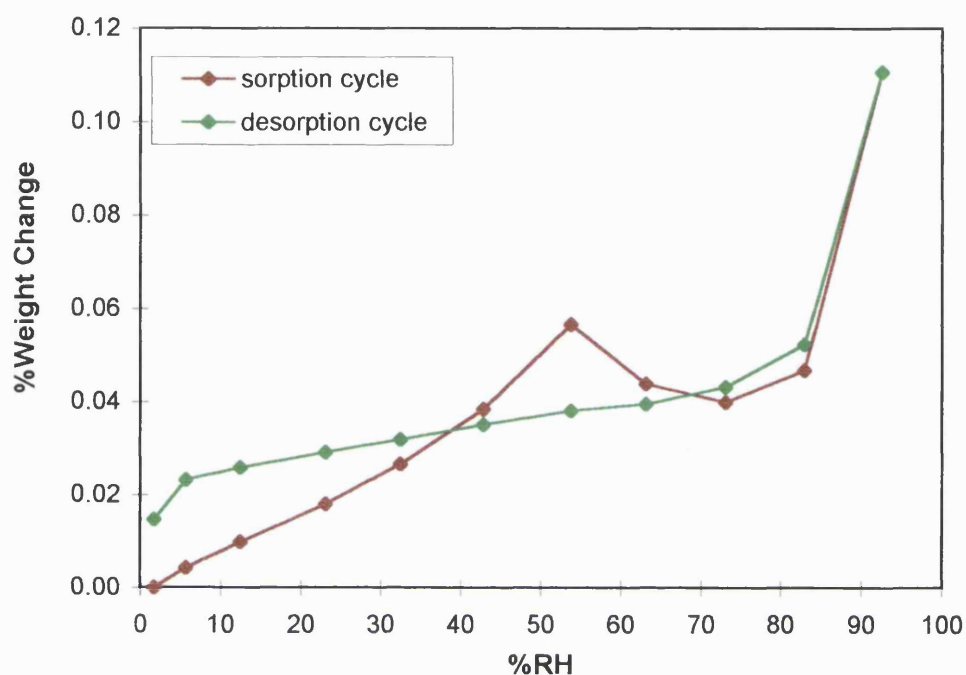


Figure 4.9 Water sorption / desorption isotherms for mix containing 0.5% amorphous lactose.

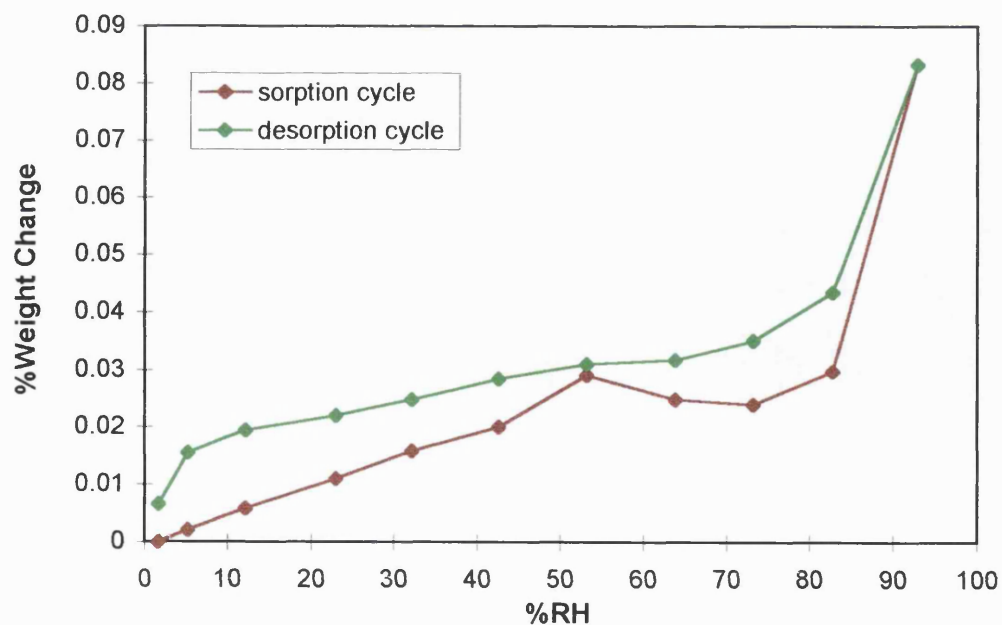


Figure 4.10 Water sorption / desorption isotherms for mix containing 0.25% amorphous lactose.

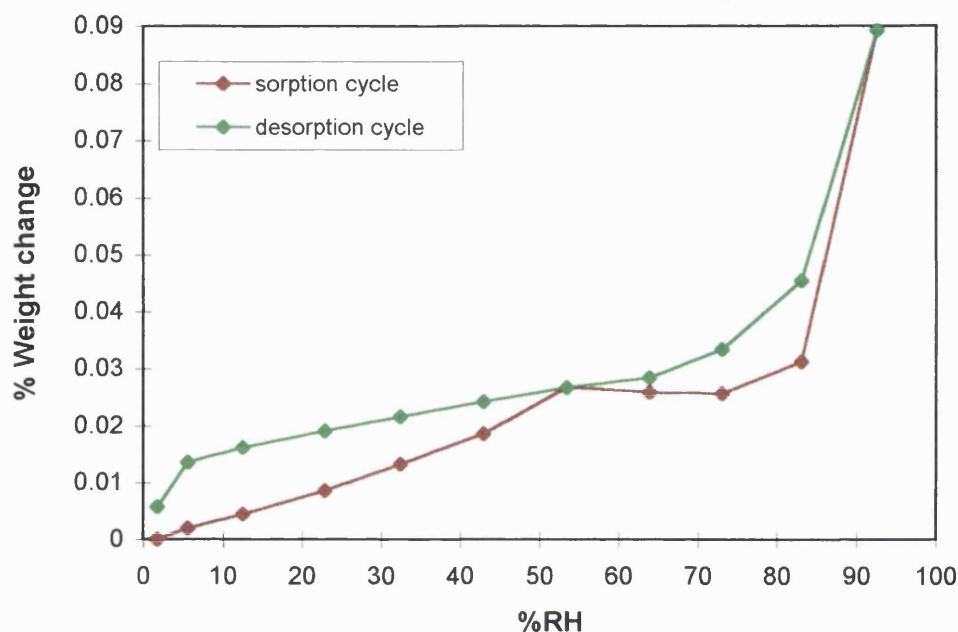


Figure 4.11 Water sorption / desorption isotherms for mix containing 0.125% amorphous lactose.

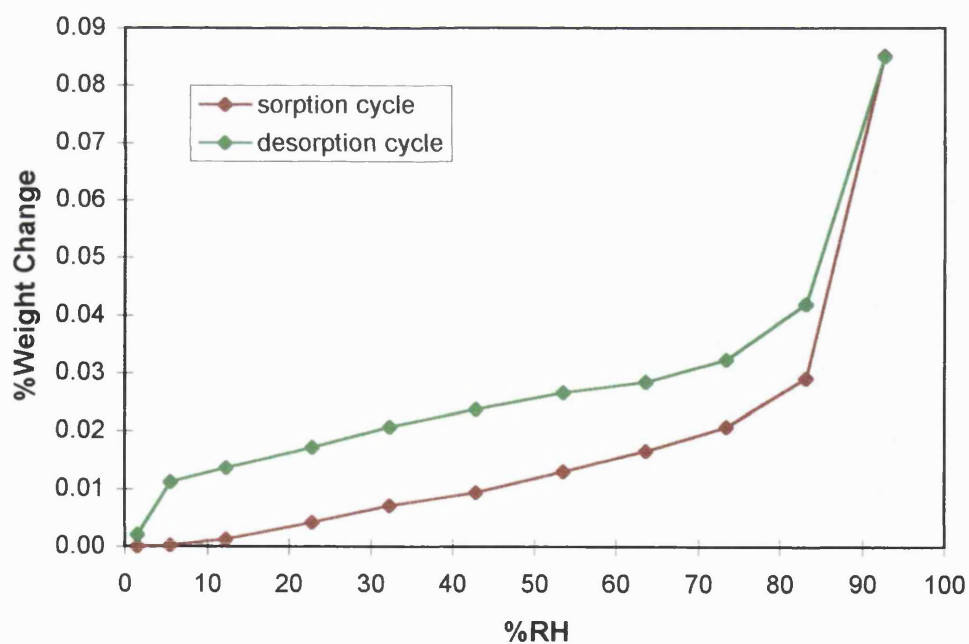


Figure 4.12 Water sorption / desorption isotherms for mix containing 0.05% amorphous lactose.

4.3.3.2 Water sorption and desorption isotherms for amorphous / crystalline mixes.

Typical sorption and desorption isotherms for 4 repeat cycles for the mix containing 0.125% amorphous lactose are shown in Figure 4.13. It can be seen that the first sorption cycle is different from subsequent cycles due to water being absorbed into the amorphous regions as well as being adsorbed onto the surface of the crystalline lactose. This pattern was observed for all the mixes investigated.

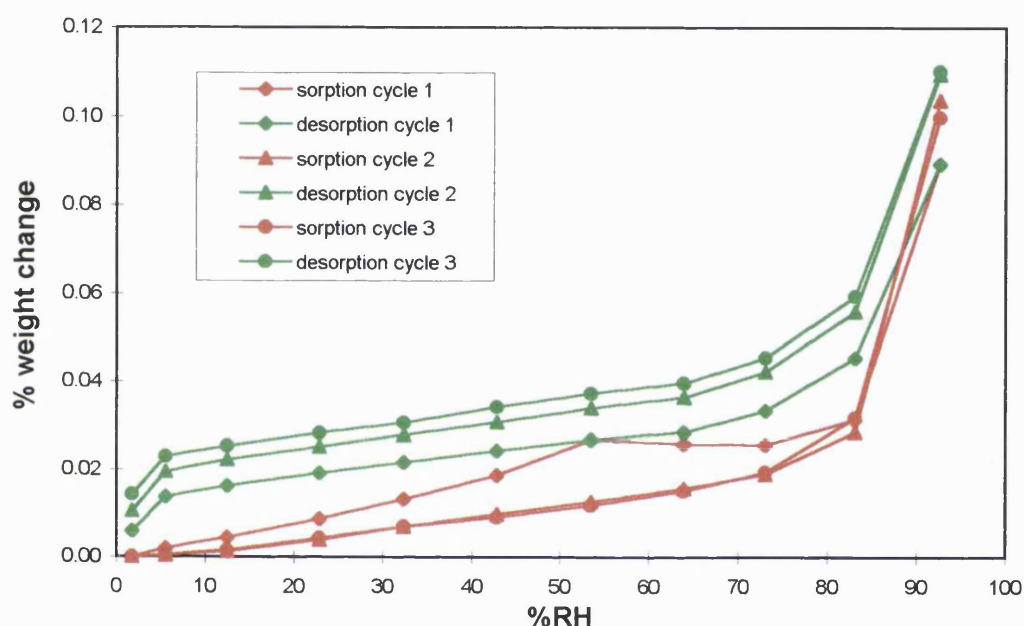


Figure 4.13 Water sorption and desorption isotherms for 3 repeat cycles on the sample containing 1.25% amorphous lactose.

4.3.4 QUANTIFICATION OF AMORPHOUS CONTENT FROM SORPTION AND DESORPTION ISOTHERMS.

Typical sorption isotherms for the first cycle for each mix (0.5%, 0.25%, 0.125% and 0.05%) are superimposed in Figure 4.14 and the mean of all subsequent sorption isotherms (i.e. 2nd, 3rd and 4th cycles) have been averaged for each of the mixes (irrespective of amorphous content) and are also shown. Mean sorption and desorption values for each of the mixes are listed in Table 4.2 (for n=3 along with standard deviations). It can be seen from this data and from the graph that there are clear differences between the responses, in that the greater the amorphous content the greater the area enclosed between the first sorption cycle and the subsequent sorption

cycles. However, since the first sorption cycle is a composite of absorption followed by water loss after crystallisation, the area between the first and subsequent sorption cycles reflects a kinetic contribution for the crystallisation as well as the amount of amorphous material that was present.

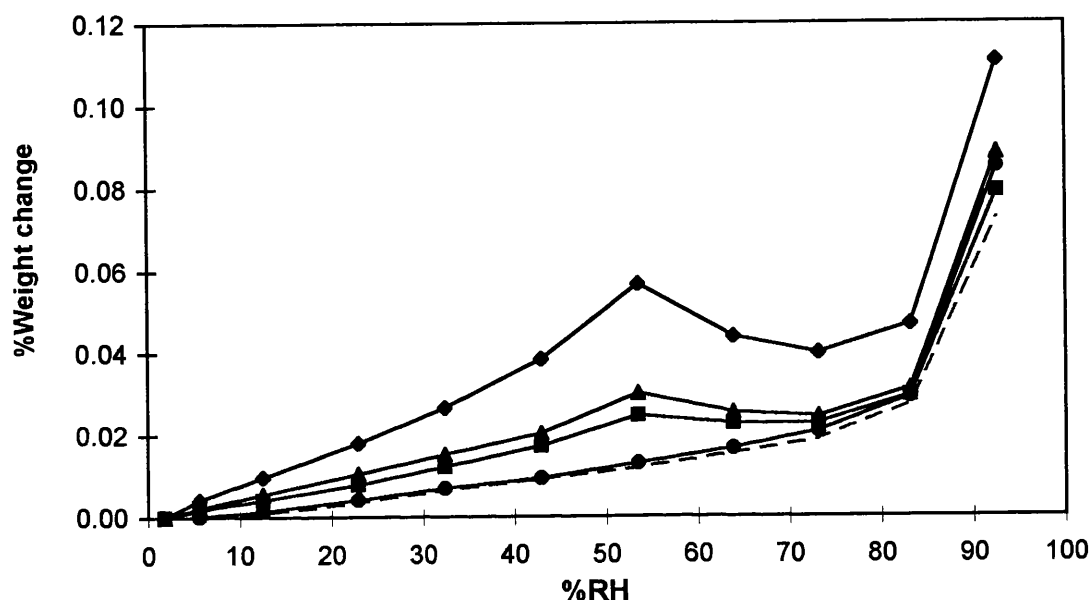


Figure 4.14 Sorption and desorption isotherms for mixes containing small amounts of amorphous material (diamonds 0.5%, triangles 0.25%, squares 0.125%, circles 0.05%, broken line is desorption).

Therefore it is probably not an accurate method to estimate the amorphous content by taking a weight difference at any one humidity. However, the increase in weight from the starting point of the first sorption run to the end of the first desorption run as shown in Figure 4.15 by the asterisk, was the approach chosen to be used here to quantify the amorphous content of the mixes. This is because the amorphous lactose crystallises to form the monohydrate under the conditions of this experiment. The molecular weight of lactose is 360g, of which 18g (5%) is water of crystallisation. Therefore if a totally amorphous sample were investigated and if it crystallised to form the monohydrate, then the weight gain after desorption of physically adsorbed water but not hydrate water would be a 5.26% increase over dry weight. The expected weight increases due to addition of water of crystallisation to the mixes investigated here have been calculated and are listed in Table 4.3.

%RH	0.05% amorphous		0.125% amorphous		0.25% amorphous		0.50% amorphous	
	Sorption	Desorption	Sorption	Desorption	Sorption	Desorption	Sorption	Desorption
1.8	0.0000 (0.000)	0.0061 (0.0098)	0.0000 (0.000)	0.0029 (0.0021)	0.0000 (0.000)	0.0047 (0.0022)	0.000 (0.000)	0.0147 (0.0004)
5.6	0.0009 (0.0011)	0.0128 (0.0106)	0.0015 (0.0006)	0.0096 (0.0042)	0.0021 (0.0003)	0.0136 (0.0018)	0.0042 (0.0007)	0.0232 (0.0002)
12.5	0.0028 (0.00220)	0.0154 (0.0105)	0.0036 (0.0011)	0.0127 (0.0037)	0.0056 (0.0002)	0.0169 (0.0020)	0.0098 (0.0003)	0.0258 (0.0003)
22.9	0.0061 (0.00330)	0.0192 (0.0103)	0.0069 (0.0018)	0.0156 (0.0037)	0.0106 (0.0006)	0.0199 (0.0018)	0.0180 (0.0002)	0.0292 (0.0004)
32.4	0.0096 (0.0042)	0.0226 (0.0095)	0.0111 (0.0024)	0.0181 (0.0038)	0.0152 (0.0005)	0.023 (0.0016)	0.0267 (0.0012)	0.0319 (0.0005)
42.9	0.0127 (0.0049)	0.0255 (0.0095)	0.0151 (0.0045)	0.021 (0.0037)	0.0204 (0.0004)	0.0265 (0.0017)	0.0384 (0.0008)	0.0351 (0.0009)
53.5	0.0168 (0.0058)	0.0283 (0.0095)	0.0210 (0.0074)	0.0238 (0.0033)	0.0301 (0.0013)	0.0295 (0.0015)	0.0567 (0.0011)	0.0382 (0.0003)
63.9	0.0202 (0.00730)	0.0303 (0.0095)	0.0202 (0.0052)	0.0251 (0.0038)	0.0255 (0.0006)	0.0310 (0.0011)	0.0439 (0.00007)	0.0396 (0.0012)
73.1	0.0237 (0.0081)	0.0340 (0.0097)	0.0208 (0.0043)	0.0291 (0.0044)	0.0244 (0.0013)	0.0346 (0.0011)	0.0400 (0.0007)	0.0433 (0.0009)
83.1	0.0325 (0.0083)	0.0432 (0.0106)	0.0279 (0.0025)	0.0387 (0.0061)	0.0312 (0.0017)	0.0437 (0.0008)	0.0469 (0.0014)	0.0524 (0.0015)
92.6	0.0815 (0.0166)	0.0815 (0.0166)	0.0779 (0.0154)	0.0779 (0.0125)	0.0884 (0.0045)	0.0884 (0.0037)	0.1107 (0.0021)	0.1107 (0.0021)

Table 4.2. Equilibrium water content values (1st cycle data) for lactose mixes containing varying amounts of amorphous and crystalline lactose.

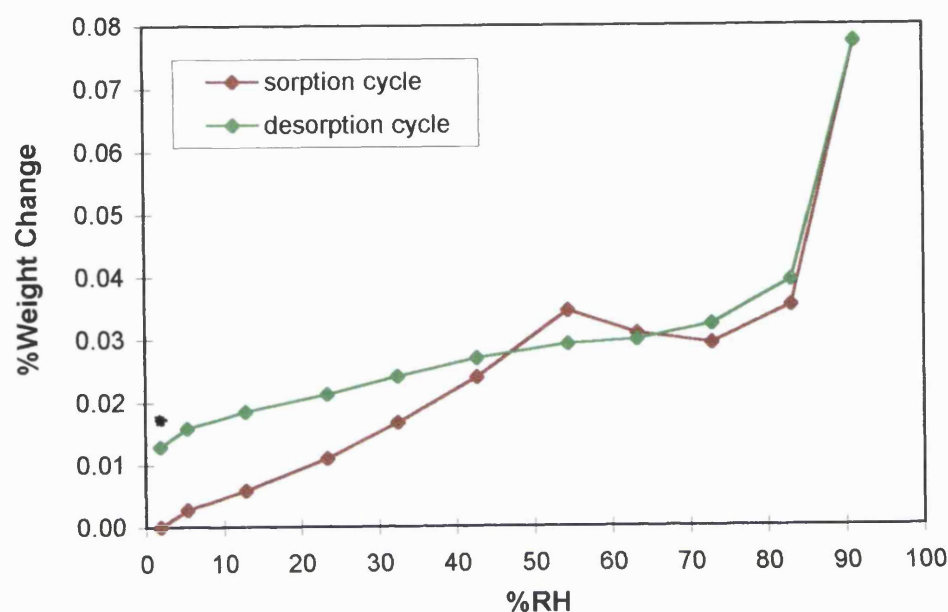


Figure 4.15 Sorption and desorption isotherms for the 1st cycle of a sample containing 0.25% amorphous lactose showing the weight gain at the end of the desorption cycle.

Amorphous content (%)	Theoretical increase (%)	Measured increase (% \pm SD)	Calculated amorphous content
0.5	0.026	$0.025 \pm 1.6 \times 10^{-3}$ (n = 2)	0.475
0.25	0.013	$0.012 \pm 2.5 \times 10^{-3}$ (n = 7)	0.228
0.125	0.007	$0.010 \pm 4.3 \times 10^{-3}$ (n = 7)	0.190
0.05	0.003	$0.005 \pm 4.7 \times 10^{-3}$ (n = 3)	0.095

Table 4.3 Theoretical and measured weight changes after crystallisation.

The actual weight increases due to incorporation of water of crystallisation were taken as the weight at equilibrium at 5%RH on the desorption cycle for each mix. These are also listed in Table 4.3. This represented the residual weight change after all the sample had crystallised. The reason why the 5%RH value was chosen instead of the 0%RH value was that this gave more reproducible values. It can be seen that for mixes containing <0.1% amorphous content there is reasonable agreement between the expected and the measure values, e.g. for the 0.5% mix the measured amorphous content was 0.475%. The standard deviation for the measured values (as shown in Table 4.3) increases as the %amorphous content decreases, as does the difference between the theoretical and measured values. This is probably not unexpected since the error due to weighing, mixing and sampling of such small amounts of material was probably greater as the amount of amorphous material present was reduced.

This method of quantifying the amorphous content does not however appear to hold for samples containing >1% amorphous content. The 100% amorphous sample typically reached an increase in residual weight at the end of the desorption cycle of around 1.4%. This is much lower than the expected 5.26% and agrees with the earlier observations from DSC and TGA that this sample does not crystallise to a complete hydrate. The mixtures containing 10% and 1% amorphous content reached typical weight increases of around 0.26 and 0.03%, respectively, at the end of the first desorption cycle, compared to expected values of 0.53% and 0.05%. These values again indicate that this quantification approach appears to work better for samples containing small amounts of amorphous material.

Another approach for quantification of amorphous content (which wasn't investigated in great depth here) would be to measure the equilibrium water uptake at either 40 or 50%RH on the first sorption cycle before the onset of crystallisation. A calibration curve could then be constructed in order to investigate unknown samples.

4.3.5 SORPTION AND DESORPTION DATA FOR A PARTIALLY AMORPHOUS LACTOSE MATERIAL (PHARMATOSE).

Since, as discussed earlier the samples investigated here constitute a 'two-state' model, it was decided to investigate Pharmatose (partially amorphous lactose) which has already been investigated by microcalorimetry above. The sorption and desorption data for a 20mg sample of this material is shown in Figure 4.16.

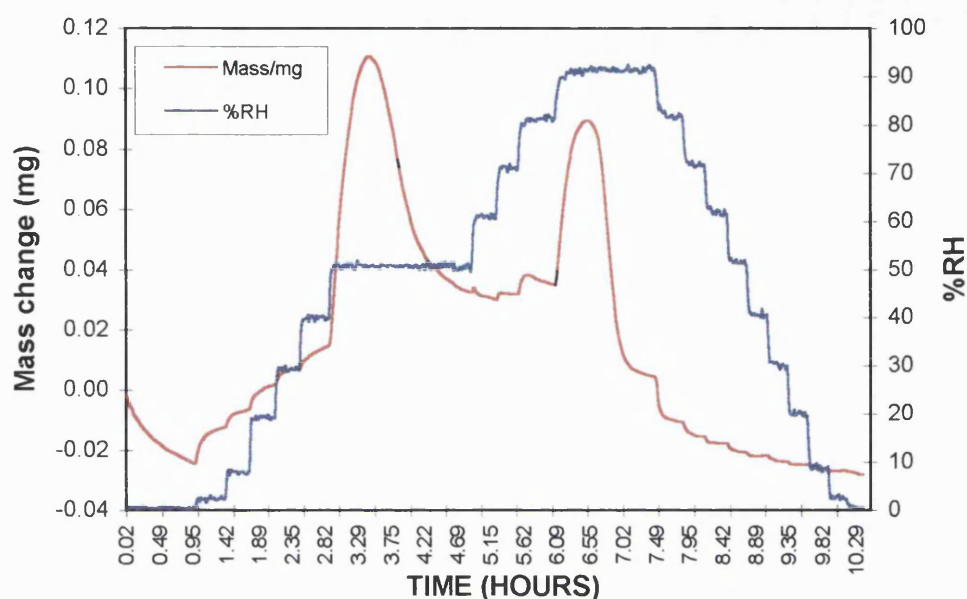


Figure 4.16 Sorption and desorption data for the 1st cycle of a sample Pharmatose (partially amorphous spray dried lactose)

This material is specified by the manufacturers to contain in excess of 10% amorphous character, and as such would be expected to show a sharp weight loss on crystallising here. The sorption cycle is different for this sample from that observed for the 100% amorphous material and the mixes investigated earlier, in that there is a gradual weight loss at increasing %RH steps from 60%RH up to 90%RH as can be seen from Figure 4.16, rather than at one %RH as was observed for the 100% amorphous lactose or for the amorphous / crystalline mixes. This indicated that the sample is crystallising gradually rather than spontaneously as observed for this sample in the microcalorimeter. The probable explanation for this is the faster supply of water vapour to the sample in the DVS rather than the microcalorimeter where the supply of water vapour to the sample will be governed by the rate of evaporation from the saturated salt solution. The surface of this sample in the DVS will become saturated quite rapidly and therefore begins to crystallise as observed by the weight loss at 60%RH. However, as the %RH is further increased from 60%RH up to 90%RH, a weight loss is observed at each of these humidities after an initial weight increase, indicating that the sample is still crystallising. In order to measure the % amorphous content of this sample it would not be possible to use the method employed by

Schmitt et al (1996) and described in section 4.4.2, since the weight loss observed here was gradual with increasing %RH. The amount of monohydrate formed therefore would be a more accurate method, as used above for the physical mixes investigated, for the quantification of amorphous content in these samples. For the sample in Figure 4.16 the expected weight gain due to hydrate formation (based on 5% hydrate formation) would be 0.63%, however, the actual weight change for this sample was – 0.03%. This is much lower than expected and again indicates that 5% hydrate is not being formed. This net negative weight change will be investigated and discussed further in Chapter 7 (section 7.4).

4.3.6 CONCLUSIONS

DVS was able to detect down to 0.1% amorphous character in samples containing physical mixes of amorphous spray dried lactose and crystalline lactose. The amount of water retained in the formation of the monohydrate was used to estimate the amorphous content of the mixes, and this yielded data that was reasonably close to the theoretical values. Vapour sorption studies using the DVS system employed here were found to be the most effective method to study small degrees of amorphous content in unknown samples.

4.4 REVIEW OF RECENTLY PROPOSED QUANTIFICATION METHOD OF AMORPHOUS CHARACTER (USING CALORIMETRIC AND VAPOUR SORPTION DATA) FROM LITERATURE.

4.4.1 Microcalorimetric and DSC approach.

Philips (1997) described how the amorphous content of an ‘unknown’ sample could be estimated without actually having to prepare amorphous and crystalline calibration standards. The energy output from the amorphous to crystalline transformation, as measured from the microcalorimeter (using the saturated salt solution approach employed here) and the heat of fusion of a purely crystalline material as measured from DSC melting exotherms were applied to an equation developed by Hoffman (1958). The amorphous content of a sample was then investigated by taking the ratio of the enthalpy of crystallisation for the amorphous fraction to the heat of fusion of the purely crystalline fraction. This method proposed to eliminate the need for preparing

calibration standards, and reported results which were very similar to literature values for the materials investigated. However, based on the findings reported earlier in both this chapter and chapter 3 for lactose, notably the fact that the amorphous material does not always crystallise to the same form, this approach would appear to have severe limitations. Furthermore, the use of DSC to quantify the heat of fusion of a crystallised material not only requires a pure 100% crystalline material, but the heating of such a material may result in enthalpic changes (such as dehydration of α lactose monohydrate to the anhydrous form at around 150°C) which would interfere with heat of fusion values. It is also necessary to note that if the crystalline solid which is formed in the microcalorimeter is of a different form to that which is melted in the DSC then the enthalpic changes which are recorded cannot be directly compared. Consequently, it would appear that the use of calibration standards is indeed necessary to determine the amorphous content of pharmaceutical powders.

4.4.2 Vapour sorption approach.

Schmitt et al (1996) estimated the amorphous content of their material by measuring the weight loss due to water being expelled from the amorphous regions on crystallising, and constructed a calibration curve. The differences in normalized water content (mg H₂O/100mg solid) at the %RH just prior to crystallisation were plotted as a function of % amorphous content. The problem with applying this approach to the samples investigated here is that the water content at 60%RH does not reach equilibrium until the sample has already crystallised, i.e. the weight begins to increase and then drops dramatically as the crystallisation occurs. Schmitt et al obtained a better linear relationship using this approach than using the equilibrium water content water at any particular %RH. This approach was not used here but may also be a possible method for quantifying small amounts of amorphous content in unknown samples.

CHAPTER 5

COLLAPSE PHENOMENA IN AMORPHOUS SPRAY DRIED LACTOSE.

5.1 INTRODUCTION

It has been discussed and shown in Chapter 3 how water sorption by amorphous lactose resulted in the T_g being lowered, due to the plasticising properties of water. As the T_g is lowered the viscosity is reduced and molecular mobility of the amorphous material increases significantly which resulted, in this case, in the eventual spontaneous crystallisation of the amorphous lactose. This 'sorption of water' ability by the amorphous lactose therefore greatly affects the physical form and stability of the amorphous structure.

Numerous studies and investigations have been carried out especially in the food science field looking at the polymer-chemical characterisation of various food ingredients such as sugars (Flink, 1983 and To and Flink, 1978a, 1978b, and 1978c) and low molecular weight carbohydrates and starches (Levine and Slade, 1986; White and Cakebread, 1966 and Tsourouflis *et al* 1976). These materials all behave as systems of amorphous or partially crystalline materials which are soluble in and/or plasticised by water, hence the interest in exploring the physical changes that are observed in such systems, on exposure to water vapour. Of major interest and importance therefore in this area of water sorption by such materials is the nature and mobility of the sorbed water. These studies led into the phenomenon known as collapse which is regarded as "diffusion controlled consequences of a structural relaxation process characteristic of a material, which reflects the microscopic and macroscopic manifestations of an underlying and pre-required molecular 'state' transformation from a metastable amorphous solid to liquid which occurs at T_g " (Levine and Slade, 1986). In effect, the amorphous material on absorbing large amounts of water becomes more liquid-like and is unable to support its own weight under gravity which occurs over a time frame so that it is visible.

The physical effects of collapse on the processing and storage of foods along with a general physiochemical mechanism for the process are discussed in Chapter 1. However, it is necessary to highlight a few important ideas in relation to the studies to be undertaken in the following piece of work. Whenever the glass transition and the resultant collapse phenomenon share a common time frame (Franks, 1982), T_g equals the minimum onset temperature for all collapse-related phenomena. Franks also postulated from the same studies that a system is therefore stable from collapse,

within the time period of the experimental measurement of T_g and T_c (collapse temperature), at , $T < T_g$. The equivalence and relationship between T_g , T_c and T_r (crystallisation temperature) has also been a source of investigation and To and Flink (1978) acknowledged that although collapse and glass transition are phenomenologically similar events, they differentiated between T_g and T_c by pointing out that while glass transitions in polymeric materials are generally reversible, the collapse of freeze dried matrices are irreversible. Levine and Slade (1986), while in agreement with To and Flink, further stated that that at a molecular level the glass-to-rubber transition for a thermoplastic material is reversible (which is also true for a completely amorphous freeze-dried material), the reason collapse is said to be irreversible for a porous matrix is nothing to do with reversibility between molecular states. Irreversible loss of porosity, they stated, is simply a macroscopic, morphological consequence of viscous flow in the rubbery state at $T > T_g$, whereby the porous glass relaxes to a fluid, incapable of supporting its own weight against flow, thus becoming more dense. Subsequent recooling to $T < T_g$ yields a non-porous glass of the original composition, which can thereafter be temperature-cycled reversibly. The only irreversible aspect of T_g -governed collapse is loss of porosity.

A number of aims and objectives were therefore identified to be addressed in the following study:

- to measure the water content necessary to lower T_g below T thus causing crystallisation of amorphous lactose
- to investigate the water mobility at and close to the point where T_g approaches the experimental temperature.
- to examine the collapse of amorphous lactose and the nature of water retention within the collapsed amorphous structure
- to attempt physical characterisation of the collapsed material using DSC and TGA and visual characterisation using a DVS-video technique
- to investigate the stability of the collapsed lactose samples by exploring the effects of drying (at 0%RH), heating and compaction.

5.2 EXPERIMENTAL

5.2.1 DYNAMIC VAPOUR SORPTION (DVS) STUDIES

Samples (15 - 20mg) of amorphous spray dried lactose (as prepared in Chapter 2) were placed on the sample pan of the DVS microbalance, having been stored at 20°C over silica gel since preparation. The samples were initially maintained at 0%RH for 4 hours in order to remove any absorbed water which was present, and were then treated in one of the following ways:

(1) Investigating the water mobility close to where $T_g = T$.

The relative humidity was increased to 40%RH or 50%RH and the sample was maintained at this %RH until it reached equilibrium moisture content as determined by a dm/dt value of 0.001. After equilibration was reached, the relative humidity was returned either to:

(a) 20%RH and then to 0%RH or (b) directly to 0%RH

and the rate of desorption of water was determined.

(2) The impact of exposing the amorphous lactose to 50%RH for different lengths of time.

In this case the samples were held at 50%RH for 5, 60, 75 and 90 minutes, and 2, 3, 4.5, 6 and 9 hours, after which time they were either removed directly from the DVS microbalance for further investigation or they were returned to 0%RH and maintained until equilibrium was reached, and they were then removed for further analysis.

5.2.2 ISOTHERMAL MICROCALORIMETRY

In order to check the crystallinity of the amorphous lactose, before and after exposure to 40 or 50%RH, samples from the DVS were exposed to sodium dichromate salt solution (54%RH) in a sealed glass ampoule in the microcalorimeter at 25°C, as described in Chapter 3. The magnitude of the heat output for the crystallisation response was used to quantify the amorphous content of the lactose samples.

5.2.3 DIFFERENTIAL SCANNING CALORIMETRY AND THERMOGRAVIMETRIC ANALYSIS.

Samples which had been held at 50%RH for varying lengths of time in the DVS, were removed and investigated by DSC using ca 4mg in non-hermetically sealed aluminum pans and scanning at 10°C /min from 25 - 250°C under a nitrogen atmosphere. Simultaneously, ca 4mg samples were placed in open aluminum pans and were scanned at 10 °C /min from 25 - 250 °C in the TGA. To ensure that no water loss occurred on removing the samples from the DVS and transferring either to the DSC, TGA or the microcalorimeter, rapid and careful sample handling was used. In order to relate DSC, TGA and microcalorimetry data, the samples for these investigations were taken from the same DVS sample, therefore no pooling or mixing of samples occurred.

5.3 RESULTS AND DISCUSSION.

5.3.1 WATER SORPTION AND MOBILITY OF AMORPHOUS LACTOSE AT 40% AND 50% RH.

5.3.1.1 Rate of water sorption at 40% RH.

Figure 5.1 shows a typical water uptake response for the amorphous lactose which was exposed to 40%RH until equilibrium. It can be seen that a biphasic profile is occurring. There is an initial sharp uptake of water until it reaches approximately 3% of the original mass, which is followed by a slower region of water sorption up to an inflection point at approximately 5% weight increase. This is then followed by a faster rate of sorption. It is unclear as to why this inflection point occurs and why different water uptake rates are observed, however, it may be related to the fact that the water sorption behaviour may be changing as the T_g is being lowered due to plasticisation by the water. Levine and Slade (1986) pointed out that sorption experiments for amorphous materials do not usually represent a true thermodynamic equilibrium situation, since the polymer substrate is changing structurally, due to plasticisation by sorbed water. Furthermore, since T_g decreases during sorption, such experiments are not isothermal with respect to the ΔT -governed viscoelastic properties of the material, because ΔT changes over the sorption time course, therefore both the extent of

sorption and the mobility of sorbed molecules generally increases with increasing plasticisation by water.

Alternatively, the inflection point at around 3 - 5% water uptake may be due to an initial faster uptake rate at the surface, then a slower region may follow as water diffuses into the bulk of the sample, which will result in increased viscosity and greater molecular mobility, hence water sorption will speed up once more. It was also noted that at the 5% inflection point, the mole ratio of water to amorphous lactose is 1:1. The sample reaches equilibrium after approximately 6 hours at 40%RH as shown in Figure 5.1.

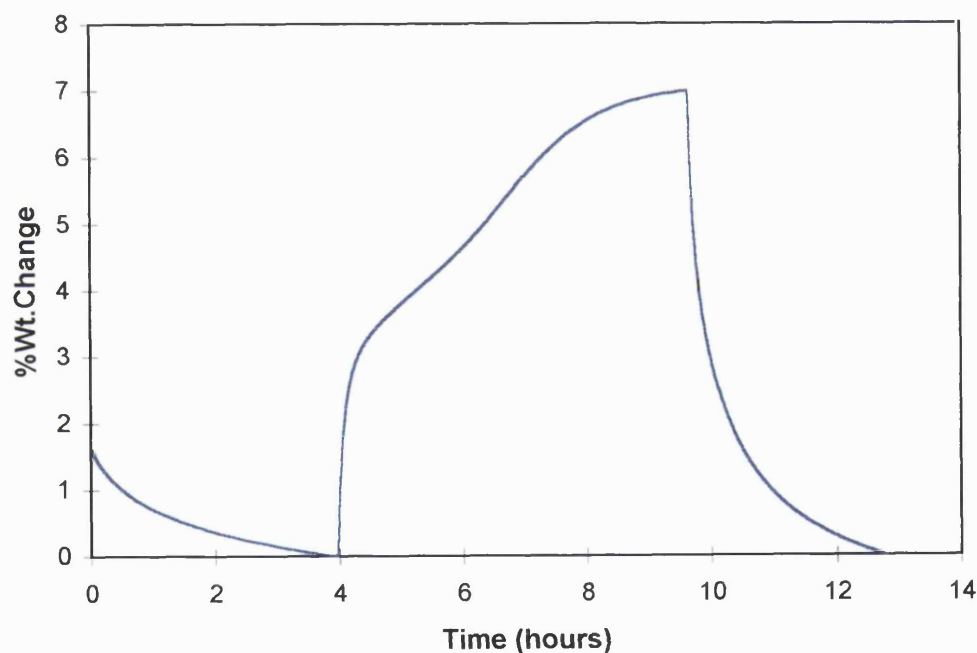


Figure 5.1 Water uptake in amorphous lactose at 40%RH until equilibrium (after initially drying at 0%RH for 4hours), followed by desorption as the relative humidity is returned to 0%RH, until equilibrium.

A further observation on the biphasic sorption profile at 40%RH is that when the sample is dried i.e. returned to 0%RH until equilibrium, as shown in Figure 5.2, and is

then subsequently exposed to 40%RH for a second time, this biphasic type of sorption does not occur. This can be seen in Figure 5.2 where a rapid sorption profile is observed until the sample reaches equilibrium at exactly the same water content as on the first sorption cycle (having lost all the initial sorbed water on the 1st desorption cycle). It would appear from this repeat cycling that although no structural transition appears to have occurred, the sample has changed in some way which has resulted in a much faster sorption rate on the second sorption cycle. This may represent a wetting effect since the sample on the second cycle (although dry) has already absorbed about 5% water which may have decreased its viscosity and hence changed the molecular mobility leading to some degree of flow occurring.

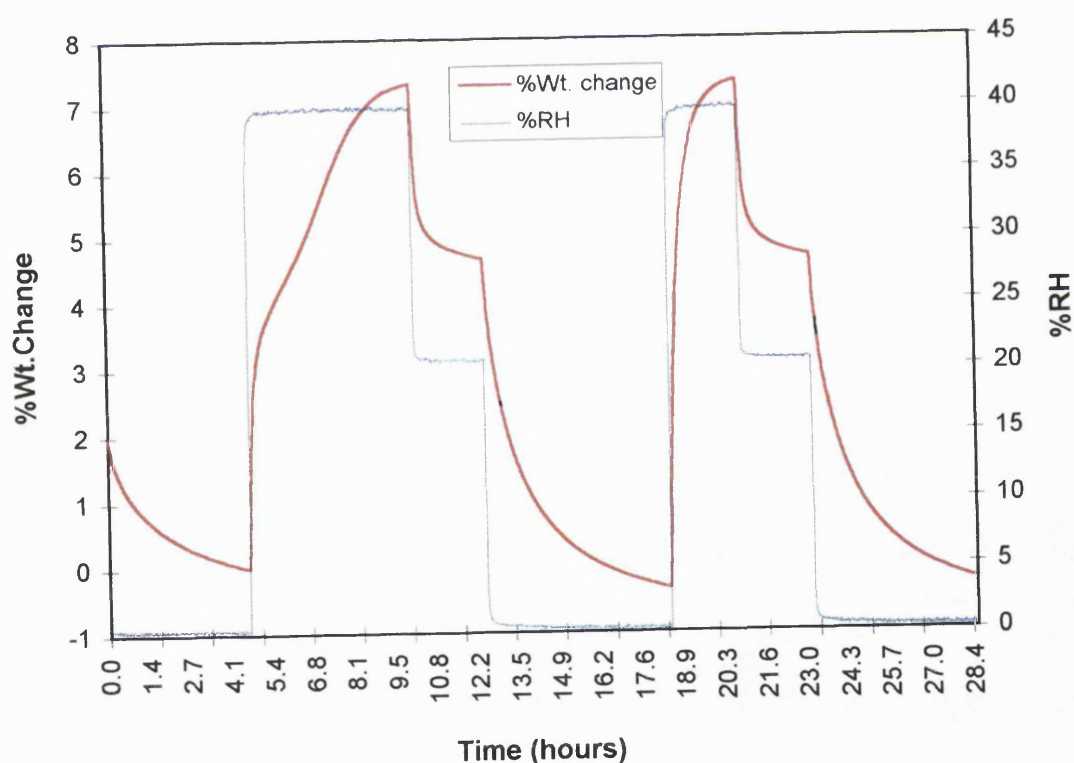


Figure 5.2 Two repeat sorption/desorption cycles for amorphous lactose, at 40%RH until equilibrium (after initially drying at 0%RH for 4hours) followed by desorption at 20%RH and finally 0%RH.

5.3.1.2 Amount of water sorbed at 40% RH.

These samples which were exposed to 40%RH reached equilibrium at approximately 7% water uptake, over the original sample mass, as can be seen in Figure 5.1. Since 15mg samples were used here this was an increase of 1.05mg, resulting in a total sample weight of 16.05mg, of which water is 6.54% w/w.

By application of the Gordon-Taylor equation:

$$T_g = (w_1 T_{g1} + k w_2 T_{g2}) / (w_1 + k w_2) \quad \text{..... equation 5.1}$$

where w represents the weight fraction of the two materials (subscript 1 and 2) and k is a constant, it can be predicted that 6.54% water would lower the T_g of the amorphous lactose to 26.7 °C, which is just above the temperature of the experiment i.e. 25°C. The values for T_g of lactose and water are 101°C and -135°C respectively, and the value of k is 6.56 (Roos, 1993). Since it is accepted that the amorphous material will crystallise when T_g has been lowered below T , it is not surprising that the sample is still amorphous. This also agrees with the observation reported in Chapter 3 that the crystallisation did not occur when the amorphous lactose samples were exposed to a saturated salt solution which gave a relative humidity of 47%RH.

5.3.1.3 Rate of desorption on returning the relative humidity either to:

(a) 0%RH directly or (b) 20%RH and then 0%RH.

(a) The desorption of water from a sample which was held at 40%RH, and was first returned to 20%RH and finally to 0%RH, was very rapid, as shown in Figure 5.3. From this figure it can be seen that the rate of desorption is related to the external relative humidity i.e. the rate is faster if the external relative humidity is 0%RH than if it is 20%RH. A sharp weight loss is observed as the %RH is returned to 20%RH, with the sample reaching equilibrium after about 2 - 3 hours, and again a dramatic drop-off in weight occurs as the %RH is changed from 20 - 0%RH. This demonstrates that the rate of desorption is linked to the concentration gradient at the surface of the sample.

(b) The rate of desorption of water from a sample of amorphous lactose which had been exposed to 40%RH until equilibrium and then exposed directly to 0%RH, was equally rapid as shown in Figure 5.1. There is a dramatic weight loss as the sample is initially returned to 0%RH, with approximately 5% of the sorbed water being lost in an hour, and all the water lost after approximately 3 hours.

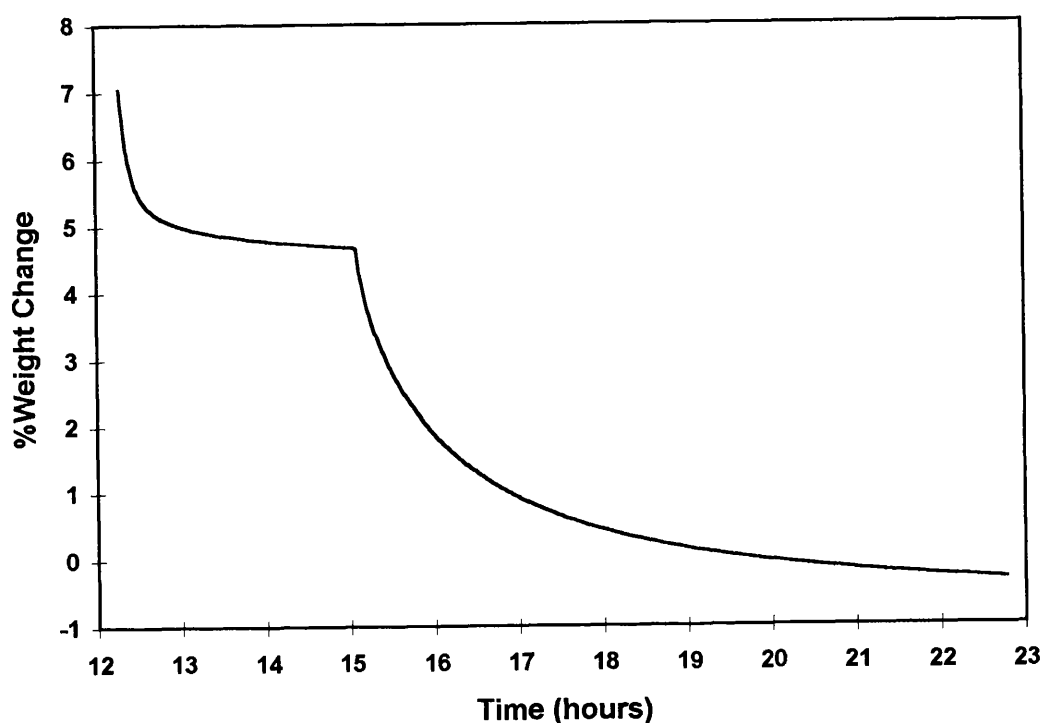


Figure 5.3 Water desorption from an amorphous lactose sample (after exposure to 40%RH until equilibrium, after drying at 0%RH for 4 hours), as the relative humidity is returned to 20%RH and then 0%RH.

5.3.1.4 Rate and extent of water sorption at 50% RH.

Figure 5.4 shows the water uptake for an amorphous lactose sample which was exposed to 50%RH until equilibrium. It can be seen that there is a sharp uptake of water which continues until the sample comes to equilibrium after approximately 3 hours at 50%RH. The sorption profile for this sample which was exposed to 50%RH

is different to that which was exposed to 40%RH in that there is no obvious inflection point for the sample exposed to 50%RH i.e. there is little evidence of the biphasic sorption profile. This is probably due to the sample becoming saturated faster at the higher %RH, unlike at 40%RH where the time frame over which the sorption behaviour alters as the sample structure begins to change, makes it possible to observe and measure.

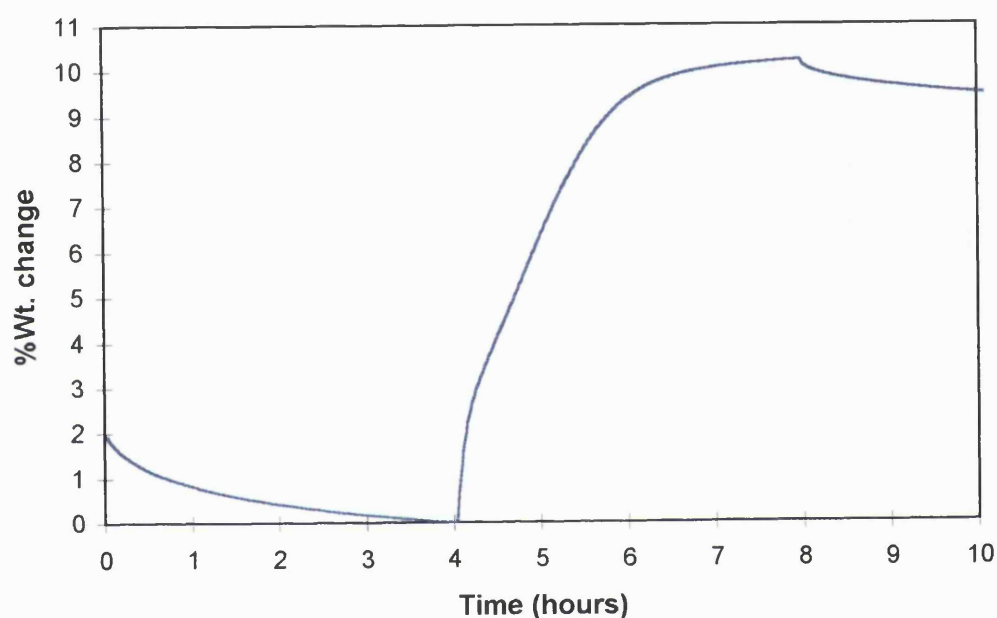


Figure 5.4 Water uptake in amorphous lactose at 50%RH until equilibrium (after initially drying at 0%RH for 4hours), followed by desorption as the relative humidity is returned to 0%RH.

The samples exposed to 50%RH, typically come to equilibrium with a moisture content of about 9.25% w/w water, which is equivalent to a 10.2% increase over dry weight. This water content of 9.25% w/w would lower the T_g of the amorphous lactose to 11.9°C (as calculated from equation 5.1), which is well below the experimental temperature (25°C), hence it is not surprising that the amorphous lactose crystallises spontaneously at this relative humidity. It must be noted however that the

samples don't crystallise instantaneously at 50%RH, but require approximately 11 hours as shown in Figure 5.5.

This lag time between water sorption and crystallisation appears to be dependent on the water content of the amorphous material, e.g. a sample exposed to 75%RH, as shown in Figure 5.5, crystallised before it reached equilibrium moisture content. It can be seen that the water uptake at 75%RH is more rapid than that at 50%RH, with the sample reaching a water uptake of almost 13% before it instantaneously crystallises after approximately 47 minutes at 75%RH, whereby the sorbed water is released. This sample reached a water uptake just prior to crystallisation of 11.2% w/w over dry weight, and from equation 5.1 this would be sufficient to lower the T_g of the amorphous lactose to 1.04°C . This is again well below the operating temperature, therefore the sample readily crystallises. These data show also how the crystallisation is a time dependent process i.e. although T_g has been lowered below T , instantaneous crystallisation does not always occur, with nucleation and crystal growth being controlling factors here also.

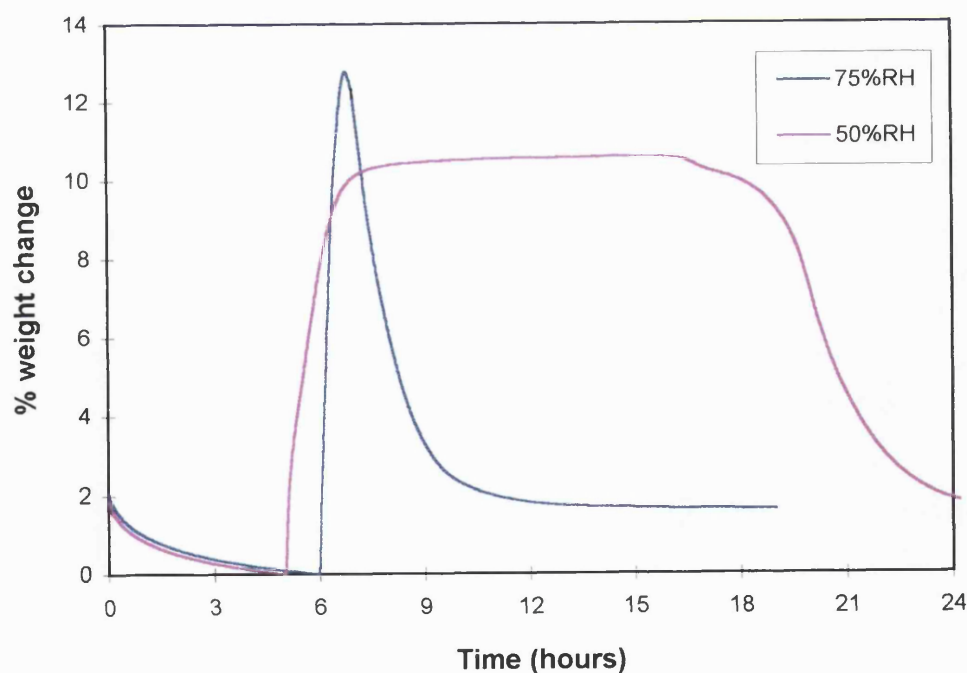


Figure 5.5 Crystallisation of amorphous lactose samples on exposure to 50%RH and 75%RH in the DVS.

These observations on the decreased delay time before crystallisation for samples exposed to increasing relative humidities agree with the observations from the microcalorimetry experiments where similar samples of 100% amorphous lactose were exposed to saturated salt solutions of specific relative humidities. The delay time before crystallisation was similarly reduced as the %RH was increased. However, the rate of supply of moisture to the samples investigated in the microcalorimeter would be much slower than that in the DVS, since the rate and onset of crystallisation are dependent on the rate of evaporation of the salt solution.

5.3.1.4 Rate of desorption on returning the sample from 50%RH to (a) 20%RH and then 0%RH (b) 0%RH directly.

The desorption of water from samples which had been allowed to equilibrate at 50%RH and then returned to a lower relative humidity was found to be very slow, unlike those which were exposed to 40%RH. This can be seen in Figure 5.6 where the desorption of water from a sample which was returned initially to 20%RH (after being exposed to 50%RH) and finally to 0%RH is shown. The desorption from a sample which was returned directly to 0%RH is shown in Figure 5.4. It can be seen from these two graphs that the desorption of water from these samples is independent of the relative humidity. This is especially obvious for the sample which was initially exposed to 20%RH and then exposed to 0%RH, since the desorption rate was not altered by changing the %RH from 20%RH to 0%RH. (The point where the %RH is altered from 20%RH - 0%RH is indicated by an asterisk on Figure 5.6).

The diffusion of water in sugar-water mixtures and glasses of carbohydrates has recently been investigated by Parker and Ring (1995) and Tromp et al (1997). Parker and Ring measured the mean diffusion coefficients, D , during desorption of water for maltose-water mixtures close to the T_g , using a gravimetric method. The mass showed a linear dependence on the square root of time. These studies revealed that at low water contents the T_g of the mixture was very sensitive to variations in water content and linear dependence of $\log D$ upon water content suggested an exponential dependence between these two factors. Parker and Ring further suggested from their studies that while there was slowing of diffusion as T_g was approached, it was not associated with the change in shear viscosity since this strongly diverged from diffusion coefficient at values of T_g/T in the region of 0.8.

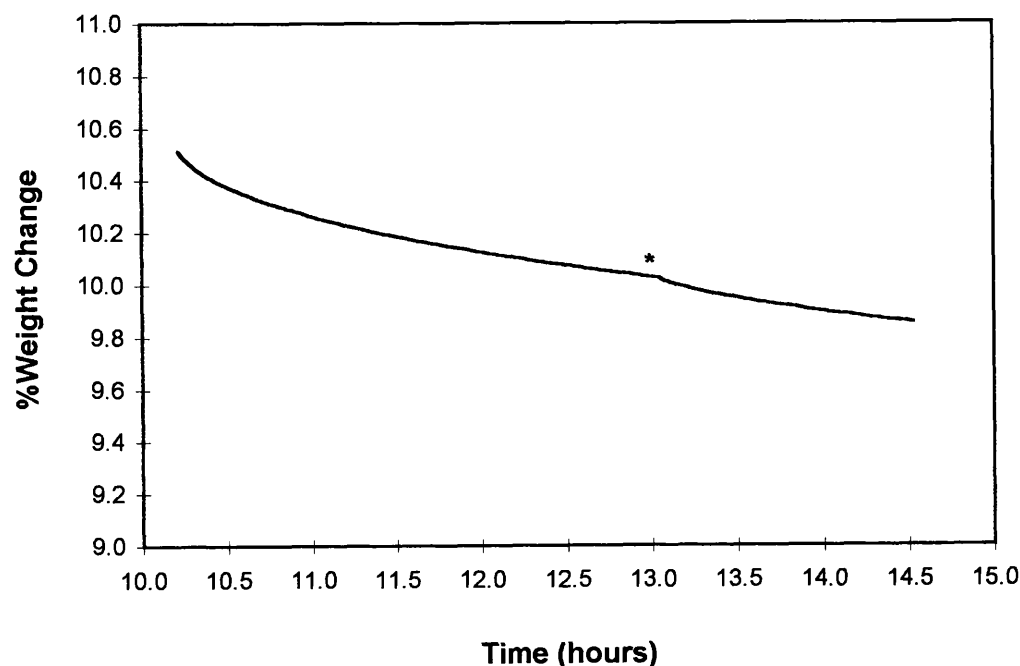


Figure 5.6 Desorption of water from samples exposed to 50%RH until equilibrium and then exposed to 20%RH and 0%RH (the point where the RH changes from 20% - 0% is indicated by an asterisk).

The lactose samples investigated here produced a linear relationship when the weight loss, on reducing the %RH from 50% to either 20% or 0%RH, was plotted as a function of the square root of time as shown in Figure 5.7. This can also be seen from Table 5.1 where the slopes of plots of %weight change as a function of square root of time for the desorption from the amorphous lactose, induced by changing the RH to 0% following exposure to 50%RH (as shown in Figure 5.7) are listed. For consistency, a linear region beginning after 5 hours at 0%RH of these curves was chosen to calculate the slopes. The slope or gradient of the desorption curve for a sample where the external %RH was 20% was $-0.107\% / \text{min}^{-0.5}$ which was followed by external RH of 0%, where the gradient of the desorption curve was $-0.108\% / \text{min}^{-0.5}$. These data indicate that the desorption of water in these samples which have been held at 50%RH until equilibrium water content was reached, is controlled by the diffusion of water through the sample.

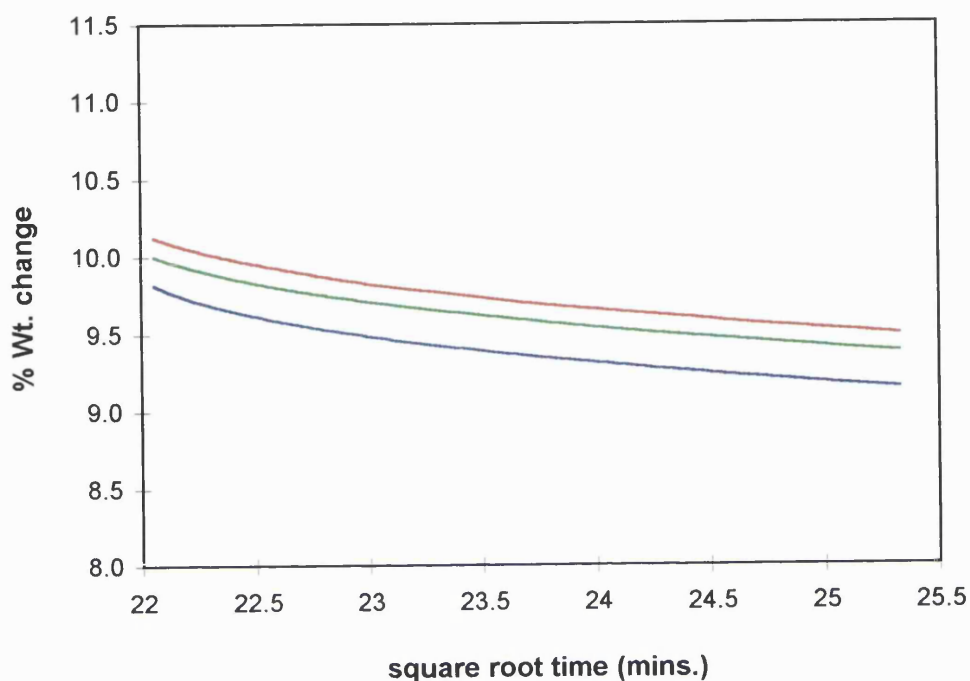


Figure 5.7 Plots of %weight change as a function of square root of time for the desorption curves (at 0%RH) for 3 samples of amorphous lactose after exposure to 50%RH until equilibrium.

Time spent at 50%RH (minutes)	Slope (% min ^{-0.5})
≤ 60	Not linear
75	0.091 ; 0.083
90	0.136 ; 0.135
120	0.100 ; 0.114
210	0.108 ; 0.127 ; 0.132

Table 5.1 Slopes of plots of %weight change as a function of square root time for the desorption from amorphous lactose induced by changing to 0%RH following exposure to 50%RH for different times.

Levine and Slade (1986), in discussing sorption-desorption hysteresis in relation to amorphous materials, stated that hysteresis is characteristically manifested by a desorption isotherm which occurs at higher moisture content than the corresponding sorption isotherm as seen here in Figures 5.4 and 5.6, such that desorption 'appears' to take place at a lower 'effective' temperature despite constant T . They also stated that if sorption by a glass produces a porous, plasticised, elastic rubber, this may lead to structural collapse (at $T > T_g$) during desorption which may result in decreased desorption rate and decreased hysteresis. This has been observed by Chinachoti and Steinberg (1986) where not only was there a sudden slope change in the sorption isotherm, but hysteresis was also observed as a consequence of irreversible collapse, which was followed immediately by sucrose crystallisation from the rubbery state. It has been concluded that hysteresis characteristically results from a moisture/temperature/time-dependent, slow, non-equilibrium, swelling-related conformational change (involving a structural relaxation, and in some cases, even a subsequent phase change), which is facilitated by increasing free volume and mobility in an amorphous material which is being plasticised.

The very slow desorption of water (Figure 5.6) from the amorphous lactose which was exposed to 50%RH until equilibrium, would therefore seem to indicate that the sample has undergone structural collapse. This is in accordance with the view that once T_g falls below T (from the discussion on the amount of water taken up by this sample the T_g was seen to have fallen to around 6°C) the increased viscosity prevents the sample from being able to support its own weight under gravity and hence it collapses i.e. the porosity is decreased and the material becomes more dense. Flink (1983) also noted from literature observations that the onset of collapse during freeze-drying resulted in no further improvements in drying rate, which was presumably due to blockage of the pores which would normally be the pathways for removal of water vapour. Thus, it could be concluded that the onset of collapse will serve as a natural limit to heat input, as further heat input will not give any improvements in drying rate. This statement and the consequences of continued drying and heating of collapsed lactose will be discussed in more detail later on in this chapter.

5.3.1.6 The effect of exposing the amorphous lactose to 50%RH for varying lengths of time.

The samples which were exposed to 40%RH until equilibrium was reached, lost all their absorbed water on drying (at 20%RH or 0%RH), unlike those which were exposed to 50%RH until equilibrium, which retained practically all their sorbed water. It was decided therefore, to look at the effect of exposing samples of the lactose to 50%RH for varying lengths of time and investigate the extent of water loss from these systems on drying at 0%RH. The data in Figure 5.8 shows the desorption behaviour of amorphous lactose samples (on returning the relative humidity to 0%RH), which were exposed to 50%RH for the following lengths of time: 5, 60, 75 and 90 minutes and 2 and 3 hours.

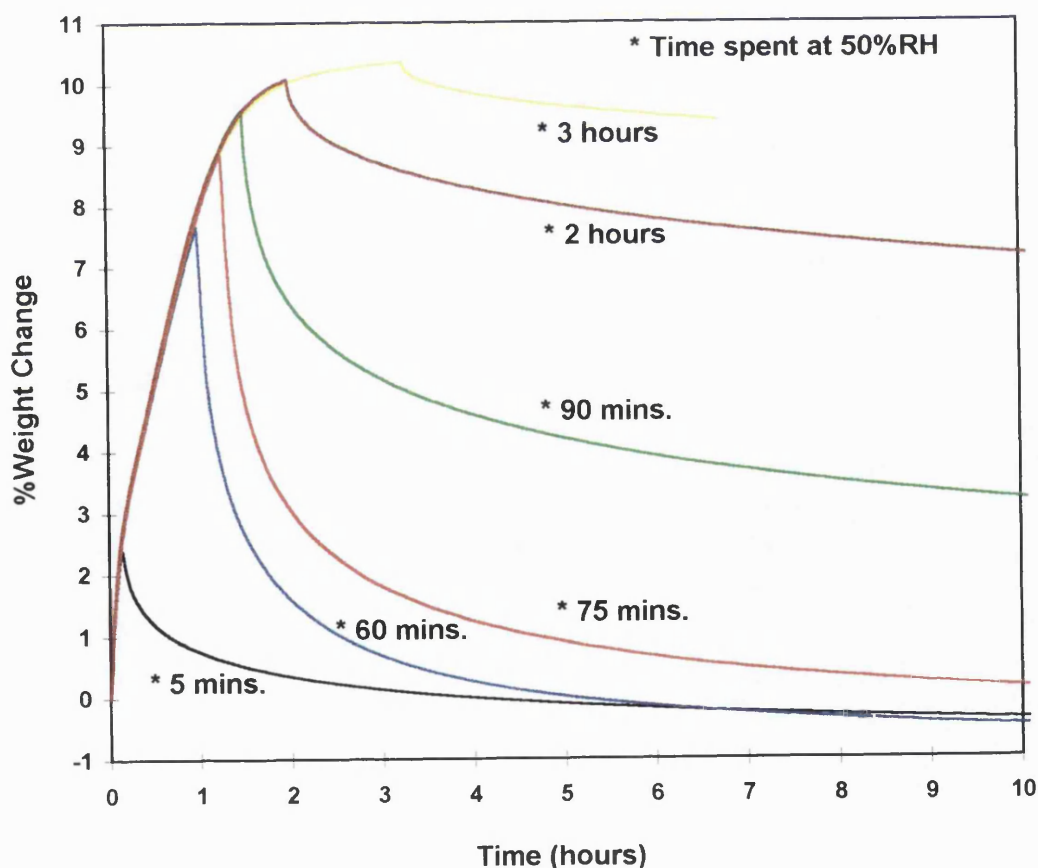


Figure 5.8 Desorption of water from samples exposed to 50%RH for varying lengths of time.

The graph shows an initial weight increase as the samples are exposed to 50%RH for a certain time, which is then followed by various degrees of weight loss for the different samples as the relative humidity is returned to 0%RH. The water sorption profile for all the samples are superimposed, indicating that the same rate of water uptake is taking place in all the different samples and also indicating the reproducibility of the system. However, the desorption profiles for these samples differ greatly depending on the length of time the sample is exposed to 50%RH. The samples which were exposed to 50%RH for 5 minutes and 60 minutes resulted in all the sorbed water being desorbed when the relative humidity is returned to 0%RH, as shown. The water uptake after 60 minutes at 50%RH was in the region of 7.7% weight increase on the original sample which is 7.12% w/w and using equation 5.1, this would be predicted to lower the T_g to 25.86°C, which is just above T (experimental temperature). For exposure times in excess of 60 minutes the water uptake reached by all the samples was sufficient to lower the T_g to (and below) T , as can be seen from Table 5.2 where the water uptake at 50%RH for the samples shown in Figure 5.8 are listed along with the corresponding value of T_g which has been calculated using the Gordon-Taylor equation. The extent to which T_g fell below T depended on the time spent at 50%RH and consequently the water uptake.

<i>Time spent at 50%RH (minutes)</i>	<i>Water uptake (%) w/w</i>	<i>T_g °C (on being lowered due to plasticisation by the sorbed water)</i>
5	2.32	69.8
60	7.12	25.9
75	8.12	19.0
90	8.73	15.5
120	9.16	12.4
210	9.39	11.0

Table 5.2 Typical water uptake values reached for samples exposed to 50%RH for varying times, and the corresponding T_g values calculated using the Gordon-Taylor equation.

The desorption isotherms for these samples on returning the relative humidity to 0%RH, varied depending on the time spent at 50%RH and consequently the extent to which T_g has fallen below T . The samples exposed to 50%RH for 75 minutes and 90 minutes show only partial desorption of sorbed water with an initial sharp weight loss as the relative humidity is returned to 0%RH, followed by a slower desorption profile. The 90 minute sample loses approximately half its sorbed water, and then the slow diffusion of water starts to dominate the desorption profile. The samples exposed to 50%RH for 2 hours lose a small amount of water also before the slow diffusion begins. The samples which were exposed to 50%RH for 3 hours and longer show very little loss of sorbed water as the relative humidity is returned to 0%RH.

The different degrees of hysteresis on the desorption isotherms indicate that these samples have collapsed to different degrees. Those exposed to 50%RH for less than 1 hour (where the T_g remained above T) do not appear to have collapsed, while those which were exposed to 50%RH for times in excess of 3 hours appear to be predominately collapsed (since virtually no desorption of water took place) and those exposed for times in between appear partially collapsed. These experiments show how the level of moisture in the amorphous sample plays a very important role in collapse. It would appear also that approximately 8% water uptake was necessary to initiate collapse in this system i.e. from Table 5.2 the %w/w water uptake for the sample held at 50%RH for 75minutes (this sample was the first to show slow release of sorbed water) was 8.12%. This is in exact agreement with Tsourouflis et al (1976) who found that the moisture content necessary to collapse amorphous lactose at ambient temperature was about 8%.

To and Flink (1978) in discussing methods for measuring degree of collapse, showed how heating freeze-dried sugars e.g. sucrose and maltose, to temperatures very close to and above the collapse temperature, the extent of collapse could be controlled and partially collapsed systems could be obtained. It was stated therefore that collapse is not an all or nothing phenomenon, rather once started, it can be stopped partway or allowed to proceed to a higher degree depending on the temperature reached. They further stated that due to different levels of internal bonding in a freeze-dried system and considering the inhomogeneity of structure (which is expected in an amorphous solid), the stability of partial collapse is not surprising as this merely reflects the situations where only a part of the matrix has adequate conditions for flow to occur.

Partial collapse has also been studied more in relation to two component systems and mixtures where collapse temperature could be altered by one of the components thus stabilizing the system. In the same way in the experiments carried out in this study different degrees of collapse have been obtained by carefully controlling the moisture levels present in the amorphous samples.

5.3.1.7 Checking the amorphous nature of the collapsed lactose using isothermal microcalorimetry

In order to investigate this collapsed material further and measure the extent to which it was still amorphous, samples which had been held at 50%RH until equilibrium was reached were removed from the DVS and were exposed to a saturated salt solution of sodium dichromate in the microcalorimeter. The result of this experiment is shown in Figure 5.9 (solid line) which is a typical microcalorimetry response for a sample which had been exposed to 50%RH for 2 hours. This sample was taken to be predominately collapsed when removed from the DVS, since desorption of water was very slow and followed the same pattern as shown in Figure 5.6.

It can be seen that the collapsed lactose sample had exactly the same sharp spontaneous crystallisation response as was observed for the original uncollapsed material, shown also in Figure 5.9 (broken line). The area under the curve for the collapsed sample was found to be 47.0 J/g, which is exactly the same heat change associated with the crystallisation of the uncollapsed amorphous material. It would appear therefore that collapse of the amorphous structure does not contribute towards the heat change accompanying the crystallisation process. This would also indicate that collapse of the amorphous sample is a distinct and unique morphological process separate from the crystallisation process.

5.3.1.7.1 Differences between microcalorimetry crystallisation responses for the uncollapsed amorphous lactose and the collapsed material.

Although it is obvious from measurements of the heat change accompanying the crystallisation of the collapsed structure that the observed microcalorimetric crystallisation response for the collapsed and uncollapsed systems were identical, there is one aspect of the overall crystallisation response which is different. It seems that the initial broad response, which was originally suspected to be due to wetting of the amorphous material, may actually be collapse of the amorphous material. The

reason for this notion is due to observations from the microcalorimetry responses shown in Figure 5.10 for 2 samples of amorphous lactose, one of which had been exposed to 50%RH for 75 minutes and the other for 120 minutes. As shown earlier in Figure 5.8, the sample which was exposed to 50%RH for 75 minutes was only partially collapsed (since only a small amount of sorbed water was lost on returning the relative humidity to 0%), but the one exposed to 50%RH for 2 hours had what appeared to be a totally collapsed structure since a slow desorption profile was observed on returning to 0%RH. In Figure 5.10 the partially collapsed sample shows an initial peak for up to 1 hour, while the collapsed sample shows no initial peak. This is also apparent in Figure 5.9 where a relatively large initial response is observed for the uncollapsed sample compared to the sample which had been exposed to 50%RH for 2 hours and was therefore predominantly collapsed.

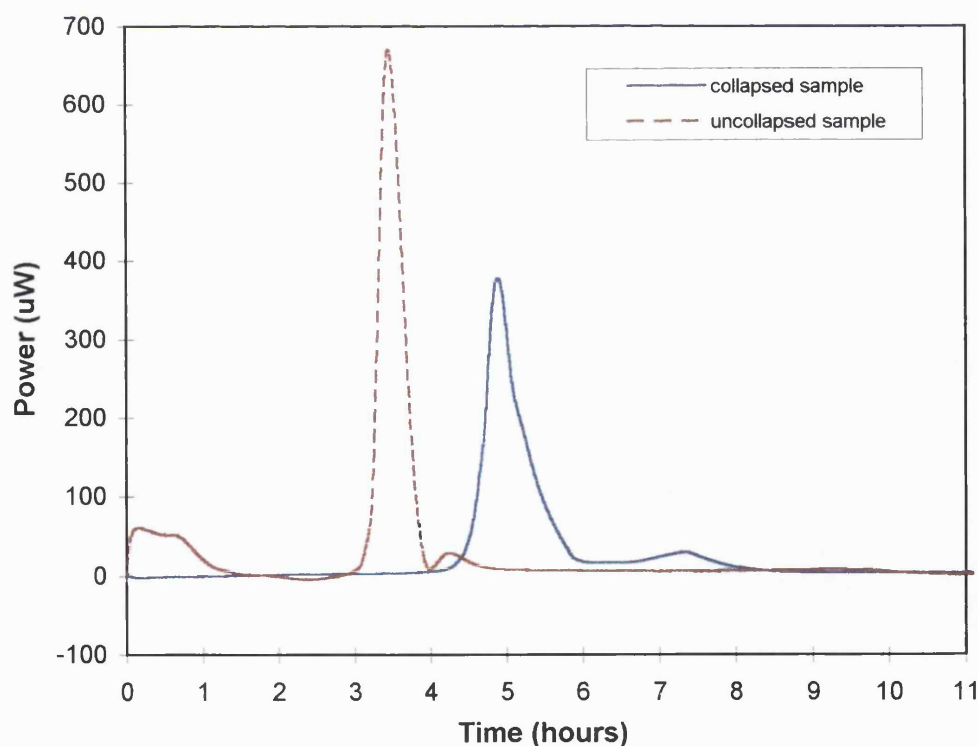


Figure 5.9 Typical microcalorimetry crystallisation responses for collapsed (solid line) and uncollapsed amorphous lactose (dotted line).

To investigate this theory further, a sample of amorphous lactose (which had been removed directly from a dry environment) was exposed to 33%RH (magnesium chloride salt solution) in a sealed glass ampoule in the microcalorimeter. The result of this experiment is shown in Figure 5.11 where it can be seen that only a very minor deviation from the baseline is observed, which is probably due to heat fluctuations on lowering the glass ampoules to the measuring position. This is as expected since the supply of water vapour at 33%RH would not be sufficient to cause structural collapse. It would appear therefore that this initial exotherm which was originally attributed to wetting of the amorphous material is more likely to be a collapse event. This again is in keeping with the view that the evaporation of the salt solution (endothermic response) should be equal and opposite to the condensation of water vapour onto the amorphous sample (exothermic response) and should consequently cancel each other out.

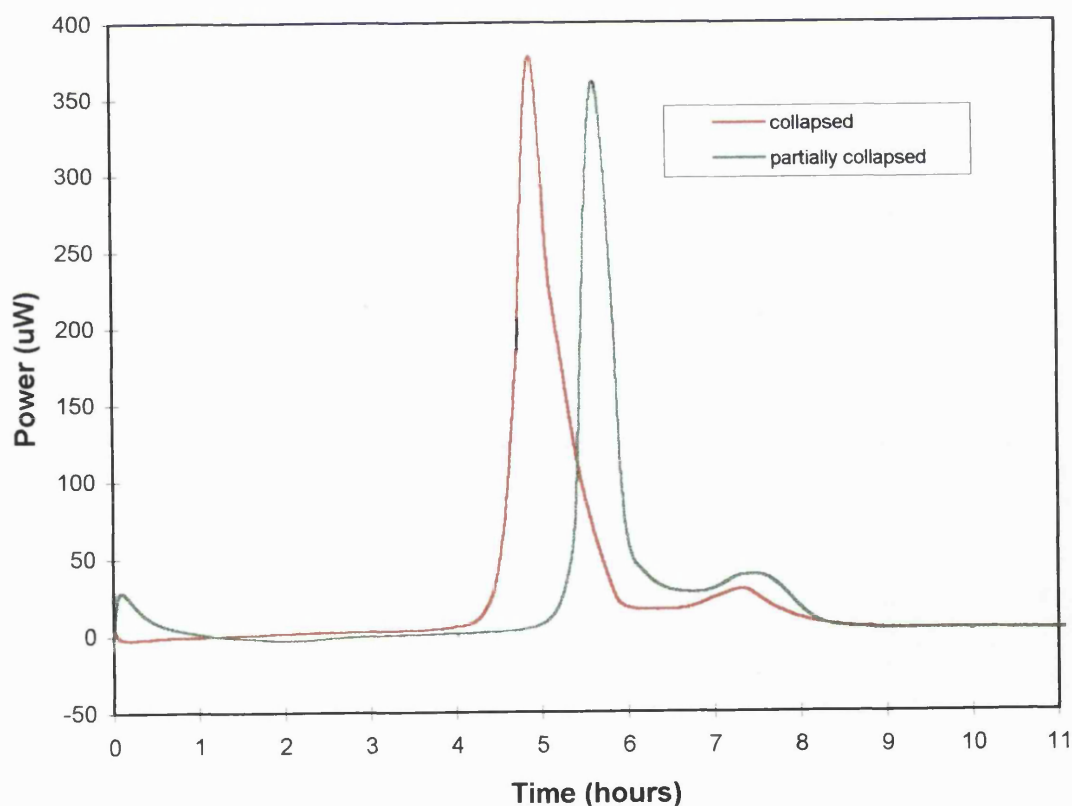


Figure 5.10 Typical microcalorimetry crystallisation responses for a collapsed amorphous lactose sample and a partially collapsed sample.

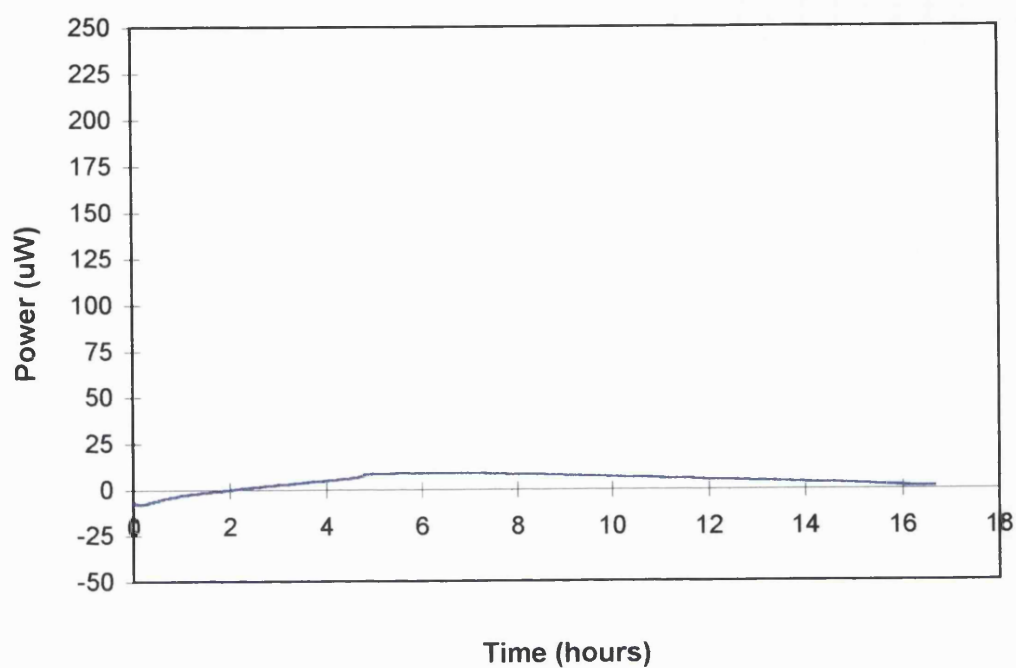


Figure 5.11 Typical microcalorimetry response for an amorphous lactose sample on exposure to magnesium chloride salt solution (33%RH).

5.3.2 PHYSICAL CHARACTERISATION BY THERMAL ANALYSIS OF AMORPHOUS LACTOSE CONTAINING VARYING DEGREES OF COLLAPSE.

5.3.2.1 Uncollapsed samples.

5.3.3.1.1 Differential Scanning Calorimetry

The superimposed graphs in Figure 5.12 show the differential scanning calorimetry results for 3 repeat samples of amorphous lactose which was exposed to 50%RH in the DVS for 60 minutes and which were then rapidly transferred to the DSC for analysis. Samples which were exposed to 50%RH and then dried at 0%RH until equilibrium were also scanned in the DSC, and a typical result for one of these samples is also shown in Figure 5.12.

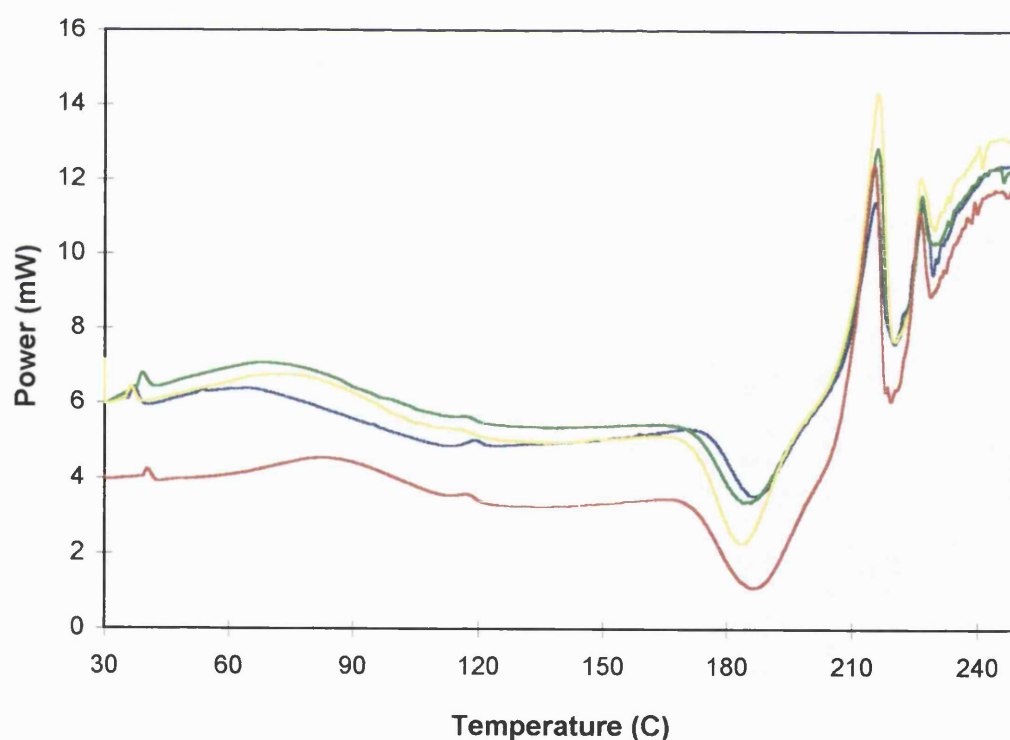


Figure 5.12 DSC data for uncollapsed amorphous lactose samples (samples were exposed to 50%RH for 60mins.)

The DSC traces show a crystallisation exotherm at approximately 185°C which corresponds to that measured for the untreated original spray dried lactose (shown by the red line in Figure 5.12). Two melting endotherms are observed at approximately 216°C and 229°C for α and β lactose melting, respectively, with α being the predominant form. No obvious differences were observed between the samples which were removed directly from the DVS after exposure to 50%RH and those which were dried at 0%RH before being analysed. These results indicate that although these samples were exposed to 50%RH for 1 hour and took up in the region of 7% water, no structural collapse appeared to have occurred since this amount of water was not sufficient to lower T_g below T .

5.3.2.1.2 Thermogravimetric analysis.

The graphs in Figure 5.13 show the TGA results for the same 3 samples which were exposed to 50%RH for 1 hour. A broad water loss peak at approximately 50°C, which is loss of sorbed water, can be observed for all the samples. The magnitude of the water loss peak for the 3 samples which were 6.9%, 6.0% and 6.5%. TGA graphs for samples exposed to 50%RH for 1 hour followed by drying at 0%RH until equilibrium showed a similar water loss peak in the same temperature region but of a slightly lower magnitude, since some of the sorbed water has been removed at 0%RH. This broad peak due to loss of sorbed water is consistent (although larger) with that obtained in this region for the original amorphous lactose, as shown earlier in Chapter 3, Figure 3.18.

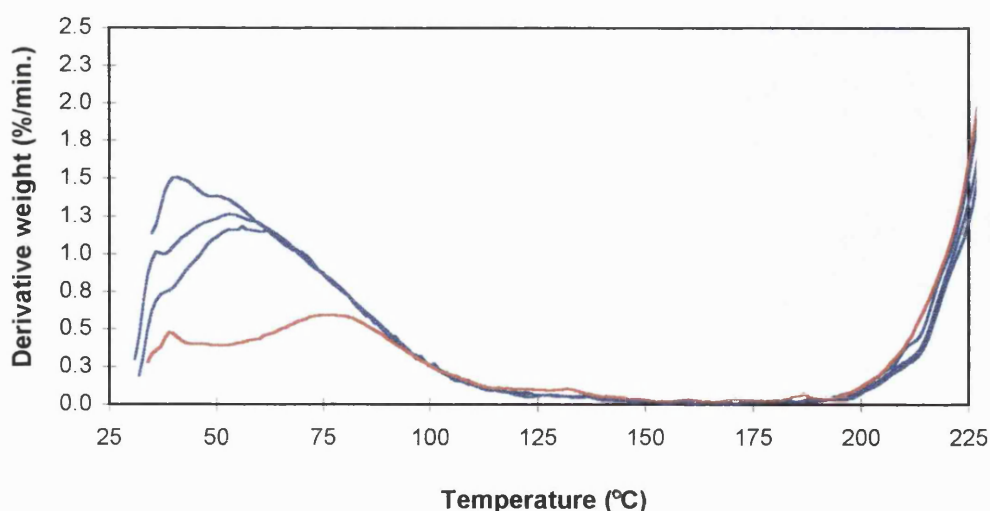


Figure 5.13 TGA data for uncollapsed amorphous lactose samples (samples were exposed to 50%RH for 60mins.)

5.3.2.2 Partially collapsed samples (exposed to 50%RH between 1 - 2 hours).

5.3.2.2.1 Differential Scanning Calorimetry

A typical DSC result for a sample that was exposed to 50%RH for 90 minutes in the DVS and then returned to 0%RH until equilibrium, is shown in Figure 5.14. As indicated in Figure 5.8 samples stored at 50%RH for 90 minutes were partially collapsed as only some of the sorbed water was lost rapidly on returning the relative humidity to 0%RH. These samples typically reached a water uptake in the region of 9.2% equilibrium water content at 50%RH. The DSC trace for this sample contains the crystallisation exotherm at 180°C and the two melting endotherms as obtained for the uncollapsed samples, however, another smaller exotherm has appeared at approximately 85°C and a small endotherm has become apparent at around 125°C.

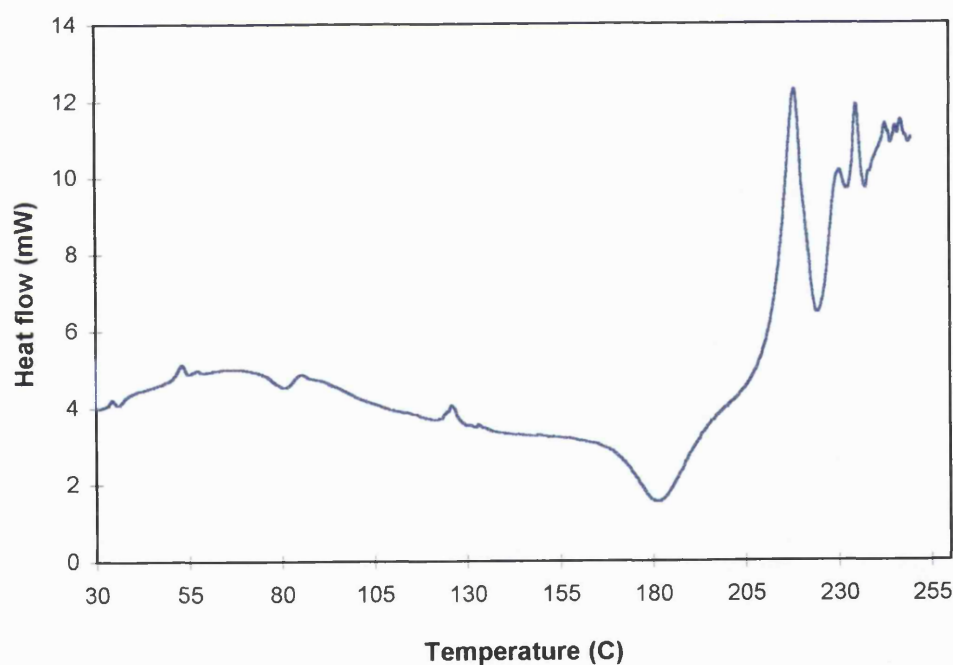


Figure 5.14 DSC data for partially collapsed amorphous lactose samples (exposed to 50%RH for 90minutes)

In order to investigate this new peak at around 85°C, a new sample of this partially collapsed material was prepared by holding the amorphous lactose at 50%RH for 90 minutes as before. The sample was then heated in the DSC until the peak at 85°C

occurred after which time the sample was then cooled to room temperature. It must be noted that this sample was heated from 0 °C here in order to locate T_g, which appears to occur around 15°C, as indicated by an endothermic shift in the baseline. The sample was then removed and was transferred to the microcalorimeter where it was sealed in a glass ampoule with sodium dichromate salt solution (54%RH) in order to check if it was still amorphous. The graph in Figure 5.15 shows the DSC heating profile for this sample i.e. heating from 0 °C - 90 °C followed by cooling to 30 °C. The exotherm at 85°C is clearly present. The graph in Figure 5.16 shows the microcalorimetry trace for this sample after it had been removed from the DSC, on cooling to room temperature, along with the response for a partially collapsed sample which hadn't been heated to 90°C in the DSC.

It can be seen that the sample which had been heated past the exotherm at 85°C in the DSC, and allowed to cool, revealed a very small crystallisation peak which had an area of 6.0J/g. The sample which hadn't been heated in the DSC, but which was placed directly in the microcalorimeter gave a crystallisation peak area of 49J/g, which is identical to that for 100% amorphous lactose. (Note however the absence of the initial wetting exotherm (now suspected to be due to collapse) in the microcalorimetry trace, since this material was partially collapsed on removal from the DVS following exposure to 50%RH for 90 minutes).

It would appear therefore from these results that the DSC exotherm at 85°C is actually crystallisation of the collapsed amorphous lactose. Since this sample was only partially collapsed, it appears that two separate crystallisation exotherms are present in the DSC scan, this one at 85°C and the original crystallisation exotherm at 187°C, as seen in Figure 5.14. A much reduced crystallisation process was measured in the microcalorimeter for this sample which was heated past the first crystallisation exotherm, since part of it was already crystalline. The peak area of 6.0 J/g is therefore an indication of the amount of material that wasn't collapsed.

The above observations and conclusions are supported by extensive DSC studies on the thermal behaviour of humidified rice starches (high molecular weight carbohydrates) by Levine and Slade (1986) and Biliaderis *et al* (1986). Their studies showed how samples with varying water contents displayed different T_g, crystallisation and melting behaviour, and it was suggested that heating in the DSC was responsible for several processes occurring simultaneously in these systems.

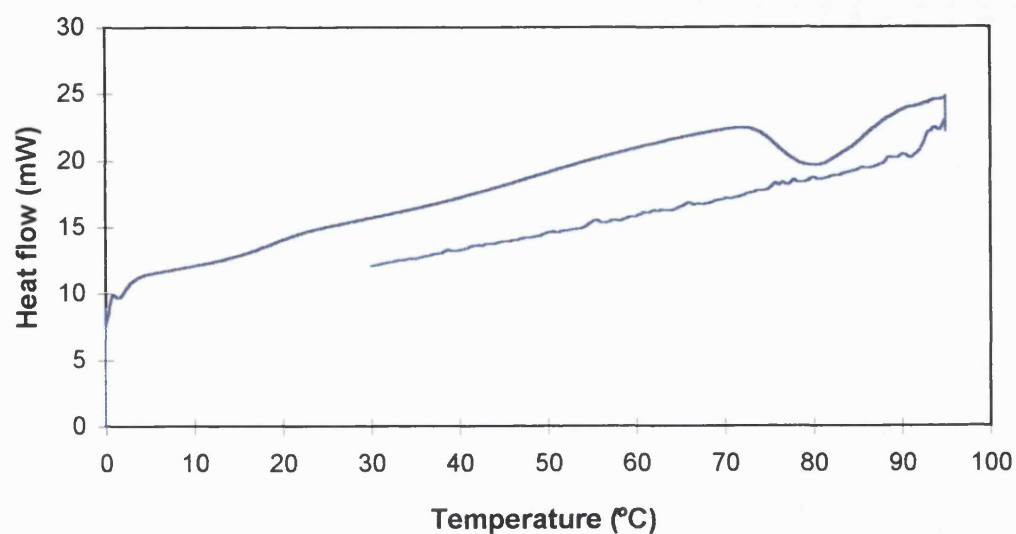


Figure 5.15 Typical DSC scan for a partially collapsed lactose sample which was heated up to 90°C and then cooled to 30°C

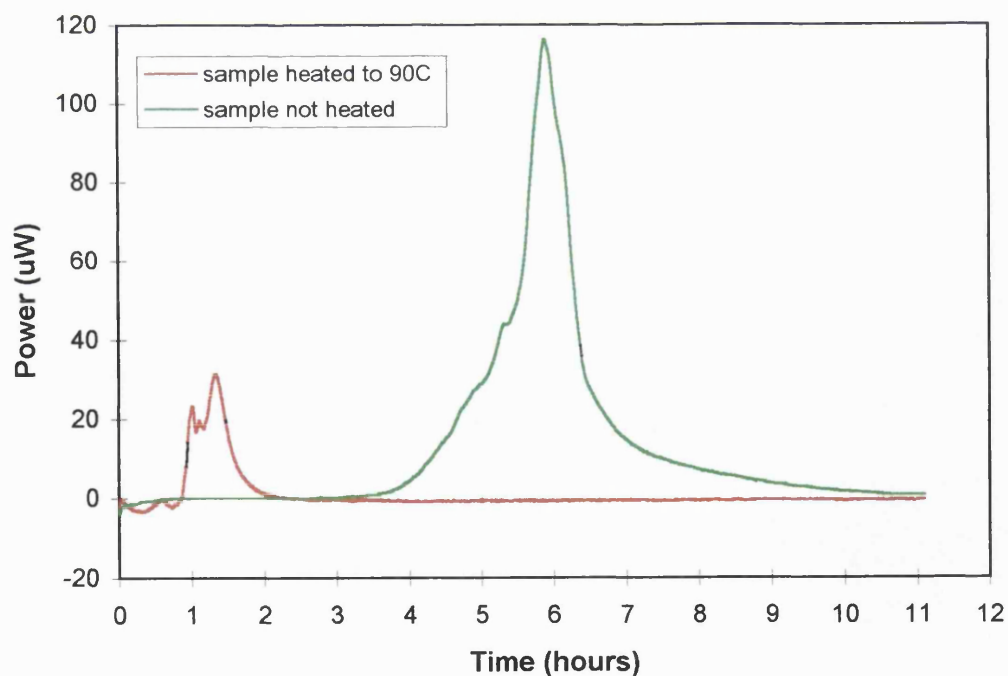


Figure 5.16 Microcalorimetry crystallisation responses for the partially collapsed lactose sample (on exposure to 55%RH) before and after heating to 90°C in the DSC.

5.3.2.2.2 Thermogravimetric Analysis.

The TGA scan for the partially collapsed sample is shown in Figure 5.17. The broad weight loss peak observed for the uncollapsed material is still present, however, a new peak has appeared which is partly superimposed on the broad peak. This new peak is present at around 85°C, which is the temperature at which the crystallisation exotherm was observed in the DSC. The magnitude of the entire water loss peak is 9.1% which is in good agreement with the water uptake value reached in the DVS which was 9.2%.

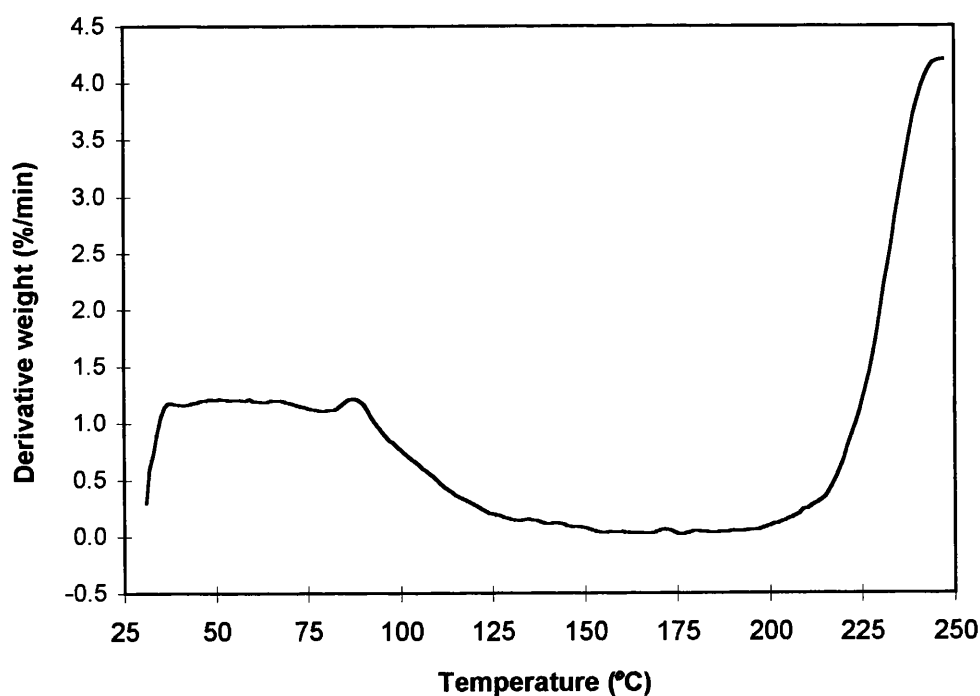


Figure 5.17 TGA data for a partially collapsed amorphous lactose sample (exposed to 50%RH for 90mins.)

5.3.2.3 Predominantly collapsed samples (exposed to 50% RH 2 hours).

5.3.2.3.1 Differential Scanning Calorimetry

A typical DSC result shown in Figure 5.18 for a sample which was exposed to 50%RH for 2.5 hours in the DVS and then returned to 0%RH until equilibrium. As indicated in Figure 5.7 samples stored at 50%RH for 2.5 hours were predominantly collapsed as only a very small amount of the sorbed water was lost on returning the relative humidity to 0%RH. These samples typically reached a water uptake in the region of 9.75% equilibrium water content at 50%RH. The DSC trace for this sample clearly shows the presence of the two crystallisation exotherms at around 73°C and approximately 173°C. The temperature at which both the uncollapsed and collapsed lactose samples crystallise appears to decrease as the sample is exposed to 50%RH for longer periods of time. The endotherm at 115°C has become more prominent. The two melting endotherms for the α - and β - lactose are still present, however, the proportion of these peaks has changed dramatically with β - now being the predominant form. It would appear therefore that the collapsed lactose is crystallising to form mainly β - lactose, unlike the uncollapsed material which has been observed to yield mainly the α - form on crystallising in the DSC.

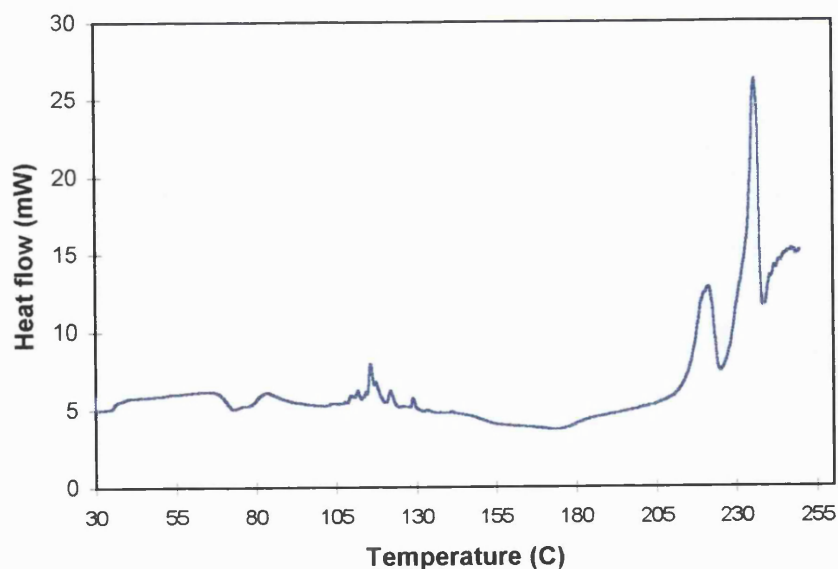


Figure 5.18 DSC data for a predominantly collapsed amorphous lactose sample (exposed to 50%RH for 2.5 hours).

5.3.2.3.2 Thermogravimetric Analysis.

The TGA scan for the predominantly collapsed sample is shown in Figure 5.19 and this now has changed significantly from that observed for the uncollapsed material. The peak at 85°C has become much sharper with a magnitude of around 2.46%. From DSC observations it would appear that this peak is due to water loss on crystallisation of the collapsed amorphous lactose. Significantly though, a much larger sharper peak has emerged at 115°C (magnitude 6.4%), which corresponds with the endotherm in the DSC at 115°C also. This again is thought to be due to water loss as the collapsed material crystallises. The TGA trace also shows the appearance of the lactose monohydrate peak at 150°C (magnitude 0.96%), which is probably being formed as the collapsed lactose crystallises on heating.

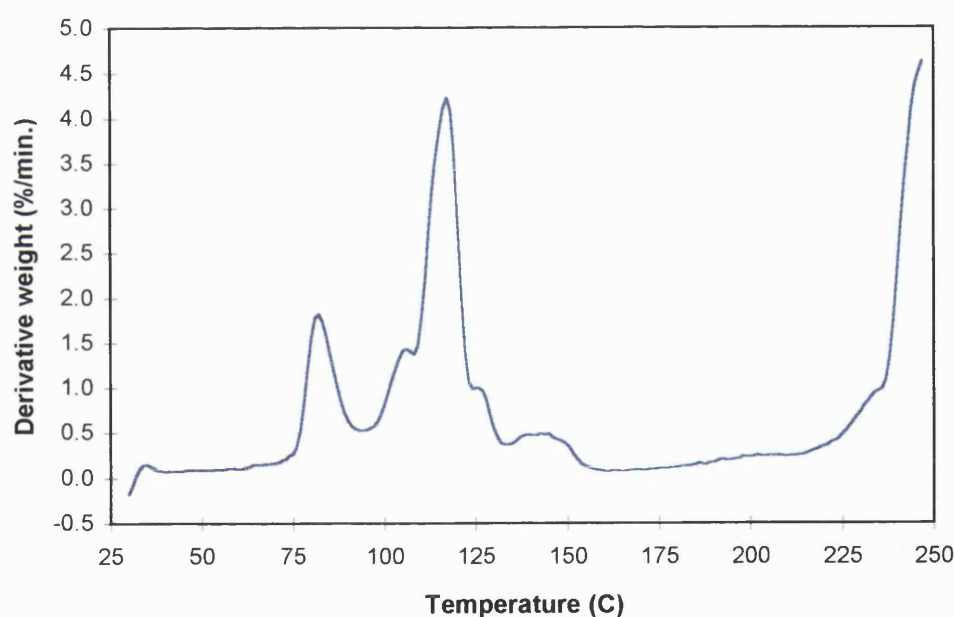


Figure 5.19 TGA data for a predominantly collapsed amorphous lactose samples (exposed to 50%RH for 2.5 hours).

In order to investigate the peak at 115°C further, lactose samples which were predominantly collapsed were prepared and heated at different heating rates in the TGA, since it was suspected that this peak was in part a kinetic event. The graphs in Figure 5.20 are for a sample which reached an equilibrium water uptake of 9.7% after

being held at 50%RH for 2.5hours and then dried at 0%RH until equilibrium. The graph in Figure 5.20 (a) is for a sample of this material which was scanned at the normal heating rate of 10°C/min. and 5.20 (b) is for a sample of the material which was scanned at 2°C/min. It would appear that this peak at 115°C represents a kinetically controlled process since it is occurring at a much lower temperature i.e. 70°C, when the sample is scanned at a slower rate.

An important observation therefore from this study is that the temperature at which these peaks occur heating in the TGA and the DSC changes depending on the scanning conditions.

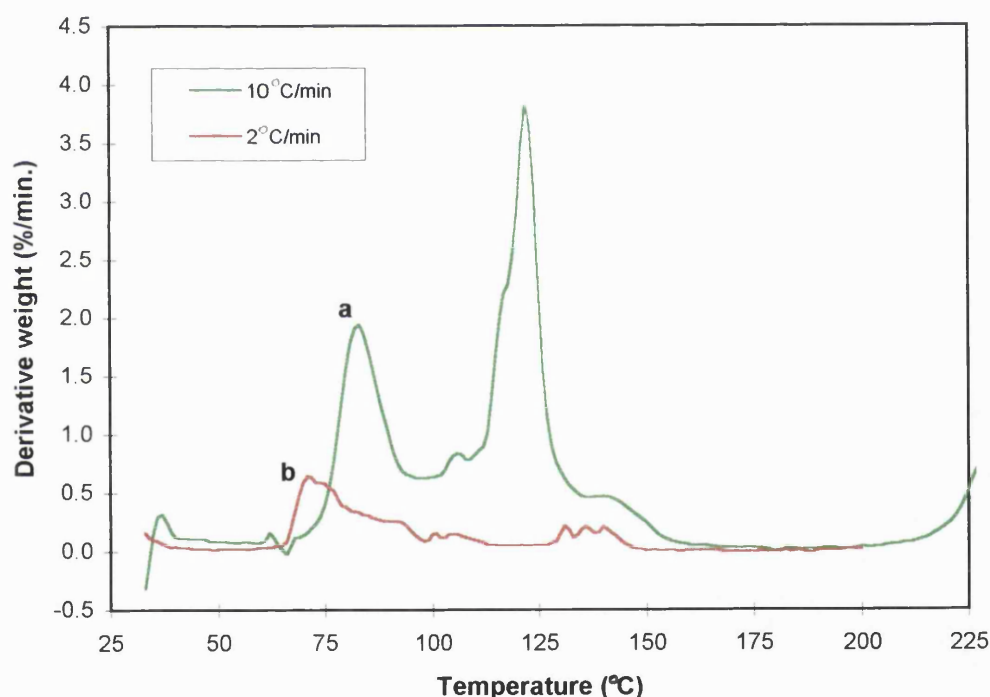


Figure 5.20 TGA data for predominantly collapsed amorphous lactose samples (exposed to 50%RH for 2.5 hours) where (a) was scanned at 10°C/min and (b) was scanned at 2°C/min.

5.3.2.4 Totally collapsed samples (exposed to 50% RH for 3 hours or longer).

5.3.2.4.1 Differential Scanning Calorimetry

DSC and TGA were also carried out on samples which had been exposed to 50%RH for various lengths of time up to 9 hours, where the samples still remained amorphous on removal from the DVS (as indicated by the presence of a crystallisation response on exposing them to 55%RH in the microcalorimeter). The DVS sorption profile for a sample exposed to 50%RH for 4.5 hours indicated that only a negligible amount of water was desorbed on returning the relative humidity to 0%RH as shown in Figure 5.21, indicating that the sample was totally collapsed. This sample reached an equilibrium water uptake in the region of 11.5% at 50%RH. The DSC result for this sample is shown in Figure 5.22 and was very similar to that obtained for the predominantly collapsed sample, Figure 5.18. The crystallisation exotherm at 70°C is clearly visible, followed by a broad endotherm at around 110 °C, the monohydrate peak at 150°C and the α - and β - lactose melting endotherms at 224°C and 240°C, respectively.

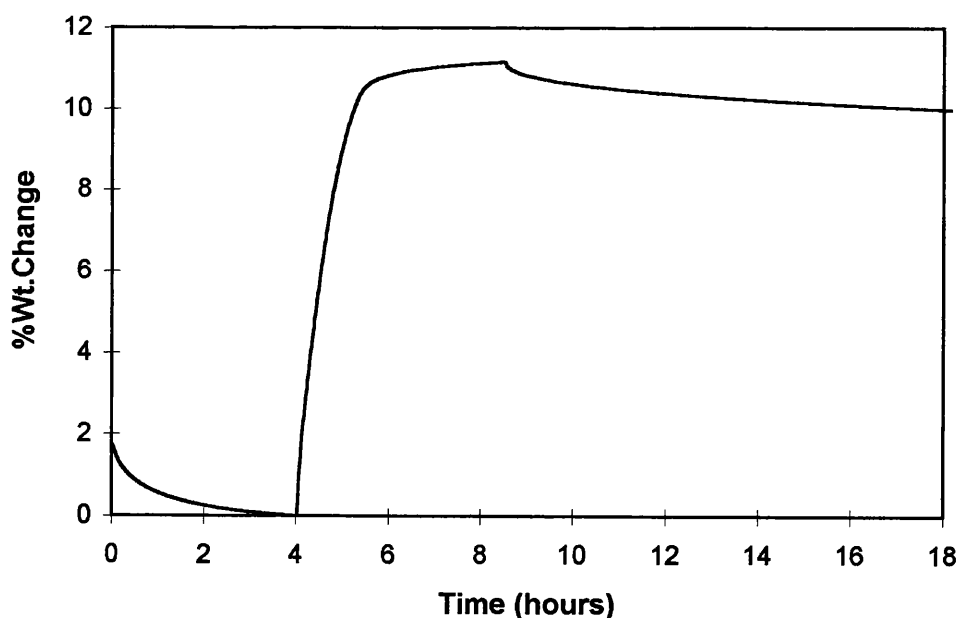


Figure 5.21 Water uptake in amorphous lactose stored at 50%RH for 4.5hours (after initially drying at 0%RH for 4hours), followed by desorption as the relative humidity is returned to 0%RH.

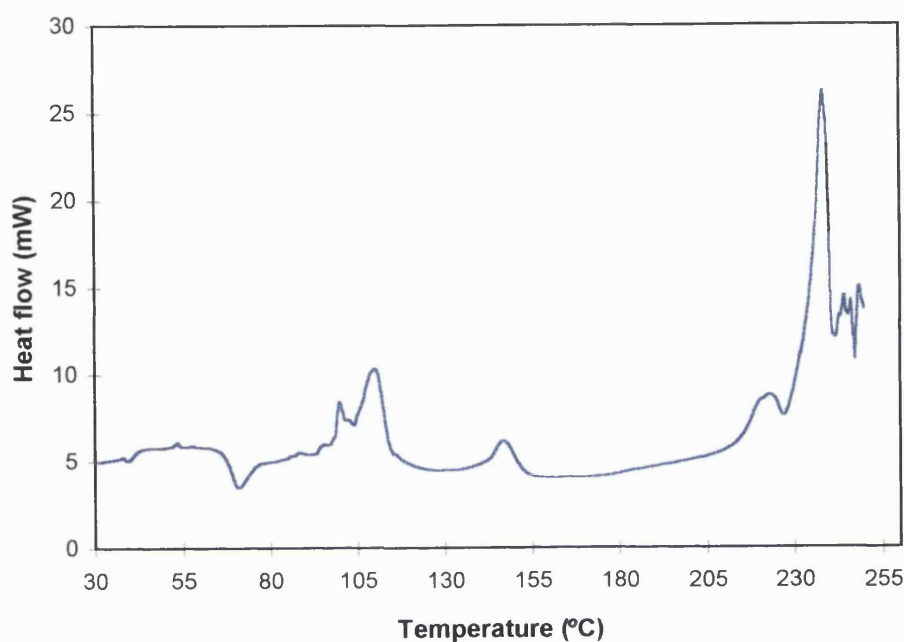


Figure 5.22 DSC data for a totally collapsed amorphous lactose sample (exposed to 50%RH for 4.5 hours).

The TGA result for this totally collapsed sample is shown in Figure 5.23 and although the 3 main peaks at 75°C, 110°C and 150°C are present, the proportions of these peaks has altered from that observed for the partially collapsed or predominantly collapsed samples. The peak at 75°C has started to reduce in size with its magnitude decreasing from 2.46% (for the sample which was at 50%RH for 2.5hours) to 1.6% for this sample. The peaks that were occurring at around 110°C in the predominantly collapsed samples are now occurring at a slightly higher temperature and are increasing in size, i.e. from 6.4% for the predominantly collapsed sample to 7.8% for this sample. The monohydrate peak has also increased in magnitude from 0.96% to 1.55%. This increase in the magnitude of the monohydrate peak indicates an increase in the amount of monohydrate in the samples that crystallise after prolonged exposure to 50%RH.

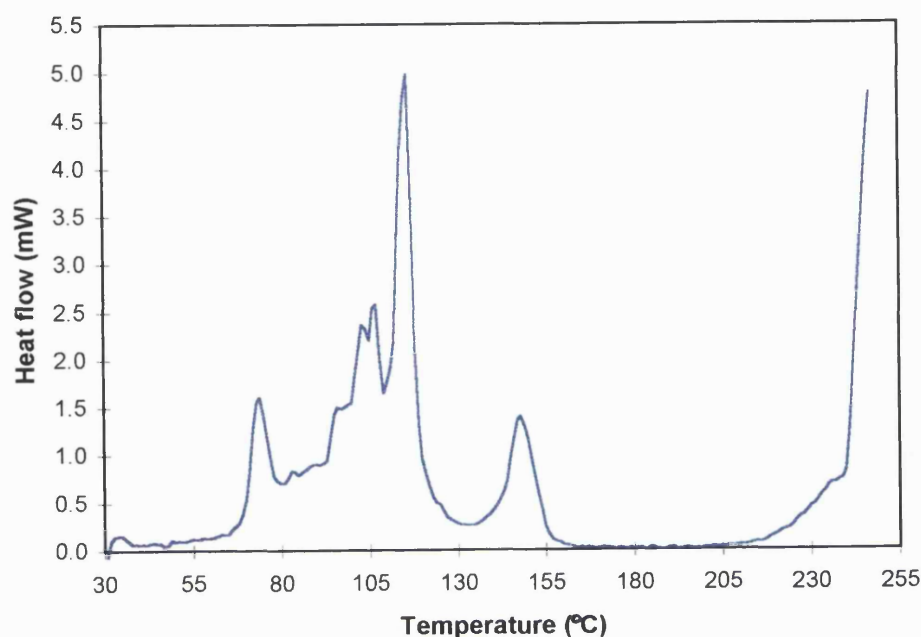


Figure 5.23 TGA data for a totally collapsed amorphous lactose sample (exposed to 50%RH for 4.5 hours).

5.3.2.5 Implications of heating collapsed amorphous lactose.

In summary, the heating involved in the physical characterisation by DSC and TGA of the amorphous lactose samples which were collapsed to varying degrees, resulted in the crystallisation of the collapsed structure at a much lower temperature than expected. It would appear therefore, that the collapsed material crystallises at a much lower temperature (around 70°C) than uncollapsed material which crystallised at around 180°C. The crystallised form is also different depending on whether the amorphous lactose was collapsed or not and also on the extent to which it had collapsed. The collapsed material yielded mainly β - lactose on crystallising (as shown also by the smaller monohydrate content), while the uncollapsed material crystallised to form mainly α - lactose. The extent of collapse of the amorphous lactose prior to heating, therefore, has a strong effect on the structure produced. This is in accordance with comments by To and Flink (1978) who noted that different final structures could be obtained for amorphous sugars depending on whether collapse and crystallisation

was temperature induced or moisture induced. Collapse of sucrose, they stated, through moisture uptake, will potentially produce a different structure to that collapsed through heat since the water of crystallisation is available in the sample collapsed through moisture. The heating rate also appeared to have an effect on the crystallisation of the collapsed structure, which seemed to proceed at lower temperatures when slower heating programmes were employed.

5.3.3 FURTHER INVESTIGATIONS AND OBSERVATIONS ON THE BEHAVIOUR AND STABILITY OF COLLAPSED AMORPHOUS LACTOSE.

5.3.3.1 Drying totally collapsed lactose at 0%RH and 25 °C.

(a) Random onset of crystallisation in the collapsed amorphous lactose.

Crystallisation of the amorphous lactose which remained at 50%RH began after approximately 12.5 hours, and occurred slowly over a further period of around 4 hours with a gradual weight loss as the sorbed water is released, as shown in Figure 5.24 (broken line). However, collapsed samples which had been exposed to 50%RH for 3 hours or more (up to 9 hours) and were then dried by returning the relative humidity to 0%RH, were also seen to crystallise. This can be seen in Figure 5.24 where a number of DVS sorption profiles for samples which were exposed to 50%RH for 3 hours and were then returned to 0%RH are shown. These samples all eventually crystallised releasing the sorbed water, however, it can also be seen that this was quite a variable and random process, with it occurring quite rapidly in some samples and occurring in discrete steps over quite long periods of time (20hours) for other samples.

The crystallisation of the collapsed material on drying would appear therefore to be a random process, probably based on molecular mobility and nucleation. The occurrence of the crystallisation process in a step-wise manner for some of the samples is shown in Figure 5.24. This was probably due to different regions of the samples crystallising at slightly different times, which may be a function of the physical form of the material i.e. whether it exists in the DVS sample pan as a number of small pieces, of large surface area, or as one or two larger pieces of material. This more gradual crystallisation process seen for these samples may also be due to the fact that the absorbed water which is liberated during crystallisation and which would

normally cause surrounding material to saturate thus causing crystallisation to continue in a domino-like fashion, may be lost to the dry surrounding atmosphere of 0%RH, hence the slower crystallisation process. This would explain the difference between the 'one-step' crystallisation and water-loss seen for the samples which remained at 50%RH and the more gradual 'step-like' process with those which were dried at 0%RH after collapse (3 hours at 50%RH).

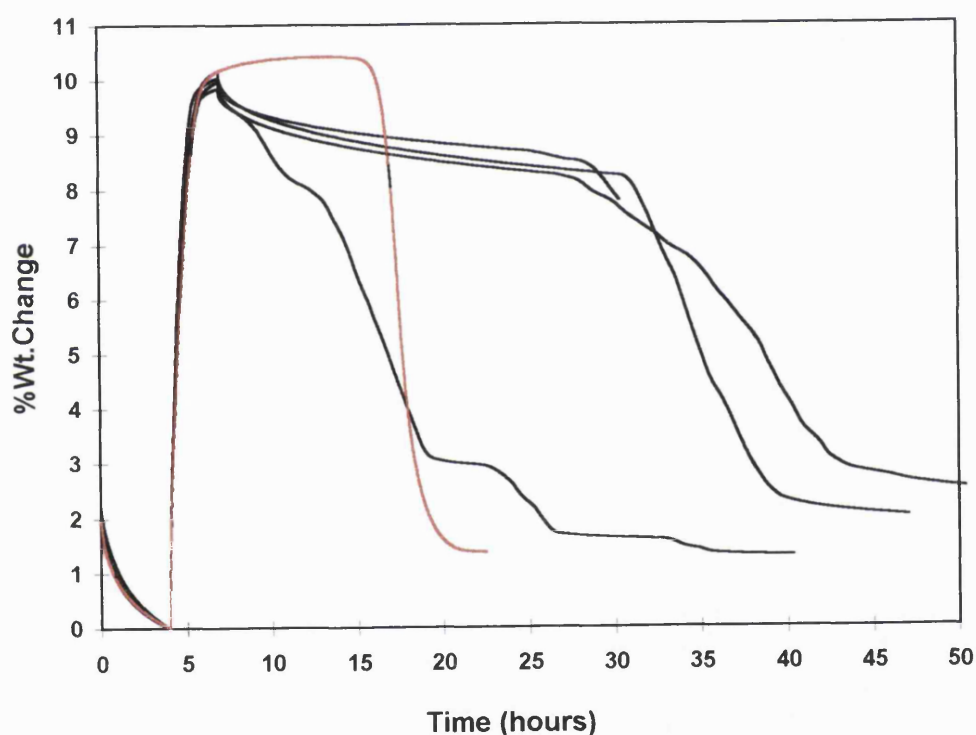


Figure 5.24 DVS sorption profiles for collapsed amorphous lactose samples (after spending 3 hours at 50%RH), with crystallisation occurring at different times on returning the sample to 0%RH. Also shown (red line) is a sample of 100% amorphous lactose exposed to 50%RH until crystallisation.

(b) Effect of Extent of Collapse on crystallisation onset during drying at 0%RH.

The data in Figure 5.25 shows how the onset of crystallisation of the collapsed amorphous lactose, during drying at 0%RH is reduced, as the time spent at 50%RH is increased. All these samples appeared to have reached a similar water uptake at 50%RH, before drying was commenced at 0%RH. The sample which was exposed to 50%RH for 4.5 hours, only crystallised after 24 hours at 0%RH, while the one exposed to 50%RH for 6 hours crystallised after 12 hours (albeit very gradually) and the one exposed to 50%RH for 9 hours began to crystallise after approximately 1 hour at 0%RH. The 9-hour sample shown here actually crystallised sooner than that which remained at 50%RH until crystallisation occurred.

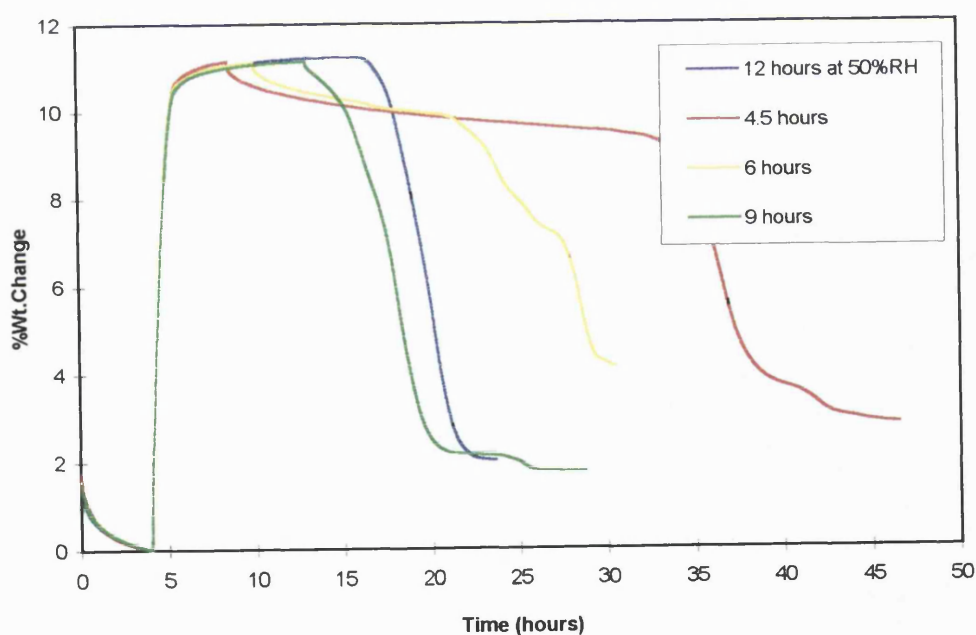


Figure 5.25 Crystallisation (during drying at 0%RH) of collapsed amorphous lactose samples which had been exposed to 50%RH for increasing periods of time (also included is a sample which crystallised without drying at 0%RH - broken line).

(c) Amount of water retained by the collapsed amorphous samples after crystallisation on drying.

Another observation on the crystallisation of the collapsed samples on drying is that the amount of water being retained after crystallisation varies for the different samples, with the samples which crystallised faster i.e. earlier, e.g. the 9-hour sample, losing more water, whereas those which crystallised gradually after a long period of time retained more water. This is illustrated in Table 5.3 which lists the amounts of water retained for samples which had crystallised on drying at 0%RH, after being exposed to 50%RH for varying lengths of time. It can be seen that a sample which crystallised after 4.5 hours had an equilibrium weight after crystallisation in the DVS of 1.3 - 2.8% water, while one which crystallised after 9 hours had an equilibrium weight of 1.2 - 1.7%. The amount of hydrate present in these samples also varied from 2 - 3%. A sample which remained at 50%RH until crystallisation occurred showed a water retention value of 1.3 - 1.4% after crystallisation was complete, and the TGA scan for such a sample indicated that it contained around 2.8% hydrate water.

As seen from Table 5.3 the samples that crystallised during drying showed a lot of variability in the amount of hydrate present, but it was generally between 2 and 3%. It is possible that more hydrate is being formed in the samples which are crystallising slowly, (e.g. the sample which was exposed to 50%RH for 4.5 hours), therefore more water is retained. Alternatively, it may be that these samples are not crystallising totally i.e. the surface is crystallising, since it will become saturated quite rapidly, and the desorbed water which would normally be absorbed by the surrounding amorphous material to continue the crystallisation is being removed by the 0%RH environment. It is further speculated that the core or centre of the sample may be remaining collapsed and is not being allowed to crystallise, due to outside of the sample fusing on crystallising and thus preventing the centre absorbing any more water. This portion of the sample that is not crystallising would however still contain a substantial amount of sorbed water, hence the higher water retention values being observed in the DVS after crystallisation. The samples remaining at 50%RH for 9 hours, on the other hand, probably have more time to become fully saturated therefore the whole sample is crystallising and a lower equilibrium weight is being observed in the DVS.

<i>Time spent at 50%RH before drying at 0%RH.</i>	<i>Water retention in the DVS (%)</i>	<i>Monohydrate weight loss in TGA (%)</i>
3	1.3, 1.5, 2.0, 2.4	2.7
4.5	2.8, 1.7, 1.3	2.7, 2.0
6	2.2, 1.3	2.8
9	1.7, 1.21	2.1, 2.9, 2.7
12.5*	1.4	2.7

Table 5.3 *The amount of water being retained after crystallisation on drying at 0%RH, by collapsed lactose samples which had been exposed to 50%RH for varying lengths of time (* sample remained at 50%RH until crystallisation).*

This theory may also be supported by data obtained for a sample which was exposed to 50%RH for 3 hours and was then held at 0%RH, where it crystallised after some 20 hours. This sample crystallised gradually in the DVS and reached a final water retention value of 2.37%, and to all intents appeared to have totally crystallised from the DVS data as shown in Figure 5.26. However, when this sample was then removed from the DVS and analysed by DSC and TGA, it appeared to have not fully crystallised. The reason for this assumption is that the DSC scan for this sample, Figure 5.27, indicated the presence of an exotherm in the region of 170°C which is where amorphous lactose (uncollapsed) normally crystallises. Therefore this exotherm may be crystallisation of amorphous lactose on heating. The surprising observation from this experiment is that the exotherm occurs at 170°C, and this is the region where the non-collapsed amorphous lactose normally crystallises. Since it is expected that this sample was totally collapsed as it remained exposed to 50%RH for 3 hours and water desorption from such samples was seen to be negligible as shown in Figure 5.26, the crystallisation (if occurring) would be expected to happen at a much lower

temperature i.e. in the region of 70°C as already observed for crystallisation of the collapsed lactose (Figure 5.22).

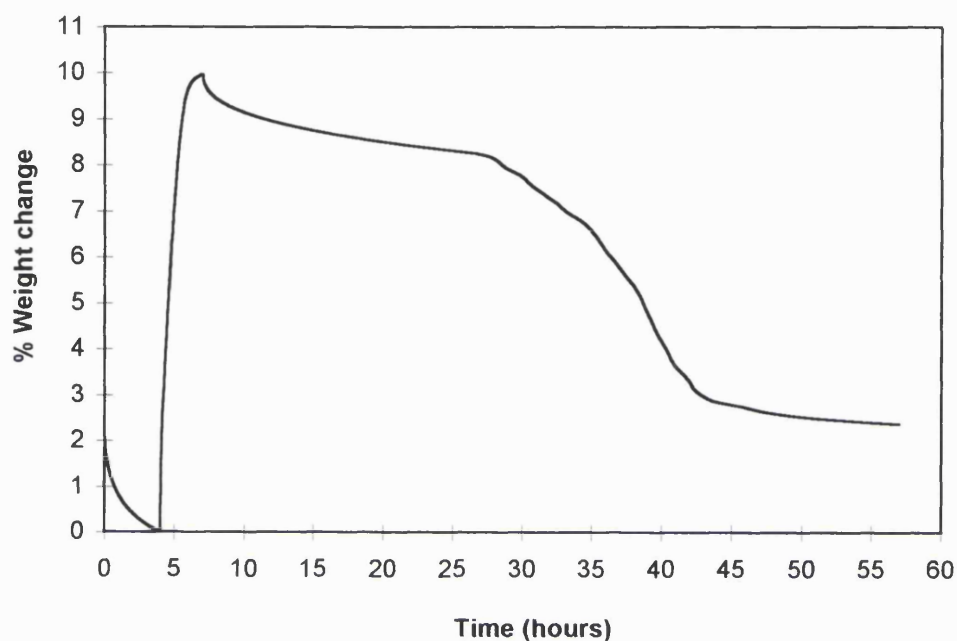


Figure 5.26 DVS data for an amorphous lactose sample which crystallised on drying at 0%RH, after being exposed to 50%RH for 3 hours.

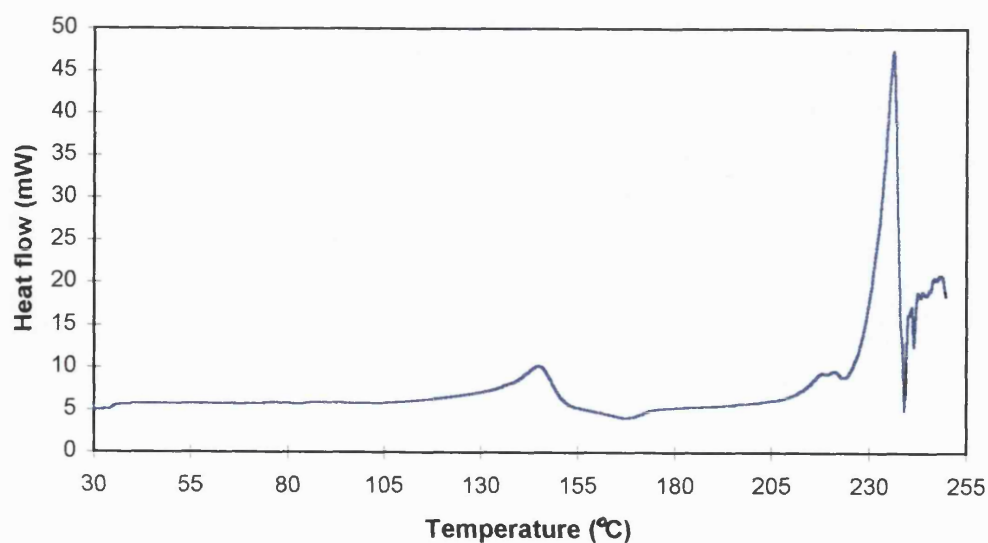


Figure 5.27 DSC scan for an amorphous lactose sample which crystallised on drying in the DVS at 0%RH, after being exposed to 50%RH for 3 hours, with a final DVS water retention value of 2.37%.

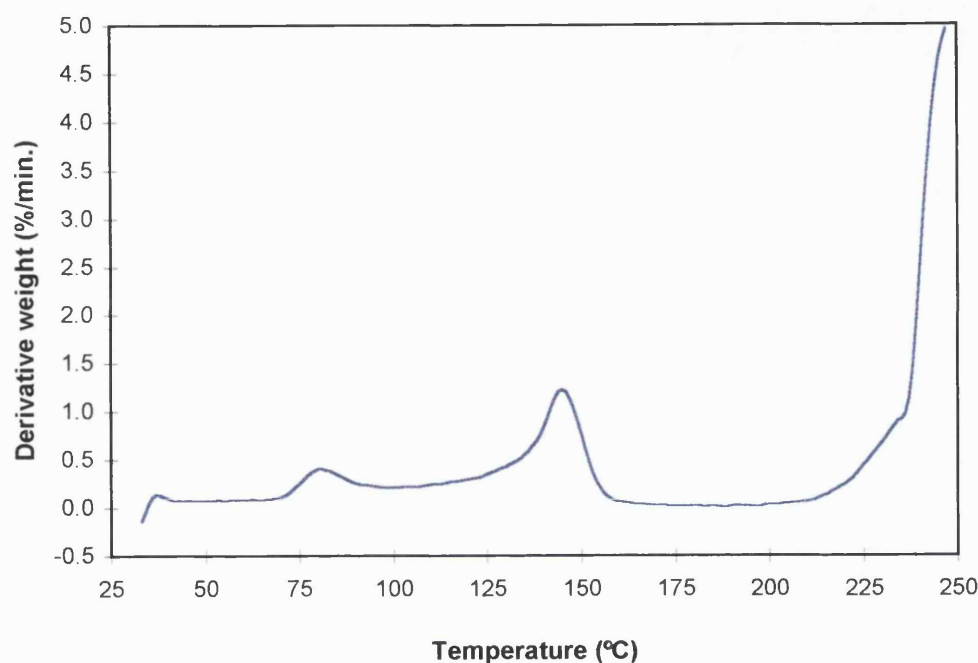


Figure 5.28 TGA scan for an amorphous lactose sample which crystallised on drying in the DVS at 0%RH, after being exposed to 50%RH for 3 hours, with a final DVS water retention value of 2.37%.

The TGA scan in Figure 5.28 also shows the presence of a small peak at 80°C, which as discussed earlier, is thought to be due to water loss as the amorphous lactose crystallises. This again backs up the DSC observations that this sample may not be crystallised fully and may contain regions which may or may not be collapsed.

Therefore it is possible that samples exposed to 50%RH for longer than 3 hours, although showing very minimal desorption of water at 0%RH, are not totally collapsed after exposure to 50%RH for 3 hours only. Samples which were exposed to 50%RH for up to 9 hours didn't crystallise until drying was initiated and samples which weren't returned to 0%RH were seen to require in the region of 12 hours at 50%RH before spontaneous crystallisation occurred.

It would appear therefore, that drying collapsed lactose samples at 0%RH and 25°C induces crystallisation in the sample which varies depending on the length of time the material had been exposed to 50%RH.

5.3.3.2 Stability of collapsed amorphous lactose at different %RH's and the implications on water content of a sample which contains collapsed amorphous material.

A sample of collapsed amorphous lactose was prepared by exposing 100% amorphous lactose to 50%RH for 6 hours in the DVS and this was then stored overnight in a desiccator containing silica gel. A normal sorption / desorption experiment was then performed in the DVS using a dm/dt value of 0.001. The resulting sorption and desorption data and isotherms are shown in Figures 5.29 and 5.30. It can be seen that no water loss occurs at 0%RH and at relative humidities up to 40% no appreciable water uptake is taking place, indicating the apparent stability of this material at low relative humidities.

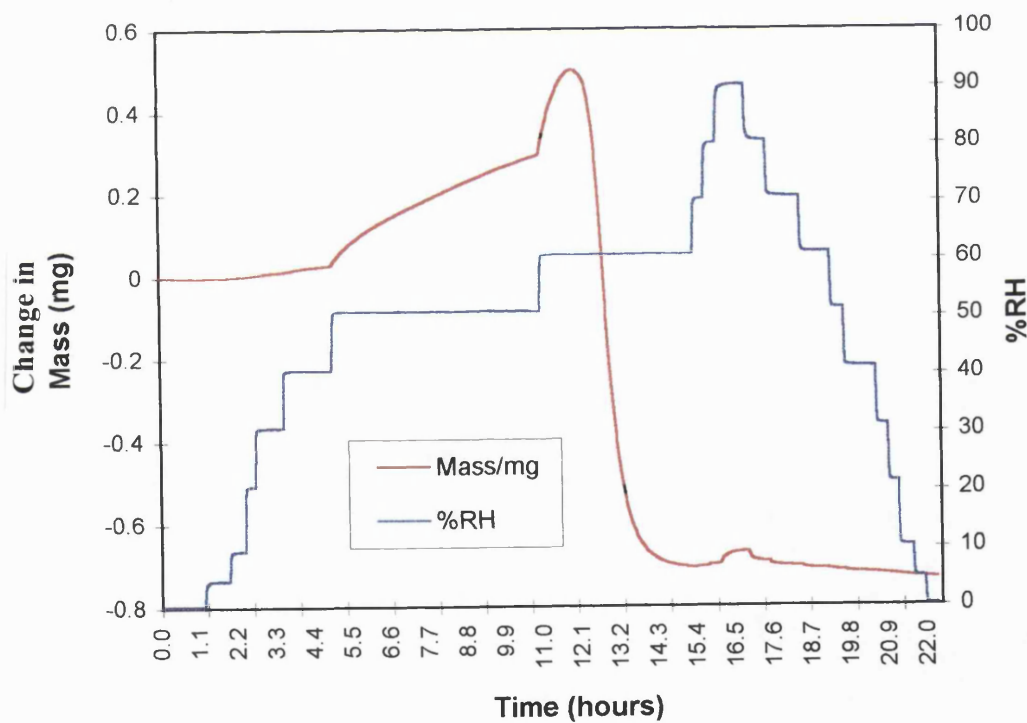


Figure 5.29 Sorption and desorption data for a collapsed amorphous lactose sample, going from 0 - 90%RH in 10% steps, and then back from 90 - 0%RH.

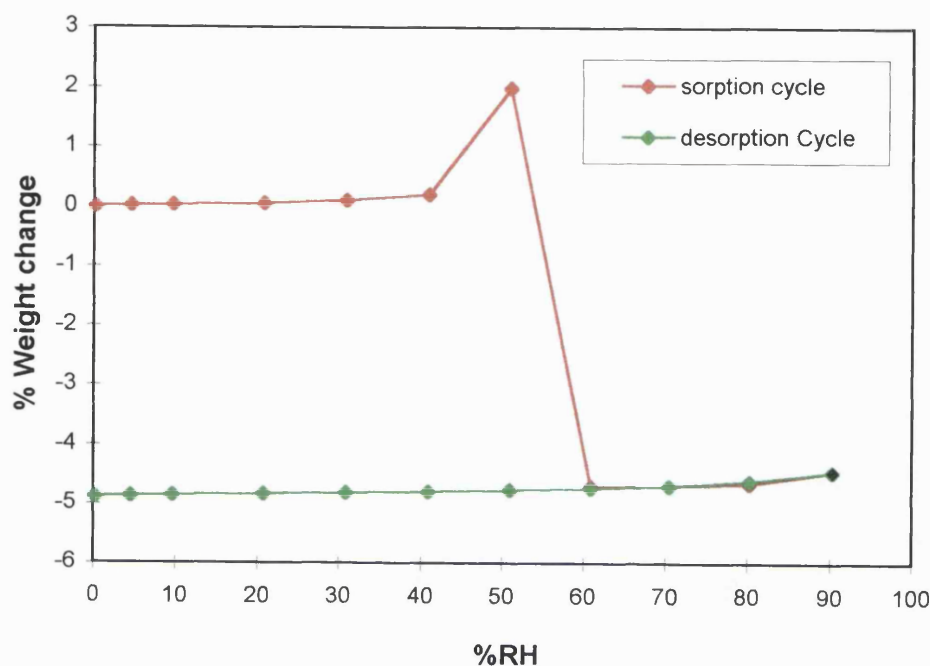


Figure 5.30 Sorption and desorption isotherms for a collapsed amorphous lactose sample, going from 0 - 90%RH in 10% steps, and then back from 90 - 0%RH.

However, as the %RH is increased from 40 – 50%RH, the water uptake begins to increase with the sample taking up approximately 2% water. This is followed by a further sharp uptake at 60%RH of another 1% water, which is followed by rapid desorption of 8% water (from the highest point reached at 60%RH before crystallisation begins) as the collapsed sample crystallises.

This experiment shows that although the material only appeared to take up a small amount of water (3%) at 50 and 60%RH, this was sufficient to induce crystallisation in the collapsed material which resulted in a total of 8% water being released on crystallisation. The fact that this occurred at normal room temperature (25°C) and close-to-ambient relative humidity (50 - 60%) indicates the importance of storage conditions on this material. This sample therefore went from being relatively dry, not losing or taking up any water in the region up to 40%RH, but on taking up approximately 2 - 3% water in the 50 - 60%RH region becomes very wet since crystallisation occurs and results in a total of 8% (maximum uptake at 60%RH from Figure 5.30) water being released into the sample bed.

5.3.3.3 Compaction / tableting studies on 100% amorphous lactose.

5.3.3.3.1 *Preparation of compacts.*

Samples (ca 100mg) of spray dried lactose were placed in a rectangular stainless steel punch and die system, which was then placed in the Specac hydraulic press assembly. The desired compaction pressure was then applied to the samples. All the samples were maintained at the desired pressure for exactly 1 minute and pressures of 0.5, 1, 2.5 and $3.5 \times 10^5 \text{ kNm}^{-2}$ were used to obtain different compacts. After compaction the samples were removed from the assembly and were stored over silica gel in a desiccator until further characterisation was carried out.

5.3.3.3.2 *Physical Characterisation of amorphous lactose compacts.*

(a) Dynamic Vapour Sorption Studies

Gravimetric moisture sorption studies were carried out on the compacts in order to determine if the lactose samples were still amorphous following compaction. Approximately 20mg of the compact was placed in the sample pan of the microbalance and water uptake and loss was investigated by carrying out a normal 0 - 90 - 0%RH experiment (in 10% increments), using a dm/dt value of 0.001. Typical sorption/desorption profiles for samples compacted at 1 and $2.5 \times 10^5 \text{ kNm}^{-2}$ are shown in Figures 5.31 and 5.32, and the corresponding isotherms for these samples are shown in Figure 5.33 along with a sorption/desorption isotherm for an uncompacted sample of amorphous lactose.

It can be seen from Figures 5.31 and 5.32 that the compacted samples are still amorphous when introduced to the DVS system, since these samples can be seen to crystallise at 60%RH (as indicated by the dramatic weight loss due to removal of absorbed water from the amorphous material during crystallisation). From the desorption isotherms for these samples it would appear that the compacted samples contain the same level of amorphous material as the 100% amorphous sample, thus indicating that no crystallisation of the amorphous lactose has occurred during compaction.

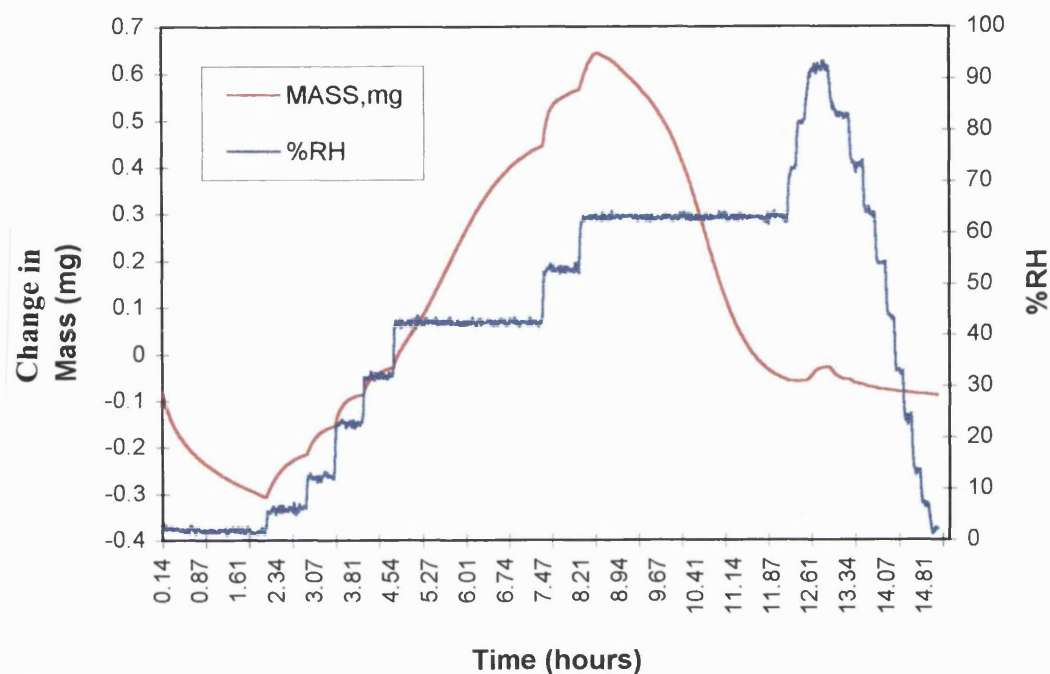


Figure 5.31 Sorption / desorption data for amorphous lactose compacted using $1 \times 10^5 \text{ kNm}^{-2}$ pressure.

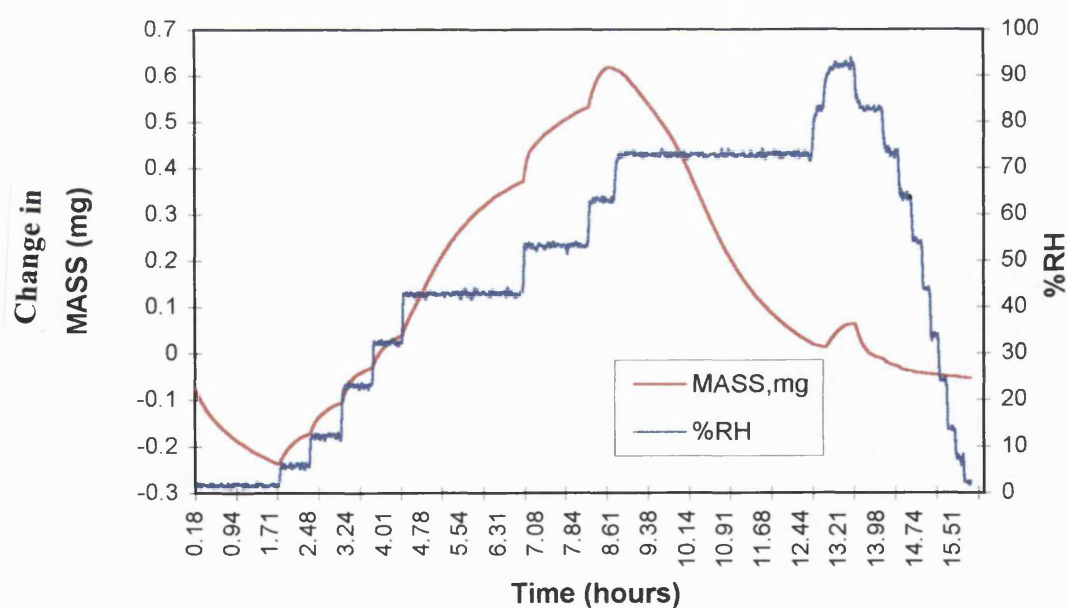


Figure 5.32 Sorption / desorption data for amorphous lactose compacted using $2.5 \times 10^5 \text{ kNm}^{-2}$ pressure.

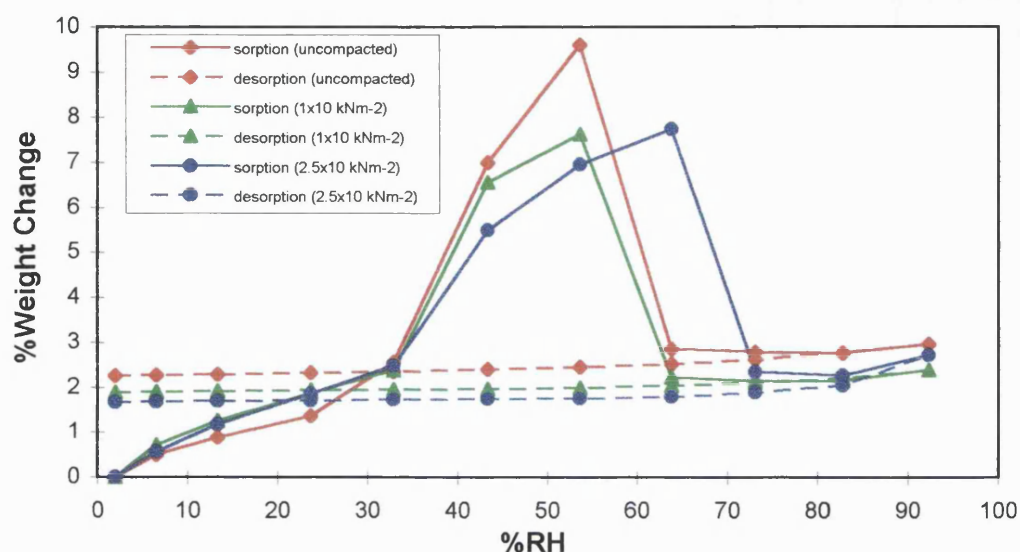


Figure 5.33 Sorption / desorption isotherms for amorphous lactose samples compacted using $1 \times 10^5 \text{ kNm}^{-2}$ and $2.5 \times 10^5 \text{ kNm}^{-2}$ pressure along with an uncompacted sample.

It appears from Figures 5.32 and 5.33 that differences in the water sorption properties do exist between the uncompacted and compacted amorphous lactose samples. The uncompacted sample and the one which was compacted at $1 \times 10^5 \text{ kNm}^{-2}$ crystallised during exposure to 60%RH, while the sample compacted at $2.5 \times 10^5 \text{ kNm}^{-2}$ crystallised slightly later, as the %RH was being increased from 60 - 70%RH. It is suspected that the reason for this behaviour may be due to the slower absorption and passage of water into the centre of the sample compacted at $2.5 \times 10^5 \text{ kNm}^{-2}$ due to the amorphous particles being compacted and held much closer together.

(b) DSC analysis.

DSC analysis was carried out on the compacts by placing approximately 5mg of sample in a non-hermetically sealed aluminium pan and scanning from 40°C - 240°C at a scan rate of 10°C/min., under an atmosphere of nitrogen. Typical DSC results for samples compacted using 0.5, 1, 2.5 and $3.5 \times 10^5 \text{ kNm}^{-2}$ pressure, along with the result for an uncompacted amorphous lactose sample, are shown in Figures 5.34 and 5.35,

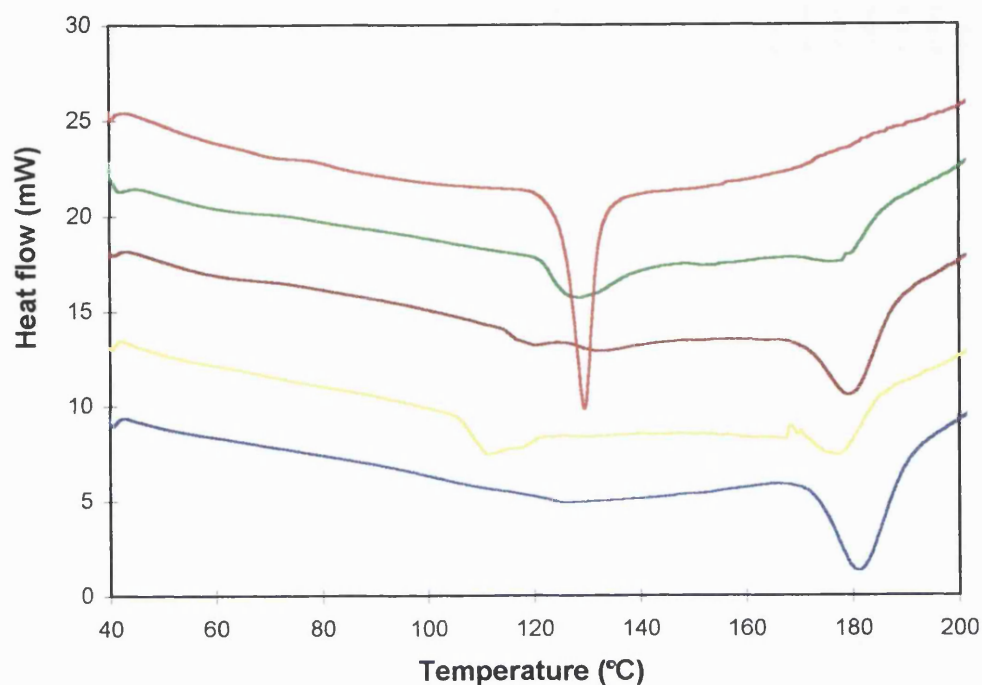


Figure 5.34 DSC results for amorphous lactose samples compacted using $0.5, 1, 2.5$ and $3.5 \times 10^5 \text{ kNm}^{-2}$ pressure along with an uncompacted sample, showing the region from $40 - 200^\circ\text{C}$.

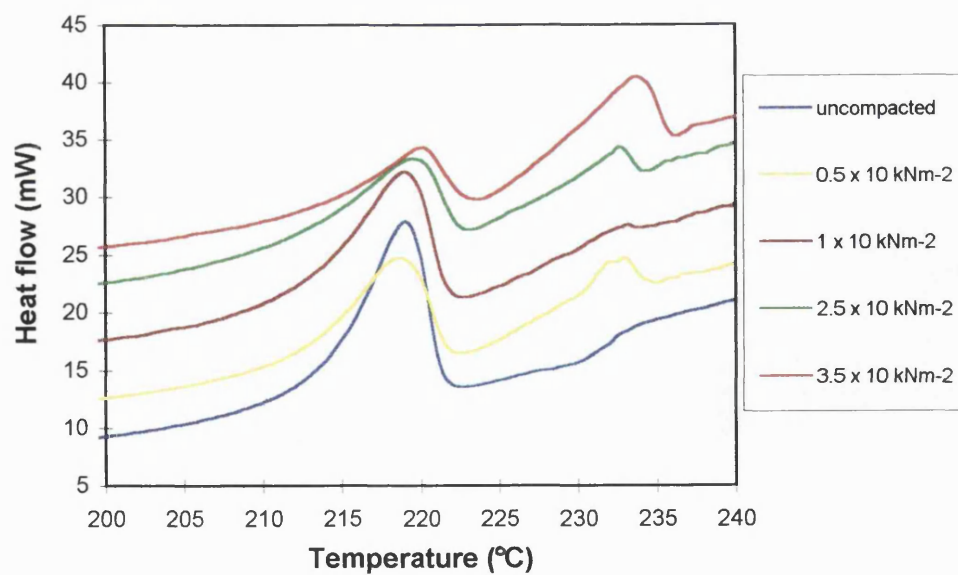


Figure 5.35 DSC results for amorphous lactose samples compacted using $0.5, 1, 2.5$ and $3.5 \times 10^5 \text{ kNm}^{-2}$ along with an uncompacted sample, showing the region from $200 - 240^\circ\text{C}$.

The regions from 40 - 200°C and from 200 - 240°C (showing the proportions of α and β lactose) are shown separately in Figures 5.34 and 5.35. The uncompact sample crystallised at around 180°C (Figure 5.34), as expected, yielding predominantly α -lactose at around 218 - 220°C (Figure 5.35). However, it can be seen that for all samples which had been compacted there is a second exotherm present, in the region between 115°C and 130°C. Furthermore, this exotherm appears to become larger and sharper with increasing compaction pressure from 0.5, 1, 2.5 and 3.5×10^5 kNm⁻², while the exotherm at 180°C actually decreases in magnitude. This can be seen in Table 5.4 where the peak temperatures and magnitudes are listed. This earlier exotherm increased from 22.3 J/g for the 0.5×10^5 kNm⁻² compact to 83 J/g for the 3.5×10^5 kNm⁻² compact, and the exotherm at 180°C decreased in magnitude from 72 J/g for the 1×10^5 kNm⁻² compact to being totally absent from the 3.5×10^5 kNm⁻² compact. It can also be seen that the temperature at which this earlier exotherm appeared varied from 111°C for the 0.5×10^5 kNm⁻² sample to 130°C for the 3.5×10^5 kNm⁻² sample. The proportions of α and β lactose present in these samples also varied, as shown in Table 5.4 with the amount of β lactose present increasing as the compaction pressure increased.

Sample kNm ⁻²	1 ST Exotherm		2 ND Exotherm		α -	β -
	T (°C)	J/g	T (°C)	J/g	J/g	J/g
Uncompact	-	-	181.4	104.5	111.4	-
0.5×10^5	111.3	22.3	177.5	32.3	79.6	-
* 1.0×10^5	120.1	20.8	179.5	77.2	95.1	-
2.0×10^5	128.6	39.2	178.0	39.9	65.0	11.4
3.5×10^5	129.6	83.0	-	-	46.2	49.4

Table 5.4 Summary of DSC results for amorphous lactose samples compacted at different pressures (* a double peak was obtained here)

5.3.3.3.3 Changes in the physical characteristics of the compacted amorphous lactose.

A number of studies have been carried out and reported in the literature on tableting characteristics and compression of spray dried lactose. Most recently Sebhatu et al (1994 and 1997), have concentrated mainly on the effects of moisture sorption on tablet strength and porosity in assessing the properties of these tablets. It was reported that amorphous lactose powders reduced in volume due to particle deformation and that powder compression was facilitated by an increased moisture content due to an increased deformability in the glassy state. Although the studies carried out here were on 'dry' amorphous lactose, it is however accepted that certain changes will have taken place in the amorphous material during compaction e.g. the particles are much closer together and during the compaction process it is likely that some heat will have been introduced to the samples. The importance of heating and increased temperature on amorphous spray dried lactose is discussed further in Chapter 6. Sebhatu et al (1994) speculated that even at low moisture contents a material that has regions in the amorphous glassy state before compaction could convert to the rubbery state due to the increased local temperature and/or pressure during the compaction process.

It was shown from DVS experiments earlier that the compacted samples were still amorphous after compaction and storage in dry conditions. However, DSC data for the compacted materials indicated that during heating, these samples (although still amorphous) behaved differently from the original uncompacted material. Those samples compacted under the lowest pressure still appear to be crystallising in the 180°C region, but it also seems that some material is present which is crystallising at a much lower temperature i.e. in the region of 120°C – 130°C. It is suspected that the exotherm between 115°C and 130°C is a crystallisation response although no detailed investigation into the exact nature and origin of this peak was undertaken in this study. This exotherm however, possibly corresponds to one reported by Berlin et al (1970) at approximately the same temperature (110°C), for lactose glass scanned in a sealed pan so that no water loss could occur. The authors stated that this exothermic peak may be associated with the solution of lactose in the released water and the mutarotation of α -lactose to the β -form. It was postulated that since removal of water vapour is prevented in a sealed capsule, a concentrated lactose solution is formed

resulting in mutarotation. This theory is supported here by the data in Table 5.4 and Figure 5.35, which both clearly show the β content to be increasing as the compaction pressure increases, indicating that mutarotation is taking place. Although the DSC pans used in this study were not hermetically sealed, it is probable that removal of water from the compacted samples will be extremely slow therefore resulting in a reduced crystallisation onset. DSC studies on uncompacted amorphous lactose in unsealed pans showed earlier that absorbed water is normally lost in the region up to 100°C, with the result that the sample crystallises at around 180°C.

From our earlier findings and reportings in this chapter regarding the crystallisation of collapsed amorphous lactose, this peak could also be crystallisation of collapsed amorphous lactose, which was found to crystallise on heating in the DSC. However, the temperature of the exotherm reported here is higher than that reported for crystallisation of the collapsed lactose, which was around 70°C. If this is crystallisation of collapsed amorphous lactose, it would appear that the samples compacted at low pressures (0.5 and $1 \times 10^5 \text{ kNm}^{-2}$) contain regions of collapsed and uncollapsed amorphous material, since these samples showed the presence of 2 exotherms in the DSC indicating that they may be partially collapsed. The samples which were compacted at the highest pressures i.e. $3.5 \times 10^5 \text{ kNm}^{-2}$, which only showed the presence of the earlier crystallisation exotherm may be totally collapsed. Sebhatu et al (1997) reported from SEM photomicrographs that a glass - rubber transition occurred initially at the surface of the amorphous lactose particles, probably due to interparticulate friction. Similarly, the DSC data showing the 2 exotherms here may be explained on the basis that the surface of these compacts was collapsed, with the core remaining uncollapsed.

5.3.3.3.4 Differences between amorphous lactose collapsed due to moisture effects or to temperature effects.

The collapsed material investigated earlier in this chapter had collapsed due to the effect of moisture uptake at 50%RH which lowered the T_g and resulted in molecular relaxation. However, although no humidification of the compacted samples occurred at any stage during the study (although they probably contained in the region of 3% residual moisture from spray drying), it is probable that during the compaction

process the amorphous lactose will have been exposed to increased temperature which in this case caused it to collapse. Flink (1978) used both heat and moisture to induce 'collapse' in the study of sugar systems such as maltose and sucrose. In fact, heating alone was used by To and Flink (1978) to obtain stable partially collapsed sugar systems. They stated from observations on their heating experiments that the degree of collapse was not a time dependent phenomenon (different heating rates were investigated) but rather was solely dictated by the temperature reached in the sample. White and Cakebread (1966) when reviewing the glassy state in sugar-containing foods, stated that collapse can occur in dry atmospheres due to, among other factors, high storage temperatures, i.e. the storage temperature is raised above T_g . Consequently, any process which results in an amorphous glass being heated e.g. milling, micronising and in this case compression, can result in collapse of the amorphous structure. This temperature effect and the importance of the temperature reached can probably explain our DSC observations where the earlier exotherm increased with increasing pressure since it is probable that the higher compaction pressure, the higher the temperature reached by the sample, as the same time period was used when preparing all the compacts.

Although it seems likely that the DSC exotherm in the region of 115°C - 130°C is likely to represent crystallisation of collapsed amorphous lactose, it must however be noted that the temperature at which this peak occurred is much higher than that reported earlier in this chapter for crystallisation of the collapsed amorphous lactose. An important fact must however be noted here, which is that the amorphous lactose in this study was collapsed due to temperature effects while the amorphous lactose studied earlier in this chapter was collapsed due to moisture effects. The important factor in this discussion is the difference between T (experimental temperature) and T_g , which determines what state the amorphous lactose will be in, i.e. T_g can be lowered due to plasticisation by water or T can be raised resulting in both cases in a reduction in $T_g - T$. Therefore, it would appear from these findings that the collapsed amorphous lactose behaves differently in the DSC depending on whether it collapsed due to temperature effects or moisture effects i.e. the sample which collapsed due to temperature effects crystallised in the region of 115°C - 130°C , while that which collapsed due to moisture effects crystallised at a much lower temperature i.e. 70°C .

This theory is entirely in line with findings by To and Flink (1978) when investigating loss of volatiles from sugar systems. They also observed that lactose behaved differently depending on whether it was collapsed due to moisture or temperature with the sample collapsed due to moisture effects crystallising earlier also. To and Flink (1978) also noted that by heating a freeze-dried sample to a temperature slightly higher than the original collapse temperature it was possible to then cool it and obtain a new T_c (collapse temperature) equal to the highest temperature previously reached, which was due to removal of water.

5.3.3.3.5 General comments on compression of amorphous lactose.

Compression of 100% amorphous lactose didn't induce crystallisation using the pressures employed in this study, however, the amorphous material did appear to collapse. This collapsed structure appeared to be more stable than the collapsed amorphous lactose studied earlier in this chapter. It appears therefore that collapsed amorphous lactose can exhibit different degrees of stability depending on whether it was collapsed due to temperature effects or to moisture effects. The material that was collapsed due to temperature effects appears to be more stable since it didn't crystallise until around 120°C in the DSC (unlike the other one which crystallised at 70°C). Furthermore, crystallisation of this material didn't occur in the DVS until a water content in the region of 8% was reached, unlike the material investigated earlier which only needed a further 2 - 3% water before crystallisation occurred since it already contained approximately 7% moisture.

5.3.4 VISUAL EXAMINATION OF THE STRUCTURAL CHANGES OCCURRING IN THE AMORPHOUS LACTOSE ON EXPOSURE TO 50% RH.

5.3.4.1 DVS-VIDEO monitoring of the amorphous lactose through drying, collapse and crystallisation.

The change in the physical appearance of the amorphous lactose was followed using a DVS microbalance with a video monitor attached. These changes can be seen in Figures 5.36 (a) - (d) which show DVS- μ graphs for a 100% amorphous lactose sample at 25°C, which was initially dried at 0%RH for 6hours, after which the %RH

was raised to 40%RH and then increased to 50%RH in 1% steps. Figure 5.36 (a) shows the original spray dried amorphous lactose powder following drying at 0%RH. The μ graph in Figure 5.36 (b) shows how the sample appears as the relative humidity has been increased to 46%RH, where the material has reached an equilibrium water content of approximately 4.5%. It can be seen that the sample has changed slightly from its dry state to a more condensed appearance and appears to be becoming more fused together taking up less space. The presence of water in the sample will result in reduced viscosity at the stage also. The water uptake of 4.5% would lower the T_g to 49.5°C which is still well above T and therefore the sample would be expected to be still totally amorphous. As the relative humidity is further increased up to 50%RH the amorphous material reaches a maximum water content of approximately 9.5%, which would be predicted to lower the T_g to 12.2°C and was shown in Figure 5.8 to result in considerable collapse of the amorphous material. The μ graph in Figure 5.36 (c) shows how this water content has resulted in a dramatic change in the physical appearance of the amorphous lactose, which has now changed to a more glassy opaque appearance and will be totally collapsed at this stage. As the %RH is then increased above this point to 52%RH, a dramatic weight loss occurs as the amorphous material crystallises releasing the sorbed water. This results in the sample becoming fused together and reducing considerably in size as shown by the μ graph in Figure 5.36(d).

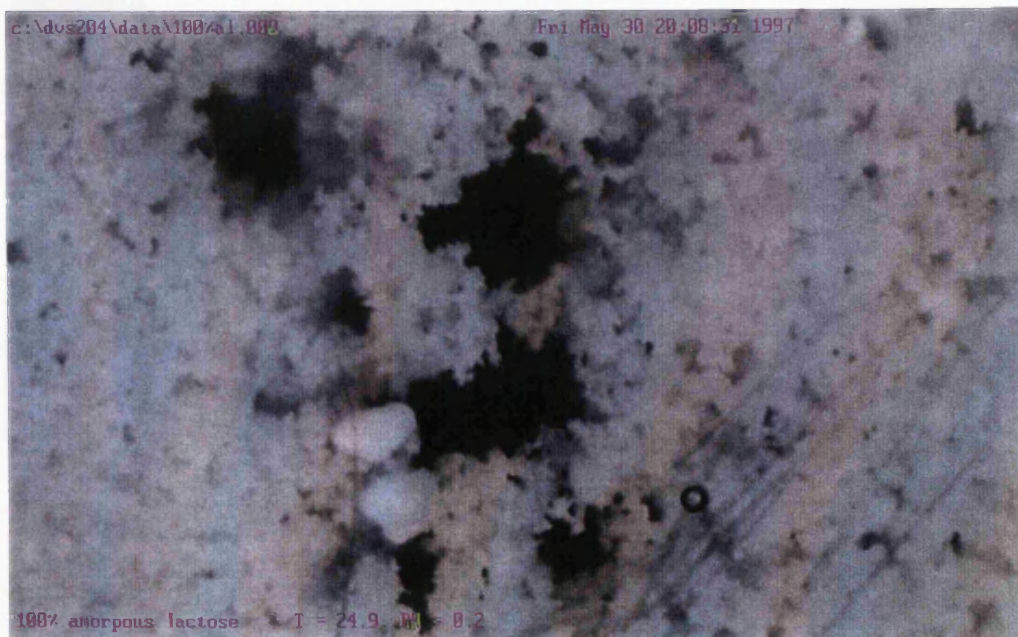


Figure 5.36 (a) DVS μ graph for 100% amorphous lactose following exposure to 0%RH for 6 hours.

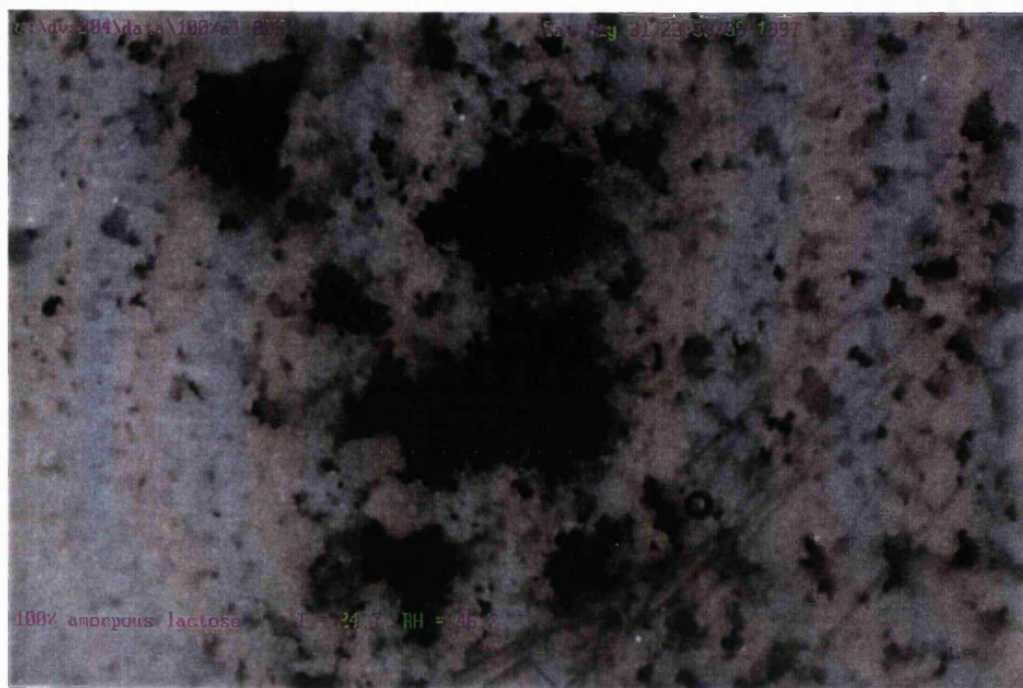


Figure 5.36(b) DVS μ graph for 100% amorphous lactose during exposure to 46%RH on increasing the RH from 40% - 50% in 1% steps (following drying at 0%RH for 6hours).

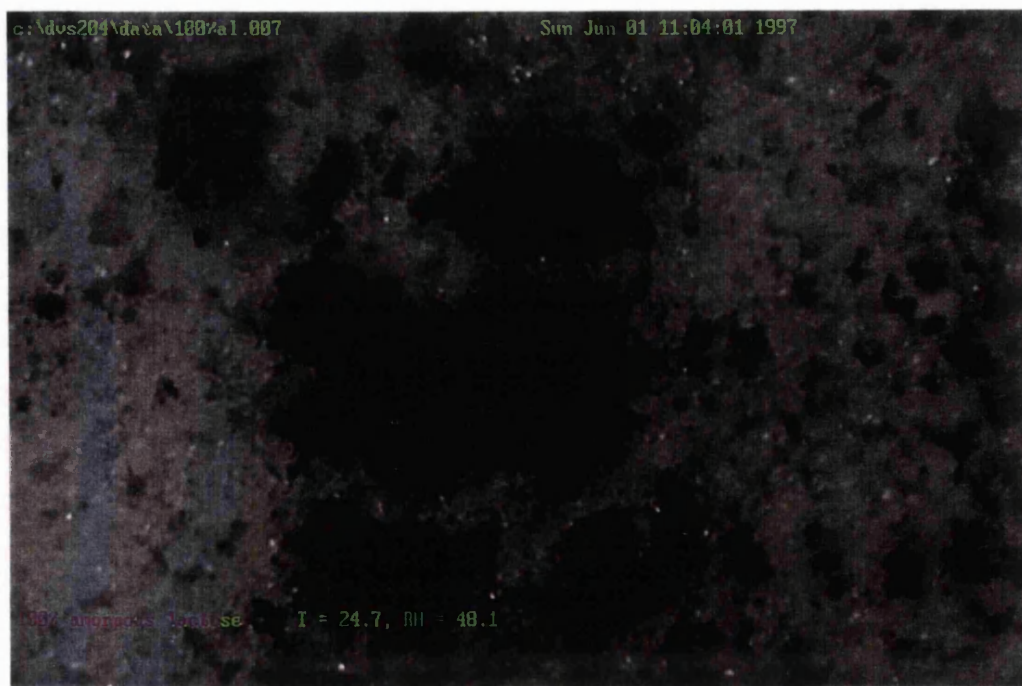


Figure 5.36 (C) DVS μ graph for 100% amorphous during exposure to 48%RH, on increasing the RH from 40% - 50% in 1% steps (following drying at 0%RH for 6hours).

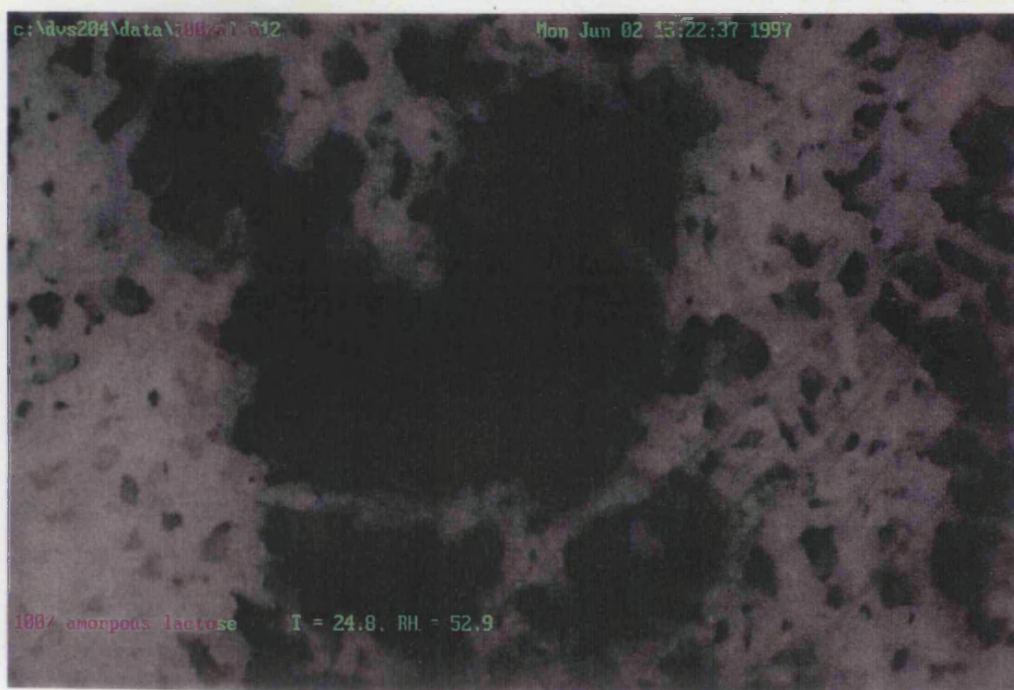


Figure 5.36 (d) DVS μ graph for 100% amorphous lactose during exposure to 52%RH, on increasing the RH from 40% - 52% in 1% steps (following drying at 0%RH for 6hours).

5.4 CONCLUSIONS

In general, the experiments carried out and discussed in this chapter have yielded important information on water mobility in amorphous lactose around and close to the point where the glass transition temperature (T_g) equals the operating temperature (T). Further information has also been obtained on the crystallisation process in amorphous lactose. The concept of collapse as it applies to amorphous lactose has been investigated in some detail and the different crystallisation behaviour and stability of the collapsed structure depending on whether it was collapsed due to temperature or moisture effects were noted.

In summary the following general comments and conclusions can be made:

- Exposure of the amorphous lactose to 40%RH resulted in an equilibrium water uptake of 7% which wasn't sufficient to lower T_g below T . Subsequent desorption of water from this sample was rapid and was dependent on the surrounding relative humidity.

- Exposure of the amorphous lactose to 50%RH resulted in water uptakes sufficient to lower T_g below T . Depending on the length of time exposed to 50%RH (i.e. water uptake reached) collapse of the amorphous material occurred, whereby subsequent water desorption was very slow.
- The water content necessary to cause collapse here was approximately 8% and samples containing different degrees of collapse were obtained again depending on the time spent at 50%RH.
- Microcalorimetry experiments showed that the collapsed structure was still amorphous and revealed that the heat output for crystallisation of the collapsed material to be identical to that for crystallisation of the uncollapsed structure. The collapsed material only crystallised after approximately 12 hours at 50%RH in the DVS.
- The amorphous lactose which had collapsed after exposure to 50%RH was found to crystallise on heating in the DSC at around 70°C, with partially collapsed samples crystallising at both this temperature and at the higher temperature of 180°C which is normally associated with crystallisation of amorphous lactose.
- Drying collapsed amorphous lactose at 0%RH and 25°C also induced crystallisation and this seemed to be a variable process depending on the degree of collapse of the sample.
- Samples of amorphous lactose which were compressed under a range of different pressures into compacts remained amorphous after compaction, but exhibited different crystallisation and melting behaviour from uncompacted samples, during heating in the DSC. This was attributed to collapse of the amorphous structure during compaction, presumably due to increased localised temperature from the compaction process.
- The amorphous lactose which had collapsed due to compression effects appeared to crystallise on heating in the DSC at around 120 - 130°C.

CHAPTER 6

HIGH TEMPERATURE CRYSTALLISATION STUDIES ON AMORPHOUS SPRAY DRIED LACTOSE.

6.1 INTRODUCTION

The DVS and microcalorimetry studies carried out in Chapter 3 indicate that for the crystallisation of amorphous lactose to occur, the T_g must be lowered to or below the experimental operating temperature, i.e. when $T=T_g$ crystallisation will occur. In these initial studies the T_g was lowered when the amorphous sample was exposed to water vapour, which acted as a plasticising agent due to it having a lower T_g (-135°C) than lactose which has a $T_g = 104^\circ\text{C}$. It was also seen that a critical water uptake was necessary to lower T_g to T , after which time a spontaneous crystallisation event occurred. The effect of changing the relative humidity to which the amorphous sample was exposed was investigated and it was observed that an inverse relationship existed between %RH and the onset time for crystallisation. This was due to the slower supply of water vapour to the sample and subsequent delay in plasticising the amorphous material, which indicates that the time required for crystallisation to occur depends on the difference between T and T_g .

Berlin et al (1970) studied the effect of temperature on water vapour sorption by dried milk products, and reported that lactose crystallisation occurred at lower RH's as temperature was increased. Elamin et al (1995) also investigated the effect of temperature and %RH on the water uptake and stability of amorphous sucrose and lactose samples and reported that temperature was a very crucial factor in determining the critical water uptake necessary to induce crystallisation in these amorphous materials. At lower temperatures much higher water uptake was necessary for crystallisation to occur.

Pikal et al (1977) investigated the thermal decomposition of β -lactam antibacterials in their amorphous and unsolvated forms as a function of temperature and water content. The behaviour of the amorphous materials deviated from the expected first-order kinetics, but no clear explanation for this was found. However, although an Arrhenius plot showed significant curvature, the effective activation energy, defined by the slope of the Arrhenius plot, decreased as the temperature was increased. This temperature dependence was thought to be the result of molecular reorientation which was assumed to be the rate determining step for thermal decomposition.

Lehto and Laine (1997) used isothermal microcalorimetry along-side differential scanning calorimetry and X-ray powder diffraction to study the crystallisation kinetics and thermodynamics of amorphous sterotex K at different temperatures. It was concluded that isothermal microcalorimetry could give valuable information on possible solid state transitions when used along with other analytical methods. The sensitivity and real-time data collection ability of the microcalorimeter enabled it to show that the crystalline transition mechanism changed as a function of temperature. This was supported by the XRPD results and DSC was used to analyse the transition quantitatively, although the precision using this approach was poor.

All the above studies indicated that temperature is a very important influencing factor in the water uptake in amorphous materials unlike crystalline materials, as shown by Shotton and Harb (1965), who showed that for a given relative humidity, temperature seemed to have little effect on the equilibrium water content for a number of materials including lactose. The object of the following study therefore was to investigate the effect of temperature on the crystallisation process in amorphous spray dried lactose, by carrying out isothermal microcalorimetry experiments using the glass ampoule set-up developed in Chapter 3. The following aims were therefore identified:

- to investigate the crystallisation response (onset time and heat change) of spray dried lactose on exposure to a range of relative humidities (22%RH - 97%RH) over a range of temperatures from 25°C to 60°C.
- to measure the water content necessary to induce crystallisation at different temperatures using Dynamic Vapour Sorption analysis (DVS) and Thermogravimetric analysis (TGA) to estimate the water uptake just prior to crystallisation.
- to further explore the basis of the net heat change which is observed during crystallisation in the humidity controlled glass ampoule, by using DSC to estimate the heats that should have been released during crystallisation and then relating this to the water uptake (just before crystallisation) as measured using DVS and TGA.
- to investigate the forms of lactose obtained on crystallisation at different temperatures and relative humidities using TGA to measure the amount of monohydrate formed and DSC to measure the proportions of α and β lactose formed.

6.2 EXPERIMENTAL

6.2.1 ISOTHERMAL MICROCALORIMETRY

Amorphous spray dried lactose, as prepared and described earlier was used in these experiments. Normally 20-30mg samples of lactose were used, however, since a rapid crystallisation response was obtained at high temperatures larger weights were used in some instances in order to slow down the response, enabling it to be measured more accurately. The samples were placed in the 3ml glass ampoules along with a tube containing a saturated salt solution, to obtain the desired relative humidity, as listed in Table 6.1. The glass ampoules and saturated salt solutions had been previously equilibrated overnight at the experimental operating temperature in order to minimise disruptions on introducing the sample to the microcalorimeter, due to temperature differences. The ampoules were sealed tightly and then introduced to the microcalorimeter and allowed to equilibrate for 30 minutes, as described earlier. They were then lowered into the measuring position and the experiment was started.

This procedure was carried out for the samples of amorphous lactose at the following temperatures: 25, 35, 45, 50 and 60°C and using the range of saturated salt solutions listed in Table 6.1, which gave a %RH at the particular temperature as indicated in this table.

6.2.2 DYNAMIC VAPOUR SORPTION STUDIES.

Samples of spray dried lactose (20mg) were held at 50%RH (after initially drying at 0%RH for 4 hours) in the DVS until crystallisation occurred. Experiments were carried out at 25, 40 and 60°C, in order to investigate and compare the water uptake necessary for crystallisation at the higher temperatures.

Salt	Temp (°C)					
	20	25	30	35	45	50
Sodium chloride, NaCl	75.5	75.3	75.1	74.8	74.7	74.7
Sodium nitrite, NaNO ₂	66.0	65.0	63.0	62.0		
Magnesium nitrate, (MgNO ₃) ₂	54.5	52.8	51.5	50.0	47.1	45.5
Magnesium chloride, MgCl ₂	33.1	32.8	32.4	32.0		

Table 6.1 Saturated salt solutions and their relative humidities over a range of temperatures (reproduced from *Pharmaceutical Handbook*, Wade (Ed.) and Nyqvist, 1983).

6.2.3 DSC AND TGA STUDIES.

The samples were removed from the microcalorimeter after the crystallisation response and were scanned in the DSC from 25°C - 250°C at a scan rate of 10°C / minute under a nitrogen atmosphere. Samples of around 4mg were used in non-hermetically sealed pans. TGA experiments were carried out under the same scanning conditions and samples in the region of 4mg were scanned in open aluminium pans.

6.3 RESULTS

6.3.1 THE EFFECT OF TEMPERATURE ON THE MICROCALORIMETRY CRYSTALLISATION ONSET TIME FOR A RANGE OF RELATIVE HUMIDITIES.

The crystallisation responses at 85%RH occurred too quickly to be measured, and at 22%RH the sample didn't crystallise within the time scale of the experiment (60 hours). Therefore, the results which will be presented and discussed here are for sample which crystallised on exposure to salt solutions which gave relative humidities from 75%RH to 33%RH at 25°C. Typical microcalorimetry crystallisation responses for 30mg samples of spray dried lactose which were exposed to sodium nitrite and magnesium nitrate saturated salt solutions over a range of temperatures are shown in Figures 6.1 and 6.2. It can be seen that the effect of increasing the experimental temperature is to decrease the delay time before crystallisation occurs. The sample at

45°C crystallised after approximately 30 minutes while at 25°C crystallisation didn't occur until after 8 hours, when sodium nitrite solution was used. This much reduced time delay before crystallisation at the higher temperatures is due to the smaller difference between T (operating temperature) and the T_g , therefore the sample doesn't have to be plasticised to the same extent. The much faster supply of water vapour (due to increased evaporation) to the sample at elevated temperatures is probably also a contributory factor to the faster crystallisation rate.

The shape of the crystallisation peak also changed slightly over the temperature range investigated, with the peak becoming much sharper and narrower at the higher temperatures. Again, this is thought to be due to the powder bed becoming fully saturated at a much faster rate and consequently crystallising at a faster rate.

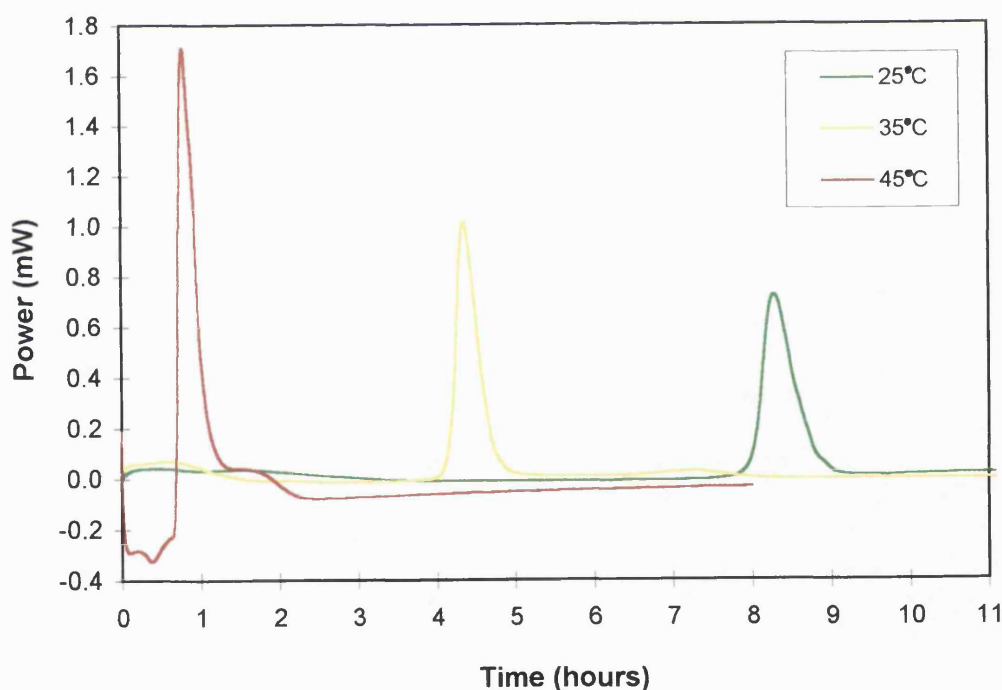


Figure 6.1 Typical microcalorimetry crystallisation responses for spray dried lactose samples (30mg) exposed to **sodium nitrite salt** solution at a range of temperatures.

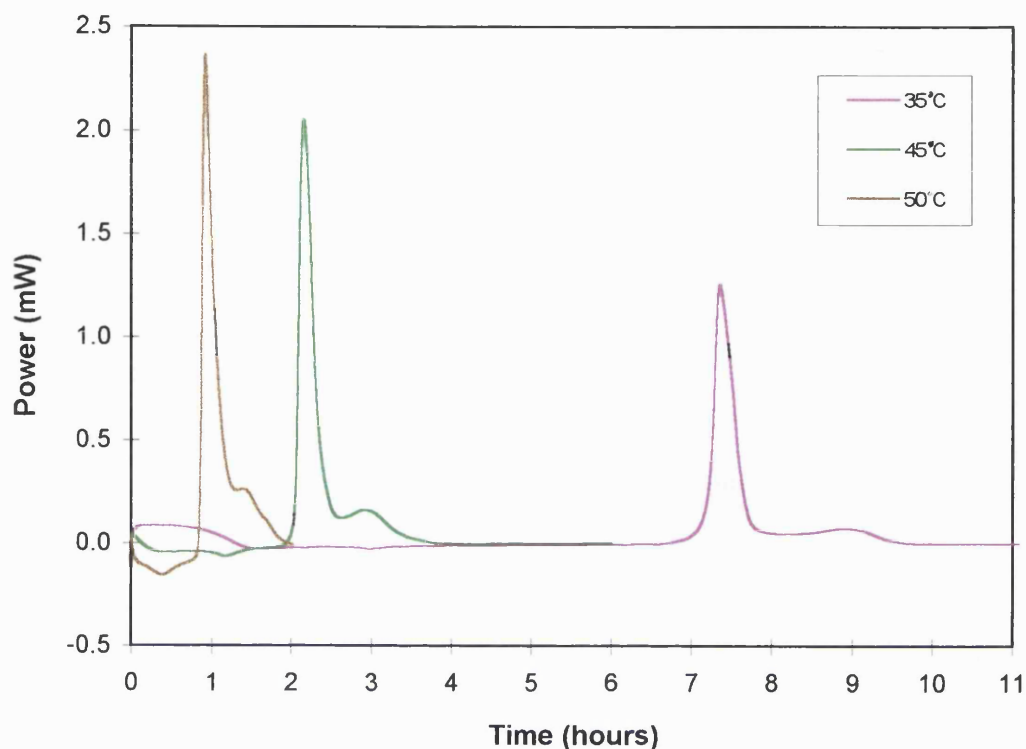


Figure 6.2 Typical microcalorimetry crystallisation responses for spray dried lactose samples exposed to **magnesium nitrate salt** solution at a range of temperatures.

Typical DSC traces for spray dried lactose samples crystallised after exposure to the sodium nitrite saturated salt solution at 25, 30, 45 and 50°C are shown in Figure 6.3. It can be seen that for each of the crystallised samples similar traces are obtained, with a hydration peak (loss of monohydrate water) at 150°C, which is followed by two melting endotherms at approximately 218°C and 238°C for α and β lactose respectively. These traces indicate that the amorphous lactose is crystallising to produce the same form of lactose at the different temperatures, although the amounts of monohydrate present along with proportions of α and β lactose are changing slightly, which is indicated in Table 6.3 and is discussed in further detail in section 6.4.2.

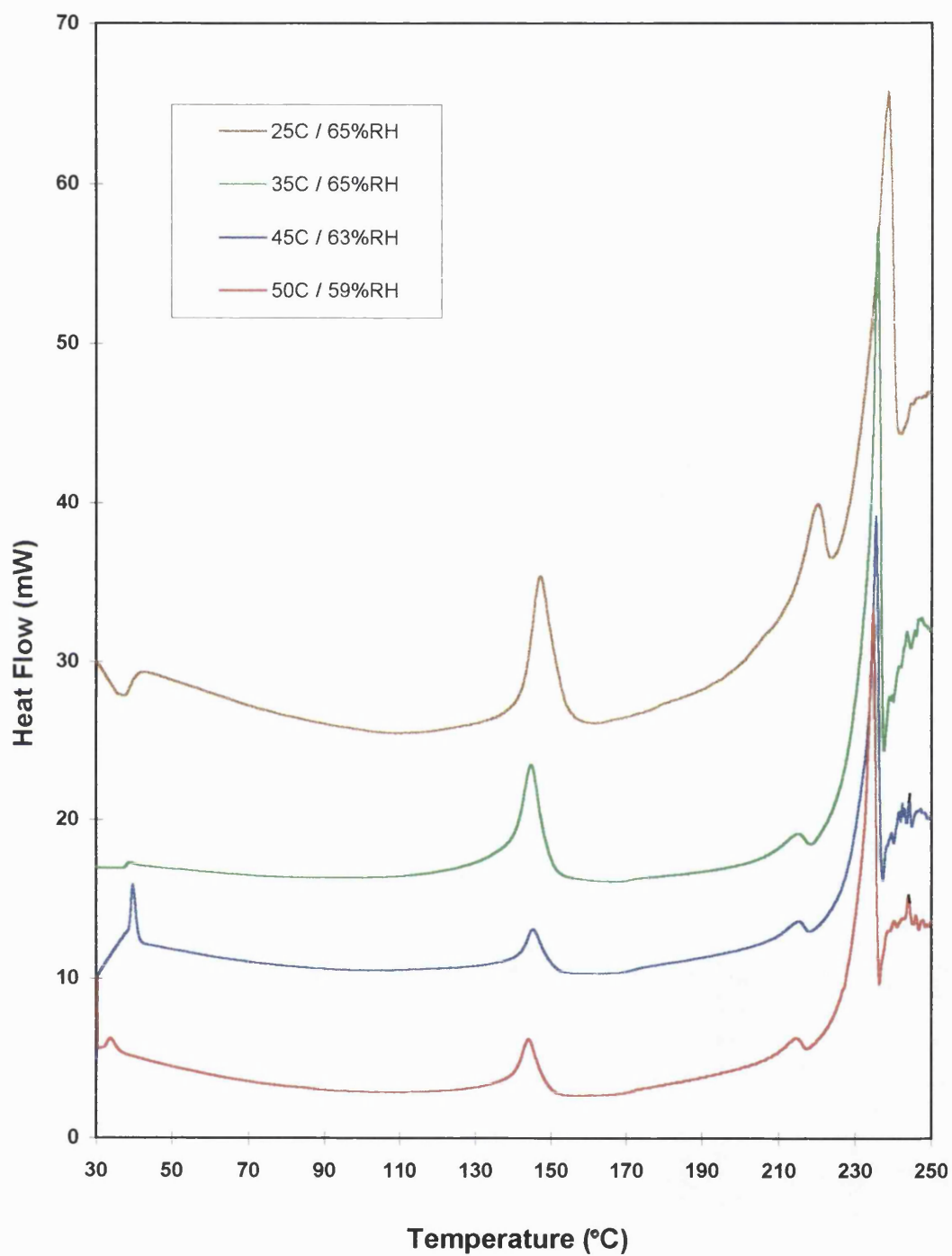


Figure 6.3 Typical DSC traces for spray dried lactose samples crystallised on exposure to sodium nitrite salt solution at 25, 30, 45, and 50°C.

The data in Table 6.2 shows the effect of temperature on the crystallisation peak time at the range of relative humidities investigated. At 25°C no crystallisation response was measured below 53%RH (i.e. at 47%RH no peak was obtained), however, using the magnesium nitrate salt solution which gave 50%RH at 35°C and 48%RH at 45°C, crystallisation responses were observed. A crystallisation response was also measured at 43%RH and 45°C. As the temperature was increased to 50°C, a crystallisation peak was obtained using the magnesium chloride salt solution which gave 31%RH at 50°C. This response was extremely variable with crystallisation occurring for some samples of similar weights after 6 hours, while others didn't crystallise for up to 50hours.

Temp(°C)	Sodium chloride	Sodium nitrite	Magnesium nitrate	Magnesium chloride
25	3.05 ± 0.03	5.12 ± 0.12	6.85 ± 0.09	no response
35	1.25 ± 0.01	2.20 ± 0.30	7.45 ± 0.05	no response
45	0.50 ± 0.16	1.00 ± 0.04	2.20 ± 0.05	variable response
50	response too fast	0.46 ± 0.05	1.67 ± 0.42	11.6 ± 3.0 (variable)
60	response too fast	response too fast	0.45 ± 0.04	2.07 ± 0.25

Table 6.2 Microcalorimetry crystallisation onset times (hours) for spray dried lactose samples at a range of %RH's and temperatures.

(Note: Different weights were used at some of the temperatures, i.e. 20mg samples were used for the 25 and 35°C studies, while 30mg samples were used for the 45, 50 and 60°C experiment referred to in this table).

The peak shape using these conditions, as shown in Figure 6.4, also deviated from the single sharp peak observed previously to a broader response which appeared to contain a number of smaller peaks, and was also variable for different samples of the same weight. The peak areas were also extremely variable as shown in Table 6.3. It is

likely that the crystallisation process is occurring at a much slower rate under these conditions due to the very slow supply of water vapour from the magnesium chloride salt solution. It is probably very close to the critical water level necessary for crystallisation to occur, hence the variability in the crystallisation onset and the peak areas and the occurrence of the process very slowly over a very long time period. It is possible that different parts of the sample are crystallising at slightly different times.

Elamin et al (1995) reported a similar observation for amorphous sucrose exposed to 33%RH, where a relatively long induction time was required by the sucrose sample before crystallisation occurred. This was interpreted to be the time required for a build up of sufficient nuclei to initiate an appreciable rate of crystallisation (Makower and Dye, 1956). It was further suggested that this delay time was probably due to the high viscosity caused by the relatively small amount of water vapour absorbed, which slowed the molecular rearrangement and thus stabilized the amorphous state for a relatively long time.

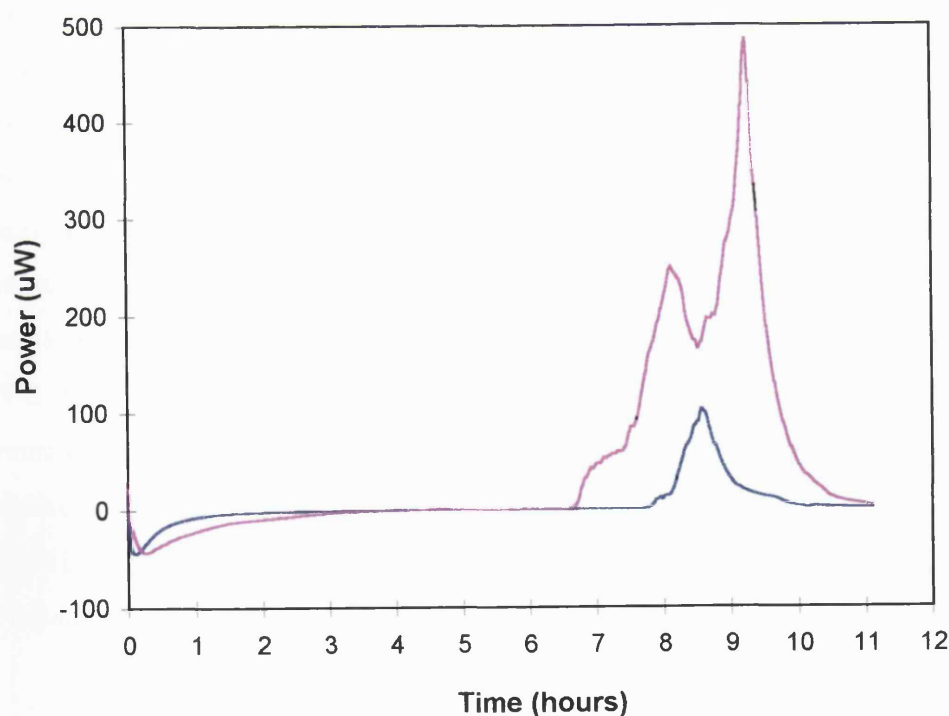


Figure 6.4 Typical microcalorimetry crystallisation responses for spray dried lactose samples exposed to magnesium chloride salt solution (30%RH) at 50°C.

When these samples were removed from the microcalorimetry cell after crystallisation at 31%RH and 50°C and scanned in the DSC, those which gave reduced peak areas (eg. 29 J/g for the smaller response in Figure 6.4) were seen to have two crystallisation exotherms at approximately 90°C and 170°C, as shown in Figure 6.5. This indicates that the part of the sample which hadn't crystallised in the microcalorimeter was collapsed and subsequently crystallised around 90°C (as discussed in Chapter 5 for collapsed amorphous samples) when heated in the DSC. This collapsed material would probably have crystallised eventually in the microcalorimetry cell, which was probably the case with the samples which only crystallised after long periods at similar conditions.

The TGA scans for these samples as shown in Figure 6.6, also had a peak present at approximately 90°C which is due to crystallisation of collapsed amorphous material (as also discussed in Chapter 5 for collapsed lactose), and agrees with the DSC observations that the sample had partly crystallised and the remainder which was collapsed subsequently crystallised on heating in the TGA. Hence, it is probable that under these conditions i.e. 31%RH and 50°C, that we are approaching the limit of the process and the crystallisation is occurring at a much slower rate than has normally been observed and measured.

As the temperature was further increased to 60°C, the crystallisation response was measured at 43%RH where the standard sharp crystallisation peak was once again obtained, and also at 30%RH where a slightly variable response was measured. The peak shape at 30%RH was more consistent for different samples than was observed at 50°C (as shown in Figure 6.7) which was probably due to the crystallisation process occurring at a faster rate due to the faster evaporation rate from the saturated salt solution at the higher temperature. The measured peak areas were also more consistent as can be seen from Table 6.3, indicating that the sample was probably all crystallising at the same time.

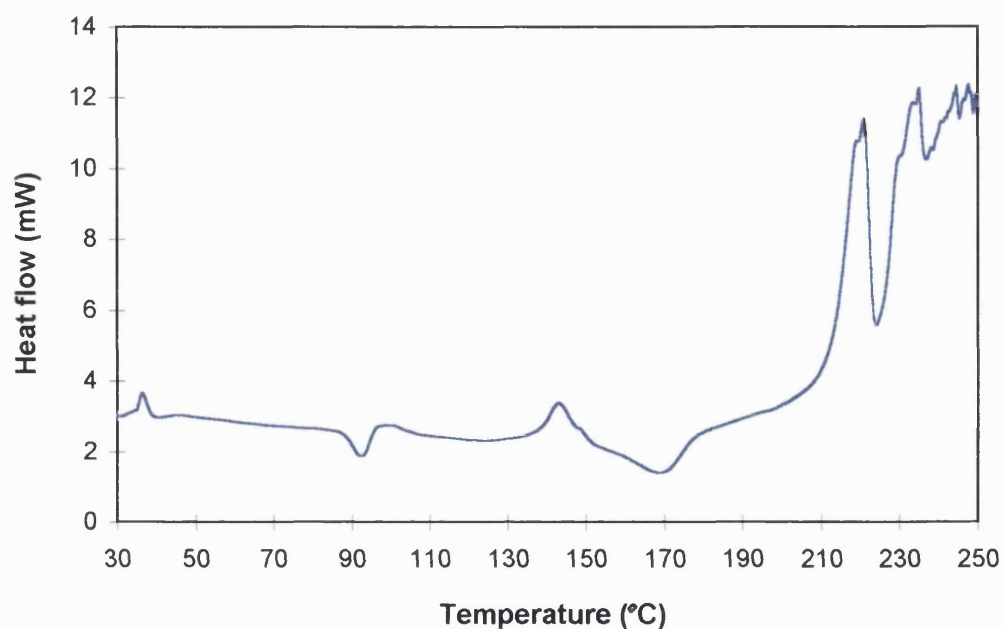


Figure 6.5 A typical DSC scan for an amorphous lactose sample exposed to 30%RH at 50°C.

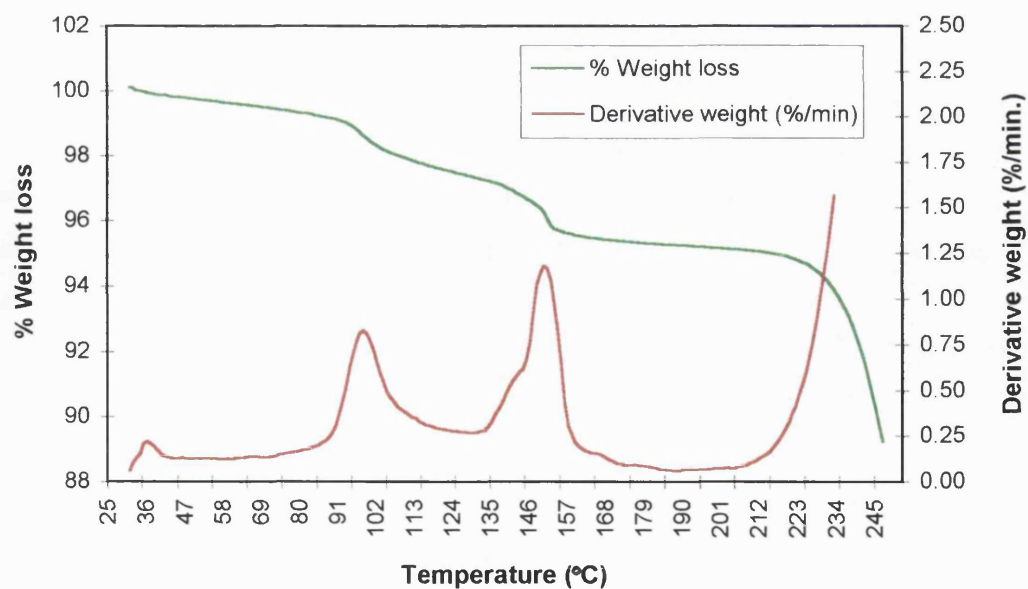


Figure 6.6 A typical TGA scan for an amorphous lactose sample exposed to 30%R at 50°C.

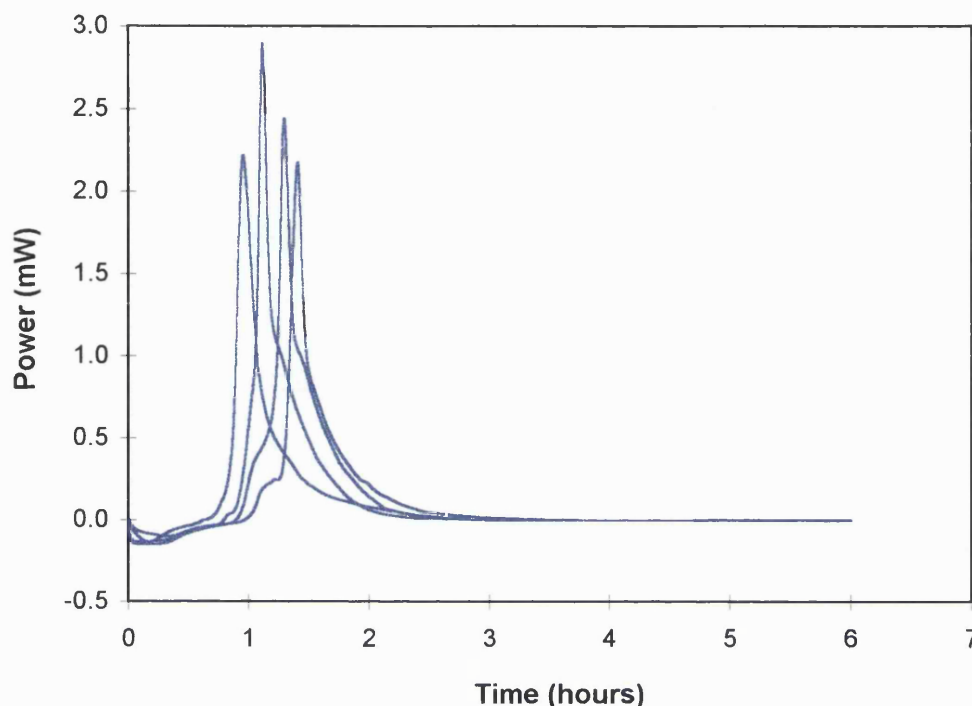


Figure 6.7 Typical microcalorimetry crystallisation responses for spray dried lactose samples exposed to magnesium chloride salt solution (30%RH) at 60°C.

A plot of temperature against the log of time before crystallisation occurred for the lactose samples showed an inverse relationship at the lower temperatures as shown in Figure 6.8. However, this linear relationship deviated at higher temperatures especially at 50°C, which may be due to the additional changes in the viscosity of the sample at high temperatures and indicating possibly different kinetic behaviour at the higher temperatures. Elamin et al (1995) also observed for samples of amorphous sucrose containing equal amounts of sorbed water, the time required for crystallisation (assessed using a DSC operated in isothermal mode) followed an exponential relationship with the temperature above T_g at which the experiment was performed. This indicated that the time for crystallisation to occur depended on the difference between T and T_g . This is in agreement with the observations here for crystallisation of amorphous lactose in the microcalorimeter, as shown in Figures 6.1 and 6.2. The samples exposed to higher %RH crystallised faster, indicating that once the critical water uptake is reached at a certain temperature, the onset of crystallisation is

kinetically controlled. Oksanen et al (1990) also observed that water vapour sorption isotherms for PVP took on a more sigmoidal shape with increasing temperature in contrast to those at lower temperatures which were linear, again indicating that the sorption kinetics were deviating at the higher temperatures. A more significant increase in absorption was also noted at higher relative humidities.

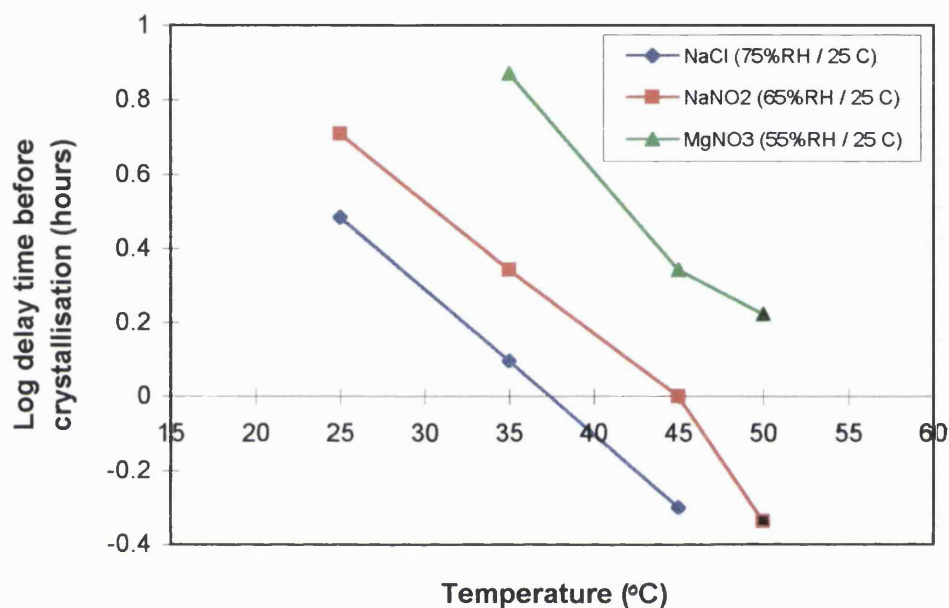


Figure 6.8 Plot of temperature against log of time delay before crystallisation occurred at varying %RH's.

6.3.2 THE EFFECT OF TEMPERATURE ON THE MEASURED CRYSTALLISATION PEAK AREAS (J/G) AT DIFFERENT RELATIVE HUMIDITIES.

The areas under the crystallisation curves as measured in Chapter 3 for parts 2 and 3 of the crystallisation response, are listed in Table 6.3 for the various combinations of temperature and %RH which were investigated. It can be seen that the heat changes are lower at lower temperatures. It can also be seen that the heat changes produced during crystallisation of the amorphous lactose at 25°C were identical for all saturated salt solutions i.e. 48.0J/g. The responses however, increased with increasing temperature for each %RH e.g. the crystallisation response increased from 48.0 J/g at

25°C to 72.6 J/g at 60°C on exposure to the magnesium nitrate salt solution and it increased from 48.0 J/g at 25°C to 62.0 J/g at 45°C on exposure to the sodium chloride salt solution. It can also be observed from this data that at temperatures in excess of 25°C, there was a general trend for the heat change to increase as the %RH decreased, e.g. at 45°C the heat change increased from 62.0 J/g when exposed to the sodium chloride salt solution (75%RH at 45°C) to 66.5 J/g on exposure to the magnesium nitrate salt solution (46%RH at 50°C). This increasing trend for the crystallisation heat change (J/g) with increasing temperature has also been observed by Giron et al (1997), while following the crystallisation of a purine derivative using a similar calorimetric method to the one employed in these studies.

Temp(°C)	Sodium chloride	Sodium nitrite	Magnesium nitrate	Magnesium chloride
25	48.9 (1.9)	47.9 (0.9)	48.2 (1.1)	no response
35	53.0 (0.6)	47.1 (3.3)	59.4 (0.3)	no response
45	62.1 (0.8)	63.2 (3.0)	66.5 (1.1)	variable response
50	response too fast	58.3 (3.1)	70.9 (0.8)	variable response
60	response too fast	response too fast	72.6 (1.3)	74.9 (1.0)

Table 6.3 Isothermal microcalorimetry heat changes (J/g) for amorphous spray dried lactose samples at a range of temperatures and relative humidities.

(*n* = 5(minimum), (sd)).

6.3.3 THE WATER UPTAKE NECESSARY FOR CRYSTALLISATION TO OCCUR AT DIFFERENT TEMPERATURES.

In order to measure the actual water uptake necessary to induce crystallisation in the amorphous lactose and see how this varies with temperature, samples were exposed to approximately 50%RH (this varied slightly with changing temperature) at 25, 40, and 60°C in the DVS until they crystallised. Typical responses for 20mg samples crystallising under the fore mentioned conditions are shown in Figure 6.9.

These samples differ in (1) the maximum water uptake reached before crystallisation and (2) the onset and timescale of the crystallisation process. It can be seen from Figure 6.9 that on lowering the experimental temperature the water uptake by the amorphous lactose increased. This data agrees with the observations of Berlin et al (1970) for foam-spray-dried whole milk where crystallisation occurred at lower water contents as the temperature was increased. It also agrees with the recent findings of Dalton et al (1997) who similarly reported a decreased water sorption with increasing temperature for a range of amorphous sugars including sucrose, raffinose and trehalose. At 25°C the lactose sample here reached a maximum water uptake in the region of 10.2%, while at 40°C it reached a maximum value of 8.7%, and at 60°C it reached 6.8% before spontaneous crystallisation occurs.

It is obvious therefore that a much lower water uptake is necessary for crystallisation to proceed at higher temperatures, which is illustrated in Figure 6.9 where it can be seen that as the temperature is increased the water uptake prior to crystallisation decreases. This is as expected since the difference between T_g and T is much smaller and hence the sample doesn't have to be plasticised to the same extent at the higher temperatures. A plot of % moisture uptake prior to crystallisation as a function of temperature showed a linear inverse relationship as shown in Figure 6.10.

Oksanen and Zografi (1990) reported similar observations in their study of the relationship between the glass transition temperature and water vapour absorption by Poly(vinylpyrrolidone). They observed that the amount of water vapour absorbed at a particular relative humidity, increases with decreasing temperature and is accompanied by a significant change in the shape of the isotherm. Elamin et al (1990) also found that the water vapour sorption prior to crystallisation of spray dried sucrose and lactose samples exposed to 57%RH decreased substantially as the temperature was decreased.

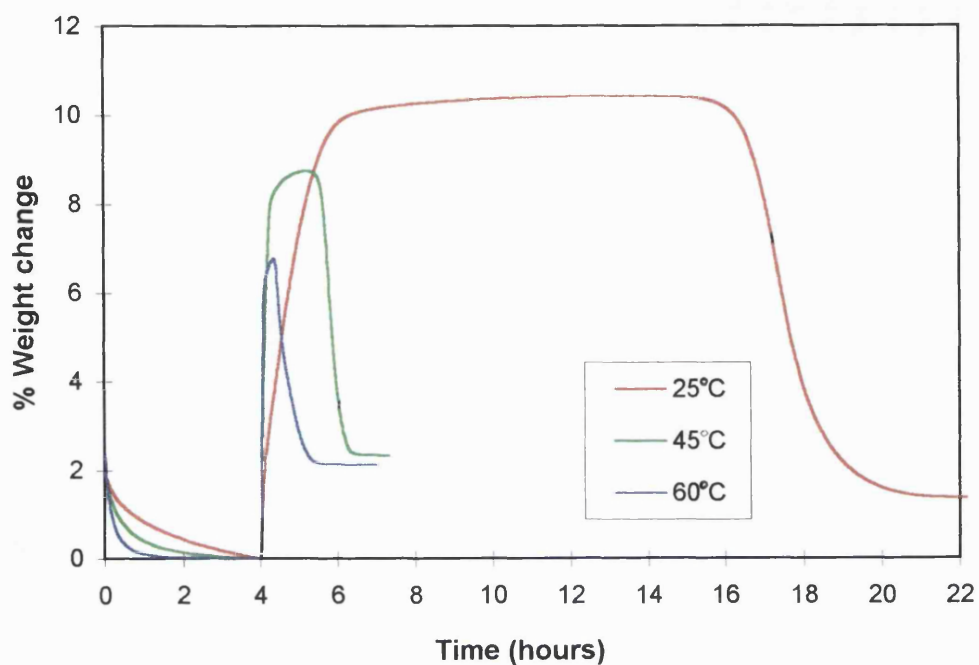


Figure 6.9 Typical DVS data for amorphous lactose samples exposed to 50%RH at 25, 40, and 60°C until crystallisation occurred.

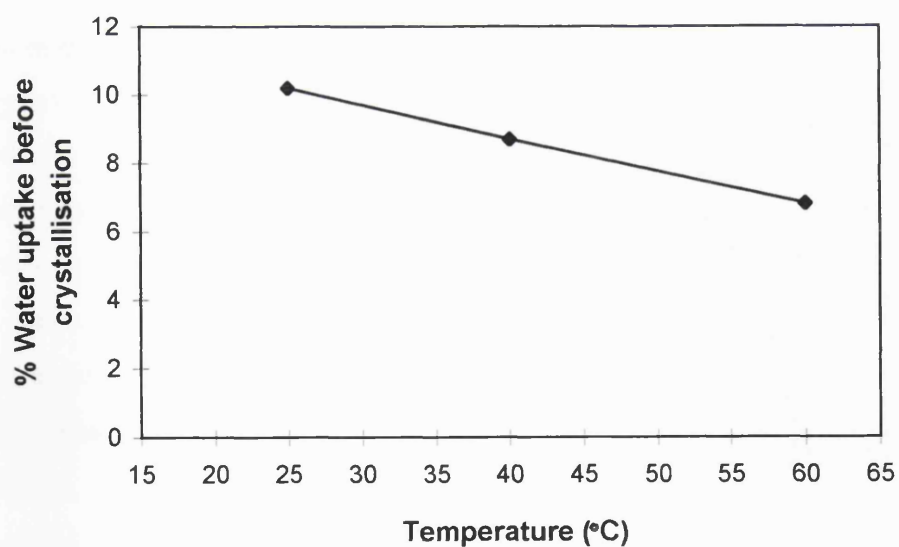


Figure 6.10 The relationship between temperature and % water uptake before crystallisation.

Using the Gordon and Taylor (1952) equation:

$$T_g = (w_1 T_{g1} + k w_2 T_{g2}) / (w_1 + k w_2) \quad \text{.....equation 6.1}$$

which was developed for polymer systems from free volume theory discussed earlier in Chapter 5, it is possible to calculate the T_g of the amorphous lactose with varying water contents at the different temperatures. The water contents which were measured in the DVS i.e. 10.2% at 25°C, 8.7% at 40°C and 6.8% at 60°C would lower the T_g to 1°C, 3°C and 23°C respectively, as shown in Table 6.4. These values are all well below the operating temperature of the experiment and the samples therefore contain much more water than would be necessary for crystallisation to occur. The samples were however not at equilibrium as can be seen from Figure 6.9, especially the one at 60°C which crystallised very rapidly. Interesting here also is the fact that $T - T_g$ was 37°C when crystallisation occurred at both 40°C and 60°C, whereas it was 24°C for the experiment at 25°C.

These findings also suggest that the moisture uptake rate was also increased as a function of temperature, which again agrees with Oksanen and Zografi (1990) that a significant change in the sorption isotherm occurs with increasing temperature. The rate of change of T_g for PVP was initially quite rapid and then more gradual after an apparent inflection in the isotherm, which was thought to be most likely associated with the glass to rubber transition, due to absorbed water. It was subsequently postulated that the plasticising effects of water appeared to produce a morphological change in the polymer which then allowed a disproportionately greater amount of water to be absorbed, despite the more unfavourable thermodynamics at higher temperatures.

The crystallisation process at 25°C didn't occur until after approximately 12 hours at 50%RH, as shown in Figure 6.9, whereby a dramatic weight loss is observed due to absorbed water being expelled from the sample. At 40°C, the sample crystallised after approximately 1.5 hours and at 60°C crystallisation occurred after approximately 30 minutes at 50%RH. It is probable that the difference between T and T_g will be crucial in determining the exact onset of crystallisation, as indicated already by the fact that at the higher temperatures $T - T_g$ was 37°C when crystallisation occurred.

Temp. (°C)	Water uptake prior to cryst. (%)	Delay time prior to cryst. (hours)	T _g (°C)	T – T _g
25	10.2	12	1	24
40	8.7	1.5	3	37
60	6.8	0.5	23	37

Table 6.4 Water uptake and T_g values prior to crystallisation.

A plot of the reciprocal of the temperature against the log of the delay time before crystallisation showed a direct linear relationship, as shown in Figure 6.11. This delay is probably due to the amorphous molecules taking a much longer time to reach the critical molecular mobility levels necessary to induce crystallisation. At the higher temperatures both the absorbed moisture and the increased temperatures lead to change in free volume and subsequent much increased molecular mobility (Ahlneck and Zograf, 1990) and hence a much more rapid rate of crystallisation. The microcalorimetry crystallisation peaks agree with this observation, since much sharper peaks were obtained at the higher temperatures, as can be seen in Figures 6.1 and 6.2, indicating that a more rapid crystallisation process is occurring.

The samples allowed to crystallise in the microcalorimeter probably achieved a lower water uptake before crystallisation and were probably closer to equilibrium than those in the DVS, due to the slower supply of water vapour in the microcalorimetry cell. This slower supply of water vapour in the microcalorimetry cell is due to the water supply being governed by the rate of evaporation of the saturated salt solution.

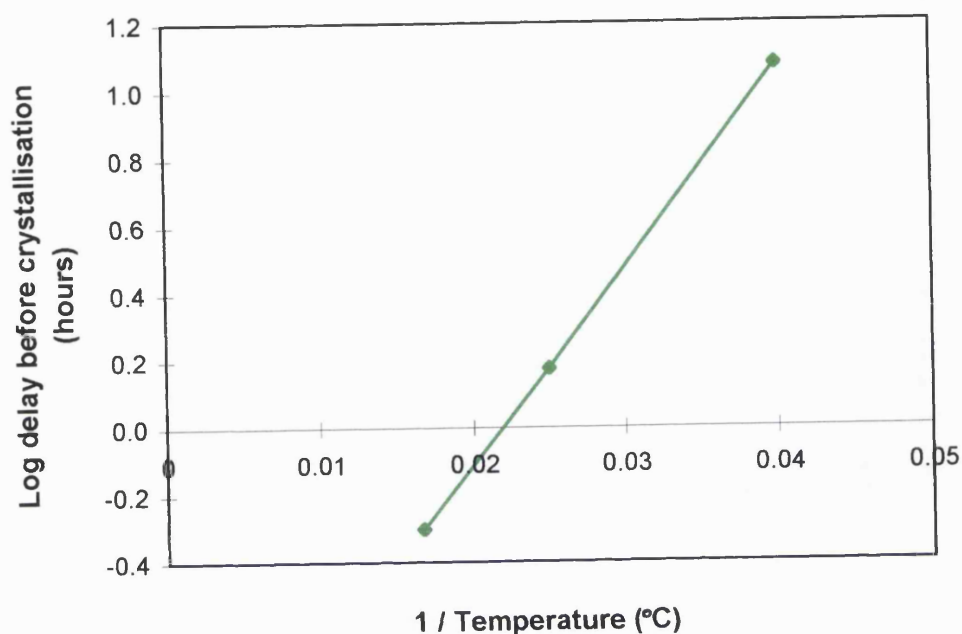


Figure 6.11 The relationship between reciprocal temperature and log delay time (hours) before crystallisation.

6.4 DISCUSSION

6.4.1 INTERPRETATION OF THE EVENTS CONTRIBUTING TO THE MEASURED MICROCALORIMETRY HEAT CHANGE.

Since isothermal microcalorimetry measures all processes which occur in the sample cell, it is necessary to consider the exact events taking place in the sealed glass ampoule in order to understand and explain the observed variation in the crystallisation heat change with varying temperature and %RH.

The microcalorimetry crystallisation process (in the sealed glass ampoule) can be split into the a number of stages as follows:

- As the saturated salt solution evaporates (an endothermic heat change) moisture is taken up by the amorphous sample (an exothermic heat change), which will cause more evaporation to occur. These two stages will result in a small overall exothermic response i.e. the initial wetting / collapse peak.

- The absorbed water acts as a plasticiser lowering the T_g of the amorphous lactose which results in structural relaxation and molecular reorientation (Ahlneck and Zografi, 1990). This is followed by a lag period during which no significant moisture changes occur in the sample. This corresponds to the near baseline region leading up to the crystallisation peak.
- Once the sample starts to crystallise water will be expelled from the amorphous regions, some of which will form the monohydrate and the remainder will be desorbed.
- Some of the desorbed water will be absorbed by neighbouring amorphous material where it will cause crystallisation to continue, and thus desorb again. This continuous process gives rise to the sharp crystallisation response which is observed. The crystallisation process itself is exothermic and the desorption of water is endothermic.
- The desorption of water will result in condensation back into the salt solution in order to establish equilibrium in the glass ampoule. This condensation will result in more of the salt dissolving.

It is obvious therefore that a number of different events are contributing to the overall microcalorimetry heat change that is measured.

6.4.2 USING DSC DATA TO CORRELATE LOSS OF CRYSTALLINE FORM WITH CRYSTALLINE FORMATION IN THE MICROCALORIMETER.

The sum of the endotherms for a typical DSC scan on the crystallised amorphous lactose (25°C) is 209.2 J/g, as listed in Table 6.5 and shown in Figure 6.3 (consisting of 60.0 J/g for the hydrate water loss, 5.5 J/g for the α lactose melting peak and 143.7 J/g for the β melting peak). There is a large difference between this heat change associated with loss of the crystalline material and that which was measured for the formation of the crystalline lactose in the microcalorimeter i.e. 48.0J/g.

It was seen earlier that the amorphous lactose absorbed approximately 10% water vapour when it was exposed to 50%RH in the DVS, before crystallisation occurred. This crystallised sample when analysed by TGA was found to contain only 3% water of hydration, as shown in Table 6.6, indicating that it was only partially hydrated. It

appears therefore that approximately 7% of the sorbed moisture is lost by evaporation during crystallisation.

Temperature (°C)	Monohydrate peak	α melting peak	β melting peak	Total area (J/g)
25	60.0	5.5	143.7	209.2
35	52.8	8.0	130.0	190.8
45	72.5	7.5	133.2	213.2
50	59.5	17.1	97.3	173.9
60	60.3	12.4	104.8	177.5

Table 6.5 Typical areas under the curve (J/g) for DSC traces for lactose which has crystallised using magnesium nitrate salt solution at different temperatures in the microcalorimeter, showing a general increase in the measured α and decrease in the β content as the crystallisation temperatures were increased.

The enthalpy of evaporation for water is 40.6 kJ/mol (Merck Index). Therefore, the heat change associated with evaporation of 7% water would be 157.9 J/g i.e.

$$\frac{40.6 \text{ kJ/mol} \times 1000 \times 7\%}{18 \text{ g (molecular wt. water)}}$$

Since as mentioned earlier the total endotherms for typical loss of crystalline form were measured to be 209.2 J/g (at 55%RH / 25°C, Table 6.5) therefore the net heat change would be 51.2 J/g (209.2 - 158.0), which is very similar to the 48.0 J/g measured in the microcalorimeter. This indicates that the measured water uptake in the DVS at 25°C of 10% is an accurate value and that the sample had reached equilibrium before crystallising.

However, it would be expected that on exposure to higher %RH, the water uptake would be greater for the amorphous material, which would result in different net heat changes being measured in the microcalorimeter. This however does not appear to be the case at 25°C, since a similar heat change (48.0J/g) was measured over the range of

saturated salt solutions which were used. The probable explanation for this is that the rate of supply of water vapour to the amorphous samples in the microcalorimeter at 25°C is sufficiently slow to allow them to start to crystallise once the critical water uptake has been reached. This would explain why samples exposed to high %RH crystallise faster and would indicate that the same water uptake is being reached before crystallisation at 25°C, irrespective of the saturated salt solution being used. The fact that the same heat change is measured in the microcalorimeter over the range of relative humidities investigated and the observation that the difference between the heat change measured by DSC and the enthalpy of vapourisation of water are very close, agrees with the above theory.

Temperature (°C)	%RH	Monohydrate weight Loss (%)
25	55	2.90
35	55	2.70
45	46	3.00
50	46	2.60
60	43	2.40

Table 6.5 TGA weight loss (%) for lactose samples crystallised using the magnesium nitrate salt solution at a range of temperatures.

6.4.3 THE INCREASING MEASURED HEATS OF CRYSTALLISATION WITH INCREASING TEMPERATURE

As shown in Table 6.3, the crystallisation heat change generally increased for each relative humidity with increasing temperature. It is accepted that the amount of water needed to plasticise the amorphous lactose is reduced as the temperature is raised as observed in the DVS studies (due to the smaller difference between T and T_g) therefore the materials will crystallise with a lower water load. Consequently the water desorption (evaporation) contribution, as discussed earlier, would therefore be expected to be lower as the experimental temperature is increased.

TGA also revealed that the samples crystallised at higher temperatures contained a smaller amount of hydrate water compared to those which had crystallised at lower temperatures, as shown in Table 6.5.

At 45°C and 45%RH the hydrate content produced was typically 3% which is the same as was observed at 25°C. A typical microcalorimetry heat change for crystallisation under these conditions was 65.1 J/g. Using the difference between the total DSC heat change (213.2 J/g as shown in Table 6.5), and the calculated heat change for evaporation of 7% water (as already discussed) the measured heat change would be expected to be in the region of 55.2 J/g i.e. $213.2 - 158.0$.

The difference between 55.2 J/g and the measured 65.1 J/g could either be due to an exotherm due to condensation or due to the fact that the sample had a lower water content prior to crystallisation, hence less vapourisation would occur. This means that the negative response due to evaporation / desorption of sorbed water would be less, hence a higher measured heat change for crystallisation would be measured in the microcalorimeter. If the water uptake value measured in the DVS prior to crystallisation at 40°C is taken i.e. 8.7%, and accounting for 3% hydrate formation, this would result in 5.7% water evaporating. The enthalpy for vapourisation of water would be 128.6 J/g. This would result in a difference of $213.2 - 128.6 = 84.6$ J/g which is substantially higher than the microcalorimetry value. This may be because the sample in the microcalorimeter contained less water prior to crystallisation than the DVS sample, which crystallised before reaching equilibrium, as shown in Figure 6.9 and therefore probably contained an excess of water.

A typical microcalorimetry response at 60°C and 43%RH was 71.7 J/g and the corresponding DSC enthalpy change due to loss of hydrate and melting of the α and β lactose forms was 177.5 J/g. TGA experiments revealed a typical hydrate content for a sample crystallised under these conditions to be 2.4%. To achieve the measured microcalorimetry response of 71.7 J/g, the initial water content of the sample would have to be in the region of 7% to account for evaporation of 4.6% ($7.0\% - 2.4\%$) water.

This value of 7% agrees very well with the water uptake measured in the DVS before crystallisation at 60°C and 45%RH (as discussed earlier) which reached 6.8%. It is

probable that the actual water uptake in the sealed ampoules in the microcalorimeter is lower than that achieved in the DVS (due to the slower rate of water supply as discussed earlier). In order to check if the microcalorimetry samples did indeed contain less water prior to crystallisation samples were removed from the microcalorimetry glass ampoules after the first wetting / collapse peak but just before crystallisation had begun at 25°C. This was done for samples exposed to 75%, 65%, and 55%RH and it was found that the water content was approximately 7.8%. Since desorption from the collapsed amorphous samples is extremely slow, no appreciable water loss would be anticipated on transferring the samples from the microcalorimeter to the TGA pan.

Using the equation:

$$T_g = (w_1 T_{g1} + k w_2 T_{g2}) / (w_1 + k w_2) \quad \text{.....equation 6.1}$$

this water content of 7.8% would lower the T_g to 23°C, which would be expected. If the water uptake just prior to crystallisation is 7.8% at 25°C and taking into account the fact that 3% hydrate is being formed, this means 4.8% water is evaporating after crystallisation. The enthalpy of vapourisation of 4.8% water would be 108.27 kJ/mol and if we subtract this value from the DSC heat change (209.2J/g for a sample which crystallised at 25°C) an expected heat change of 100.93 J/g is obtained. When compared to the measured microcalorimetry heat change at 25°C (48.0J/g), this suggests that the net difference (52.93J/g) is due to an exotherm, probably from condensation of desorbed water back into the salt solution. This further explains why larger heat changes are measured at higher temperatures, i.e. since less water is needed then a smaller endotherm due to evaporation will be measured, hence a larger net heat change. It appears therefore, that the difference in measured heat can be explained on the basis of a change in quantities of absorbed water and slight changes in hydrate formation.

6.4.4 THE INCREASED MEASURED ENTHALPY OF CRYSTALLISATION ON LOWERING %RH AT ELEVATED TEMPERATURES.

It can also be observed from Table 6.3 that the heat changes generally increased as %RH was decreased at elevated temperatures. The crystallisation of the amorphous lactose samples with a lower water content at elevated temperatures may also be used to explain this observation. The samples will have absorbed less water before collapse and subsequent crystallisation and therefore the endothermic response due to evaporation of desorbed water will be lower resulting in larger net heat changes. The samples exposed to the higher RH solutions e.g. sodium nitrite solution (59%RH at 50°C) which gave a heat change of 58.3 J/g would appear to reach higher absorbed water contents before crystallisation than those exposed to lower RH, e.g. magnesium nitrate solution (46%RH at 50°C) which gave a heat change of 71 J/g. At the higher temperatures evaporation of the salt solutions will be faster, hence there is a faster supply of water vapour to the samples. The different microcalorimetry responses demonstrate that sample stored at high relative humidities do have time to reach higher absorbed water contents than those stored at lower RH's. At 25°C the rate of evaporation will be slower and hence there will be a slower supply of water vapour to the samples. This lower rate of vapour supply has made water supply the rate limiting step in the crystallisation process and the material will crystallise before it reaches saturation point, as is the case with samples exposed to high %RH at 25°C.

6.5 CONCLUSION

The amorphous spray dried lactose crystallised much faster at elevated temperatures when it was exposed to a range of relative humidities. The water content necessary to induce crystallisation decreased as the temperature was increased, which was attributed to the smaller difference between T and T_g , therefore less was needed to plasticise the sample. The microcalorimetry measured heat of crystallisation for amorphous lactose generally increased with increasing temperature at each relative humidity. The microcalorimetry responses also increased on lowering the %RH at the higher temperatures, but this was not the case for the crystallisation response at 25°C where the measured response was 48.0 J/g for all relative humidities investigated.

These variations in the measured response are assumed to be due to the sample crystallising with a lower water content at the higher temperatures, resulting in the endothermic contribution due to evaporation of sorbed water being reduced. The variation in the heat change for the crystallisation process may also be due to lower amounts of the hydrate being formed.

The consistency of the data at 25°C indicates that this is the best temperature at which to follow the crystallisation process, especially for quantitative investigations. At higher relative humidities any variation in amorphous content between samples may result in different levels of absorbed water in the amorphous regions prior to collapse and crystallisation, thus giving different measured net heat changes due to the more rapid supply of water vapour at higher temperatures. At 25°C the slow rate of water vapour ensures that the sample will collapse with a consistent water content (irrespective of the %RH) which will be determined by the rate of supply of water vapour rather than the kinetic events within the sample.

CHAPTER 7

CRYSTALLISATION IN BULK SAMPLES OF TWO PARTIALLY AMORPHOUS LACTOSE MATERIALS.

7.1 INTRODUCTION.

The experiments carried out to date in this study have concentrated on looking at the crystallisation process in small samples (<1g) of amorphous material. In these samples spontaneous crystallisation occurred within the whole sample when it was fully saturated with water vapour, after which, the sorbed water was released instantaneously from the sample. The movement and transport of water in these samples has been earlier discussed in Chapter 3, and it was proposed that water vapour is taken up at the surface of the sample and then absorbed into the bulk of the amorphous material until the whole sample is fully saturated. This results in a lowering of the T_g , thus increasing the free volume of the material and when there is sufficient molecular movement crystallisation will occur. It was therefore considered appropriate to investigate the transport of water vapour in larger samples of amorphous material, and to measure its influence on the crystallisation process.

The aims of this study were twofold:

- to monitor and compare the weight changes occurring (due to water uptake and loss) in a range of sample sizes of two partially amorphous lactoses when stored at 75%RH and 20°C.
- to measure the time taken for these samples to crystallise.

7.2 MATERIALS AND METHODS.

7.2.1 MATERIALS.

Two commercially available partially amorphous lactoses were chosen to be investigated in this study, since it was not possible to obtain and store sufficiently large quantities of 100% amorphous lactose. The materials chosen were Zeparox (Borculo Whey Products, UK) and Pharmatose (DMV, Netherlands). Zeparox is specified by the manufacturers to be 15% amorphous, while Pharmatose is also specified to be 15% amorphous. The Pharmatose bulk material was stored at 20°C and < 30%RH when not being studied and

appeared to remain stable (i.e. it didn't crystallise) under these storage conditions. The Zeparox, however, was stored at ambient temperature (25°C) and %RH (ca 45%RH) which resulted in it taking up moisture from the atmosphere and eventually the whole sample crystallised.

7.2.1.1 Amorphous content of Pharmatose and Zeparox.

The amorphous nature of these materials was checked by exposing them to either 54% or 75%RH in a sealed glass ampoule in the microcalorimeter and measuring the crystallisation peak areas. The % amorphous content was then calculated by comparing these to the response obtained for a 100% amorphous sample. The heat output due to crystallisation of the Pharmatose sample was 5.6J/g indicating it to be 11.7% amorphous and the heat output due to the crystallisation of Zeparox was 5.13J/g indicating it to be 10.7% amorphous. The microcalorimetry responses for these samples are shown in Figures 7.1 and 7.2.

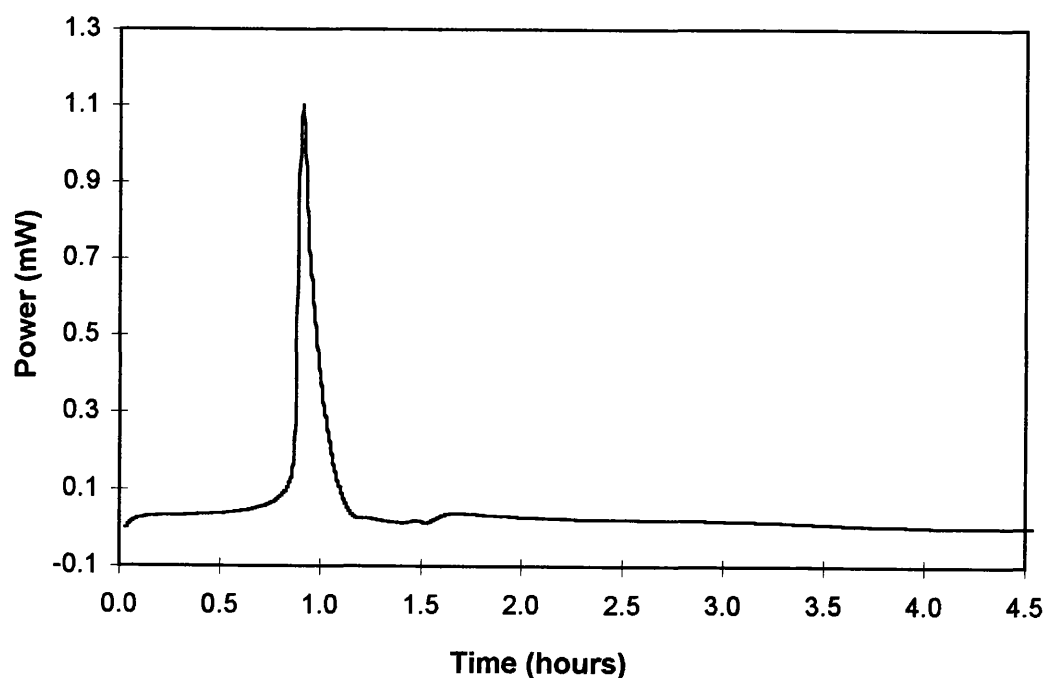


Figure 7.1 Typical microcalorimetry crystallisation response for Pharmatose.

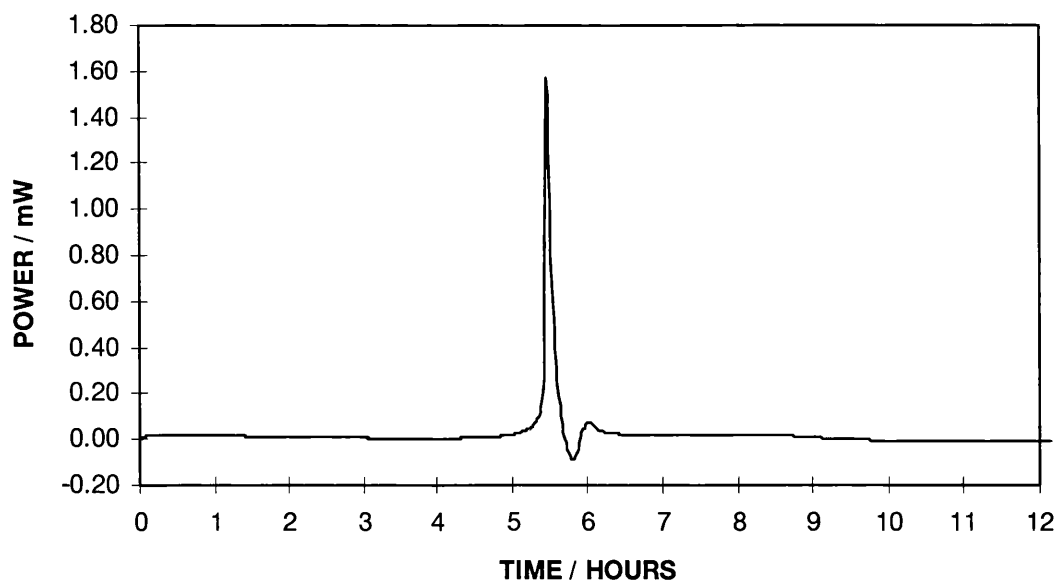


Figure 7.2 Typical microcalorimetry crystallisation response for Zeparox.

7.2.1.2 Residual water content of Pharmatose and Zeparox.

The residual water content of the Pharmatose sample was measured to be 0.3% water on holding it at 0%RH in the DVS for 16 hours and no further weight loss occurred as shown in Figure 7.3.

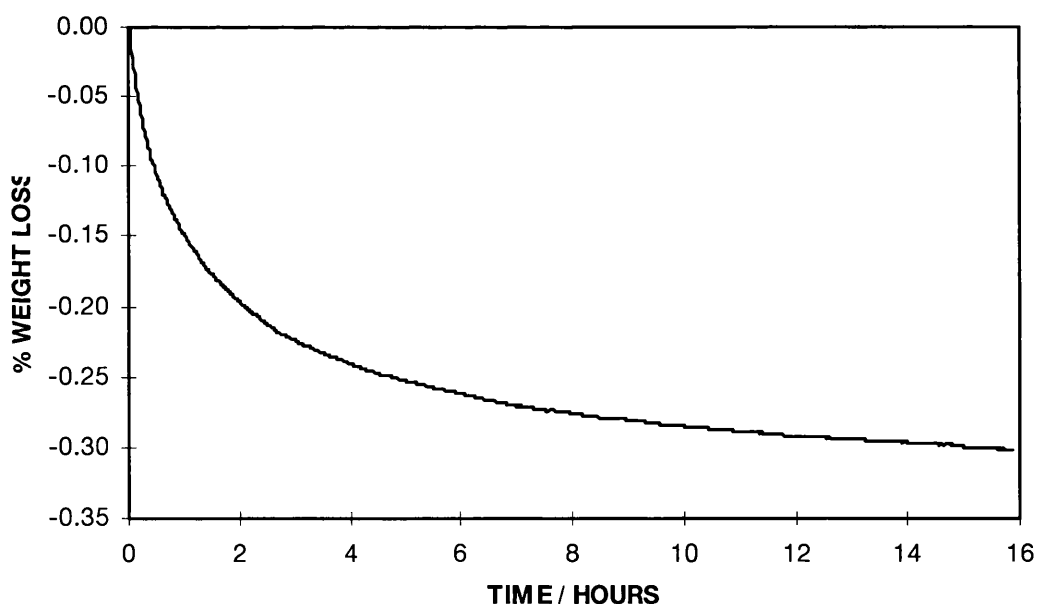


Figure 7.3 Water loss on drying at 0%RH for Pharmatose.

The presence of this residual water is an important influencing factor in the water uptake and subsequent crystallisation of these samples as will be discussed later on in

this chapter. This material is specified by the manufacturers to have a typical ‘loss on drying’ of 0.5%. The Zeparox sample was specified by the manufacturer to have a typical ‘loss on drying’ of 1.0% maximum. This was not checked prior to carrying out this study.

7.2.2 METHODS.

7.2.2.1 Measurement of changes in sample weights.

Different amounts of the lactose (ranging from 18g to 100g, as indicated in Tables 7.1 and 7.2) were weighed into 5 plastic cylinders (250ml), such that the distance between the sample and the top of the cylinder was the same for each cylinder, as shown in Figure 7.4

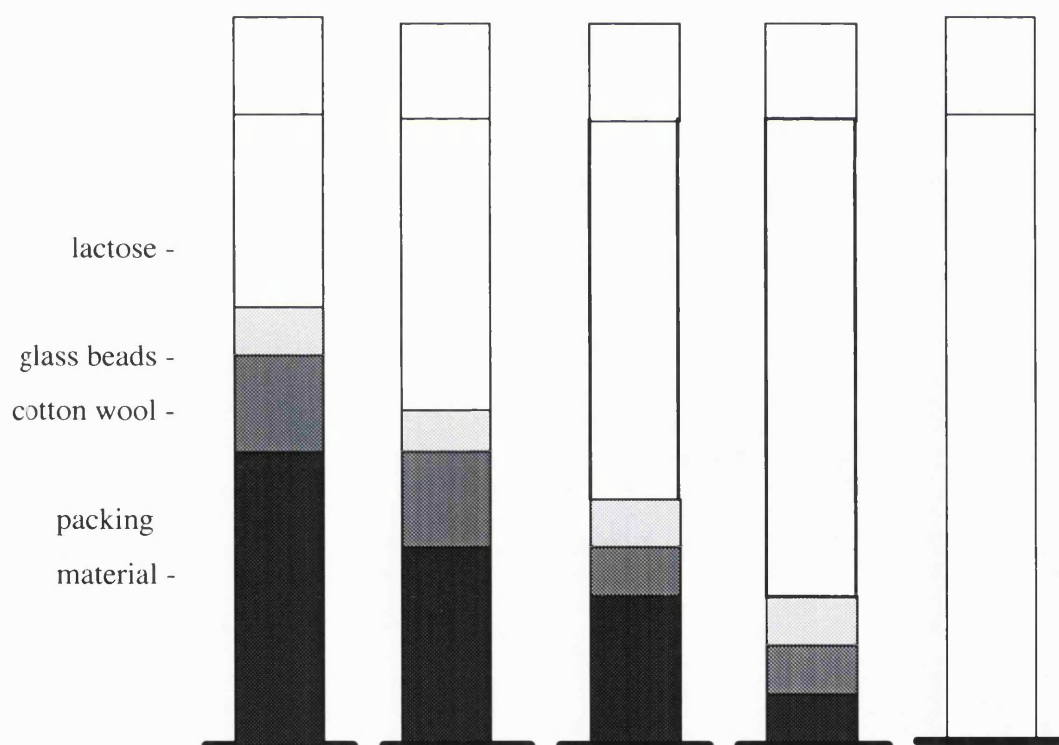


Figure 7.4 Experimental set-up for the cylinders with the top layer of lactose on a bed of glass beads, cotton wool and packing material.

This importance of ensuring that all the samples were the same distance from the top of the cylinder was to minimise any differences in moisture uptake due to the different

sample loads being at different levels in the cylinders. The cylinders were therefore pre-packed with a bottom layer of an inert packing material, a layer of cotton wool and a layer of glass beads. The lactose was then weighed directly onto the glass beads, as shown in the experimental set-up (Figure 7.4). It was hoped that by using different sample sizes it would be possible to investigate and compare weight changes and water transport at different stages throughout the sample bed. The surface area of these samples was $7.1 \times 10^{-4} \text{ m}^2$. An initial weight at time $(t) = 0$ was taken and the samples were then placed in a pre-equilibrated glove-box which contained beakers of saturated sodium chloride solution. This produced a 75%RH environment (Nygqvist, 1983) which was monitored using a hygrometer. The samples were then stored at this %RH and their weights recorded at regular intervals until no further weight changes were observed. All the experiments in this study were carried out at 20°C.

(Surface area refers to the powder surface directly exposed to 75%RH).

7.2.2.2 Weight control experiments.

Empty cylinders and cylinders containing the packing material, cotton wool and glass beads were investigated for weight changes in this study, by storing them under the same conditions as the test samples and measuring their weights at the same time intervals. All measured weight changes were then corrected by subtracting the weight change for the control cylinders which was measured to be 0.0055g after storage at 75%RH for 21days.

7.2.2.3 Monitoring the rate of crystallisation.

Lactose samples were weighed directly into the cylinders, which contained no packing material since it was desirable only to monitor the crystallisation rate in the largest sample possible. The weight at time $(t) = 0$ was recorded. The surface area for these samples was also $7.1 \times 10^{-4} \text{ m}^2$. These were then stored for a range of times at 75%RH, as above, after which they were removed from the controlled humidity environment and a final weight was recorded. Samples (ca 100mg) were then taken at different depths in the powder bed, and these were investigated for amorphous content by exposing them to 75%RH in a sealed glass ampoule in the microcalorimeter. From these microcalorimetry experiments it was then possible to determine how much of the powder bed had crystallised after each storage time.

7.2.2.4 Thermogravimetric analysis.

Samples of Pharmatose were removed from the cylinders after crystallisation and were scanned from 25°C - 250°C at a scan rate of 10°C / min. in the TGA, in order to investigate the amount of monohydrate in the crystallised form after crystallisation.

7.3 RESULTS.

7.3.1 TIME TAKEN FOR BULK SAMPLES OF THE PARTIALLY AMORPHOUS LACTOSE TO CRYSTALLISE.

Once the cylinders had been stored for different time periods at 75%RH, they were removed from the glove box. With some samples it was possible to observe a gap in the powder bed, where the powder appeared to have separated into two distinct layers. The position of this gap was noted and samples (ca 100mg) were then taken throughout the powder bed and were exposed to 75%RH in sealed glass ampoules and placed in the microcalorimeter in order to ascertain how much of the sample had crystallised. It was found from these microcalorimetry experiments that material above the gap had already crystallised, but samples taken below the gap were still partially amorphous.

7.3.1.1 Pharmatose.

The graph in Figure 7.5 shows the amount of Pharmatose (weight 95g, depth 19cm and surface area $7.1 \times 10^{-4} \text{ m}^2$) which was crystallised, after storage at 75%RH for specific periods of time. The gap in the powder bed (as described above) was used as an indication of the depth of sample crystallised. However, the data for the graph in Figure 7.5 was obtained by taking samples in the region of the gap and placing in the microcalorimeter as described above, to check for amorphous content.

It appears from the graph in Figure 7.5 that the sample begins to crystallise slowly and the crystallisation then becomes more rapid since there are two different regions to the graph. The first section of the graph up to approximately 55 hours shows a more gradual response and the slope of this section is 2.28 cm/hours, while the second part from 55 to 75 hours is more steep with a slope of 5.22 cm/hours. This indicates that once the sample begins to crystallise, the rate at which this occurs may become faster as it progresses further into the sample bed. This is probably due to a more abundant

supply of water to the lower regions of the sample, since there will probably be a delay in desorbed water being transported back up the powder bed after crystallisation. This phenomenon will be discussed later in explaining the more gradual weight loss in increasing sample size. It appears therefore from this data that larger samples of partially amorphous lactose crystallise at a gradual rate unlike small samples of totally amorphous lactose, which crystallise spontaneously when the whole sample bed is fully saturated with water vapour. Larger samples therefore behave differently to small samples and the crystallisation will proceed in these larger samples depending on the continued supply of water vapour to the sample. If the sample is removed from the high %RH environment, when only part of it has crystallised, no further crystallisation of the sample will occur.

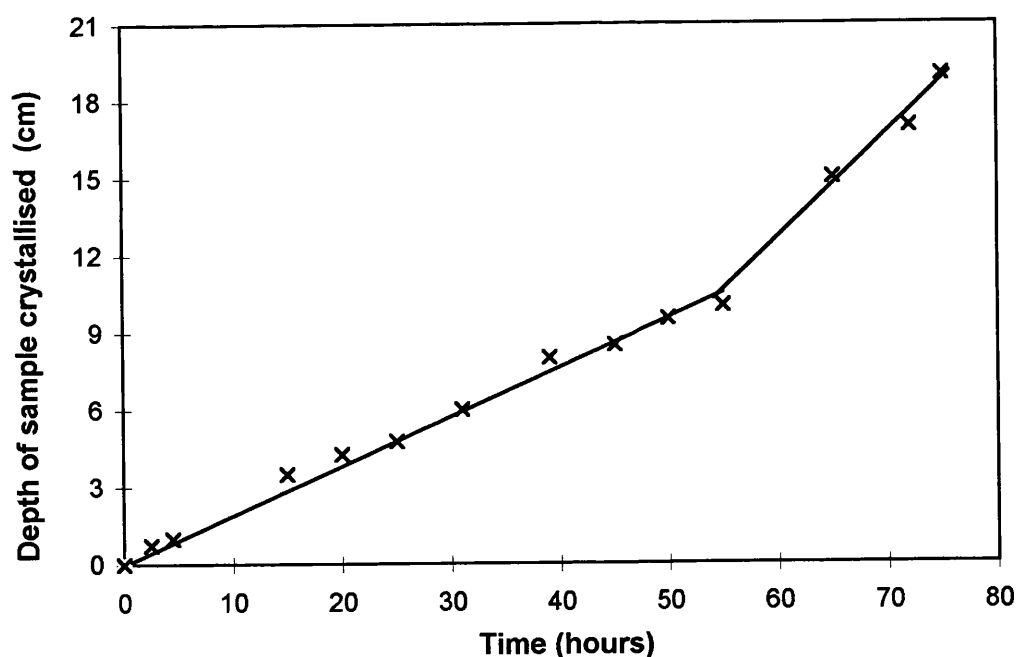


Figure 7.5 Rate of crystallisation of Pharmatose samples at 75%RH

7.3.1.2 Zeparox.

The graph in Figure 7.6 shows the time taken for the 80g sample of Zeparox of surface area of surface area $7.1 \times 10^{-4} \text{ m}^2$ and depth 19cm to crystallise. It can be seen that this sample also crystallised gradually with the whole sample taking up to 50 hours to become totally crystalline. A gap in the powder bed was also observed here,

between the top crystallised material and the bottom partially amorphous uncrystallised material as was observed for the Pharmatose samples. Samples were taken in the region of the gap and these were then placed in the microcalorimeter as described earlier, to check for amorphous content.

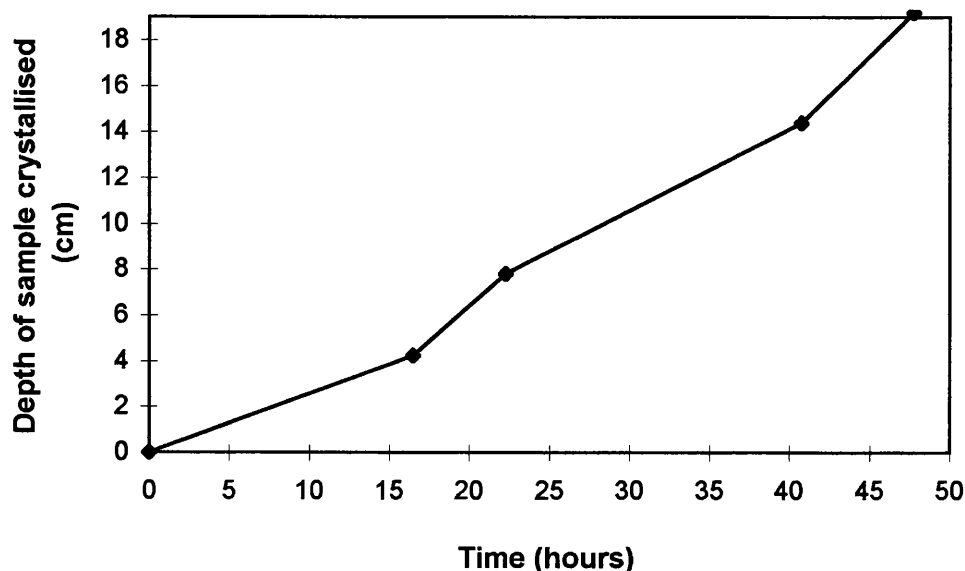


Figure 7.6 Rate of crystallisation of Zeparox samples at 75%RH.

Since not enough data points were obtained, it wasn't possible to determine accurately if the rate of crystallisation in the Zeparox sample increased with time as was the case for the Pharmatose samples. However, it does appear from Figure 7.6 that the initial rate of crystallisation is slower and becomes faster as we move into the sample bed, since the slope of the graph up to 16 hours is 2.18 and the slope from 16 to 50 hours is 4.55. This suspected increase in the rate of crystallisation in the lower regions of the powder bed is probably again the result of an abundant supply of moisture to the lower regions due to a reduced transfer of desorbed water back up the powder bed as the crystallisation process continues. It is apparent from this data, as for the Pharmatose samples, that the samples crystallised at a gradual rate unlike small samples of totally amorphous lactose which crystallise spontaneously when the whole sample bed is fully saturated with water vapour. It is probable that the crystallisation will only proceed in these samples so long as they remain exposed to the high %RH, and the entire powder bed is saturated.

7.3.2 WEIGHT CHANGES OCCURRING IN BULK SAMPLES OF THE PARTIALLY AMORPHOUS LACTOSE MATERIALS ON STORAGE AT 75%RH.

7.3.2.1 Experimental

The weight monitoring experiments were carried out as described in section 7.2.2.1 and Tables 7.1 and 7.2 show the weights, depths and volumes used for the Pharmatose and Zeparox samples in these experiments. It is important to note that the Zeparox material prior to being investigated was stored at ambient temperature (20°C) and relative humidity (ca 45%), which resulted in it absorbing moisture from the atmosphere. This eventually resulted in the material all crystallising making it impossible to fully complete the study on Zeparox. The samples used in this study therefore contained an unmeasured amount of residual water at the start of the experiment, which will probably have an affect on the results obtained.

7.3.2.2 Weight change profile.

The graphs in Figures 7.7 and 7.8 show a plot of the weight changes for the Pharmatose and Zeparox samples which were measured until no further changes were observed. There was an initial sharp increase in weight for all the sample loads, due to water being absorbed into the amorphous regions of the material. The Pharmatose samples reached maximum water uptake after about 2 days, while the Zeparox samples reached maximum weight after approximately 24 hours. There then followed a small region where a plateau was reached. The samples then began to lose weight and they eventually all reach an equilibrium weight. It would appear therefore from these results that once the samples reached a critical water uptake, they began to crystallise and we see a subsequent drop-off in weight, which is assumed to be due to due to desorption of water from the sample as it crystallises.

This weight loss continued until well after all the sample has crystallised, which was found to be approximately 80 hours for the largest (95g) sample load of Pharmatose as indicated in Figure 7.5, and 20 hours for the Zeparox samples (Figure 7.6). The larger samples continued to lose weight over long time periods with the 95g Pharmatose sample continuing to decrease in weight up to 10 days, and the 65g Zeparox sample losing weight for up to 7 days. Once the samples ceased to lose weight, they then

begin once again to increase in weight very slowly as can be seen from Figures 7.7 and 7.8. This very gradual increase in weight may be due to mutarotation of β lactose to α lactose after crystallisation (Angberg, 1991a), as the samples continue to be exposed to 75%RH. Eventually an equilibrium weight is reached for all the samples. (The volume values indicate the weight of powder(g) per cm depth of powder.)

Cylinder	Sample weight (g)	Sample depth (cm)	Volume (g/cm ³)
C1	22	4.0	5.50
C2	42	8.5	4.97
C3	60	12.5	4.85
C4	76	15.0	5.09
C5	95	19.0	5.01

Table 7.1 Weights and volumes of Pharmatose used in these experiments.

Cylinder	Sample weight (g)	Sample depth (cm)	Volume (g/cm ³)
C1	18	4.0	4.5
C2	32	8.5	3.76
C3	50	11.5	4.34
C4	65	15.0	4.33

Table 7.2 Weights and volumes of Zeparox used in these experiments.

7.3.2.3 Initial weight increases for Pharmatose and Zeparox samples.

It can be seen from Table 7.3 that all the Pharmatose samples reached a similar maximum water uptake of approximately 0.06g, apart from the smallest sample which reached a maximum uptake of 0.04g. This would indicate that the initial water uptake in these samples is independent of sample size.

The maximum water uptake in these samples is much lower than expected for a 12% (as measured by microcalorimetry) amorphous lactose sample. The water uptake would be expected to be in the region of 1.08%, since 100% amorphous lactose reaches a water uptake value of approximately 9% before recrystallising (as observed and discussed in Chapters 3 and 4 from microcalorimetry and vapour sorption studies). The total expected water uptake values for these samples, which are based on a water uptake of 1.08% are listed in Table 7.3.

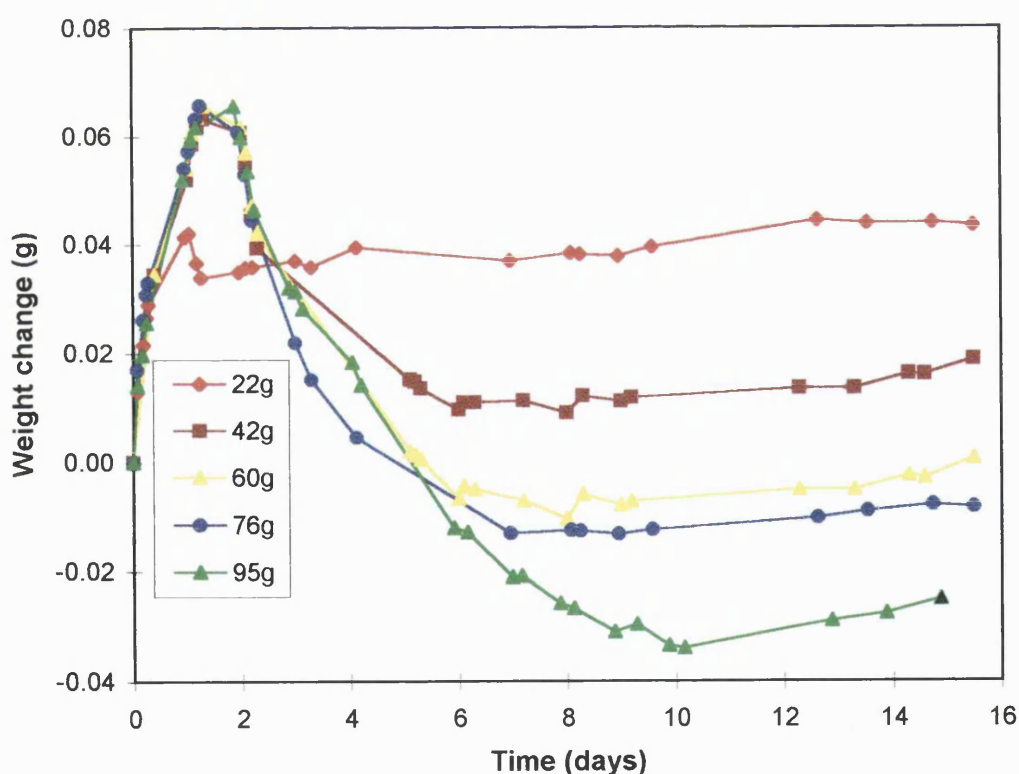


Figure 7.7 Weight changes for Pharmatose samples after storage at 75%RH until crystallisation

The maximum % uptake for the 42g Pharmatose sample was only 0.15%, while for the 95g sample it was only 0.07% as shown in Table 7.3. This indicates that the water is not uniformly distributed in these samples. Table 7.4 shows the maximum measured water uptake values and % water uptake for all the Zeparox samples along with the expected water uptake values. The maximum weight increase for all these samples is again much lower than expected, since as mentioned earlier an 11% amorphous material (as calculated from the microcalorimetry crystallisation response in Figure 7.2) would be expected to reach a water uptake level of approximately 0.88% before crystallising. Apart from the 18g sample, the other samples appear to be reaching a similar maximum uptake in the region of 0.02g water. This only amounted to 0.05% weight increase in the 32g sample compared to an expected value of 0.99%, and 0.03% weight increase in the 65g sample compared to an expected 0.99%. It appears therefore that this initial weight increase was again not dependent on sample mass.

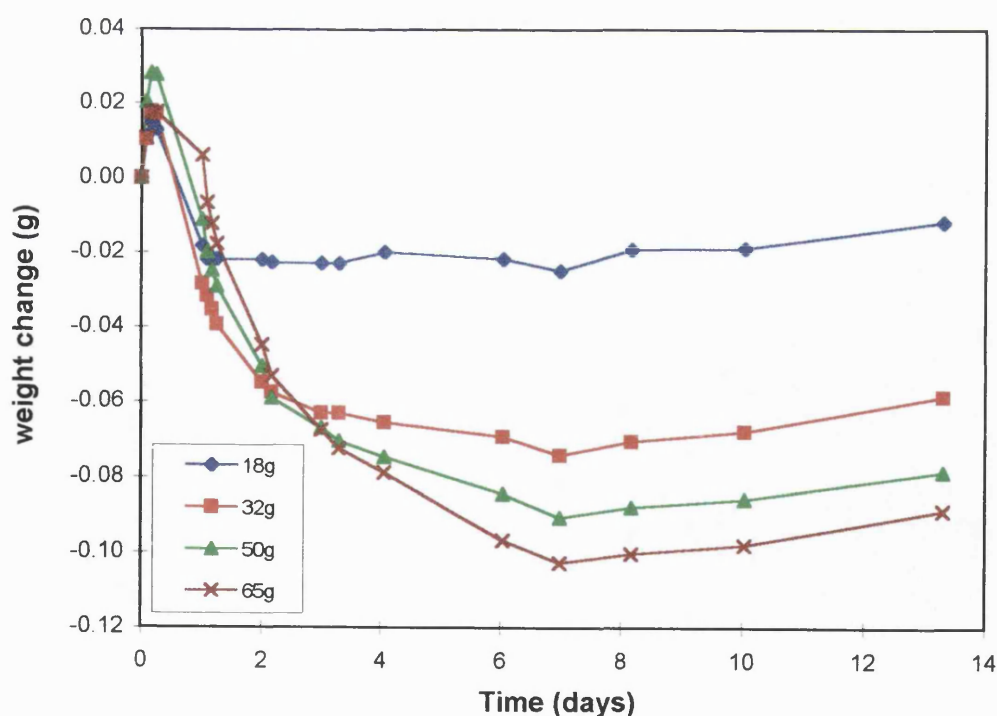


Figure 7.8. Weight changes for Zeparox samples after storage at 75%RH until crystallisation.

The uptake and movement of water in these larger samples is therefore quite different to that in much smaller samples of the same material (as can be seen from the DVS data shown in Figures 7.9 and 7.10). It would appear that in these samples there is a pulse of high water content moving through the powder bed with a small amount of sorbed water ahead and a small amount of water behind. It was observed also that on removal of the cylinders from the 75%RH environment, no further crystallisation occurred in the samples. This indicates that once the samples remain in equilibrium with the high %RH, this pulse of water will move through the powder bed until all the sample has crystallised.

Sample weight(g)	Measured Weight Increase(g)	Expected weight increase(g)	Measured % weight increase	Expected % weight increase
22	0.042	0.238	0.191	1.08
42	0.063	0.454	0.150	1.08
60	0.066	0.648	0.110	1.08
76	0.066	0.821	0.087	1.08
95	0.066	1.026	0.069	1.08

Table 7.3 Measured and expected weight increases due to water uptake for Pharmatose samples stored at 75%RH

Sample weight(g)	Measured Weight Increase(g)	Expected weight increase(g)	Measured % weight increase	Expected % weight increase
18	0.0149	0.178	0.083	0.99
32	0.0178	0.317	0.056	0.99
50	0.0281	0.495	0.056	0.99
65	0.0175	0.644	0.027	0.99

Table 7.4 Measured and expected weight increases due to water uptake for Zeparox samples stored at 75%RH

7.3.2.4 Water uptake prior to crystallisation in small samples (<200mg) of Pharmatose.

A 20mg sample of Pharmatose reached a maximum water uptake of 1.1% on storage at 70%RH in the DVS before crystallising as shown in Figure 7.9. This water uptake is in very good agreement with the predicted value of 1.1% listed in Table 7.3, based on 100% amorphous lactose taking up in the region of 9% water before crystallising. This 20mg sample was first exposed to 0%RH, where it lost 0.3% water. When compared to the samples being studied here (which weren't dried at 0%RH initially) this would still result in a weight increase of 0.8%, which is still much higher than that observed for the samples in this study.

The water uptake and subsequent crystallisation for a 120mg Pharmatose sample was also measured in the DVS as shown in Figure 7.10. This sample wasn't pre-dried at 0%RH (as for the samples used in the cylinder experiments here) and a slower flow rate of 20 sccm (standard cm³) was used instead of the usual value of 150 sccm, in order to mimic the cylinder experiments as closely as possible. The water uptake in this sample reached 0.78% before crystallisation occurred.

This indicates that:

- The same water uptake/transport mechanisms and crystallisation processes are taking place in the 20mg and 120mg samples in the DVS, since the whole sample crystallised spontaneously in both cases when they reached a water uptake of 1.1% (or 0.8% for the 120mg sample which already contained 0.3% sorbed water).
- That by exposing the sample directly to 75%RH without first exposing it to a drying step at 0%RH, results in a lower % water uptake (by an amount equivalent to the amount of water lost on drying at 0%RH) prior to crystallisation. This highlights the significance of residual water content on the water uptake and loss prior to and after crystallisation.

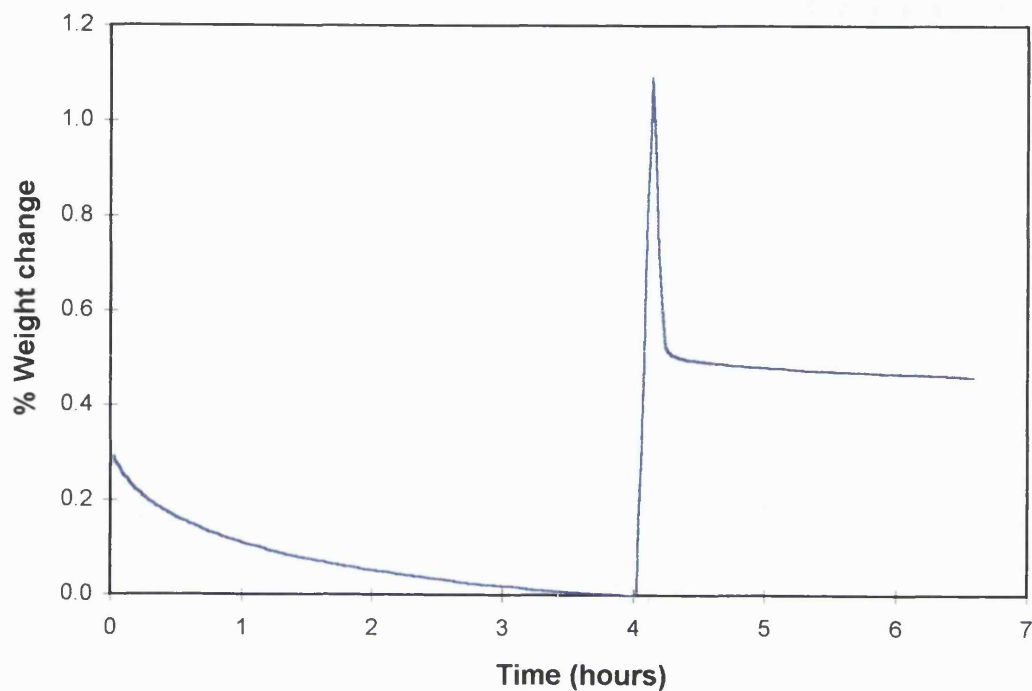


Figure 7.9 Water uptake and crystallisation for a 20mg Pharmatose sample at 70%RH (after first drying at 0%RH) in the DVS.

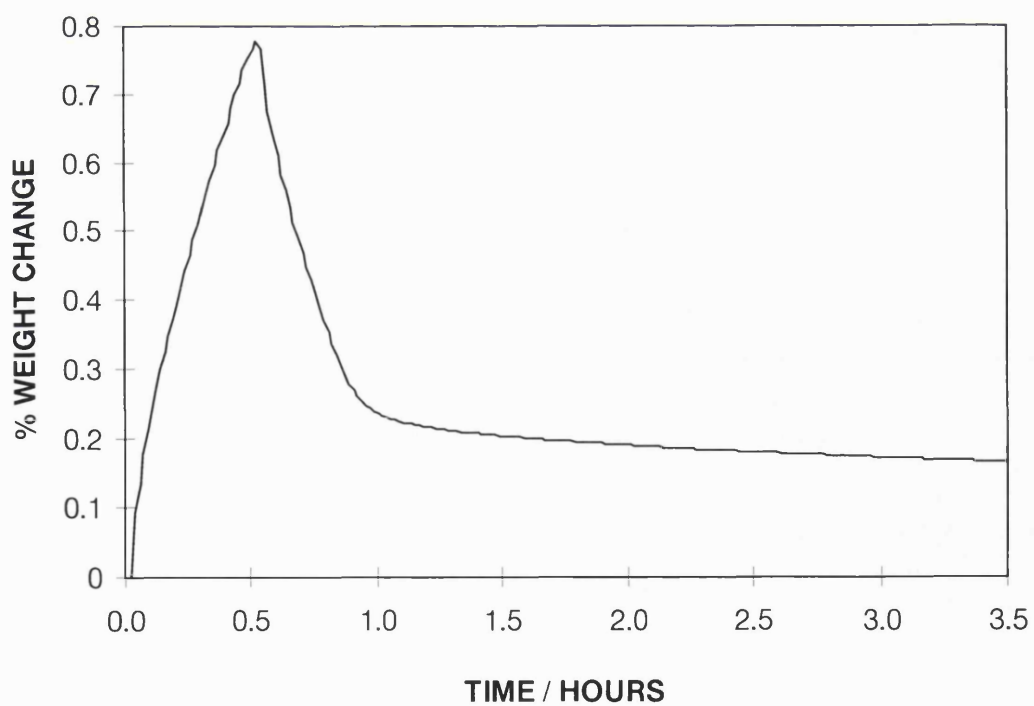


Figure 7.10 Water uptake and crystallisation for a 120mg Pharmatose sample exposed directly to 70%RH in the DVS.

7.3.2.5 Weight loss

It can be seen from Figures 7.7 and 7.8 that there is a sharp drop-off in weight after the maximum weight has been reached. This is assumed to be due to water desorption from the sample as the amorphous material begins to crystallise. The total measured weight losses (g) and %weight losses along with the net overall weight change for the Pharmatose and Zeparox samples are shown in Tables 7.5 and 7.6 respectively. The measured weight loss values were calculated by subtracting the minimum value reached on the weight profile curve from the maximum value reached, for each cylinder.

Unlike the maximum water uptake values measured, which were similar for all samples, the overall net weight loss values increased slightly with increasing sample size, from 0.008g for the 22g Pharmatose sample to 0.1g for the 95g sample. This can be seen in Figure 7.11 where the water uptake on storage at 75%RH and the water loss (as shown in Table 7.5) are plotted.

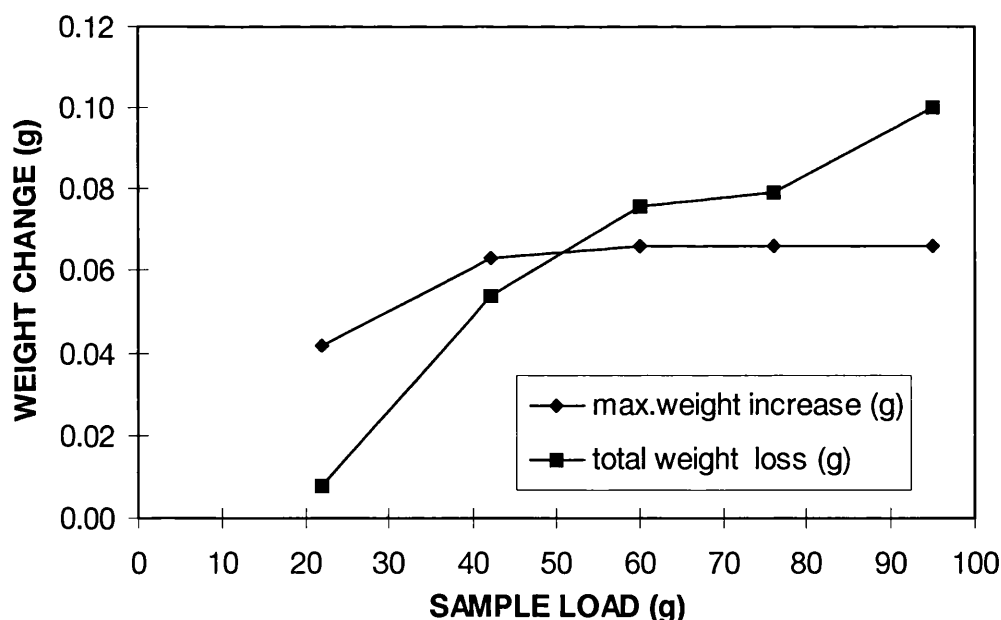


Figure 7.11. Comparing the maximum water uptake and the total water loss from the Pharmatose samples on crystallisation.

Similarly for the Zeparox samples where the 18g sample lost 0.04g while the 65g sample lost 0.12g. Initially, this would not seem to appear unreasonable since more water will be needed to plasticise the larger sample loads in order for the whole sample to crystallise and therefore more water will be desorbed on crystallisation.

However, the more surprising observation from this data is that there is a negative net overall weight change for the larger samples, as can be seen from Figures 7.7 and 7.8. With both the Pharmatose and Zeparox materials the trend is that the larger the sample the more negative the final weight.

Sample load (g)	Measured weight loss (g)	% Weight loss	Net final weight change (g)
22	0.008	0.037	0.0434
42	0.054	0.128	0.0188
60	0.076	0.125	0.0006
76	0.079	0.123	-0.0083
95	0.100	0.105	-0.0249

Table 7.5 Measured weight losses and final net weight changes for Pharmatose samples.

Sample load (g)	Measured weight loss (g)	% Weight loss	Net final weight change (g)
18	0.0398	0.02273	-0.020
32	0.0919	0.2833	-0.075
50	0.1187	0.2397	-0.080
65	0.1201	0.1864	-0.090

Table 7.6 Measured weight losses and final net weight changes for Zeparox samples.

7.4 DISCUSSION

7.4.1 THE TOTAL AMOUNT OF ABSORBED WATER PRESENT IN THE PHARMATOSE SAMPLES PRIOR TO CRYSTALLISATION.

It was seen earlier in Table 7.3 that the Pharmatose samples reached a lower water uptake prior to crystallisation, than was expected on storage at 75%RH, i.e. 0.04g for the 22g sample and 0.06g for all other samples.

However, as mentioned earlier, the material already contained 0.3% residual water which meant a lower water uptake from the surrounding atmosphere was required in order to reach the critical water content required to induce crystallisation. This was the case for the 120mg sample which was exposed directly to 70%RH in the DVS (see Figure 7.8) without first drying at 0%RH to remove residual water. This sample reached a water uptake of 0.78% compared to the dry sample, which reached a water uptake of 1.1% before spontaneous crystallisation occurred. These two samples both lost 0.6% water on crystallising, resulting in the sample which wasn't dried initially ending up with a water content after crystallisation which was 0.3% lower than the sample which was dried at 0%RH initially.

The presence of residual water is a very important parameter in materials which contain even small amounts of amorphous character as the results of this study have shown. Ahlneck and Zografi (1990) described how disordered amorphous material, because it is in an 'activated' state will take up much more water than crystalline material where only surface adsorption of water will occur. This concentration of water in the amorphous regions of the sample will therefore result in the water content being amplified in these regions.

If we take, for example, the 95g Pharmatose sample here and we use the DVS value of 0.3% (section 7.2.1.2) for absorbed water, this corresponds to 0.285g water in the entire sample as shown in Table 7.7. However, from the discussion above it is probable that this water will be concentrated in the amorphous regions of the material, and the actual water content will therefore be amplified to 2.5% (since this material was calculated to be approximately 12% amorphous from the microcalorimetry response) in these regions. This is a substantial amount of water and will lead to a considerable lowering of the T_g , producing significant increases in molecular mobility, and will ultimately cause the sample to crystallise as occurred in this study.

Therefore, what would appear to be a reasonable water content when distributed uniformly throughout the entire sample is in actual fact a very substantial amount when concentrated in the amorphous regions. It is necessary therefore, to adjust the measured water uptake values in these samples prior to crystallisation, by adding the amount of absorbed water already concentrated in the amorphous regions (12% of the sample) to the amount absorbed on exposure to 75%RH. The total water uptake in these samples is listed in Table 7.7.

Sample load (g)	Amorphous content (g) (based on 12%)	Initial moisture content in amorphous region (g) (based on 0.3%)	Measured moisture uptake at 75% RH	Total moisture in amor. Regions (g)
22	2.64	0.066	0.042	0.106
42	5.04	0.126	0.063	0.186
60	7.20	0.180	0.066	0.240
76	9.12	0.228	0.066	0.288
95	11.4	0.285	0.066	0.345

Table 7.7 Total moisture present in the amorphous regions of the Pharmatose samples prior to crystallisation.

7.4.2 ESTIMATING THE EXPECTED NET WEIGHT CHANGE AFTER CRYSTALLISATION.

It was possible to calculate an expected final weight after the crystallisation process for each sample, by subtracting the total amount of absorbed water present in the material prior to crystallisation from the expected weight gain due to monohydrate formation on crystallisation. In order to calculate the expected weight gain due to monohydrate formation, it was necessary to perform Thermogravimetric analysis on the crystallised samples. The weight increases due to monohydrate formation were investigated by measuring the magnitude of the peak at 150 °C in the TGA scans, and comparing this to the original Pharmatose sample before crystallisation. The data in Table 7.8 shows the %weight loss values at 150 °C due to loss of the monohydrate

peak for samples taken at different regions in the 5 cylinders after crystallisation, along with that for the original Pharmatose material.

Sample load (g) (sampling point)	% weight loss at 150 °C (mean, \pm sd)	Overall mean (\pm sd)
original Pharmatose	4.70 ($n=9, \pm 0.26$)	
22 - top	5.16 (0.015)	
- bottom	4.98 (0.0043)	5.07 (0.091)
42 - top	5.20 (0.09)	
- bottom	4.99 (0.06)	5.10 (0.105)
65 - top	5.12 (0.015)	
- bottom	5.11 (0.068)	5.11 (0.004)
76 - top	5.17 (0.072)	
- middle	4.91 (0.122)	
-bottom	5.09 (0.136)	5.06 (0.109)
95 - top	5.16 (0.06)	
- middle	5.14 (0.163)	
-bottom	5.15 (0.113)	5.15 (0.008)

Table 7.8 Thermogravimetric Analysis data for Pharmatose samples prior to and after crystallisation ($n=3$ for all samples except original Pharmatose).

The magnitude of the peak at 150 °C for the original Pharmatose sample was 4.70%. All the crystallised samples contained in the region of 5% hydrate water. Some small differences were observed between the monohydrate content for the different cylinders, as shown in Table 7.8, however, no apparent trend is obvious. Since, α lactose monohydrate normally contains 5% hydrate water, the expected weight increases for the Pharmatose samples (based on the amorphous material crystallising to give 5% monohydrate) are listed in Table 7.9.

The final net expected weight changes are also shown and were calculated by subtracting the absorbed water (which is desorbed after crystallisation), from the expected increase due to hydrate formation. The measured final weight changes are

also listed. It can be seen that major differences exist between the measured and the expected values.

Sample load (g)	Total absorbed water (g)	Expected monohydrate weight gain (g)		Expected net weight change (g)		Measured net weight change (g)
		5%	3%	5%	3%	
22	0.106	0.132	0.079	0.026	-0.027	0.0434
42	0.186	0.252	0.151	0.066	-0.035	0.0188
60	0.240	0.360	0.216	0.0120	-0.024	0.0006
76	0.288	0.460	0.274	0.170	-0.014	-0.0083
95	0.345	0.570	0.342	0.225	-0.003	-0.0249

Table 7.9 Measured and expected net weight changes for Pharmatose samples after crystallisation

Although α lactose monohydrate normally contains 5% monohydrate, it was shown earlier in Chapter 3 (section 3.7) that 100% amorphous lactose crystallised (when exposed to 75%RH) to form α lactose monohydrate which was only partially hydrated i.e. 3% rather than the expected 5%. Therefore it is possible that the Pharmatose samples studied here also crystallised to yield a partial hydrate i.e. 3% rather than 5%. The expected weight gain if the Pharmatose samples studied here crystallised to give 3% hydrate were also calculated and are also listed in Table 7.9. The expected weight change of 0.026g for the 22g sample agrees quite well with the measured value of 0.0434g, which is based on this sample crystallising to form 5% hydrate. However, the largest (95g) sample appears to be forming only 3% hydrate, since the measured value was much lower than expected if 5% hydrate were being formed. It would appear therefore from the data presented in Table 7.9 that the reason for the negative overall weight changes in the larger samples is due to different amounts of monohydrate being formed on crystallisation of the amorphous material.

The TGA data did not highlight significant differences in the amount of hydrate formed in these samples. This was due to the small difference between % weight

change if 5% hydrate were formed (where the weight change would be 0.6%) and if 3% hydrate were formed (where the weight change would be 0.36%). This is a difference of 0.24%, but TGA studies of the original Pharmatose sample (as shown in Table 7.8) revealed a hydrate peak of 4.70 +/- 0.26%, therefore this technique is probably not sensitive enough to detect such small weight changes. It is probable that over a long period of time, all the samples will form the same amount of hydrate, if they remain in contact with the high RH environment, due to mutarotation of β to α lactose (Angberg et al, 1991). This was also observed for the 100% amorphous lactose samples investigated in Chapter 3, where mutarotation occurred as the samples continued to be exposed to high relative humidity. The weights of the larger samples do actually appear to be increasing towards the end of the 15 day storage period, as can be seen from Figure 7.7.

It is unclear as to why the smallest (22g) sample behaved differently to the larger ones, in apparently forming more hydrate. This may be because the desorbed water is preferentially re-absorbed by the lower regions of the larger samples, and consequently the water is available to cause total hydration on crystallisation.

7.5 CONCLUSIONS

The results from this study show that the crystallisation process in larger samples of partially amorphous material differs substantially to that in small samples of 100% amorphous material, and the following general points were noted:

- The larger samples crystallised gradually over an extended period of time i.e. days depending on the sample size and surface area, and the rate of crystallisation appeared to increase as the samples reached 100% crystallinity. This was assumed to be due to the higher availability of water at lower levels in the sample bed, as loss of desorbed water back up the powder bed would probably be delayed as the sample size increased.
- It seems likely, therefore that once a critical water uptake has been reached at the top of the powder bed, crystallisation will commence. It is then probable that a

pulse of high water content moves through the sample, with a small amount of water ahead and a small amount behind.

- The amount of water necessary to induce crystallisation in these large samples was surprisingly low compared to the expected amounts for a 12% amorphous material, i.e. water uptake in the region of 0.06g (0.07% for the 95g sample compared to an expected value of 1.1%) was measured for all samples apart from the smallest one which took up 0.04g.
- The presence of residual water in the samples also had an effect on the crystallisation onset, since it resulted in the samples having a higher amount of absorbed water concentrated in the amorphous regions, hence the samples crystallised faster, as was the case with the Zeparox samples.
- The net final weight change was negative for the larger samples investigated, indicating that these samples lost more water than they took up. An estimate of expected weight change for the samples was obtained by taking the amount of water desorbed from the expected weight increase due to hydrate formation. It appeared that the large samples were forming less hydrate, since the weight changes were progressively more negative for these samples.

CHAPTER 8

SUMMARY AND FUTURE WORK

8.1 SUMMARY

The isothermal microcalorimetry method developed in Chapter 3 proved to be an extremely useful method with which to follow the amorphous to crystalline transformation in spray dried lactose. By manipulating and controlling the weight of the sample used and the %RH to which it was exposed, it was possible to obtain optimum conditions for detecting and quantifying the crystallisation process. The delay time before crystallisation increased as the weight was increased and as the %RH was decreased. This delay time was due to the time taken for the sample to become saturated with water vapour such that the glass transition temperature (T_g) fell below the operating temperature. A sharp and rapid crystallisation response was measured and the accompanying heat change for crystallisation for amorphous lactose was calculated to be in the region of 48 J/g.

The events leading to crystallisation of the amorphous lactose were explained on the basis of water vapour being taken up at the powder surface and then being transported into the rest of the sample bed. Once the surface of the powder begins to reach saturation crystallisation will begin and water that is released during crystallisation will be rapidly absorbed by the rest of the sample resulting in a spontaneous crystallisation process occurring. Consequently, only when the entire sample is fully saturated will complete crystallisation occur.

The microcalorimetry studies carried out investigating the influence of additives on the crystallisation process provided further information on the kinetics of the process. These experiments revealed that the presence of a layer of either an inert, hydrophobic or hydrophilic material between 2 distinct layers of spray dried lactose caused significant differences in the crystallisation onset time. Minor delays were observed when the additive was either an inert or a hydrophobic material, but a single crystallisation response was still measured. The presence of a hydrophilic additive resulted in a much longer delay time before crystallisation, due to its higher capacity for water sorption. On varying the weight of amorphous lactose in the top and bottom layers it was possible to conclude that water vapour is absorbed into upper layers of the sample and is then transferred away yielding a concentration gradient through the entire sample. As the water content gradually increases in the lower layers, the rate of water absorbed can become more rapid than the rate of water transfer away from the

surface. After this time the surface saturates and starts to crystallise thus liberating an excess of water vapour which is sufficient to cause the lower layers to become saturated and also crystallise.

Gravimetric experiments using the Dynamic Vapour Sorption (DVS) technique provided further information regarding the critical %RH around which the amorphous to crystalline transformation occurs. It also provided an indication of the water uptake prior to crystallisation and the residual water content of the crystallised sample. The critical %RH was identified to be in the region of 50%RH and a water uptake in the region of 9% was necessary before spontaneous crystallisation occurred. The constant supply of water vapour in the DVS system resulted in the amorphous lactose reaching saturation and crystallising faster than in the microcalorimetry ampoule experiments using saturated salt solutions, where the supply of water vapour to the sample was governed by the rate of evaporation of the saturated salt solution.

Physical characterisation of the crystallised lactose using X-ray diffraction data confirmed the microcalorimetry response was indeed crystallisation of the spray dried lactose. A typical halo type response was observed for the amorphous sample prior to the microcalorimetry studies, while a series of sharp peaks, characteristic of crystalline lactose obtained for the sample after the sharp microcalorimetry peak. DSC indicated the presence of α lactose monohydrate and a large melting endotherm for the β - form also. TGA results indicated that only 3% hydrate was present, hence the spray dried lactose did not form a complete hydrate (5% for lactose monohydrate) on crystallising. The studies carried out in Chapter 4 investigating small amounts of disorder in predominantly crystalline samples revealed both the microcalorimetric technique and the DVS system to be able to detect down below 0.5% disorder. Physical mixes of amorphous and crystalline lactose were used. A lower detection limit of 0.3% amorphous content was obtained for the microcalorimetric technique and %amorphous content was calculated using the heat response for the 100% amorphous standard. A lower detection limit of 0.1% was obtained for the DVS technique, however no definitive method of quantifying the % amorphous content was established. One method of determining % amorphous content which was considered, was to record the weight gain after crystallisation due to monohydrate formation. However, this is not deemed an accurate method, especially in the case of samples containing a large

amorphous content, since it would only be applicable if a complete hydrate were formed and as already mentioned the spray dried lactose did not form a complete hydrate on crystallising.

One concern in this study was the use of the 'two-state' model i.e. the use of discrete amorphous and crystalline particles which probably doesn't reflect a situation where a material has been rendered partially amorphous through comminution where the amorphous character will probably only be present at the surfaces of particles. Microcalorimetry and DVS experiments were therefore performed also, using two partially amorphous spray dried lactose materials which were specified to contain in the region of 15% amorphous content. These techniques were readily able to detect the amorphous character of these two materials, with microcalorimetry probably being more useful in the actual quantification since a sharp crystallisation peak was obtained and the calculated amorphous content agreed quite well with specified value.

The detection and quantification of unknown amounts of disorder in processed materials is definitely an area for more investigation along with the need for an accurate and reliable method of quantification. The use of standard materials as 100% amorphous or crystalline may not be possible for some materials.

In Chapter 5 the process of water sorption and the mobility of water in the amorphous lactose at the point where T_g approaches T were investigated. Water desorption from the amorphous lactose at water contents where T_g was above T was rapid and controlled by the external %RH, while desorption at water contents where T_g was close to or below T was seen to follow a slow diffusion pattern. This was explained on the basis of the amorphous material undergoing structural collapse as T_g fell below T but prior to crystallisation occurring. Collapse is an irreversible loss of porosity which is a consequence of the decreased viscosity of the sample at high water contents where it can no longer support its own weight under gravity. The water content necessary to cause collapse was found to be approximately 8%. Samples containing different degrees of collapse were prepared by exposing the amorphous lactose to 50%RH for varying periods of time. Microcalorimetry experiments indicated that the collapsed material was still amorphous and the same heat change was measured for the crystallisation process as for the uncollapsed samples.

The behaviour of the collapsed material was such that it crystallised on heating in the DSC at a much lower temperature (70°C) than that observed for the uncollapsed material (180°C). The collapsed material also crystallised on heating in the TGA where 3 distinct regions of weight loss were observed, one of which was loss of hydrate water (150°C), and the other two at approximately 80°C and 110°C were suspected to be related to crystallisation of the collapsed structure. Samples of the amorphous spray dried lactose were also compacted at a range of pressures ranging from 0.5 to 3.5 kNm⁻². These samples retained their amorphous nature after compaction but again revealed a crystallisation exotherm on heating in the DSC which occurred between 115°C and 130°C. It is unclear as to the exact reason for this much reduced crystallisation temperature which is suspected to be a combination of the effects of compaction, namely the introduction of localised heat and the effects of pressure on the amorphous material. This also is another area where further work could be carried out to investigate the exact nature of the changes in the amorphous lactose on compaction and whether the material undergoes structural collapse. The use of the microcalorimetry technique in observing the collapse of the amorphous lactose could be further investigated in this study.

Experiments investigating the effects of temperature on the crystallisation of the spray dried lactose at a range of relative humidities showed how crystallisation occurs much sooner at elevated temperatures and a much lower water content is necessary to bring it about. The microcalorimetry measured heat of crystallisation for amorphous lactose generally increased with increasing temperature at each relative humidity. The microcalorimetry responses also increased on lowering the %RH at the higher temperatures, but this was not the case for the crystallisation response at 25°C where the measured response was 48.0 J/g for all relative humidities investigated. These variations in the measured response were assumed to be due to the sample crystallising with a lower water content at the higher temperatures. These studies, along with DSC investigations, provided further evidence that the microcalorimetry crystallisation process in the combination of a number of processes including evaporation of the salt solution, water sorption by the amorphous lactose, wetting, collapse and crystallisation of amorphous sample which is then followed by desorption of water and condensation

back into the salt solution. Consequently the measured heat response is the net overall result of a mixture of endothermic and exothermic processes.

Crystallisation of the spray dried lactose yielded a mixture of α - lactose monohydrate, β - lactose and it is possible that some of the anhydrous α - form was also present. The proportion of these different forms appeared to change depending on how the sample was treated prior to crystallisation and on the crystallisation conditions. This was shown by the varying proportions of the α - and β - melting endotherms obtained after crystallisation during heating in the DSC. Further investigations into determining the crystallisation conditions which determine the production of a particular form, and how the water content and pre-treatment of the amorphous material prior to crystallisation affect the product would be of interest to this study.

Investigations into uptake and transport of water in larger samples (20g – 100g), of two commercial spray dried lactoses, revealed differences between the behaviour of these materials and that of smaller samples (100mg) on exposure to water vapour. It was observed for these samples that the crystallisation process occurred gradually, with the top of the sample beginning to crystallise initially and eventually all the sample crystallised as it was exposed to 75%RH. It appeared therefore that water was taken up at the surface and once a critical water uptake had been reached crystallisation began. It is probable that a pulse of high water content was then present which moved down the sample bed as desorbed water was released from the top layers after crystallisation. The amount of water necessary to initiate crystallisation in these bulk samples was much lower than expected, again indicating that the water was not evenly distributed throughout the powder bed before crystallisation commenced. Another surprising observation from these studies was the net negative weight changes after the samples had crystallised. This was thought to be due to differences in the amount of hydrate being formed in the larger samples. Further studies to investigate these unexpected weight changes and the exact amounts of α - lactose monohydrate and the β - form in these bulk samples also needs to be carried out.

FINAL COMMENTS

Crystallisation of the amorphous spray dried lactose was induced during DVS and microcalorimetry experiments due to exposure of the sample to humid air. However, the rate of supply of water vapour and the experimental set-up for these two techniques was quite different. In the DVS experiment, a constant flow of humid air is maintained which ensures that as the amorphous sample absorbs water vapour from the surrounding environment, this is immediately replaced with more humid air. This results in a rapid and constant uptake of water vapour by the sample. The microcalorimetry experimental set-up however differs from the DVS in that the supply of water vapour to the amorphous sample depends on the rate of evaporation of the saturated salt solution. As water vapour is taken up by the sample, more of the salt solution evaporates and the process continues in this manner until the top layers of the sample become saturated and crystallisation begins. It is obvious therefore that the rate of supply of water vapour in the sealed glass ampoule of the microcalorimeter is much slower than in the DVS system.

The rate of crystallisation of the amorphous lactose in the microcalorimeter depends therefore on the supply of water vapour to the sample. Increasing the sample weight and decreasing the relative humidity both resulted in an increased delay time before crystallisation commenced. If the surface area of the saturated salt solution were increased, the delay time would also be expected to change, since an increase in the surface area of the salt solution would result in a more rapid supply of water vapour to the sample.

The microcalorimetry heat output of 48.0J/g for the crystallisation of the amorphous lactose, was the output for crystallisation under a specific set of experimental conditions. The experimental temperature was 25°C and the saturated salt solution in the Durham tube had a liquid surface area of $19.6 \times 10^{-6} \text{ m}^2$. The crystallisation heat outputs increased as the temperature was increased due to the sample crystallising with a lower water load.

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