THE UNIVERSITY OF HULL

The Co-Crystallisation of Sugars by the Supersaturation Process

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by

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Abstract

Co-crystallising sugars by the supersaturation process was investigated using sucrose and lactose as the matrix sugars. The components to be co-crystallised with either sucrose or lactose were a variety of mono- and di-saccharides along with sweeteners such as saccharin.

Analysis of the materials yielded from the supersaturation process was done primarily by differential scanning Calorimetry (DSC) and powder x-ray diffraction (PXRD). DSC analysis allowed information to be obtained on the thermal behaviour of the various co-crystalline materials, whilst PXRD permitted information on the structural aspects of the materials to be gained. These two forms of analysis were complimentary to each other, each revealed unique characteristics of the co-crystalline materials.

To unambiguously differentiate the difference between a material that is co-crystalline and one that is not, physical blends of the sugars to be co-crystallised were analysed by DSC and PXRD. This approach allowed for identification of all the components in the mixture, more importantly, this identification was achievable at all levels of the second component in the mixture.

Co-crystallising either sucrose or lactose, with various sugars, yielded solely co-crystalline material up to a certain level of the added sugar. Once this level has been reached, two distinct phases appear in both DSC and PXRD analysis. A co-crystallised and a phase relating to the added sugar can be observed. The formation of a potentially co-crystalline material appears to result from a direct inclusion of the added sugar into the matrix sugar. DSC analysis of the co-crystallised material revealed thermal behaviour that is suggestive of a doping of the matrix sugar by the added component. PXRD analysis did provide some data to further this argument, axis elongation for co-crystallised material is suggestive of a sopping of the main phase. However, determination of the unit cell volumes did not yield conclusive evidence to help prove this hypothesis though. This behaviour in both forms of analysis was generally proportional to the quantity of the second component that has become included into the matrix sugar. The formation of solely co-crystalline materials appears to rely on the structural similarity between the matrix sugar and the component to be included. A higher degree of similarity is reflected by a high level of inclusion of the added

component. Co-crystallisation appears to rely on a degree of intermolecular sugar-sugar recognition.

The inclusion of a second component is not solely down to structural similarities between materials however. It appears that there are kinetic factors potentially involved to. Varying the method of co-crystallisation allowed for higher, or lower, amounts of various second components to be included within a matrix sugar.

The appearance of co-crystalline materials may be due to the inclusion of the second component in an amorphous state. Analysis by Autosorb of various co-crystalline materials has dispelled this idea. All co-crystalline materials behaved in a manner that was indicative of a crystalline material.

During the course of the work with sucrose, it was noted that a unique melting point was observed. From previous work, this unique phase was thought to be a hydrated form of sucrose. Further analysis on this material has allowed for further postulation on its formation and on new methods of its synthesis.

Overview

Co-crystalline materials are becoming of greater interest within the scientific community and within industry. The direct inclusion of a secondary component into a host phase has the potential to enhance the properties of the original material.

Within the pharmaceutical industry, such co-crystals are being studied as a means to enhance the stability of various active pharmaceutical ingredients (API's). Furthermore, co-crystallising an API into a suitable host material (via co-evaporation in a suitable solvent) has been shown to elevate the solubility of previously poorly soluble drugs, e.g. intraconazole. The ability for enhancing the properties of API's via co-crystal formation has not been fully explored yet. It has also been shown that forming a co-crystal with an API and a suitable host material also confers stability to the API. Both of these factors mean that the field of pharmaceutical co-crystals is one worthy of further exploration.

Co-crystal materials are not only isolated in the field of pharmaceutical formulation. Co-crystalline sugars have also been shown to have superior properties compared to single sugars on their own. These co-crystalline sugar materials have been shown to exhibit superior solubility, flowability, compressibility, and possess better anti-caking properties compared to single sugars. At the moment, such sugar co-crystals are thought consist of an agglomeration of sugar crystals with a secondary component distributed through this agglomeration. This incorporation of secondary ingredients into sugar agglomerates has not been limited to solely other sugars. Flavourings are another class of materials that can be included into the resulting sugar agglomerates yielded from co-crystallisation. It has been postulated that any material that exhibits a degree of heat stability is a candidate for inclusion into a co-crystalline sugar material.

There is not a complete understanding of sugar co-crystal formation. The purpose of this study is to try to obtain an understanding of how these materials form, and what factors influence their formation. It is thought that co-crystals form *via* hydrogen bonding occurring between suitable functional groups. This notion is a distinct possibility in sugar co-crystallisation, however, this has not been proven as of yet. If sugar co-crystals can be shown to form between structurally different materials, then co-crystal formation may result

from recognition and bonding between the host and the introduced material, rather than entrapment in an agglomerate of the host sugar. An aim of this study is to gain insight into this possibility, and if the above idea can be shown, then the possibility of rationally designing co-crystal sugars *via* candidate selection based on structure is a prospect. The key to utilising a process is to understand it, and through this, further its applications.

As such, this thesis aims to provide a basis of understanding of co-crystallising sugars. The proposed method for achieving this is to introduce various mono- and di-saccharides into supersaturated sugar solutions, followed by their crystallisation. These materials will be analysed by differential scanning Calorimetry (DSC) and powder X-ray diffraction (PXRD) in order to reveal their physical characteristics.

Contents

1. Introduction

Within this chapter, an overview of the theories of sugar crystallisation from solution is to be outlined along with the nuances that affect this process. Furthermore, an understanding of these concepts will be useful in the context of this thesis. Gaining insight into results obtained from experimental work will be facilitated *via* knowledge of the behaviour of sugar crystallisation from solution.

1.1. Sugar Crystallisation in Food and Pharmaceutical Products

Sugar is a generic word that encapsulates a wide range of carbohydrates; these molecules vary from mono-, di- or oligosaccharides. Sugar is commonly associated with the confectionary industry but sugars play a vital role in many biological processes within nature. The most common sugar, sucrose, is the most widely used saccharide. However, there are many other sugars that are commercially important such as glucose, fructose, lactose and maltose to name but a few. Sugar, in its many guises, can be deployed within foodstuffs in a variety of roles. Typically and the best known, is the role it plays as a sweetener. Sucrose and glucose are two of the most commonly used sugars that fulfil this role. Examples of their use range from chocolate and mints right through to soft drinks, the physical properties of sugar in the product are also made use of. Chewing gum requires that the product does not have a gritty mouth feel. Consequently, the sucrose used in the manufacturing process has to be of a very small crystal size, typically these crystals are in the range of 20-40μm in size.[1] Indeed, the initial crystal size of sucrose has a direct impact on what process it can be used within the confectionary industry.[1] Toffee and fudges can be manufactured from all grades of sucrose crystals, whilst the manufacture of liquorice demands a certain size of sucrose crystals for its formation.[1]

It is not only the initial size of sugar crystals that can influence the final manufactured product. The vast majority of sugars containing products are manufactured from a liquid state. The crystallisation of the sugars within these products from the liquid state to the solid state has a direct impact on the variety and quality of the final product.[2]

As sugar is such a versatile material, it also finds itself being used within the pharmaceutical industry. Bowe has written about the potential and current usage, of sugar as excipients for active pharmaceutical ingredients (API's).[3] Utilisation of sugars within this role is possible due to their taste properties, and crucially, their ability to be formed into a suitable physical form *via* controlled crystallisation from solution.

As such, an understanding of the principles involved in sugar crystallisation and the solid state of crystals is vital to the formulation of a desired product.

1.2. Crystallisation Theory

1.2.1. Crystal Phase Equilibrium

The crystal form of a material can be obtained *via* its crystallisation from a solvent. For this to occur, a driving force for the formation of the solid phase from solution must exist. This driving force occurs due to a difference in the chemical potential between phases; such a change is usually instigated by an alteration in the physical conditions of the system, e.g. pressure or temperature. For a single component system, the Clausius-Clapeyron equation (1.1) can illustrate how external factors affect the relationship between phase transitions of a material.[4]

$$\frac{\Delta P}{\Delta T} = \frac{\Delta H}{T\Delta V} \qquad (1.1)$$

Where P is equal to pressure, T equates to temperature, ΔV is the change in volume between physical states and ΔH is equal to the enthalpy change occurring when a material moves between states, e.g. solid to liquid or vice versa.

For a single component system, a phase diagram can be plotted using this equation and the values of P and T as the axis. A typical plot can be seen in figure one.

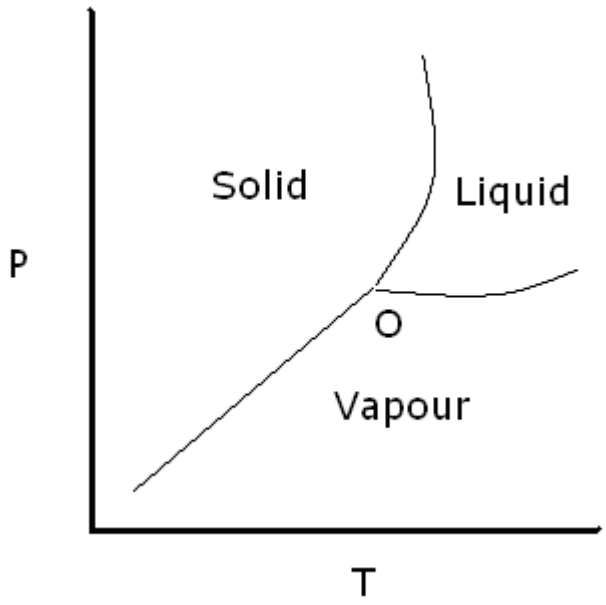

Figure 1. Simplified Plot for a Single Component System Using the Clausius-Calpeyron Equation

Equilibrium can exist between two physical states, the conditions under which this occurs are illustrated by the lines on the plot in figure 1. At point *O* in this figure, all three phases are in equilibrium and this point is known as the triple point.

For equilibrium between two phases to be satisfied, the chemical potential values, μ, must be equal. For these values to be equal the below equation needs to be satisfied (1.2).[5]

$$\mu_a(P,T) = \mu_b(P,T) \quad (1.2)$$

Where *P* equals the pressure and *T* relate to the temperatures of each phase respectively. When two phases are in equilibrium, the values for temperature and pressure are identical. Consideration of equation (1.2) show that the equilibrium can be swiftly removed by altering pressure or temperature, this will yield a difference in the values of μ for each phase. Applying this idea to the plot in figure one, such a loss of equilibrium between phases will result in deviation from the lines of physical equilibria. As such the formation of one phase over the other will occur. This difference in the values of chemical potential between phases is the driving force for a phase transitions to occur. Any

difference in the chemical potential between a mother phase and a new phase is termed a *supersaturation*.[6]

1.2.2. Supersaturation

In sugar solutions, the term supersaturation can be used to refer to a solution that contains more sugar molecules than is thermodynamically allowed. Forming a supersaturated sugar solution can be achieved by a variety of methods as outlined in figure two.

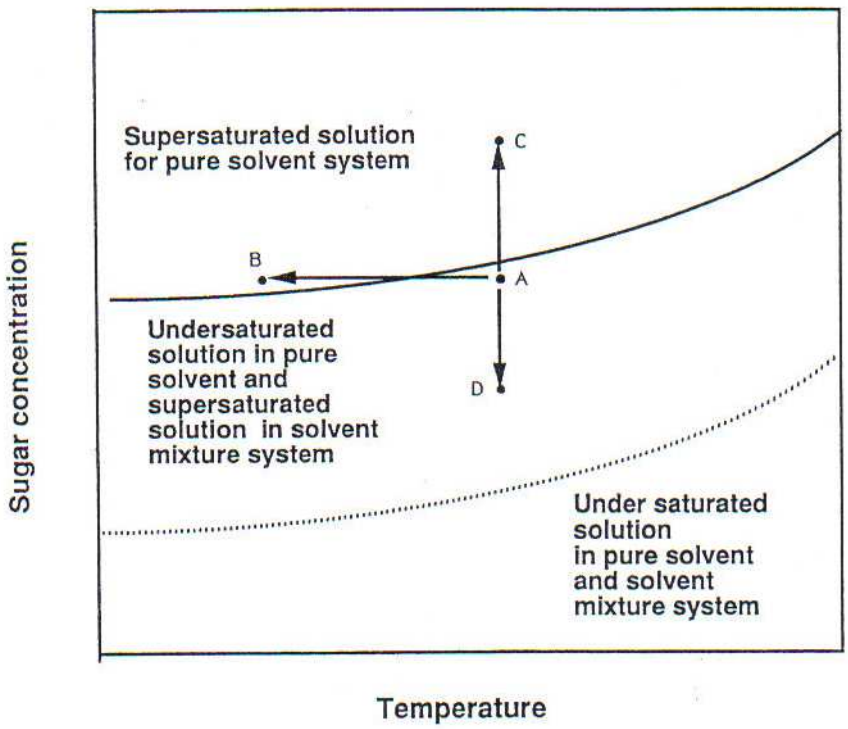

Fig 2. Supersaturation of Sugar Solutions by Evaporation, Cooling and Solvent Addition[7]

In figure two, the line A-B represents the effects of the cooling of an under-saturated solution, cooling results in the total amount of sugar a solution can hold decreasing as sugar solubility depends on temperature. As the solution temperature drops, the sugar concentration increases and thus, drives the concentration towards supersaturation. Removing water from a sugar solution, as represented by the line A-C in figure two; like the previous example this yields an increase in sugar concentration. Adding a second solvent, in which the sugar is insoluble, to the solution causes the mixture to have a lower solubility for the sugar; this is represented by the line A-D in figure two. As all sugars have

varying solubility in water and other solvents, the conditions necessary for forming supersaturated solutions for each sugar will differ. A supersaturated sucrose solution will form under different conditions to those required to form a lactose supersaturated solution.

Forming a supersaturated solution results in a difference in chemical potential ($\Delta\mu$) arising. The equilibrium as defined in equation (1.2.) is removed and a driving force for a phase transition exists. For a supersaturated solution, the value of $\Delta\mu$ for crystallisation from a supersaturated solution is defined in equation 1.3.[5]

$$\Delta\mu = kT \ln \frac{C}{C_o} \quad (1.3.)$$

In sugar solutions, the value of $\Delta\mu$ can be calculated accurately using values for sugar activity. However, whilst this method is accurate, little data exists for these values. Consequently, a simplified measure of supersaturation has been developed and is defined in equation 1.4.

$$S = \frac{C}{C_s} \quad (1.4)$$

Where C is the concentration of the sugar in solution and C_s is the concentration of the saturated sugar solution at the same temperature. The value of S has an inherent effect on the crystallisation of sugar solids from a supersaturated sugar solution. Depending on the sugar, the values of S will determine if, at a given temperature, a solution will crystallise from the addition of a seed crystal, spontaneously, or not at all. The values of S at which these occur can be illustrated graphically using a supersolubility diagram. A simplified version of such a diagram is shown in figure three.

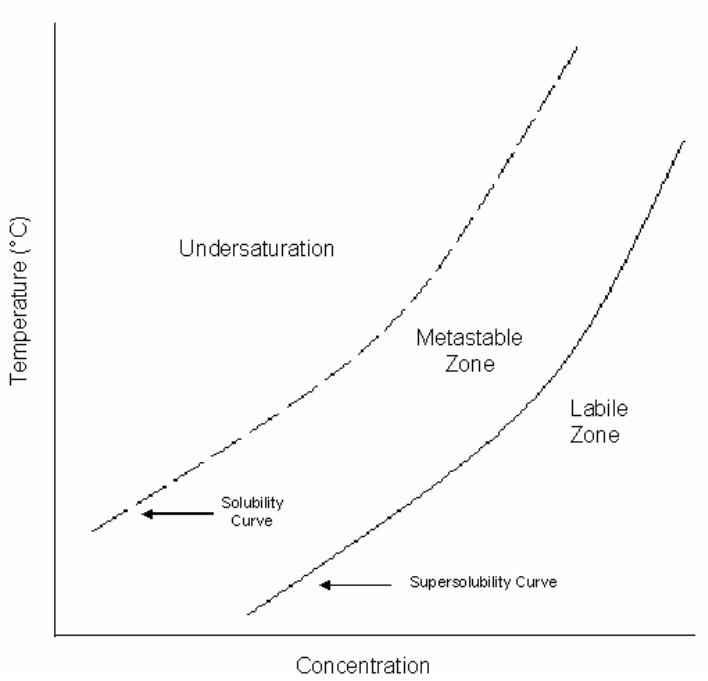

Figure 3. Simplified Supersolubility Diagram for a Sugar Solution

In the above diagram, the area on the graph labelled 'undersaturation' represents the solution conditions under which no nucleation or crystal growth will occur. Solutions residing in the labile region will undergo spontaneous crystallisation whilst any solutions represented by the metastable region are stable to nucleation and subsequent crystallisation.[8]

For a metastable solution to undergo crystallisation, the driving force present within a supersaturated solution needs to be sufficient to surmount the energy barrier defining the metastable condition. Achievement of this criterion relieves the metastable condition. The system then passes on to a state of lower energy, and a more thermodynamically stable state is yielded. This process can be more easily understood schematically, figure four outlines this process.

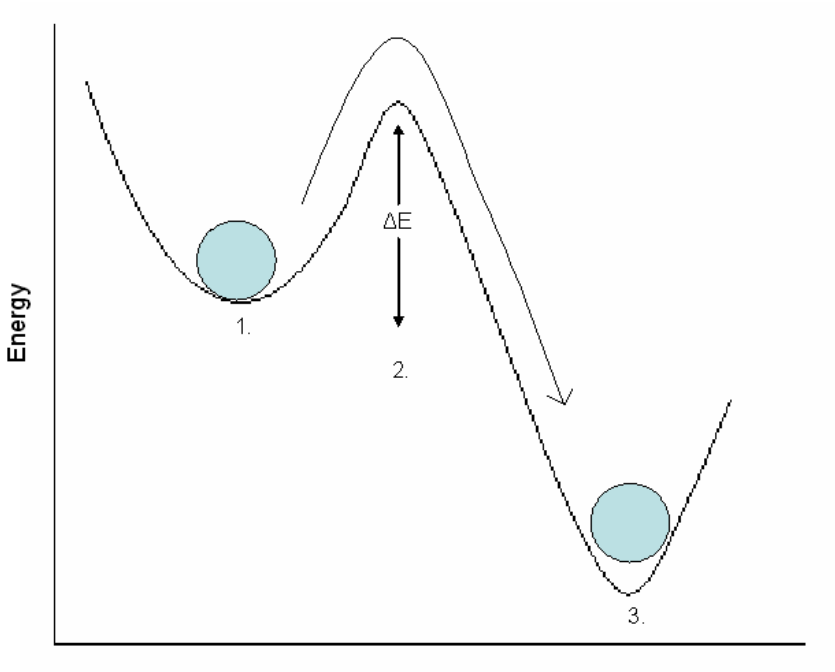

Figure 4. Simplistic Schematic Showing the Transition from a metastable state (1) via surmounting the Energy Barrier defining the state (2) and the transition to a state of lower energy (3)

The energy required to overcome the barriers to the metastable state vary and is dependent on the material. A metastable state does not change with time; ergo it is in equilibrium. Any changes pertaining to the system are related to transitions induced by external factors. Transformations from the metastable state may be induced by changes in temperature, pressure occurring *via* irradiation or milling for example.[9, 10]

Crystallisation from the metastable state proceeds according to the rules set out by the Oswald in his Law of Stages. In essence, this law states that crystallisation proceeds via the formation of thermodynamically unstable phases initially. This is followed by re-crystallisation to a thermodynamically more stable phase.[11] Streuer describes such transformations of various alloys from their amorphous states as occurring *via* a two step procedure during annealing. This occurs by a low temperature nucleation yielding a metastable quasicrystalline state, followed by crystallisation of the crystalline state at high temperature.[9] Further incidences of this phenomenon are well documented in literature. It has been shown that cyclohexanol can move between forms from a metastable state depending on the experimental conditions.[12, 13] Due to this

process, crystalline compounds can exist, as stable forms, in a variety of different forms otherwise known as polymorphs. These forms can be yielded by variations in the crystallisation conditions. A compound such as benzophenone, has been demonstrated to form polymorphs depending on the crystallisation conditions.[12, 13, 14]

Formation of a crystalline material from solution occurs via overcoming any energy barriers to crystal growth. In practical terms, this can be likened to nucleation of a solution followed by crystal growth.

1.2.3. Nucleation

Nucleation of a solution to form a crystalline material is the most common first-order transition.[14] The nucleation of a supersaturated solution is a prerequisite step to the formation of a crystalline product. The growth of a crystal structure cannot occur without the formation of a stable platform from which to proceed. Nucleation can occur in two different forms: primary or secondary with further subdivisions in either category (fig 5).

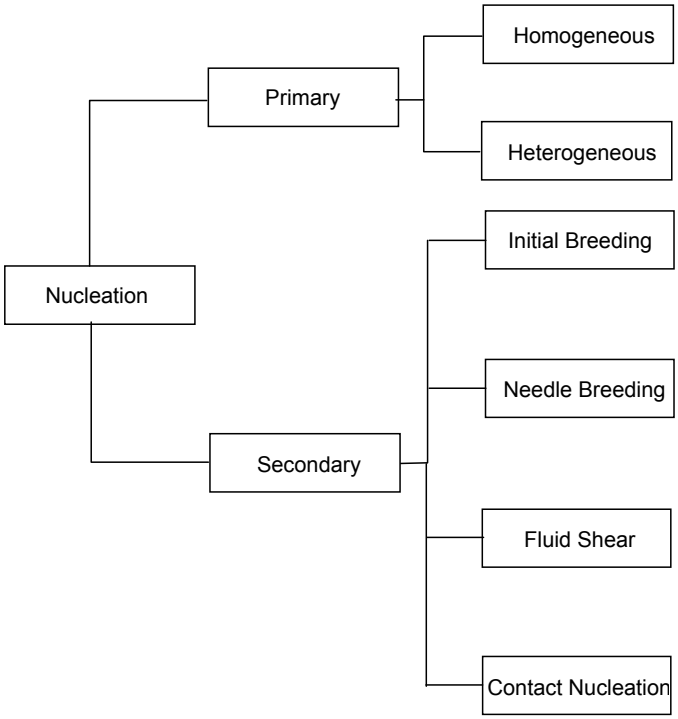

Figure 5. Classification of Nucleation Mechanisms

1.2.3.1. Primary Nucleation

Primary nucleation can occur via two different mechanisms. Homogeneous or heterogeneous nucleation can be the mechanism by which crystals will precipitate from a supersaturated solution.

Homogeneous nucleation, otherwise known as capillary or classical nucleation, can be attributed to the formation of molecular clusters within a supersaturated solution. Fluctuations in the supersaturated solution lead to areas that become 'denser' with respect to the molecules in the solution. As such, these areas may form nuclei that may lead on to growth of the crystal phase. This will only occur when the nuclei formed in these 'denser' areas are of a sufficient size. Formation of nuclei with this critical size will only occur if the equilibrium as in eqn. 1.1 is upset and the chemical potential value of for the new phase (i.e. crystalline) is lower than the chemical potential value (i.e. $\mu_{liquor} > \mu_{crystal}$) of the mother phase.[5] Creation of a stable nucleus requires the system to expend a quantity of energy sufficient enough to relieve the boundaries that are defining the system as a solution. Using figure four as a means of illustration, the quantity of energy necessary is equivalent to moving from point 1 to point 2.

If a supersaturated solution is formed where the equilibrium between the values of μ lies in favour of the new phase (i.e. $\mu_{liquor} < \mu_{crystal}$) then the fluctuations will give areas of greater density. However the clusters will tend to fall apart and not grow. It can be easily conceived that, in sugar solutions, these clusters will be spherical in nature. As such, the critical size for these nuclei can be calculated using equation 1.5.[2]

$$r_c = \frac{2\sigma_s v}{[kT(\ln S)]} \quad (1.5)$$

Where σ_s is the interfacial energy of the droplet, v is the molecular volume, k is the Boltzmans constant, T is the absolute temperature and S is the supersaturation ratio.

Sugar solutions having a supersaturation value of ~ 4.0 will undergo homogeneous nucleation. Supersaturation ratio values over this value are more stable and take longer to crystallise.[15]

Homogeneous nucleation rarely occurs in supersaturated sugar solutions due to the presence of impurities acting as nucleation sites. Such foreign bodies catalyse the formation of nuclei. Consequently, nuclei formation can occur at lower supersaturation levels than is usually required for homogenous nucleation.

As homogeneous nucleation is a rare occurrence in solutions, it can be said that heterogeneous nucleation is the mechanism by which the vast majority of crystallisation from solution occurs.[15] It differs from homogeneous nucleation in the fact that nucleation is promoted by the presence of foreign particles or contact with vessel walls. Such nuclei tend to form at the boundaries between phases. Clusters stable for growth proceed from droplets gathering on the foreign substrates. Droplets are able to gather on foreign substrates due to differences in surface tension between the droplet edge and the substrate on which it has formed. The differences in surface tension between the substrate and droplet promote a characteristic wetting angle (θ) which can be calculated using equation (1.6) which is known as the Young equation.[6]

$$\sigma_s = \sigma_i + \sigma \cos\theta \quad (1.6)$$

Where σ_s is the surface energy of the substrate, σ_i equates to the surface energy of the substrate-droplet interface, σ equals the surface energy of the droplet and θ is the contact angle between the droplet and the substrate. This relationship between the droplet and the substrate is illustrated in the figure below.

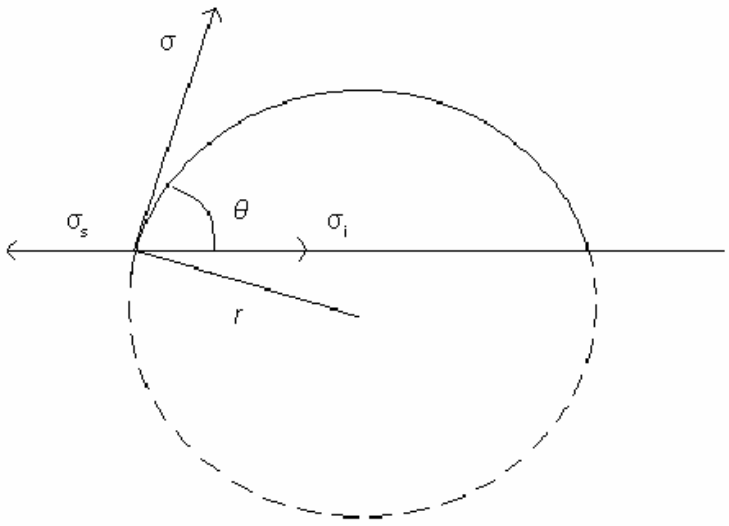

Figure 6. Schematic of a Droplet Forming on a Substrate

The wetting angle, θ, is directly related to the radius of the droplet forming on the substrate. A low wetting angle results in a large radius for the droplet; the higher the angle, the smaller the value of *r*. The radius of the droplet, as in the case for homogeneous nucleation, is directly linked to its propensity to remain as a stable site for continued growth. In heterogeneous nucleation, this value has to be above a critical size for continued growth.[5, 6]

The presence of a foreign substrate, in a supersaturated solution, means that initiation of nucleation heterogeneously demands lower energy than if nucleation were to proceed homogeneously. As such, it can be further understood why crystallisation from solution evolves heterogeneously more readily than homogeneous nucleation.[15]

1.2.3.2. Secondary Nucleation

Simply put; this method of nucleation results from the introduction of seed crystals of the crystalline phase into a supersaturated phase. This type of nucleation can occur at much lower levels of supersaturation compared to primary nucleation. Secondary nucleation can proceed *via* a myriad of different mechanisms.

For both primary and secondary nucleation, once the initial, stable nuclei have been formed, the solution will go on to crystallise. This occurs *via* building on the initial nuclei according to the theories of crystal growth.

1.2.4. Crystal Growth

Once the metastable condition in a supersaturated solution has been relieved, the passage to a lower state of energy can be envisaged as the growth of the final crystal. Early work on crystal growth drew, clearly, a line between nucleation and crystal growth. It was only with Kossels consideration that a relationship between the two was realised.[11] Kossels theory suggests that crystal growth is perpetuated by continued nucleation on the growing crystal. This occurs *via* irregularities on the crystals surface acting as sites for nucleation. The Kossel crystal model outlines several idealised locations for units (sourced from the supersaturated solution) to be incorporated into the growing lattice. Figure seven gives a depiction of a model Kossel crystal with these sites identified.

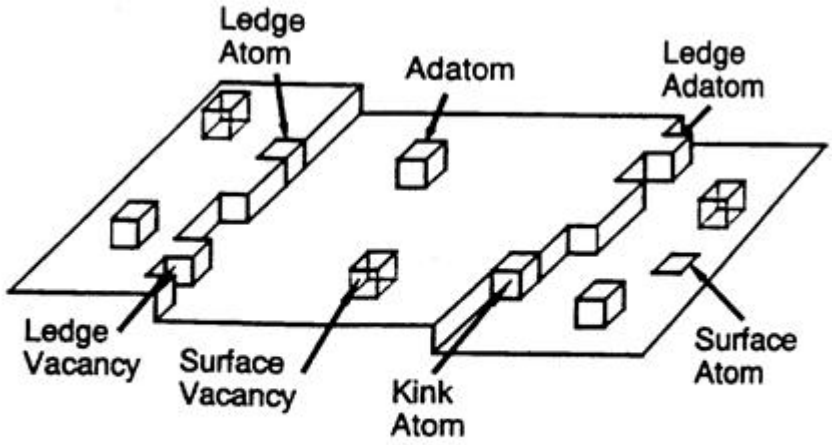

Figure 7. Simplified Diagram of Nucleation Sites on a Kossel Model

The Kossel theory of growth suggests that the most favoured site for addition is the kink site. This position is the most probable for attachment due to its influence on the specific surface energy of the growing crystal. An atom adsorbed in this position has an equal number of saturated and unsaturated bonds. Ergo, either its attachment or removal equates to the same change in surface energy.[11] Consequently the change in free energy remains the same and the adsorbed unit occupies the position of lowest energy. The site is the most energetically favoured position for adsorption and growth. Inclusion of molecules onto this site allows for the step-by-step development of a crystals

structure in a layered manner. This style permits the periodic structure of a crystal to form.[17]

Prior to the inclusion of a molecule into the growing crystal lattice, a growth unit has to arrive at a position where it is capable of becoming included into the growing lattice. This process is governed by mass-transfer (or diffusion) of growth units from solution to the growing crystal face. Mass-transfer is driven by a difference in the bulk solution concentration and the concentration at the crystal surface. The rate of mass-transfer is directly influenced by the rate at which the solution flows over the growing crystal face. In a stagnant solution it is purely reliant on diffusion of molecules from solution to the crystal.[8]

The inclusion of a molecule from solution onto, and into, a growing crystal can be summarised in two steps. Firstly a molecule has to arrive at a growing crystal face, *via* diffusion from solution, and secondarily its incorporation.

Inclusion of a unit onto a growing crystal occurs *via* their adsorption onto an appropriate growth site. The example Kossel crystal (fig 7) gives the most probable locations for these attachments to occur.[11] As already stated, adsorption will occur more readily at sites which are energetically favourable. Such circumstances arise due to attaching growth units being bonded on more than one side.[17] It is worth noting that the rate at which a crystal grows influences the sites at which adsorbing growth units attach at. Faster growth rates results in the favourable sites for inclusion becoming passed over, with attachment occurring at less energetically favoured sites.[18, 19] This in turn leads to vacancies within the crystal lattice. It is worth noting that elevated growth rates also lead to insufficient time for impurities to diffuse away from attachment sites. As such, this leads to their inclusion into the final structure, along with rough crystal surfaces.[18-20] Allowing a crystal to grow in an orderly fashion allows for a regular, repeating crystal lattice to form. This means that the preferred positions for attachment dictate the final form of the crystal. Opposing this, i.e. rapid growth, would result in imperfect crystals to form. Evidence for this can be seen from crystals grown in forced conditions. Such crystals often differ from their preferred equilibrium form. In these crystals, certain faces are overdeveloped, whilst others are retarded in their growth.[18, 20]

An important fact to consider when using the above model for crystal growth is; what happens when a layer is completed and there are no more sites for attachment? The crystal surface can be considered smooth and there are no sites for attachment. The crystal has to generate a suitable site for further additions. This problem was discussed in the Burton, Frank, Cabrera, (BFC) model. In this, a screw dislocation on the crystal surface allows for the constant generation of suitable sites for attachment of incorporable molecules at certain supersaturations.[5, 11] An alternative to this theory is the one of 2-D nucleation on a flat surface. The crystal surface provides sufficient impetus for the organising of molecules from solution. As such, a nucleus is formed on the crystal surface and acts as a site for further additions and subsequent growth.[5, 11]

What can be further inferred from the adsorption of growth units onto, and into, a growing crystal is the stereochemical restraints imposed by the various attachment sites. The nature of these sites is very stereospecific, as in a similar manner to enzyme-substrate interaction. Consequently, the adsorption of molecules occurs more readily if a molecule to be attached parallels the structure of the crystal lattice. From this, it can be inferred that molecules that are similar, but not identical to the host lattice may become incorporated into the growing structure. This is true and will be discussed later in the chapter.[21]

1.2.5. Factors Affecting Crystal Growth

A myriad of external factors can affect the rate of crystal growth from solution. Typically these include factors such as the solution temperature, supersaturation level and cooling rate. Factors such as these influence the rate by having a direct impact on the rate of mass-transfer and nucleation rate.

1.2.5.1. Supersaturation

The supersaturation level in a solution is directly liked to the rate at which both nucleation and crystal growth occur.

As already stated, the driving force for crystallisation is linked to the degree of supersaturation. A higher degree of supersaturation results in a larger driving force for crystallisation to occur. Ergo, higher levels of supersaturation typically induce higher levels of crystalline growth from solution.[22]

The influence of supersaturation level on the rate of nucleation differs depending on the mechanism. Homogeneous nucleation occurs by formation of clusters of sufficient size *via* density fluctuations in solution It can be extrapolated from this that, solutions of higher supersaturation levels contain a greater number of molecules in proximity to one another. As such, it can be inferred that, the higher the level of supersaturation, the greater the rate of nucleation. In solutions with a higher level of supersaturation there will be a higher probability of nuclei forming that will possess the size necessary for continued growth.

With heterogeneous nucleation, the trend of increasing nucleation correlating with increasing levels of supersaturation stands. However this only applies up to a certain point. Once this critical level of supersaturation is reached (and further elevated) the heterogeneous nucleation rate begins to drop off till it reaches zero. This zero point of nucleation rate also applies to homogeneous systems as well. But, rather than tailing off, a homogeneous nucleating system will stop abruptly upon reaching a critical level of supersaturation. The effect of supersaturation on both forms of nucleation can be neatly summarised in figure eight.

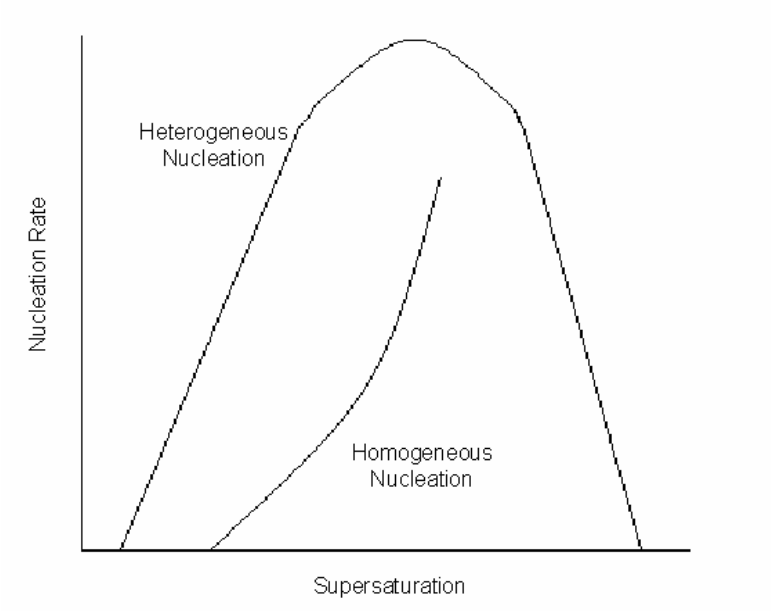

Fig 8. Nucleation Rate *vs*. Supersaturation Level for Homogeneous and Heterogeneous Nucleation

The reaching of a zero point in nucleation in both mechanisms of nucleation is concordant with an inhibition of molecular mobility.

1.2.5.2. Temperature

The influence of temperature on the rate of nucleation and crystal growth centres around its effect on the viscosity of solution. Temperature also has a direct influence on the solubility of the material being crystallised.

As the temperature of a solution is elevated, the viscosity of the solution decreases. As such, the molecules in solution can move more freely. Due to this, the mass-transfer of molecules to the growing crystal surface can occur more readily. Growth will proceed at a faster rate compared to a solution at a lower temperature. In the matter of nucleation, temperature affects the degree of supersaturation in a solution. Varying temperatures produce varying levels of supersaturation. As such, if a solution is allowed to equilibrate at a certain temperature, the solution will nucleate at the rate at which the level of supersaturation dictates as in section 1.2.6.1.

1.2.5.3. Cooling Rate

The formation of a crystalline lattice from solution is a kinetic event. It requires time for molecules to orientate themselves into the growing structure. If a rapid rate of cooling is applied to a supersaturated solution, there will be insufficient time for the organisation of the molecules into a crystal structure. Consequently, a rapid rate of cooling will yield an amorphous product as there is insufficient time for adsorbing and including molecules into a structure. As such, the viscosity of solution will not allow the required molecular mobility for the formation of a crystal structure. It can be stated that a fast rate of cooling has a negative effect on nucleation along with growth rate. The mobility of the molecules in solution is hampered and, consequently, the events necessary to both nucleation and growth cannot occur.

1.2.5.4. Agitation Rate

Agitation rate generally elevates the rate of nucleation in supersaturated solutions. This process helps input energy into the system, thus helping the formation of stable nuclei. In the terms of crystal growth, the rate of agitation

elevates the rate in the case of system that is dependent on the effects of mass-transfer for growth. Agitation increases the flow across the crystal surface, thus increasing the rate of mass-transfer and thereby elevating the rate of deposition on the crystal surface. This heightens the rate of crystal growth. For systems in which the growth rate is determined by the rate of surface incorporation, an elevated level of agitation has no effect on the rate of growth.

1.2.5.5. Impurities

As mentioned in section 1.2.5. the successful addition of growth units to the crystal surface is influenced by stereochemical factors. This relates to the orientation of bonds within the potential site for attachment. A substrate exhibiting similar properties to the crystal structure may become included within the final structure, rather than a unit of the host material.

This class of substrates are those similar in structure to the growing crystal. They are termed 'tailor made' additives as they are able to incorporate irreversibly into the crystal structure. Generally, all other substrates operate via adsorption mechanisms.[21, 23] Upon incorporation or adsorption, further inclusions onto the crystal are inhibited as relevant terrace, ledge or kink sites are blocked. This is turn hinders subsequent adsorptions and inclusions and thus, slows the rate of growth.[24]

As any soluble component may be considered as a potential additive, the studies of the effects of additive addition have been widely investigated. The study of sucrose crystal growth in the presence of raffinose has been studied and has shown that the presence of raffinose reduces the rate of growth.[25-27] Reduction of the growth rate was postulated to occur by disruption of the growth layer by the included raffinose. Raffinose is though to include due to its molecular composition (figure 9).

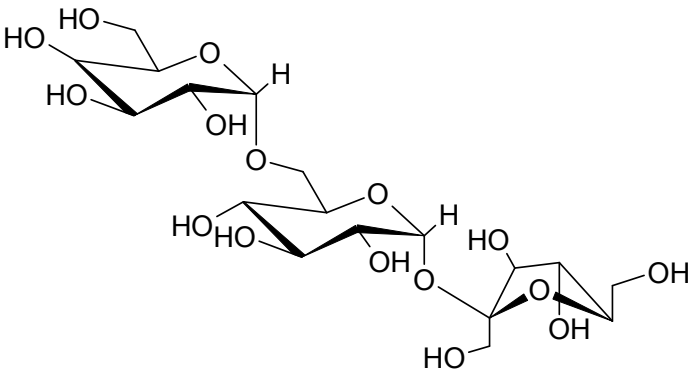

Fig 9. Chemical Structure of Raffinose

Raffinose is a tri-saccharide consisting of galactose joined to a sucrose moiety. It is due to the presence of the sucrose moiety that raffinose includes into a sucrose crystal structure. Adsorption occurs via H-bonding of the sucrose moiety to an appropriate position on the growing sucrose crystal. This leaves the galactose moiety emerging from the surface of the growing crystal. It is this projection that disrupts even addition of further layers, thus slowing the rate of growth. Inclusion of raffinose has a subsequent effect on the final morphology of the final crystal. Raffinose adsorbs on specific faces and as such the growth on those faces is reduced. On the faces where no adsorption occurs, growth is normal, this phenomenon is not limited to solely raffinose (fig 10).[28]

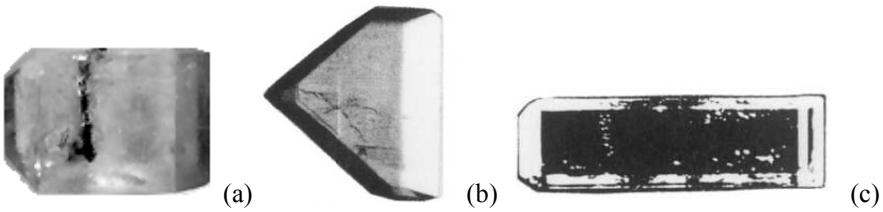

Fig 10. Morphology of sucrose crystals grown from (a) pure solution, (b) in the presence of *neo*-ketose and (c) in the presence of raffinose[28]

The alteration of crystal morphologies is not solely the domain of organic compounds. Inorganic materials are also susceptible to this form of habit modification. The crystallisation of gibbsite ($Al(OH)_3$) in the presence of various polycarboxylic acids highlighted differences in gibbsite morphology. These differences were dependent on the variety of polycarboxylic acid added to the growth solution. The work by Seyssiecq *et al.* hypothesised that the additives

form a molecular complex with the gibbsite growth unit, thus yielding a different structure.[29] The addition of any additive to a growth solution inevitably leads to an alteration in the crystal habit crystals grown from the solution.[30-32]

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2. Sugars

Within this chapter, an overview of the sugars to be studied as the components for co-crystallisation is the intention. Particular emphasis will be placed on sucrose and lactose. These two di-saccharides will be predominantly used as the matrix to which other components will be included. An insight into their solubility and crystallisation behaviour is necessary so as to allow an understanding of any results obtained.

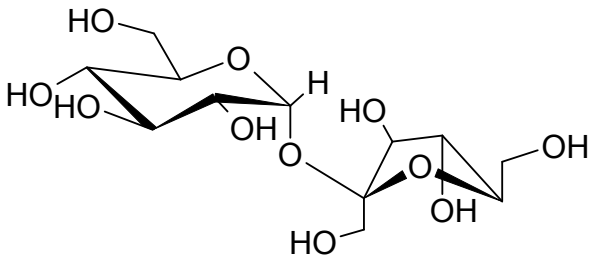
2.1. Sucrose

2.1.1. Introduction

Sucrose is a widely used sugar with applications ranging through a wide variety of foodstuffs and drinks. It is also one of the most abundant pure organic chemicals available. Naturally, sucrose is found in the sugar-cane plant that resides in tropical areas of the world. Its formation is the major product of photosynthesis and the product is stored as a foodstuff for the plant.

The extraction of sugar from sugar-cane, or in milder climates, sugar-beet; occurs *via* pressing to remove the sugar syrup. Sugar removal occurs by boiling the juice till crystallisation occurs then centrifuging to separate the remaining syrup and crystals. Finally before retail sale, sugar is transported to a sugar refinery where the raw crystals are washed and filtered to yield the final product. Extraction from beet sugar occurs by sluicing the raw sugar from sliced beets using hot water rather than pressing. This is followed by filtration and drying steps to yield the final product.

The chemical formula of sucrose is $C_{12}H_{22}O_{11}$, it has a molecular weight of 342.30g mol^{-1} and its nomenclature reads as; α-D-glucopyranosyl-β-D-fructofuranoside. As can be inferred from the nomenclature it consists of a glucose and fructose moieties. The structure can be seen in figure one.



Glucose Fructose

Fig 1. Chemical Structure of Sucrose Molecule

The two monosaccharides are linked by the glycosidic bond linking the C1 carbon of glucose with the C2 carbon of fructose. As both the anomeric carbons are used to link the monosaccharides, sucrose is a non-reducing sugar.

Sucrose can be hydrolysed, this breaks the glycosidic bond and yields a mixture of glucose and fructose. This is commonly known as invert sugar and is the main ingredient of honey. The label of invert sugar derives from the solutions ability to change the specific rotation of polarised light from positive whilst the solution consists of sucrose, to negative upon its hydrolysis. Another common foodstuff, caramel, may also be formed by heating of crystalline sucrose to beyond its melting point. Crystalline sucrose decomposes into a variety of decomposition products and this water soluble mixture is known as caramel.

2.1.2 Sucrose Crystallinity

Sucrose is a naturally crystalline material and has been extensively studied. The crystals belong to the $P2_1$ monoclinic crystallographic system, the unit cell has a Z number of two and exhibits the following dimensions:[1]

$$a = 10.8631(9)\ \text{Å},\ b = 8.7044(6)\ \text{Å},\ c = 7.7624(7)\ \text{Å};\ \alpha = \gamma = 90°,$$
$$\beta = 102.938(7)°;$$
$$U = 715.3\ \text{Å}^3$$

Within the unit cell, the sucrose molecule interacts with others surrounding *via* hydrogen bonding between hydroxyl groups. In the sucrose asymmetric unit there are seven hydrogen bonds, two are intramolecular whilst the remaining five are intermolecular.[2] Within the crystal structure of sucrose, the

two intramolecular bonds exist between the C6'-OH to the C5-O and the C1'-OH to the C2-O as shown in figure two.[3]

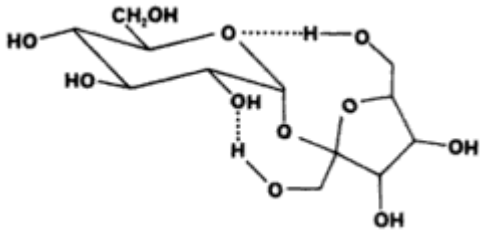


Fig 2. Position of Intramolecular Bonds in the Sucrose Molecule[3]

Sucrose crystals have been reported to melt at varying temperatures, though it is generally accepted to be 186°C.[4] However, a range of melting points for sucrose has been documented by Hurreta *et al.*.[5] Furthermore in this paper, the effect of the heating rate was also shown to influence the temperature at which sucrose melts.[5] Additionally, it has been shown that sucrose can exhibit more than one melting point. A melting transition at ca. 150°C has been reported that has been attributed to the presence of a hydrated form of sucrose.[6]

2.1.3. Sucrose Crystal Growth

The growth of sucrose crystals from solution has been extensively studied. A thorough understanding is critical when operating under industrial conditions for sugar manufacture.

Growth of sucrose crystals from supersaturated solutions can be thought of as a two-step procedure. Typically this is thought to be mass-transfer of growth units from solution to the growing face and a surface reaction step. The limiting step between these two is dependent on the crystallisation conditions. It has been shown that mass-transfer is the determining factor for growth rate in solutions that are stirred at <5 cm min^{-1}. Up to this value, the faster the rate of stirring, the greater the rate of diffusion and hence a faster rate of growth occurs.[7] Above this rate of solution velocity, no further elevations in crystal growth were observed. Growth rate in these conditions was shown to be reliant on a surface-reaction step.[7] It has been postulated that a third factor has an influence on growth from solution that interplays with the surface-reaction step. This is thought to be the disassociation of the water of hydration of sucrose. Sucrose, in

solution, is surrounded by seven molecules of water. Prior to the final inclusion of a molecule into a sucrose structure, the sucrose molecule has to be de-solvated.[8] This occurs once a sucrose molecule has diffused from the bulk solution to the crystal surface. Bennema proposed at this stage there are four energy barriers to the final inclusion of a molecule.[9] These consist of diffusion from the bulk to the crystal surface, desolvation, a 'diffusion jump' and final incorporation.[9] Mathlouthi *et al*. hypothesised that the desolvation of sucrose is the rate determining step for growth from solution. Their hypothesis is based on experimental crystallisation thermograms obtained from sucrose crystallisation.[10, 11] The thermograms showed an un-explained endothermic peak. Mathlouthi postulates that this occurs as a function of the necessary desolvation of sucrose prior to its inclusion into the final structure.[10] As this step requires more energy compared to any of the other energy barriers proposed by Bennema, it can be thought of as the rate limiting step for the growth of sucrose from solution.

The presence of impurities within a sucrose solution has an effect on the rate at which sucrose crystals grow. It has been shown that common monosaccharides induce a retarding effect on the rate of sucrose growth from solution.[12] This effect has been examined by Kubota *et al*. and this behaviour has been attributed to changes in the rate-determining process. In the Kubota models a competition between the adsorption of impurities and growth units occurs during crystallisation.[13, 14] Increasing levels of impurities generally have a proportional effect on the rate of growth, this effect disappears at a critical level of supersaturation however.[13]

The effects of supersaturation levels on sucrose crystal growth are concordant with that for crystallisation of many materials from solution. Growth rate generally increases with higher levels of supersaturation. This holds till high levels of supersaturation where growth declines. This can be attributed to a loss in molecular mobility thus hindering the transport of new growth units to the growing crystal face.[4]

2.1.4. Sucrose Solubility

Sucrose is readily soluble in water and displays a relationship with the solution temperature. The solubility of the di-saccharide increases with elevating

solution temperature.[15] Sucrose solubility is of great importance, the levels of dissolved sucrose have a direct effect on the supersaturation ratio. Ergo, the level of supersaturation will then influence the driving force for crystallisation.[16] Sucrose is readily soluble in water, the heightened number of hydroxyl groups on the sucrose molecule allows facile interaction with water. Sucrose is therefore readily hydrated and taken up into solution.[16] Sucrose is also reasonably soluble in other polar solvents and alcohols, it is relatively insoluble in non-polar solvents.[17] The precipitation of sucrose from solution can be induced by the addition of solvent, in which sucrose is insoluble, to a supersaturated sucrose solution.

Like most materials, the solubility of sucrose is diminished by the presence of impurities within solution. Hartel states that impurities that are readily hydrated hinder sucrose solubility, whilst other materials, such as salts, improve the solubility of sucrose.[4]

2.1.5. Amorphous Sucrose

Sucrose can be formed into its amorphous state by various methods such as, spray-drying, freeze-drying from aqueous solutions and dry-milling. This material is of interest due to its high viscosity and the low diffusion co-efficient of materials included within the amorphous material. Due to these properties, the incidence of reactions leading to food degradation are reduced.[18] Sucrose is also been utilised as a cyroprotectant due to its properties in the amorphous state. In this role, sucrose is thought to act as an agent protecting proteins from denaturation.[18, 19] Amorphous sucrose has a glass transition (T_g) temperature reported as -34°C in literature.[20] This value can be influenced by the rate at which sucrose is cooled to the amorphous state and its water content.[20]

Sucrose can be re-crystallised readily from its amorphous state. This temperature (T_c) of re-crystallisation can be influenced by the relative humidity of the environment, along with the presence of additives within amorphous sucrose.[21] This temperature of T_c is also influenced by how the amorphous material is prepared. Mathlouthi *et al.* has shown the difference in re-crystallisation behaviour of amorphous sucrose formed *via* different methods.[22]

2.1.6. Utilisation of Sucrose

Sucrose is one of the most prevalent natural materials used in the food industry; finding uses from a natural sweetener in drinks, chocolate and many other products. Sucrose has a sweetness that is used as the benchmark by which all other sugars are judged. Sucralose (fig 3) a sweetener based on a chlorinated sucrose molecule is judged to be 600 times sweeter than sucrose alone for example.

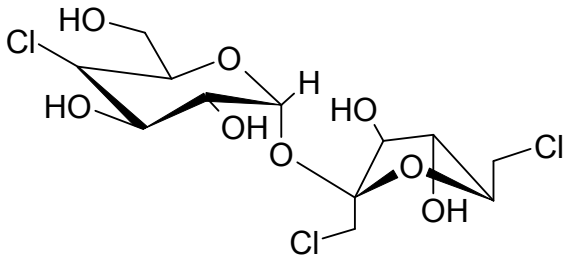


Fig 3. Structure of Sucralose

As sucrose exhibits a pleasant sweet taste, it is widely used within the pharmaceutical industry in formulations; namely as a tableting agent. Sucrose in its natural crystalline state does not compress well. However, dry blending with modified dextrins or with sorbitol has yielded a sucrose material that is more easily compressible compared to solely sucrose. Recently, a new sucrose product, formed from a sucrose and invert sugar blend has become available. This material has been shown to offers superior tabletting properties compared to purely crystalline sucrose.[23] Sucrose is also becoming investigated with respect to its cryoprotectant properties. Either solely sucrose, or combined with other polyols such as sorbitol, have been shown to exhibit cryoprotectant properties.[24]

2.2. Lactose

2.2.1. Introduction

The disaccharide, lactose, is another important ingredient in food and pharmaceutical manufacture. It is the predominant sugar found in milk and is common to all mammalian milk. In humans, the formation of lactose occurs in the mammary glands from the reaction between UDP-galactose and glucose, this reaction is catalysed by galactosyl transferase.[25] A protein, called α-lactalbumin, has to bind to the galactosyl transferase to ensure it is specifically geared to

catalysing the formation of lactose. The levels of this protein in mammary glands is under the control of the hormone prolactin.[25]

Industrially, lactose powder is often produced from whey which is a by product from the milk industry. Lactose is extracted from this raw material by concentration of the whey, crystallisation of the lactose content, and then separation by centrifugation.[26] This leaves the lactose in a raw state from which it can be purified to various grades depending on the final usage of the material.

Lactose has the identical chemical formula to sucrose, $C_{12}H_{22}O_{11}$, and it has a molecular weight of 342.34 g mol^{-1}. The IUPAC nomenclature is β-D-galactopyranosyl-(1-4)-D-glucopyranose. Lactose consists of a galactose and a glucose moiety with the structure of α-lactose monohydrate shown in figure four.

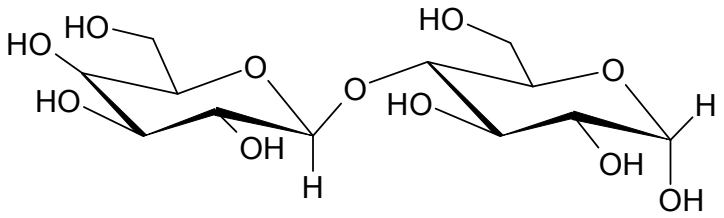


Fig 4. The Chemical Structure of α-D-lactose monhydrate

Unlike sucrose, lactose exists in different crystalline states. It can exhibit both alpha- and beta- configurations as well as exist as a monohydrate or anhydrous material in various ratios. As lactose is classed as a reducing sugar, it can exist in a variety of forms in solution. Typically, these are the cyclic α-form, the cyclic β-form and the open chain structure through which these two forms interconvert.

Due to the open nature of the hemiacetal group on the glucose moiety, lactose is classed a reducing sugar as well. The galactose anomeric carbon is bound *via* the glycosidic 1-4 linkage to glucose. This bond can be hydrolysed to yield the two individual monosaccharides. In digestion, this allows the absorption of the sugars for use as an energy source. This process is mitigated by the enzyme lactates. A lack of this enzyme in humans is the root cause of lactose intolerance. This affliction results in un-cleaved lactose passing into the colon where bacterial action induces lactose fermentation. The discomfort associated with lactose intolerance arises from the build up of these fermentation gases.

2.2.2. Crystalline Lactose

Lactose exists in three different crystalline forms, α-monohydrate, α-anhydrous and the β-anhydrous form. Lactose α-monohydrate exist in the monoclinic, $P2_1$ system with an occupancy of two. The unit cell of this material has been reported to show the following dimensions:[27]

$$a = 7.815\text{Å}, b = 21.567\text{Å}, c = 4.844\text{Å}; \alpha = \gamma = 90°, \beta = 106.2°;$$
$$U = 784.022\ \text{Å}^3$$

The stable α-lactose anhydrous form exists in the triclinic P1 system with an occupancy of two, the unit cell has the following reported dimensions:[28]

$$a = 7.6522(2)\ \text{Å}, b = 19.864(1)\ \text{Å}, c = 4.9877(1)\ \text{Å}; \alpha = 92.028(1)°, \beta = 106.261(1)°, \gamma = 97.153(1)°;$$
$$U = 720.18(3)\ \text{Å}^3$$

The β-anhydrous form of lactose exists in the monoclinic $P2_1$ system and the unit cell has an occupancy of two. Hirotsu and Shimada report the unit cell as follows:[29]

$$a = 10.839(6)\ \text{Å}, b = 13.349(6)\ \text{Å}, c = 4.954(5)\ \text{Å}; \alpha = \gamma = 90.0°, \beta = 91.31(9)°; U = 716.7\ \text{Å}^3$$

The melting of the α-monohydrate form of lactose exhibits two transitions in differential scanning calorimetry (DSC) analysis. Itoh *et al.* reported the dehydration of lactose occurs at 154°C and the melting of the α-form occurring at 221°C.[30] Drapier-Berche *et al*. reported the melting point of the α-anhydrous form at 216°C with the β-anhydrous form melting at 225°C. This paper reports a slightly lower melting point for α-lactose monohydrate of 215°C however.[31]

It is interesting to note that lactose can also take the form of a mixed crystal. Materials have been reported that exhibit varying ratios of the α- and β-forms of lactose.[31, 32] These encompass 1:1, 5:3, 3:2 and 4:1 ratios of α:β. Such

varieties of lactose crystals have been termed molecular crystals of lactose by Drapier-Berche *et al*.[31]

Interchanging between these different forms of lactose is achievable by many methods. The drying of the monohydrate form over P_20_5 at various temperatures, shaking of α-lactose monohydrate with acid or by refluxing with methanol in the presence of potassium methoxide are a few examples of these from literature.[30, 31, 33]

2.2.3. Lactose Crystal Growth

Lactose crystallises slowly from solution. Crystallisation from solutions below 93.5°C yields solely the α-form, whilst the β-form is obtained at higher temperatures. The mechanism of growth for lactose from solution is not controlled by mass-transfer from solution. The rate of growth from solution is not elevated by increasing the flow rate in growth solutions.[34] Lactose crystal growth from solution is greatly affected by the supersaturation level within the growth solution. This suggests that growth of lactose from solution is dependent on surface-incorporation of growth units.[35, 36]

Investigation of nucleation within lactose solutions is not as well documented compared to that of sucrose. An investigation into the mechanism of lactose nucleation by Shi *et al* has shown that nucleation by contact nucleation produces a dispersion of viable nuclei for growth. It was shown that the phenomenon of growth rate dispersion (GRD) occurred.[36] The growth of lactose crystals appears to be reliant on the size of seed crystals present in solution. Further work by Dincer *et al* recently has provided further data to affirm this.[37]

The subsequent growth of lactose is dependent on various factors that include temperature, mutarotation, viscosity, pH and impurities.[38] The rate of crystal growth can also be effected by the presence of sugar-phosphate impurities. Such a presence within growth solutions has been shown to inhibit the adsorption of α-lactose onto growing faces, thus, the crystal growth is retarded and the final crystal shape altered.[39, 40] Indeed, deviation from the expected tomahawk morphology is possible by the inclusion of the β-anomer of lactose into the growing crystal lattice.[41] Interference of crystal growth alters the final morphology. Altering the level of supersaturation can also result in the formation of different lactose morphologies.[42] The growth rate of lactose crystals

is affected by mutarotation at temperatures below 30°C. With increasing temperature however, the rate of mutarotation becomes too great for any observable influence on growth rate.[43] Such elevations of temperature also impart an increase in the growth rate of lactose crystals.[44] The rate of crystal growth can be reduced by the presence of impurities, as is the case of lactose grown in the presence of sucrose. Also the attendance of β-lactose within a solution exhibits an inhibition on the growth of α-lactose crystals.[45, 46]

2.2.4. Lactose Solubility

Though lactose is not an easily dissolved sugar, it still exhibits increasing solubility with elevating solution temperature though.[15] When in solution, lactose is optically active and the degree of optical activity is governed by the ratio of the two forms.[47] The ratio between alpha and beta-lactose in solution lies in favour of the alpha form in roughly the ratio of 2:1 (α:β). This equilibrium is strongly temperature dependent, elevating temperatures move the equilibrium in favour of the alpha form.[47] Mutarotation is not a rate limiting step for lactose crystallisation, though the ratio of the two materials does have an impact on the final morphology of the lactose crystal.[34, 41]

2.2.5. Amorphous Lactose

Amorphous lactose is formed by a rapid quenching of a lactose solution. In this way, lactose molecules are frozen *in situ* and a disordered structure results. Industrially, this is done by spray drying of a lactose solution. The product from this is often used as a direct-compaction powder for pharmaceutical tablets.

2.2.6. Usage of Lactose

Dried milk powders are a common form of lactose, baby milk formed from powder is a major usage of dried crystalline lactose for example. The amorphous form is of excellent potential as a carrier, and absorbent, of flavours. Lactose monohydrate is used widely within the pharmaceutical industry as a tabletting agent, its role as a carrier for drugs is also being explored. Recently, the drug carbamazepine has been shown to have superior dissolution properties in solution when lactose is utilised as the carrier.[48]

2.3. The Potential for Acceptance

2.3.1. Introduction

In this section, potential sugars to be incorporated into a sucrose, or lactose, crystalline matrix are to be outlined. The materials to be investigated will range from monosaccharides and disaccharides to artificial sweeteners. A wide range of materials will be used in order to elucidate their implications on sugar co-crystal formation.

2.3.2. Fructose

Fructose belongs to the ketose family of sugars, it has the chemical formula of $C_6H_{12}O_6$ and consequently is a hexose. The crystals have the IUPAC nomenclature of β-D-fructofuranoside. Like all monosaccharides, it is a reducing sugar and the structure can be seen in figure five.

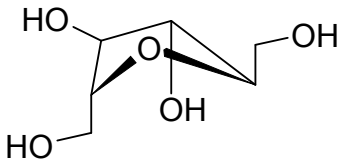


Fig 45 The Structure of β-D-fructofuranose

Fructose crystals belong to the orthorhombic system $P2_1P2_1P2_1$, with an occupancy of four, the unit cell dimensions have been reported as follows:[49]

$$a = 8.088\ \text{Å},\ b = 9.204\text{Å},\ c = 10.034\text{Å};\ \alpha = \beta = \gamma = 90°;\ U = 746.96\text{Å}^3$$

Fructose can exist in two forms, its commonly known five-membered ring, known as β-D-fructofuranose, or as a six-member ring referred to as β-D-fructopyranose. In solution, fructose can exist in its furanosic form along with the pyranosic form. These two forms interconvert *via* the open chain ketose form of the sugar. Furthermore, both the hemiketal and hemiacetal form can exist in either their α- or β-anomers. Fructose crystals have been reported to melt at 112.7°C.[50, 51] The solubility of fructose is also extremely high compared to other sugars. Consequently, fructose can be used as an inhibitor to crystallisation in food products.[52]

Fructose has the added advantage of being suitable for use in diabetic products as it possesses a desirable sweetness. Work by Abraha *et al.* has shown that ingestion of fructose decreases the postprandial glucose response when fructose is the predominant source of carbohydrates.[53] However, this was shown to only be valid when fructose was administered in low levels. [54] Elevated levels of fructose are hypothesised to contribute to the development of obesity and its subsequent problems.[55] Fructose, when replacing sucrose in food, yields a product that is less likely to induce tooth decay.

2.3.3. Galactose

Galactose, an aldose sugar, belongs to the hexose group of sugars. It has the IUPAC nomenclature of D-(+)-galactose (α-D-galactopyranose), the sugar is a C-4 epimer of glucose. Its structure is shown in figure six.

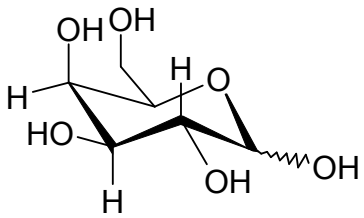


Fig 6. Structure of α-D-galactopyranose

Crystalline galactose belongs to the orthorhombic system with space group $P2_12_12_1$ and the unit cell has a occupancy of four. The dimensions of the unit cell have been reported as follows:[56]

$$a = 15.7806(38)\ \text{Å},\ b = 7.8783(15)\ \text{Å},\ c = 5.9436(20)\ \text{Å};\ \alpha = \beta = \gamma = 90°;$$
$$U = 738.4\ \text{Å}^3$$

The crystals of galactose have been reported to melt in the range of 168-170°C.[57] In solution, galactose can mutarotate between its two anomeric forms, the molecule can exist in either its α- or β- configuration. The β-configuration has been reported to be more stable in solution at 25°C.[58]

Galactosemia is a rare metabolic disorder, the afflicted person cannot metabolise galactose properly. Consequently, toxic levels build up in the blood.

Without treatment, this condition is fatal and even if diagnosed and treated, severe complications can result such as speech disorders and brain damage.

2.3.4. Maltose

Maltose consists of two glucose moieties linked by a 1-4 glycosidic bond, it has the IUPAC nomenclature of (4-*O*-(α-D-glucopyranosyl)-D-glucopyranose) It is a reducing sugar as an anomeric carbon is unused in bonding to another moiety and its structure can be seen in figure seven.

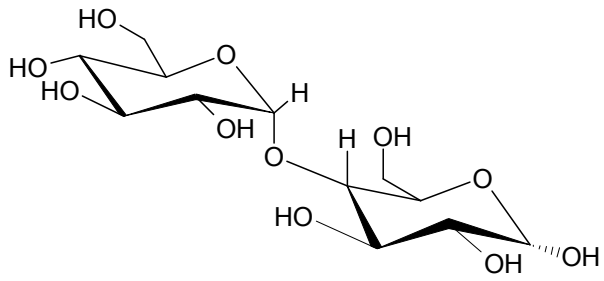


Fig 7. Structure of α-maltose

Crystalline maltose belongs to the orthorhombic, $P2_12_12_1$ system with a occupancy of four. The unit cell dimensions for α-D-maltose monohydrate have been reported as:[59]

$$a = 12.667(3)\ \text{Å},\ b = 13.380(5)\ \text{Å},\ c = 8.400(2)\ \text{Å};\ \alpha = \beta = \gamma = 90°,$$
$$U = 1471.5(7)\ \text{Å}^3$$

The melting point of maltose monohydrate has been reported to be in the range of 119-121°C.[57] Maltose is not widely used in the food industry, it has no real sweetness and it uses are not fully understood. However, maltose does play an important role within the brewing industry. Maltose is the preferred source of sugar for the production of alcohol via the enzymic action of yeast. Maltose is yielded via the breakdown of the grain used (typically barley or wheat) via enzymes (notably α- and β- amylase) present within the starch source.

2.3.5. Glucose

Glucose is the single most important sugar in biological systems; all cells use it as metabolic intermediate and as a primary energy source. It is the main

constituent of starch and cellulose. Glucose has the IUPAC nomenclature D-glucopyranose and it exists in three crystalline forms; α-D-glucose monohydrate, β-D-glucose monohydrate and anhydrous-$_D$-glucose. The structures of these three crystalline forms can be seen in figure eight. Glucose is an aldohexose sugar; consequently, it is also a reducing sugar.

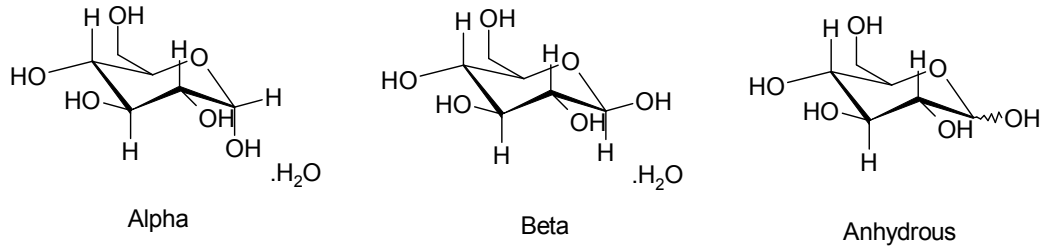


Fig 8. Structure of α-D-Glucose Monohydrate, β-D-Glucose Monohydrate and Anhydrous Glucose

The crystals of the three forms of glucose have been reported to belong to the following crystalline systems and their unit cells have been shown to be as follows.

The crystal structure of α-D-glucose monohydrate belongs to the monoclinic $P2_1$ system, with a Z = 2; and the following dimensions:[60]

a = 8.803 Å, b = 5.085 Å, c = 9.708 Å; α = γ = 90°, β = 97.67°, U = 430.7 Å^3

The unit cell of anhydrous β-D-glucose belongs to the orthorhombic system $P2_12_12_1$ with a Z = 4, and the following dimensions:[61]

a = 9.29 Å, b = 12.65 Å, c = 6.70 Å; α = γ = β = 90°, U = 787 Å^3

The unit cell of anhydrous α-D-glucose belongs to the orthorhombic system $P2_12_12_1$ with a Z = 4, and the following dimensions:[62]

a = 10.36 Å, b = 14.84 Å, c = 4.97 Å; α = γ = β = 90°, U = 764 Å^3

The three forms of glucose have been reported to melt at 146°C (α-D-glucose monohydrate), 150°C (β-D-glucose) and 146°C for anhydrous glucose.[57]

The solubility of glucose is strongly dependent on temperature, and it has a direct impact on which form crystallises out from solution.[15] Allowing a solution to crystallise above 50°C yields a material containing predominantly the anhydrous form of glucose, below this temperature, the α-form dominates.[15]

Glucose, as a reducing sugar, will undergo mutarotation in aqueous solution. Depending on the solution conditions, different ratios of the α- and β-anomers will be present. The pyranose structure of glucose typically dominates within solution. It has been reported that only 1% of D-glucofuranose is found at equilibrium and any crystallisation of either glucose anomer is subsequently followed by mutarotation in order to re-establish the equilibrium.[63] The α-anomer becomes favoured upon elevation of either the solution temperature or the glucose concentration. Indeed, higher temperatures have been shown to increase the rate of mutarotation.[63]

Glucose is widely used in the food industry but attention is being turned to other area for the application of glucose. Poor solubility of active pharmaceutical ingredients (API's) is a problem which dogs the pharmaceutical industry. This problem is usually overcome by stabilising the API with components with desirable properties. Recently a new technique has been developed whereby the quantity of stabilising substances required can be reduced by addition of the API and stabiliser solution to an aqueous glucose solution.[64] Glucose has also been recently utilised to enhance the bioavailability of lipophilic ketones. Glucose behaves as a solubilising agent for the substrate and, as a result, the substrate solubility was increased by fifty times.[65] Orally administered drugs are often formulated with amorphous glucose due to its pleasant taste and relative solubility. Indeed the alleviation of low-blood sugar levels has long been remedied by formulations containing glucose, e.g. Lucozade.

2.3.6. Saccharin

Saccharin is the oldest artificial sweetener, it was first synthesised in 1879 from work undertaken on toluene derivatives. The discovery of the sweetness of saccharin was an accident, both researchers noted the taste on their hands during dinner; consequently, the synthetic route toward saccharin was published.[66] Saccharin exists as a white, crystalline powder that crystallises in a

monoclinic, $P2_1/C$ system with occupancy of four. The unit cell exhibits the following dimensions: [67]

$$a = 9.55 \text{ Å}, b = 6.91 \text{ Å}, c = 11.80 \text{ Å}; \alpha = \gamma = 90°, \beta = 103.9°, Z = 4$$

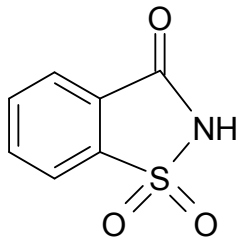


Fig 9. Chemical Structure of Saccharin

Saccharin exists in a several forms and, depending on which, the solubility varies. Saccharin in the acidic form is not very soluble in solution. However, its sodium or calcium salt is extremely soluble in aqueous solution with a value of 0.67g per mL of water.

The sweetening power of saccharin is approximately 300 times that of sucrose. Its utilisation as a foodstuff became widespread during World War One due to sugar shortages. Saccharin is suitable for consumption by diabetics as it is not metabolised within the body. However, due to an unpleasant metallic aftertaste, it is usually used in conjunction with a synergistically suitable sweetener.

Early reports on saccharin consumption in rats showed that with high consumption levels, incidences of bladder cancer were observed.[68] Concurrently, saccharin was banned in both Canada and the US due to concerns of saccharin consumption within humans. This ban was lifted upon further studies showing that saccharin consumption by humans is not related to incidents of bladder cancer. The levels required to induce cancer in humans was unfeasibly large and would require massive consumption of the sweetener. Further to this, a study on primates ingesting only 5-10 times the allowable daily intake of saccharin showed no instances of a carcinogenic effect on the primate urinary tract.[69, 70]

Saccharin is still in use today, blended with cyclamate, another artificial sweetener. The unwanted metallic aftertaste of saccharin is un-noticeable and this blend of the two sweeteners is available as the product ‘Sweet ‘n’ Low’.

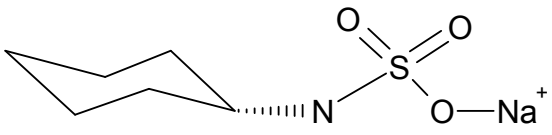


Fig 10. Structure of Sodium Cyclamate

2.3.7. Aspartame

Aspartame is a non-carbohydrate, artificial sweetener with the IUPAC nomenclature, aspartyl-phenylalanine-1-methyl-ester. Its structure can be seen in figure eleven. The sweetener was discovered by accident during the screening for an anti-ulcer drug candidate where its sweetness was noted. Anhydrous aspartame has a molecular weight of 294.3 g mol^{-1} and it crystallises in the monoclinic, $P2_1$ space group. The unit cell exhibits the following dimensions:[71]

$a = 19.1034$ Å, $b = 4.9608$ Å, $c = 15.6565$ Å; $\alpha = \gamma = 90°$, $\beta = 94.875°$;
$V = 1502.14$ $Å^3$

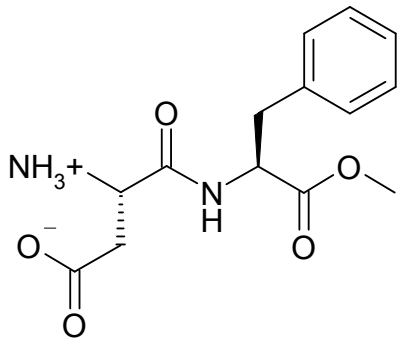


Fig 11. Chemical Structure of Aspartame

Aspartame is suitable for consumption by diabetics and is widely used as a low-calorie sweetener. Aspartame is 200 times sweeter than sucrose and it does possess calorific value. However, due to the low quantity required to provide the desired sweetness, it can be regarded as negligible. The taste of aspartame is not like conventional sugar. The onset of its sweetness is slower and lasts longer which consumers find unappealing. To counteract this, the sweetener is blended with a synergistically suitable counterpart, acesulfame-K. It is in this form that

aspartame is found in a wide variety of products such as drinks, chewing gums and as a table top sweetener.

As aspartame is a peptide derivative, it can become hydrolysed under conditions of elevated temperature or pH. As such it is unsuitable for baked goods and in drinks it is stabilised with saccharin if the pH level is around 7.0. At lower levels of pH, aspartame is relatively stable.

Aspartame has long been the subject of scrutiny with regard to its potential carcinogenic properties. It is thought to possibly be a cause of brain tumours, lymphoma and genotoxic effects. Several studies have been conducted with regard to this concern and it has been shown that consumption of the sweetener is not directly linked to the manifestation of carcinogenic effects.[72, 73] The levels of aspartame needing to be consumed to induce carcinogenic effects are high compared to the recommended levels of aspartame consumption in humans.[74] It is worth noting that research has been published that does show a direct correlation between aspartame consumption and the incidence of unwanted effects.[75] As such, the controversy still surrounds aspartame and its usage.

2.3.8. Acesulfame-K

Acesulfame-K is an artificial sweetener that has no calorific value and is the potassium salt of 6-methyl-1, 2, 3-oxathiazine-4*(3H)*-one-2, 2-dioxide. The structure can be seen in figure twelve.

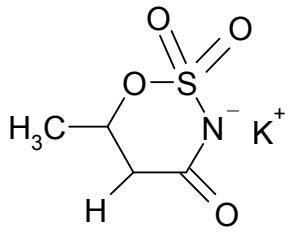


Fig 12. Structure of Acesulfame-K

Like aspartame, acesulfame-K was discovered by accident from a reaction between 2-butyne and fluorosulfonyl isocyanate that yielded a sweet tasting compound.[76] This work was driven by a need to discover new low-calorie sweeteners due to cyclamate and saccharin becoming banned by the American FDA. Acesulfame-K has a sweetening power of 180-200 times that of sucrose,

approximately an equal sweetness to aspartame. It has half the sweetness of saccharin and a quarter that of sucralose. The compound displays a bitter aftertaste like saccharin; this is countered by blending with a suitable synergistic sweetener such as aspartame or sucralose. Acesulfame-K is stable at higher temperatures, under basic or acidic conditions and thus, can be used in baked goods or products requiring a long shelf life.

As with all artificial products consumed within the human diet, acesulfame-K has been met with concerns over its safety, namely the carcinogenic implications. However, there is insufficient data available to prove the presence of carcinogenic properties of acesulfame-K, preliminary studies suggest that there is no significant risk.[73]

2.3.9. Glucosamine

Glucosamine is an amino sugar and is commonly used as a medicinal treatment for the condition osteoarthritis. Glucosamine possesses the chemical formula $C_6H_{13}NO_5$ and has the IUPAC nomenclature, (*3R, 4R, 5S, 6R*) -3-amino-6-(hydroxymethyl)oxane-2, 4, 5-triol. Glucosamine has a molecular mass of 179.17 $gmol^{-1}$ and the chemical structure can be seen in figure thirteen. The amino sugar is not approved by the American FDA for consumption, but it can be given as dietary supplement. This can only be done as long as any possible medicinal properties are not advertised. European law dictates that glucosamine is approved as a medicinal drug and is sold in its sulphate form.

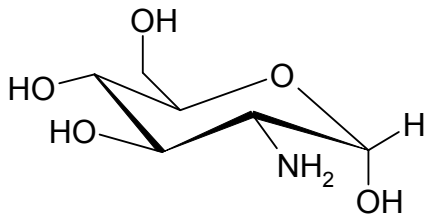


Fig 13. Chemical Structure of Glucosamine

Glucosamine is the precursor for all nitrogen containing sugars in the body. It is utilised in the hexosamine biosynthesis pathway which yields UDP-N-acetylglucoamine. This is utilised as a precursor to form glycosaminoglycans, proteoglycans and glycolipids. The usage of glucosamine in a therapeutic vein stems from glycoaminoglycans forming a significant portion of cartilage tissue.

Administering glucosamine has been postulated to help with rebuilding cartilage as it is the precursor for glycoaminoglycans. However, the usefulness of glucosamine is countered by possible harmful side effects. Glucosamine has been shown to raise insulin resistance in animals. A study has shown in humans that subjects with already underlying insulin sensitivity are at higher risk of raising insulin resistance.[77] Whilst there are many studies commenting on the harmful nature of glucosamine, there is still insufficient data to conclusively prove the harmful or beneficial nature of glucosamine.

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3. Sugar Crystal Modification

3.1. New Roles for Old Materials

Sucrose, one of the most widely used natural materials on the earth, has long been used in a variety of formats. Whether that being as a food ingredient, or as an excipient within the chemical industry. What can be inferred from this is that natural products, such as sucrose, can be utilised in a wide variety of means. To that end, a material that can be widely used is a viable contender for research into improving its performance, as well as finding new areas for its use.

Sugars are already widely used. An example of their versatility can be drawn from their use as cryoprotectants. Materials used within this role help preserve the structure of cells and proteins for their study. Recent work has shown that both sucrose and trehalose inhibit the formation of ice crystals, thus rendering them suitable for use as cryoprotectants.[1] The inhibition of growth of ice crystals in the presence of sucrose, or trehalose, was attributed to their interaction with the water molecules in solution. This results in a hindering of the growth of ice crystals from solution.[1]

The interaction of sugar molecules in the solid-state is a source of interest for improving the performance of food and pharmaceutical products. Bringing about the interaction of sugar molecules in the solid state with other molecules typically occurs *via* crystallisation from a liquid state.

The re-crystallisation of crystals, using a suitable solvent, has long been used as a method to yield a pure phase of a crystalline material. Materials formed in this manner are relatively free of impurities. Prior to this process, it is logical to assume that any impurities within a solid, crystalline material have become integrated within the crystalline lattice during the growth of said crystals.

Typically, the presence of impurities within a crystal structure is unwanted. Altered morphology, reduced crystal size, along with hindered growth from solution, are phenomena that can be induced by foreign material. Such inclusions are possible due to the nature of crystal growth. Any material that exhibits marked structural similarity to the host structure can be come included within the final structure. Sites for adsorption and inclusion on a growing crystal lattice are highly stereospecific. Any material showing stereochemical likeness can conceivably become included into the final structure. As such, impurity

inclusion can be seen as a negative aspect in crystallisation. However, the notion that an impurity only confers negative characteristics to a material should not be taken as the sole truth.

Co-crystallisation is a relatively new method of including a secondary component into a primary structure.[2] The process involves the heating of a material to high temperatures to form supersaturated solutions. At this point a secondary component is introduced and crystallisation is induced. Chen detailed the potential of co-crystallised sugar products, along with a methodology, within the sphere of functionalising sugar with specific properties.[3] Within this paper, Chen described co-crystallised sugar products to exhibit enhanced physical properties. These included solubility, compressibility and flowability to name but a few.[3] Details of other advantages obtained from co-crystallising sugars have been detailed by Maillet, these included:[4]

- Co-crystallising with xylitol yields material that are less hygroscopic along with possessing superior tabletting qualities
- Improving solubility characteristics of ingredients including, fumaric acid, proteins and starch

The process of included foreign components into a sugar structure is thought to occur as a function of the porous nature of sugar crystals. The porous nature occurs when crystallisation proceeds from a sugar solution formed at a high temperature. It is thought that secondary components become encapsulated within this porous structure. Materials of this nature can still be considered as two separate materials, rather than the process yielding a new material composing of the initial materials. From this structure, it has been elucidated that an aqueous solution can penetrate easily into the structure, thus dispersing the material rapidly.[5, 6]

The list of materials that have been co-crystallised have been detailed as mentioned above. Work from this laboratory by Maillet and Maulny has further contributed to the list of ingredients incorporable within a sugar solutions.[4, 7] In order to gain an understanding of the phenomenon of co-crystallisation, a review

of the findings from the above thesis is critical to an improved understanding of co-crystallisation.

3.2. Co-Crystallisation of Sugars

Furthering the work performed by Bandhari, [2] co-crystallisation of sucrose with honey was investigated with respect to the behaviour of the components in the solid state. Through the course of this work, an extra peak at ~ 150°C was observed via DSC that was not relevant to any of the components present. Isolation of this phase was successfully achieved and the presence of the phase was shown to be influenced by the presence of inorganic impurities.[8] This work highlighted the difference in behaviour of sucrose obtained from different sources. For the continuing work on co-crystallisation, commercial sugar was used due to the relevance of the process to industry.

Co-crystallisation of sugars *via* a co-precipitation method has been achieved successfully with a range of commercial sugars. The sugars utilised to generate the supersaturated solutions were sucrose, lactose and glucose, into which a variety of mono-, di- and poly-saccharides were successfully incorporated.

The methodology for the formation of co-crystalline materials remains virtually identical for each combination of sugars and is based on the work performed previously. A supersaturated solution is formed from the matrix sugar, the quantity of solvent (H_2O) and the temperature required for this to occur differ from sugar to sugar. The solvent quantity and temperature used are the only two variables in the methodology. For example, to form a sucrose supersaturated solution, 30g of sucrose in 6 ml of H_2O is heated to 128°C; for lactose, 30g of sugar requires 12 ml of H_2O and a temperature of 110°C. Upon reaching supersaturation, a sugar is incorporated, the solution re-heated to the temperature necessary for supersaturation, then cooled and agitated to induce nucleation. The co-crystalline materials are then dried to remove any water that is not inherent with the crystallisation process.

The co-crystalline materials were then subjected to analysis by DSC and PXRD to ascertain their physical characteristics.

Sucrose was the first sugar to be investigated with regard to its ability to form co-crystalline materials as an existing methodology was available.

3.2.1. Co-Crystallisation of Sucrose with Monosaccharides.

A Sucrose supersaturated solution was achieved by heating 30g of sucrose in 6 ml of H_2O to 128°C. At this temperature various monosaccharides were added, including: glucose, mannose, galactose, fructose, xylose, glucose pentaceate and a mixture of glucose plus fructose. Each of these individual sugars incorporates into a sucrose solution and yields co-crystalline material (in an analogous manner to the work by Bhandari and Hartel[2]) up too a level of the added monosaccharide. Above this level of incorporation, two phases are observed. Both the co-crystalline phase and a phase relating to the added component can be observed. The varying levels of incorporation for the monosaccharides outlined above into a supersaturated sucrose solution are summarised in table one.

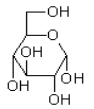
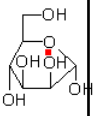
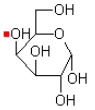
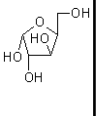
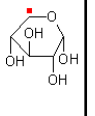
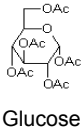
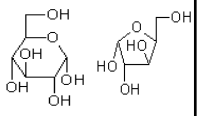
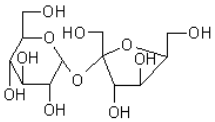
Matrix Sugar \ Incorporated Component	 Glucose	 Mannose	 Galactose	 Fructose	 Xylose	 Glucose Pentacetate	 Glucose + Fructose
 Sucrose	7%	4%	2%	30%	2%	2%	Glucose 15% Fructose 15%

Table 1. Incorporation Levels of Monosaccharides into Sucrose.[4]

The difference between co-crystalline materials and materials exhibiting two phases is achieved by comparison of physical blends (in an analogous ratio) of the two sugars to be co-crystallised. The subsequent analysis (fig.1 and fig.2) of the physical blends allows characteristic peaks for the incorporated material to be identified. This aids the process of co-crystalline material identification as if a material is not co-crystalline, peaks relating to the incorporated material will be observable.

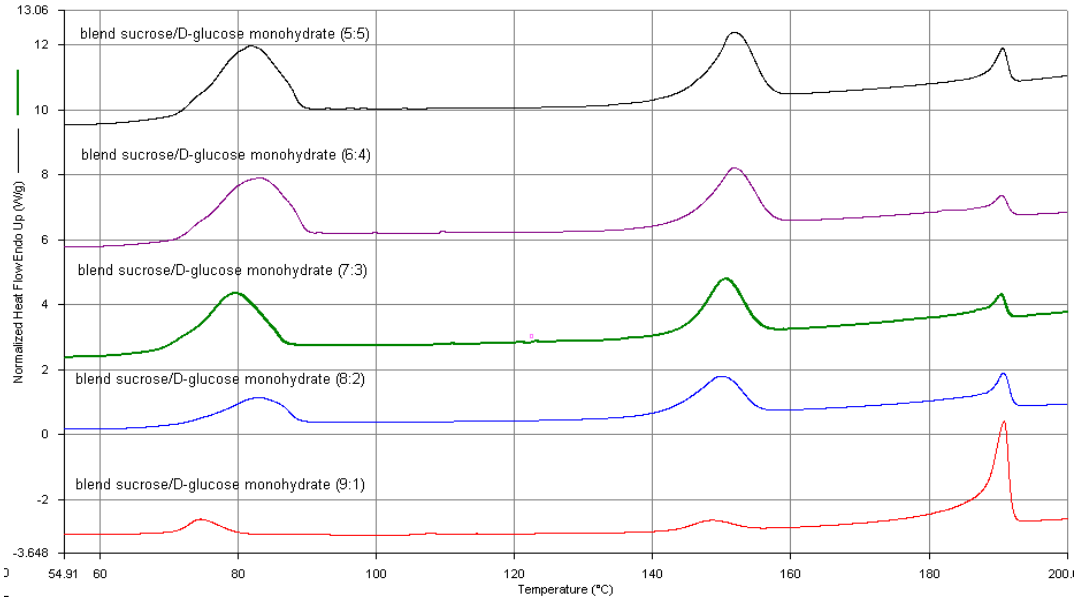


Fig 1. DSC traces of Sucrose and Varying levels of Glucose Monohydrate.[4]

DSC analysis of physical blends of sucrose with increasing levels of glucose monohydrate (10-50% w/w) clearly shows the two melting endotherms for the two forms of glucose (the monohydrate form melts at ~70°C and the anhydrous form melts at ~145°C) along with the sucrose endotherm at ~190°C as seen in figure 1. Levels of added glucose monohydrate in such physical blends are detectable at low levels; DSC analysis of a physical blend containing 1% w/w of glucose monohydrate exhibited both characteristic endotherms for glucose.

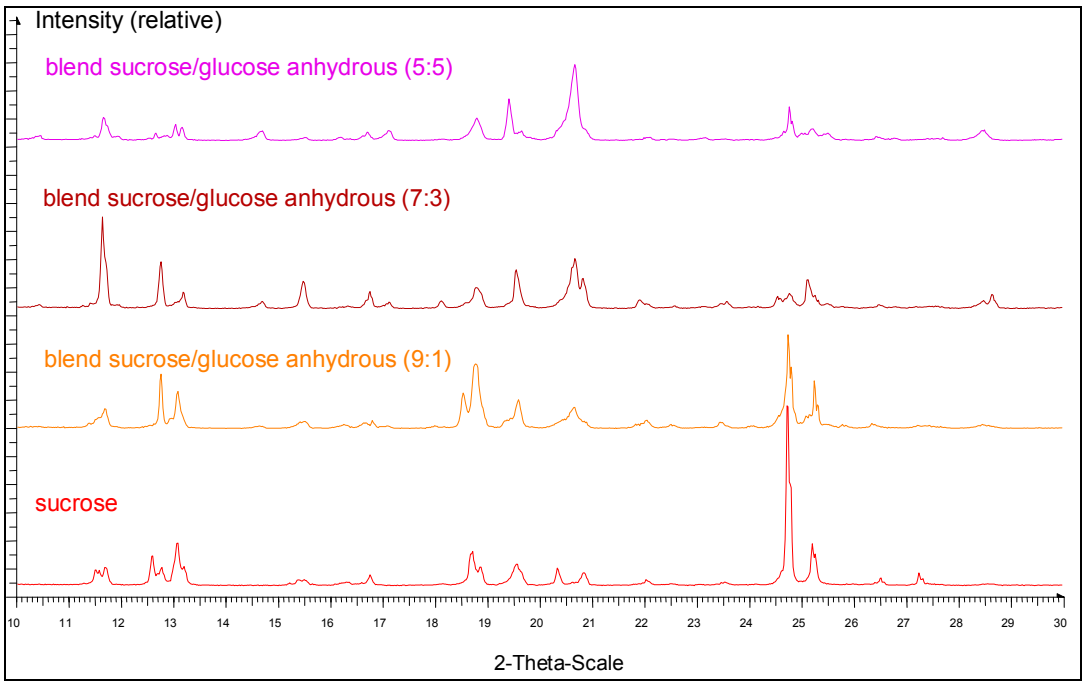


Fig 2. PXRD Diffractograms of Sucrose and Varying levels of Glucose.[4]

PXRD analysis for physical blends of sucrose and varying levels of glucose monohydrate (10-50%, figure 2) showed easily identifiable, characteristic peaks for both phases. Unlike DSC, PXRD did not show the presence of glucose at the 1% w/w level. This is due to the high intensity of the sucrose peaks overshadowing the glucose peaks.

This process of identification of the characteristic peaks in both forms of analysis was successfully repeated for every combination of sucrose and the monosaccharide to be investigated. A clear example of the difference between co-crystalline material and material showing more than one phase can be seen in fig 3. This data originates from preliminary work by the author. For co-crystalline material containing 1-4% w/w of glucose, solely the sucrose endotherm at 190°C is observed. A subsequent elevation to 7% w/w of glucose results in an endotherm emerging at ~ 125°C, this corresponds to the presence of glucose in its anhydrous form. Further evidence for the inclusion of the added component into the lattice of the matrix sugar is provided by analysis of the enthalpy values for the sucrose endotherm. Contamination of a phase by an impurity is observable by a decrease in ΔH values for the peak relating to the main phase. This decrease in ΔH is proportional to the quantity of the contaminant. A decrease in ΔH is also coupled with a broadening of the peak

relevant to the main phase. Both of these observations are classical behaviour in DSC analysis for contamination of a phase and this behaviour is displayed by all co-crystalline materials.[9]

Characterisation by PXRD supports the data gathered from DSC analysis. Below 7% w/w of included glucose, solely peaks corresponding to a sucrose phase are observed, above this level, peaks emerge at 2θ values of ~14°, 17° and 20°.

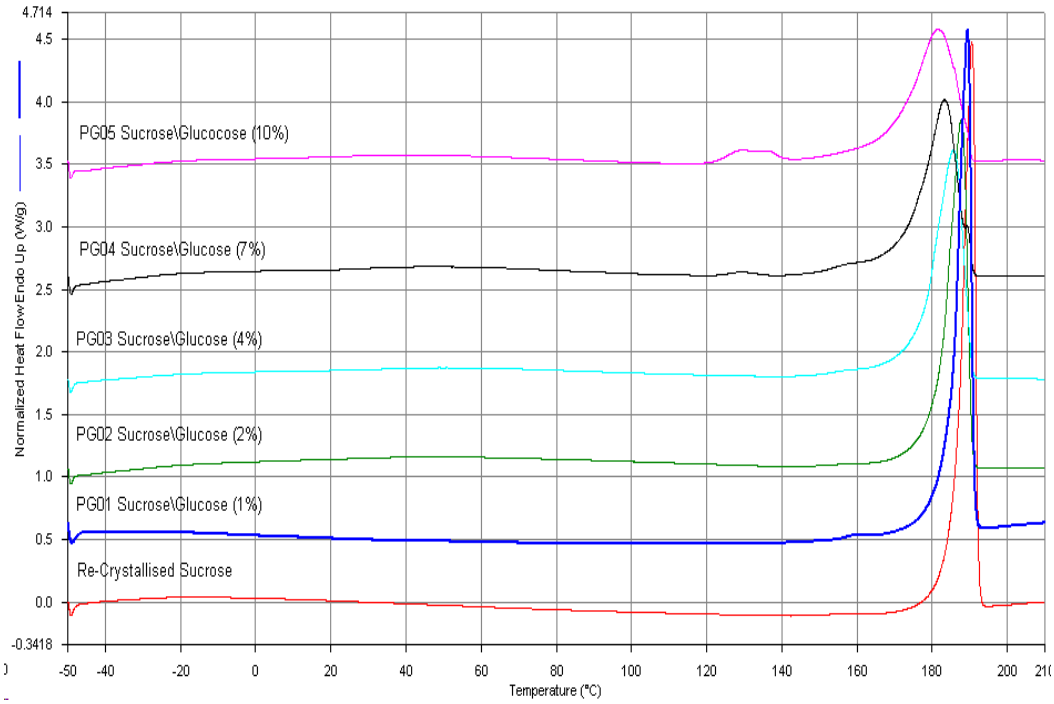


Fig 3. DSC Traces of Co-Crystalline Sucrose and Glucose Materials from Preliminary work by the Author.

This trend is applicable to all the combinations of sucrose and the various monosaccharides and the limits of incorporation for each of the monosaccharides are summarised in table 1.

It can be clearly observed that there is a marked difference in the limit of incorporation for each monosaccharide. This variance can be attributed to differences within the individual monosaccharides.

3.2.2. Differences in the Levels of Monosaccharide Incorporation.

All of the monosaccharides investigated have different structures; it appears the level of incorporation is dependent on the structure of the individual

monosaccharide. This can be illustrated by comparing three of the monosaccharides with similar structures; glucose, mannose and xylose as shown in table 1.

The limit of incorporation decreases as follows:

Glucose > Mannose > Xylose

The differences between the three sugars are:

1. Orientation of the hydroxyl groups
2. The number of hydroxyl groups

Taking glucose first, from table 1, the limit of incorporation is 7% w/w, which is the highest of the three sugars. As sucrose is a disaccharide consisting of glucose and fructose, if a monosaccharide is to become incorporated into the crystal structure, a component of that structure would logically fit. This is the observed case. Consideration of the theories of crystal growth furthers this hypothesis. Kossel [10] postulated that a lattice of a crystalline material is built by incorporation of each individual component into the lattice. However, each component may not be disposed to immediate incorporation due to steric factors (e.g. unfavourable orientation of the component to be incorporated).

To expand on this idea further, the incorporation of mannose into sucrose was performed. Mannose, a C2 epimer of glucose, does not incorporate to as a high level as glucose and a limit of 4% w/w is observed (table 1). It appears that during nucleation and the subsequent crystallisation, mannose does not fit well into the growing sucrose lattice. This is logical considering the differences between glucose and mannose. One (glucose) is a component of the predominant sugar (sucrose) so would become involved more readily in the building of the sucrose lattice, whilst the other is not.

Though not mentioned above, galactose, a C4 epimer of glucose, incorporates (2% w/w) to a lesser degree than mannose. The orientation of hydroxyl groups on the sugar to be incorporated appears to be directly related to

how well it incorporates into the sugar crystallising from the supersaturated solution.

The sugar used to generate the supersaturated solution therefore appears to display an affinity for sugars exhibiting similar crystal structure. An extreme example would be to generate a supersaturated sucrose solution and add more sucrose too it. Obviously solely crystalline sucrose would result. The limit of incorporation for monosaccharides relates to how similar in structure it is to the sugar used to generate the supersaturated solution. Greater dissimilarity results in lower levels of incorporation. This notion of 'like likes like' is furthered by evidence from the incorporation of glucose and fructose (1:1 ratio) into sucrose. Each of the monosaccharides incorporates up to a level of 15% w/w when simultaneously included. Normally, glucose includes up to a level of 7% w/w, in this case the amount included has been increased to 15% w/w. Considering that sucrose consists of these two monosaccharides it is not unexpected that a significant level of incorporation is obtained. The hypothesis for this (increased level of incorporation) is that the two monosaccharides can be likened to a 'broken' sucrose molecule. As such, it is an excellent mimic of sucrose and, therefore, will include well into the constructing lattice. So, a recognition factor appears to play an important role in the co-crystallisation of sugars.

This recognition between sugars is further exemplified by consideration of the incorporation of xylose into sucrose. Xylose possesses one less hydroxyl group than glucose but an identical configuration with regard to the remaining hydroxyl groups. As such; it is a slightly smaller molecule than glucose.

If the crystalline lattice is built from individual components (from the matrix sugar and the incorporated sugar) adding directly onto the initial nuclei, then for each subsequent addition to the growing lattice there is a preference for a component that fits. The growing lattice presents sites with a specific size, and orientation of hydroxyl groups for additional components to incorporate onto.

Co-crystallising xylose into a supersaturated sucrose solution reveals a low limit (2% w/w, lower than mannose) of incorporation for xylose. This evidence further shows the reliance of co-crystallisation on recognition between the sugars used. This recognition appears to rely on the orientation of the hydroxyl groups on the constructing lattice. The importance of the hydroxyl groups is further exemplified by the incorporation of glucose pentacetate, a sugar

with the same configuration as glucose but with acetate groups present. A low level (2% w/w) was achieved, thus displaying the need for recognition between hydroxyl groups, though steric factors need to be taken into account to.

Another factor that appears to be applicable to co-crystallisation of sugars is the rate of crystallisation of the added component. With sucrose, the maximum limit of incorporation is 7% w/w, except for fructose, which incorporates up too a level of 30% w/w. Fructose is known to crystallise slower than the other mono-saccharides used. This slower rate of crystallisation appears to allow the added ingredient to insert itself into the building lattice more effectively. This theory is furthered by evidence from work performed on allowing co-crystalline sucrose and glucose to crystallise at 80°C (rather than 55°C). The level of incorporation increases from 7% w/w too 10% w/w. The higher temperature allows a proportion of the co-crystalline material to remain more mobile, thus allowing more time for components that would not quite fit to find a way of orientating itself more favourably into the building lattice. This increase in the level of incorporation as a function of a slower crystallisation rate appears to have an effect on other incorporated monosaccharides. The co-crystallisation of sucrose with glucose and fructose results in an increase in the level of glucose incorporation. As mentioned above, this is, in part, likely to be due to a recognition factor. But consideration of the slow crystallisation rate of fructose (and its ability to incorporate in high proportion) it is not unlikely that this rate facilitates the incorporation of glucose into the sucrose lattice.

From the observed data, it appears that co-crystallisation of sucrose with various monosaccharides involves a direct incorporation of the added component into the lattice of the supersaturated sugar. Analysis of the co-crystalline material by PXRD reveals detailed information about the structure of the material: using the Checkcell software, the dimensions of the unit cells can be determined. If a direct incorporation of the added component in the lattice of the sugar is the method of inclusion, a change in the unit cell should be detectable. Calculation of the unit cell parameters for co-crystalline sucrose and glucose materials reveals a trend (fig 4) in the unit cell volume as the portion of glucose increases.

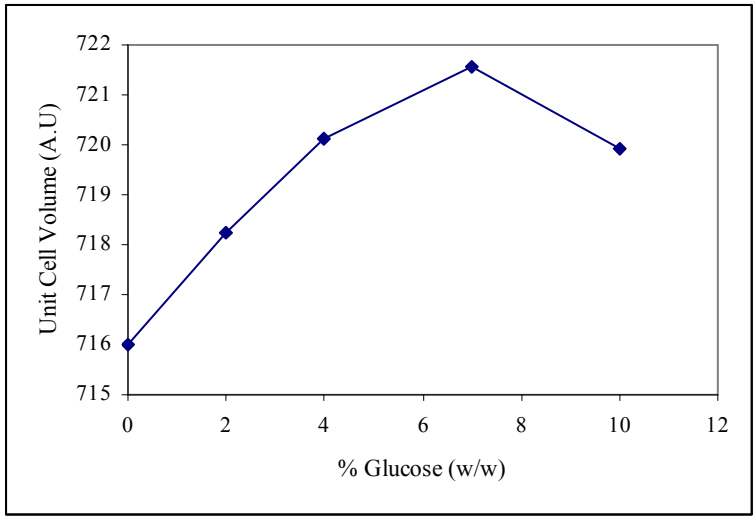


Fig 4. Graph of Unit Cell Volume versus % Incorporated Glucose[4]

Calculation of the unit cell volume reveals a general increase in the unit cell volume as the percentage of the secondary component increases. This suggests that the included component is incorporated directly into the lattice of the supersaturated sugar. This phenomenon is observed for co-crystallisation with other monosaccharides incorporated into sucrose.

Therefore, it appears that trends of co-crystallising sucrose with monosaccharides can be established. Further investigations were undertaken in order to study the effects of co-crystallisation with di- and tri-saccharides.

3.2.3. Co-Crystallisation of Sucrose with Di and Triaccharides

A summary of the findings from the co-crystallisation with disaccharides can be seen in table 2.

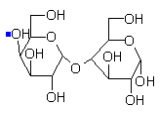
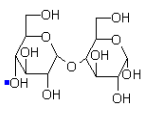
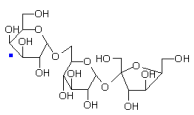
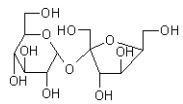
Incorporated Component / Matrix Sugar	 Lactose	 Maltose	 Raffinose
 Sucrose	4%	10%	> 30%

Table 2. Incorporation levels of Disaccharides in Sucrose[4]

In an analogous manner, the incorporation of di- and trisaccharides into sucrose yields varying limits of incorporation. Again, this appears to relate to the structure of the incorporated component and its relation to the sucrose structure.

Lactose incorporates at a relatively low level (4% w/w) and this is similar to the level of incorporation of galactose (2% w/w) into sucrose. Lactose consists of a galactose linked to a glucopyranose unit; the presence of the glucopyranose unit appears to facilitate the incorporation of lactose into the sucrose lattice.

Maltose, which consists of glucose linked to a glucopyranose unit, incorporates to a similar level to glucose (7-10% w/w). Again, it appears the presence of glucose moieties within the maltose structure, which are also within the sucrose structure, facilitate incorporation of maltose into the sucrose lattice.

Raffinose, a trisaccharide consisting of a sucrose core linked with a galactopyranosol unit, incorporates highly into the sucrose lattice. Even with the presence of the galactopyranosol unit, raffinose incorporates well (unlike lactose). This high level of incorporation can be due to the presence of an accessible fructofuranosyl unit.

All of the incorporated sugars above display the same characteristics upon analysis by DSC and PXRD. DSC analysis shows a decreasing of ΔH values with increasing amounts of added second ingredient and a broadening of the sucrose endotherm peak. PXRD shows an increasing in unit cell volume. This behaviour is indicative of incorporation of an impurity into a lattice and provides further evidence that co-crystallisation involves the direct incorporation of the added component into the lattice of the matrix sugar.

Sucrose has the ability to be co-crystallised with a wide variety of secondary components and these findings indicate that the level of incorporation is dependent on similarity of their structure to the sucrose structure. Successful incorporation is displayed by DSC behaviour (decreasing ΔH and peak broadening) and PXRD patterns (increasing unit cell volumes). Investigation into the ability of lactose in co-crystallisation was undertaken in order to further the understanding of co-crystallisation and widen its applications.

3.2.4. Co-Crystallisation of Lactose with Mono- and Disaccharides

A summary of the findings from the co-crystallisation of lactose with a variety of sugars can be seen table 3.

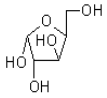
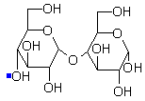
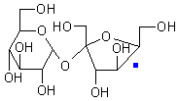
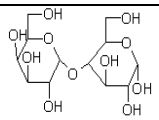
Incorporated Component / Matrix Sugar	 Glucose	 Fructose	 Maltose	 Sucrose
 Lactose	10%	15%	20%	7%

Table 3. Co-Crystallisation of Lactose with Mono- and Disaccharides.[4]

As can be clearly seen, the behaviour of lactose as the matrix is similar to the behaviour of sucrose. The limit of incorporation is dependent on the similarity of the incorporated component to the matrix sugar and, possibly the incorporated components rate of crystallisation.

Glucose (a part of the lactose dimer) incorporates well; this is again mirrored by maltose. Further evidence is provided of the need for the incorporated component to exhibit similarities in structure to the matrix sugar. Though fructose is not a part of the lactose dimer, as seen with sucrose, its slow rate of crystallisation allows higher levels of incorporation.

Incorporating glucose singularly, as well as fructose, results in significant levels of incorporation. Theoretically, sucrose should incorporate well; however this is not observed experimentally. Sucrose shows the same space group as lactose ($P2_1$) and this should lead to a high level of incorporation. This observation suggests that, with lactose as the matrix, the availability of an anomeric hydroxyl group could be a factor for deciding how the level of incorporation of the secondary component into the lattice of the matrix.

As with sucrose, the incorporation of a secondary component into the lactose lattice is accompanied by the expected indicators arising from DSC and PXRD analysis.

Co-crystallisation can therefore be achieved using a different disaccharide other than sucrose as the matrix. However, the ability of a monosaccharide to be utilised as the matrix sugar had not been investigated.

3.2.5. Co-Crystallisation of Glucose with Mono- and Disaccharides

Co-crystallisation of glucose with a range of mono- and disaccharides is summarised in table 4.

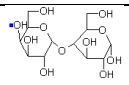
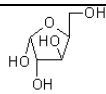
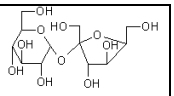
Incorporated Component / Matrix Sugar	 Lactose	 Fructose	 Sucrose
Glucose	4%	10%	7%

Table 4. Co-Crystallisation of Glucose with Mono- and Disaccharides.[4]

As seen with co-crystallisations involving sucrose and lactose, varying levels of incorporation of the added component are observed depending on the chemical nature and structure of the added component. As seen with sucrose and lactose, the presence of a fructofuranosyl ring facilitates the incorporation of the added ingredient into the crystalline lattice. This is exemplified by the difference in the levels of incorporation between lactose and sucrose.

Analysis by DSC and PXRD revealed the usual indicators for the presence of co-crystalline material.

3.2.6. Summary of Co-Crystallisation

The incorporation of various components into supersaturated solutions yields co-crystalline materials. Co-crystalline material is formed up to a level of the added component, above which a mixture of crystalline materials is obtained. Both the co-crystalline phase and the crystalline form of the added component are observed. The ability of the included component to incorporate into the crystalline lattice is determined by its similarity in structure to the sugar used to form the supersaturated solution. Co-crystallisation appears to rely on an inter-molecular recognition between the sugars used. The presence of a fructofuranosyl moiety on the added sugar facilitates the incorporation of the included component into a crystalline lattice. The formation of co-crystalline materials is confirmed by DSC and PXRD and the data suggests that the added

component is included as a solid-solution within the crystalline lattice of the sugar used to form the supersaturated solution.

The phenomenon of sugar-sugar interaction is not one that is limited to solely the field of co-crystallisation; biological processes rely on this recognition between sugars to communicate subtle messages within all living organisms.

3.3. Sugar Interactions in Nature

Every living organism is described by a code of four letters signifying the four bases within a DNA molecule; adenine (A), thymine (T), guanine (G) and cytosine (C). The function of all molecules is determined by the order in which these bases appear; as such, the sequence is a code that conveys information on what the function of the resulting cell will be. Consideration that a vast variety of cells exist within nature, such a subtle code cannot convey the many interactions necessary for the continuance of life within an organism. Forwarding of such a hypothesis is possible via analysis of the interactions between nucleic and amino acids; both these classes of biomolecules interact via solely two linkage points resulting in a limited number of permutations for each code. As a result, another level of code exists within nature to communicate between molecules and that code utilises carbohydrates; such molecules offer substantially more than two linkage points. Consequently, the number of permutations has been shown to be in excess of 1.05×10^{12} potential linkages (fig 5) possible between two carbohydrate residues.[11] Variation on solely linking between carbohydrates is possible considering the effects of anomeric stereochemistry, ring size and sub-unit modification (e.g. sulphonation, phosphorlyation and methylation) and the code that exists between carbohydrates, termed glycocode, develops further.

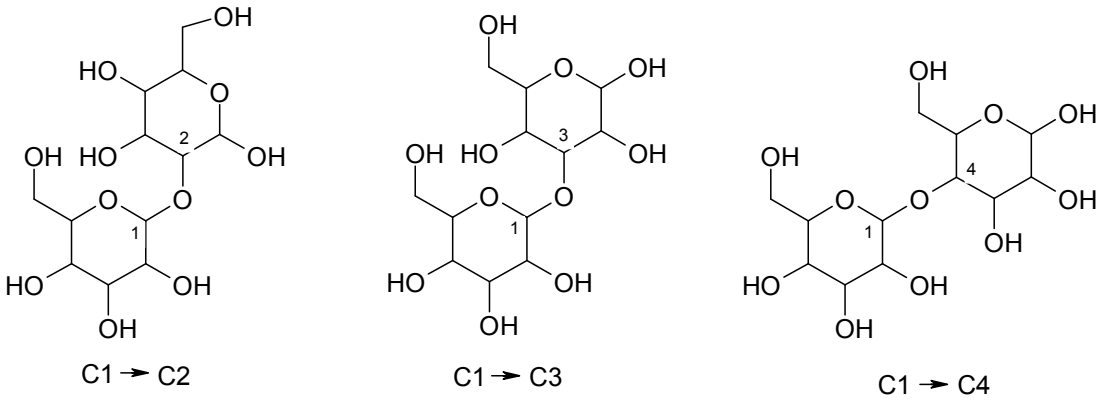


Fig 5. Three Possible Linkages between Pyranose Moieties

The interaction between carbohydrates within an organism is achieved by sugar binding proteins known as lectins.[12] Lectins are ubiquitous throughout nature and are highly specific in their recognition of sugar moieties; indeed, the name lectin, derived from the Latin word *legere*, means 'to select'. Lectins are typically involved in recognition events with components within the glycocode (the interacting moieties are known as glyco-conjugates) and these appear mainly in two forms. *N*-linked, where the sugar is bound to the side chain of asparagine residues (fig 6a) and *O*-linked where the sugar is bound to the side chain of serine or theronine ligands (fig 6b). These resulting proteins are located at the cell surface membranes or in a soluble form. A third form of lectin glyco-conjugate exists; glycolipids, these involve a liphophilc 'head' that is within the cell membrane attached to a carbohydrate portion (fig 6c).

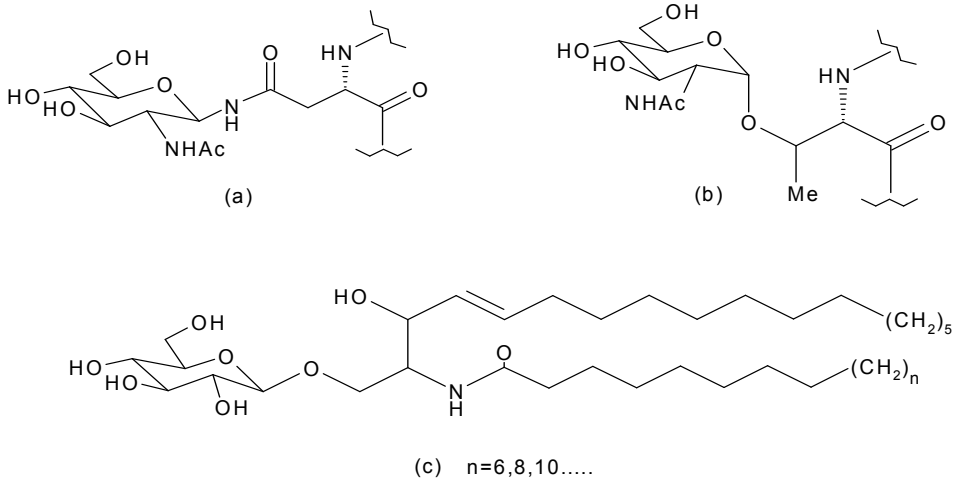


Fig 6. Glycoconjugate Interacting Moieties

Such lectin structures possess a region known as the carbohydrate recognition domain where incoming carbohydrates are bound. Typically this binding involves both H-bonding from amide group donors to lone pair oxygen acceptors. Carbohydrate hydroxyl groups are thought to donate to carbonyl groups on the lectin site. Furthermore, protein bound calcium ions can also play a role in co-ordinating carbohydrate hydroxyl groups and reinforcing the lectin structure. This further reinforces the correct position of H-bond donors and acceptors. These interactions are amplified by non-polar van der Waals interactions between aromatic side chains and hydrophobic patches on the binding carbohydrates. These factors ensure a high degree of spatial constraint on which ligands can bind to the lectin and are the source of their specificity.

Lectins are held in place on the surface of cells by oligosaccharides or glycoproteins. Either of these two functionalities can be construed as a sugar-hand holding the lectin in position. Furthermore, the arrangement of lectins on the cell surface can occur in a variety of ways, from the presence of numerous single binding lectins on a cell surface to the arrangement of lectins possessing multiple binding sites. Other factors that help reinforce the highly selective nature of lectin sites include how the oligosaccharides bond (multiple ways are possible between carbohydrates, see fig 5). This, in turn, influences a myriad of other factors within the cell or on the cell wall surface. Whilst the role of lectins is to recognise carbohydrates within nature, the manner in which this occurs result in a variety of functions being fulfilled by lectins.

3.3.1. Handshaking

The crucial role undertaken by lectins within the body is that of highly specific ‘handshaking’ interactions. ‘Handshaking’ interactions occur in a similar manner to the ‘lock and key’ mechanism of enzyme and substrate interaction. These interactions can be considered highly specific in nature. Such events highlight the key role played by sugars in nature. Recognition between lectins and carbohydrates initiates beneficial processes in the body but it is also the mechanism by which harmful bacteria ingratiate into tissue. Nethertheless, this selectivity between lectin and sugar is crucial. Within early childhood, an adaptive immune response to pathogens has not been developed in the body and the body is reliant upon lectins for defence.[13] This defence utilises the high

selectivity of lectin binding to appropriate groups (see fig 7) on pathogenic cells, this then inactivates the pathogenic cell and thus stops any infection occurring.[14] Lectins are also crucial when the adaptive immune response is compromised; HIV infection is one such example of this.[15]

The complexation of lectins with appropriate groups induces a response within the body to treat a wide range of trauma within the body. A well studied event is the inflammatory response in the body. Any damage to tissue initiates a complex process to recruit white blood cells to the damaged area.[16, 17] To treat the damage, white blood cells must leave the blood vessels and pass into the affected tissue. As a function of this, the blood vessel walls become leaky; this facilitates a build up of fluid into the tissue and inflammation results. This process occurs as a function of selectins (a class of sugar binding lectins) and occurs in a three-step process.[18] An injury results in E-selectins being expressed on the inner surface of the blood vessel in the vicinity of the injury and P-selectins are present on platelets within the blood stream. As a function of this, interactions between the selectins and carbohydrates present on cell surfaces encourages white blood cells to roll along the blood vessel wall toward the damaged site. Reaching the injury, the white blood cells adhere to the vessel wall via protein-protein interaction. This process is completed by the expression of L-selectins by the white blood cells and the final passing of the white blood cells into the tissue. The exact nature of the carbohydrate that binds to the selectins is not precisely known; the tetrasaccharide sialyl Lewis x (sLe^x) (fig 7) is bound by all varieties of selectin and can be taken as a guide.

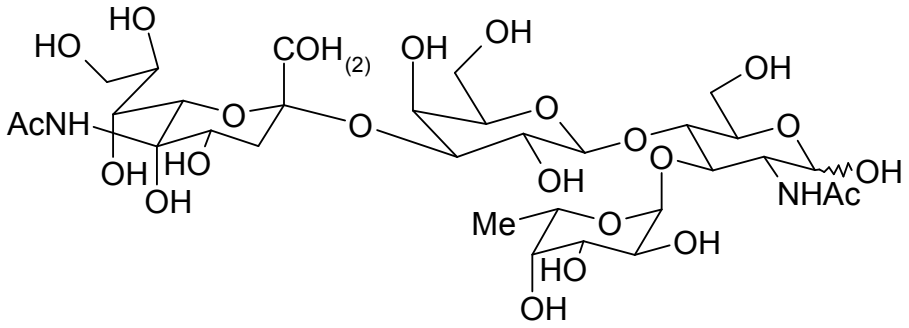


Fig 7. Structure of tetrasaccharide sialyl Lewis x (sLe^x)

Whilst this process is a well controlled process in healthy individuals, if too many white blood cells pass through the blood vessel wall they can cause damage to healthy tissue. This can result in a range of ailments including, septic shock, arthritis, asthma and heart disease. As sLe^x is known to bind universally to all selectins, therapeutic avenues are opened for the regulation of the inflammation process. A sLe^x derivative (fig 8) has been shown to be 800 times more effective as a selectin binding agent compared to sLe^x (fig 7). This allows early inhibition of the selectin-blood interaction thus facilitating the regulation of the preliminary stages of inflammation.[16]

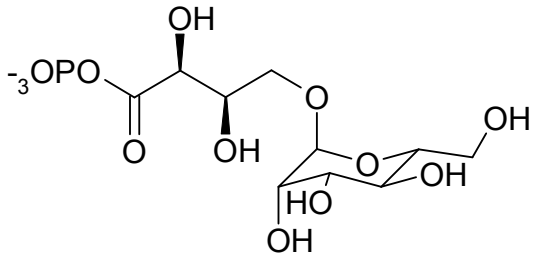


Fig 8. sLe^x derivative

The bacteria, *Heliobacter pylori*, which causes gastric ulcers interacts with the gut cells during the initial stages of infection. This occurs *via* binding to extracellular glycoproteins containing the sugar sialic acid. Treatment can be achieved by inhibiting this interaction by administering conjugates glycosylated with 3'-sialyllactose residues (fig 9).[19]

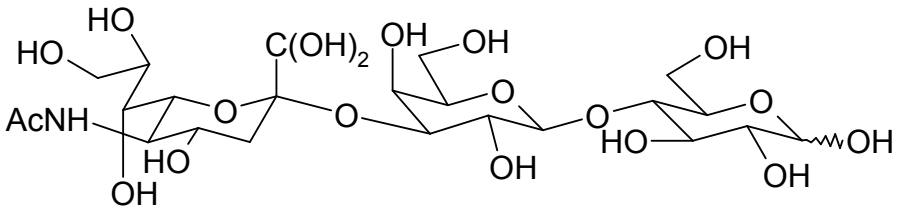


Fig 9. A 3'-sialyllactose residue

Fatal toxins such as ricin and abrin enter cells and tissue via this sugar handshaking system, the toxins bind to the sugars on the cellular surface. Internalisation of the toxins occurs after binding, followed by release into the cell cytoplasm. This release is coincident with inactivation of the cellular ribosomes followed by cell death. The bacteria, *Escherichia coli*, gains access to kidney

cells by binding to the surface of kidney red blood cells. But as the toxin can access this binding site, therapeutic treatments have been developed that exploit this specificity. A treatment known as *Synsorb Pk* has been developed and works by binding the toxin in a selective manner and clearing it from the body.[20]

Such interactions have also been explored with respect to other conditions within the body. Therapeutic agents are being developed using glycocode as the basis for design. Agents used to help regulate the effects of type I and type II diabetes are currently in usage; such agents act via prevention of poly- or oligosaccharide degradation.[21, 22] A crucial aspect in these interactions is that they do not kill the harmful bacteria. As such, the emergence of more resilient strains to this therapy will be reduced compared to the appearance of strains that become resistant to anti-biotic treatment. The usage of anti-adhesion theraputics is a growing field that is being explored to treat and prevent a wide variety of conditions within the body.[23]

3.3.2. Lectins in a Defensive Role

As lectins have such specificity within the body, they also facilitate in the defence of cells. This role relies on the physical properties of lectins protecting proteins from thermal degradation and hydrolysis by enzymes or peptidases. Such defence stems from protection via the polarity of the lectins or the steric hindrance that protects vulnerable areas from attack. In this context, the lectin structure protects by virtue of their size or changing the polarity of local regions by using polar hydroxyl fingertips. An example of lectins disrupting interactions within nature is within the blood of deep sea fish. These disrupt ice crystallisation thus facilitating survival at temperatures as low as -4°C.

3.3.3. Utilising Glycocode

The specific interaction between lectins and sugars, know as glycocode, in the sphere of therapeutics is vast. It can be used to send or block messages and processes within the body in a highly specific manner. Such an acute specificity results in a significant lack of unwanted side-effects in the body, thus allowing the treatment or prevention of ailments in the body in a more effective manner.

Whilst the development of glycocode based therapeutics is progressing, another similar field of treatment is emerging that also relies on the recognition

and subsequent interaction of various moieties. The resulting compounds formed from these interactions are known as pharmaceutical co-crystals.

3.4. Pharmaceutical Co-Crystals

Active pharmaceutical ingredients (API's) are among the most commercially viable within the chemical industry. Pharmaceutics consist of the API and a formulation designed to carry the API to its desired location. Any technique that can improve this formulation and enhance the functionality of the product would be desirable. APIs occur as solids within their formulations; their crystalline forms are preferred due to their relative ease of isolation, they also tend to reject impurities inherent with the crystallisation process. Furthermore the crystalline state provides enhanced stability. However, there are problems associated with using APIs in their crystalline state; typically these show poor solubility and the existence of more than one crystalline form.

Application of crystal engineering to this problem is a relatively new approach [24] to the development of new classes of APIs, and it has yielded a new class; the pharmaceutical co-crystal.

Crystal engineering can be defined as the application of supramolecular chemistry to the solid state with particular emphasis on the idea of self-assembly of molecules to form crystalline solids. As such, crystal structures can be regarded as the result of a series of weak bur directional molecular recognition events. In a supramolecular context, pharmaceutical co-crystals are related to one another in that at least two components interact by hydrogen bonding, and possibly other non-covalent interactions. The definition of an API being co-crystalline stems from the nature of the final material. If one component is liquid at room temperature it is deemed as a solvate, if both (or more) components are solid then it is defined as a co-crystal.

3.4.1. Interactions in Pharmaceutical Co-Crystals

Due to the complex structure of APIs, they contain exterior functional groups that can engage in molecular recognition events. It is the presence of these functionalities that bestows the ability to interact with the human body in biological events. There are two basic interactions within pharmaceutical co-

crystals; supramolecular homosynthon and supramolecular heterosynthon interactions. Homosynthons occur between self-complementary functional groups, heterosynthons occur between different but complementary functional groups. Carboxylic acid and amide moieties form homodimers via a two point donor-acceptor recognition path (fig 10) and both moieties can interact (fig 11) through the formation of a heterosynthon. The heterosynthon has been studied more extensively than the homosynthon in the context of co-crystals. [25]

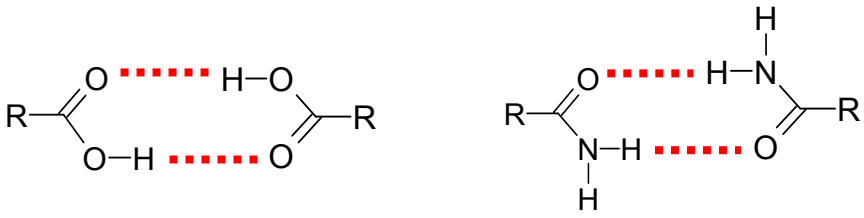


Fig 10. Homosynthons between Carboxylic and Amide Dimers

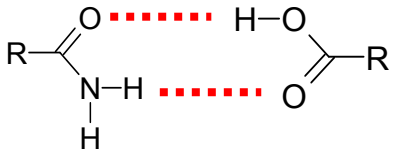
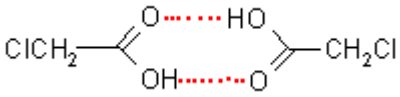


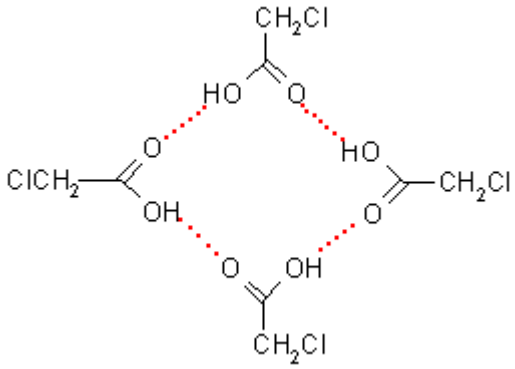
Fig 11. A Heterosynthon between a Carboxylic and an Amide Moitiey

3.4.2. Structures Involving Carboxylic Acids

Carboxylic acid moieties are the most widely studied functional groups in terms of hydrogen bonding in both the solution and solid state. [26] Carboxylic acids display a range of diversity in their supramolecular chemistry, thus they can form a variety of polymorphs with simple chemical structures. There are two ways that carboxylic acids can organise in the form of homosynthons; the dimer and the catemer. Chloroacetic acid is a compound that can display both forms of the homosynthon (fig 12).[27, 28]



Dimer Homosynthon



Catemer Homosynthon

Fig 12. Dimer and Catemer Arrangement of Carboxyic Homosynthon in Chloroacetic Acid

2-(-2-methyl-3-chloroanilio)-nicotinic acid, a molecule that exhibits analgesic and anti-inflammatory properties is an example of an API that organises via the carboxylic acid homosynthon. [29] The molecules organise as centrosymmetric dimers which are sustained by the dimer form of the homosynthon.

Not all carboxylic acid polymorphs are a result of isomerism in supramolecular homosynthons. They can arise from factors such as different crystal packing arrangements, or torsional flexibility which can yield conformational polymorphism.

3.4.3. Structures Involving Amide Moieties

Amide groups self-assemble in the dominant homosynthon as represented in fig 10. This homosynthon contains complementary hydrogen bond donors and acceptors; as such they are capable of further self-assembly which yields supramolecular tapes or sheets. Piracetam, a learning process drug, is an API that can organise itself in three different forms, two forms are sustained by the amide homodimer (fig 10) and NH---O=C (carboxamide) hydrogen bonds.[30] The third

form is maintained by catemer chains that are crosslinked by NH---O=C (carboxamide) hydrogen bonds.[31]

3.4.4. Structures Involving Heterosynthons

There are over 4000 entries in the Cambridge Structural Database (CSD) of crystal structures in which at least one carboxylic acid moiety is present. Also amides are well represented in the CSD with 1152 entries. It is understandable that, rather than the homosynthon being the dominant form found within crystal structures, it is the heterosynthon that dominates. APIs often exhibit numerous moieties that contain either the carboxylic or amide moiety and, when coupled with structures with either functionality, they show a high potential for the discovery of new pharmaceutical co-crystals.

Supramolecular synthons are structural units within supermolecules that can be assembled by either known or conceivable synthetic operations. Such assemblies occur by intermolecular interactions. Consequently, the possibility of rational design of co-crystals is real. As mentioned above, there are many combinations available between an API and a crystal structure and an analysis of the API's structure can highlight regions that can be exploited for interaction with a synthons. This leads to narrowing the field of search for a suitable component to co-crystallise with APIs.

This approach to rational design and exploitation of the acid-amide heterosynthon has already being utilised to form several co-crystals such as; succinic acid: benazamide [32], urea: glutaric acid [33] and ibuprofen: 4-4'-bipyridine. [34]

3.4.5. Pharmaceutical Co-Crystal Advantages

The term co-crystal is related to all components of the co-crystal being in the crystalline state. However other pharmaceutical products are not necessarily in the solid state. APIs that interact with solvents are termed solvates, these solvates usually arise from serendipity rather than rational design and re-producing a form of a solvated API is not guaranteed. Solvent use has inherent problems; firstly the list of acceptable solvents is limited, secondly, solvents are more mobile and have higher vapour pressures. Due to this, dehydration or desolvation of hydrates can occur in the solid form of solvates depending on the

storage conditions. This loss of solvent frequently leads to amorphous compounds, which are generally less stable then crystalline forms.

Pharmaceutical co-crystals avoid all of the problems mentioned above and often provide enhanced functionality to the API. Itraconazole, an anti-fungal agent, has poor solubility in aqueous solution. Co-crystallisation with 1, 4-dicarboxylic acid results in a heterosynthon forming between the triazole of each pair of drug molecule and carboxylic acid moieties on a single diacid molecule. This interactions confers enhanced solubility upon itraconazole.[35]

Pharmaceutical co-crystals are novel compounds, co-crystallisation with either hetero- or homosynthons results in a new form of the API. These new forms can be characterised in a variety of ways, single crystal diffraction, PXRD, DSC and solid-state NMR are a few examples. Characterisation confers new intellectual property on the co-crystalline material formed. Consequently, materials displaying new intellectual property can be assigned patents, giving an edge over the competition within industry. As all pharmaceutical co-crystals give a new form to APIs, all new co-crystals are patentable.

An analogy can be drawn between pharmaceutical co-crystals and co-crystalline sugars. Both materials appear to form due to discrete recognition events in the solid state and this recognition is related to H-bond formation. Whilst there are only hydroxyl groups present in sugars, it is logical to suggest that a degree of H-bonding takes place in the solid state. Recently, this interaction has been noted to occur in a co-crystal of saccharin and the API carbamazepine. The resulting, crystalline product has been said to be a viable alternative to the traditional form of the drug.[36] Furthermore, enhancement of the physical properties of a poorly soluble anti-HIV (loviride) drug by forming a co-crystal with sucrose. The loviride-sucrose co-crystal was shown to have superior dissolution properties compared to a physical blend of the two and to the drug on its own.[37] In both cases, the drug was reported to be encapsulated within a matrix of the respective sugar.

Sugars can also be used as a defence for molecules that are susceptible to degradation. P450 enzymes are of great interest as biocatalysts and drug metabolism, exposure to air however results in lyophilization and their activity is lost. The co-lyophilization of the molecule with sucrose or trehalose allows the enzymic activity to be retained with the enzyme in an anhydrous state. The

resulting material is easier to handle and store compared to the traditional storage method for these enzymes.[38] Such protein protection has also been observed in work produced on Super Critical Fluid (SCF) drying of sucrose with lysozyme and myoglobin. The end product of this process was shown to exhibit characteristic thermal behaviour of solely sucrose and was not amorphous.[39] Due to the results garnered from physical characterisation, the SCF dried sucrose with lysozyme or myoglobin is very much like a co-crystalline material as all the criteria for such materials are met.

The reasons for utilisation of sugars to form co-crystalline products are many, the foremost being that sugar is the most widely consumed substance that is generally regarded as safe (GRAS). As such, once a suitable API has been found, a high-throughput screening process with sugars (to find a suitable excipient) if successful will give a guaranteed product that is suitable for human consumption. Furthermore, the enhanced dissolution characteristics conferred upon the encapsulated component are of obvious benefit to a pharmaceutical product. This enhanced solubility does not solely stem from the encapsulation of APIs into a co-crystalline matrix. The blending of salmeterol xinafoate (an API administered *via* inhalation) with varying ratios of fine and coarse grains of sugars has been shown to improve the dispersability of the active component.[40]

Further investigation into sugar usage in the sphere of co-crystals will reveal potentially more applications, not solely within the pharmaceutical industry but also in the role of the food industry.

3.5. References

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4. Results and Discussion

4.1. Co-Crystallisation of Sucrose

Co-crystallisation using sucrose as a host material is becoming more widely used. The co-crystallisation methodology has been shown to impart favourable characteristics upon additive materials.[1] Furthermore, co-crystallisation is now being explored with respect to protecting added components. This has been shown to improve handling of certain materials.[2] The co-crystallisation methodology using sucrose involves the formation of a supersaturated sucrose solution. This is achieved *via* the addition of sucrose to water followed by heating to the required temperature. At this juncture, a second component is then included followed by allowing the solution to crystallise.[3-6] Investigation into a wide variety of components added to a supersaturated sucrose solution has previously been performed by Maillet, this work showed variation in how well sugars will include within a host sucrose structure.[7] The work contained herein tries to expand on this by attempting to determine what causes particular sugars to include well. Furthermore, if a poor inclusion is observed, explore methods to raise the quantity of incorporation.

Within the literature, the generation of a supersaturated sucrose solution is achieved by boiling the solution to a temperature of 128°C. Use of temperatures higher than this result in caramelising of the sucrose and no further improvements in the co-crystallisation process are observed.[3-6, 8] On the basis of this, the temperature of 128°C was initially used to form the supersaturated solution prior to inclusion of materials.

4.2.1. Re-Crystallisation of Sucrose

Sucrose was re-crystallised from water using the methodology outlined in Section 8.2.1 and DSC analysis (Fig 1) shows the material to exhibit melting behaviour typical to that of sucrose.

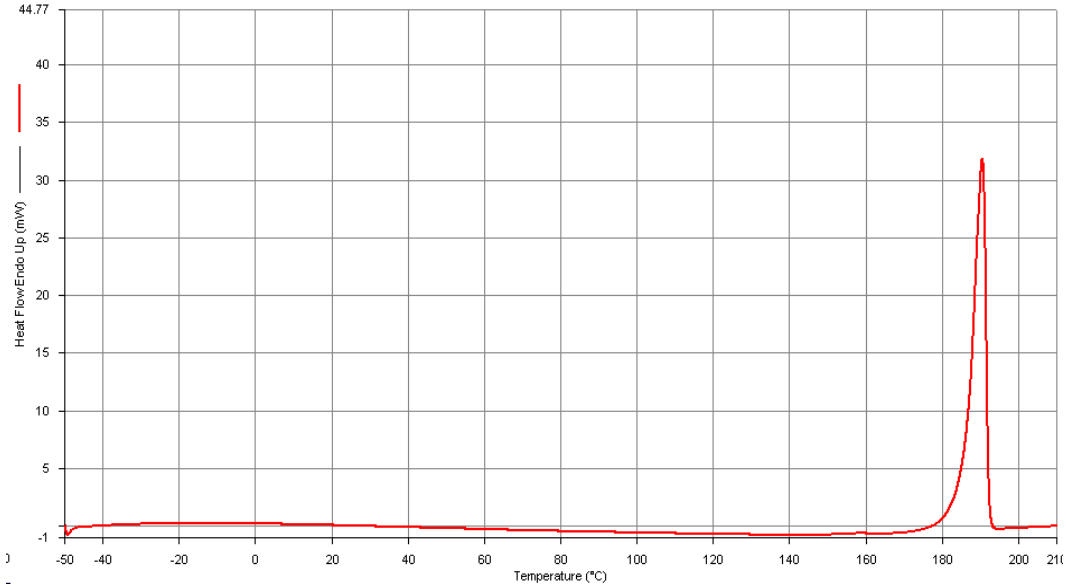


Fig 1. DSC Trace of Re-Crystallised Sucrose

	ΔH (Jg^{-1})	Onset (°C)
Re-Crystallised Sucrose	123.315	186.004

Table 1. DSC Data for Re-Crystallised Sucrose

PXRD analysis (Fig 2) yielded a diffraction pattern that matched well for a sucrose structure: no peaks were discernable that were not related to sucrose. The PXRD data allowed calculation of the unit cell and its parameters, which were as follows:

a = 10.8702 (± 0.0016), b = 8.7136 (± 0.0012), c = 7.7751 (± 0.0017),
α = γ = 90°, β = 103.0° (± 0.0015), V = 717.57$Å^3$ (± 2.8817)

These values will be used for comparative purposes during the discussion of the results found from co-crystallisation.

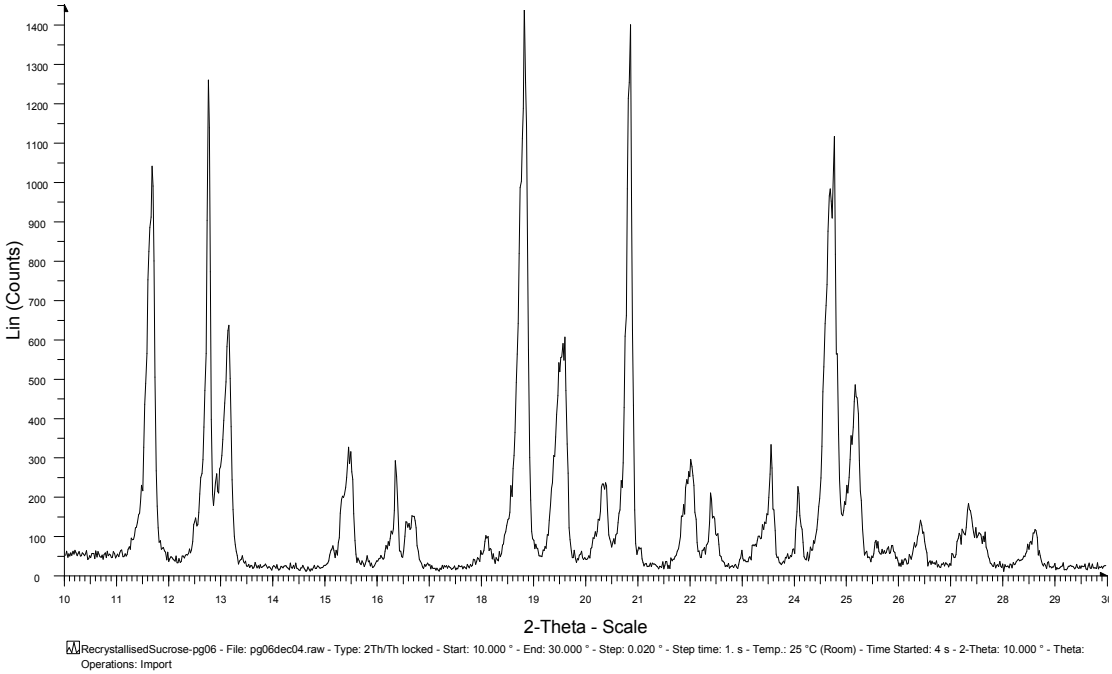


Fig 2. PXRD Diffractogram for Re-Crystallised Sucrose

4.2.2. Co-Crystallisation of Sucrose with Glucose

4.2.2.1. Physical Blends of Sucrose and Glucose Monohydrate

Physical blends of glucose, in varying ratios, involving materials mixed physically ground with sucrose have been studied by Maillet.[7] This work showed clearly a discernable phase for glucose monohydrate, anhydrous glucose and sucrose melting *v*ia DSC analysis, PXRD data showed predominantly reflections relating to sucrose; however, characteristic peaks relating to glucose could be observed at the 2θ positions of ~ 12°, 14° and 18°. Importantly, these data allow for a clear difference to be drawn between what is a co-crystalline material and what is a mixture of phases. What is also evident is that with increasing amounts of glucose present in the physical mixtures, the enthalpy of melting associated with glucose increases proportionally. Higher amounts of glucose equate to higher values for the enthalpy of melting.

4.2.2.2. Co-Crystalline Sucrose and Glucose: Series I

As the combination of sucrose and glucose is ubiquitous throughout confectionary products, the attempted inclusion of glucose into a sucrose matrix was seen a useful starting point, as outlined in Section 8.2.1. To the supersaturated sucrose solution varying amounts of α-D-glucose monohydrate (1, 2, 4, 7 and 10% w/w) were added to give materials that were white, dry and crystalline. The procedure was carried out three separate times in order to determine the reproducibility of the method. All materials were subjected to DSC scans (fig 3) at a rate of 10°C min^{-1} over a temperature range of -50°C-210°C.

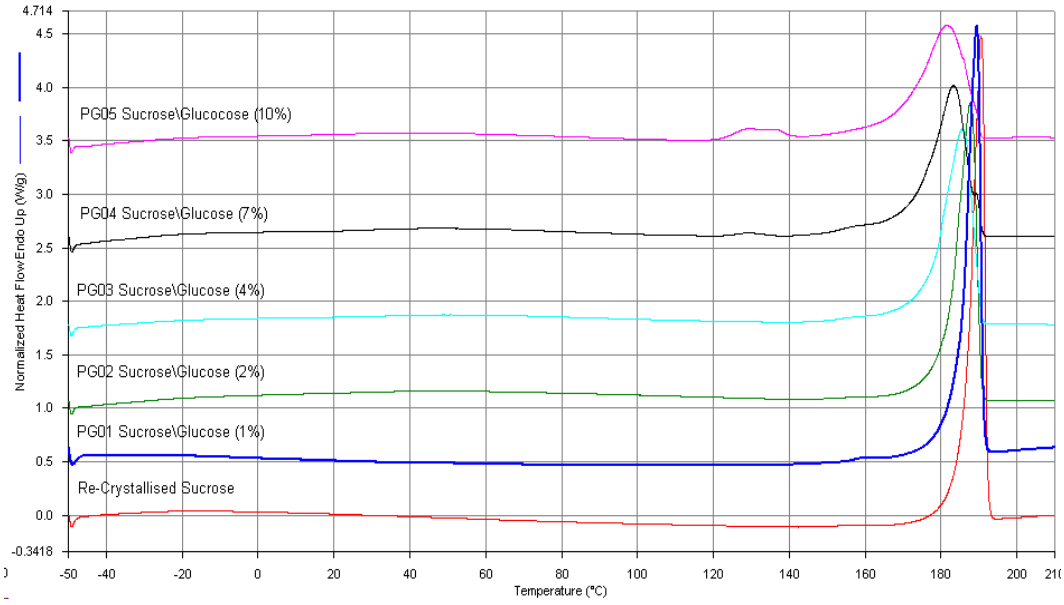


Fig 3. DSC Scans of Co-Crystalline Sucrose and Glucose Series I (1-10% w/w)

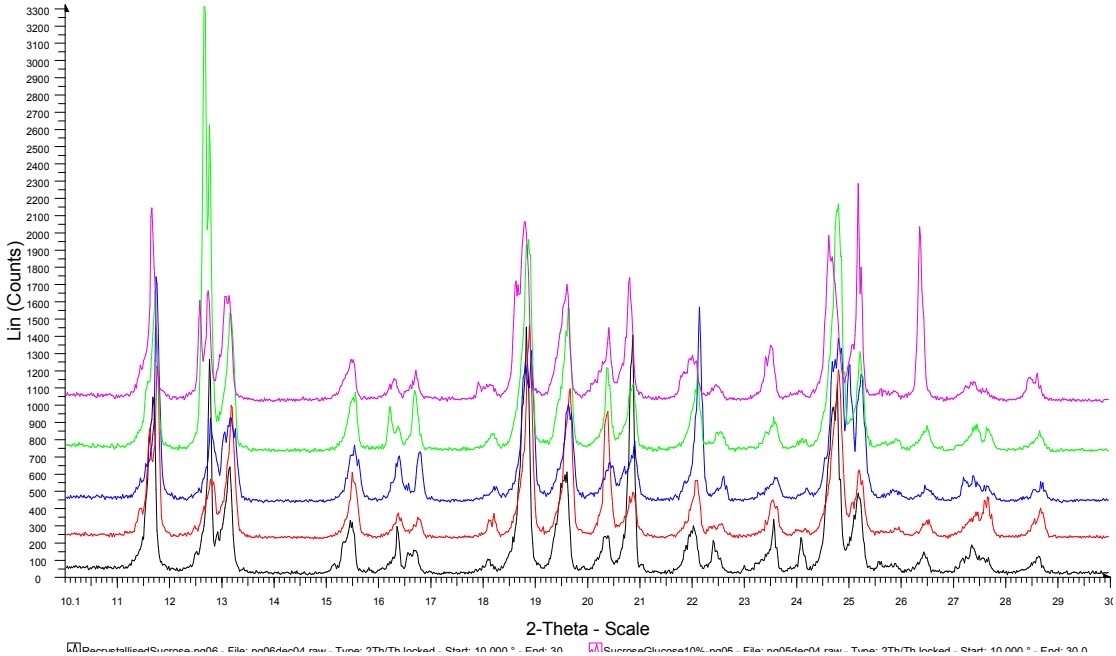
	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Re-crystallised Sucrose	----------	----------	186.004	123.334
Suc/Glu (1%)	----------	----------	184.845	118.173
Suc/Glu (2%)	----------	----------	180.926	110.989
Suc/Glu (4%)	----------	----------	176.468	103.745
Suc/Glu (7%)	124.720	0.890	173.758	92.203
Suc/Glu (10%)	122.574	6.308	169.181	94.434

Table 2. DSC data for Sucrose/Glucose Series I

The DSC traces (Fig 3) show that for co-crystallised sucrose and glucose, solely phases related to sucrose appear up to 7% w/w of glucose. Up to this level a single peak is observed with thermal behaviour that is indicative of only

sucrose melting. With increasing amounts of added glucose, the melting behaviour of sucrose alters. The onset and the values of ΔH become lower, which is accompanied by a broadening of the endotherm at ~ 190°C. Upon elevation of the quantity of included glucose to 7% w/w, a second endotherm appears at ~ 125°C that corresponds to glucose in its anhydrous form. The value of ΔH for this endotherm increases upon elevation of the amount of added glucose to 10% w/w.

Analysis by PXRD (Fig 4) of co-crystallised sucrose and glucose materials gave data that were in good agreement with DSC results. The addition of glucose up to a level of 7% w/w yielded diffractograms that are indicative of the presence of solely sucrose. This is confirmed by comparison of diffractograms obtained from analogous physical blends and re-crystallised sucrose. Upon reaching a level of 7% w/w of incorporated glucose, a separate anhydrous glucose phase was observed with a characteristic peak at the 2θ position ~ 12.6°. This peak can be attributed to the presence of a separate anhydrous glucose phase by comparison with physical blends and re-crystallised sucrose.



Black: 1% w/w Glucose, Red: 2% w/w Glucose, Blue, 4% w/w Glucose, Green: 7% w/w Glucose, Purple: 10% w/w Glucose

Fig 4. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose Materials

This emergence of a separate phase can be clearly seen upon magnification of the relevant area (Fig 5) of the diffractograms pattern.

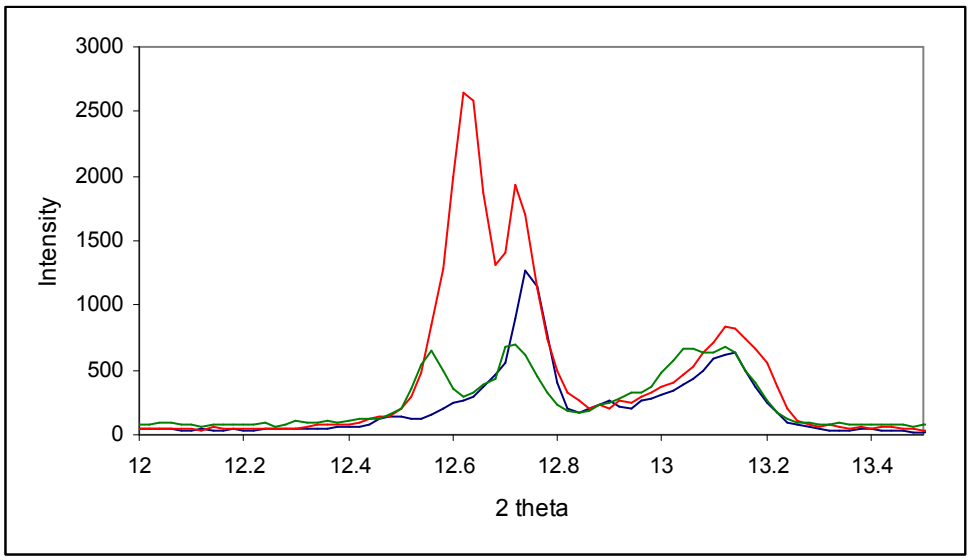


Fig 5. PXRD of Re-crystallised Suc (blue), Suc/Glu ((7% w/w) green) and Suc/Glu ((10% w/w) red)

Determination of the unit cell parameters was achieved by comparison with the sucrose unit cell calculated for re-crystallised sucrose, which was determined using literature values.[9] A plot of unit cell volumes (Fig 6) for the sucrose co-crystallised materials showed that the unit cell volumes were of a similar magnitude up to a level of 7% w/w of added glucose and a larger unit cell was observed for material containing 10% w/w of glucose. The material containing 10% w/w of glucose exhibited a possibly significantly larger unit cell as the calculated significance fell within a 95% confidence region.

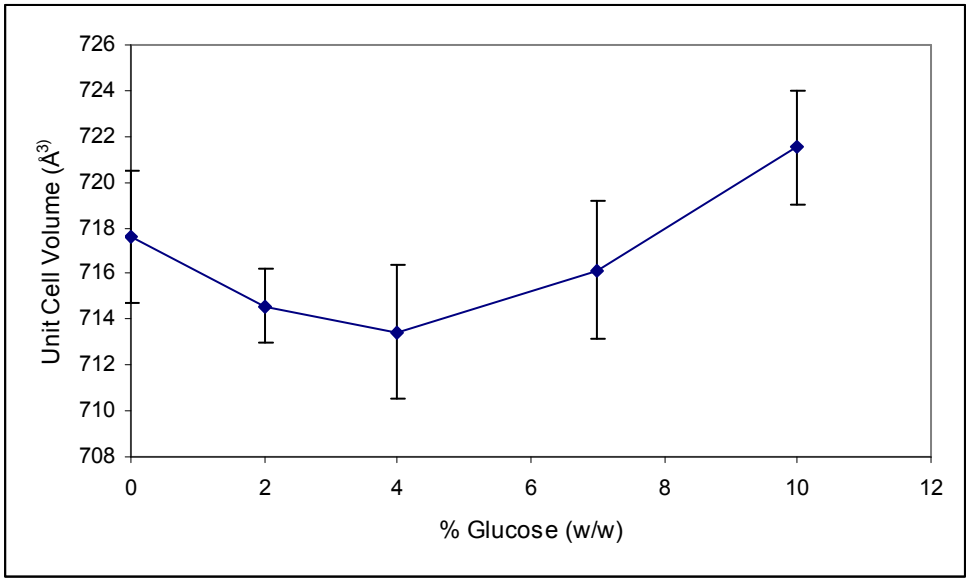


Fig 6. Unit Cell Volume *vs* Level of Incorporated Glucose for Sucrose-Glucose: Series I

4.2.2.3. Co-Crystalline Sucrose and Glucose: Series II

A second repetition of co-crystallising sucrose with glucose gave materials that were white, dry and crystalline. All the materials were subjected to DSC analysis (Fig 7) at a scan rate of 10°C min^{-1} over a range of -50-210°C.

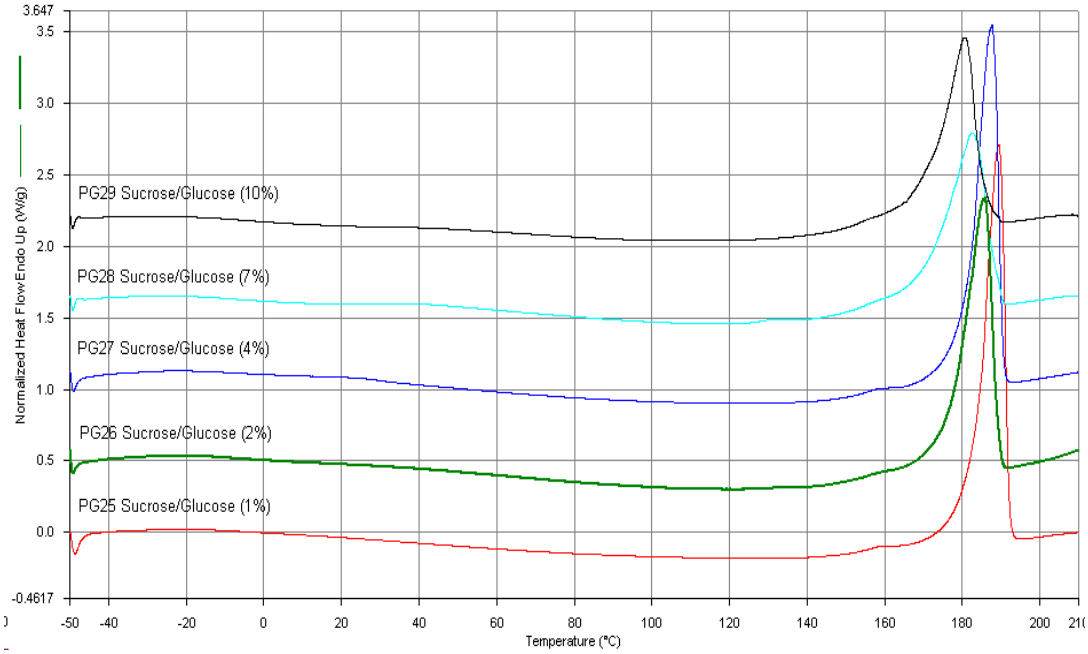


Fig 7. DSC Scans of Co-Crystalline Sucrose and Glucose: Series II (1-10% w/w)

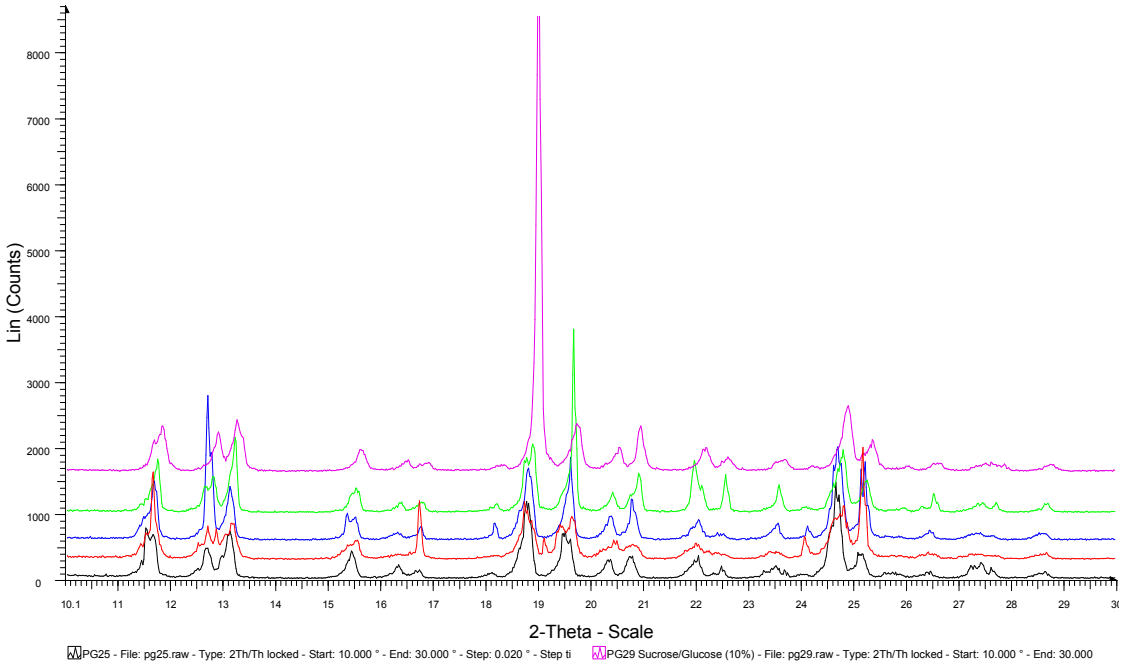
	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Suc/Glu (1%)	----------	----------	182.992	112.937
Suc/Glu (2%)	----------	----------	180.551	107.822
Suc/Glu (4%)	----------	----------	177.860	97.358
Suc/Glu (7%)	126.045	0.630	171.447	86.295
Suc/Glu (10%)	125.987	1.034	170.548	92.203

Table 3. DSC Data for Co-Crystalline Sucrose and Glucose: Series II

Data gleaned from analysis of the DSC traces showed that up to a level of 7% w/w of added glucose, the melting behaviour of the co-crystallised material was indicative of sucrose. The observation of solely an endotherm at ~ 190°C is indicative of sucrose. With the addition of glucose at a level of 7% w/w and above, a second endotherm emerges at ~ 125°C that is representative of anhydrous glucose. The enthalpy values for the endotherm relating to the melting of sucrose decreased with increasing amounts of glucose. This is accompanied by depression of the onset melting temperature and a broadening of the peak for material containing 1-7% w/w of glucose. Elevation to 10% w/w of added glucose resulted in an increase in ΔH compared to the material containing 7% w/w of glucose. The value of ΔH for the endotherm at ~ 125°C increased in value when the quantity of added glucose was elevated from 7% w/w to 10% w/w.

Analysis by PXRD (Fig 8) of co-crystallised sucrose and glucose materials gave data that were in good agreement with DSC results and with the

previous attempt at co-crystallising sucrose with glucose. The addition of glucose up to a level of 7% w/w gave diffractograms that were indicative of the presence of solely sucrose. This was confirmed by comparison to diffractograms obtained from analogous physical blends and re-crystallised sucrose. Upon reaching a level of 7% w/w of added glucose, a separate anhydrous glucose phase was observed with a characteristic peak at the 2θ position ~ 12.6°. This peak can be attributed to the presence of a separate anhydrous glucose phase by comparison with physical blends and re-crystallised sucrose.



Black: 1% w/w Glucose, Red: 2% w/w Glucose, Blue, 4% w/w Glucose, Green: 7% w/w Glucose, Purple: 10% w/w Glucose

Fig 8. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose Materials

Determination of the unit cell parameters was again achieved by comparison with the sucrose unit cell calculated for re-crystallised sucrose. A plot of unit cell volumes (Fig 9) for the sucrose co-crystalline materials showed that the unit cell volumes do not alter significantly compared to that for re-crystallised sucrose. The two unit cells that was larger than that for re-crystallised sucrose (sucrose containing 1% and 10% w/w of glucose) are not

siginificantly different. The calculated confidence levels fell outside of the 95% confidence region.

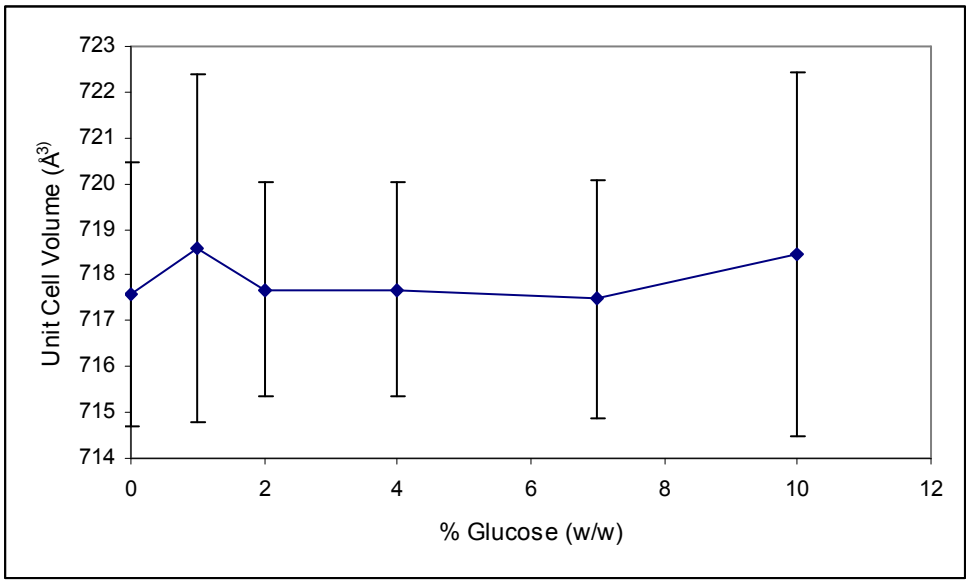


Fig 9. Unit Cell Volume vs. Level of Incorporated Glucose for Sucrose-Glucose: Series II

4.2.2.4. Co-Crystalline Sucrose and Glucose: Series III

A third repetition of co-crystallising sucrose with glucose yielded white, dry, crystalline materials that were analysed by DSC (Fig 10) at a scan rate of 10°C min^{-1} over a range of -50-210°C. In this repetition, the inclusion of 1% w/w of glucose was omitted as the material yielded was extremely similar to re-crystallised sucrose and as such was deemed un-necessary for analysis.

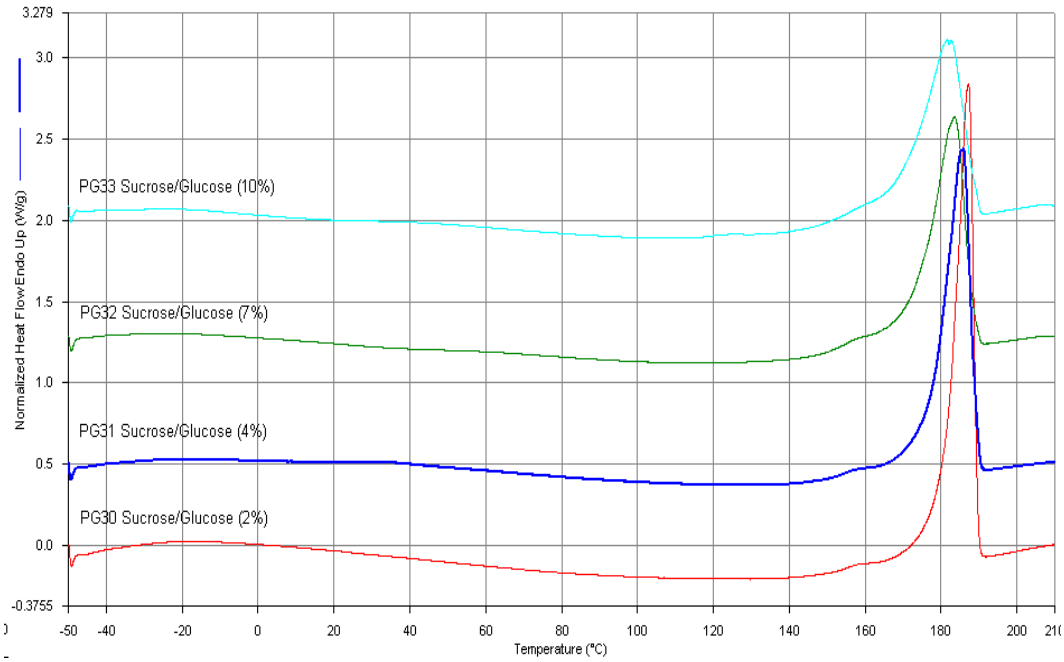


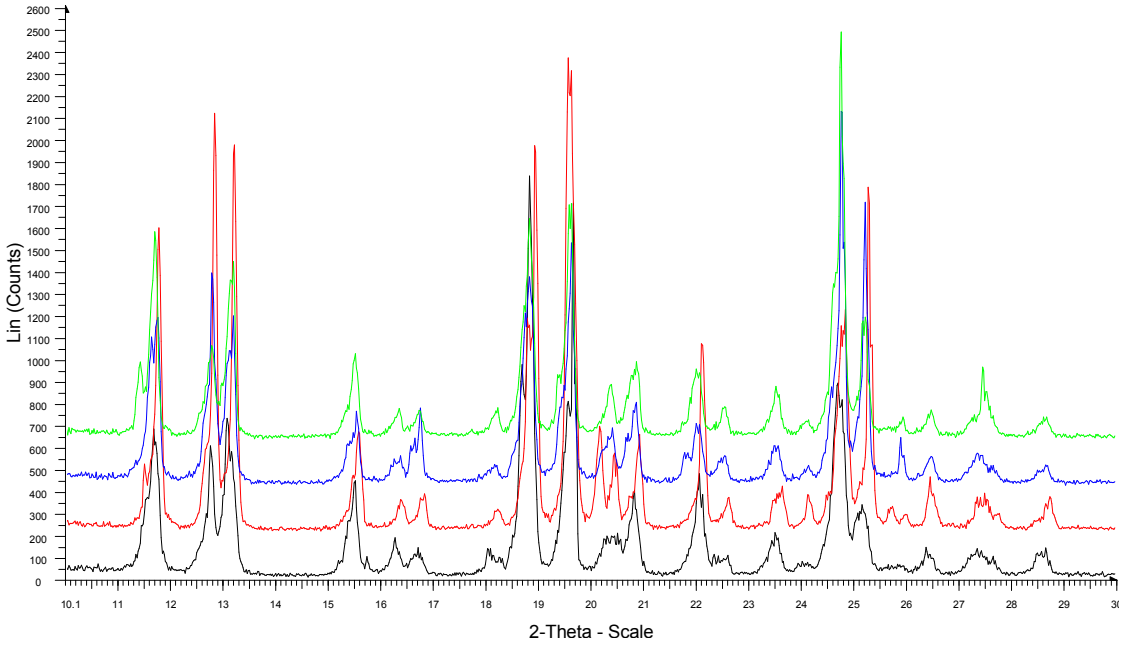
Fig 10. DSC Scans of Co-Crystalline Sucrose and Glucose Series III (1-10% w/w)

	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Suc/Glu (2%)	----------	----------	181.423	108.957
Suc/Glu (4%)	----------	----------	177.449	100.575
Suc/Glu (7%)	118.701	0.060	173.240	84.769
Suc/Glu (10%)	118.492	0.439	169.553	81.268

Table 4. DSC Data for Sucrose/Glucose: Series III

Data obtained from analysis of the DSC traces showed that up to a level of 7% w/w of added glucose, the melting behaviour of the co-crystallised material was indicative of sucrose with solely an endotherm at ~ 190°C. As observed previously, with the first two repetitions, the addition of glucose at a level of 7% w/w and above gave a second endotherm that emerged at ~ 125°C which is representative of anhydrous glucose. Again, enthalpy values for the endotherm relating to the melting of sucrose decreased with increasing amounts of glucose. This was accompanied by depression of the onset melting temperature and a broadening of the peak. The value of ΔH for the endotherm at ~ 125°C increased in value when the quantity of added glucose was elevated from 7% w/w to 10% w/w.

Subsequent analysis by PXRD (Fig 11) of co-crystalline sucrose and glucose materials gave data that were again in good agreement with DSC results and with the previous attempt (see sections 4.2.2.2 and 4.2.2.3) at co-crystallising sucrose with glucose. The addition of glucose up to a level of 7% w/w gave diffractograms that were indicative of the presence of solely sucrose, this is confirmed by comparison to diffractograms obtained from analogous physical blends and re-crystallised sucrose. Upon reaching a level of 7% w/w of added glucose, a separate anhydrous glucose phase was observed with a characteristic peak at the 2θ position ~ 12.6°. This peak can be attributed to the presence of a separate anhydrous glucose phase by comparison with physical blends and re-crystallised sucrose.



Black: 2% w/w Glucose, Red: 4% w/w Glucose, Blue: 7% w/w Glucose, Green: 10% w/w Glucose

Fig 11. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose Materials

Calculation of the unit cells (Fig 12) for materials obtained from the third repetition of the co-crystallisation of sucrose with glucose was achieved by comparison with the unit cell calculated for re-crystallised sucrose. The plot of unit cell volumes showed that with elevating amounts of glucose the volumes were tended to be smaller in size compared to solely sucrose, except for material

containing 2% w/w of glucose. However, whilst larger in volume, this increase is not significant as it falls outside of the 95% confidence region.

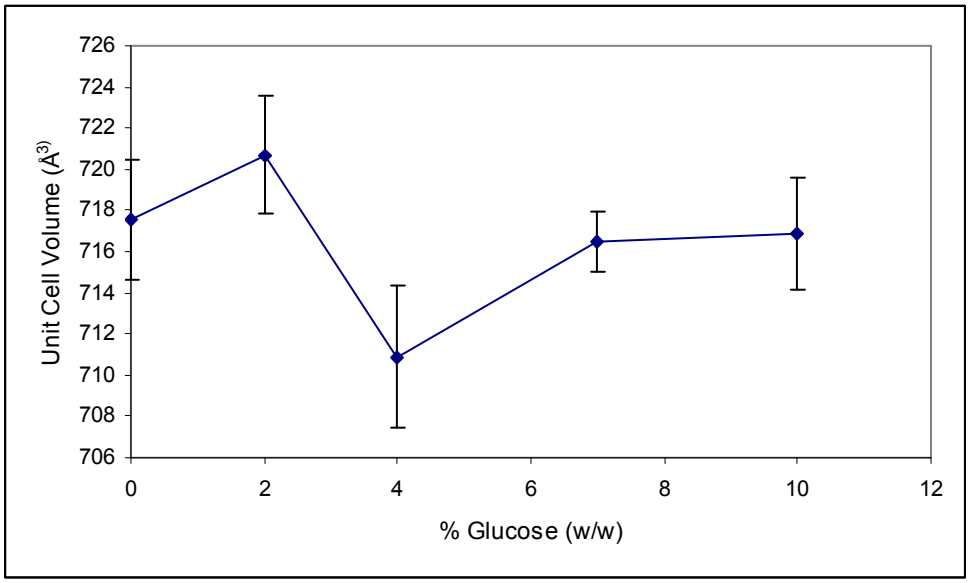


Fig.12 Unit Cell Volume vs. Level of Incorporated Glucose for Sucrose-Glucose: Series III

4.2.3. Co-Crystallisation at Different Temperatures

The supersaturation ratio and temperature of crystallisation influence the size, shape and properties of the sucrose crystals obtained from re-crystallisation solutions.[10] In order to investigate the effects that alter the final sucrose crystal and the subsequently formed co-crystalline material, the temperature at which the sucrose solution was formed was elevated and also lowered.

4.2.3.1. Co-Crystallisation of Sucrose and Glucose at 110°C

Co-crystallised material was formed using the methodology outlined in Section 8.2.3. The materials yielded were white, dry and crystalline, all materials were analysed by DSC at a scan rate of 10°C min^{-1} over a range of -50-210°C. The DSC data (Fig 13) for co-crystallised material formed at 110°C showed that up to a level of 7% w/w of added glucose, the melting behaviour of the material was indicative of sucrose alone.

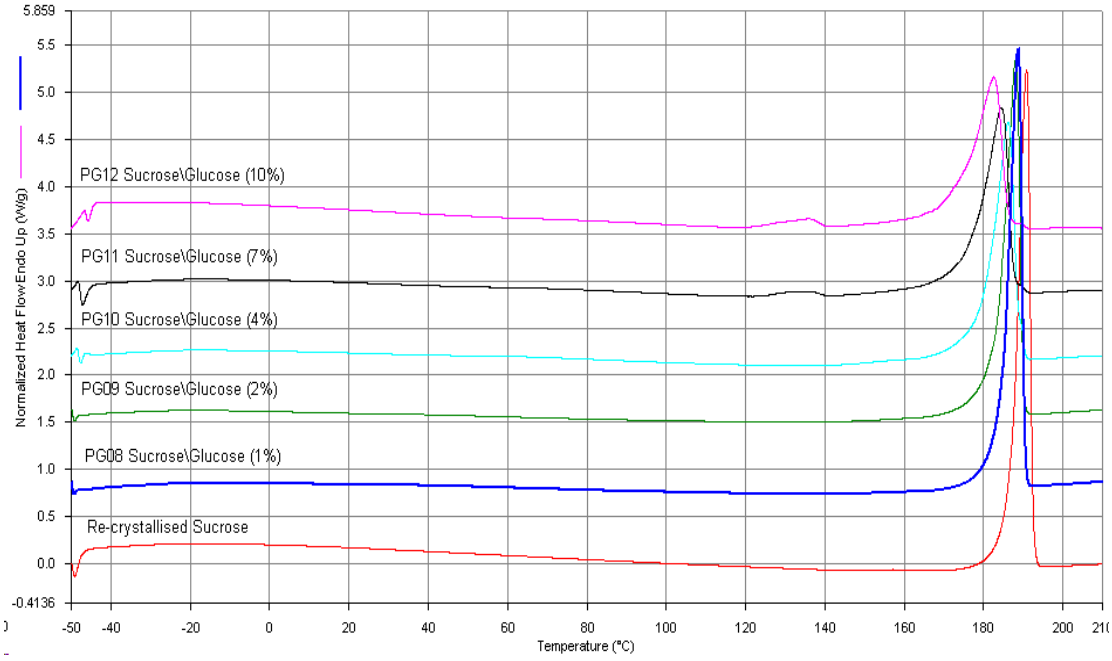


Fig 13. DSC Scan of Co-Crystalline Sucrose and Glucose Formed at 110°

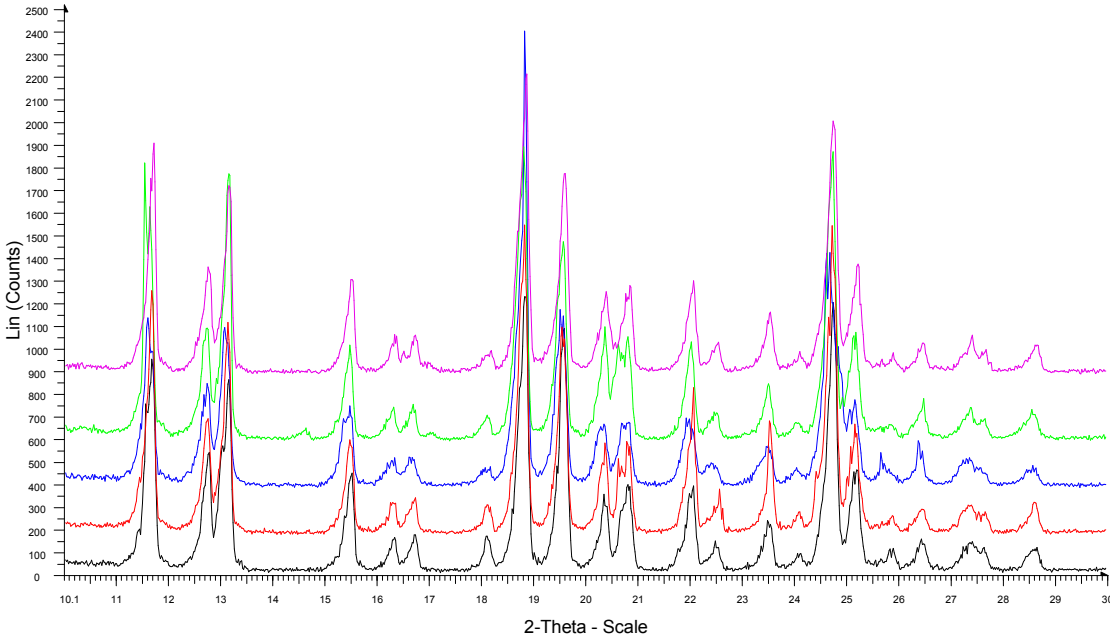
	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Re-crystallised Sucrose	---------- ----------	---------- ----------	186.969	126.550
Suc/Glu (1%)	----------	----------	184.407	122.629
Suc/Glu (2%)	----------	----------	183.190	116.105
Suc/Glu (4%)	----------	----------	179.435	104.934
Suc/Glu (7%)	125.080	2.853	176.313	103.993
Suc/Glu (10%)	124.066	4.359	173.423	95.768

Table 5. DSC Data for Co-Crystalline Sucrose and Glucose Formed at 110°C

At a level of 7% w/w and above of added glucose, DSC analysis revealed the presence of an emerging endotherm at ~ 125°C that corresponded to a separate anhydrous glucose phase. The enthalpy value for this peak increased upon elevation of the level of included glucose from 7 to 10% w/w. The enthalpy values for the endotherm relating to the melting of sucrose decreased with increasing amounts of glucose. This was accompanied by depression of the onset melting temperature and a broadening of the peak.

Analysis by PXRD (Fig 14) gave diffractograms that were in alignment with the observations noted in DSC analysis. The addition of up to 7% w/w of

glucose yielded diffractogram patterns that were indicative of crystalline sucrose. The addition of 7% w/w of glucose and beyond produced a diffractogram that was primarily crystalline sucrose, with the exception of peaks emerging at the 2θ positions ~ 12.6° and 14.0°. The appearance of these peaks suggests the presence of a separate anhydrous glucose phase accompanying the predominant crystalline sucrose phase.



Black: 1% w/w Glucose, Red: 2% w/w Glucose, Blue:4% w/w Glucose, Green: 7% w/w Glucose, Purple: 10% w/w Glucose

Fig 14. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose Formed at 110°C

Calculation of the unit cell volume (Fig 15) for the co-crystalline materials obtained, allowed comparison with solely re-crystallised sucrose. The plot showed that the unit cell volume increased generally with elevating levels of included glucose. Whilst these volumes tend to be larger, calculation of the significance of these elevations showed them to be not significant increases.

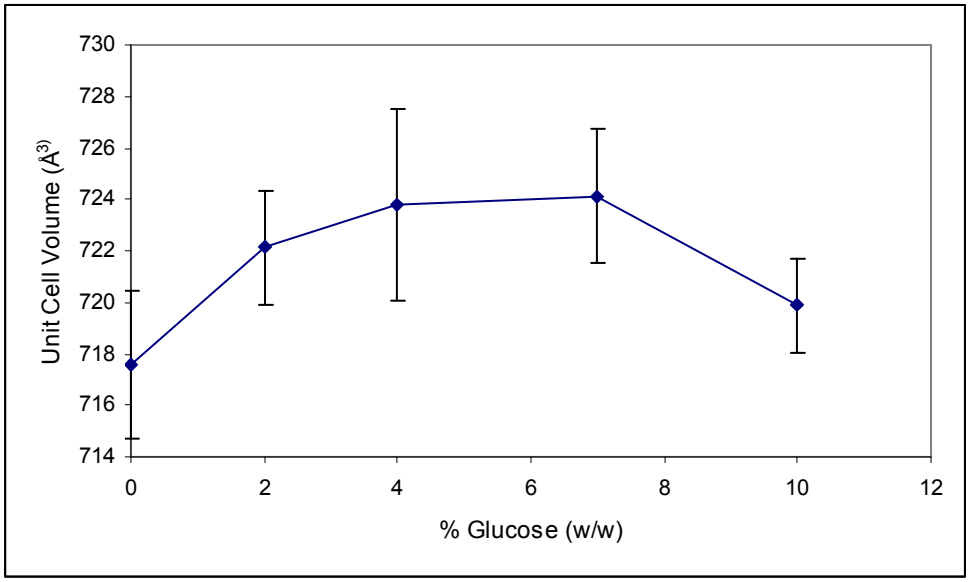


Fig 15. Unit Cell Volume vs. % Glucose for Co-Crystalline Materials formed at 110°C

4.2.3.2. Co-Crystallisation of Sucrose and Glucose at 120°C

Co-crystallised material was formed using the methodology outlined in Section 8.2.4. The materials yielded were white, dry and crystalline. All materials were analysed by DSC at a scan rate of 10°C min^{-1} over a range of -50-210°C. The DSC data (Fig 16) for co-crystalline material formed at 110°C showed that up to a level of 7% w/w of included glucose, the melting behaviour of the material was indicative of solely sucrose.

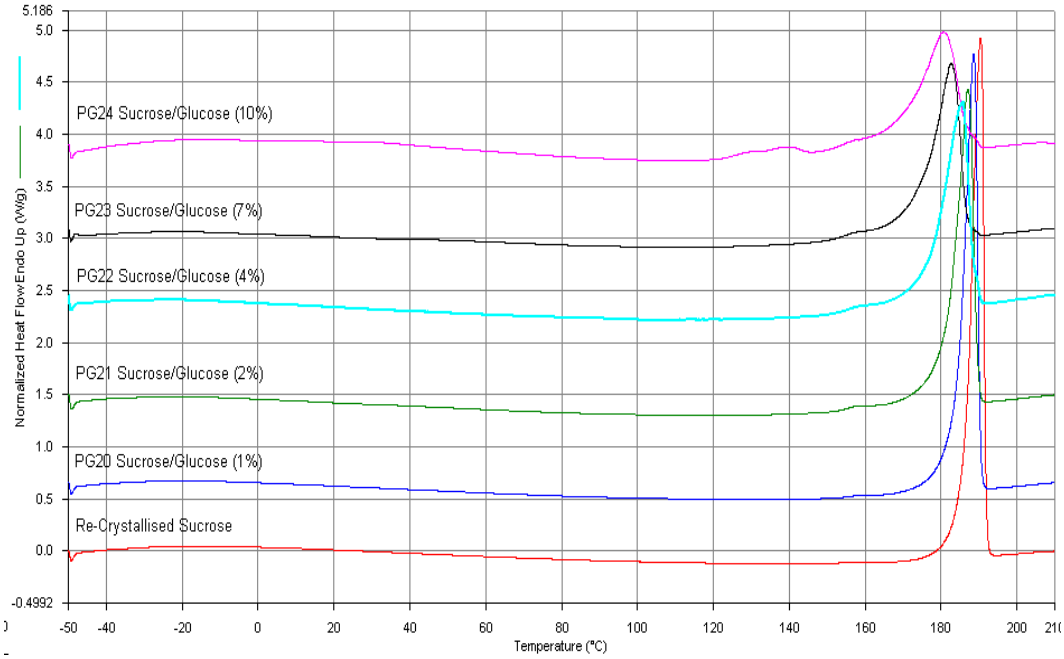


Fig 16. DSC Scan of Co-Crystalline Sucrose and Glucose Formed at 120°C

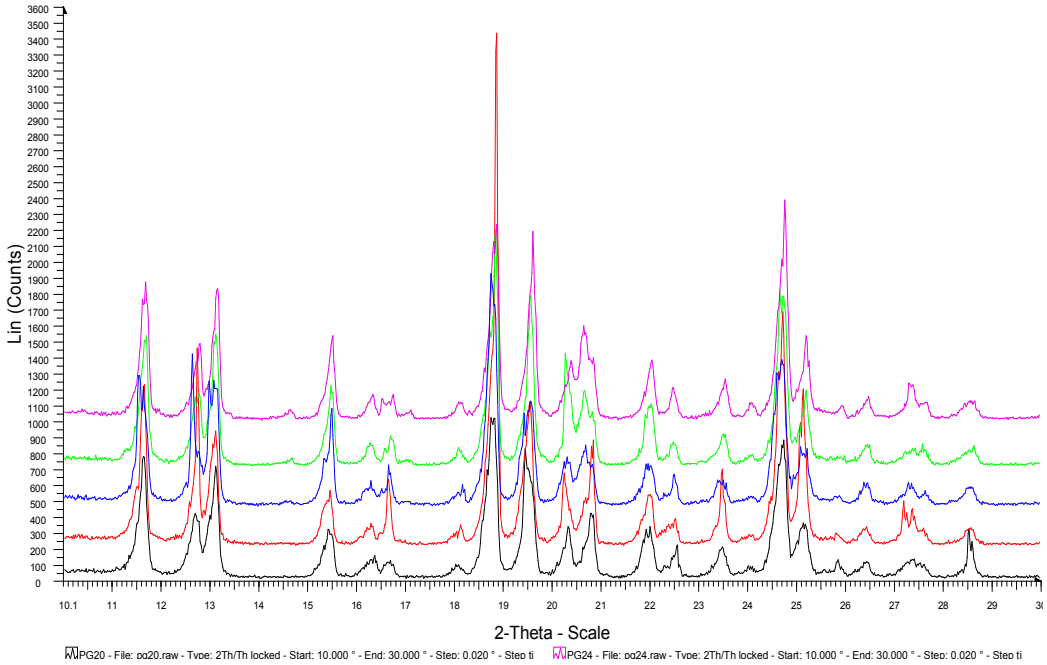
	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Re-crystallised Sucrose	----------	----------	186.098	125.241
Suc/Glu (1%)	----------	----------	183.995	118.394
Suc/Glu (2%)	----------	----------	182.062	110.465
Suc/Glu (4%)	----------	----------	177.110	98.419
Suc/Glu (7%)	124.125	1.789	175.760	87.760
Suc/Glu (10%)	123.046	3.645	170.482	76.928

Table 6. DSC Data for Co-Crystalline Materials Formed at 120°C

At a level of 7% w/w and above of added glucose, DSC analysis revealed the presence of an emerging endotherm at ~ 125°C that corresponded to a separate anhydrous glucose phase. The enthalpy value for this peak increased upon elevation of the level of included glucose from 7 to 10% w/w, though it must be noted that these values were lower than the ones obtained for the same endotherm when the materials were formed at 110°C. The enthalpy values for the endotherm relating to the melting of sucrose decreased with increasing amounts of glucose. This is accompanied by depression of the onset melting temperature and a broadening of the peak.

PXRD analysis (Fig 17) of the co-crystalline materials gave data that were in good agreement with DSC data. Solely materials indicative of sucrose were formed up to a level of 7% w/w of added glucose. At this level and above,

characteristic reflections emerge at the 2θ position of ~ 12.0°, which suggests the presence of glucose as a separate crystalline phase.



Black: 1% w/w Glucose, Red: 2% w/w Glucose, Blue, 4% w/w Glucose, Green: 7% w/w Glucose, Purple: 10% w/w Glucose

Fig 17. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose Materials formed at 120°C

PXRD data allowed the calculation of the unit cells for the co-crystallised materials (Fig 18) and they showed that all the materials exhibit larger unit cells compared to solely sucrose. However, these increases are not significantly different to the unit cell volume for re-crystallised sucrose. Calculation of the levels of significance showed that all the values for the larger unit cells fell outside of the 95% confidence level.

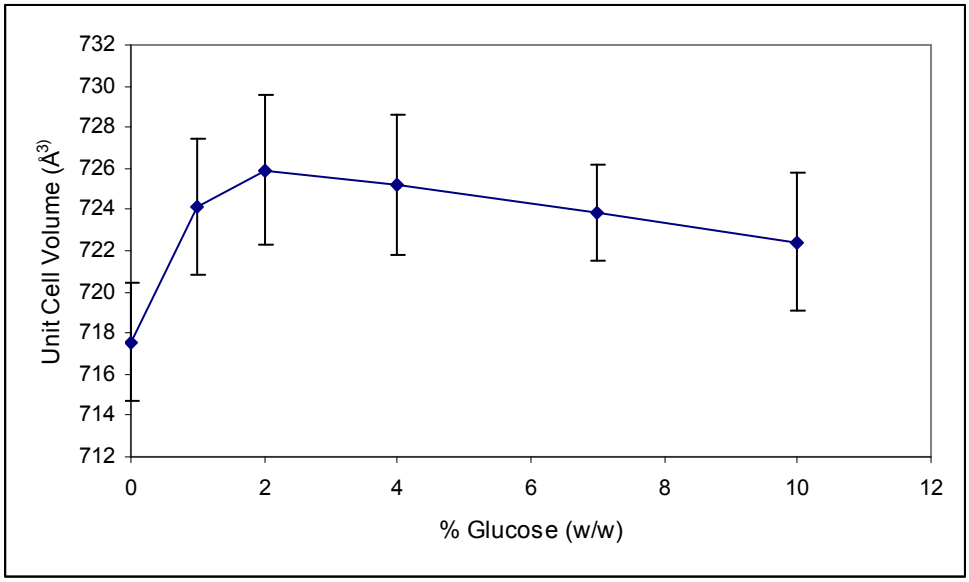


Fig 18. Unit Cell Volume vs. % Glucose for Co-Crystalline Materials formed at 120°C

4.2.4. Co-Crystallisation of Sucrose and Glucose on a Smaller Scale

In an effort to isolate crystals of the co-crystalline sucrose and glucose material, rather than a mixture of co-crystalline material and crystalline sucrose, the scale of the co-crystallisation was scaled down (see Section 8.2.5.)

The materials yielded were white, dry and crystalline; all materials yielded were subjected to DSC analysis (Fig 19) at a scan rate of 10°C min^{-1} over a range of -50-210°C.

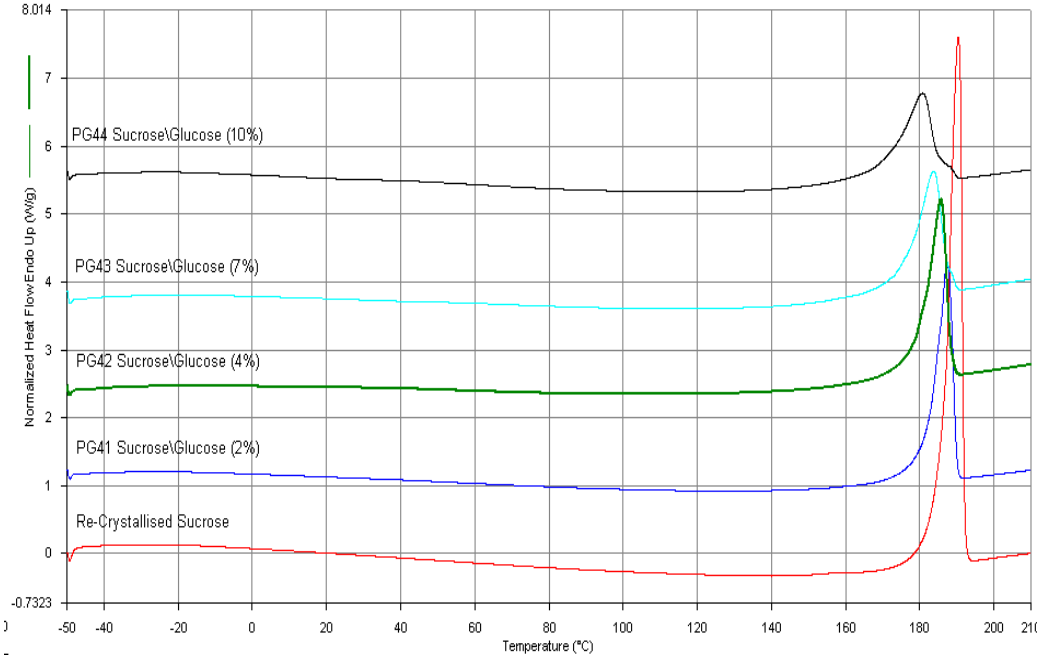


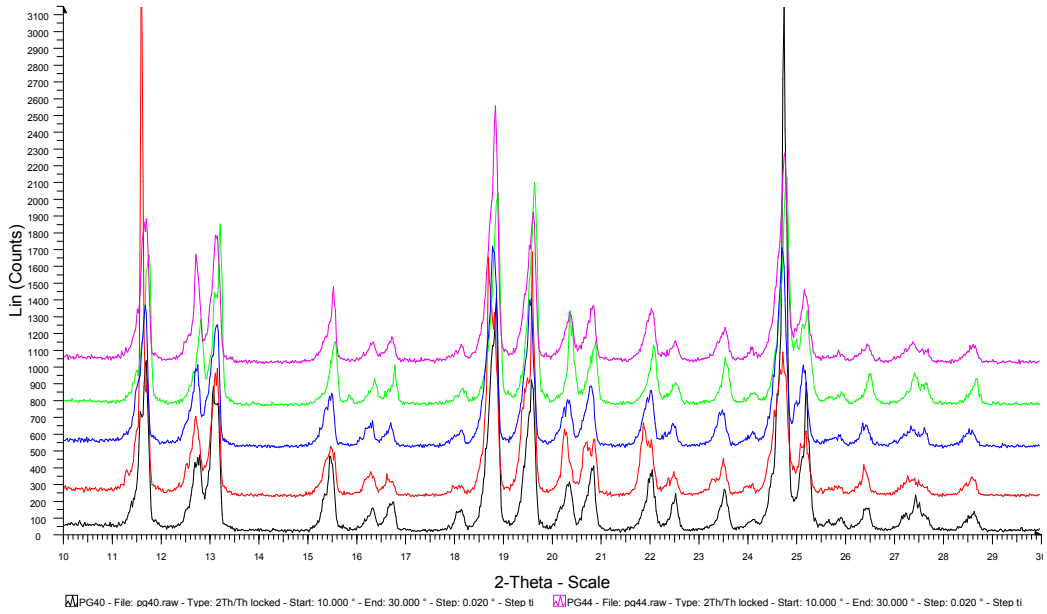
Fig 19. DSC Scan of Co-Crystalline Sucrose and Glucose Materials Formed on a Smaller Scale

	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Re-crystallised Sucrose	----------	----------	186.110	217.432
Suc/Glu (2%)	----------	----------	183.137	111.944
Suc/Glu (4%)	----------	----------	179.488	106.588
Suc/Glu (7%)	----------	----------	175.568	93.456
Suc/Glu (10%)	----------	----------	170.506	88.411

Table 7. DSC Data for Co-Crystalline Sucrose and Glucose formed on a Smaller Scale

Unlike co-crystallisations performed on a larger scale, utilisation of this methodology appeared to form solely sucrose related material. No melting of anhydrous glucose was observed when the level of added glucose either at 7% w/w 10% w/w. A slight undulation was noted at ~ 125°C for the material containing 10% w/w of glucose. However, no enthalpy could be calculated for this. All the DSC traces exhibited melting behaviour for sucrose with the expected endotherm peaking at ~ 190°C. The sucrose endotherm decreased in enthalpy with elevating levels of added glucose. This was accompanied by a depression of the onset melting temperature and a broadening of the endotherm.

Analysis of this range of materials by PXRD (Fig 20) confirmed the data obtained from DSC analysis. On the small scale, it appears if solely sucrose related materials were formed. No characteristic peaks suggesting the presence of anhydrous glucose could be identified from the observed diffractograms. All diffractograms gave reflections that were indicative of crystalline sucrose.



Black: Re-Crystallised Sucrose, Red: 2% w/w Glucose, Blue: 4% w/w Glucose, Green: 7% w/w Glucose, Purple: 10% w/w Glucose

Fig 20. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose formed on a Small Scale

Calculation of the unit cells for the materials (Fig. 21) prepared showed there was no linear trend between the amount of glucose added and the volume of the unit cell. The majority of the materials did exhibit a larger unit cells compared to solely sucrose however, except for the material containing 7% w/w of glucose. Calculation of the confidence levels for the observed increase in unit cell volume for materials containing 2% and 4% w/w of glucose showed these to be not significant elevations. Both of these fell values fall outside of the 95% confidence levels, as such they can not be deemed significant.

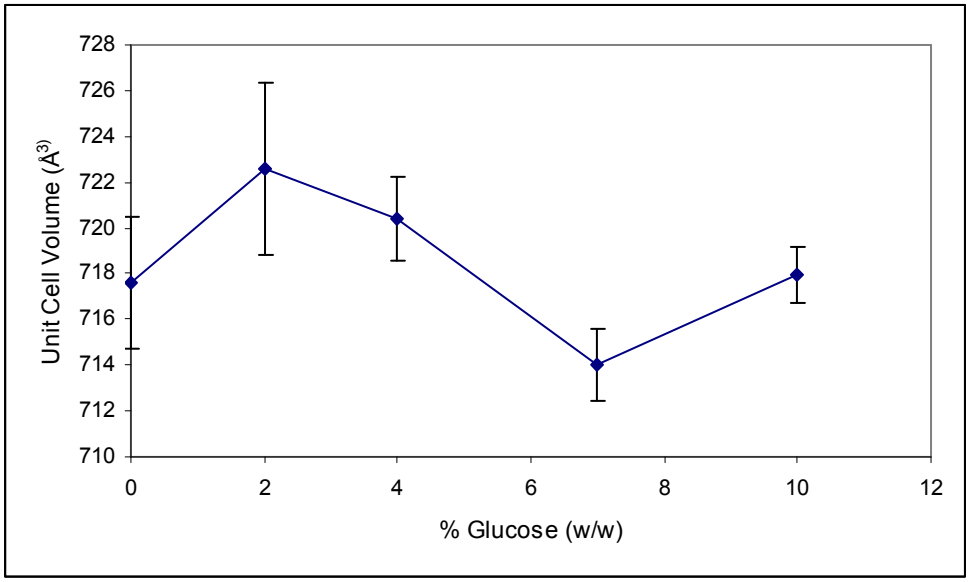


Fig 21. Unit Cell Volume vs. % Glucose for Co-Crystalline Materials formed on a Smaller Scale

4.2.5. Co-Crystallisation of Sucrose and Glucose from a Physical Blend

Modification of the co-crystallisation methodology in accordance with Section 8.2.6, showed differences in the character of the materials formed. DSC analysis (Fig 22) showed that solely materials indicative of sucrose were formed containing 4-13% w/w of glucose. Elevation of the quantity of glucose present to 15% w/w of glucose resulted in the emergence of an endotherm that corresponded to glucose along with the endotherm relating to sucrose.

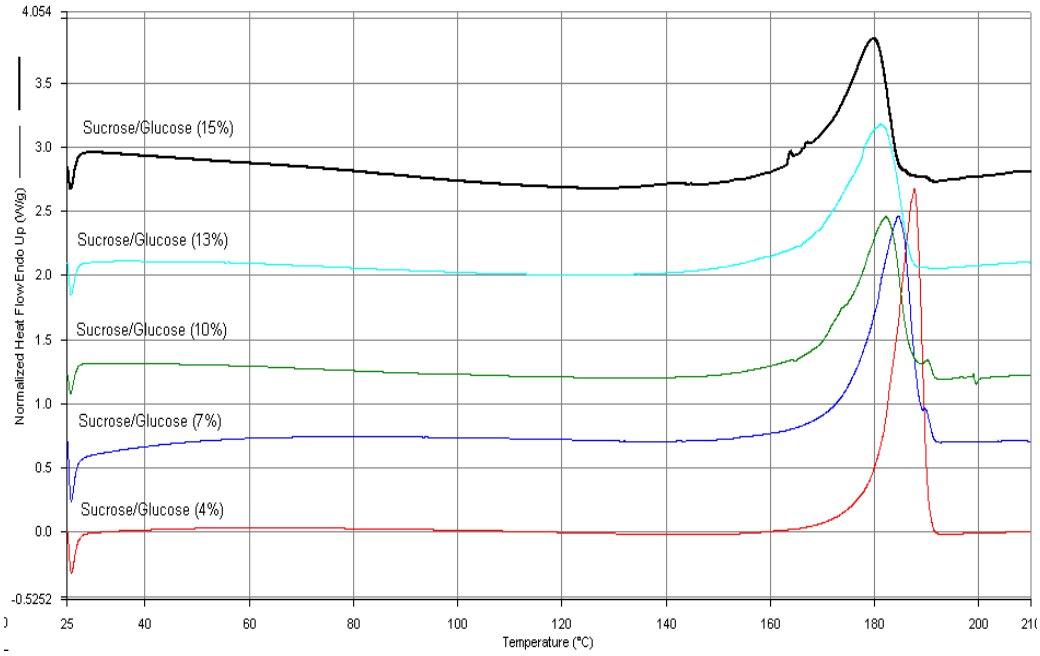


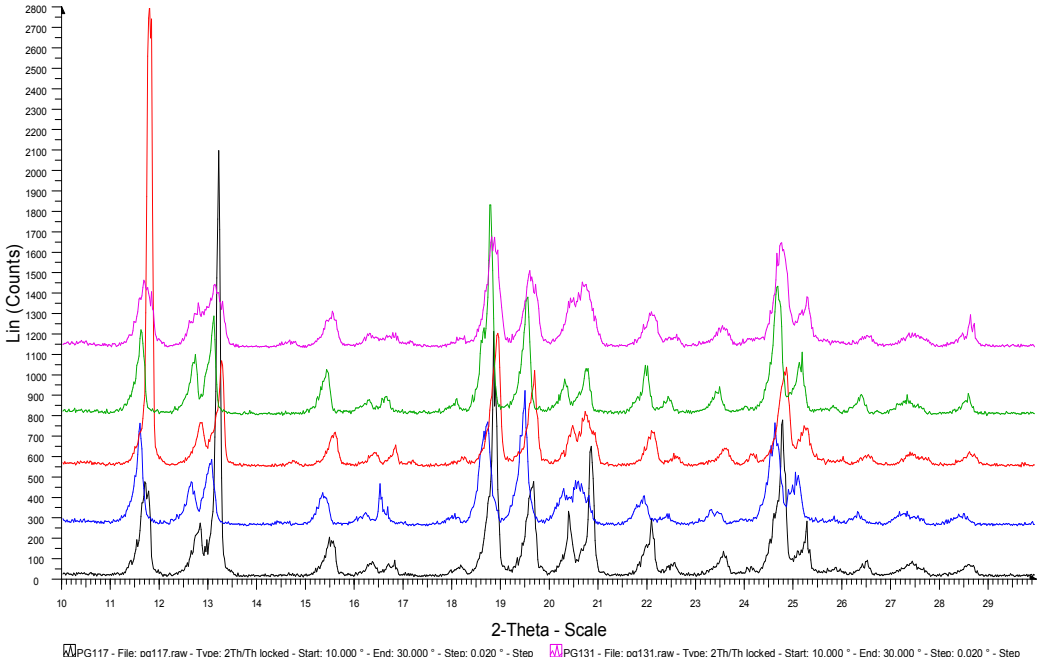
Fig 22. DSC Scans of Co-Crystalline Sucrose and Glucose Materials formed from a Physical Blend

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)
Suc/Glu (4%)	----------	----------	107.317	181.463
Suc/Glu (7%)	----------	----------	95.940	177.328
Suc/Glu (10%)	----------	----------	84.901	172.326
Suc/Glu (13%)	----------	----------	76.006	172.829
Suc/Glu (15%)	0.711	124.144	68.517	163.158

Table 8. DSC Data for Co-Crystalline Sucrose and Glucose Materials formed from a Physical Blend

The DSC data showed that a decrease in the value of ΔH for the melting of sucrose was proportional to the quantity of glucose within the material. Higher amounts of glucose induce lower enthalpy values.

PXRD analysis (Fig 23) gave results that were concordant with those obtained from DSC analysis. Glucose levels of 4-13% w/w gave diffraction patterns that were indicative of solely sucrose. Within these patterns, no peaks could be observed that suggested the presence of glucose as a separate phase. Elevation of the level of glucose present to 15% w/w resulted in a characteristic reflections emerging at the 2θ positions of ~ 12° and 14.5°. These peaks are symptomatic of a glucose presence.



Black: 4% w/w Glucose, Blue: 7% w/w Glucose, Red: 10% w/w Glucose, Green: 13% w/w Glucose, Purple: 15% w/w Glucose

Fig 23. PXRD Diffractograms of Co-Crystalline Sucrose and Glucose formed from a Physical Blend

PXRD data allowed the calculation of the unit cell (fig 24) volumes for materials sucrose and glucose materials formed from a physical blend.

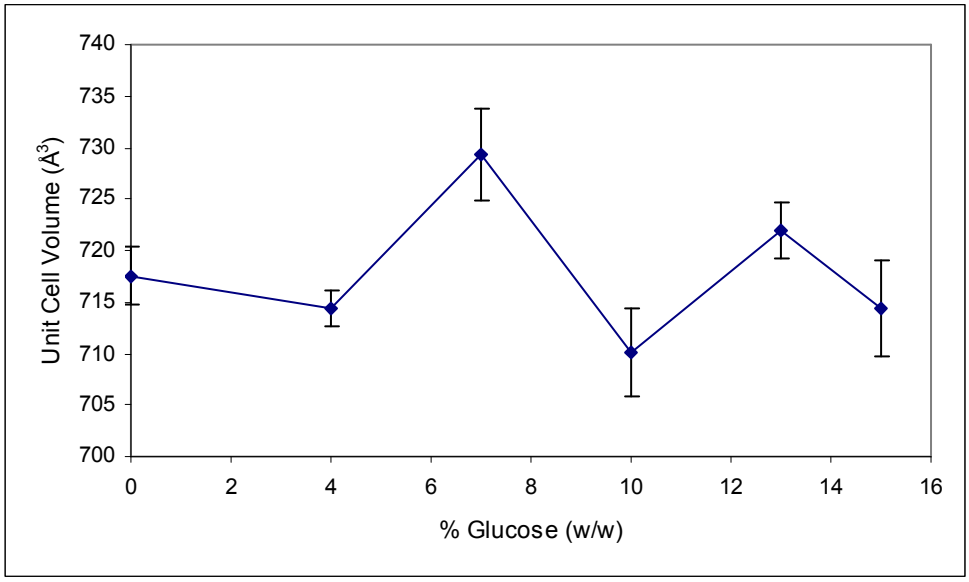


Fig 24. Unit Cell Volume vs. % Glucose

As can be seen from the above diagram, there is no real pattern to the unit cell volumes with changing amounts of glucose present. Sucrose with 7% and 13% w/w of glucose did show an increase in the unit cell volume, however this cannot be construed as significant. Calculation of the level of significance for these elevations showed that they both fell outside of the 95% confidence interval. All other materials showed similar unit cell volumes to co-crystallised sucrose.

4.2.6. Co-Crystallisation of Sucrose with Glucose Addition at 110°C

Addition of glucose at 110°C followed by subsequent heating to 128°C resulted in materials indicative of sucrose being formed up to 10% w/w of added glucose. Elevation of the amount of glucose to 13% and 15% w/w resulted in a unique form of sucrose to be formed.

DSC analysis (Fig 25) showed that for materials containing 4-10% w/w of glucose, solely the melting of sucrose was observed. Above this level, a sucrose phase was seen to melt that corresponds to a possibly unique form of sucrose. It appears that from the DSC data that this phase appears to related to a new form of sucrose found from work performed within this laboratory.[11]

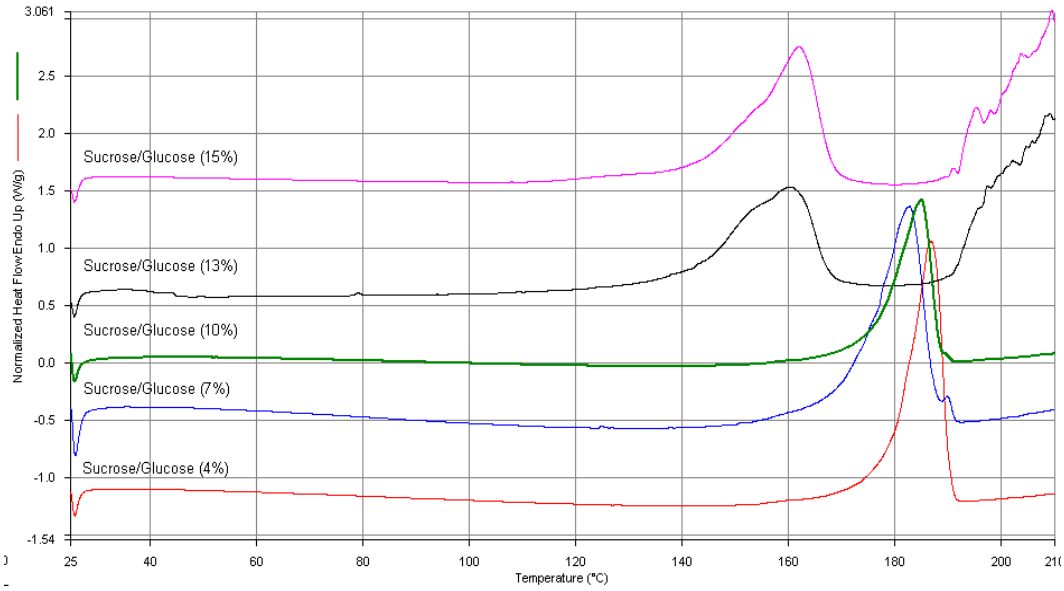


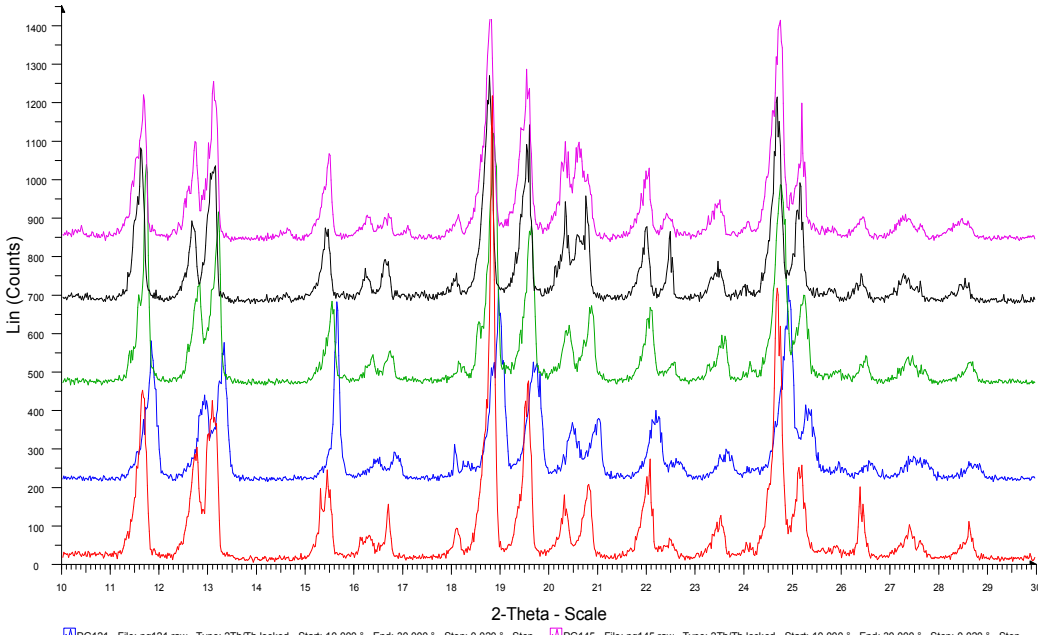
Fig 25. DSC Scans of Sucrose and Glucose Materials formed from Glucose Addition at 110°C followed by Heating to 128°C

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)
Suc/Glu (4%)	----------	----------	103.548	180.719
Suc/Glu (7%)	----------	----------	98.762	179.821
Suc/Glu (10%)	----------	----------	66.050	176.314
Suc/Glu (13%)	----------	----------	74.339	143.852
Suc/Glu (15%)	----------	----------	87.057	149.477

Table 9. DSC Data for of Sucrose and Glucose Materials formed from Glucose Addition at 110°C followed by Heating to 128°C

Before the formation of a possible sucrose hydrate,[11] the melting of sucrose decreased in enthalpy and onset temperature was proportional with the amount of added glucose. Elevation of the glucose level to 13% w/w and above yielded a hydrated form of sucrose. The enthalpy associated with this melt increases proportionally to the quantity of glucose added.

PXRD analysis (Fig 26) showed that all of the materials in this series exhibited a pattern that corresponded to sucrose and no characteristic peaks for the presence of glucose could be observed.



Red: 4% w/w Glucose, Blue: 7% w/w Glucose, Green: 10% w/w Glucose, Black: 13% w/w Glucose, Purple: 15% w/w Glucose

Fig 26. PXRD Diffractograms for Co-Crystalline Sucrose and Glucose Materials with Glucose Addition at 110°C

PXRD data allowed the calculation of the unit cell volumes (Fig 27) for these materials. The majority of materials showed an increase in unit cell volume compared to re-crystallised sucrose except for those with 7% and 10% of glucose present. The unit cell volumes seen to increase cannot be seen as significant elevations, all the data points related to these fall outside of the 95% confidence region.

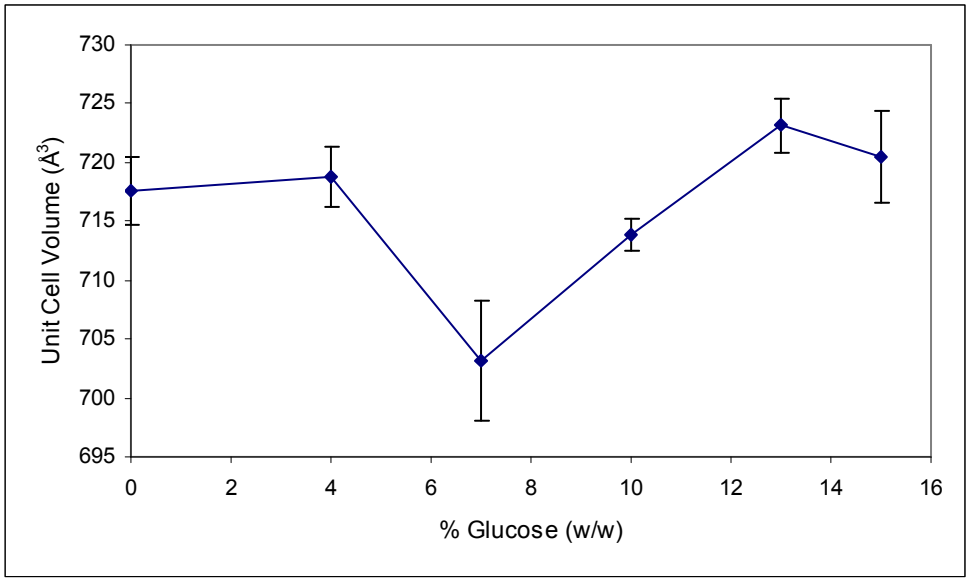


Fig 27. Unit Cell Volume vs. % Glucose for Co-Crystalline Sucrose and Glucose Materials with Glucose Addition at 110°C

4.2.7. Co-Crystallisation of Sucrose with Glucose and Fructose

Work performed by Maillet on the co-crystallisation of sucrose with a 1:1 w/w mixture of glucose and fructose showed that up to 15% w/w of each sugar was incorporable.[7] It appears that fructose facilitates the inclusion of glucose, to this end. Therefore, a study on how much fructose is required to induce facilitated uptake of glucose was undertaken. As shown in Section 4.2.1.2., the limit of inclusion for glucose was 7% w/w. This study endeavoured to show how much fructose is required to form solely sucrose relared materials.

4.2.7.1. Physical Blends of Sucrose with Glucose and Fructose

Physical blends of sucrose with glucose (7% w/w) and fructose (2-6% w/w) were subjected to DSC analysis (Fig 28) and all the traces allowed for facile differentiation between the three sugars present.

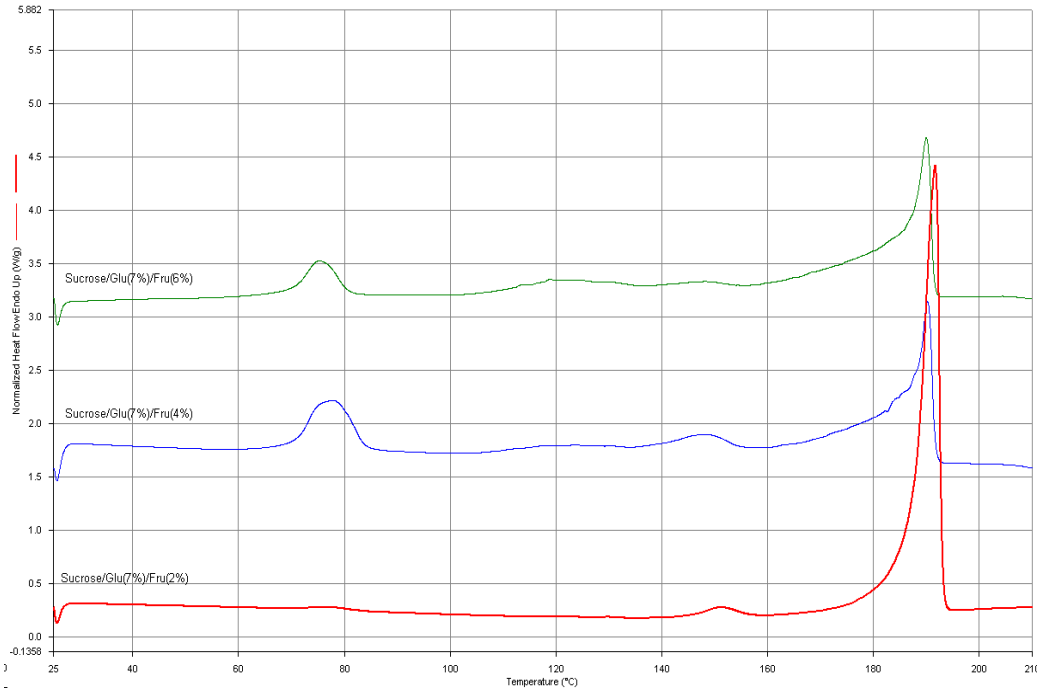


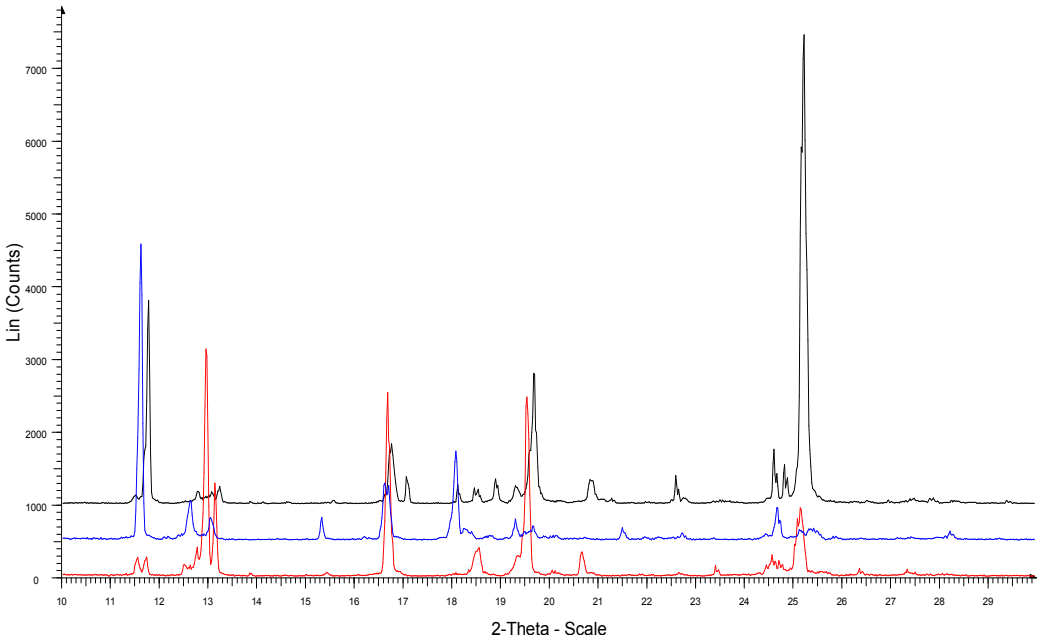
Fig 28. DSC Scans of Physical Blends of Sucrose with Glucose and Fructose

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Suc/Glu(7%) /Fru(2%)	0.513	75.502	2.316	147.292	112.413	187.453
Suc/Glu(7%) /Fru(4%)	2.195	71.1	5.447	140.618	59.941	187.412
Suc/Glu(7%) /Fru(6%)	9.999	70.953	1.946	138.506	59.622	186.589

Table 10. DSC Data for Physical Blends of Sucrose with Glucose and Fructose

DSC analysis clearly showed characteristic melting of glucose monohydrate at ~ 70°C which was followed by fructose at ~ 140°C and finally sucrose melting at ~ 187°C.

The PXRD analysis (Fig 29) of the physical blends of sucrose, glucose and fructose gave diffractograms that allowed for an easy differentiation between the three phases. Whilst the majority of the reflections could be accorded to sucrose, peaks at the 2θ positions of ~ 12.5, 14.5° and 17.5° were suggestive of glucose, whilst at the 2θ positions of ~ 14°, 17.2° and 28.2° were symptomatic of fructose.



Red: 7% w/w Glucose & 2% Fructose w/w, Blue: 7% w/w Glucose & 4% Fructose w/w, Black: 7% w/w Glucose & 6% Fructose w/w

Fig 29.PXRD Diffractograms of Physical Blends of Sucrose, Glucose and Fructose

The diffractograms obtained were unrelated to sucrose, this is highly unusual and unexpected. Due to the complexity of the multiple phases present in the diffractograms, no meaningful data on the unit cell volumes could be extracted. The important observation from this data is the ability to discern between sucrose, glucose and fructose from peak positions.

4.2.7.2. Co-Crystallisation of Sucrose with Glucose and Fructose

DSC analysis (Fig 30) of co-crystallised sucrose with glucose (7% w/w) and fructose (2-6% w/w) showed that all materials exhibited melting behaviour of solely sucrose.

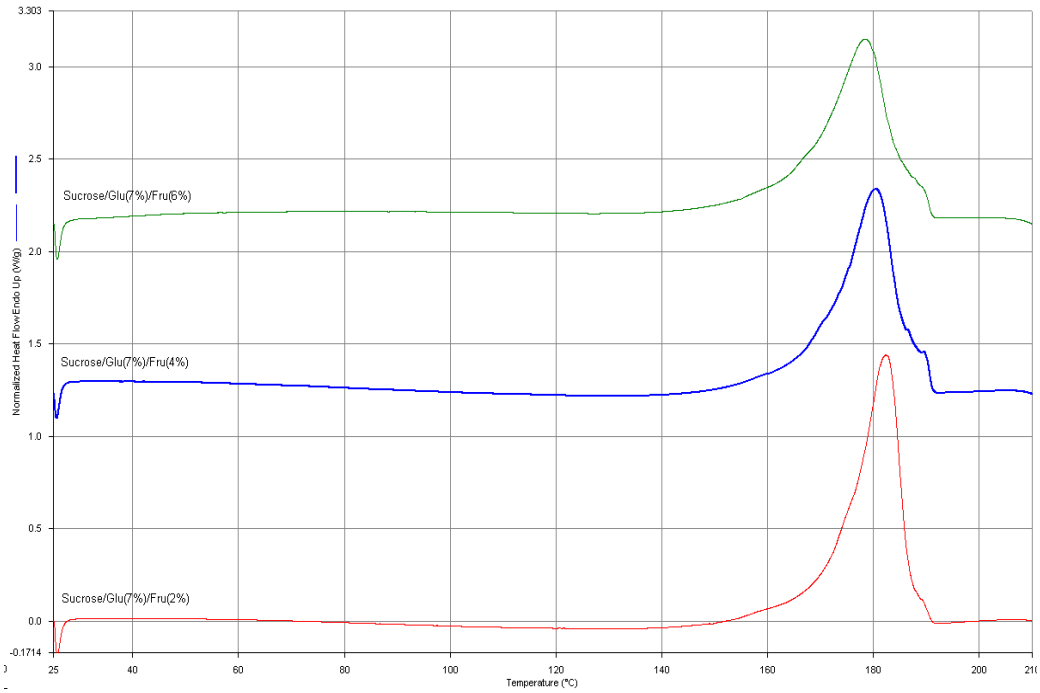


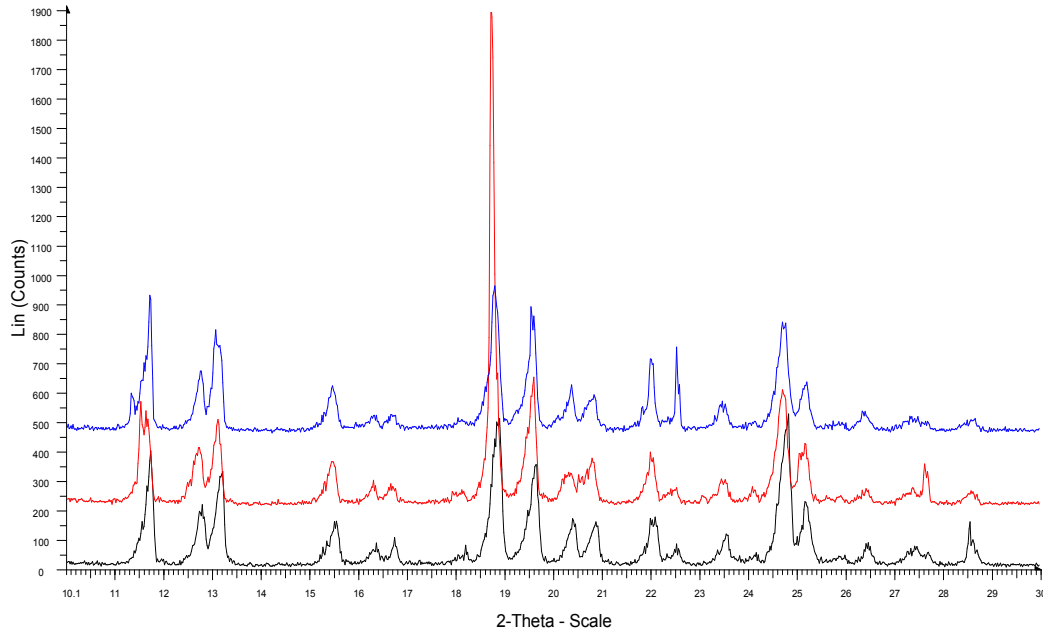
Fig 30. DSC Traces of Co-Crystalline Sucrose, Glucose and Fructose Materials

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Suc/Glu(7%) /Fru(2%)	--------	----------	--------	----------	91.142	170.413
Suc/Glu(7%) /Fru(4%)	--------	----------	--------	----------	85.207	170.013
Suc/Glu(7%) /Fru(6%)	--------	----------	--------	----------	76.312	166.225

Table 11. DSC Data for Co-Crystalline Sucrose, Glucose and Fructose Materials

With increasing amounts of the added monosaccharides, the decrease in the value of ΔH and the onset temperature was more pronounced. No melting of either glucose or fructose could be observed.

PXRD analysis (Fig 31) gave results that agrred with those obtained from DSC analysis. For all levels of added glucose and fructose, solely sucrose related materials were observed. The diffractograms generated from the materials showed reflections that were indicative of sucrose. No peaks could be observed that suggest the presence of either glucose or fructose.



Red: 7% w/w Glucose & 2% Fructose w/w, Blue: 7% w/w Glucose & 4% Fructose w/w, Black: 7% w/w Glucose & 6% Fructose w/w

Fig 31. PXRD Diffractograms for Co-Crystalline Sucrose, Glucose and Fructose Materials

From the PXRD data the unit cell volumes for the co-crystalline materials were calculated (Fig 32) and showed that materials containing 4% and 6% of fructose exhibited larger unit cells than solely sucrose. These elevations cannot be seen to be significant. The data associated with it can be calculated to fall outside of the 95% confidence region.

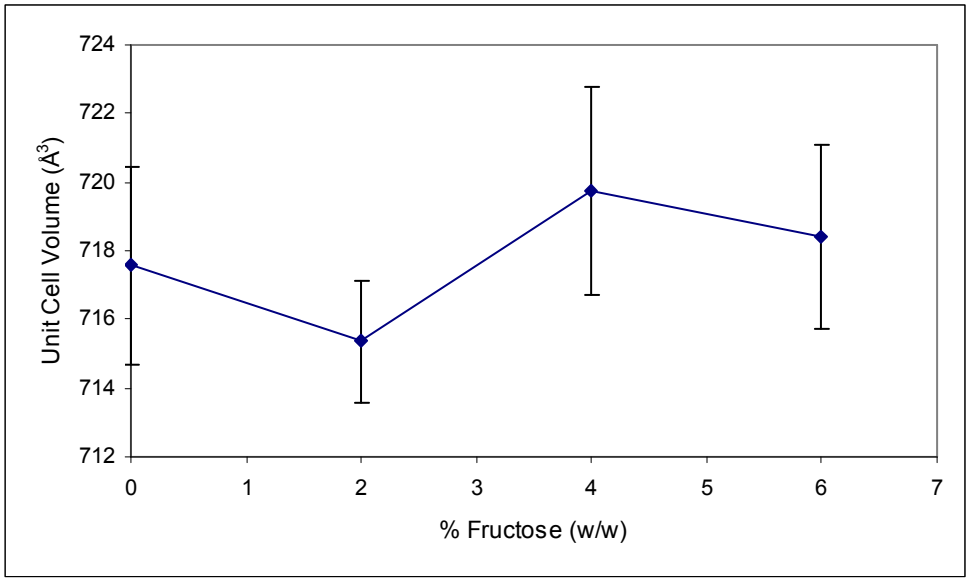


Fig 32. Unit Cell Volumes vs. % Fructose for Co-Crystalline Sucrose, Glucose and Fructose Materials

4.2.8. Co-Crystallisation of Sucrose with Galactose from a Physical Blend

Co-crystallisation from a physical blend appears to allow higher levels of added sugars whilst retining single phases. The extent to how much this can influence the addition of a monosaccharide component unrelated to sucrose was investigated. Galactose was chosen as the candidate for this study. It is a C4 epimer of glucose and has been shown to include within a sucrose structure poorly by Maillet.[7] To this end, the modification was tested to ascertain how well it can be included.

4.2.8.1. Physical Blends of Sucrose and Galactose

Prior to co-crystallisation, physical blends of the two materials were formed in order to enable the distinction between a co-crystalline material and a mixture obtained from physically mixing compounds together. DSC analysis (Fig 33) allowed differentiation between galactose and sucrose. Galactose was seen to be melting at ~ 145°C and sucrose was seen to melt at ~ 170°C. Also, the melting enthalpy for galactose increased with higher amounts of the monosaccharide present.

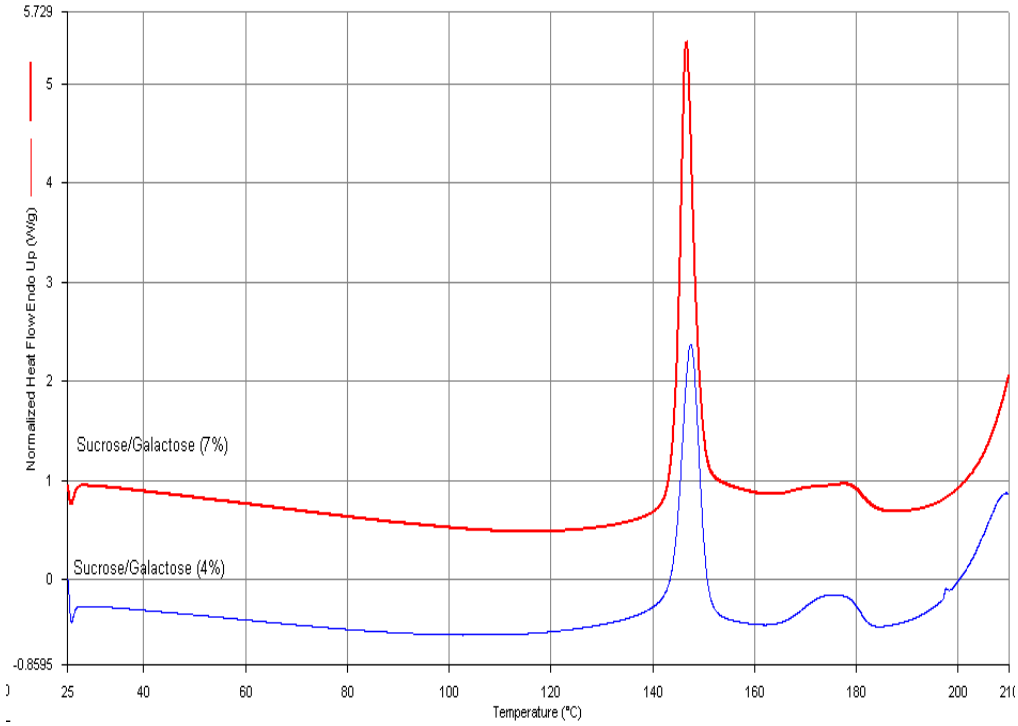


Fig 33. DSC Scans for Physical Blends of Sucrose and Galactose

	ΔH 1 (Jg^{-1})	Onset 1 (°C)	ΔH 2 (Jg^{-1})	Onset 2 (°C)
Suc/Gal (4%)	64.402	144.068	20.573	166.134
Suc/Gal (7%)	98.193	144.368	14.632	164.562

Table 12. DSC Data for Physical Blends of Sucrose and Galactose

Due to a mix up with sample labelling, PXRD data for physical blends of sucrose and galactose were rendered meaningless. It appears that a mixture of lactose and galactose was analysed inadeverdently. However, by using Powdercel, suggestive peaks for galactose can be ascertained. Overlapping model patterns for both sucrose and galactose shows that characteristic peaks for galactose are likely to emerge at the 2θ positions of 11.2° and 15.4°.

4.2.8.2 Co-Crystallisation of Sucrose and Galactose from a Physical Blend

Co-crystallisation of sucrose with galactose proceeding from a physical blend of the two sugars yielded results that were completely unexpected. Typically, co-crystallisation yields materials that are either; indicative of solely the host phase (e.g. sucrose) or a mixture of the host phase and a phase relating to

the added material.[7] DSC analysis (Fig 34) of these materials showed the formation of a suspected hydrated form of sucrose.[11] The melting behaviour did not resemble the melting of either sucrose or galactose.

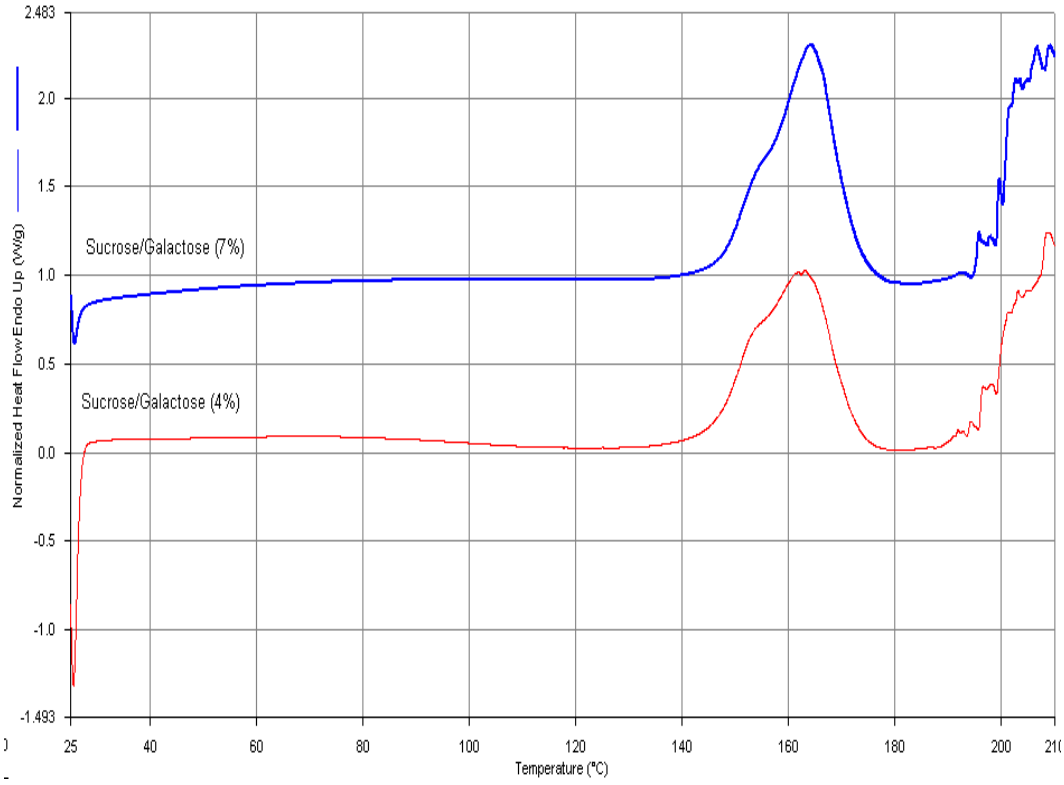


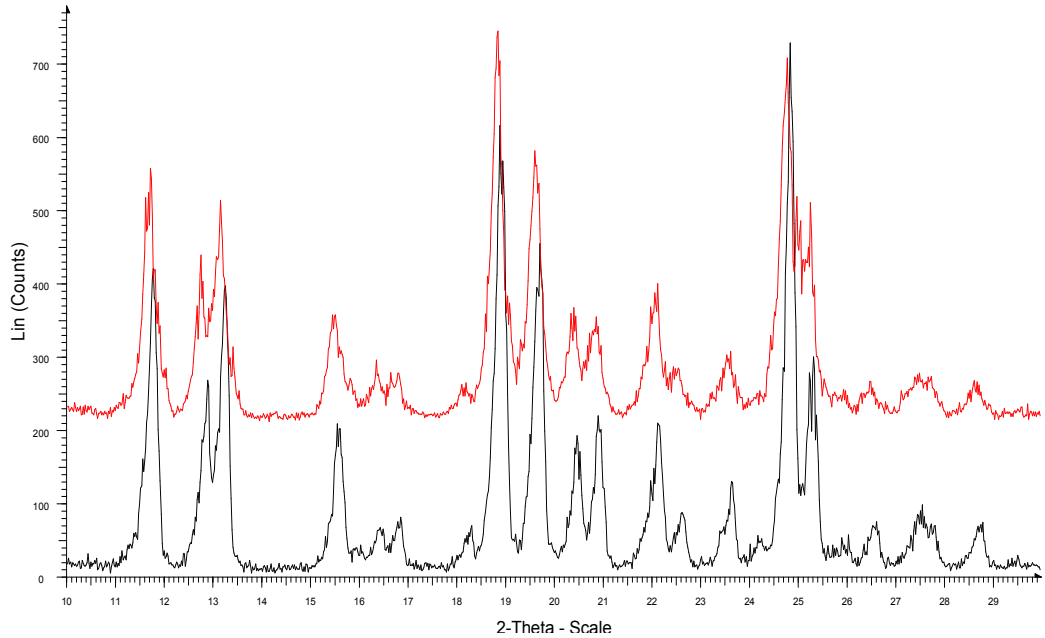
Fig 34. DSC Traces of Co-Crystalline Sucrose and Galactose Materials formed from a Physical Blend

	ΔH 1 (Jg^{-1})	Onset 1 (°C)	ΔH 2 (Jg^{-1})	Onset 2 (°C)
Suc/Gal (4%)	----------	----------	96.645	146.864
Suc/Gal (7%)	----------	----------	112.844	151.380

Table 13. DSC Data for Co-Crystalline Sucrose and Galactose formed from a Physical Blend

PXRD analysis (Fig 34) showed that the materials exhibited reflections that are highly suggestive towards the presence of solely sucrose and no reflections for galactose could be observed. Data obtained from the diffractograms allowed the calculation of the unit cell volumes for these materials (fig 35). This showed that the co-crystalline material containing 4% w/w of galactose had a smaller unit cell volume compared to sucrose. Elevation

of the level of added galactose to 7% w/w resulted in a similar unit cell volume to re-crystallised sucrose.



Black: Galactose 4% w/w, Red: 7% Galactose w/w

Fig 34. PXRD Diffractograms for Co-Crystalline Sucrose and Galactose Materials formed from a Physical Blend

Fig 35. Unit Cell Volume vs. % Galactose for Co-Crystalline Sucrose and Galactose Materials formed from a Physical Blend

4.3. Discussion

4.3.1. Re-Crystallisation of Sucrose

The re-crystallisation of sucrose from water yielded a material that showed little difference in the expected melting points by DSC analysis (see section 4.2.1). Furthermore, the reflections seen from PXRD analysis were identical in position to those expected for sucrose. From this it can be confidently stated that re-crystallisation of sucrose in this manner induces little change in the physical properties of the material.

4.3.2. Co-Crystallisation of Sucrose and Glucose

For all three studies (sections 4.2.2.2, 4.2.2.3 and 4.2.2.4) of the addition of glucose to a supersaturated sucrose solution, a limit of addition of 7% w/w of added glucose was observed by both DSC and PXRD analysis.

Analysis of data obtained from DSC analysis showed (see sections 4.2.2.2, 4.2.2.3 and 4.2.2.4) that when glucose is introduced to a supersaturated sucrose solution, the enthalpy associated with the melting of sucrose decreases proportionally with added glucose. Higher amounts of added glucose generally induce higher decreases in the value for ΔH for the sucrose endotherm compared to solely sucrose. This decline in enthalpy for the melting of sucrose is suggestive of a doping of the sucrose crystalline structure with the added glucose.[12, 13] This reduction in the enthalpy of melting for sucrose may be induced by increased strain within the sucrose lattice arising from the introduction of glucose.[14] Such evidence suggests the inclusion of glucose directly within the sucrose crystal structure is a possibility.

Upon reaching the limit of addition at 7% w/w of added glucose, an endotherm emerges at ~ 125°C that corresponds to the presence of anhydrous glucose (see section 4.2.2.2 Fig 3, 4.2.2.3 Fig 7 and 4.2.2.4 Fig 10). What is noticeable from the data relating to this phase is that elevation of the added glucose from 7% to 10% w/w results in a higher value for the enthalpy of melting. This suggests a higher quantity of unincorporated glucose present as a separate phase. However, the quantity of glucose present is lower when compared to the physically blended materials containing analogous amounts of glucose. From this it can be inferred that even when the limit of addition is

breached, a quantity of glucose could possibly incorporate within the sucrose structure.

PXRD data were in good agreement with those obtained by DSC analysis (Figures 3, 7 and 10 in sections 4.2.2.2, 4.2.2.3 and 4.2.2.4 respectively) Diffractograms obtained from samples containing 1-4% w/w of glucose displayed patterns indicative of solely sucrose. Elevation of the quantity of glucose to 7% and 10% w/w resulted in a phase unrelated to sucrose emerging at the 2θ position of ~ 12.6° that is indicative of the present of glucose. From the unit cell volume calculations, it appears that when glucose is added to a supersaturated sucrose solution, the added glucose may include within the sucrose structure. A significant proportion of materials containing glucose exhibited unit cell volumes that were larger compared to that for re-crystallised sucrose. However, from the data garnered from these calculations it is clear that any increase is not significant. All data fell outside of the 95% confidence level. The observation of solely sucrose phases for co-crystallised material *via* PXRD analysis does not provide conclusive evidence for the direct incorporation of glucose into the sucrose strcture. This does not provide a clear picture of what occurs to glucose added to a supersaturated solution. DSC data is strongly suggestive of incorporation. Unfortunately, this is not reinforced by PXRD data.

Whilst it cannot be conclusively proved from the data herein, it can be postulated that the added glucose may become an intra-crystalline species in the sucrose crystal structure. It cannot be elucidated however, where the position of glucose could include from these results. Consideration of the structure of a crystal lattice, allows some postulation on this however. There are two likely positions for any included glucose to reside within, either in the periodic bond chain that form the layers within a crystal or sandwiched between these chains and held in place by hydrogen bonding between hydroxyl groups. Due to glucose being a reducing sugar, there is also the possibility of it existing in its acyclic form when it is taken up into solution. Interactions between this form and sucrose are a possibility and these interactions may continue into the final crystalline state.

As sucrose is a disaccharide consisting of glucose and fructose moieties it is not inconceivable that glucose and sucrose are interchangeable during crystal growth.[15-19] Work has been performed on the study of sucrose crystal growth

from solution in the presence of various impurities and their impact upon the growth rate and morphology of the final sucrose crystal.[15-19] The work performed in these studies showed that the addition of impurities resulted in a reduced rate of growth for sucrose crystals from solution. This was attributed to impurity adsorption onto suitable sites of the growing crystal surface. As a result, the adsorption and subsequent incorporation of sucrose into the crystal lattice becomes hindered and the overall growth rate is retarded compared to a pure sucrose solution.[18] Though it must be stated that a reduction in the growth rate can also be imparted by impurities via an alteration in the rate of mass transfer from solution. Impurities increase the viscosity of solution, consequently the rate at which sucrose is transferred to the growing crystal is reduced.[18] What is interesting to note is that the differentiation between these two mechanisms of growth retardation. For a reduction imparted by adsorption, the added impurity has to exhibit structural similarities to the sucrose structure. A slowing of growth rate by an increase in viscosity can be induced by a wide variety of materials.[19]

The adsorption of impurities onto a growing sucrose crystal results (depending on the impurity introduced) in the retardation or stopping of the growth of certain crystal faces. Due to this, the growth of the sucrose crystal will proceed more predominantly along an axis where there is no (or reduced) impurity adsorption. This behaviour induces a different morphology upon the final sucrose crystal that is evident from observable elongation along either the *a*, *b* or *c* axis of the sucrose crystal.[20] Another observable phenomenon that is indicative of inclusion of an impurity is variance in the unit cell parameters of a material. Variance of calcite unit cell parameters, and the subsequent lattice distortion, has been shown to be related to the presence of intra-crystalline organic species.[21] The presence of organic species within biogenic calcite was shown to elongate the axis of this material.[21] Indeed, in metallurgy, Vergard's Law shows clearly the relationship between changing unit cell parameters with altering composition of metal alloys. The variance in the lattice parameter of a Ni-Zn-Ferrite material were shown to change with an alteration in the ratio of nickel to iron.[22] Another example of this can be seen from the formation of a nickel-doped iron deficient cobalt ferrite. The variation in length of the *a* axis of this material was shown to vary linearly with increasing amounts of added nickel, higher amounts of nickel resulted in shorter lengths of the *a* axis.[23] The

contraction occurred as nickel possesses a smaller ionic radii compared to cobalt, as such substitution of cobalt for nickel induces a contraction in the length of the *a* axis.[23] These changes in the unit cell parameters can be both additive or subtractive to the initial value of the changing unit cell parameter.[21-23] It must be noted that the unit cell volume fluctuates with these changing parameters as well, and is typically a reliable indicator of inclusion within a material.

Whilst these examples are predominantly inorganic in nature, and Vegard's Law relates solely to metal alloys, it is not inconceivable that such phenomenon can be demonstrated by co-crystal sugars. As such, analysis of the unit cell parameters for sucrose co-crystalline materials may reveal any induced distortion by added glucose.

From PXRD data of co-crystalline sucrose and glucose materials the unit cell volumes were shown to be not significantly larger than solely sucrose (see Figs 6, 9 and 12 in sections 4.2.2.2, 4.2.2.3 and 4.2.2.4 respectively). From these calculations, there does appear to be a degree of variance (fig 39) around the value of *a* calculated for re-crystallised sucrose. In order to ascertain if these changes are significant, the values for the *a* axis (table 14) in the material containing 4% w/w of glucose are taken as an arbitrary point. The data suggests that in series I and III, the change in the value of *a* is significant. The data from these two series fell within a 95% confidence interval.

	a (Å)	a_{diff} (Å)
Re-Crystallised Sucrose	10.8702 (± 0.0016)	----------
Sucrose/Glucose I	10.8558 (± 0.0018)	0.0144
Sucrose/Glucose II	10.8679 (± 0.0016)	0.0023
Sucrose/Glucose III	10.8404 (± 0.0025)	0.0298

Table 14. Values of the *a* axis in Re-crystallised Sucrose and Co-crystalline Sucrose and 4 % w/w Glucose Materials

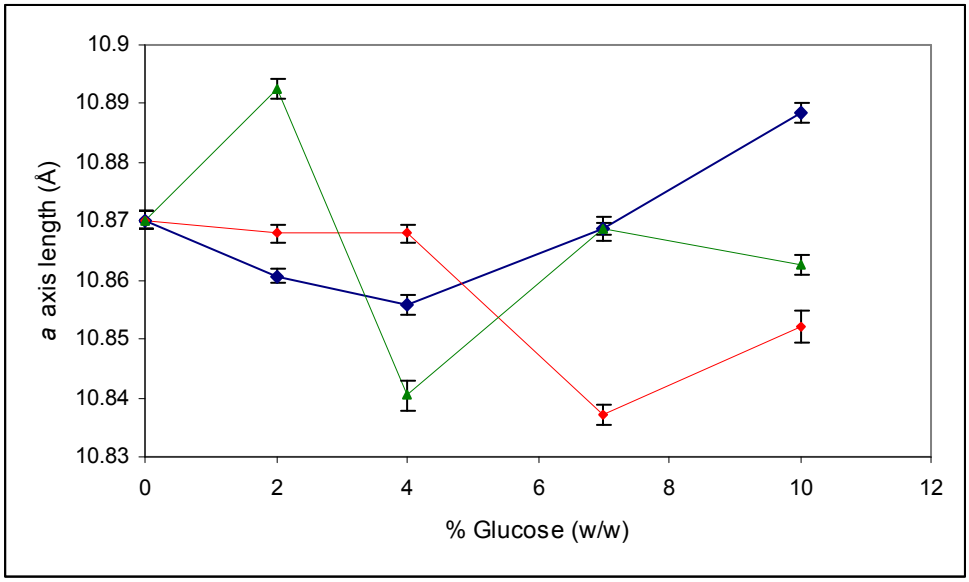


Fig 39. Length of the *a* Axis in Co-Crystalline Sucrose and Glucose Materials Series I (Blue), II (Red) and III (Green)

Comparison of the *b* (Fig 40) and *c* axis (Fig 41) for the co-crystalline materials with values found for re-crystallised sucrose showed a degree of variance again.

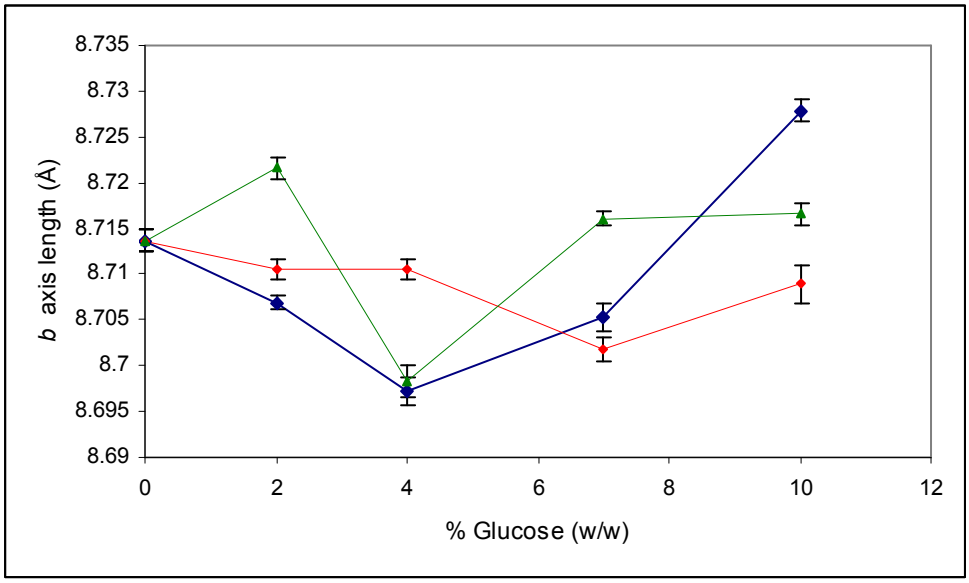


Fig 40. *b* Axis Length in Co-Crystalline Sucrose and Glucose Materials Series I (Blue), II (Red) and III (Green)

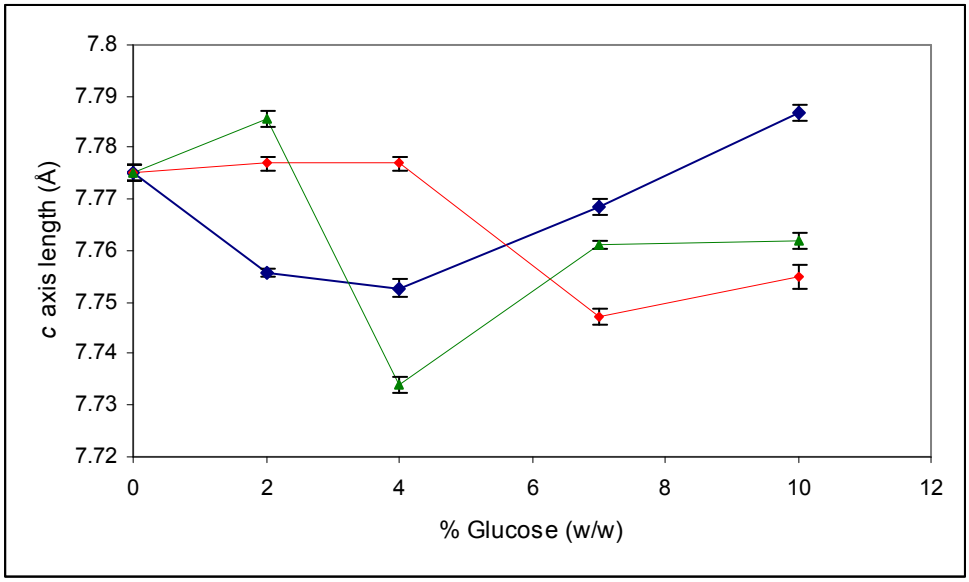


Fig 41. *c* Axis Length in Co-Crystalline Sucrose and Glucose Materials Series I (Blue), II (Red) and III (Green)

Taking the values for both the *b* and *c* axis for materials containing 4% w/w of glucose (table 15) it can be seen that this variance from the value for re-crystallised sucrose is significant for all except for the *c* axis of the second series.

	b (Å)	b_{diff} (Å)	*c* (Å)	c_{diff} (Å)
Re-Crystallised Sucrose	8.7312 (±0.0012)	---------- ----------	7.7751 (±0.0017)	---------- ----------
Sucrose/Glucose I	8.6972 (±0.0015)	0.034	7.7528 (±0.0017)	0.0223
Sucrose/Glucose II	8.7105 (±0.0011)	0.0207	7.7769 (±0.0014)	0.0018
Sucrose/Glucose III	8.6983 (±0.0018)	0.0329	7.734 (±0.0017)	0.0411

Table 15. Values for *b* and *c* axis in Re-crystallised Sucrose and Co-Crystalline Sucrose with 4% w/w of Glucose

The above data does suggest that the addition of glucose to a supersaturated sucrose solution yields a material that differs from sucrose. For material that is 'co-crystalline' (i.e. sucrose with 4% w/w of added glucose) the

lattice parameters display significant variance from re-crystallised sucrose. This is potentially suggestive of a disruption of the expected sucrose crystal structure. However, such disruption is usually accompanied by significant changes in the unit cell volume. The inclusion of added material into a host lattice should be accompanied by both a unit cell volume increase and axis elongation.[21] The data obtained for these materials does not exhibit a significant change in unit cell volume unfortunately. The addition of glucose to sucrose does not give a final material that has a significantly smaller or larger unit cell volume in this instance. As such, the data obtained from DSC, PXRD and unit cell calculations cannot conclusively prove the formation of a sucrose glucose co-crystal. Whilst there is evidence to suggest this is the case, a lack of significance in the crucial unit cell volume data points is lacking. It can be postulated that he addition of glucose to a supersaturated sucrose solution potentially yields a co-crystalline material using the methodology employed. Further work on reducing experimental error, and any associated with the method, is necessary to conclusively prove this point.

Whilst there is insufficient evidence to truly prove co-crystal formation, the appearance of a separate phase relating to glucose above the level of 4% w/w is interesting. It appears (see sections 4.2.2.2, 4.2.2.3 and 4.2.2.4) that the co-crystallisation of sucrose with glucose results in a quantity of 1-4% w/w of glucose potentially becoming adsorbed and incorporated into the resulting sucrose crystals. As noted from PXRD and DSC analysis, elevation above this to 7% w/w results in the appearance of an anhydrous glucose and a potentially co-crystalline phase. A possible reason for this can be found by the consideration of the events that unfold when sucrose crystallises from solution and the effect that added glucose induces.

In a pure supersaturated sucrose solution, induction of nucleation results in sucrose growth units becoming integrated onto the growing nucleus, which continues until crystallisation halts. With the introduction of an impurity into a supersaturated sucrose solution, a chance of the impurity becoming included within the final lattice *via* adsorption onto the growing lattice arises. The successful inclusion of an impurity is dependent on the strength at which it adsorbs onto the growing crystal. If a strong adsorption is apparent then any subsequent desorption is more difficult compared to when an impurity adsorbs weakly. A reluctance to depart the growing crystal *via* desorption from the

growing crystal increases the chances of it becoming irrevocably bound within the host lattice. A slow desorption will result in another layer of growth units becoming deposited which traps the impurity. Kubota *et al*. hypothesised that this process of adsorption and desorption of growth units and impurities is not instantaneous or final.[24] As a result, this has implications for the inclusion of impurities.[24] The work by Kubota suggested that, at high levels of supersaturation (*S*), adsorption of host growth units is preferred over the adsorption of impurities; this trend becomes reversed at low levels of *S*.[24] These ideas were built on data obtained from growth studies of paraffin, sucrose and potassium di-hydrogen phosphate in the presence of impurities.[25-27] Furthermore, the tenacity of bonding of an impurity to the growing crystal has implications for the rate of growth and the inclusion of an impurity. A strong interaction allows for deposition of impurities and their subsequent inclusion. A weak interaction allows for competition between the host molecules and the impurity thus allowing for differences in how well an impurity includes.[28]

Application of these theories, to co-crystallising sucrose with glucose, and the appearance of a secondary phase with the addition of 7% w/w and above of glucose allows the forwarding of a possible explanation.

Firstly, the value of *S* within the sucrose solution has to be considered; using the methodology outlined in Section 8.2.1., at 128°C the sucrose solution has a *S* value of ~ 0.948. Upon reaching the *S* value obtained by heating the solution to 128°C, the impurity (glucose) is added. Utilisation of the model proposed by Kubota means that at this level of *S*, the adsorption of glucose is extremely rapid and as such, glucose may become included within the host sucrose structure.[24] As the solution cools and begins to crystallise, the supersaturation level will increase (from ~ 0.948) until it reaches the level at which the samples crystallise (~ 1.13). With this increase, the preference will shift to the adsorption of sucrose growth units over glucose adsorption. Application of this to the data obtained from co-crystallising sucrose with glucose allows the supposition that in the levels of 1-4% w/w of glucose, the time between a preference for glucose adsorption and the shift to preferential sucrose adsorption is sufficient to allow full inclusion of the added glucose. Elevation of the level of added glucose to 7% w/w results in a quantity of glucose that is too large to be completely incorporated into the host sucrose

structure. It is highly likely that a proportion is adsorbed and included in the sucrose structure but a portion appears to remain un-incorporated and will crystallise; this postulation fits well with evidence obtained from DSC and PXRD analysis (see sections 4.2.2.2, 4.2.2.3 and 4.2.2.4). Furthermore, it is possible that upon addition to the supersaturated solution (in the level of 7% w/w), not all of the added glucose is taken up into solution. Consequently, undissolved glucose may induce nucleation rather than it occurring *via* molecular collisions. Due to this prompting of nucleation, the time between low *S* levels and high *S* levels will be shorter compared to supersaturated solutions containing quantities of glucose below 7% w/w. As such, the complete inclusion of glucose is more unlikely as the rate of crystallisation will be higher.[15]

4.3.3. Co-Crystallisation of Sucrose and Glucose at 110°C

Modification of the temperature at which the supersaturated sucrose solution is formed from 128°C to 110°C did not change the limit at which glucose is addable (see section 4.2.3.1). Both DSC and PXRD analysis showed (section 4.2.3.1) the formation of solely phases related to sucrose containing glucose in the range of 1-4% w/w. Above this level, a secondary phase appears that corresponds to the anhydrous form of glucose.

DSC analysis showed (Fig 13, section 4.2.3.1) that the introduction of glucose to a supersaturated sucrose solution had an effect upon the enthalpy associated with the melting of sucrose. Enthalpy values decreased proportionally with added glucose; higher amounts of added glucose generally induced higher decreases in the value for ΔH for the sucrose endotherm compared to solely sucrose. Such a decline in enthalpy is suggestive of a doping of the sucrose crystalline structure with the added glucose.[12, 13] This reduction in the enthalpy of melting for sucrose is induced by increased strain within the sucrose lattice that arises from the introduction of glucose within the sucrose crystal structure.[14] Such evidence suggests the potential inclusion of glucose directly within the sucrose crystal structure.

Upon reaching the limit of addition at 7% w/w of added glucose, an endotherm emerges at ~ 125°C that corresponds to the presence of anhydrous glucose (Fig 13, section 4.2.3.1). What is noticeable from the data relating to this phase is that elevation of the added glucose from 7% to 10% w/w results in a

higher value for the enthalpy of melting. This suggests a higher quantity of unincorporated glucose. However, the quantity of un-incorporated glucose present is lower when compared to the physically blended materials containing analogous amounts of glucose. From this it can be inferred that even when the limit of addition is breached, a quantity of glucose is still possibly incorporated within the sucrose structure. What is interesting to note is that even though a proportion of glucose is still included under these conditions, the amount included appears not to be as significant compared to when the sucrose solution is formed at 128°C. This observation is drawn from comparison of ΔH values for the glucose endotherm that emerges when the sugar is included in the quantity of 7% w/w. Addition at 128°C gives an enthalpy value of 0.890 Jg^{-1} for this endotherm (table 5, section 4.2.3.1). The addition of 7% w/w of glucose at 110°C results in a melting enthalpy of 2.853 Jg^{-1}. This suggests that the utilisation of a lower temperature to form the sucrose solution, whilst not altering the actual limit of addition, does effect how well glucose includes into the host sucrose structure.

PXRD data showed (Fig 15, section 4.2.3.1) that the unit cells for the co-crystalline sucrose and glucose materials formed at 110°C were larger compared to solely sucrose. However, this increase was calculated to be not significant, the calculated levels of significance fell outside of the 95% confidence level. As already stated in section 4.3.2., this does not help with providing a good argument for co-crystal formation. However, analysis of the unit cell parameters may provide more evidence toward the formation of a co-crystal.

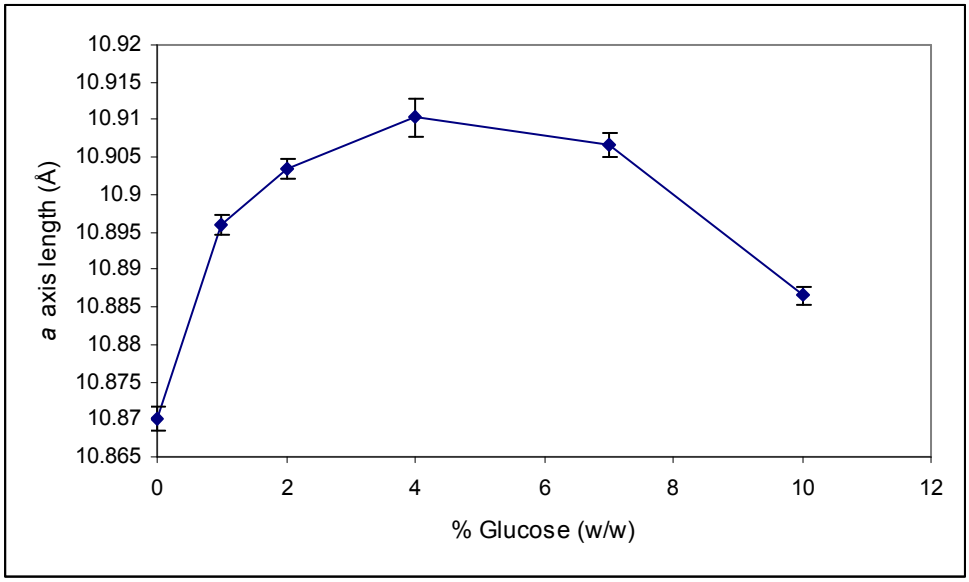


Fig 42. *a* Axis Length vs. % Glucose for Co-Crystalline Sucrose and Glucose Materials Formed at 110°C

Figure forty two shows that for all the materials formed at this temperature were significantly larger. All the data points for this parameter can be construed as significant increases as they all fall within the 95% confidence interval. Analysis of the *b* and *c* axis lengths revealed a similar (figs 43 & 44) trend.

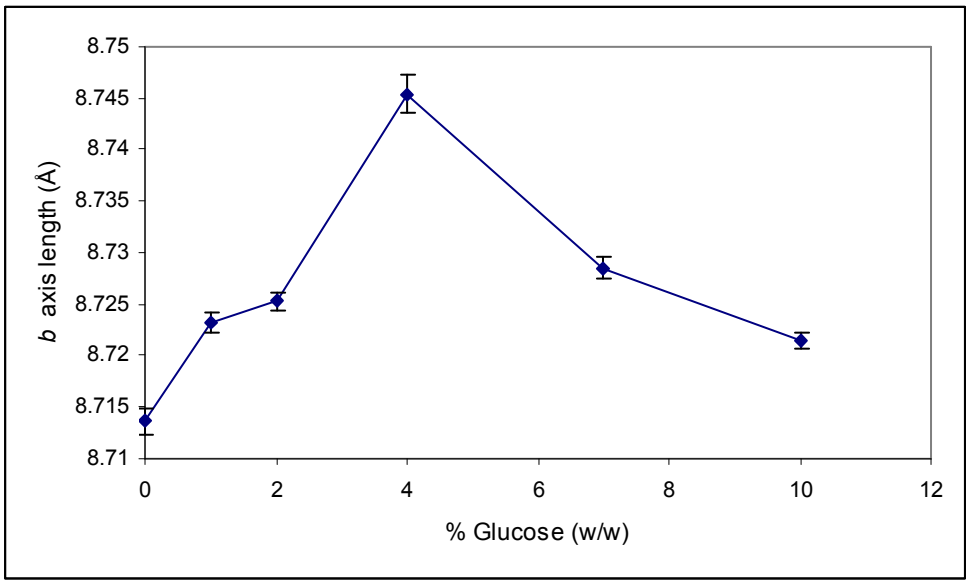


Fig 43. Variation in *b* axis length for Sucrose/Glucose Materials formed at 110°C

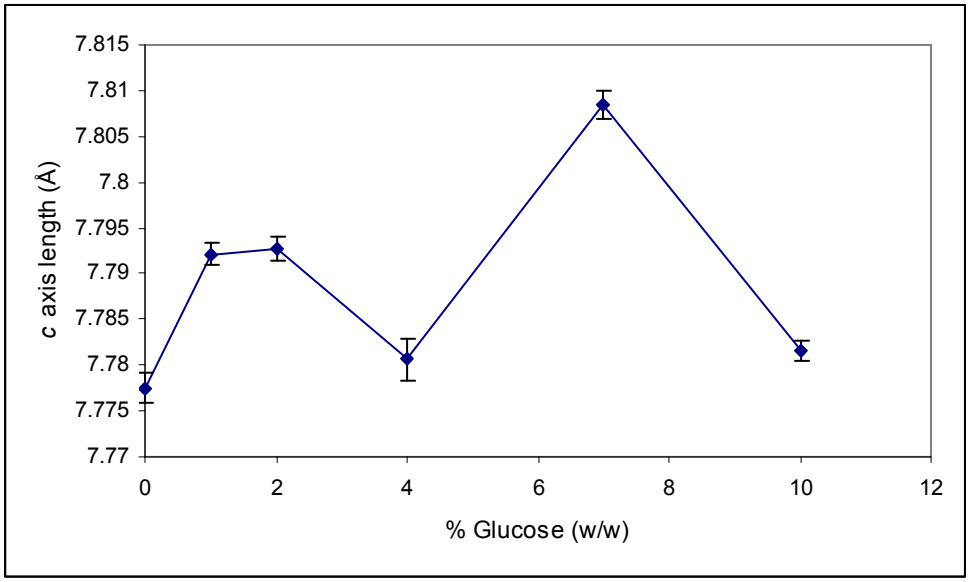


Fig 44. Variation in *b* axis length for Sucrose/Glucose Materials formed at 110°C

As can be seen from the above two plots, both the *b* and *c* axis increase in length significantly. All data points reside within the 95% confidence interval and therefore can be construed to have increase significantly. The above observations lend themselves strongly to the possibility of co-crystal formation, and whilst this can be strongly suggested, it cannot be conclusively proved due to the lack of significance in the increase of the unit cell volumes.

However, the combination of DSC data and the analysis of the unit cell axis parameters lend weight toward the argument for a sucrose and glucose co-crystal. Interestingly, the increases in the *a*, *b* and *c* axis tend towards linearity. Higher amounts of added glucose generally induce a longer elongation along the axis. This observation lends itself further to the possible formation of a sucrose and glucose co-crystal. Further work on reducing the experimental error for calculating the unit cell volume is necessary. A reduction of these may help to conclusively tie all the evidence together for the formation of co-crystalline sucrose and glucose materials.

The formation of a secondary phase relating to the presence of glucose is likely to be attributable to the ideas set forth in Section 4.3.2. The time between the generated *S* level and the level of *S* reached during crystallisation is insufficient to allow full inclusion of 7% w/w of added glucose. However it is notable that this level of supersaturation will change with the solution temperature dropping from 128°C to 110°C. Formation of a supersaturated

sucrose solution at 110°C will give a supersaturation ratio of ~ 0.987; this level is higher than the one found in a solution at 128°C. The implications of this can be clearly seen when considering the enthalpy values for the glucose endotherm that appear when 7% w/w of the monosaccharide is introduced. At a higher *S* level (~ 0.987), a greater amount of glucose remains un-incorporated when introduced in the level of 7% w/w compared to when this quantity of glucose is introduced at a lower *S* (~ 0.948) level. When this observation is considered in the light of the model put forward by Kubota *et al*., it appears logical that a higher degree of glucose will not become incorporated within the sucrose structure.[24] At 110°C the value of *S* is higher than at 128°C. Consequently, the time between preferential glucose adsorption and the shift to a preference for sucrose growth unit adsorption is shorter. Ergo, there is less time for glucose adsorption than before; therefore, a lower degree will become included within the sucrose structure. Though the time is shorter for the inclusion of glucose, the window of opportunity for inclusion is still big enough to potentially allow 4% w/w of glucose to become included into the host sucrose structure.

4.3.4. Co-Crystallisation of Sucrose and Glucose at 120°C

Materials formed at 120°C were very similar to co-crystallised sucrose and glucose materials formed at 128°C and 110°C. DSC and PXRD analysis showed (section 4.2.3.2) that 1-4% w/w of glucose is addable. Above this level a separate glucose phase is observable.

DSC analysis showed (Fig 16, section 4.2.3.2) that the introduction of glucose had an effect upon the enthalpy associated with the melting of sucrose. The data showed (Table 6, section 4.2.3.2) higher amounts of added glucose generally induce higher decreases in the value for ΔH for the sucrose endotherm compared to solely sucrose. This behaviour is indicative of a doping of the sucrose crystalline structure with the added glucose.[12, 13] This reduction in the enthalpy of melting for sucrose is induced by increased strain within the sucrose lattice that arises from the introduction of glucose within the sucrose crystal structure.[14] Such evidence points towards the possibility of glucose inclusion within the sucrose crystal structure.

Elevation of the amount of added glucose to 7% w/w resulted (Fig 16, section 4.2.3.2) in an endotherm emerging at ~ 125°C that corresponds to the

presence of anhydrous glucose. What is noticeable from the data (Table 6, section 4.2.3.2) relating to this phase is that elevation of the added glucose from 7% to 10% w/w results in a higher value for the enthalpy of melting which suggests a higher quantity of un-incorporated glucose. However, the quantity of un-incorporated glucose present is lower when compared to the physically blended materials containing analogous amounts of glucose. From this it can be inferred that even when the limit of inclusion is breached, a quantity of glucose is still possibly incorporated within the sucrose structure. What is interesting to note is that even though a proportion of glucose is still included under these conditions, the amount included appears not to be as significant compared to when the sucrose solution is formed at 128°C. Furthermore, though the quantity is not as high as when added at a solution at 128°C, it is greater compared to when glucose is added to a solution at 110°C. This observation is drawn from comparison of ΔH values for the glucose endotherm that emerges when the sugar is included in the quantity of 7% w/w; addition at 128°C gives an enthalpy value of 0.890 Jg^{-1} and 2.853 Jg^{-1} when added at 110°C. When 7% w/w of glucose is added to a sucrose solution at 120°C, the enthalpy of melting for the glucose phase was shown to be 1.789 Jg^{-1}. This suggests that altering the temperature at which the sucrose solution is generated, whilst not altering the actual limit addable glucose, it does appear to effect how much glucose can be potentially become intra-crystalline.

PXRD data showed (Fig 18, section 4.2.3.2) that the unit cells for the co-crystalline sucrose and glucose materials formed at 120°C were larger compared to solely sucrose. However, these elevations in unit cell volume were not significant; all the data points fell outside of the 95% confidence level. Analysis of the individual unit cell parameters however lends weight towards the possible formation of a co-crystal. Figure forty-five shows a plot of the *a* axis length versus the amount of added glucose. As can be clearly seen, the addition of glucose induces a significant elongation of the *a* axis. Analysis of the values calculated (fig 46 and 47) for both the *b* and *c* axis reveals a similar trend, all of the data points in this series fall within the 95% confidence level.

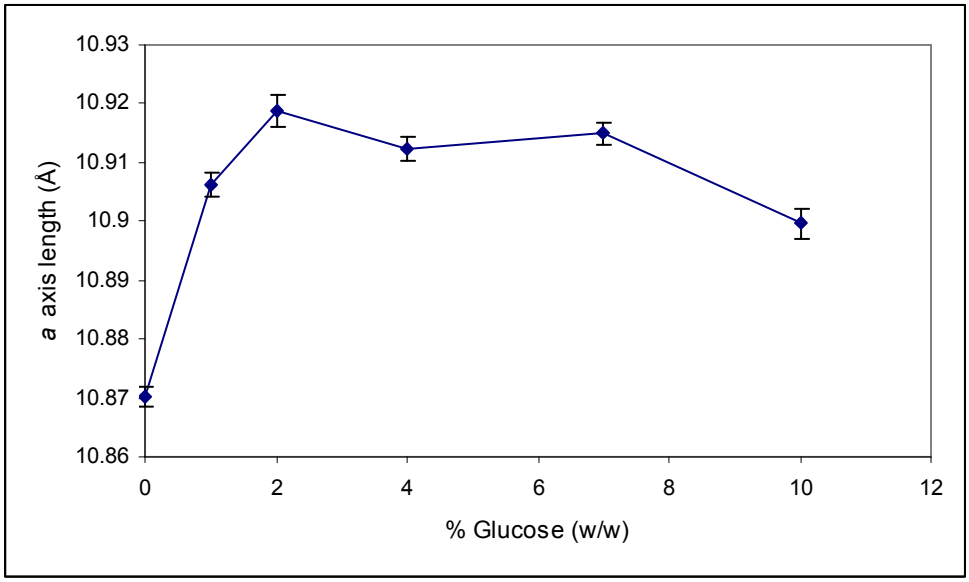


Fig 45. *a* Axis Length *vs* % Glucose for Co-Crystalline Sucrose and Glucose Materials Formed at 120°C

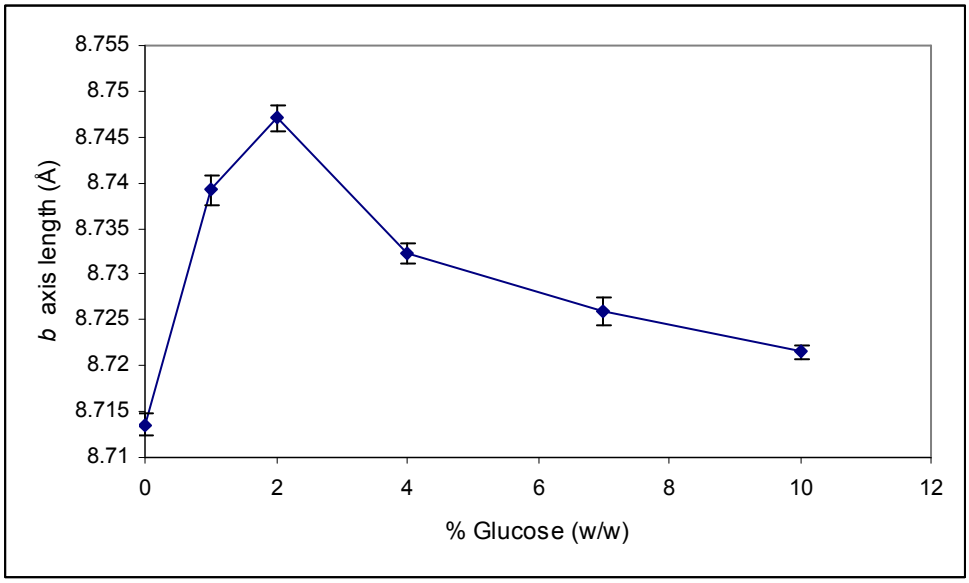


Fig 46. *b* Axis Length *vs* % Glucose for Co-Crystalline Sucrose and Glucose Materials Formed at 120°C

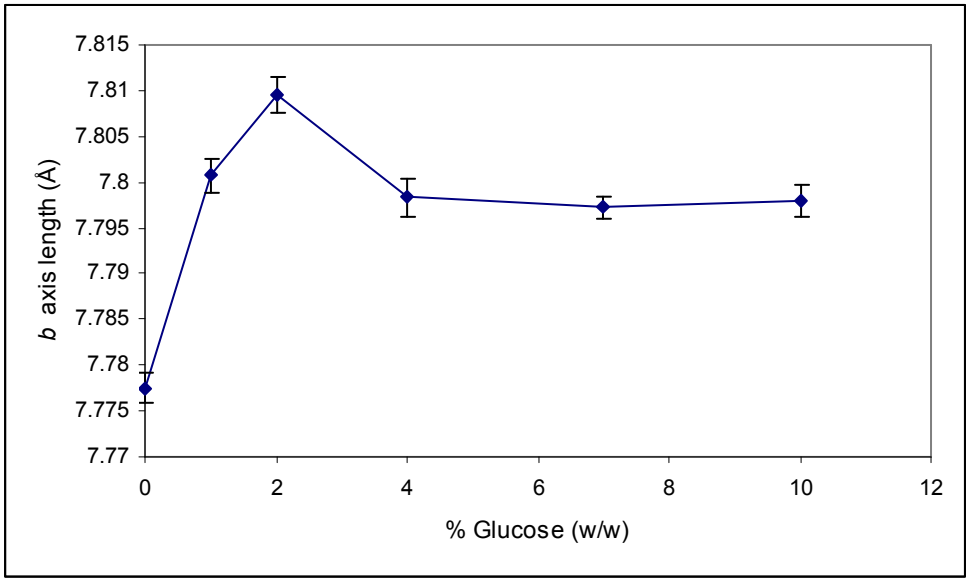


Fig 47. *c* Axis Length *vs* % Glucose for Co-Crystalline Sucrose and Glucose Materials Formed at 120°C

The above data, coupled with that obtained from DSC analysis is strongly suggestive of the formation of a sucrose and glucose co-crystal. However, this cannot be conclusively stated due to the lack of significant increase in the volumes of the unit cells for this series of materials.

However, the combination of DSC data and the analysis of the unit cell axis parameters lend weight toward the argument for a sucrose and glucose co-crystal. Further work on reducing the experimental error for calculating the unit cell volume is necessary in order to conclusively prove co-crystal formation.

The formation of a secondary phase relating to the presence of glucose is likely to be attributable to the ideas set forth in Section 4.3.2.: the time between the generated *S* level and the level of *S* reached during crystallisation is insufficient to allow full inclusion of 7% w/w of added glucose. What should be discussed is that the changing level of *S* that will occur when the sucrose solution is heated to 120°C. Formation of a supersaturated sucrose solution at 120°C will give a supersaturation ratio of ~ 0.965. This level is higher than the one found in a solution at 128°C though lower than *S* in a solution at 110°C. The implications of this can be clearly seen when considering the enthalpy values for the glucose endotherm that appear when 7% w/w of the monosaccharide is introduced. As already mentioned, at a higher *S* level (~ 0.987), a greater amount of glucose remains unincorporated when introduced at the level of 7% w/w compared to

when this quantity of glucose is introduced at a lower *S* (~ 0.948) level. The value observed for the melting of glucose when present in a quantity of 7% w/w in a material formed at 120°C (*S* level of ~ 0.965) is between the values found at 110°C and 128°C. Consideration of this in the light of the model proposed by Kubota *et al*., it appears logical that a higher degree of glucose will not become incorporated within the sucrose structure.[24] At 120°C the value of *S* is higher than at 128°C, consequently, the time between preferential glucose adsorption and the shift to a preference for sucrose growth unit adsorption is shorter. Ergo, there is less time for glucose adsorption in sucrose solutions formed at 120°C than in sucrose solutions formed at 128°C, therefore, a lower degree will become included within the sucrose structure. Juxtaposing this with the result found at 110°C shows that more glucose is adsorbed at 120°C than at 110°C. In a sucrose solution formed at 120°C there is more time for the preferential adsorption of glucose as the shift to sucrose adsorption will take longer, consequently this will allow for a greater quantity of glucose to be included.

Though the time is shorter for the inclusion of glucose, the window of opportunity for inclusion is still big enough to allow 4% w/w of glucose to become included into the host sucrose structure.

4.3.5. Co-Crystallisation of Sucrose and Glucose on a Smaller Scale

In an effort to isolate crystals of a co-crystalline material, the co-crystallisation methodology was scaled down in accordance with the procedure outline in Section 8.2.5.

DSC results for these materials suggested the formation of solely sucrose related materials in the range of 2-10% w/w of added glucose. The enthalpy associated with the melting of sucrose decreased proportionally with the quantity of added glucose; higher amounts of the added monosaccharide induced larger declines in the value of ΔH. This suggests a doping of the sucrose host phase by the glucose. Such a reduction in ΔH is induced by elevated strain imposed upon the sucrose lattice by the presence of glucose within it.[11-13] As such, the added glucose can be regarded as being potentially present within the sucrose structure.

PXRD data reinforced the observations obtained from DSC analysis for all levels of added glucose. Diffractograms showed only reflections pertaining to sucrose and no characteristic reflections for glucose could be identified. The

calculation of the unit cell volumes showed that the materials exhibited larger unit cells compared to solely sucrose. However, these elevations cannot be construed as significant as all of the data points fell outside of the 95% confidence interval.

As previously seen, co-crystal formation cannot be conclusively proved due to the unit cell volume data. However, weight can be lent towards this possibility by analysis of the unit cell axis. Figure forty-eight shows a plot of the *a* axis length against the quantity of glucose. Materials containing 2-4% w/w of glucose exhibit longer *a* axis lengths compared to re-crystallised sucrose, furthermore, these elongations are significant. The lack of elongation of the *a* axis for materials containing 7-10% w/w of glucose does not point toward possible co-crystal formation.

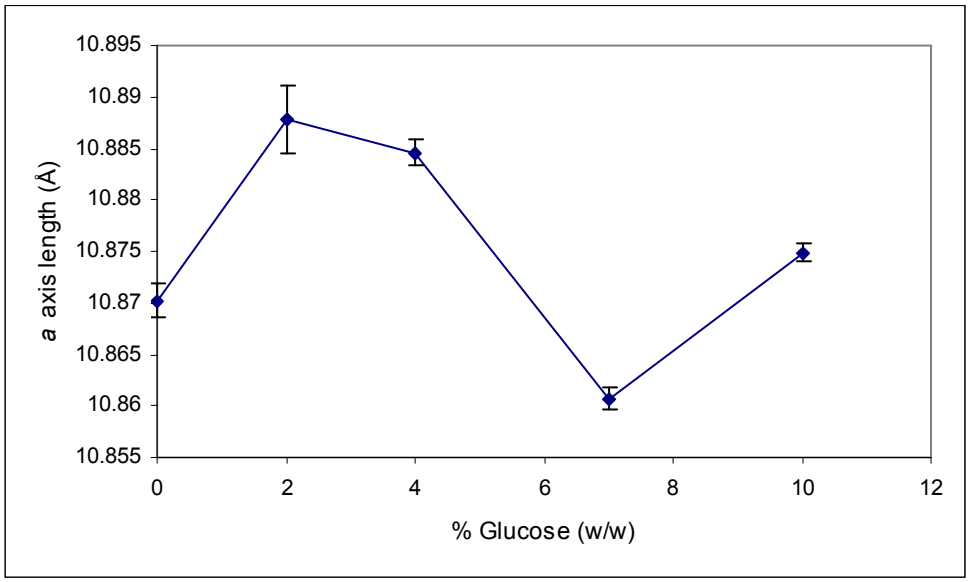


Fig 48. *a* Axis Length *vs* % Glucose for Co-Crystalline Materials Formed on a Smaller Scale

This trend is also observed in the lengths for both the *b* and *c* axis of materials formed using this method. Sucrose, with 2-4% w/w of added glucose exhibits significant elongation along both the *b* and *c* axis. Elevation of the level of added glucose above 4% w/w does not result in any elongation along the *b* and *c* axis. Again, it appears there may be some evidence to suggest possible co-crystal formation, but the lack of significance in the elevation of the unit cell volume hinders this. Whilst there is evidence suggesting that glucose may dope a sucrose lattice, further work on reducing the experimental error for calculating

the unit cell volume is necessary. Achievement of this may help to conclusively prove co-crystal formation.

The lack of an identifiable phase attributable to glucose is unusual. Previously (see sections 4.2.2.2, 4.2.2.3, 4.2.2.4, 4.2.3.1 and 4.2.3.2), the limit was up to 7% w/w of added glucose. It seems unlikely that the limit of inclusion has been raised by producing the materials on a smaller scale. A possible explanation is that the limit is still 7% w/w, but the quantity of glucose that is present as a separate phase is too small for the detection by the techniques employed. This idea is plausible as the DSC scan (of material containing 10% w/w) does show a very slight peak at ~ 125°C that would correspond to the melting of anhydrous glucose. However, this peak is far too small to have any quantifiable enthalpy. As has already been observed when co-crystallising sucrose and glucose (section 4.2.2.2, 4.2.2.3 and 4.2.2.4), the amount of glucose present as a separate phase increases when the amount added to the supersaturated sucrose solution rises from 7% to 10% w/w. Considering that the amount of glucose present as a separate phase (when included at the level of 10% w/w) is barely observable, it is logical to suggest that the amount present as a separate phase when added at 7% w/w will be extremely difficult to detect by the methods employed herein. Therefore, co-crystallising sucrose and glucose, on a smaller scale, will not result in a higher amount of incorporable glucose.

It can therefore be surmised that the model hypothesising (see section 4.3.2) reasons for the imposition of a limit of inclusion in co-crystalline materials, will still hold for these materials. The addition of 2-4% w/w of glucose forms a co-crystalline phase; the period of time between the level of *S* at 128°C and the level of *S* reached during crystallisation is sufficient to allow complete inclusion of the added monosaccharide. Upon reaching a level of added glucose of 7-10% w/w, this window for inclusion is not sufficient to allow complete incorporation of the added glucose. It is likely that a portion will become a part of the sucrose structure (as reflected by PXRD and DSC data, Figs 19 and 20 respectively in section 4.2.4) but a fraction will crystallise as a separate phase. Again, it would be worth mentioning that the quantity of glucose (7 and 10% w/w) may induce nucleation by a distinct change in local concentration, thus further shortening the time for incorporation as outlined in Section 4.3.2.

4.3.6. Co-Crystallisation of Sucrose and Glucose from a Physical Blend

Altering the co-crystallisation methodology from adding glucose at a desired temperature, to starting from a physical blend of the two sugars resulted in an elevation of the limit of addable (from the level seen in sections 4.2.2.2, 4.2.2.3 and 4.2.2.4) glucose. DSC analysis showed (Fig 22, section 4.2.5) that 4-13% w/w of glucose can be successfully added to a supersaturated sucrose solution. Increasing this amount to 15% w/w resulted in a slight endotherm emerging at ~ 125°C that represents anhydrous glucose.

From DSC analysis (Table 8, section 4.2.5), the enthalpy of melting for sucrose was shown to be proportional to the amount of glucose added. Increasing levels of glucose induce bigger drops in ΔH for the melting of sucrose. This observation, accompanied by dropping onset temperatures for sucrose melting, is indicative of a doping of the host sucrose phase by glucose.[12-13] The lower values of enthalpy are indications of increasing lattice strain imposed by the possible inclusion of glucose impurities within a sucrose structure.[14]

Analysis by PXRD gave analogous results to those seen by DSC (Fig 23, section 4.2.5). Solely sucrose phases were formed containing 4-13% w/w of glucose and above 13%, peaks relevant to glucose were observed. From the PXRD data (Fig 24, section 4.2.5), unit cell volumes for the materials were calculated and it was shown that they showed no real trend. Sucrose materials with 7% and 13% w/w of additional glucose showed larger unit cell volumes, while the remaining were smaller. The increases can be thought to be of insignificant due to the data points falling outside of a 95% confidence region. However, from these calculations, the axis lengths were obtained and, in keeping with the notion that the glucose present may possibly adsorb into the growing sucrose structure, a plot of the *a* axis length (Fig 49) was undertaken.

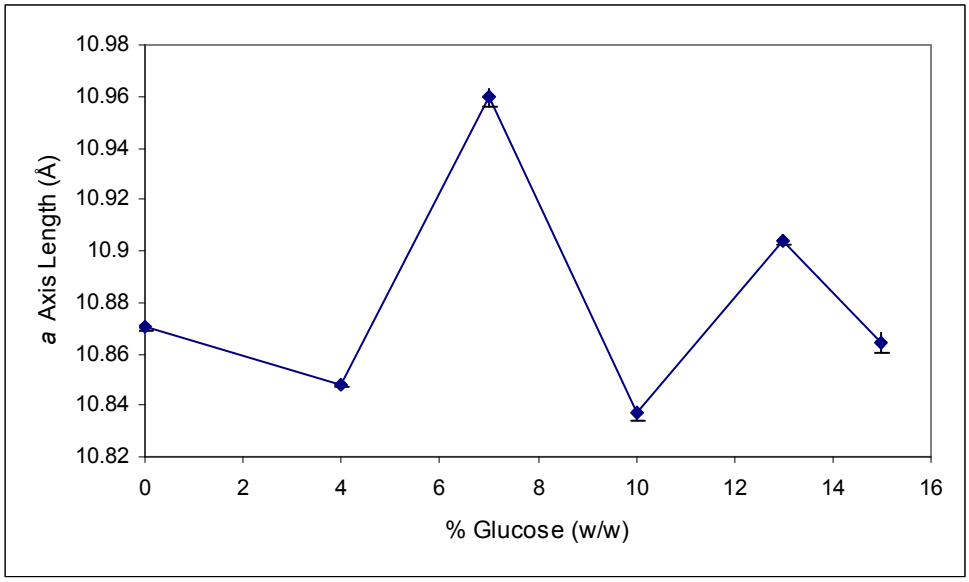


Fig 49. *a* Axis Length *vs* % Glucose for Co-Crystalline Sucrose and Glucose Materials formed from a Physical Blend

As the above diagram shows, there is no real trend in the *a* axis length; this data mirrors that seen in the unit cell volumes. However, materials containing 7% and 13% w/w of glucose exhibit unit cell with significantly longer *a* axis lengths compared to re-crystallised sucrose. These elongations can be thought to be significant as the data points associated with these fall within a 95% confidence interval. The analysis of the *b* and *c* axis lengths (fig 50 and 51) show that this trend is perpetuated by the *b* axis for both and only by the *c* axis for material containing 7% w/w of glucose. Materials containing 7% and 13% w/w of glucose display significant (i.e. the data points can be thought to fall within a 95% confidence interval) elongations compared to re-crystallised sucrose.

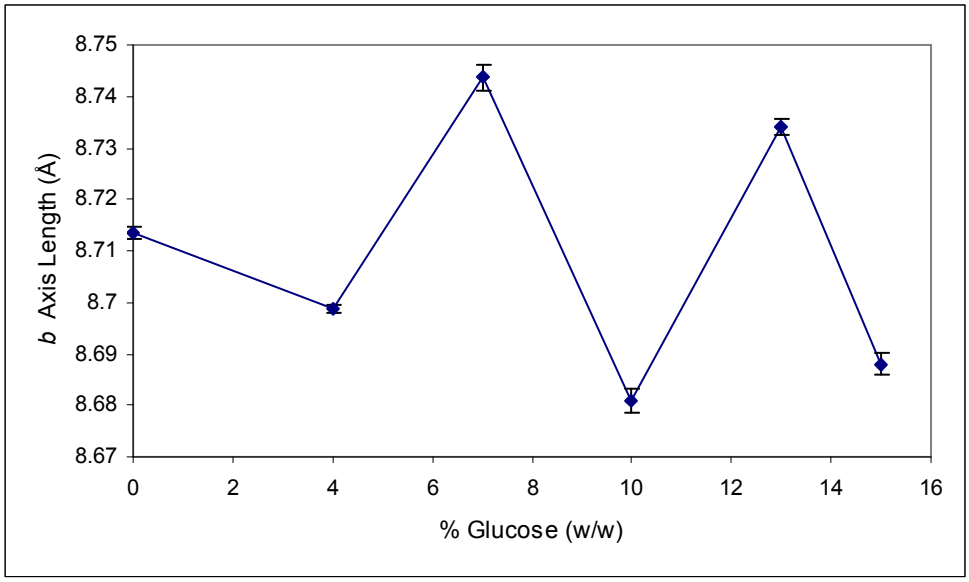


Fig 50. *b* Axis Length *vs* % Glucose for Co-Crystalline Sucrose and Glucose Materials formed from a Physical Blend

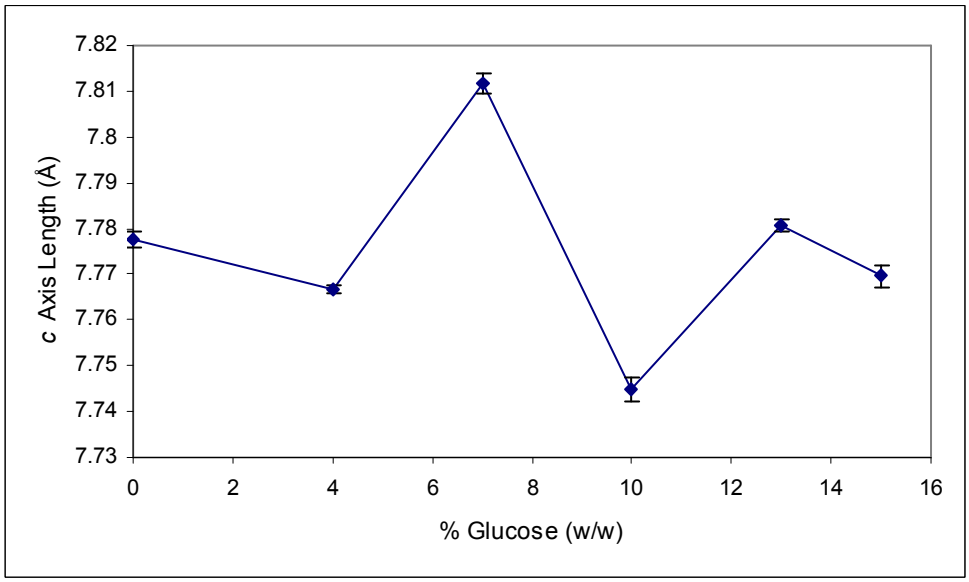


Fig 51. *c* Axis Length *vs* % Glucose for Co-Crystalline Sucrose and Glucose Materials formed from a Physical Blend

There is no elongation of any axis for materials containing 4%, 10% or 15% w/w of glucose. Typically, these materials show a declination which is in line with the decrease in unit cell volume noted for these materials. DSC data, along with PXRD data shows that materials with both 4% and 10% of glucose exhibit solely phases related to sucrose in both forms of analysis. The lack of unit cell volume increase for these materials is unusual (it is typically observed, but is

not significant) and can only be attributed to experimental error. For materials formed with 4-13% w/w of additional glucose, the lack of any observable phase relating to glucose in both DSC and PXRD is suggestive of co-crystal formation. There is further evidence to suggest co-crystal formation noted in materials containing 7% and 13% w/w of glucose. Both of these materials show significant elongation along the *a* and *b* axis, and in the case of material containing 7% w/w of glucose, the *c* axis. Whilst such evidence can possibly suggest the formation of a co-crystal, it is not conclusive. The lack of significant unit cell volume increases for all materials does not support this. What can be drawn is that a concerted effort to reduce experimental error may provide data that can prove this theory.

What is interesting to note is that modification of the co-crystallisation method to starting from a physical blend allows an increase in the level of addable glucose to (15% w/w) that of what was previously (see section 4.2.2.2, 4.2.2.3. and 4.2.2.4) found (7% w/w) for glucose. A possible reason for this could be attributable to the effects of seeding. As already mentioned (see section 4.3.2) glucose in the quantity of 7% w/w may result in nucleation inducement. As such, the time for inclusion becomes reduced as the rate of crystallisation is being raised.[29] Starting from a physical blend may remove the occurrence of nucleation by seeding and as such, there will be a longer time between the level of *S* formed at 128°C and the level at which the material crystallises at. As already mentioned, this period of time appears to be relevant to the inclusion of impurities into a sucrose structure; if this is prolonged (i.e. nucleation occurs in a more homogeneous fashion) a greater amount of glucose appears to be incorporable as suggested (section 4.2.5) from the data herein. The hypothesis of starting from a physical blend of sucrose and glucose will result in both sugars being in solution will have ramifications for the rate of crystal growth.

Smythe showed that higher quantities of impurity present in solution induce slower rates of sucrose growth.[18] This observation is also supported by the work of Bhandri and Hartel on the crystallisation of sucrose in the presence of glucose.[30] As the added glucose can be considered to be in solution using this methodology, the rate of crystal growth is retarded. In practical terms this means that the rate at which supersaturation increases will be slower, consequently the time between the level of *S* reached at 128°C and the level at which the samples

crystallise at will be much longer. Logically, the implications for this are that the glucose present in solution will have a greater chance of becoming included within the sucrose structure. The emergence of a glucose phase when the quantity of glucose is elevated to 15% w/w can be attributed to the rate of sucrose growth being too rapid for complete incorporation of this quantity of glucose.

What can be concluded from this is that the rate of crystallisation in co-crystallisation appears to be a significant factor. This parameter could influence the amount of added sugar that is incorporated.

4.3.7. Co-Crystallisation of Sucrose with Glucose Addition at 110°C

It has been observed (see sections 4.2.2.2, 4.2.2.3, 4.2.2.4, 4.2.3.1 and 4.2.3.2) that the seeding of supersaturated sucrose solutions could influence the quantity of addable glucose. Addition of glucose at 110°C followed by subsequent heating to 128°C was performed in order to investigate this effect. The purpose was to see if the effects of seeding could be negated by adding glucose at a lower temperature. This investigation produced results that were extremely unexpected, with glucose addition in the range of 4-10% w/w; solely material related to sucrose was yielded. However, above this level of added glucose, a possible hydrated form of sucrose was yielded.[11]

DSC analysis (Fig 25, section 4.2.6) showed that the introduction of 4-10% w/w of glucose to a sucrose solution at 110°C, followed by heating to 128°C, gave a material exhibiting melting behaviour of sucrose. Values of ΔH for these materials decreased as a function of added glucose and higher amounts of added glucose induced lower values for ΔH. Such behaviour is suggestive of a doping of the host sucrose phase by the introduced glucose; reduction in enthalpy results from increased lattice strain imparted by glucose residing within the sucrose structure.[11-13]

As mentioned, the addition of 13% and 15% w/w of glucose, respectively, give neither of the two results expected, i.e., neither a solely sucrose related material nor a mixture of sucrose related material in combination with a separate glucose phase. Rather, a suspected hydrated form of sucrose appears to be formed; this phase has recently been reported from this laboratory and has been observed (and is further investigated in chapter 6) appearing from sucrose

re-crystallisation solutions.[11] This is highly irregular as proceeding from a physical blend of sucrose and 13% w/w of glucose gives a material exhibiting both melting and crystalline properties of sucrose. What can be drawn from this point is that the phase observed from co-crystallisation under these conditions is that the melting behaviour does not stem from a severely doped form of sucrose. As shown in Section 4.3.6. where sucrose containing 13% w/w of glucose exhibits melting behaviour that is identifiable as sucrose.

Further evidence for the identification of the unique nature of the phase formed can be obtained by comparison with work performed by Maulny.[31] Work on this hydrated phase culminated in the development of a method that isolated this hydrated form of sucrose. A comparison of the melting of this material with the one formed by the addition of glucose to a sucrose solution at 110°C (followed by heating to 128°) showed an excellent likeness (Fig 52). The reason for the formation of this phase is not clear, though it can be stated that a slow rate of growth from solution is crucial. Further discussion on the formation of this phase can be found in Chapter 6.

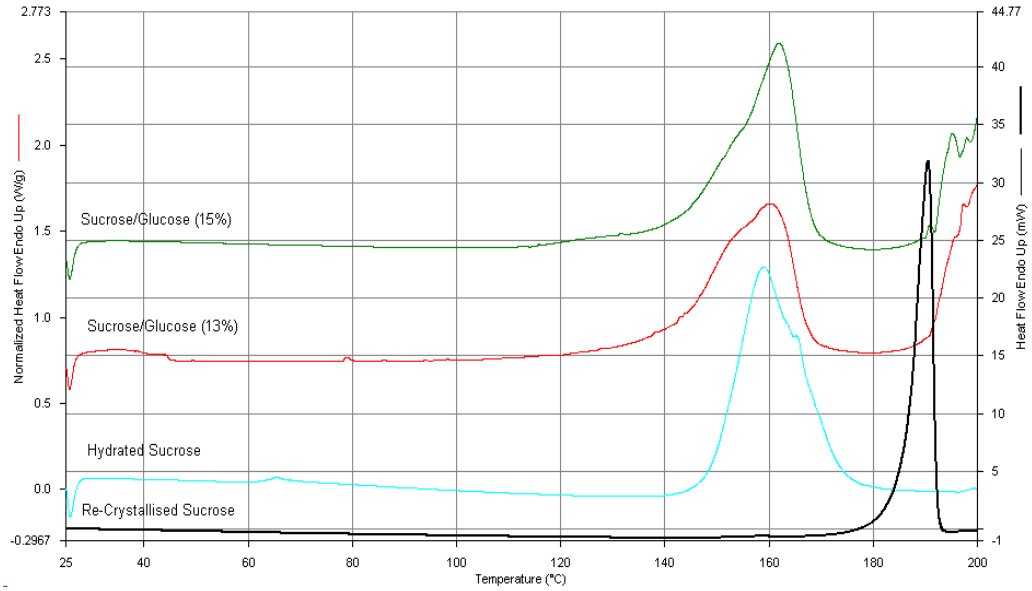


Fig 52. DSC Scans of Re-Crystallised Sucrose, a Hydrated Form of Sucrose and Co-Crystalline Materials with Glucose Addition at 110°C

4.3.8. Co-Crystallisation of Sucrose with Glucose and Fructose

Work on the addition of a 1:1 ratio of glucose and fructose to a supersaturated sucrose solution showed that up to 15% w/w of each sugar could be incorporated into a sucrose structure.[7] Fructose has been shown to include within a sucrose structure extremely well whereas glucose does not, from this it can be inferred that fructose helps facilitate glucose incorporation. To this end, the quantity of fructose required to induce elevated uptake of glucose into a sucrose structure was investigated.

DSC analysis of materials (Fig 30, section 4.2.7.2) containing 7% w/w and 2-6% w/w of fructose showed that all materials exhibit melting behaviour that is indicative of solely sucrose. No melting could be observed for either glucose or fructose. Calculation of the enthalpy (Table 11, section 4.2.7.2) for the melting of sucrose showed that it decreases proportionally with the quantity of added monosaccharides. Higher amounts of included glucose and fructose induce greater reductions in the value of ΔH. This behaviour suggests a doping of the host sucrose phase with the added monosaccharides; enthalpy reduction is a function of increased lattice strain induced by the presence of glucose and fructose within the sucrose lattice.[12-14] PXRD diffractograms (Fig 6, section 4.2.2.2.) supports this as for all materials there is a distinct lack of any peaks related to either glucose or fructose. However, calculation of the unit cell volumes (Fig 32, section 4.2.7.2.) does not go on further to suggest this. All materials exhibit unit cells that are markedly similar to the one found for re-crystallised sucrose. Due to the lack of evidence to further support the notion of co-crystal formation from the unit cell volume data, a closer look at the axis lengths associated with these may provide more weight to the idea of co-crystal formation.

A plot of the *a* axis lengths (Fig 53) shows that for materials that show a slight increase in unit cell volume (materials with 4% and 6% w/w of fructose) there is a significant increase in the elongation of this axis. Data points for these two materials fall within a 95% confidence interval, as such they can be thought of as significant elongations.

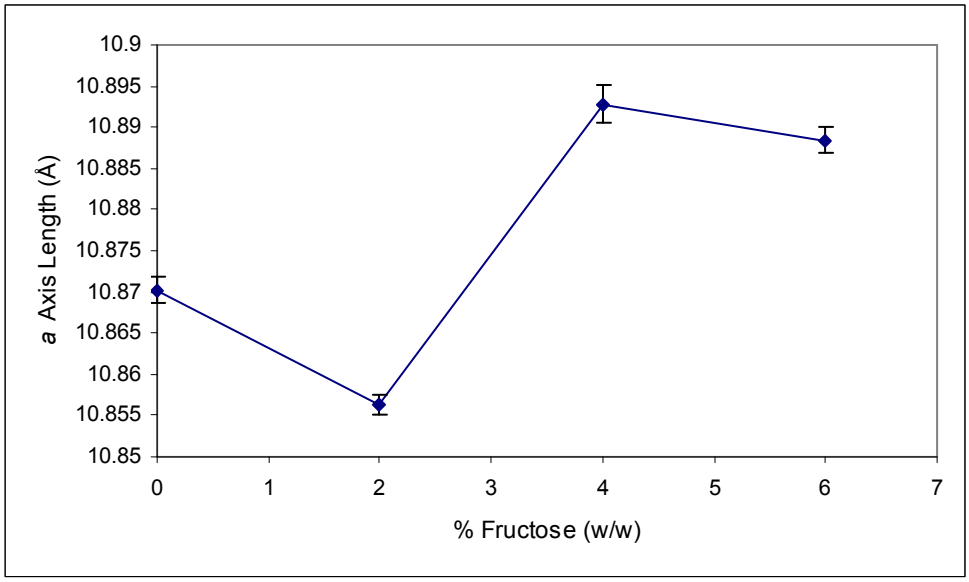


Fig 53. *a* Axis Length *vs* % Fructose for Co-Crystalline Sucrose, Glucose and Fructose Materials

This trend is continued for both the *b* axis (Fig 54) but not for the *c* axis (fig 55) for these materials.

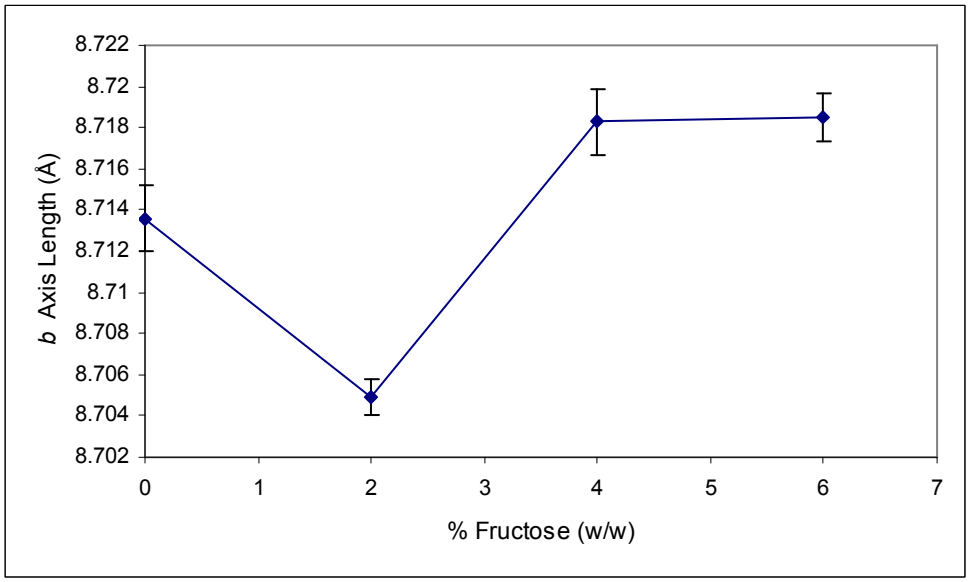


Fig 54. b Axis Length *vs* % Fructose for Co-Crystalline Sucrose, Glucose and Fructose Materials

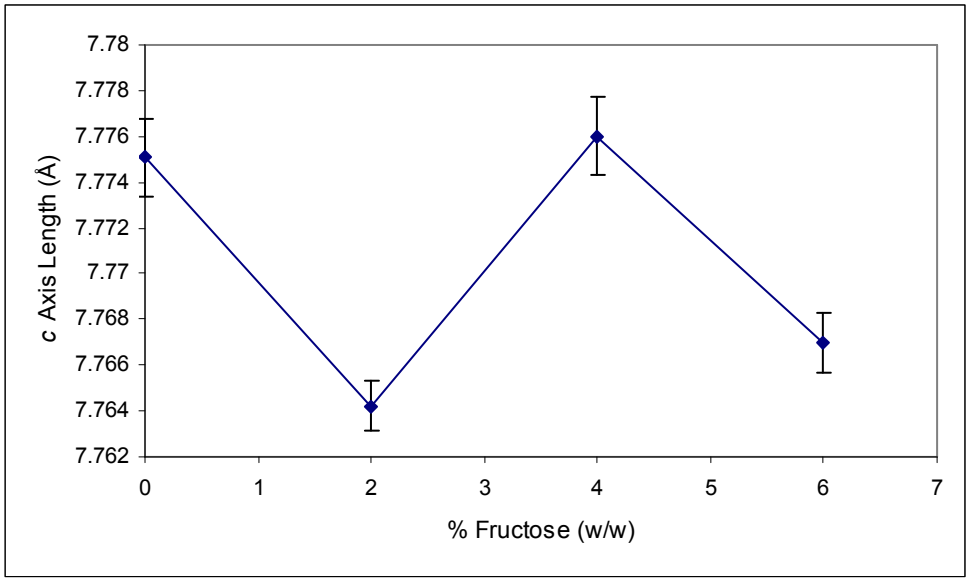


Fig 55. *c* Axis Length *vs* % Fructose for Co-Crystalline Sucrose, Glucose and Fructose Materials

The lack of observable phases relating to either glucose or fructose in DSC and PXRD analysis is suggestive of a single phase relating to a possible sucrose-glucose-fructose co-crystal. With the evidence from the unit cell volumes, and their axis, there is some evidence for a potential co-crystal formation. The lack of significant data from the unit cell volumes hinders conclusively proving this however. What is evident is the need to reduce experimental error in an effort to provide data that may give greater support to the idea of co-crystal formation.

What is interesting to note is that a 1:1 ratio of the two sugars is not required to potentially facilitate a higher amount of glucose into a sucrose structure. Normally, co-crystallisation of 7% w/w of glucose at 128°C yields a material that shows melting of both a sucrose and glucose phase. However, with 2% w/w of fructose present, this is not the case and solely sucrose melting is observed. It appears that usage of fructose allows for improvements in the level of glucose incorporation. The possibilities for this can be found when considering the interaction of the two sugars upon adsorption to the growing sucrose structure. Furthermore, the behaviour of fructose in solution will also have ramifications for the inclusion of itself and glucose into the host sucrose structure.

In aqueous solution, fructose can mutarotate through four anomeric forms; two of which consist of the α and β fructofuranose anomers, two others

are accounted for by the α and β fructopyranose forms.[32] Sgualdino *et al.* showed that in a sucrose solution containing fructose and glucose, the preference for adsorption is in favour of the added monosaccharides. Fructose is the impurity adsorbed primarily (in the fructofuranose form) due to its ability to form a high number of H-bonds with the sucrose substrate.[17] This adsorption slows down crystal growth; hence there is sufficient time for further adsorption of fructose. Due to the reduction in growth rate, fructose has adequate time to enter the favourable fructopyranose form for adsorption.[18, 17] This hypothesis could account for the excellent incoporability of fructose, but it will also allow for higher amounts of glucose to become included. This idea is feasible when consideration of the similarities in the structures of fructose in its pyranose form and glucopyranose, along with the effect impurities have on crystal growth.[18-19]

The structures of fructopyranose and glucopyranose are markedly similar (Fig 56), as such, it can be suggested that either fructose in the fructopyranose form or glucose may become adsorbed after the initial adsorption of fructose in its furanose form. Indeed, this is furthered when, after the preferential adsorption of fructofuranose, the penchant for adsorption is equal between fructopyranose and glucopyranose.[17]

Fig 56. Structure of D-fructopyranose (left) and D-glucose (right)

The key to the heightened amount of glucose inclusion (ca. the expected amount of 7% w/w) may be the primary adsorption of fructofuranose inducing a sufficient reduction in the rate of crystal growth. This could allow for a greater amount of glucose to be included. This reduction in growth rate will also be amplified due to the presence of both fructose and glucose in solution. Invert sugar (an equimolar mixture of fructose and glucose) has been shown to significantly slow down the growth of sucrose from solution.[18] What can be

drawn from these hypotheses is that two factors might influence successful inclusions of added materials within a host material:

i. The structural similarity between the added material and the substrate; the greater similarity will mean a higher number of H-bonds being formed. A high number of H-bonds results in a strong adsorption and a high chance of potential inclusion within the host structure.
ii. The rate of crystal growth from solution; a slow growth rate will allow for a higher amount of inclusion in accordance with the model proposed by Kubota *et al*.[24] Slow growth will mean prolonged time for preferential impurity adsorption over host growth units.

What can be concluded from the investigation of this combination of sugars is that fructose is an excellent additive. Its ability to assume two forms that are extremely relevant to sucrose renders it a powerful tool for potential glucose inclusion (as seen from heightened uptake of glucose when only 2% w/w of fructose is present as seen in section 4.2.7.2) as well as enabling the monosaccharide to potentially ingratiate itself successfully within a host sucrose structure.

4.3.9. Co-Crystallisation of Sucrose and Galactose from a Physical Blend

Previously, Maillet showed that the addition of galactose to a supersaturated sucrose solution gave only 2% w/w of included monosaccharide and above this a separate phase was observed which related to galactose.[7] This is perhaps logical since galactose is not overly similar to sucrose in structure, consequently it will form a relatively low number of H-bonds when adsorbing to a growing sucrose structure. Due to this, it will desorb easily and its chances for inclusion are severely limited.

Co-crystallisation (section 4.2.5) starting from physical blend of sucrose and glucose results in double the amount of addable glucose over starting from a more traditional protocol of adding glucose to a (section 4.2.2.1). The former methodology was applied to this combination of other carbohydrates, in

particular galactose, in an effort to raise the amount that could be added whilst retaining single sucrose phases.

DSC analysis (Fig 36, Table 14 in section 4.2.8.2) of the materials yielded from this co-crystallisation appeared to be a hydrated from of sucrose.[11] Comparison of the co-crystalline sucrose and galactose materials with an isolated sample of the hydrate form (Fig 57) revealed marked similarities in melting.

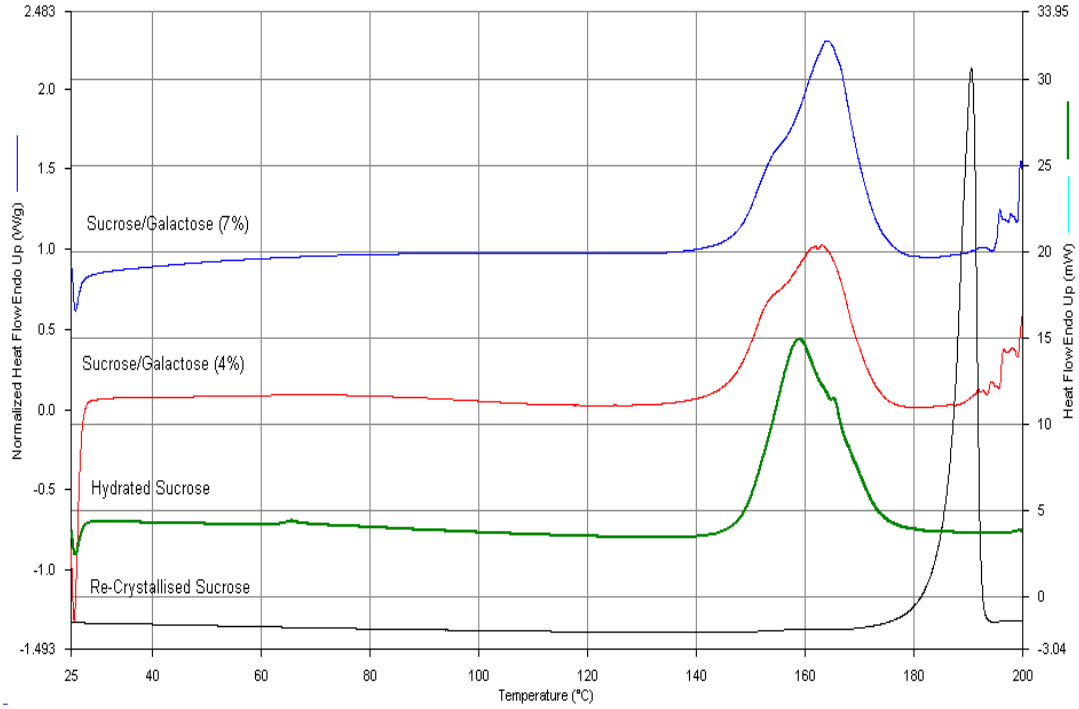


Fig 57. DSC Comparison of Re-Crystallised Sucrose, a hydrated form of Sucrose and Co-Crystalline Sucrose and Galactose Materials formed from a Physical Blend

As can be clearly seen, the expected melting of sucrose is not apparent at all and only a single phase appears to be present. No melting of galactose is observable. Confirmation of the sucrose nature of the material was obtained by PXRD analysis (Fig 37, section 4.2.8.2); the diffractograms revealed the presence of a phase related to sucrose but no peaks were discernable for the presence of galactose. Calculation of the unit cells for these materials showed no clear trend. The galactose presence did not induce elevated unit cell volumes and elevation to 7% w/w of galactose resulted in a material with a smaller unit cell volume compared to sucrose. Further analysis of the axis lengths obtained from

these calculations show that there is little elongation along any axis, which points towards a lack of incorporation of galactose.

For an impurity to be adsorbable onto a host structure during crystal growth, the impurity has to exhibit structural similarities of the host.[18] If the impurity is similar, adsorption occurs and the rate of crystal growth is retarded as the adsorption of host growth units is hindered. However, if an impurity is not alike to a host, reduction in the rate of crystal growth occurs but this arises from an increased viscosity interfering with the mass transfer of growth units to the growing structure. In this case, no clear alteration in crystal morphology is discernable as the crystal will grow in the expected manner albeit slower.[18, 19] Impurities acting in this manner may become incorporated within the host structure, but there inclusion is akin to an encapsulation rather than a direct inclusion within the structure of the host structure.

From the above data, it appears that galactose is sufficiently different to sucrose to induce reduced growth rate due to a higher viscosity. However, the galactose present appears to be captured within the final sucrose structure. Evidence from both DSC and PXRD supports this postulation and no identifiable melting or reflections could be observed. What can be drawn from these data for this combination of materials is that as crystallisation appears to be impeded by a reduction in the rate of mass transfer, resulting in the formation of a hydrated form of sucrose (with encapsulated galactose). This is extremely unusual and such a unique form of sucrose has only been previously isolated using a specialised crystallisation environment.[31] Whilst the mechanism for this formation is not clear, a key factor appears to be a slow rate of crystal growth and it appears that these conditions are present when utilising the methodology and materials discussed in this Section.

4.4. Conclusions

The utilisation of a supersaturated sucrose solution has allowed a clear identification of parameters that affect co-crystallisation. When employing the methodology of heating the sucrose solution to 128°C followed by glucose addition, potentially co-crystalline material can be formed containing up to 7% w/w of glucose. At the level of 7% w/w of glucose, a separate phase relating to

this monosaccharide is observable from DSC and PXRD analysis. Whilst there is unincorporated glucose when included at a level of 7% w/w, the amount of un-included material can be influenced by modification of the *S* level of the sucrose solution

A reduction of the solution temperature to 110°C showed a higher amount of un-included glucose compared to the amount not incorporated in the material formed at 128°C. Reducing the solution temperature to 120°C showed a higher amount of un-included glucose compared to material formed at 128°C, though the quantity included is higher compared to the material formed at 110°C. From this it can be concluded that the level of *S* influences how glucose incorporates. In accordance with the model proposed by Kubota *et al*., the adsorption of impurities is preferred at low levels of *S*; this preference shifts towards host material adsorption at higher levels of *S*.[24] The time between these two preferences is a window in which impurities can include; a bigger window will therefore result in greater incorporation. As dropping the temperature will raise the level of *S* in solution, the time between the preference for impurity adsorption and the shift to host material adsorption is reduced; this is reflected by higher amounts of un-included glucose being concordant with lower solution temperatures. What may influence the appearance of a phase relating to glucose is the possible effects of seeding; in the quantity of 7% w/w, glucose may nucleate the solution and induce crystallisation. Due to this, the rate of crystallisation is faster and the time for preferential impurity adsorption is reduced.

From this, the removal of this induced nucleation factor was attempted by starting co-crystallisation of sucrose and glucose from a physical blend. This allowed a 100% rise in the amount of addable glucose. The co-crystallised material can be formed with up to 15% w/w of glucose added. It appears that using this methodology removes this factor and consequently the amount of addable glucose is heightened. This factor was also shown to be circumventable when glucose was added at 110°C followed by continued heating to 128°C. The amount of glucose incorporated was shown to be raised to 10% w/w.

The adsorption of glucose could be considered to result in its direct inclusion within the host sucrose structure. Whist there is certainly data that is suggestive of this, it cannot be conclusively proved due to the lack of significant

elevations in the unit cell volumes. Any increase in unit cell volume could not also be accorded with this being significant, all such data points fell outside of a 95% confidence interval. However, whilst this stops the theory of an inclusion of added components into a sucrose structure being robust, there is some evidence for this. Analysis of the axis parameters for materials that exhibit unit cell volume elevations (unfortunately, all insignificant) does provide some weight to the argument. What this mix of results shows that a concerted effort is required to reduce experimental error. A reduction of this may help provide adequate data to further the hypothesis that any added components include into the sucrose structure. It has been shown that substitution and/or addition of impurities or various substituents is synonymous with variation in unit cell volume and its parameters. A reduction in experimental error may provide data that falls in line with these reported observations.

Whilst it cannot be proven that added saccharides include within a sucrose structure there is also further evidence obtained from DSC. This data shows that the inclusion of glucose resulted in reduced enthalpy values for the melting of sucrose. This was accompanied by decreasing onset temperatures and peak broadening. Such behaviour is concordant with the doping of a host phase by an impurity; the lower enthalpy values arise from heightened lattice strain within the host material induced by the impurities present.[12-14] For an impurity to adsorb, it has to be related, structurally, to the host materials structure; the higher this degree of correlation the greater the strength adsorption as higher number of H-bonds are formed.

This idea can be seen from the work on the inclusion of fructose along with glucose to a supersaturated sucrose solution. Even low amounts of fructose allow the total inclusion of an amount of glucose that is normally not totally incorporable. The two monosaccharides constitute a sucrose molecule hence have the stereochemical features to match well. Furthermore, the power of fructose to enhance the inclusion of glucose is related to its capability to transform into the most favourable anomeric and ring size forms in solution when adsorbing.[17, 32]

If added materials are not structurally related to the host material, a reduction in crystal growth rate results from a reduction in the mass transfer of host material to the growing structure.[18, 19] The formation of materials formed

from a physical blend of sucrose and galactose appears to result in this phenomenon occurring; a hydrated form of sucrose is yielded and it has been postulated that a slow rate of crystal growth is necessary for the formation of this phase.[11, 31] This unique form of sucrose was also noted to be formed when 13-15% w/w of glucose was added to a sucrose solution at 110°C, followed by heating to 128°C and allowing crystallisation to take place.

4.5. References

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5.1. Co-Crystallisation of Lactose

Lactose is an important component of many foodstuffs, as such, investigation into how the co-crystallisation protocol affects this disaccharide is a valid sphere of investigation. The sugar is available in its α-D-lactose monohydrate form and previous work by Maillet has shown that a supersaturated solution can be formed by following the methodology as outlined in section 8.3.1. The extent to which saccharides can be included within a lactose matrix by the co-crystallisation is not clearly known; various mono- and disaccharides, in varying ratios, have been co-crystallised in order to provide a clearer picture.

Lactose is suitable for diabetic consumption; however it lacks the necessary sweetness for usage in foodstuffs on its own hence the co-crystallisation with various commonly used sweeteners was investigated in this study. Although the co-crystallisation method yields materials that are suggested to be co-crystalline, the notion that the added component is present in the amorphous state is a possibility. Subjection of various co-crystalline lactose materials to autosorb analysis was undertaken to ascertain the nature of the included material in the lactose matrix.

5.2.1. Re-Crystallisation of Lactose

α-D-lactose monohydrate was subjected to the re-crystallisation protocol as outlined in Section 8.3.1. DSC analysis (Fig 1) of the material showed the expected endothermic transitions for the material. A dehydration endotherm can be observed at ~ 145°C whilst the melting of the α-form is evident at ~ 210°C, these observed melting points correlate well with those reported within literature.[1]

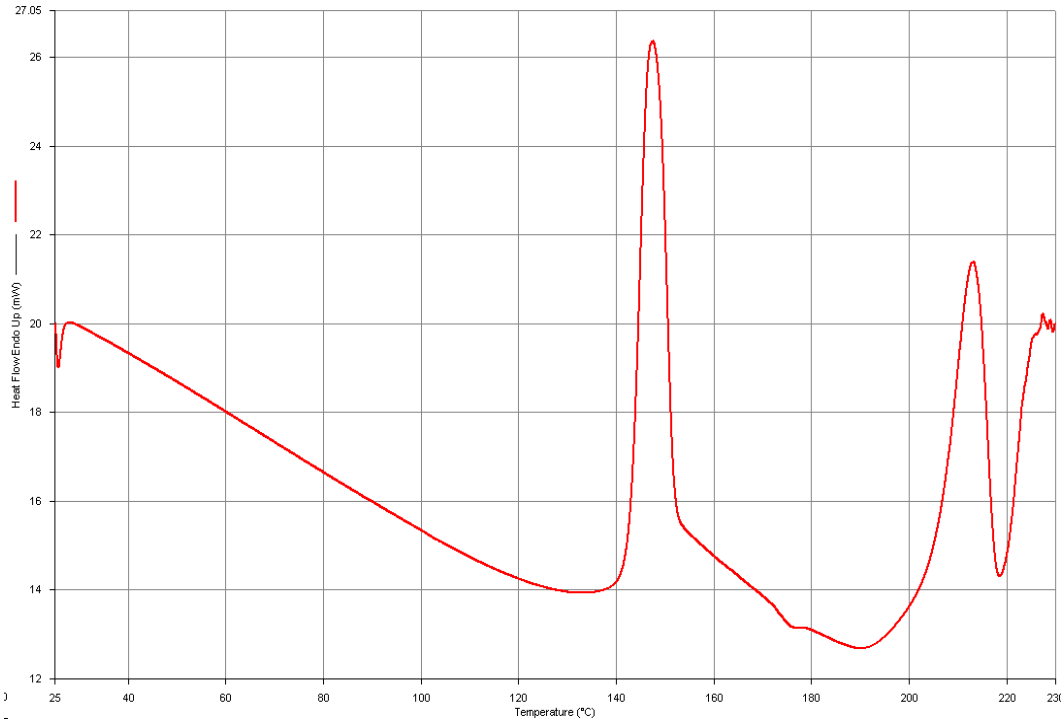


Fig 1. DSC Trace of Re-Crystallised Lactose

	Onset 1 (°C)	ΔH1 (Jg^{-1})	Onset 2 (°C)	ΔH2 (Jg^{-1})
Re-Crystallised Lactose	143.060	67.140	205.281	60.339

Table 1. DSC Data for Re-Crystallised Lactose

PXRD analysis of the material (Fig 2) yielded a diffractogram that indicate that the material is a mixture of the two anomers of lactose. The vast majority of the peaks conform to the α-anomer of lactose. However, a reflection at the 2θ position of ~ 10° suggests the presence of the β-anomer of lactose.

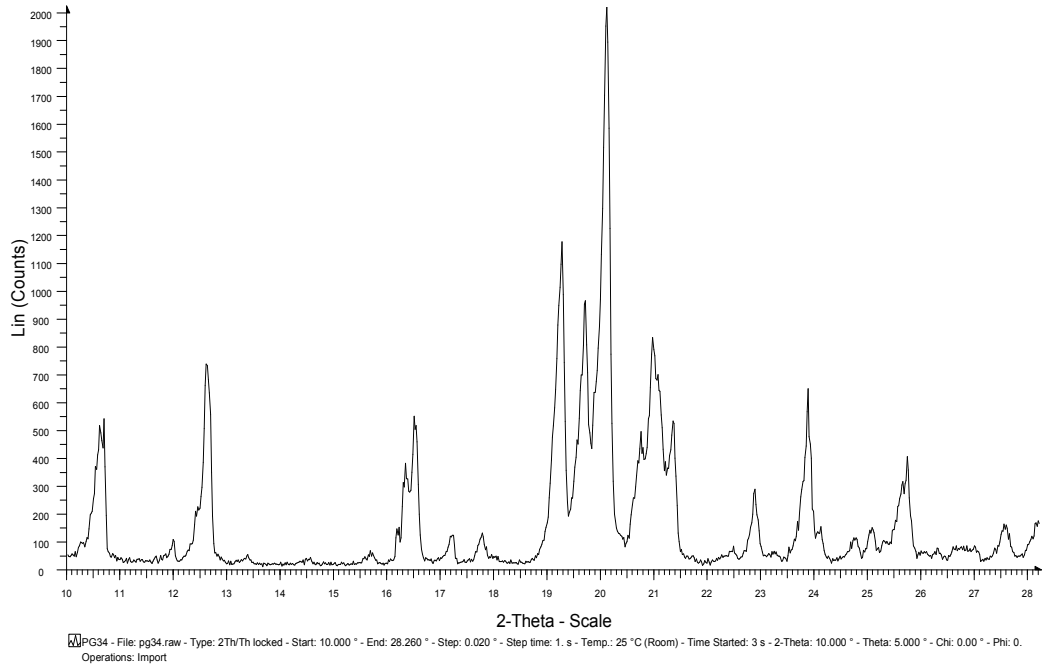


Fig 2. PXRD Diffractogram of Re-Crystallised Lactose

In order to elucidate the possibility of the presence of β-lactose, a Le Bail extraction was performed on the data obtained. The extraction showed that lactose re-crystallised in this manner is predominantly the α-monohydrate form. The ratio of alpha to beta was 96.3% to 3.7%. As such, PXRD data were used to calculate the unit cell dimensions for the re-crystallised lactose in its monohydrate form. The volume of the unit cell was 777.39 (± 13.869) $Å^3$ with the axis being; a = 7.836 (± 0.0048)Å, b = 21.516 (± 0.0093) Å and c = 4.839 (± 0.0027)Å, β = 107.689 (± 0.051)°. The unit cell displays monoclinic, $P2_1$ symmetry. As was the case with sucrose materials, these values will be used for comparison with other lactose materials.

5.2.2. Co-Crystallisation of Lactose with Maltose

5.2.2.1. Physical Blends of Lactose and Maltose

In order to unambiguously determine if co-crystalline materials had been formed using the co-crystallisation protocol, comparisons were drawn between the work performed by Maillet on the physical blends of lactose and maltose.[2] These showed that a characteristic endotherm for maltose emerges at ~ 120°C in

DSC analysis whilst within PXRD analysis, peaks at the 2θ positions of ~ 14° and 21° are suggestive toward the presence of maltose.

5.2.2.2. Co-Crystallisation of Lactose with Maltose

A range of materials were prepared using lactose as the matrix to which a proportion of maltose (7, 10, 15, 17 and 20% w/w) was included in accordance with the methodology outline in Section 8.3.2. This blend of sugars is of interest as it is suitable for consumption (in confectionary) by diabetics; the two sugars however do not have the desired sweetness. To this end, a third component was included to enhance the sweetness. All materials prepared were subjected to DSC analysis at a rate of 10°C min^{-1} over a range of -50-230°C and to PXRD analysis.

The DSC traces from the co-crystallised lactose and maltose materials (Fig 3) show that up to a level of 20% w/w of maltose can be added into a supersaturated lactose solution whilst exhibiting a single phase. The melting behaviour of the co-crystallised material is indicative of both the dehydration endotherm of lactose emerging at ~ 145°C followed by the α-anomer melting at ~ 210°C. The enthalpy associated with the melting of the α-form decreases with increasing levels of added maltose. This behaviour is also concordant with a slight depression of the onset melting temperature and a broadening of the melting endotherms. Upon elevation of the level of added maltose to 20% w/w, an endotherm emerges at ~ 120°C that is suggests the presence of a separate, crystalline maltose phase

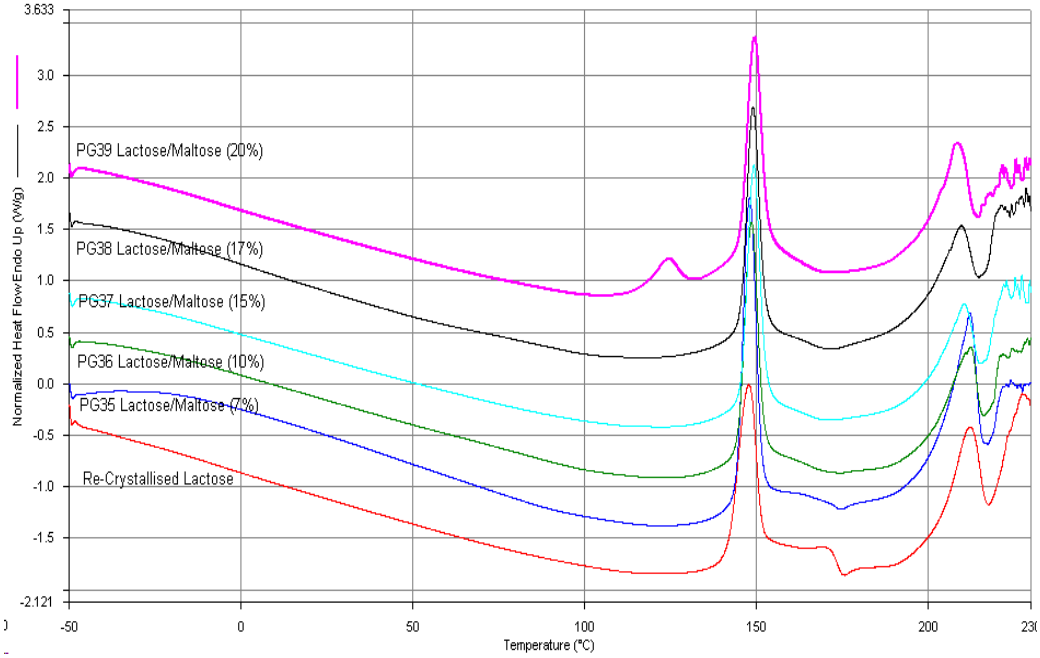
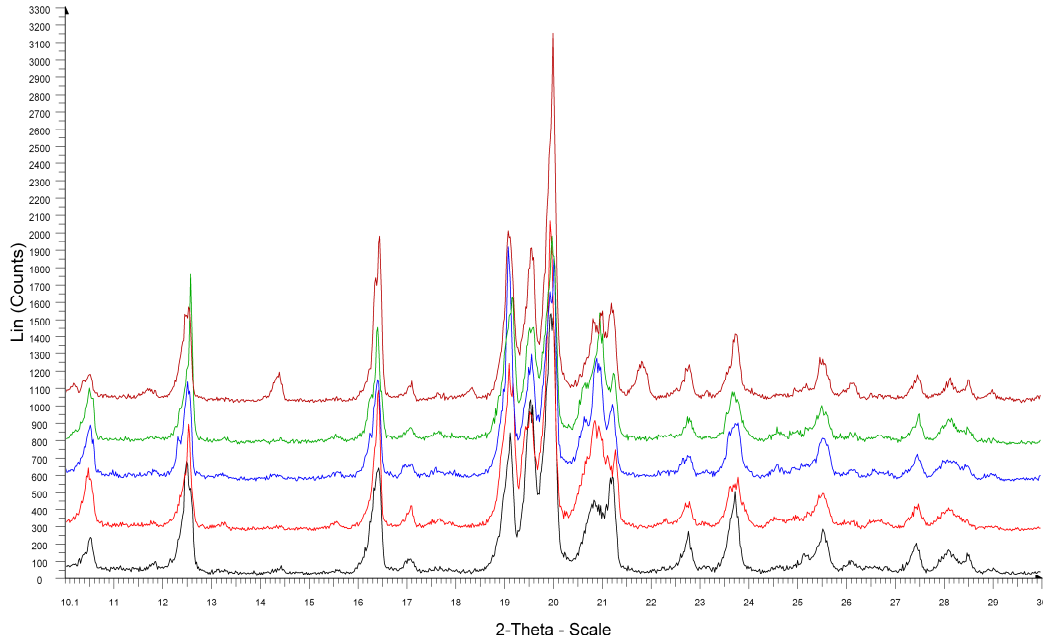


Fig 3. DSC Traces for Co-Crystalline Lactose and Maltose Materials

	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})	Onset 3 (°C)	ΔH 3 (Jg^{-1})
Re-crystallised Lactose	----------	---------	141.974	72.162	203.835	42.943
Lac/Mal (7%)	----------	---------	144.574	77.539	205.904	53.462
Lac/Mal (10%)	----------	---------	144.829	69.130	201.332	38.473
Lac/Mal (15%)	----------	---------	145.730	67.203	202.306	34.635
Lac/Mal (17%)	----------	---------	145.651	61.047	202.476	32.710
Lac/Mal (20%)	117.135	11.933	145.594	69.822	202.511	45.121

Table 2. DSC Data for Co-Crystalline Lactose and Maltose Materials

PXRD analysis (Fig 4) yielded results that were in good agreement with those obtained from DSC analysis. Up to 20% w/w of maltose gave diffractograms that are representative of crystalline lactose. Elevation of the incorporated maltose to 20% w/w resulted in a peaks appearing at the 2θ positions ~ 14.6° and 21.8°, these reflections are suggestive of the presence of maltose as a separate phase from the co-crystalline material.



Black: 7% Maltose w/w, Red: 10% Maltose w/w, Blue: 15% Maltose w/w, Green: 17% w/w Maltose, Purple: 20% Maltose w/w

Fig 4. PXRD Diffractograms for Co-Crystalline Lactose and Maltose Materials

Upon the incorporation of maltose into a lactose matrix, the unit cell for each of the co-crystalline material show an increase in volume compared to re-crystallised lactose. However, none of the increases are significant, all the data points fall outside of the 95% confidence interval.

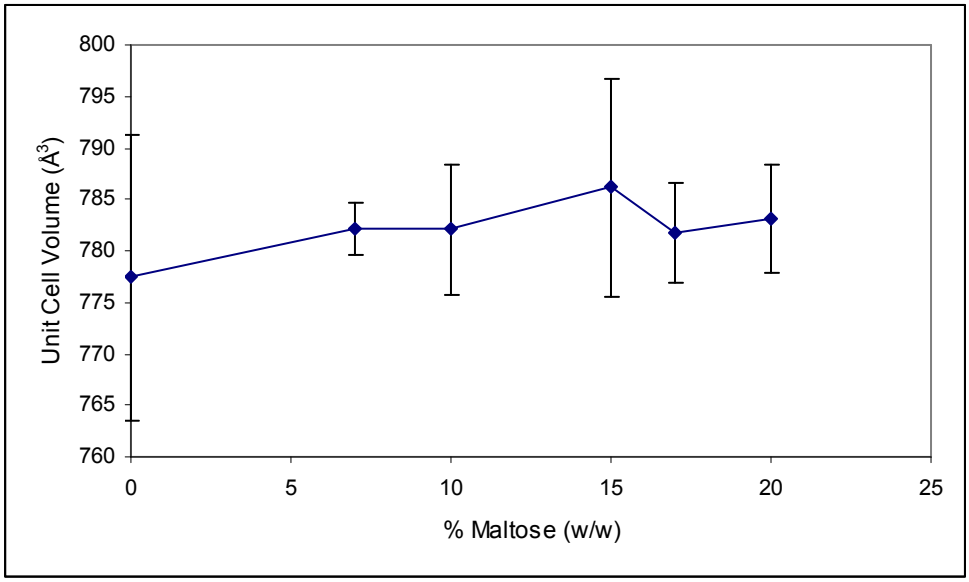


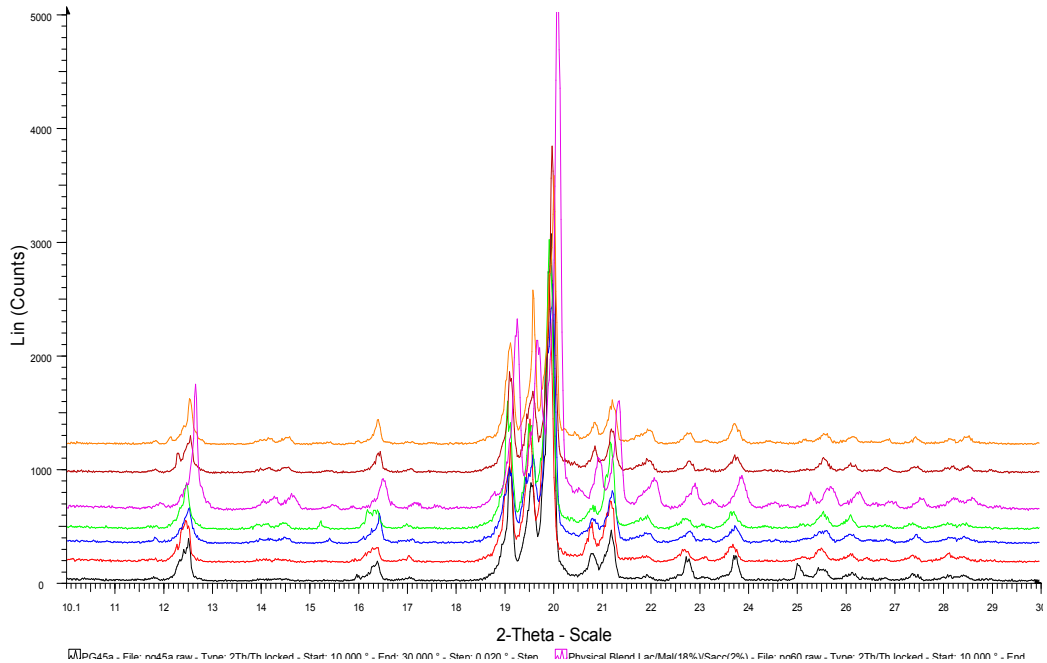
Fig 5. Unit Cell Volume vs. Level of Incorporated Maltose.

5.2.3. Co-Crystallisation of Lactose with Maltose and Saccharin

5.2.3.1. Physical Blends of Lactose with Maltose and Added Saccharin

DSC analysis (Fig 6) of this lactose blended with maltose (5-25% w/w) and saccharin (2% w/w) showed the dehydration of lactose occurring at ~ 145°C and the melting of the α-anomer at ~ 210°C, the melting of maltose monohydrate is observed at ~120°C but a peak for saccharin could not be positively identified. This may result from either the quantity being to low for detection by DSC analysis or, as it has a similar melting point to the α-anomer of lactose it becomes lost in this melt. From the DSC data (Table 3) it is observed that with increasing amounts of maltose present in the mixture, the enthalpy for the melting of maltose increases thus showing that a higher quantity of the disaccharide is present.

PXRD analysis (Fig 7) for the range of materials showed characteristic diffraction patterns for α-lactose monohydrate, peaks at the 2θ positions of ~ 14° and 21° confirmed the presence of maltose as a separate phase. Unlike DSC analysis, PXRD allowed characteristic peaks for saccharin to be identified; reflections at the 2θ positions of ~ 14.9° and 15.1° were indicative of crystalline saccharin.



Black: Re-Crystallised Lactose, Red: α-Lactose Monohydrate, Blue: 5% Maltose, 2% Saccharin w/w, Green: 8% Maltose, 2% Saccharin w/w, Purple: 13% Maltose w/w, 2% Saccharin, Brown:15% Maltose, 2% Saccharin, Orange: 18% Maltose, 2% Saccharin w/w

Fig 7. PXRD Diffractograms for Physical Blends of Lactose, Maltose (5-25% w/w) and Saccharin (2% w/w)

5.2.3.2. Co-Crystallisation of Lactose with Maltose and Added Saccharin

DSC analysis of this series of materials (Fig 8) showed that addition of maltose (up to 18% w/w) and saccharin (2% w/w) yielded only single phases. The enthalpy of fusion values for the materials dropped dramatically compared to re-crystallised lactose, values for the onset temperature do not alter to a large extent (table 2). Unlike co-crystallised lactose and maltose, the dehydration endotherm is barely present when saccharin is incorporated into the system. The melting of this material strongly resembles β-lactose. In this combination of materials it appears that a larger quantity of addable components is possible. The addition of 20% w/w of maltose yielded a material that exhibits two phases (see Section 5.2.2.2.) whereas in this study, when 20% w/w *in total* had been included, only a phase related to lactose results. Conformation of the presence of solely lactose nature of these materials was achieved by comparison with physical blends of sugars in analogous ratios.

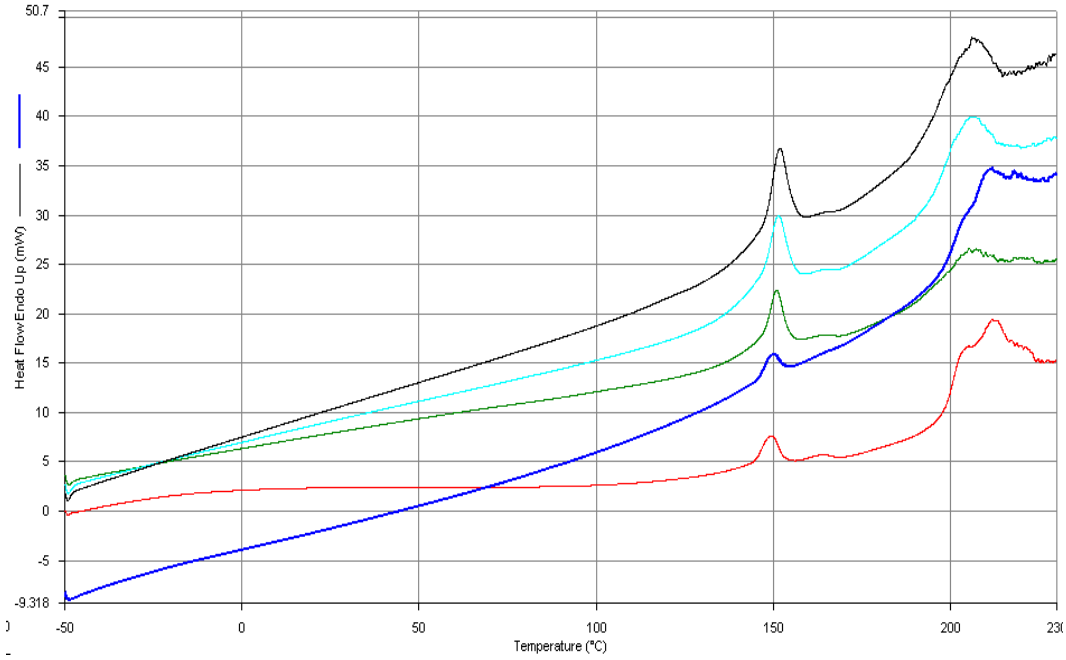
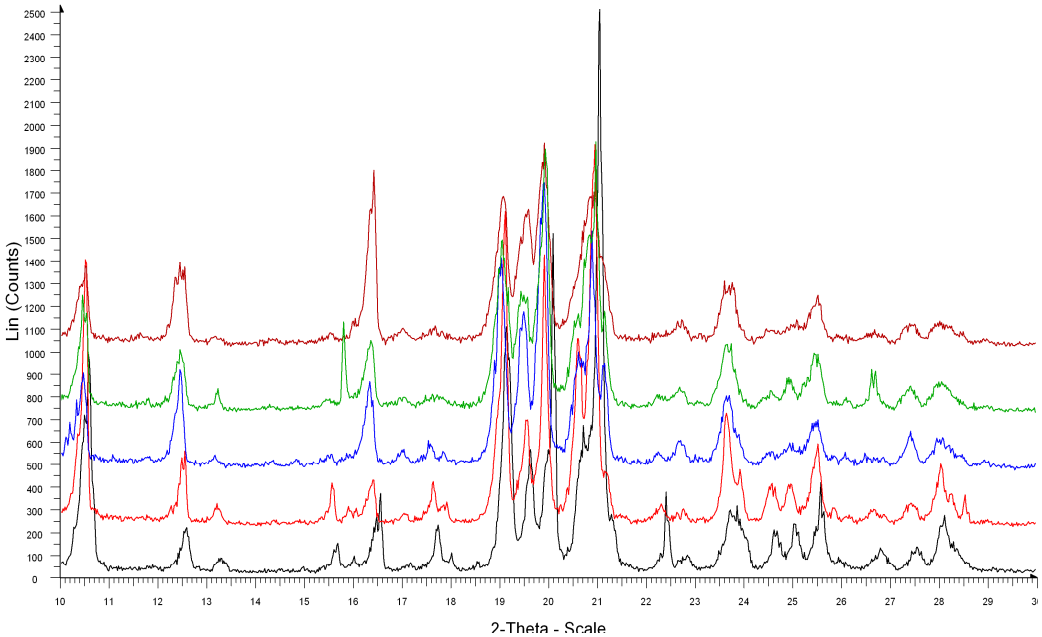


Fig 8. DSC Traces for Co-Crystalline Lactose, Maltose and Added Saccharin Materials

	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Re-crystallised Lactose	141.974	72.162	203.835	42.943
Lac/Sacc(2%)/Mal(5%)	145.046	14.822	202.768	40.567
Lac/Sacc(2%)/Mal(8%)	146.439	9.938	200.234	35.673
Lac/Sacc(2%)/Mal(13%)	146.554	46.168	201.562	33.908
Lac/Sacc(2%)/Mal(15%)	146.181	43.065	197.560	31.876
Lac/Sacc(2%)/Mal(18%)	147.207	41.863	199.654	28.987

Table 4. DSC Data for Co-Crystalline Lactose, Maltose and Added Saccharin Materials

PXRD analysis (Fig 19) provided conformation of the observations noted from DSC analysis. Up to 18% w/w of added maltose and 2% of added saccharin can be successfully added to a supersaturated lactose solution whilst still retaining single phase attributable to lactose.



Black: 5% Maltose, 2% Saccharin w/w, Red: 8% Maltose, 2% Saccharin, Blue: 13% Maltose, 2% Saccharin w/w, Green:15% Maltose, 2% Saccharin w/w, Purple:18% Maltose, 2% Saccharin w/w

Fig 9. PXRD Diffractograms for Co-Crystalline Lactose, Maltose and Saccharin Materials

No peaks relating to the presence of a separate maltose or saccharin phase could be observed. Interestingly, the diffraction patterns indicate the presence of both anomers of lactose. Fitting of the patterns confirms this, all the materials contain significant quantities of β-lactose: table five details the ratios found from fitting of the patterns.

	α-lactose content	β-lactose content
Re-crystallised Lactose	96.3%	3.7%
Lac/Sacc(2%)/Mal(5%)	85.6%	14.4%
Lac/Sacc(2%)/Mal(8%)	61.2%	38.8%
Lac/Sacc(2%)/Mal(13%)	60.8%	39.2%
Lac/Sacc(2%)/Mal(15%)	76.2%	32.8%
Lac/Sacc(2%)/Mal(18%)	70.3%	39.7%

Table 5. Ratios of α:β Lactose in Co-crystallised Lactose, Maltose and Saccharin Materials

It appears that the addition of maltose and saccharin to a supersaturated lactose solution yields a final material that is a mix of the two anomers of lactose.

Fitting of the patterns showed that the co-crystalline material is still predominantly α-lactose in nature. This observation fits with the data obtained from DSC analysis. The significantly reduced values of ΔH (see table 4) for the dehydration of lactose suggest there is a lesser presence of this material within the co-crystalline lactose, maltose and saccharin. For the purposes of unit cell volume comparison, the volumes will be calculated using both the model for α-lactose monohydrate (fig 10) and the β-lactose anomer (fig 11).

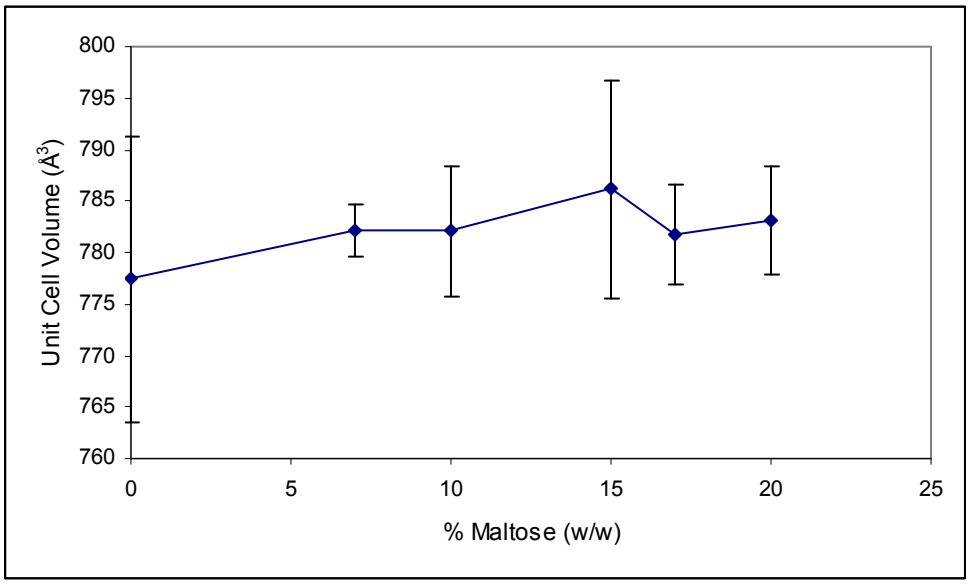


Fig 10. Unit Cell Volumes for Co-Crystalline Lactose, Maltose and Saccharin Materials Based on a α-Lactose Monohydrate Model

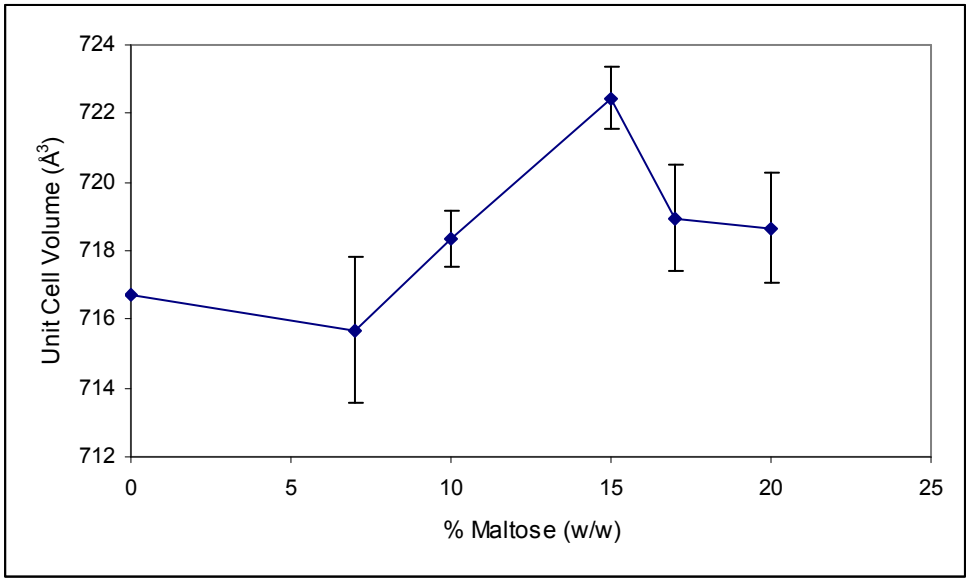


Fig 11. Unit Cell Volumes for Co-Crystalline Lactose, Maltose and Saccharin Materials Based on a β-Lactose Anhydrous Model

As can be seen from figures ten and eleven, utilisation of either model as the basis for unit cell volume determination reveals a similar pattern in the data. Overall, there is an observable increase in the unit cell volume, compared to re-crystallised lactose. However, these elevations in volume can not be construed as significant in either case; all of the data points in figures ten and eleven fall outside of the 95% confidence interval.

In order to investigate the effects of how large a quantity *in total* of maltose and saccharin can be successfully included into a lactose matrix, elevated amounts of maltose were attempted to be incorporated. The DSC traces (Fig 12) of co-crystalline lactose, maltose (20-25% w/w) and saccharin (2% w/w) show that elevation of the level of added maltose too 20% and above results in the formation of two phases. Both a phase related to lactose and a maltose phase is observable. Both sets of materials exhibit an endotherm at ~ 117°C that is relevant to the presence of maltose. This observation is confirmed by comparison with physical blends of the materials and interestingly, no endotherm for saccharin could be observed. Lactose endotherms were present at ~ 145°C and ~ 200°C that corresponds to the dehydration of lactose monohydrate and the α-form of lactose respectively.

DSC data (Table 6) shows that the endotherms relevant to lactose display lower ΔH values with higher levels of maltose incorporation. This is reinforced

by comparison of ΔH values for the maltose phase. A higher degree of maltose present as a separate phase is possible due to the higher value of ΔH for the maltose phase.

An interesting point to note is the differences in the value of ΔH for the maltose endotherm for the co-crystallised material containing solely 20% w/w of maltose and material containing an identical quantity of maltose and the addition of 2% w/w of saccharin. Upon addition of saccharin, the value for the endotherm in question dropped significantly from 11.933 Jg^{-1} to 0.67 Jg^{-1}.

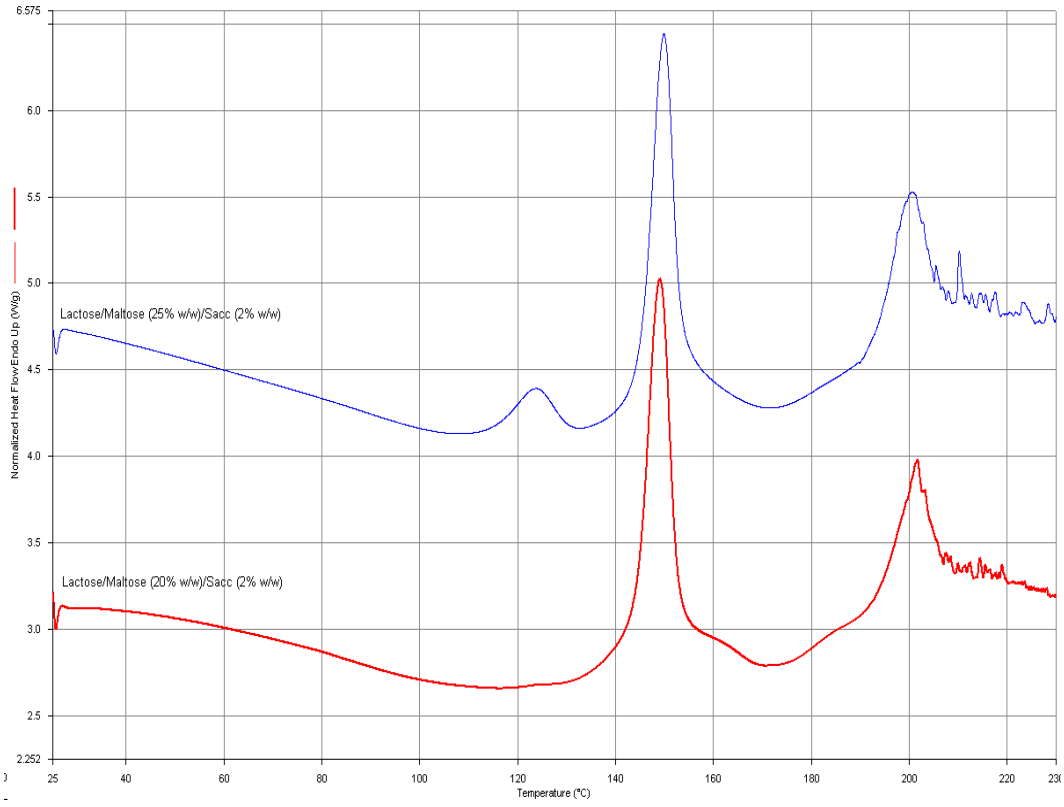


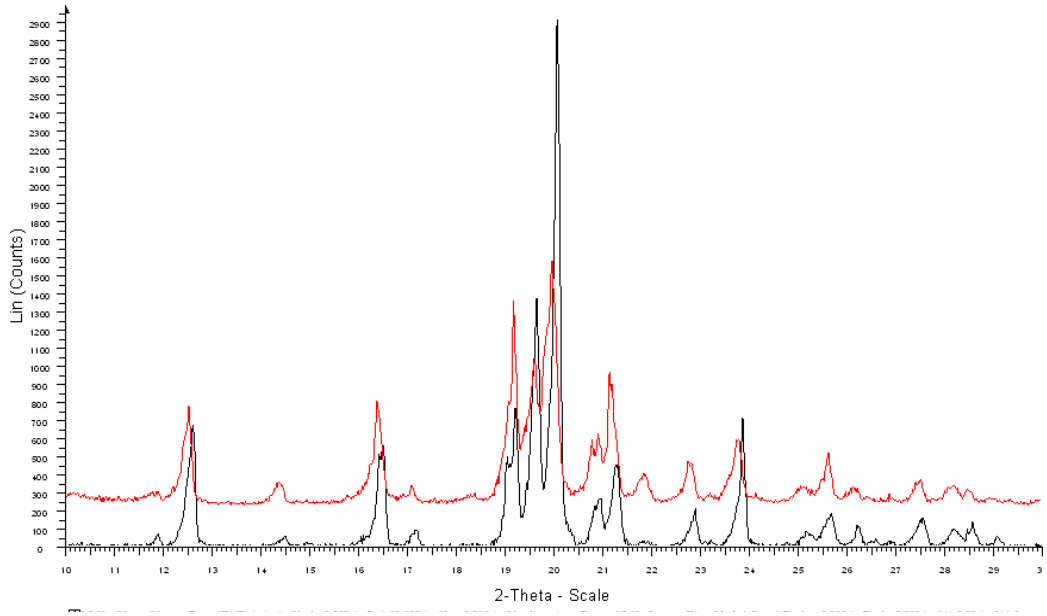
Fig 12. DSC Traces of Co-Crystalline Lactose, Maltose and Added Saccharin

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Lac/Malt(20%)/Sacc(2%)	0.067	119.039	76.904	144.298	27.959	196.854
Lac/Malt(25%)/Sacc(2%)	13.301	115.385	87.844	145.071	26.987	195.639

Table 6. DSC Data for Co-Crystalline Lactose, Maltose and Added Saccharin

Co-crystallised lactose, saccharin (2% w/w) and maltose (20-25% w/w) were subjected to PXRD analysis (Fig 13) and the diffractograms suggested that

maltose, when co-crystallised with saccharin, exhibits two phases when included at a level of 20% w/w and above.



Black: Maltose 20%, Saccharin 2% w/w, Red: Maltose 25% w/w, Saccharin 2% w/w

Fig 13. PXRD Diffractograms for Co-Crystalline Lactose, Maltose and Saccharin Materials

At this level and above, two phases are observable in the diffractograms, a phase relating to lactose and a phase suggesting the presence of maltose can be observed. PXRD diffractograms for the material containing 20-25% w/w of maltose displayed peaks at the 2θ positions of ~ 14.3° and 21.9° suggesting the presence of a crystalline maltose phase. Comparison of analogous ratios of materials in a physical blend confirmed that these peak positions are indicative of a maltose phase. No peaks relating to saccharin could be observed within the diffractograms. Interestingly, when maltose and saccharin were added at these levels, the form of lactose obtained was the α-monohydrate anomer rather than the β-anomer when the added sugars were included in lower levels. This was confirmed by fitting of the pattern with models for alpha- and beta-lactose. The ratio of the anomers was shown to be 96% α-lactose monohydrate to 4% of β-lactose. The diffractograms enabled the calculation of the unit cell volumes (Fig 14) as a function of included maltose for the materials.

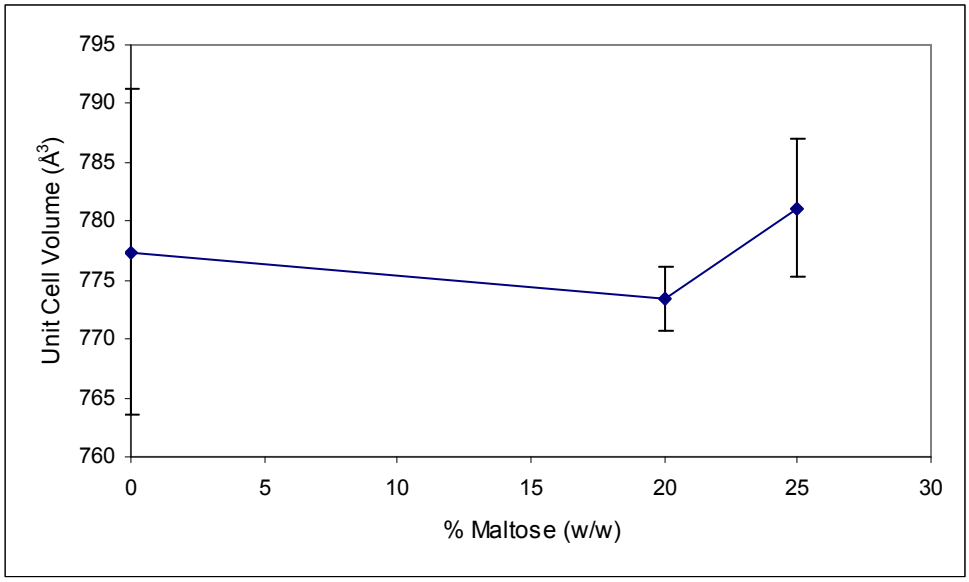


Fig 14. Unit Cell Volume vs. % Incorporated Maltose

Unit cell volumes for the material containing 20% w/w of maltose exhibited a slightly smaller unit cell volume. Elevation of the level of added maltose yielded a material with a larger unit cell volume compared to re-crystallised lactose. This however is not a significant increase, the data for this elevation falls outside of the 95% confidence region.

5.2.3.3. Co-Crystallisation of Lactose with Maltose and Higher Amounts of Saccharin

With the notion that 2% w/w of added saccharin possibly induces higher levels of addable maltose, the investigation into how higher levels of saccharin effect this was undertaken. DSC analysis (Fig 15) of these materials revealed that with the addition of 4% w/w of saccharin and 25% w/w of maltose yielded a material that exhibited two phases. The emergence of an endotherm at ~ 116°C suggests the presence of a separate maltose phase. Elevation of the level of added saccharin to 10% w/w along with 25% w/w of maltose resulted in a material that appeared to be show only lactose melting. The DSC trace (Fig 15) shows the presence of lactose with no maltose or saccharin endotherms. The confirmation of the peak at ~ 116°C relating to maltose was achieved by comparison with physical blends of the materials. The melting behaviour of the characteristic lactose peaks differs slightly depending on the quantity of saccharin added, a higher level of saccharin results in an increase in the enthalpy associated with the

lactose endotherm present at ~ 145°C. For both sets of materials, no melting of saccharin was observed.

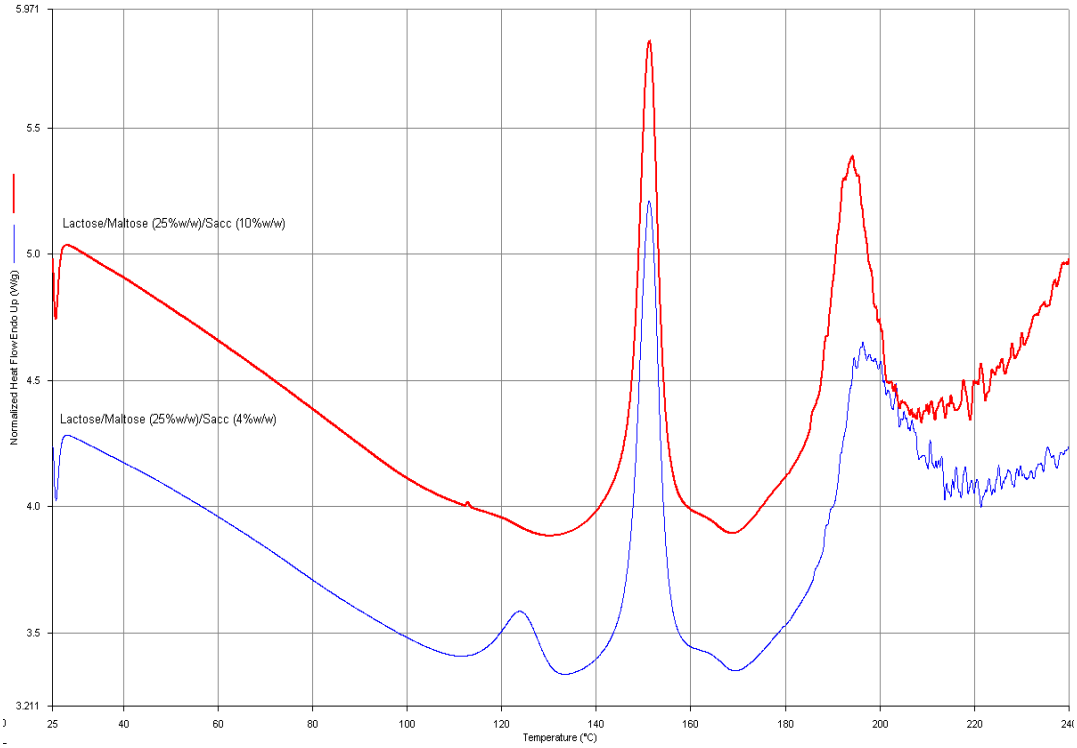


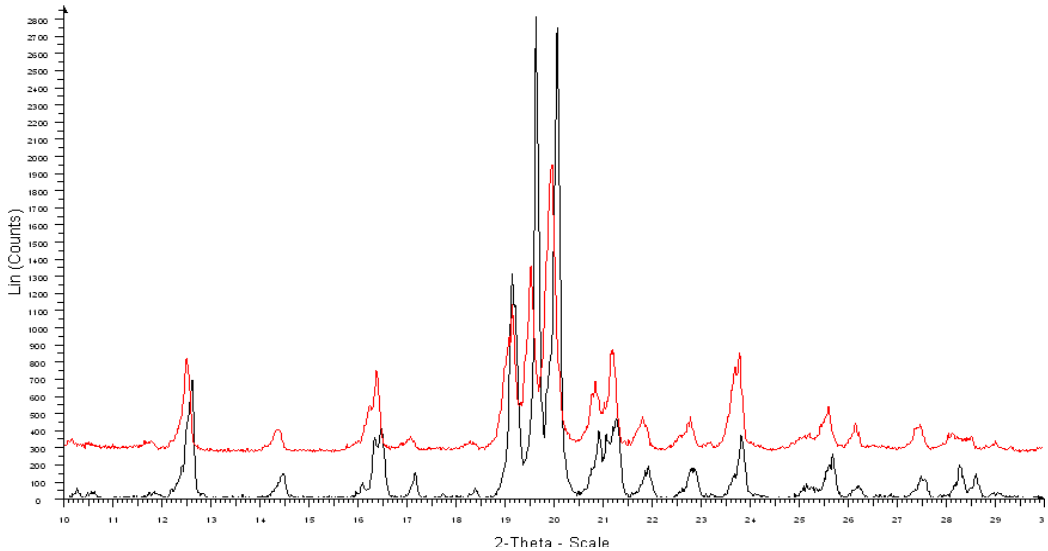
Fig 15. DSC Traces for Lactose, Maltose and Saccharin Co-Crystalline Materials

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Lac/Malt(25%)/Sacc(4%)	11.035	116.377	57.946	146.811	19.118	192.335
Lac/Malt(25%)/Sacc(10%)	--------	---------	64.982	146.943	49.856	188.892

Table 7. DSC Data for Lactose, Maltose and Saccharin Co-Crystalline Materials

The co-crystallised lactose, maltose (25% w/w) and saccharin (4-10% w/w) materials were subjected to PXRD analysis (Fig 16) and the diffractograms suggested that elevation of the level of included saccharin does not increase the level of addable maltose. Three phases are detectable in the diffractograms, a phase relating to co-crystalline material, a phase related to the presence of maltose and a saccharin phase. The emergence of peaks at the 2θ positions of ~ 14.1° and 21.7° indicates the presence of maltose and a peak emerging at 2θ position of ~ 16.7° is indicative of the presence of a phase relating to saccharin.

Comparison with analogous ratios of materials in a physical blend confirmed this observation. Calculation of the unit cells (Fig 17) was possible from the diffractograms and the data shows little variation in the unit cell volume.



Black: Maltose 25%, Saccharin 4% w/w, Red: Maltose 25%, Saccharin 10% w/w

Fig 16. PXRD Diffractograms for Co-Crystalline Lactose, Maltose (25% w/w) and Saccharin (4-10% w/w)

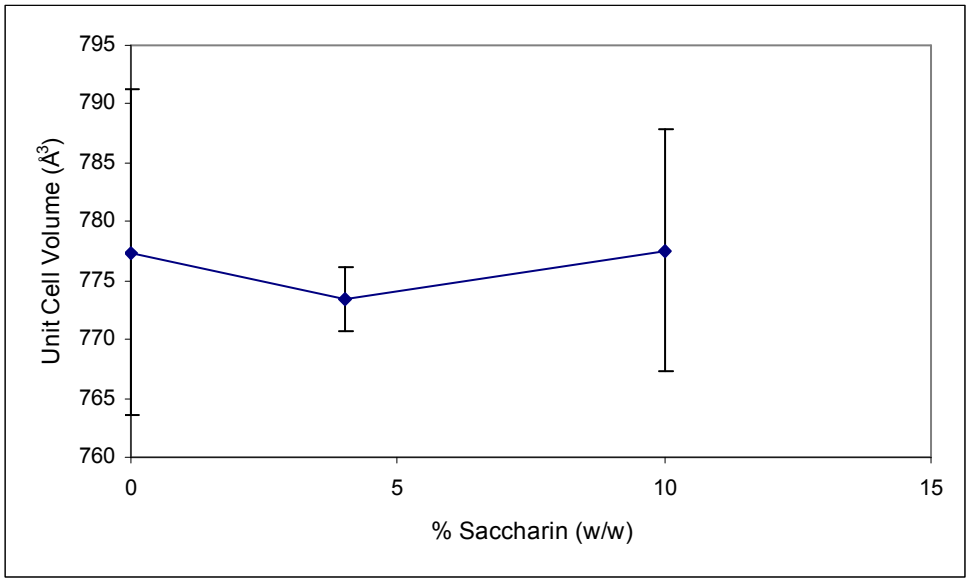


Fig 17. Unit Cell Volume vs. % Incorporated Saccharin

In order to ascertain the physical nature of the included maltose and saccharin, co-crystalline lactose, maltose (25% w/w) and saccharin (10% w/w) was subjected to an autosorb study. The plot of relative humidity (RH) against percentage weight gain obtained is shown in figure eighteen.

Autosorb data suggests that the material has no amorphous content, which indicates that the physical nature of the material to be crystalline rather than amorphous.

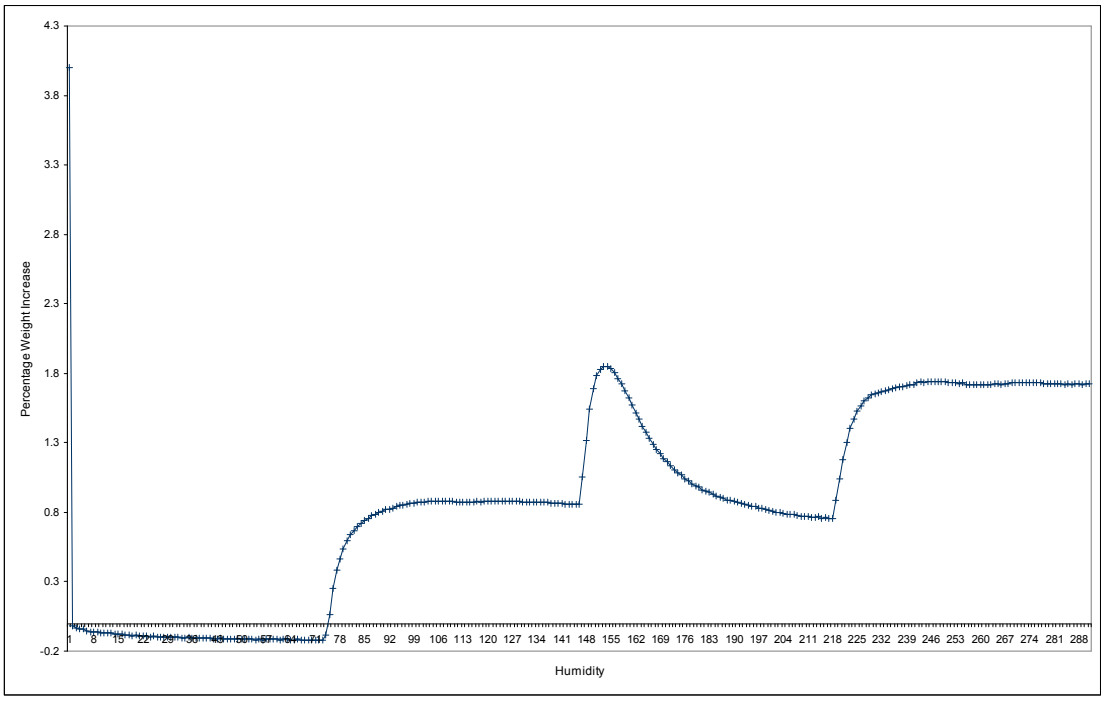


Fig 18. Relative Humidity *vs*. Percentage Weight Gain for Co-Crystalline Lactose, Maltose and Saccharin

For the co-crystallised material, it was observed that for each elevation of RH, an there was an increase in the percentage weight gain. This increase was subsequently followed by a steady decrease in the percentage weight gain; this trend continued until the samples deliquesced at ~ 90% RH.

5.2.3.4. Co-Crystallisation of Lactose with Staggered Addition of Maltose and Saccharin

Initially, the addition of maltose and saccharin to a supersaturated lactose solution was performed with simultaneous addition of maltose and saccharin. The effects of staggering this addition were investigated by altering the

methodology according to Section 8.3.5. A DSC trace (Fig 19) of co-crystallised lactose, maltose (18% w/w, added first) and saccharin (2% w/w, added second) revealed that the material yielded gave melting behaviour that was indicative of a mixture of phases.

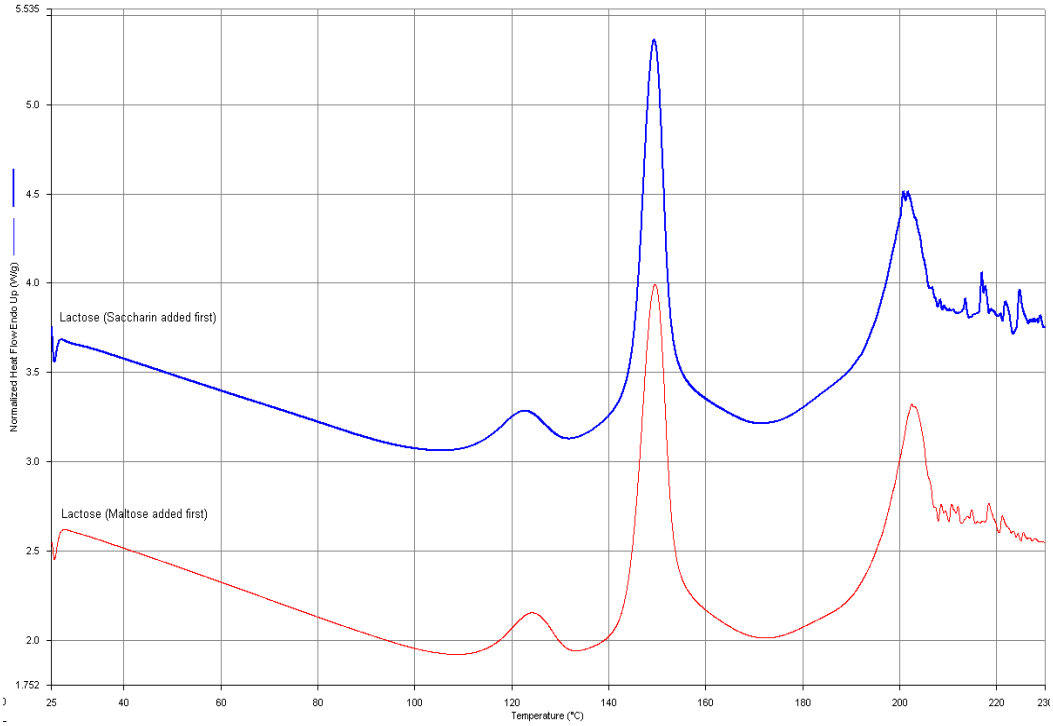


Fig 19. DSC Traces for Co-Crystalline Lactose Materials with Staggered Addition of Maltose and Saccharin

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Lac/Malt(18%)/Sacc(2%)	12.416	115.525	70.400	144.045	34.728	198.263
Lac/Sacc(2%)/Malt(18%)	8.812	114.511	68.148	144.942	34.686	196.802

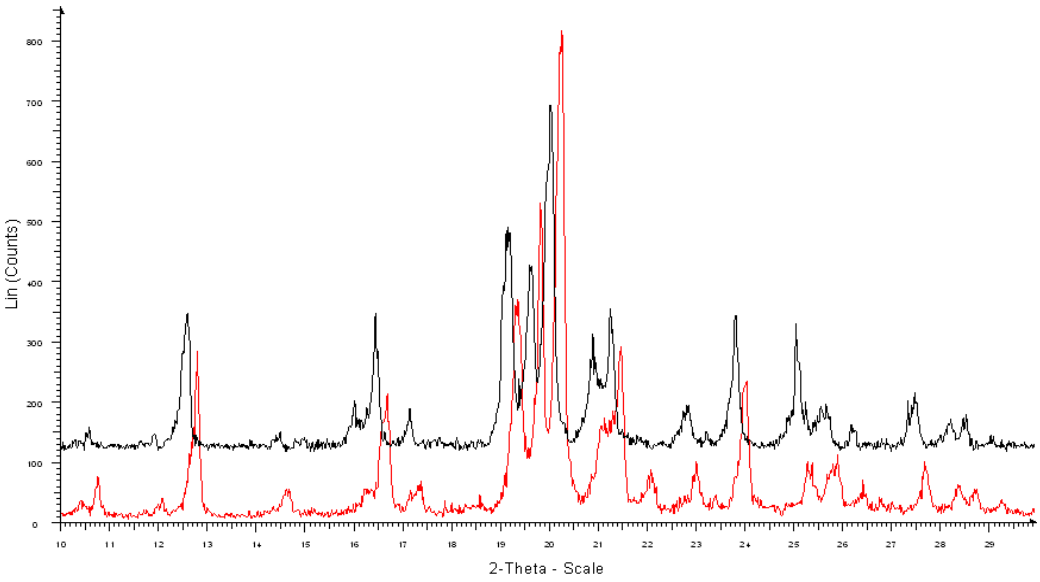
Table 8. DSC Data for Lactose, Maltose and Saccharin Materials

The DSC traces for the co-crystallised lactose, saccharin (2% w/w, added first) and maltose (18% w/w, added second) showed that the material exhibited similar melting behaviour to when the maltose was added first followed by the saccharin. An endotherm emerges at ~ 117°C that is indicative of a maltose presence in the material along with the expected lactose endothermic peaks. No saccharin could be detected in either material. Confirmation of the emerging

endotherm representing maltose was achieved by comparison with a physical blend.

The DSC data (Table 8) obtained allows the comparison of the above two sets of materials. It appears that the addition of maltose first results in a lower level of incorporation compared to the addition of saccharin first.

PXRD analysis (Fig 20) of the two materials provided diffractograms that were in agreement with the data yielded from the DSC analysis (Table 8). Thus, staggering the addition (either saccharin or maltose first) produced a material that displayed a mixture of phases.



Red: Maltose (added first) 18%, Saccharin 2% w/w, Black: Maltose 18%, Saccharin (added first) 2% w/w

Fig 20. PXRD Diffractograms of Co-Crystalline Lactose with Staggered Addition of Maltose and Saccharin

The diffractograms of co-crystallised lactose with the staggered addition of maltose (18% w/w) and saccharin (2% w/w) gave characteristic peaks for the presence of lactose along with peaks emerging at the 2θ positions ~ 14.3° and 21.4°. This suggests the presence of a separate maltose phase. Again, like in DSC the PXRD analysis (Figs 19 and 20 respectively) showed no peaks were observable that could suggest the presence of a saccharin phase. Calculation of the unit cell volume from the diffractograms for the material with maltose added

first gave a unit cell volume (774.09 (± 3.1445) $Å^3$) which is smaller compared to material containing analogous amounts of maltose and saccharin (787.06 (± 12.7469) $Å^3$) when they are co-crystallised. The unit cell volume is markedly smaller than the volume for solely re-crystallised lactose. For the co-crystallised material when saccharin was included first followed by maltose; the unit cell volume calculated is 783.04 (± 6.6896)$Å^3$ which is smaller than the material containing an analogous quantity of saccharin and maltose. However, it is still larger than that for lactose alone, but akin to the material with maltose and saccharin included simultaneously; this increase is not significant.

5.2.4. Co-Crystallisation of Lactose with Maltose & Aspartame

5.2.4.1. Physical Blends of Lactose with Maltose and Aspartame

DSC analysis (Fig 21) of this lactose blended with maltose (5-25% w/w) and aspartame (2% w/w) showed the dehydration of lactose occurring at ~ 145°C and the melting of the α-anomer at ~ 210°C, the melting of maltose monohydrate is observed at ~120°C but a peak for aspartame could not be positively identified. This may result from either the quantity being to low for detection by DSC analysis or, or it lost in the melting of the α-anomer of lactose. From the DSC data (Table 8) it is observed that with increasing amounts of maltose present in the mixture, the enthalpy for the melting of maltose increases thus showing that a higher quantity of the di-saccharide is present.

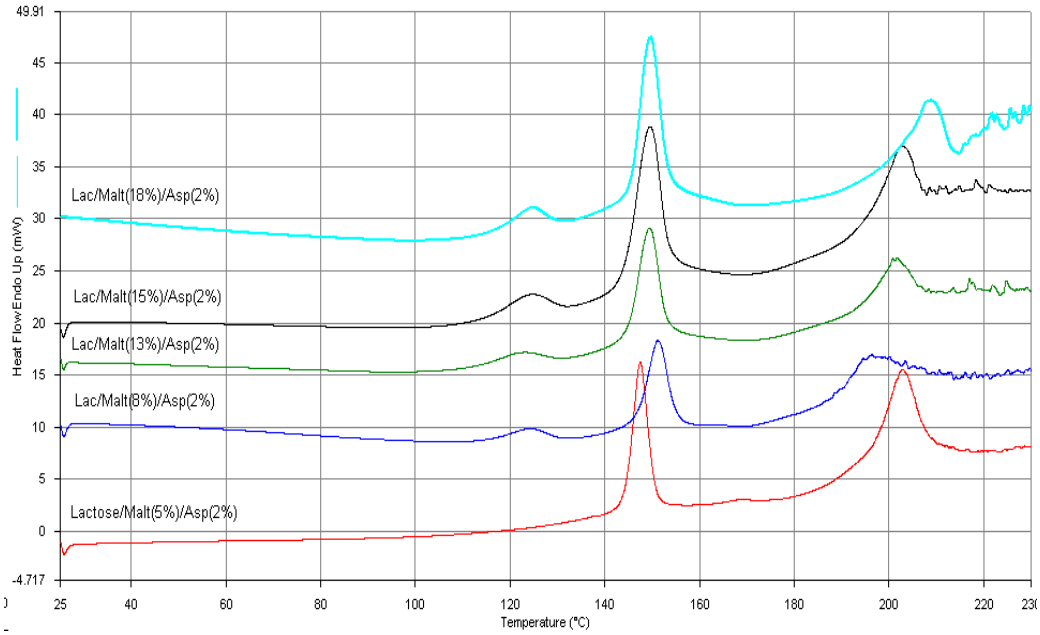
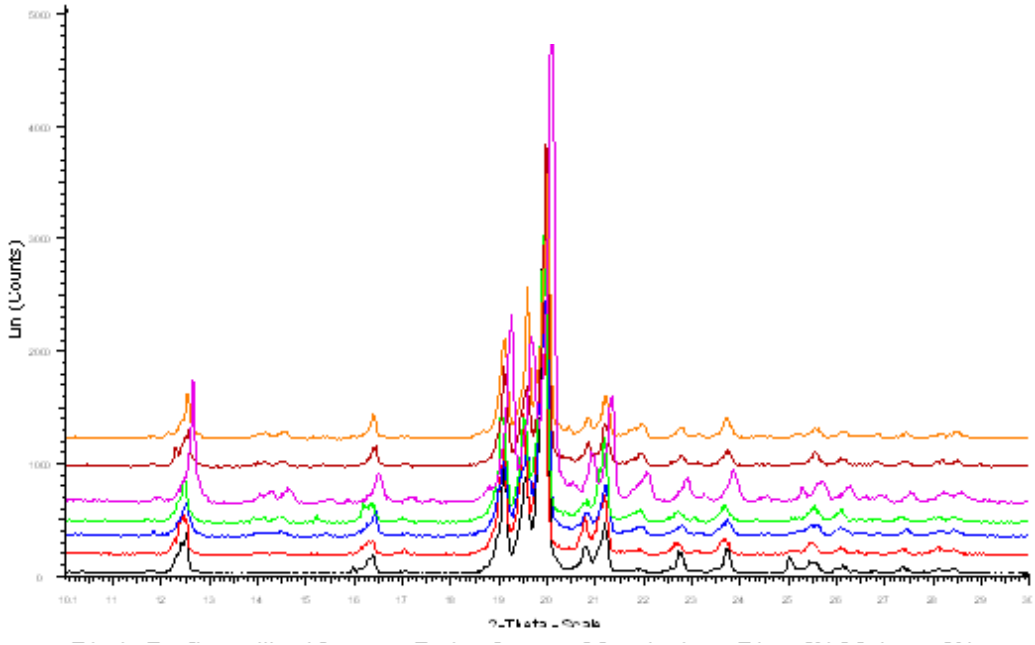


Fig 21. DSC Traces of Physical Blends of Lactose, Maltose (5-18% w/w) and Aspartame (2% w/w)

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Lac/Malt(5%)/Asp(2%)	1.648	122.427	89.700	145.002	96.543	202.346
Lac/Malt(8%)/Asp(2%)	6.691	124.850	88.563	145.192	92.517	204.635
Lac/Malt(13%)/Asp(2%)	10.531	121.859	91.929	145.911	90.094	199.082
Lac/Malt(15%)/Asp(2%)	16.876	123.092	91.902	145.825	93.903	197.994
Lac/Malt(18%)/Asp(2%)	17.267	123.808	92.510	144.724	91.563	204.346

Table 9. DSC Data for Physical blends of Lactose, Maltose and Aspartame

PXRD analysis (Fig 22) for the range of materials showed characteristic diffraction patterns for α-lactose monohydrate, peaks at the 2θ positions of ~ 14° and 21° confirmed the presence of maltose as a separate phase. In an analogous manner to DSC analysis, PXRD did not allow characteristic peaks for aspartame to be identified. However, a PXRD scan of solely aspartame suggests reflections at the 2θ positions of ~ 11.20°, 14.94° and 18.38° will suggest the presence of crystalline aspartame due to their intensity.



Black: Re-Crystallised Lactose, Red: α-Lactose Monohydrate, Blue: 5% Maltose, 2% Aspartame w/w, Green: 8% Maltose, 2% Aspartame w/w, Purple: 13% Maltose w/w, 2% Aspartame, Brown:15% Maltose, 2% Aspartame, Orange: 18% Maltose, 2% Aspartame w/w

Fig 22. PXRD Diffractograms for Physical Blends of Lactose, Maltose and Aspartame

5.2.4.2. Co-Crystallisation of Lactose with Maltose and Aspartame

Co-crystallisation of lactose with maltose and aspartame yielded materials that exhibited melting behaviour (Fig 23) that is indicative of solely lactose up to a quantities of 15% w/w of maltose and 2% w/w of aspartame being added. Above these levels of added maltose and aspartame, a second endotherm emerged at ~ 116°C that suggests the presence of a separate maltose phase. For all materials, the melting of aspartame could not be detected. All materials displayed the expected melting endotherms for lactose at ~ 145°C (lactose monohydrate) and the α-form at ~ 200°C. Again, the singularity of the phase of the samples was confirmed by comparing physical blends of the sugars that were co-crystallised.

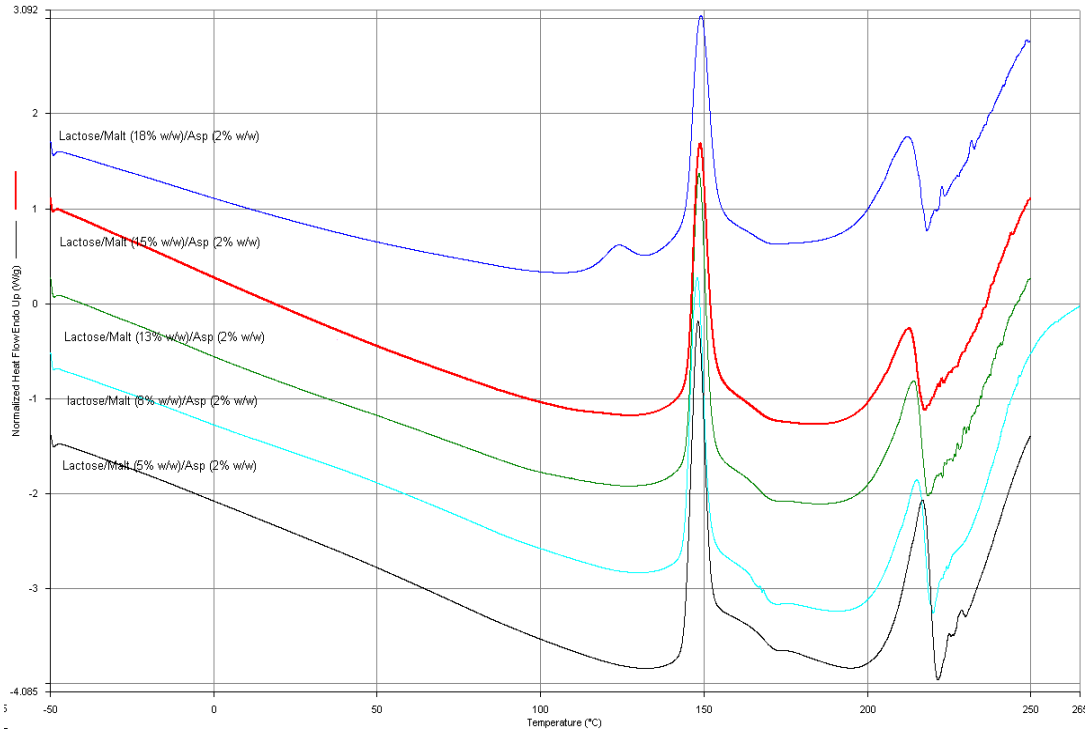
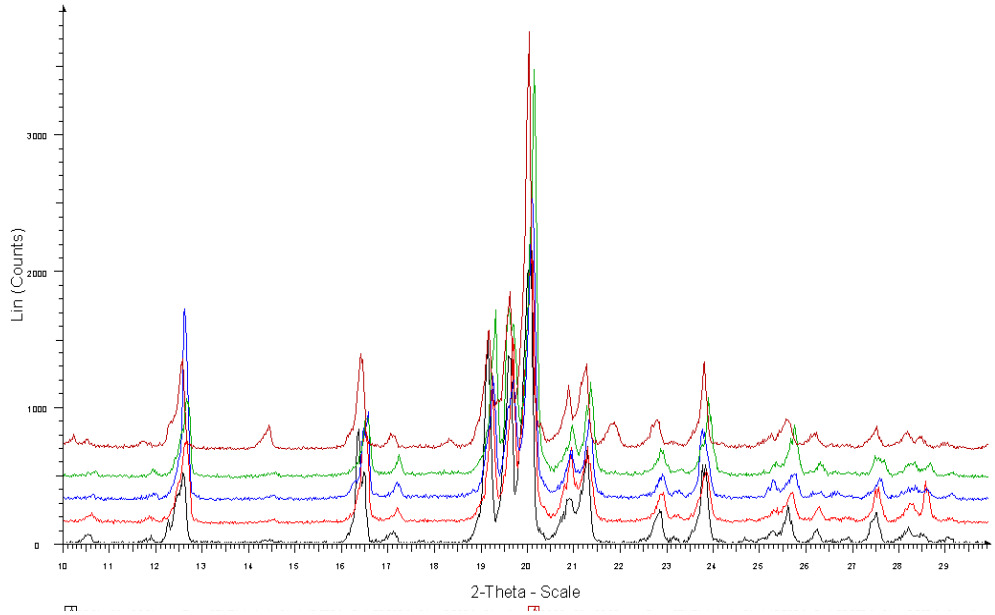


Fig 22. DSC Traces for Co-Crystalline Lactose, Maltose and Aspartame Materials

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Lac/Asp(2%)/Malt(5%)	------	---------	99.896	144.561	100.896	206.989
Lac/Asp(2%)/Malt(8%)	------	---------	84.092	144.002	68.370	206.056
Lac/Asp(2%)/Malt(13%)	------	---------	87.417	144.845	61.416	204.267
Lac/Asp(2%)/Malt(15%)	------	---------	81.129	145.107	52.481	202.384
Lac/Asp(2%)/Malt(18%)	6.926	117.208	79.307	144.698	70.282	199.947

Table 11. DSC Data for Lactose, Maltose and Aspartame Co-Crystalline Materials

Confirmation of the peak emerging at ~ 117°C being related to maltose was made by comparing the physical blends of the lactose, maltose and aspartame with the co-crystalline lactose, maltose and aspartame materials. DSC data (Table 11) shows that the lactose endotherms decrease in enthalpy as a function of elevating levels of added sugar; this is accompanied by a broadening of the peaks. The materials were further analysed by PXRD (Fig 24) and the diffractograms gained were in agreement with the results obtained from DSC analysis. It was found that a combination of maltose and aspartame could be added up to a level of 15% w/w of maltose and 2% w/w of aspartame.



Black: Maltose 5%, Aspartame 2% w/w, Red: Maltose 8%, Aspartame 2% w/w, Blue: Maltose 13%, Aspartame 2% w/w, Green: Maltose 15%, Aspartame 2% w/w, Purple: Maltose 18% w/w, Aspartame 2% w/w

Fig 24. PXRD Diffractograms for Co-Crystalline Lactose, Maltose and Aspartame Materials

Above this level, peaks emerge at the 2θ positions of ~ 14.6° and 21.5° suggesting the presence of a separate phase relating to maltose. Comparison with physical blends (see Section 5.2.4.1) confirmed this observation. The diffractograms enabled the calculation of the unit cell volumes (Fig 25) and the data does not suggest any significant elevation in unit cell volume.

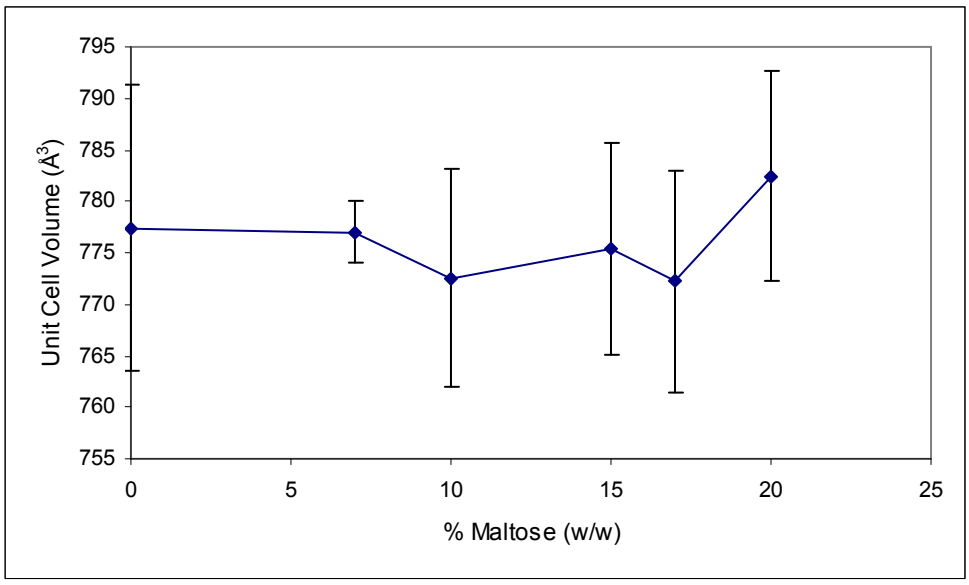


Fig 25. Unit Cell Volume vs. % Incorporated Maltose

5.2.5. Co-Crystallisation of Lactose with Galactose

5.2.5.1. Physical Blends of Lactose and Galactose

Physical blends of crystalline lactose monohydrate and crystalline galactose were prepared in accordance with the methodology outlined in Section 8.3.7. DSC analysis (Fig 25) of these blends showed three clearly defined endotherms: an endotherm emerging at ~ 145°C relating to lactose monohydrate; an endotherm appearing at ~ 170°C indicating the presence of galactose; the final endotherm emerges at ~ 210°C corresponding to α-lactose. Both phases can be easily discerned (see Fig 25 and Fig 26) from one another. As such, discriminating between a mixture of phases and a co-crystallised phase can be done easily.

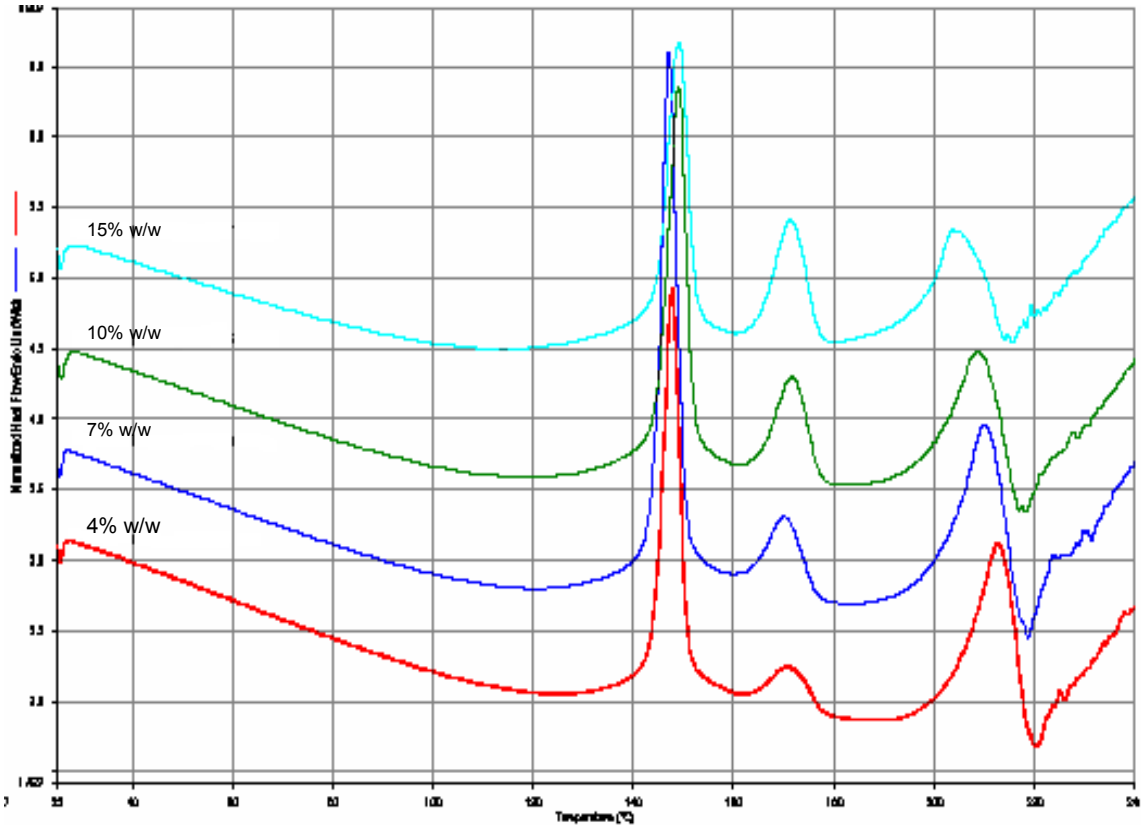


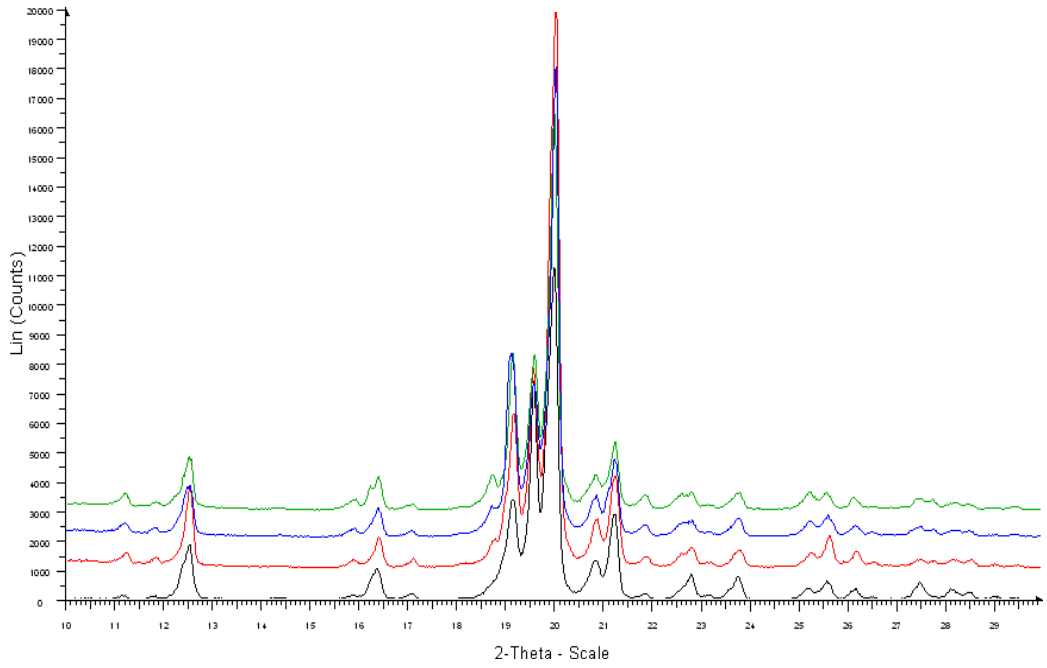
Fig 25. DSC Data for Physical Blends of Lactose Monohydrate with Varying Levels of Galactose

	ΔH1 (Jg^{-1})	Onset 1 (˚C)	ΔH2 (Jg^{-1})	Onset 2 (˚C)	ΔH3 (Jg^{-1})	Onset 3 (˚C)
Lac/Gal(4%)	93.423	144.149	12.196	164.733	80.658	204.346
Lac/Gal(7%)	85.824	144.202	22.711	163.867	77.837	201.635
Lac/Gal(10%)	83.386	144.543	30.562	165.206	75.654	199.842
Lac/Gal(15%)	84.432	144.822	38.970	165.209	74.834	197.994

Table 11. DSC Data for physical blends of Lactose monohydrate with varying levels of Galactose

The endothermic peak relating to galactose at ~ 170°C increases in the value of ΔH with increasing quantities of the sugar present in the physical blend and the peaks corresponding to lactose decrease in their values for ΔH.

PXRD analysis (Fig 26) of the physical blends of lactose and galactose yielded diffractograms that facilitated a clear difference to be observed between lactose and galactose.



Black: Galactose 4% w/w, Red: Galactose 7% w/w, Blue: Galactose 10% w/w, Green: 15% Galactose

Fig 26. PXRD Diffractograms of Physical Blends of Lactose and Galactose

Analysis of the diffractograms (Fig 26) allowed identification of characteristic peaks for the presence of a galactose phase. Peaks at the 2θ

positions of ~ 18.5° and 21.6° are indicative of the presence of a galactose phase. The majority of the reflections indicate the presence of lactose in its monohydrate state. With increasing amounts of galactose present in the blend, the intensity for the peaks relevant to this sugar grow in intensity. The diffractograms allowed calculation of the unit cells (Fig 27) for the physical blends. The unit cell volumes remain reasonably constant, it appears that the blending of increasing amounts of galactose with lactose does not alter the unit cell of the material overall.

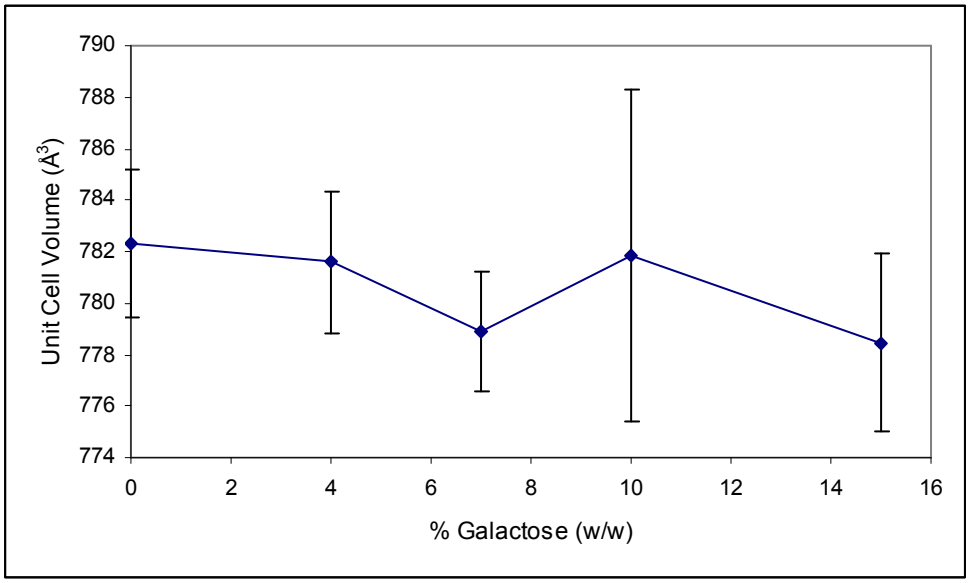


Fig 27. Unit Cell Volume vs. % Galactose (w/w)

5.2.5.2. Co-Crystalline Lactose and Galactose Materials

DSC traces (Fig 28) for co-crystallised lactose and galactose materials showed that up to a level of 4% w/w of added galactose, the melting behaviour of the material is indicative of solely lactose with the characteristic peaks present at ~ 145°C and ~ 210°C. Above this level of included galactose, an endotherm appears at ~ 157°C that corresponds to the melting of galactose. Continuation of elevation in the level of added galactose results in the enthalpy value for this peak increasing.

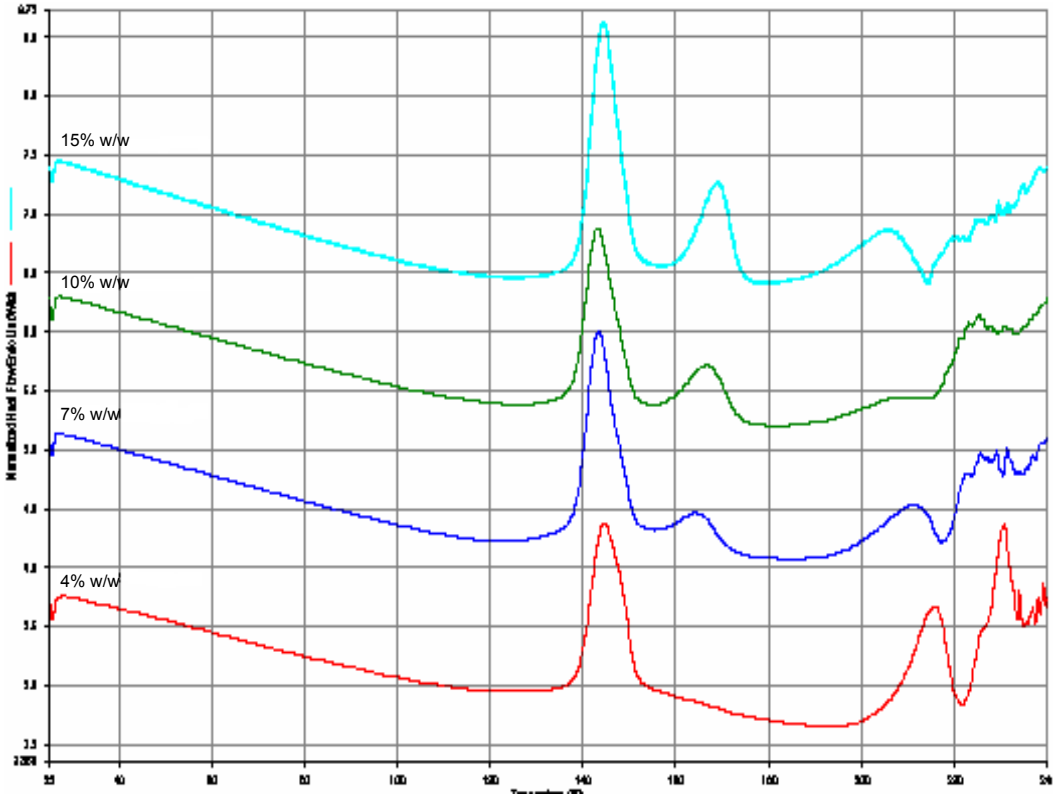


Fig 28. DSC Traces of Co-Crystalline Lactose and Galactose Materials

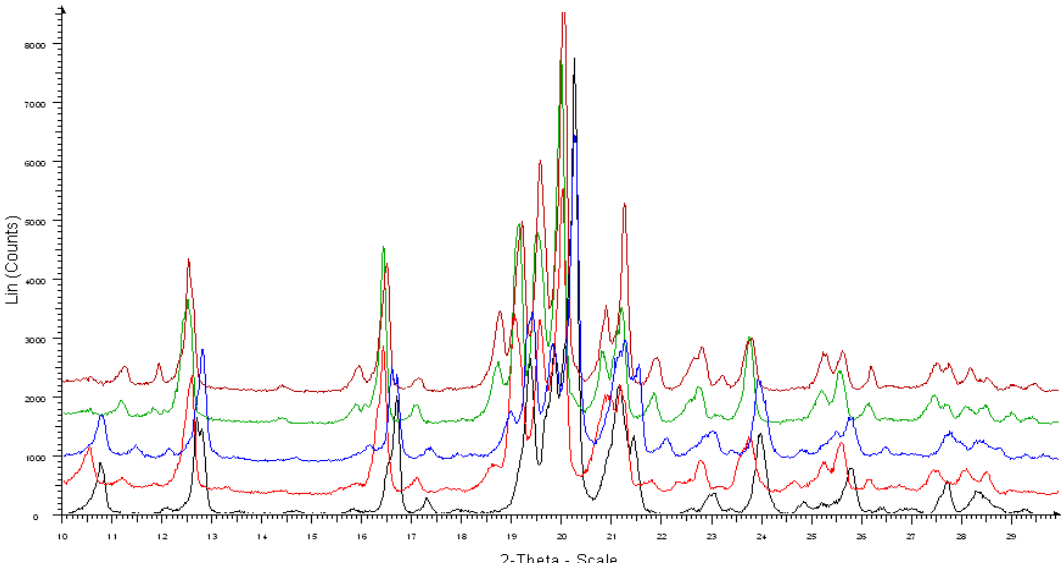
	ΔH1 (Jg^{-1})	Onset 1 (˚C)	ΔH2 (Jg^{-1})	Onset 2 (˚C)	ΔH3 (Jg^{-1})	Onset 3 (˚C)
Lac/Galac(4%)	65.441	139.188	---------	----------	46.023	206.703
Lac/Galac(7%)	70.661	138.525	10.580	157.702	22.223	198.676
Lac/Galac(10%)	62.768	138.299	20.919	158.990	4.295	196.985
Lac/Galac(15%)	87.842	139.372	35.881	161.511	34.143	191.295

Table 12. DSC Data for Lactose and Galactose Materials

With increasing amounts of galactose, the endotherms at ~ 145°C and ~ 210°C become broader with decreasing enthalpy values.

PXRD analysis (Fig 29) of the co-crystallised lactose and galactose materials did not yield data that coincided with the findings (see Fig 28, table 12) obtained *via* DSC analysis. Co-crystallised material containing 4-15% w/w of added galactose showed the presence of two phases. One related to lactose, and a second phase related to galactose was evident from the peak positions of ~ 18.6° and 21.8° 2θ. Comparison of co-crystallised material diffractograms with diffractograms (see Fig 26) yielded from physical blends (in analogous ratios)

confirmed that the emerging peaks were due to the presence of a separate galactose phase.



Black: Re-Crystallised Lactose, Red: Galactose 4% w/w, Blue: 7% Galactose w/w, Green: 10% Galactose w/w, Purple: 15% Galactose w/w

Fig 29. PXRD Diffractograms for Co-Crystalline Lactose and Galactose Materials

The diffractograms obtained allowed calculation of the unit cells of the materials yielded from the co-crystallisation process (Fig 30) and the calculation showed that the inclusion of galactose does not give any clear trend in the unit cell volume.

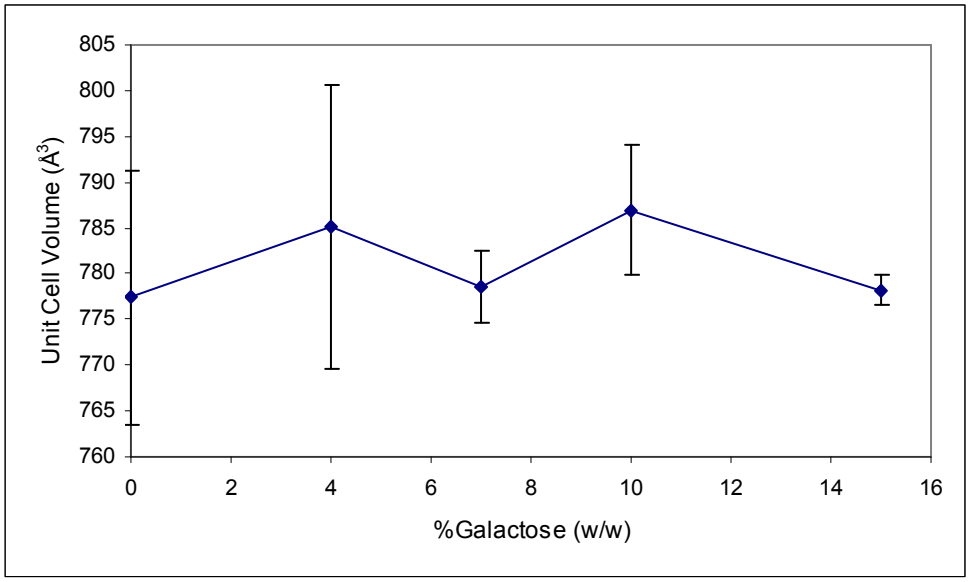
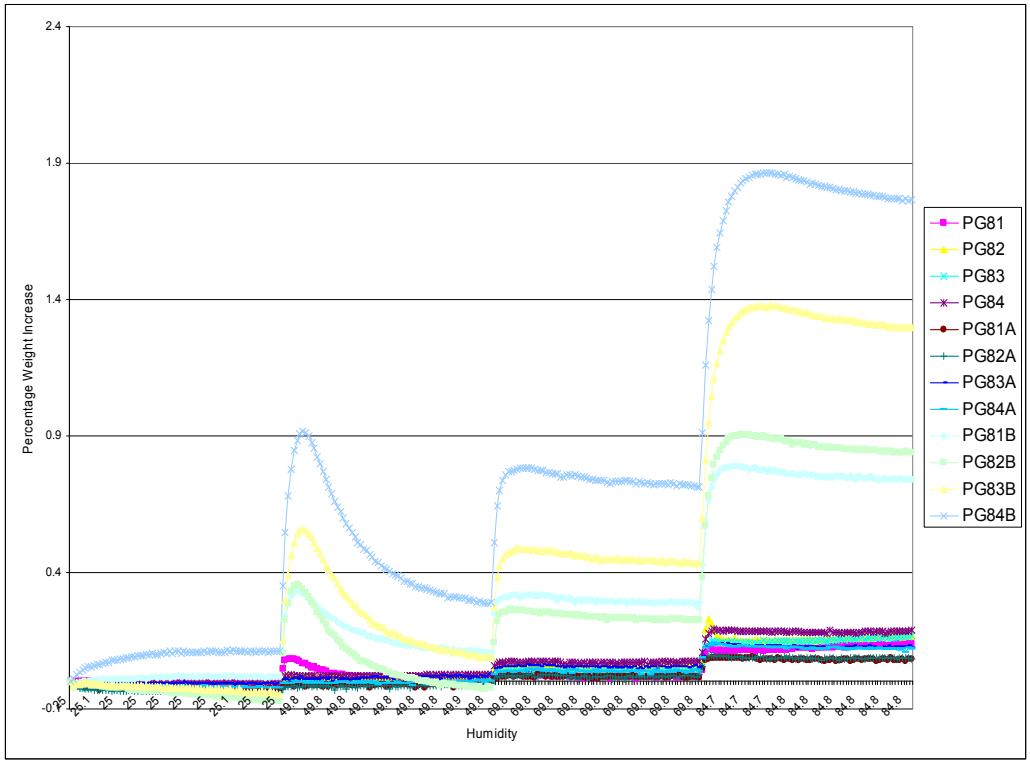


Fig 30. Unit Cell Volume *vs*. Level of Incorporated Galactose

5.2.5.3. Autosorb Analysis of Co-Crystalline Lactose and Galactose

In order to ascertain the physical nature of the material formed from adding galactose to a supersaturated lactose solution, analysis by autosorb was undertaken. Lactose with 4-15% w/w of galactose added, physical blends (4-15% w/w of galactose) of the two crystalline sugars and physical blends of crystalline lactose with amorphous galactose (4-15% w/w) were subjected to an autosorb analysis. Weight gains for each sample were recorded, and the percentage weight gain was plotted (Fig 31) against the level of humidity. Analysis of material from the co-crystallisation process by autosorb suggests this material has no amorphous content. Comparison of the material yielded from the co-crystallisation process with physical blends (in analogous ratios to the co-crystalline material) containing crystalline lactose and amorphous galactose confirmed this observation. These materials behaved as expected upon being subjected to increasing humidity whilst the co-crystalline material did not. Physical blends of the two crystalline sugars behaved in an identical manner to the materials yielded from the co-crystallisation methodology. This does suggest that this process yields crystalline material rather than material with significant amorphous content.



Key: PG81: Co-Crystalline 4% Gal w/w, PG82: Co-Crystalline 7% Gal w/w, PG83: Co-Crystalline 10% Gal w/w, PG84: Co-Crystalline 15% Gal w/w

PG81a: Physical Blend (PB) 4% Gal w/w, PG82a:PB 7% Gal w/w, PG83a: PB 10% Gal w/w, PG84a: 15% Gal w/w

PG81b: PB 4% Amorphous Gal w/w, PG82b: PB 7% Amorphous Gal w/w, PG83b: PB 10% Amorphous Gal w/w, PG84b: PB 15% Amorphous Gal w/w

Fig 31. Autosorb Data for Lactose and Galactose Materials

The material formed from the co-crystallisation process increased in mass (as a function of percentage weight gain, see fig 31) with each increase in the level of humidity. This percentage weight gain began to steadily decrease as the humidity level was held. This trend continued until the level of humidity caused the samples to deliquesce. Physical blends of the two crystalline sugars behaved in an identical manner to the material formed in the co-crystallisation process. Blends of crystalline lactose, with varying levels of amorphous galactose, increased in percentage mass with increasing levels of humidity (Fig 31). Unlike materials formed in the co-crystallisation process and physical blends of crystalline materials, material containing amorphous galactose steadily gained weigh upon being held at each level of humidity. Materials containing amorphous galactose displayed a relationship between the amount of amorphous

galactose and the quantity of moisture adsorbed. For the elevation of humidity from 25% relative humidity (RH) too 50% RH, material containing 17% w/w of amorphous galactose adsorbed ~ 0.5% of its weight whilst material containing 7% w/w of amorphous galactose adsorbed ~ 0.2% of its weight. Higher amounts of amorphous galactose adsorbed more moisture compared to material containing lower amounts of amorphous galactose.

5.2.5.4. Co-Crystalline Lactose and Galactose Materials Post Autosorb

Materials subjected to autosorb analysis were retained in order to ascertain if any physical changes had occurred due to exposure to humidity. DSC analysis (Fig 32) of the materials showed that all materials displayed a mixture of phases. Lactose and galactose endotherms could both be clearly observed. Interestingly, lactose containing 4% w/w of galactose displays only endothermic melting for lactose (Section 5.2.4.2.); being subjected to humidity causes an endotherm to appearing at ~ 157°C that relates to galactose.

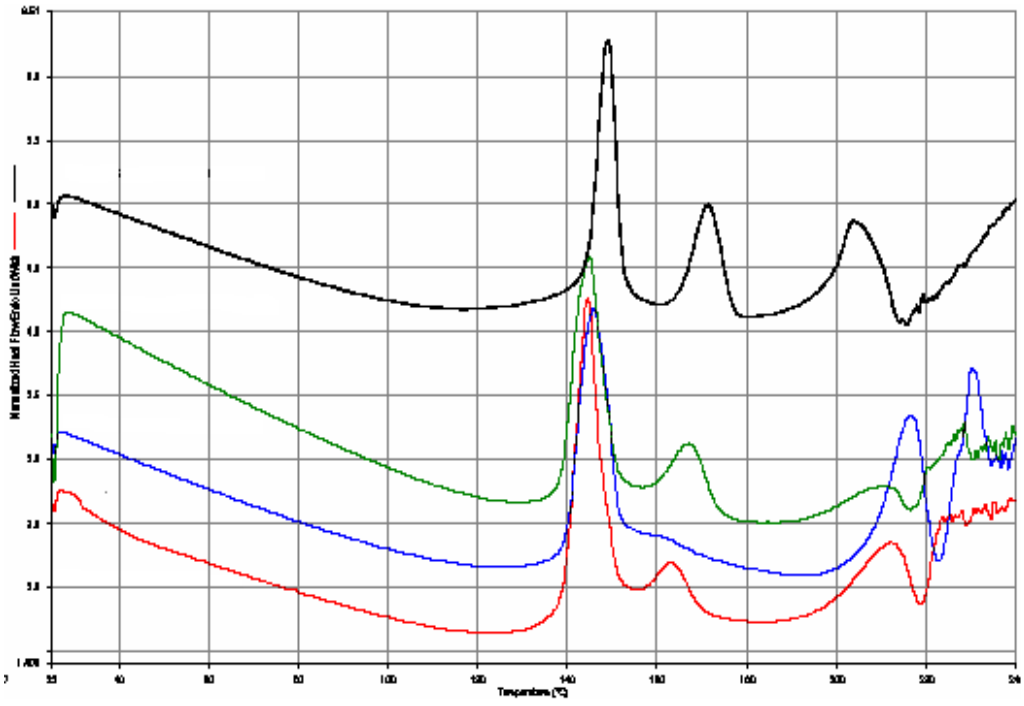


Fig 32. DSC Traces for Co-Crystalline Lactose and Galactose Materials Post Autosorb

	ΔH1 (Jg^{-1})	Onset 1 (˚C)	ΔH2 (Jg^{-1})	Onset 2 (˚C)	ΔH3 (Jg^{-1})	Onset 3 (˚C)
Lac/Galac(4%)	86.108	139.518	12.400	157.551	34.718	198.949
Lac/Galac(7%)	75.007	140.050	1.031	158.149	60.691	207.657
Lac/Galac(10%)	75.619	138.872	21.735	159.614	12.761	198.020
Lac/Galac(15%)	88.271	139.421	36.441	159.723	45.077	198.277

Table 13. DSC Data for Co-Crystalline Lactose and Galactose Materials Post Autosorb

Interestingly, the material containing 10% w/w of galactose showed a change in the enthalpy value for the endotherm related to galactose. Values for the endotherm at ~ 157°C decrease (from 10.580 Jg^{-1} too 1.031 Jg^{-1}) in ΔH, which is coupled with a decrease in ΔH values for the two lactose endotherms. A comparison between ΔH values for the lactose monohydrate endotherm in the co-crystalline material and in the material subjected to autosorb analysis can be seen in figure thirty-four.

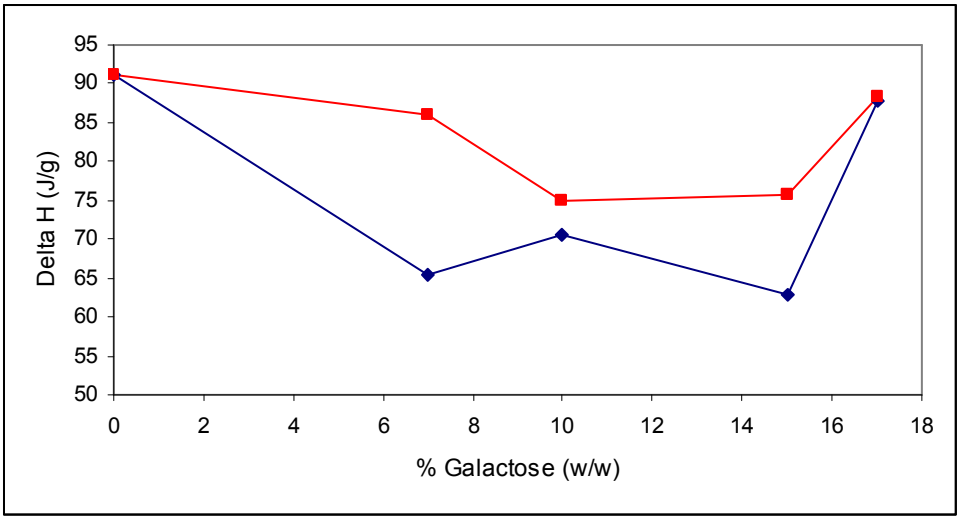
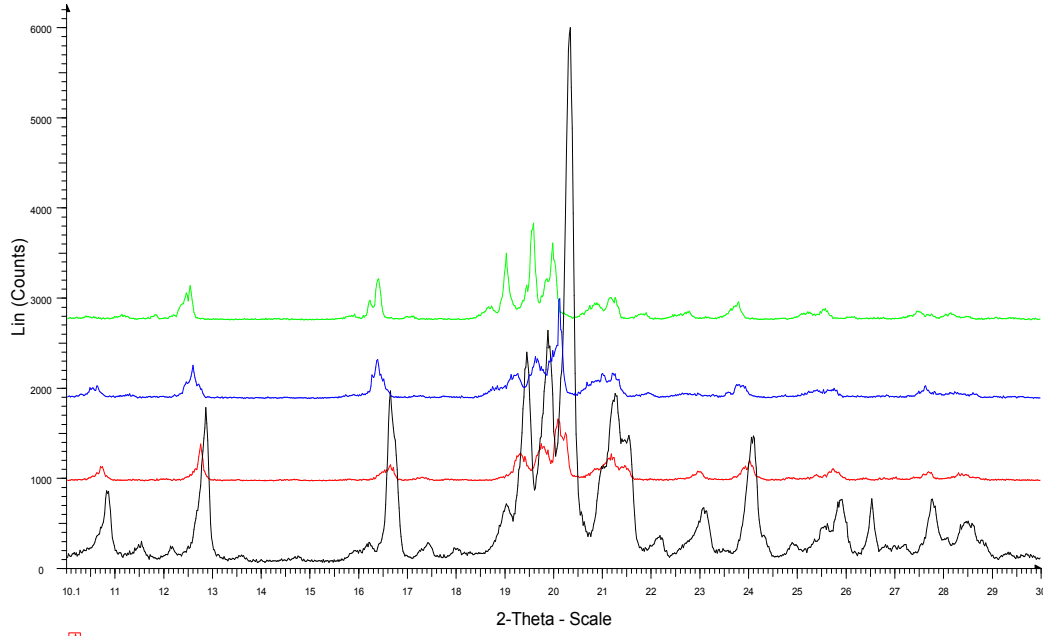


Fig 33. ΔH *vs*. % Incorporated Galactose for Co-Crystalline (blue) and Co-Crystalline Material after Autosorb Analysis (red)

The values of ΔH for the material investigated after the autosorb analysis are higher than the values for the material prior to the autosorb analysis.

Analysis of the co-crystallised lactose and galactose materials after being subjected to autosorb analysis by PXRD analysis (Fig 34) provided results in agreement with those garnered from DSC analysis (Fig 32). All of the materials displayed a mixture of phases, a lactose phase and a galactose phase. Peaks

emerging at the 2θ positions of ~ 18.4° and 21.4° are highly indicative of the presence of a galactose phase. The co-crystallised material containing 4-15% w/w of galactose did not display any physical changes. PXRD diffractograms (Fig 34) were identical in peak positions to the initial diffractograms (Fig 29) taken of the co-crystallised lactose and galactose materials. This suggests the presence of two phases.



Black: Galactose 4% w/w, Red: Galactose 7% w/w, Blue: Galactose 10% w/w, Green: Galactose 15% w/w

Fig 34. PXRD Diffractograms of Co-Crystalline Lactose and Galactose Post Autosorb

PXRD diffractograms enabled the calculation of the unit cell dimensions (Fig 35) for the materials.

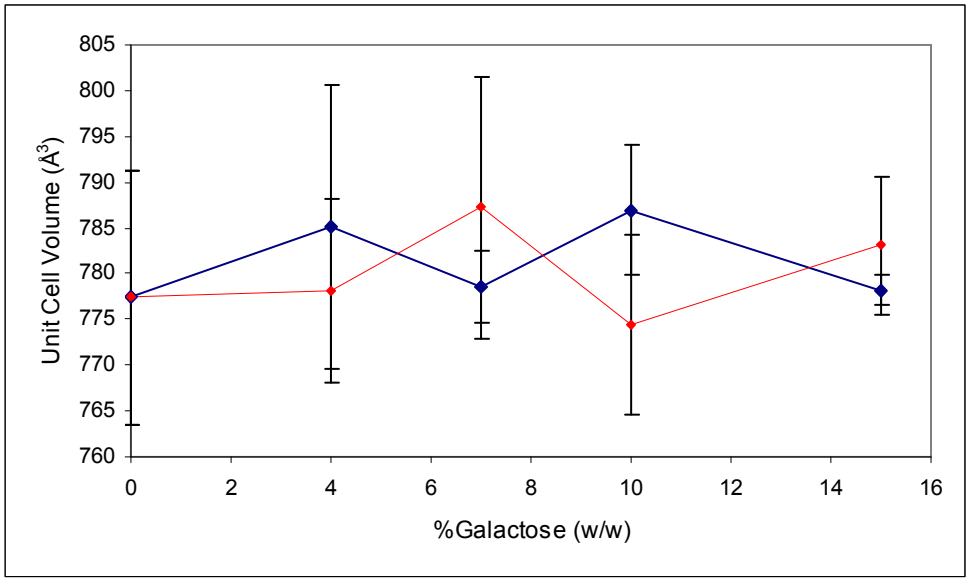


Fig 35. Unit Cell Volumes vs. % Incorporated Galactose for Materials before Subjection to Humidity (blue) and after (red)

Calculation of the unit cells using CheckCell for the remaining materials revealed no clear pattern in unit cell volumes.

5.2.5.5. Co-Crystallisation of Lactose and Galactose Starting from a Physical Blend

Modification of the co-crystallisation methodology (see Section 8.3.8) and its application to the co-crystallisation of lactose and galactose was investigated. DSC analysis (Fig 36) showed that up to 10% w/w of galactose is possibly incorporated using the method of co-crystallising from a physical blend of lactose and galactose. At this level, a characteristic endotherm for galactose appeared at ~ 159°C. The expected endotherms for the two forms of lactose appeared at ~ 145°C and ~ 210°C, the distinction between co-crystalline materials and a mixture of phases was achieved by comparison with a physical blend of the two materials (Section 5.2.4.1)

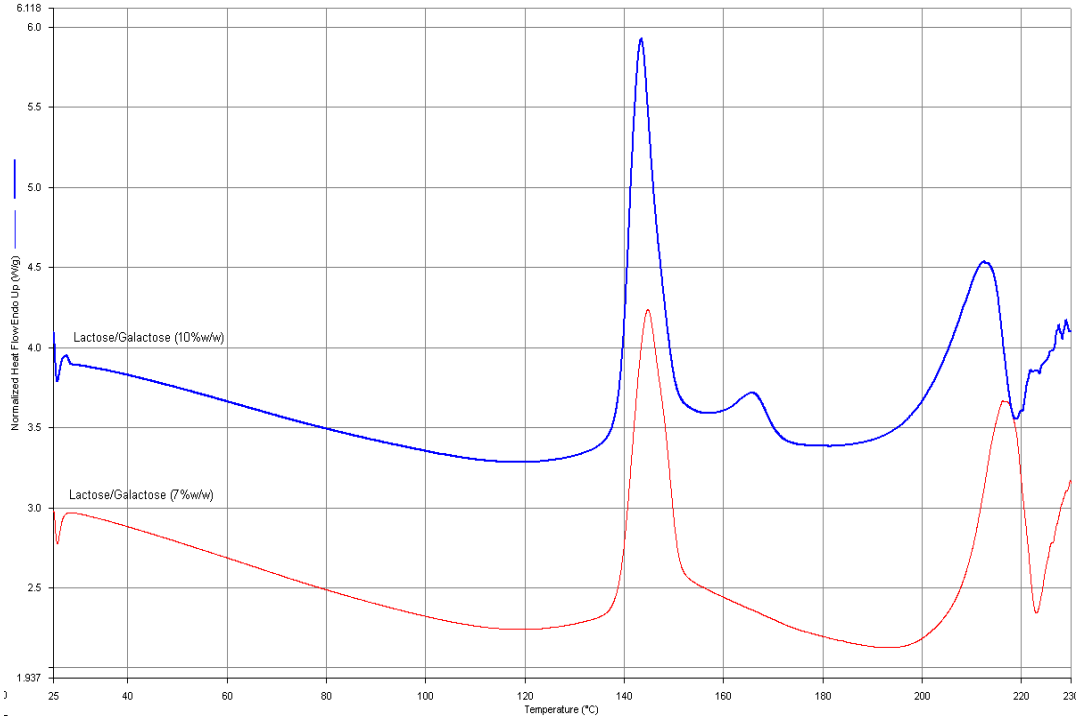


Fig 36. DSC Traces of Co-Crystalline Lactose and Galactose Materials Formed from a Physical Blend

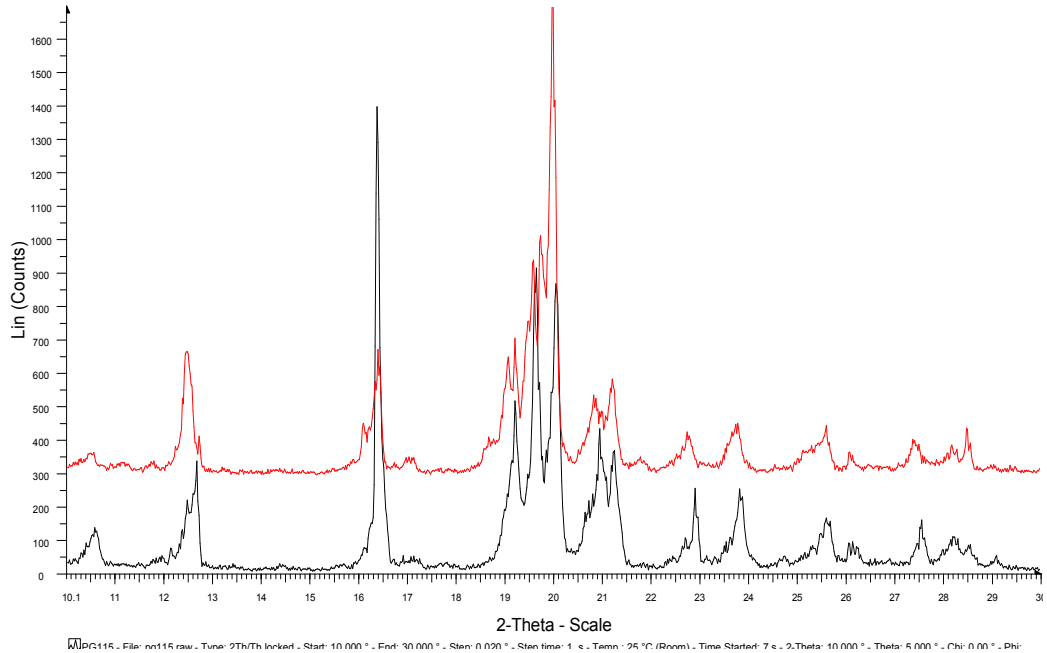
	Onset 1 (°C)	ΔH1 (Jg^{-1})	Onset 2 (°C)	ΔH2 (Jg^{-1})	Onset 3 (°C)	ΔH3 (Jg^{-1})
Lac/Gal (7%)	139.165	78.984	----------	----------	207.894	79.614
Lac/Gal (10%)	139.138	88.792	159.603	9.307	201.114	65.070

Table 14. DSC Data for Co-Crystalline Lactose and Galactose Materials Formed from a Physical Blend

Analysis of the DSC data (Table 14) shows that the enthalpy values for the endotherms relating to the co-crystallised lactose are lower compared to when the materials are together in an analogous physical blend (Section 5.2.4.1.). This reduction in enthalpy is coincident with a broadening of the lactose endotherms and a lowering of the onset temperature. Interestingly, the value of ΔH for the galactose endotherm when included at a level of 10% w/w is lower compared to when the initial method (see Section 5.2.4.2) of co-crystallisation is used. Using the initial method, the enthalpy value for the endotherm obtained

from this material is 20.919 Jg^{-1}, which drops to 9.307 Jg^{-1} for the material obtained from the modified methodology.

PXRD analysis (Fig 37) was in agreement with the results obtained (Fig 36) from DSC, up to 10% w/w of galactose can be incorporated within a lactose structure using the modified methodology (see Section 8.3.8). At the level of 10% w/w, peaks emerging at the 2θ positions of ~ 18° and 21° are indicative of a separate phase relating to galactose.



Black: Galactose 7% w/w, Red: Galactose 10% w/w

Fig 37. PXRD Diffractograms for Co-Crystalline Lactose and Galactose Materials formed from a Physical Blend

Interestingly, the diffraction pattern for the material containing 10% w/w of galactose sows a degree of peak splitting at the positions of 16.07°, 19.06°, 19.47° and 19.69° 2θ. This suggests the presence of an additional phase other than lactose or galactose. This phase could not be attributed to any anomeric form of either galactose or lactose.

The data from the PXRD diffractograms (Fig 37) allowed the unit cell volumes to be calculated for the co-crystalline lactose and galactose materials

(Fig 38). Neither material exhibited a significantly larger unit cell compared to re-crystallised lactose.

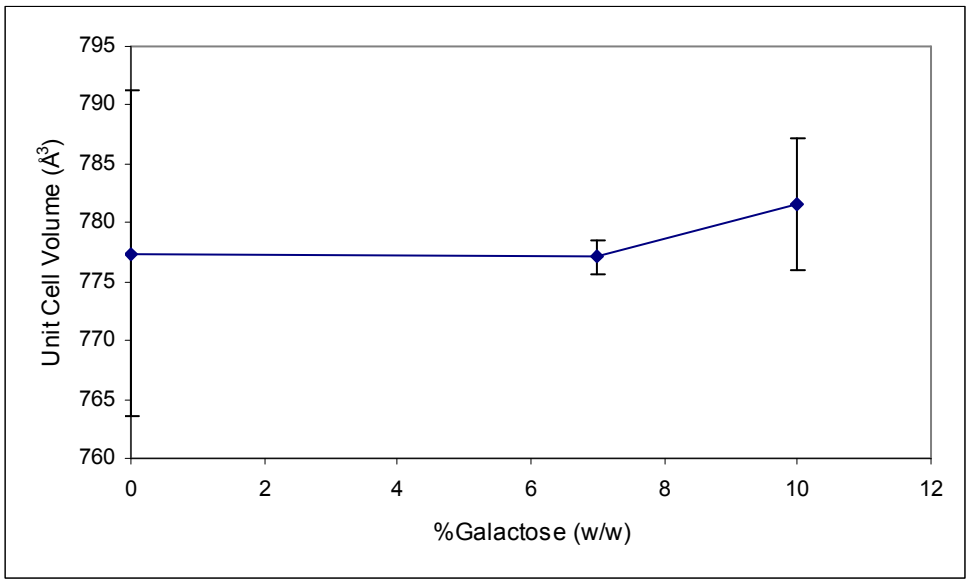


Fig 38. Unit Cell Volumes vs. % Galactose

5.2.6. Co-Crystallisation of Lactose with Galactose and Glucose

5.2.6.1. Physical Blends of Lactose, Galactose and Glucose

Physical blends of lactose, galactose and glucose were prepared in accordance with the methodology outlined in Section 8.3.11.

DSC analysis (Fig 39) of the blended material allowed facile differentiation between all of the phases in the mixture: glucose monohydrate emerges at ~ 70°C, lactose monohydrate followed, emerging at ~ 145°C, galactose melting is observed at ~ 160°C and finally the α-form of lactose was detected melting at ~ 210°C.

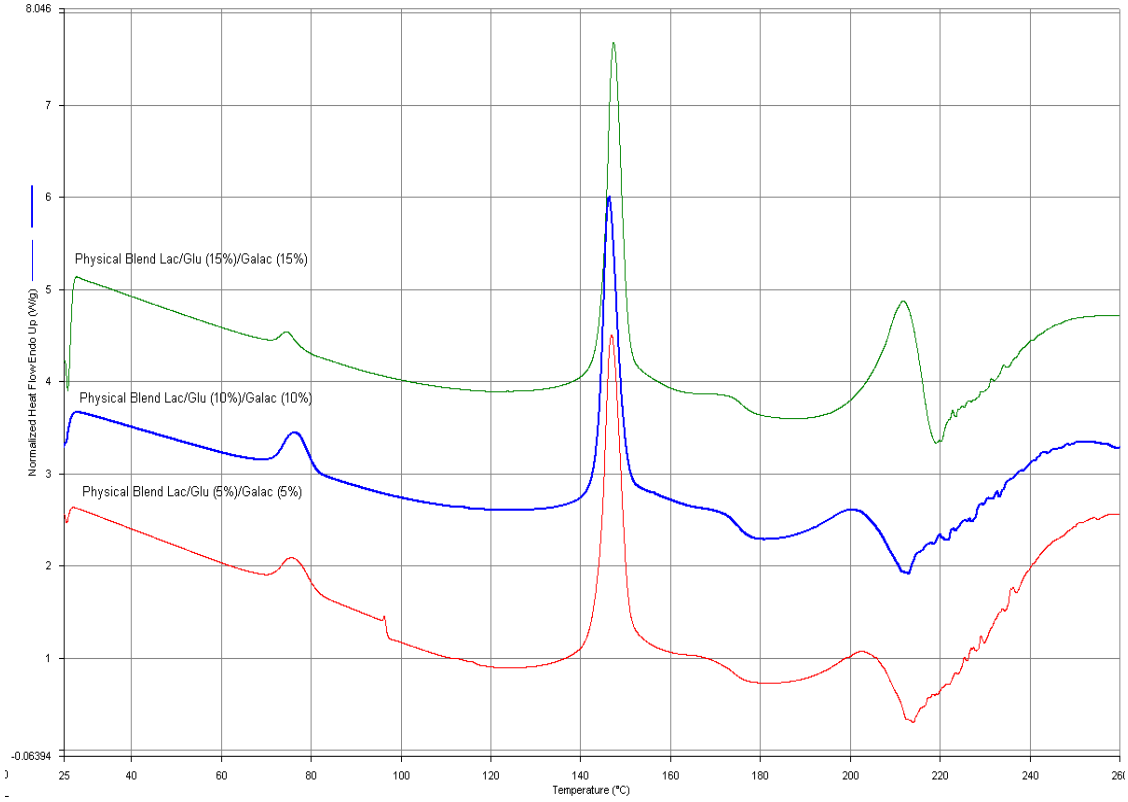


Fig 39. DSC Traces of the Physical Blends of Lactose, Galactose and Glucose

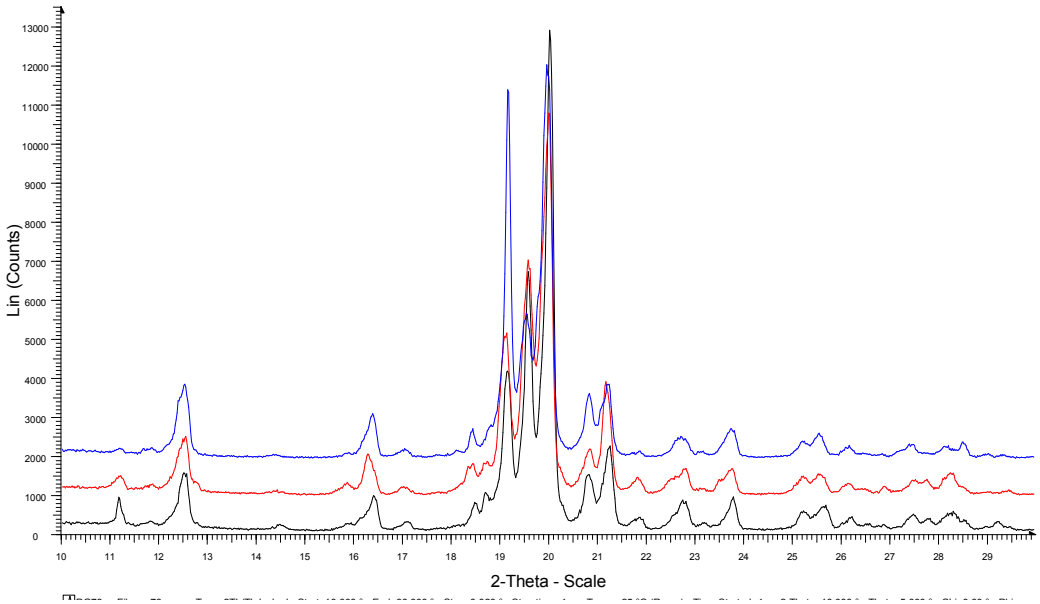
	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)	ΔH4 (Jg^{-1})	Onset 4 (°C)
Lac/Glu (5%)/ Galac (5%)	2.587	72.055	86.927	144.594	4.604	164.780	91.951	202.532
Lac/Glu (10%)/ Galac (10%)	10.846	71.460	97.633	143.798	4.490	161.413	47.982	191.78
Lac/Glu (15%)/ Galac (15%)	13.952	71.725	78.180	143.776	5.016	163.819	43.776	188.343

Table 15. DSC Data for Physical Blends of Lactose, Galactose and Glucose

The enthalpy associated with the melting of glucose monohydrate increased in the value of ΔH with increasing quantities of the sugar present in the material. This was found also to be the case for the enthalpy value relevant to galactose. The melting behaviour of the endothermic peaks relevant to lactose underwent a depression in the enthalpy value and the onset temperature that was

related to the quantity of the included glucose and galactose. Higher amounts of the blended monosaccharides resulted in a greater reduction in both of these values for the lactose relevant endotherms (see Table15).

PXRD (Fig 40) analysis of the physical blends of lactose, glucose and galactose showed that the three different types of sugars could be clearly discerned within the diffractograms. Characteristic peaks emerge for glucose at the 2θ positions of ~ 14.5° and 17.5°, peaks indicative of galactose emerge at the 2θ positions of ~ 18.6° and 21.6°. The remaining reflections were characteristic of lactose monohydrate. The diffractograms enabled the calculation of the unit cells (Fig 41) for the physically blended materials.



Black: Glucose 5% w/w, Galactose 5% w/w Red: Glucose 10% w/w, Galactose 10% w/w, Blue: Glucose 15% w/w, Galactose 15% w/w

Fig 40. PXRD Diffractograms for Physical Blends of Lactose, Galactose and Glucose Materials

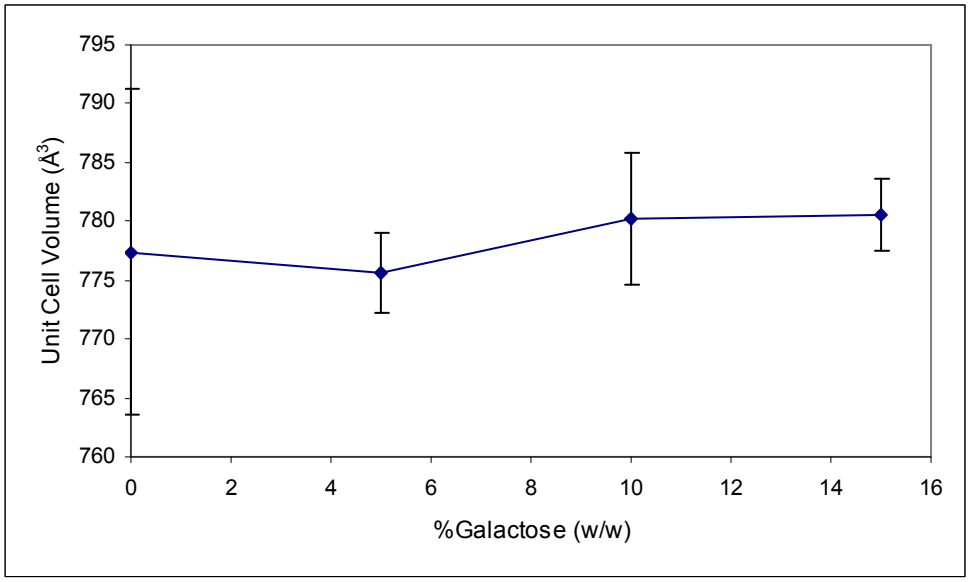


Fig 41. Unit Cell Volume vs. % Galactose for Physical Blends of Lactose, Galactose and Glucose

5.2.6.2. Co-Crystallisation of Lactose with Galactose and Glucose

DSC analysis (Fig 42) of the co-crystallised materials containing 5-15% w/w of the monosaccharides showed that they exhibited melting behaviour indicative of solely lactose. Elevation to 20% w/w of each monosaccharide led to the emergence of an endotherm at ~ 155°C that corresponded to galactose. All of the co-crystallised sugars displayed the characteristic endothermic peaks for the dehydration of lactose at ~ 145 °C and the melting of α-lactose at ~ 210°C.

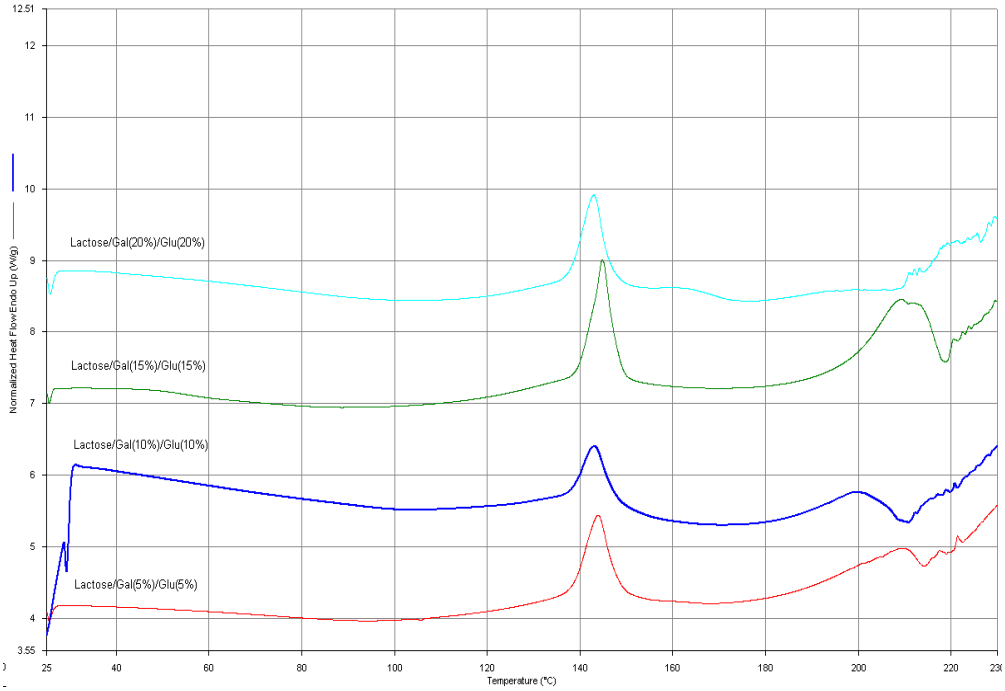


Fig 42. DSC Traces of Co-Crystalline Lactose, Galactose and Glucose

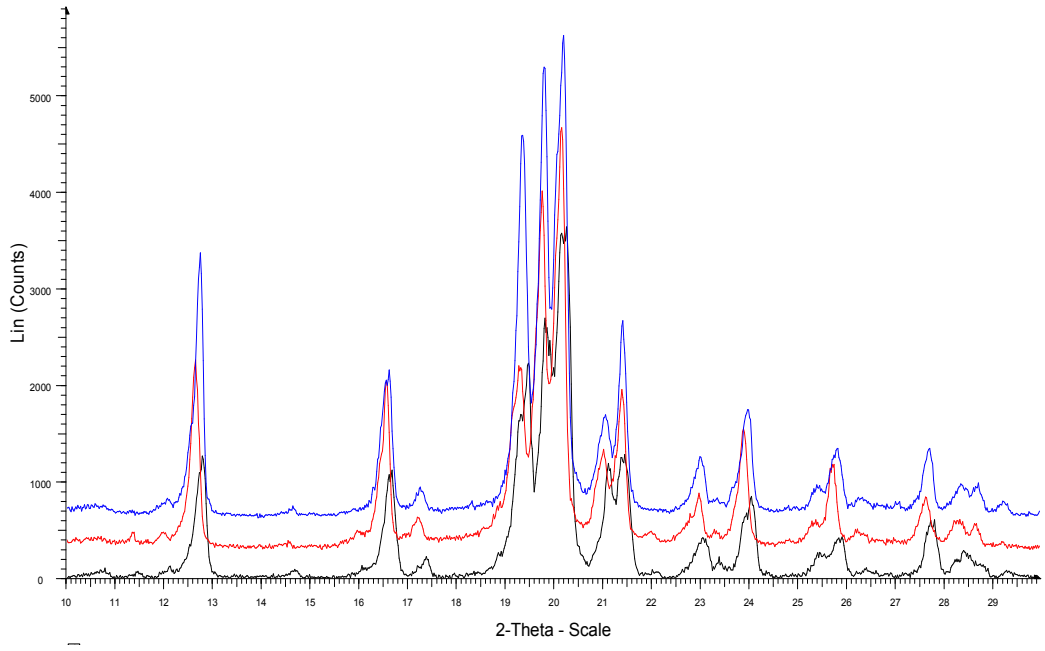
	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH 3 (Jg^{-1})	Onset 3 (°C)
Lac/Glu(5%)/Gal(5%)	49.445	141.085	------	---------	69.782	198.900
Lac/Glu(10%)/Gal(10%)	43.484	138.490	------	---------	19.244	200.017
Lac/Glu(15%)/Gal(15%)	30.873	138.382	------	---------	27.945	189.500
Lac/Glu(20%)/Gal(20%)	43.283	137.717	3.969	155.745	3.153	186.260

Table 16. DSC Data for Co-Crystalline Lactose, Galactose and Glucose Materials

Comparison with physical blends confirmed the singular phased nature of the materials containing 5-15% w/w of glucose and galactose. The physical blends showed glucose melting at ~ 70°C and galactose at ~ 160°C which are not observable in the corresponding co-crystalline materials. The co-crystallised material containing 20% w/w of each monosaccharide exhibited a typical endotherm for galactose. DSC data (Table 16) showed that the endotherms corresponding to lactose decreased in ΔH values and became broader as a function of increasing levels of included galactose and glucose.

The co-crystallised lactose, galactose and glucose materials were analysed by PXRD (Fig 43) and the diffractograms indicate the presence of solely phases related to lactose containing 5-15% w/w of the added

monosaccharides. No phases relating to either glucose or galactose could be observed.



Black: Glucose 5% w/w, Galactose 5% w/w Red: Glucose 10% w/w, Galactose 10% w/w, Blue: Glucose 15% w/w, Galactose 15% w/w

Fig 43. PXRD Diffractograms for Co-Crystalline Lactose, Galactose and Glucose Materials

Comparison with physical blends (Fig 40) confirms this observation. Characteristic peaks for glucose emerge at the 2θ positions of ~ 14.5° and 17.5° and for galactose at the 2θ positions of ~ 18.6° and 21.8°. Peaks in these positions are not present within co-crystallised materials diffractograms. Elevation to 20% w/w of each monosaccharide showed characteristic peaks emerging at the 2θ positions of ~ 14.2° and 21.6° that correspond to glucose and galactose respectively. Diffractograms enabled the calculation (fig 44) of the unit cells and this showed a slight increase for all materials compared to re-crystallised lactose. This however cannot be construed as a significant increase as all of the data points fall outside of the 95% confidence interval.

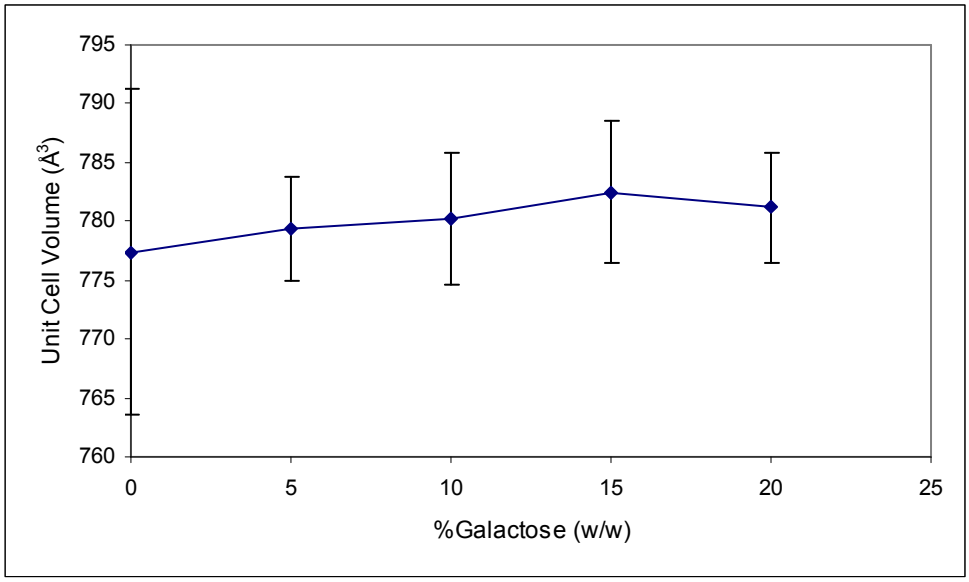


Fig 44. Unit Cell Volume vs. % Incorporated Galactose

Co-crystallised and physical blends of lactose, galactose and glucose were subjected to an autosorb analysis to determine the physical nature of the yielded material. A plot of relative humidity against percentage weight gains for each sample can be seen in figure forty-five. The autosorb data indicates that including galactose and glucose into lactose results in the potential inclusion forming in its crystalline state rather than its amorphous state. Elevation of the RH results in each sample gaining weight. This gain declines steadily whilst the samples are held at each level of RH. This trend continues until the samples reach a level of humidity (~90% RH) that causes deliquescence. Physical blends behaved in an analogous manner to the co-crystalline material. Each increase in RH yields a gain in mass followed by a decline as the samples are held at each level of RH. Again, at ~ 90% RH, the samples deliquesce.

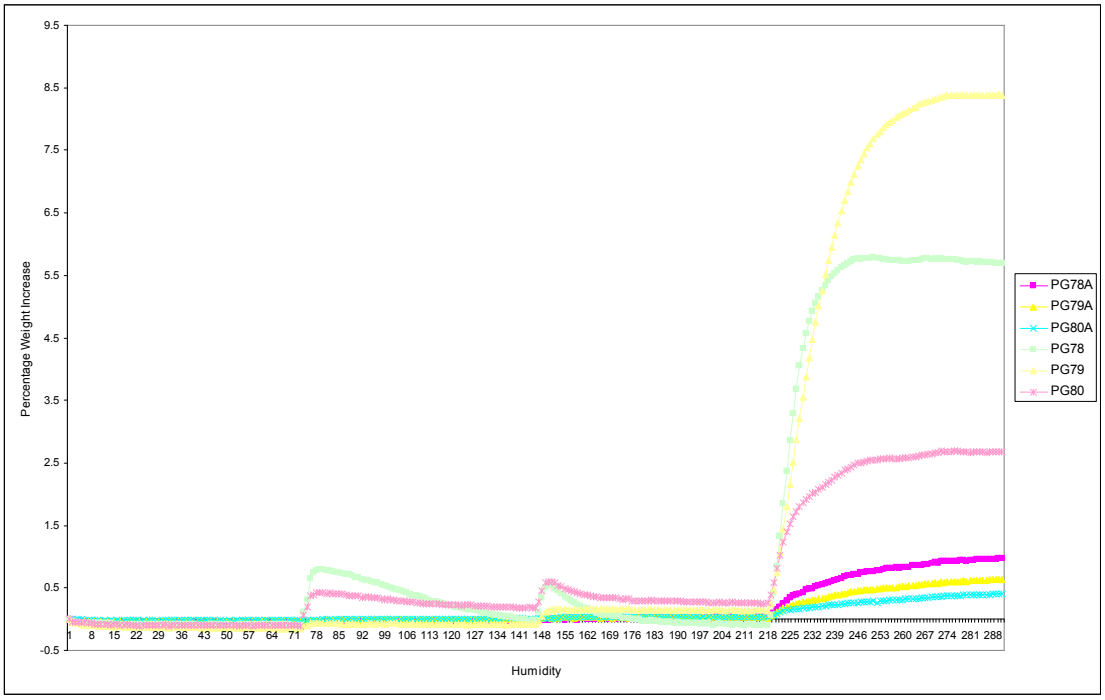


Fig 45. Relative Humidity vs. % Weight Gain for Lactose, Galactose and Glucose Materials

Within both sets of samples, there appears to be no relationship between the amount of the included sugars and the amount of moisture adsorbed. However, the co-crystalline materials adsorbed more moisture than the physical blends. For the increase of 25% to 50% RH, the co-crystalline material containing 10% each of glucose and galactose, a percentage weight gain of ~ 0.17% was obtained. For the same ratio in a physical blend, a percentage weight gain of ~ 0.01% was observed.

5.2.6.3. Co-Crystallisation of Lactose with Galactose and Glucose addition at 90°C

Changing of the temperature of addition (see Section 8.3.10.) of the included components was investigated when co-crystallising lactose with galactose and glucose. DSC analysis (Fig 46) of the materials showed that with the inclusion of 20% w/w of both galactose and glucose yielded lactose related materials. The melting behaviour is indicative of solely lactose. No characteristic endothermic peaks are observable for either galactose or glucose. Comparison with analogous physical blends of the crystalline materials (see Section 5.2.5.1) provides further confirmation of this observation.

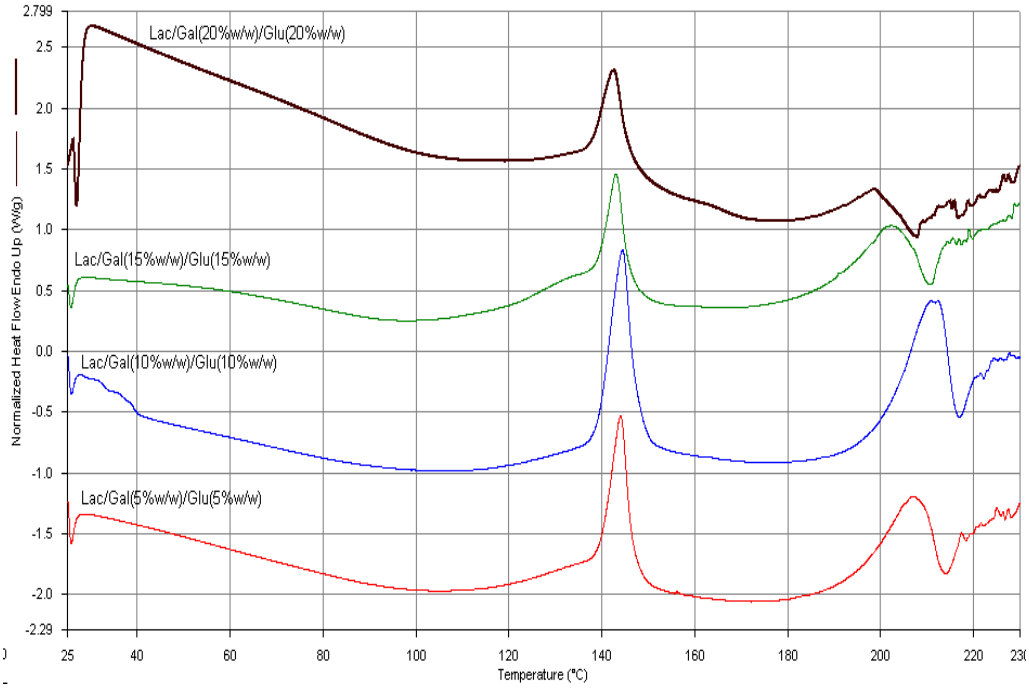


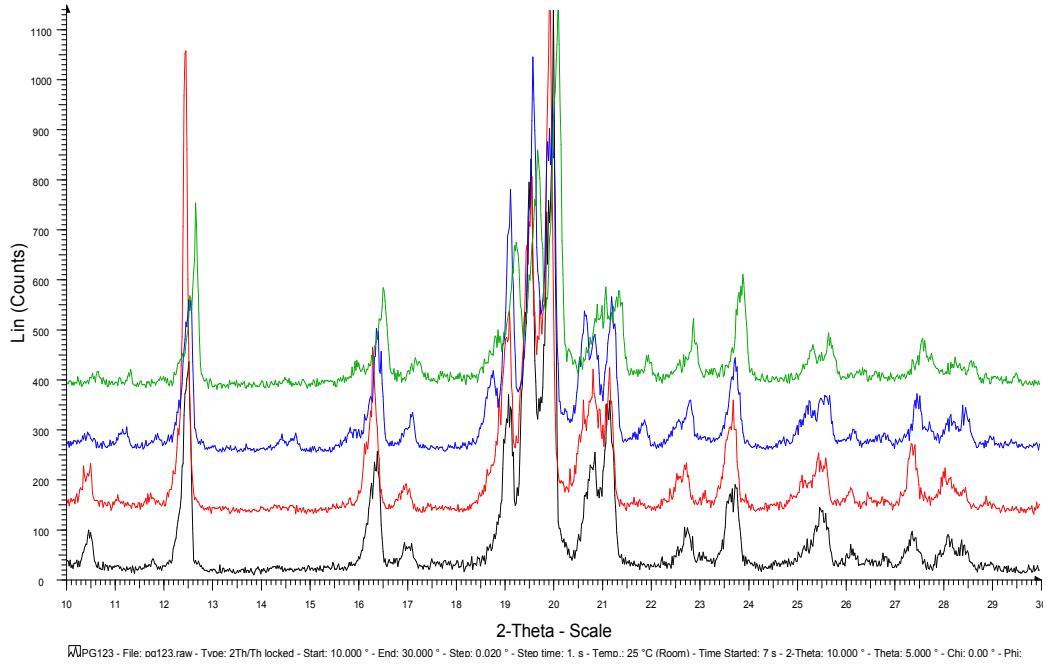
Fig 46. DSC Traces of Co-Crystalline Lactose, Galactose and Glucose Materials with Addition at 90°C

	Onset 1 (°C)	ΔH1 (Jg^{-1})	Onset 2 (°C)	ΔH2 (Jg^{-1})
Lac/Gal(5%)/Glu(5%)	139.805	33.783	198.129	40.929
Lac/Gal(10%)/Glu(10%)	139.156	51.845	201.887	58.527
Lac/Gal(15%)/Glu(15%)	139.154	41.505	189.514	36.859
Lac/Gal(20%)/Glu(20%)	137.690	25.747	189.687	19.890

Table 12. DSC Data for Co-Crystalline Lactose, Galactose and Glucose Materials with Addition at 90°C

The analysis of DSC data (Table 12) for the co-crystallised materials showed that the endotherms relating to lactose exhibit much lower ΔH values compared to the analogous endotherms in the physical blends. This depression in enthalpy appears to correlate with the quantity of the included monosaccharides. Higher amounts of added galactose and glucose yield lower values of enthalpy for the melting of the α-lactose endotherm.

PXRD analysis of the materials (Fig 47) gave some results that were in agreement with those obtained from DSC analysis (Fig 45) as material with 5-10% of the two monosaccharides showed only peaks relevant to lactose. Elevation of the level of addition to 15-20% w/w resulted in peaks emerging that correspond to the presence of galactose.



Black: Glucose 5% w/w, Galactose 5% w/w Red: Glucose 10% w/w, Galactose 10% w/w, Blue: Glucose 15% w/w, Galactose 15% w/w

Fig 47. PXRD Diffractograms for Lactose, Galactose and Glucose Materials

The PXRD data allowed the calculation of the unit cell dimensions for these materials. A plot of unit cell volume against the quantity of galactose included (Fig 48) shows that all the materials exhibit similar unit cells compared to solely lactose. There is a slight increase in unit cell volume for materials containing 10-20% w/w of glucose and galactose. However, these elevations are not significant; the data points for these increases fall outside of the 95% confidence level.

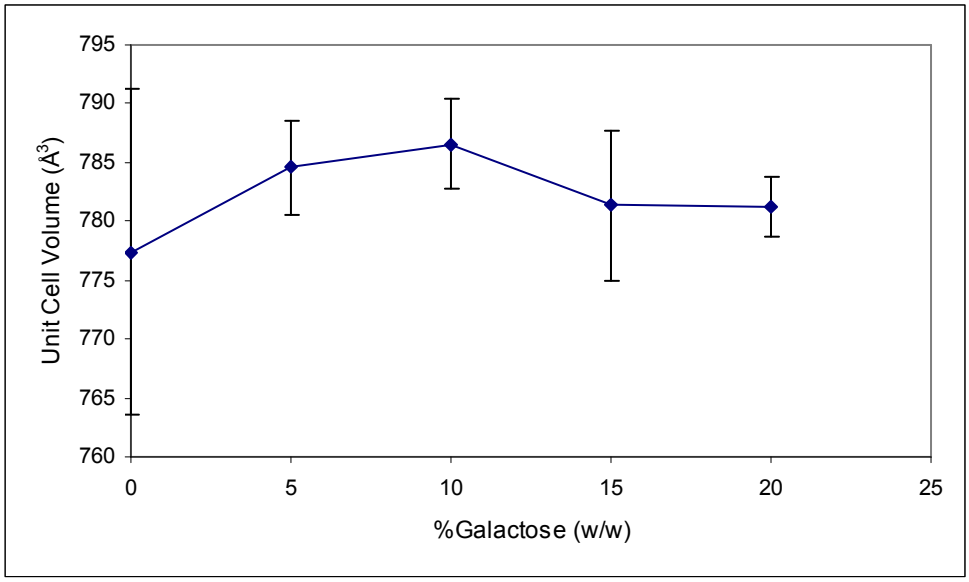


Fig 48. Unit Cell Volume vs. % Galactose for Co-Crystalline Lactose, Galactose and Glucose Materials Formed

5.2.6.4 Co-Crystallisation of Lactose with Galactose and Glucose Starting from a Physical Blend

Modification of the methodology (see Section 8.3.11) employed to form co-crystallised materials and its effect on the co-crystallisation of lactose with galactose and glucose was investigated. DSC analysis (Fig 49) shows that up to 15% w/w of galactose and 15% w/w of glucose is addable to a supersaturated lactose solution whilst retaining solely a single phase. The characteristic endotherms for either galactose or glucose are not apparent on the DSC traces obtained from analysis of the co-crystallised materials.

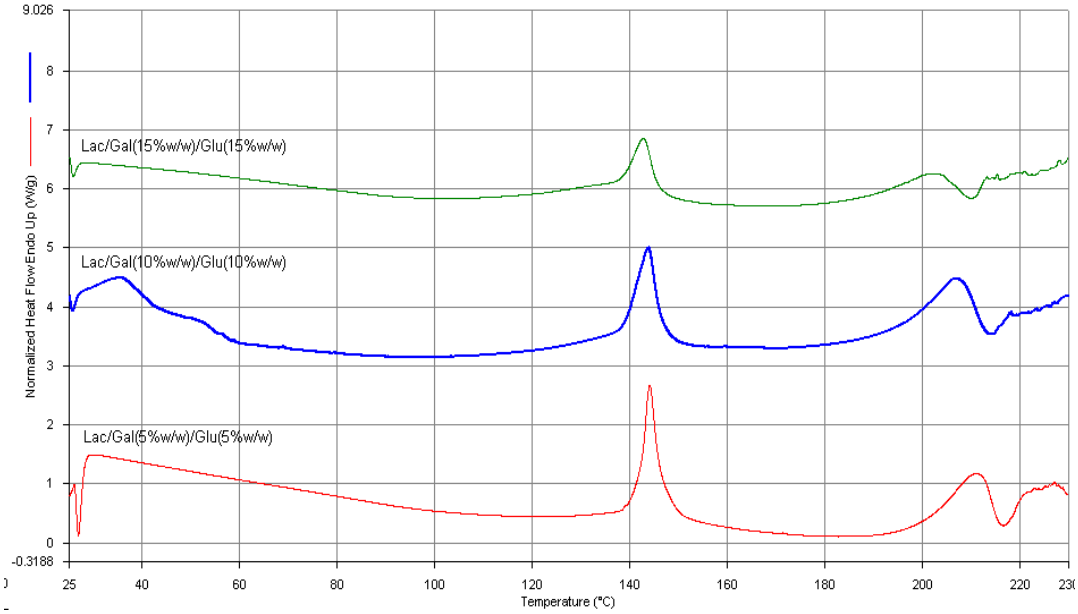


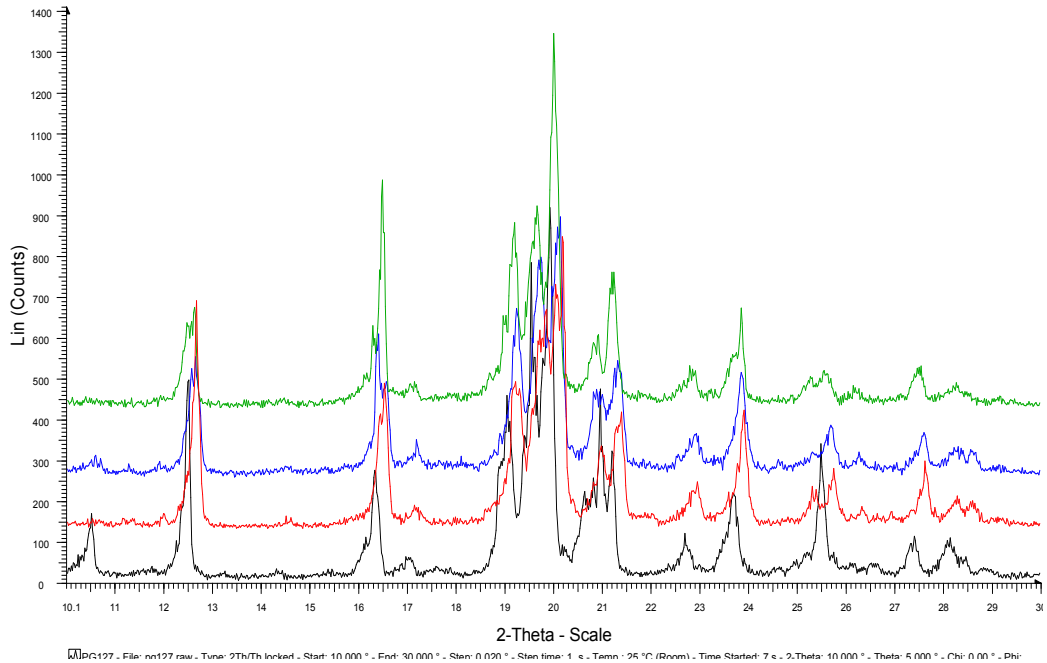
Fig 49. DSC Traces of Co-Crystalline Lactose, Galactose and Glucose Materials formed from a Physical Blend

	Onset 1 (°C)	ΔH1 (Jg^{-1})	Onset 2 (°C)	ΔH2 (Jg^{-1})
Lac/Gal(5%)/Glu(5%)	141.845	53.698	201.036	48.474
Lac/Gal(10%)/Glu(10%)	138.739	46.246	198.134	56.736
Lac/Gal(15%)/Glu(15%)	138.785	20.628	192.392	28.151
Lac/Gal(20%)/Glu(20%)	137.565	36.154	188.364	37.364

Table 13. DSC Data for Co-Crystalline Lactose, Galactose and Glucose Materials formed from a Physical Blend

The DSC data (Table 13) shows that with elevating levels of galactose and glucose, the value of ΔH for the lactose relevant endotherms becomes lower with respect to analogous physical blends. Increasing levels of the monosaccharides are concordant with higher reductions in the enthalpy value of the endotherms representative of lactose.

PXRD analysis of the materials (Fig 50) was in agreement with the results obtained from DSC analysis (Fig 51) all the materials exhibited solely a lactose phase.



Black: Glucose 5% w/w, Galactose 5% w/w Red: Glucose 10% w/w, Galactose 10% w/w, Blue: Glucose 15% w/w, Galactose 15% w/w

Fig 50. PXRD Diffractograms for Co-Crystalline Lactose, Galactose and Glucose Materials Formed from a Physical Blend

PXRD data (Fig 50) allowed the calculation of the unit cell volumes for these materials (Fig 51) and these data showed that all of the materials exhibited similar unit cell volumes compared to solely lactose. There was a slight elevation in volume for materials containing 5-20% w/w of glucose and galactose. However, this increase is not significant as the data points do not fall within a 95% confidence interval.

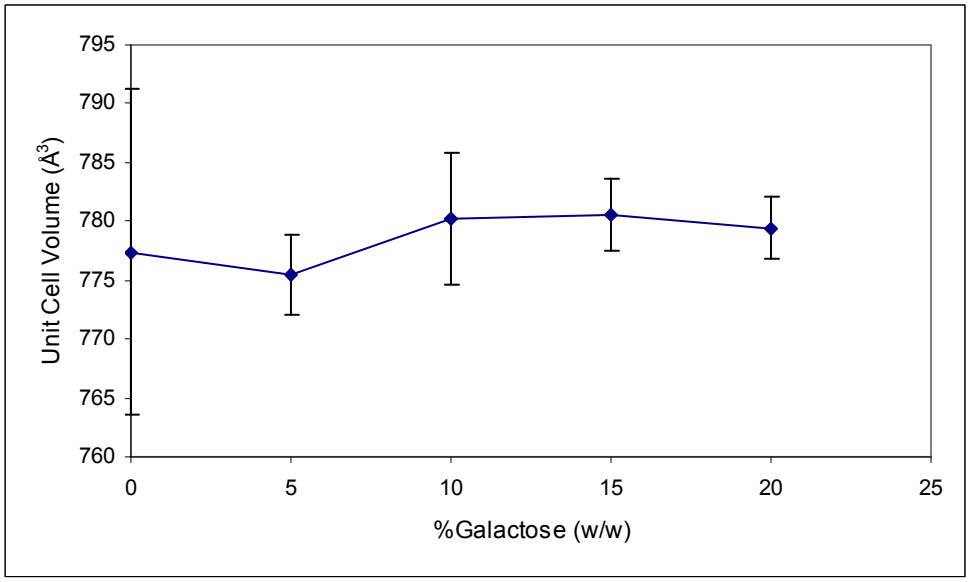


Fig 51. Unit Cell Volume *vs*. % Galactose for Co-Crystalline Lactose, Galactose and Glucose Materials formed from a Physical Blend

5.2.7. Co-Crystallisation of Lactose with Galactose and Fructose

5.2.7.1. Physical Blends of Lactose with Galactose and Fructose

The DSC analysis (Fig 52) of physical blends of lactose, galactose and fructose shows that each of the individual phases can be identified. The two expected forms of lactose are observed to melt at ~ 145°C and ~ 210°C, galactose is seen melting at ~ 160°C and fructose is seen to melt at ~ 130°C. Whilst the differentiation between the melting of lactose and galactose can be clearly seen, the difference between lactose monohydrate melting (at ~ 145°C) and fructose is not as sharp. The melting of fructose can be discerned as a slight shoulder on the peak associated with the dehydration of lactose monohydrate; however, even at the lowest level of addition (5% w/w) this is identifiable.

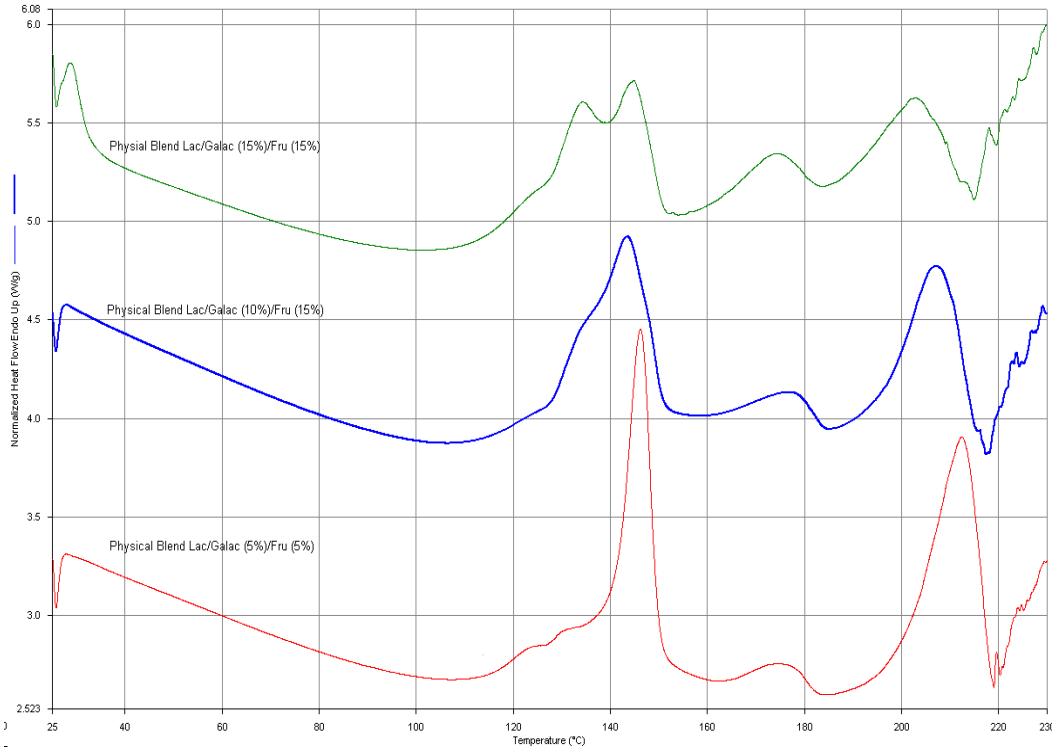
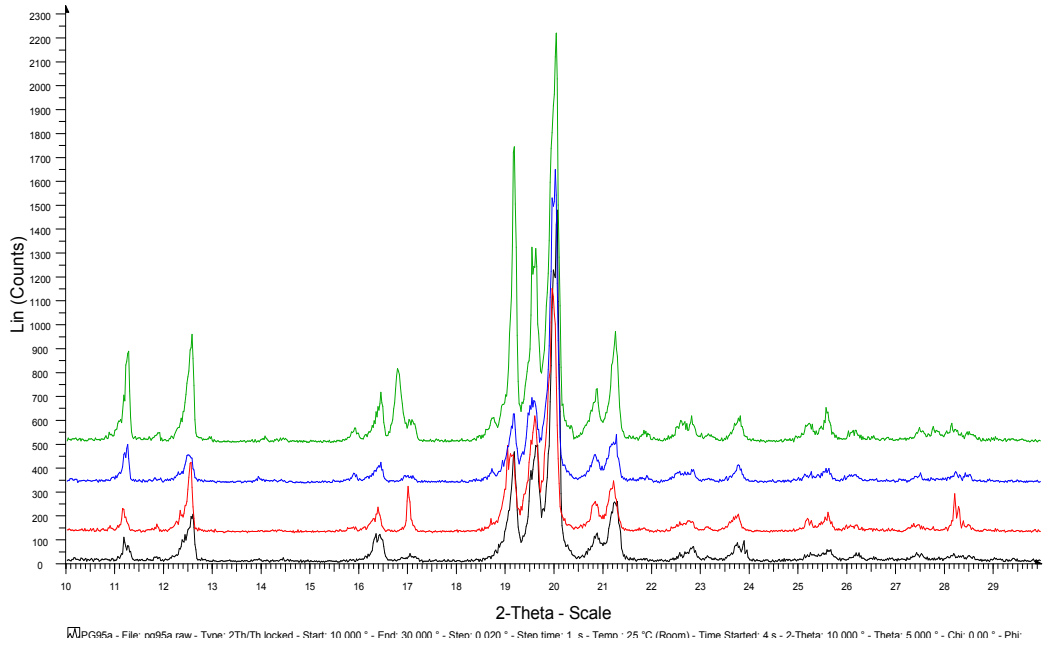


Fig 52. DSC Traces of Physical Blends of Lactose, Galactose and Fructose Materials

	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)	ΔH4 (Jg^{-1})	Onset 4 (°C)
Lac/Galac (5%)/ Fruc (5%)	8.495	128.524	13.956	140.758	8.795	166.287	13.295	196.226
Lac/Galac (10%)/ Fruc (10%)	1.416	118.374	49.733	141.421	6.679	166.634	81.396	202.137
Lac/Galac (15%)/ Fruc (15%)	1.752	129.417	21.791	138.875	8.904	166.582	49.248	197.908

Table 14. DSC Data for Physical Blends of Lactose, Galactose and Fructose Materials

PXRD analysis (Fig 53) of the physical blends allowed an easier distinction to be made between the three phases compared to DSC analysis. Characteristic peaks for fructose are identifiable at the 2θ positions of ~ 14°, 17.2° and 28.2°, whilst for galactose the characteristic peaks are observable at the 2θ positions of ~ 18.6° and 21.8°. The diffractograms enabled the calculation of the unit cells (Fig 54) for the physically blended materials.



Black: Re-Crystallised Lactose, Red: Galactose 5% w/w, Fructose 5% w/w, Blue: Galactose 10% w/w, Fructose 10% w/w Green: Galactose 15% w/w, Fructose 15% w/w

Fig 53. PXRD Diffractograms for Physical Blends of Lactose, Galactose and Fructose

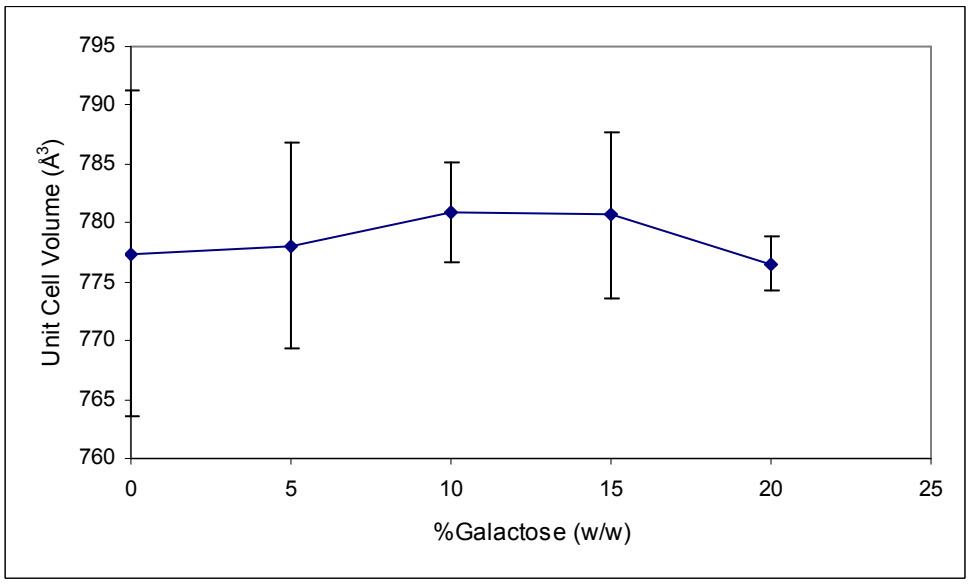


Fig 54. Unit Cell Volume vs. % Galactose for Physical Blends of Lactose, Galactose and Fructose

5.2.7.2. Co-Crystallisation of Lactose, Galactose and Fructose

DSC traces (Fig 55) of co-crystallised lactose, galactose and fructose materials showed that up to a level of 15% w/w of each monosaccharide, the melting behaviour is indicative of the presence of lactose. No endotherms can be observed indicative of the presence of either galactose or fructose as separate phases within the co-crystalline material.

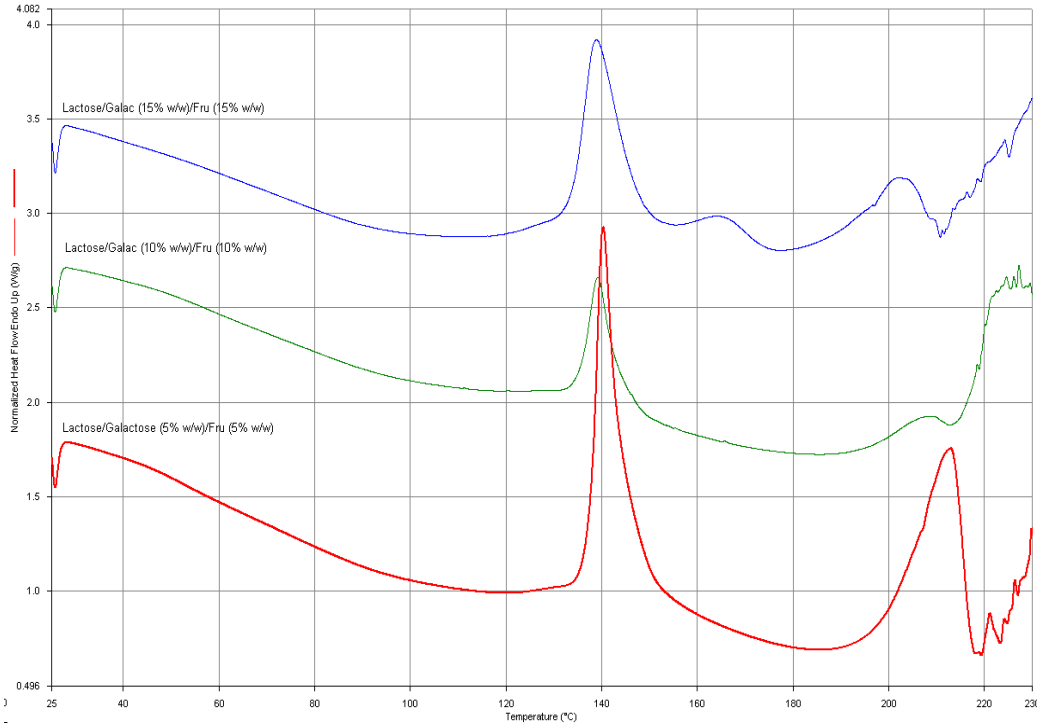


Fig 55. DSC Traces of Co-Crystalline Lactose, Galactose and Fructose Materials

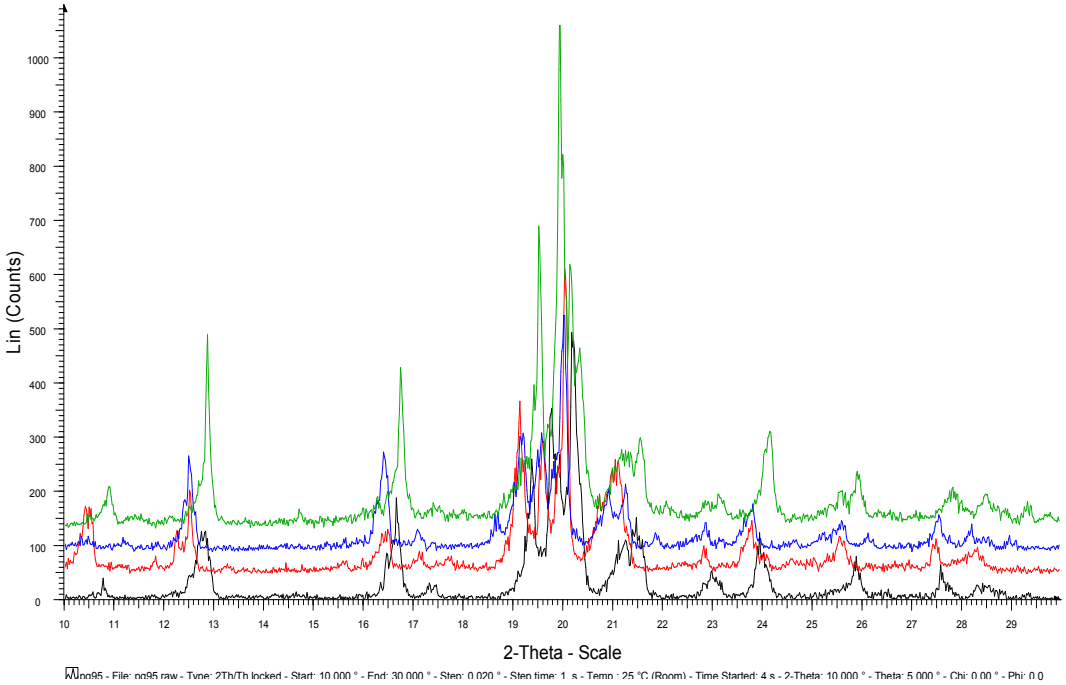
	ΔH1 (Jg^{-1})	Onset 1 (°C)	ΔH2 (Jg^{-1})	Onset 2 (°C)	ΔH3 (Jg^{-1})	Onset 3 (°C)
Lac/Galac(5%)/Fru(5%)	66.341	137.547	-----	---------	65.660	202.986
Lac/Galac(10%)/Fru(10%)	22.721	135.382	-----	---------	4.401	198.856
Lac/Galac(15%)/Fru(15%)	46.505	133.844	4.84	157.890	13.965	194.989

Table 15. DSC Data for Co-Crystalline Lactose, Galactose and Fructose Materials

At the level of 15% w/w of each of the monosaccharides, a characteristic endothermic peak emerges at ~ 157°C that is representative of galactose but no clear melting endotherm can be observed for fructose. However, a slight shoulder is observable on the lactose peak at ~ 145°C that is highly likely to be due to

fructose melting. Due to the ambiguous nature of the peak; no enthalpy value could be calculated. From the DSC data (Table 15) it can be seen that increasing amounts of added galactose and fructose, the value of ΔH for the lactose peaks lessens which is accompanied by a broadening of the said endothermic peaks.

PXRD analysis (Fig 56) of the co-crystallised lactose, galactose and fructose materials gave diffractograms that were in good agreement with the data garnered from DSC analysis (Fig 55). Materials with up to 15% w/w of the two monosaccharides exhibit patterns that are indicative of solely lactose. At this level and above, characteristic peaks emerge for galactose at the 2θ positions of ~ 18.6° and 21.8° and for fructose at the 2θ position of ~ 28.2°.



Black: Re-Crystallised Lactose, Red: Galactose 5% w/w, Fructose 5% w/w, Blue: Galactose 10% w/w, Fructose 10% w/w Green: Galactose 15% w/w, Fructose 15% w/w

Fig 56. PXRD Diffractograms for Co-Crystalline Lactose, Galactose and Fructose Materials

The diffractograms gathered enabled the calculation of the unit cells (Fig 57) for the co-crystallised materials.

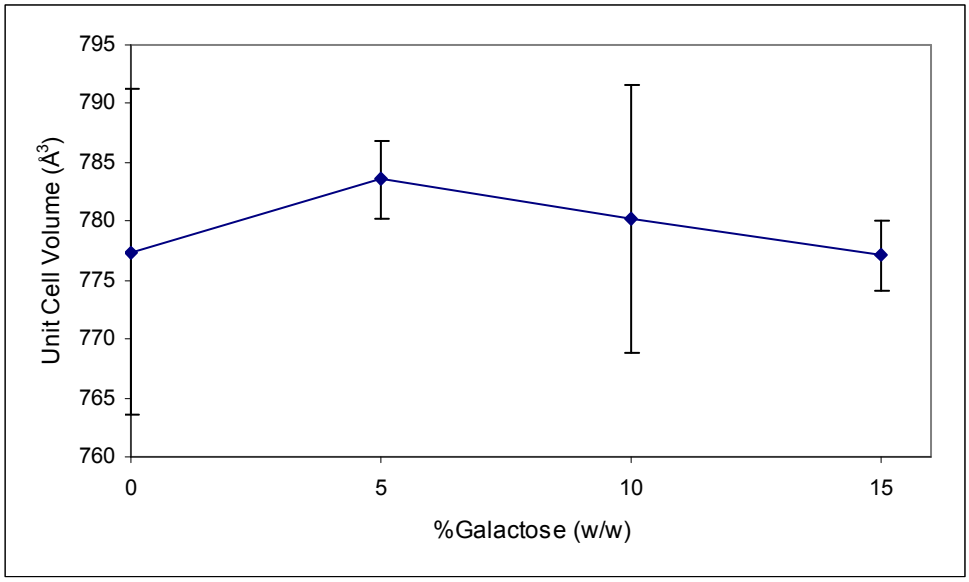


Fig 57. Unit Cell Volume vs. % Galactose for Co-Crystalline Lactose, Galactose and Fructose Materials

The calculations show no particular trend in the volume of the unit cells of the co-crystallised material. Some material does display a slightly larger unit cell volume compared to re-crystallised lactose. However, these elevations are not significant; all the data points associated with such an increase fall outside of a 95% confidence interval.

The freshly prepared co-crystallised and physical blends of lactose, galactose and fructose were subjected to an autosorb analysis. A plot of percentage weight gain against RH for each of the samples can be seen in Fig 58. The autosorb data suggests that co-crystallisation of lactose with galactose and fructose yields a crystalline material; the state of the included components (galactose and fructose) appears to be crystalline rather than amorphous. For the co-crystalline material, elevation of the RH results in an increase in the percentage weight gain which decreases as the samples are held at each level of RH. This trend continues until the samples deliquesce at ~ 90% RH. The physical blends of the sugars exhibits identical behaviour, where elevation of the RH results in an initial increase in the percentage weight gain followed by a steady decrease as the samples are held at each level of RH until the samples deliquesce at ~ 90% RH.

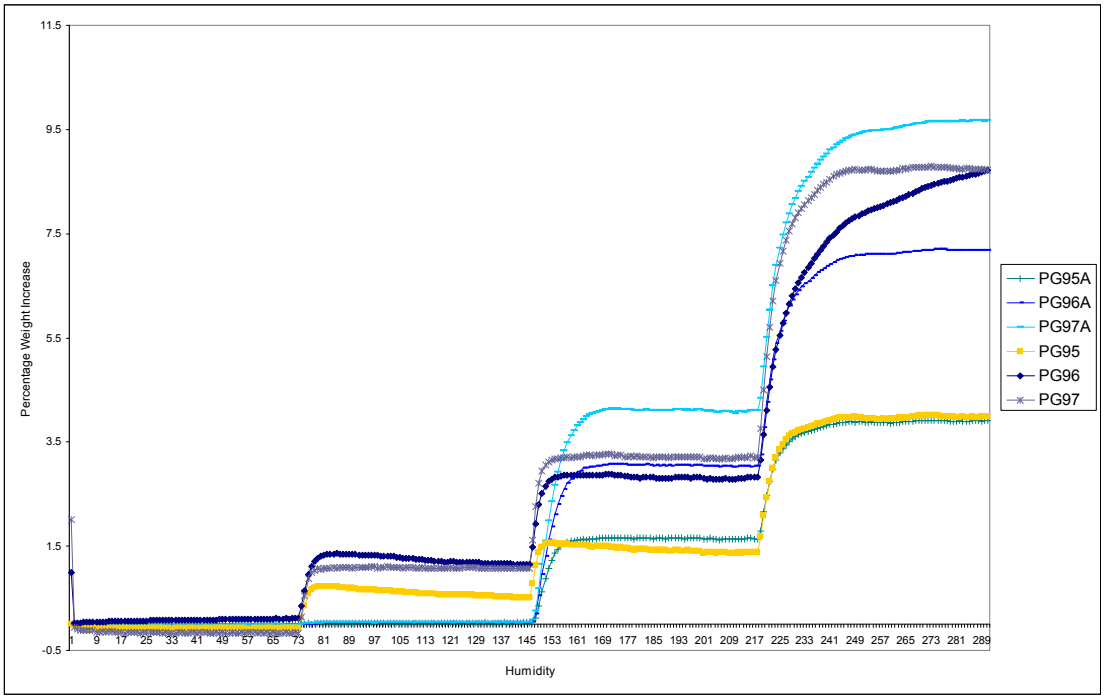


Fig 58. Relative Humidity vs. % Weight Gain for Lactose, Galactose and Fructose Materials

For the physical blends of the three sugars, there is no relationship between the amount of moisture adsorbed and the amount of included galactose and fructose. The co-crystallised material does not behave in an identical manner; these data suggest that there is a direct relationship between the quantity of included components (galactose and fructose) and the amount of moisture adsorbed. Higher levels of galactose and fructose result in elevated levels of moisture adsorbed. With an increase in RH from 25% to 50%, the material containing 5% w/w of galactose and 5% w/w of fructose has a ~ 0.19% increase in percentage weight gain compared to a ~ 0.4% increase in percentage weight gain for material containing 15% w/w of each of the two sugars.

Co-crystallised materials also adsorb more moisture compared to the physical blends for each elevation of RH. A change from 25% to 50% RH gives a ~ 0.06% increase in the percentage weight gain for a physical blend containing 5% of galactose and 5% w/w of fructose compared to a ~ 0.19% increase for the analogous co-crystallised material.

5.2.8. Co-Crystallisation of Lactose with Polydextrose

Polydextrose is a low-calorie dietary fibre that is used within the food industry as a sugar substitute, as a thickening agent or a stabiliser. It is resistant to hydrolysis and thus, cannot be broken down in the lower intestine by enzymic action. This resistance stems from an uncommon linkage (in nature) between the dextrose units and, due to this, polydextrose is regarded as a low-calorie material.[3] Polydextrose is becoming of a greater interest for health conscious consumers due to its beneficial nature and, as such, provides a viable material to study as a component in co-crystallisation. Polydextrose has a problem when on its own as a foodstuff; it has an un-desirable mouthfeel. Upon entering the mouth, polydextrose becomes gluey in its consistency and co-crystallising this material with lactose may alleviate this characteristic.

5.2.8.1. Physical Blends of Crystalline Lactose and Polydextrose

DSC analysis (Fig 59) of the physically blended materials showed that the mixture exhibits thermal behaviour of solely lactose; the two expected forms emerge at ~ 145°C and ~ 210°C. This was not un-expected since polydextrose is amorphous in its structure (as evident from DSC analysis of the material, Fig 59) and is not expected to display any endothermic peaks.[4]

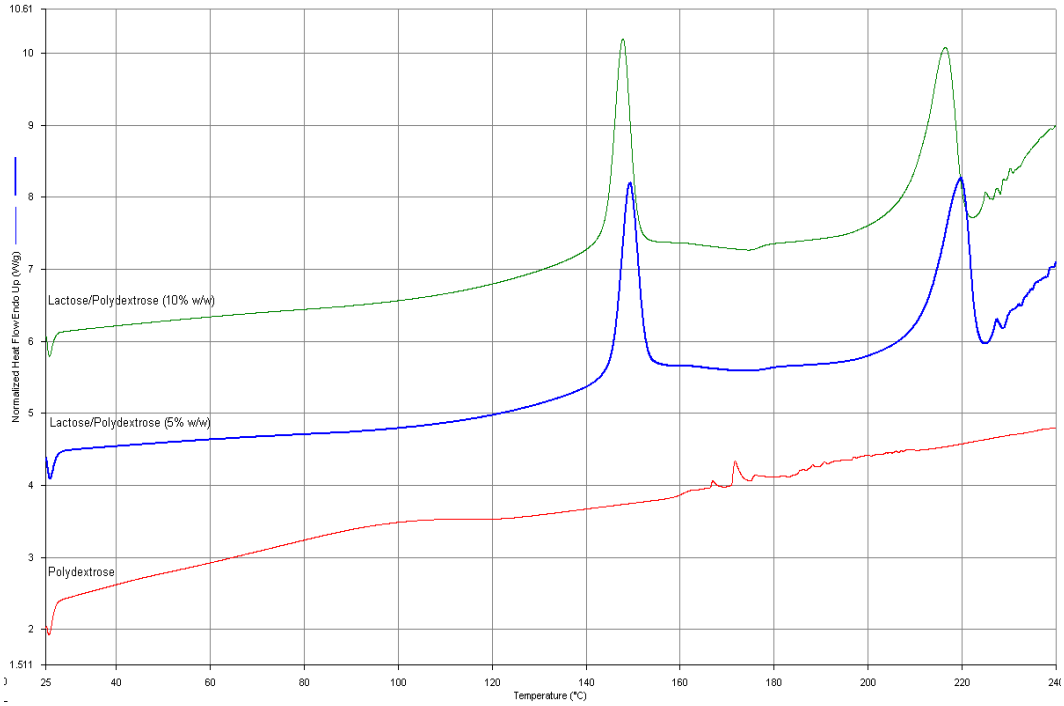
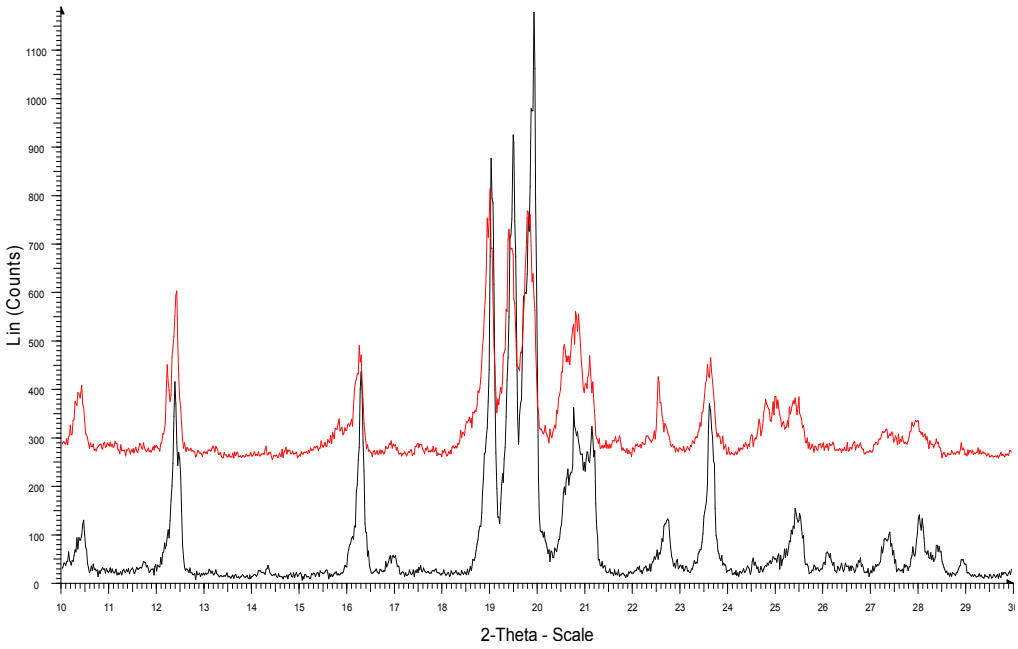


Fig 59. DSC Traces of Polydextrose and Physical Blends of Lactose and Polydextrose

	Onset 1 (°C)	ΔH1 (Jg^{-1})	Onset 2 (°C)	ΔH2 (Jg^{-1})
Lactose/Polydextrose (5%w/w)	145.722	72.762	211.467	107.033
Lactose/Polydextrose (10%w/w)	144.295	73.371	209.387	110.733

Table 16. DSC Data for Physical Blends of Lactose and Polydextrose

Further confirmation of the amorphous character of the polydextrose was obtained from PXRD analysis (Fig 60) of the physical blends of the two materials. The diffractograms suggest solely the presence of lactose in its monohydrate form.



Black: Polydextrose 5% w/w, Red: Polydextrose 10% w/w

Fig 60. Physical Blends of Lactose and Polydextrose

5.2.8.2. Co-Crystallisation of Lactose with Polydextrose

DSC analysis (Fig 61) showed that with the included levels of polydextrose, solely phases related to lactose are formed. The DSC traces suggest the presence of lactose; the expected endothermic peaks emerge at ~ 145°C and ~ 200°C, but due to the amorphous nature of polydextrose, the formation of a co-crystalline material is unlikely.

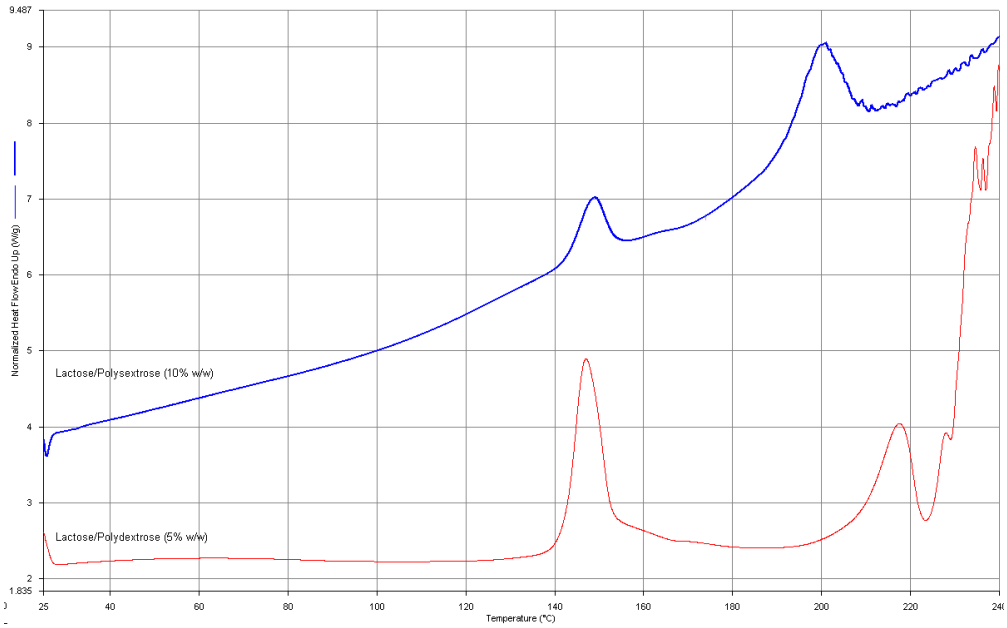
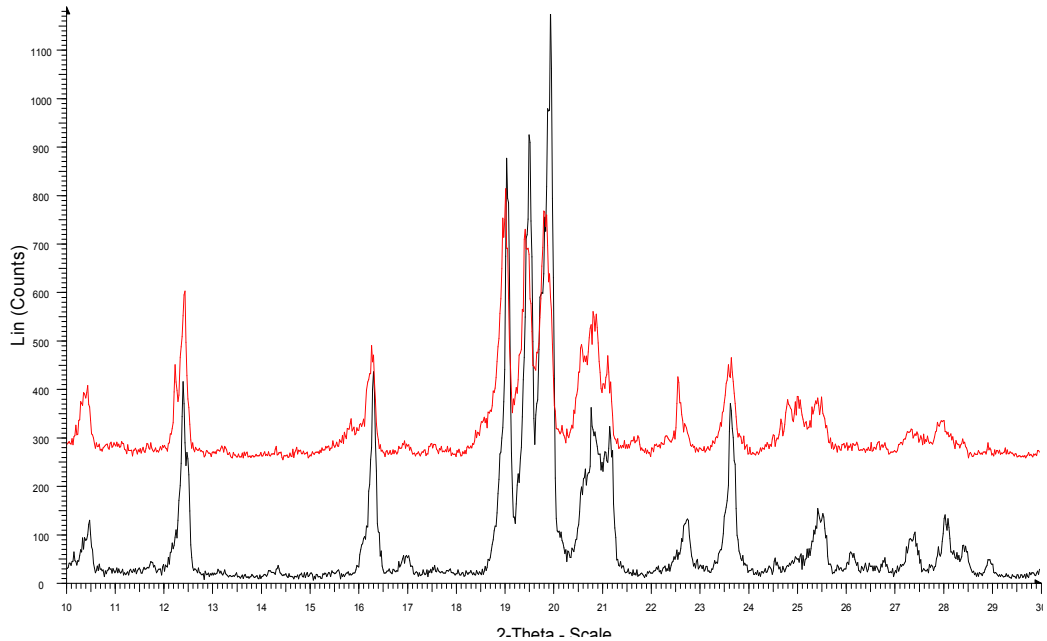


Fig 61. DSC Traces of Co-Crystalline Lactose and Polydextrose

	Onset 1 (°C)	ΔH1 (Jg^{-1})	Onset 2 (°C)	ΔH2 (Jg^{-1})
Lactose/Polydextrose (5%w/w)	142.342	117.410	208.628	73.256
Lactose/Polydextrose (10%w/w)	142.785	29.750	193.143	62.023

Table 17. DSC Data for Co-Crystalline Lactose and Polydextrose Materials

PXRD analysis (Fig 62) of the co-crystallised materials showed that the materials display characteristics of the α-lactose monohydrate structure. No other phases were discernable but this is expected as the component attempted to be included (polydextrose) is amorphous and as such, it will not signify its presence as a separate phase by PXRD analysis.



Black: Polydextrose 5% w/w Red: Polydextrose 10% w/w

Fig 62. PXRD Diffractograms for Co-Crystalline Lactose and Polydextrose Materials

PXRD data allowed the calculation of the unit cell volumes for the materials (Fig 63) and they show that all of the materials exhibit larger unit cell volumes compared to solely lactose.

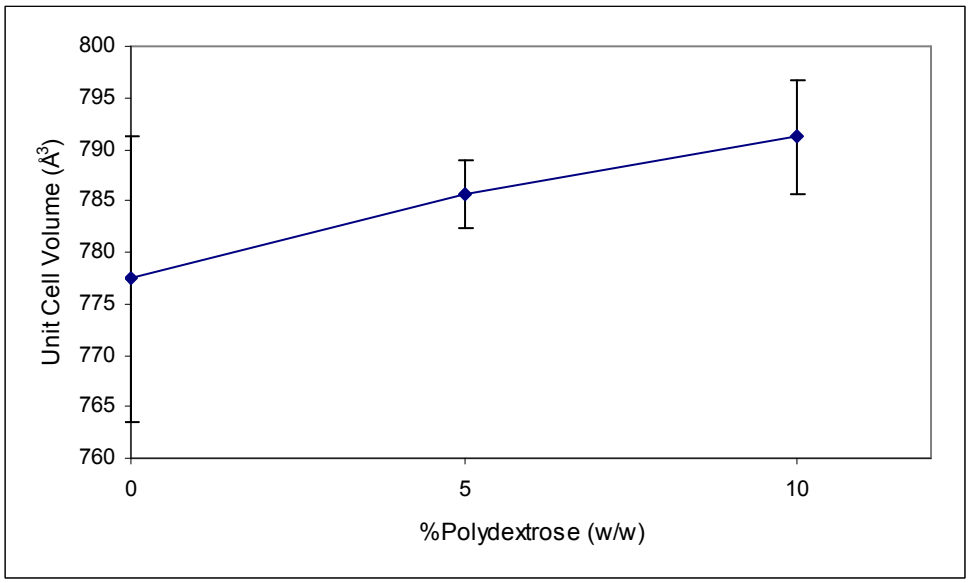


Fig 63. Unit Cell Volume vs. % Polydextrose

5.2.9. Co-Crystallisation of Lactose and Saccharin

5.2.9.1. Physical Blends of Lactose and Saccharin

DSC analysis of the blended materials (Fig 64) shows that the materials display two phases: a α-lactose monohydrate phase is identifiable from the endotherms at ~ 145°C and ~ 200°C; a separate peak is also identifiable at ~ 200°C that relates to saccharin.

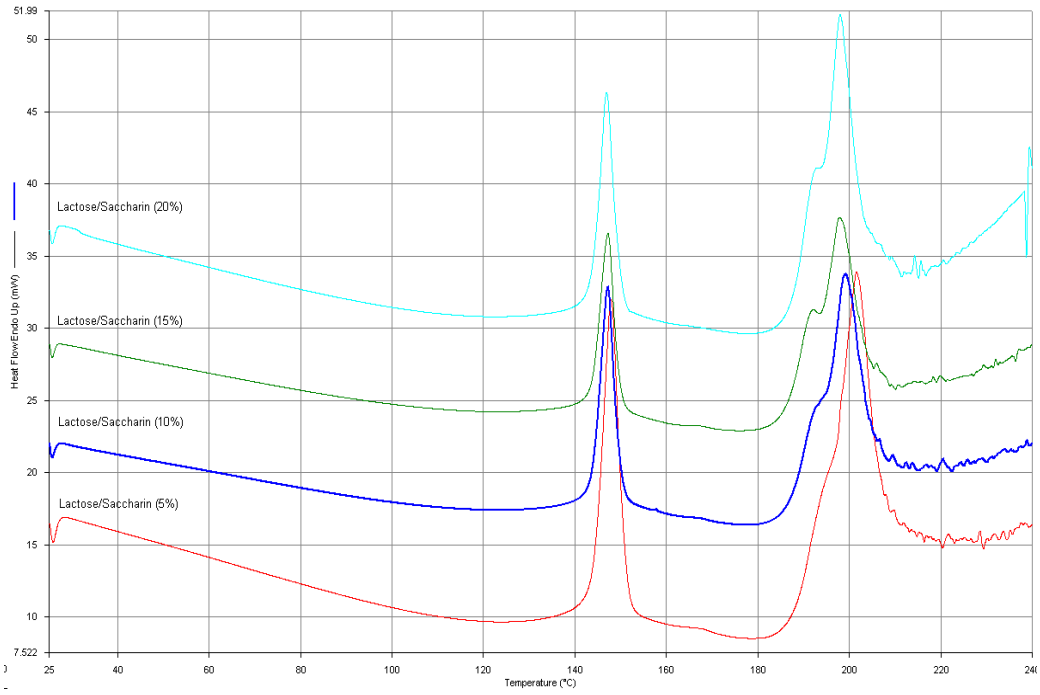
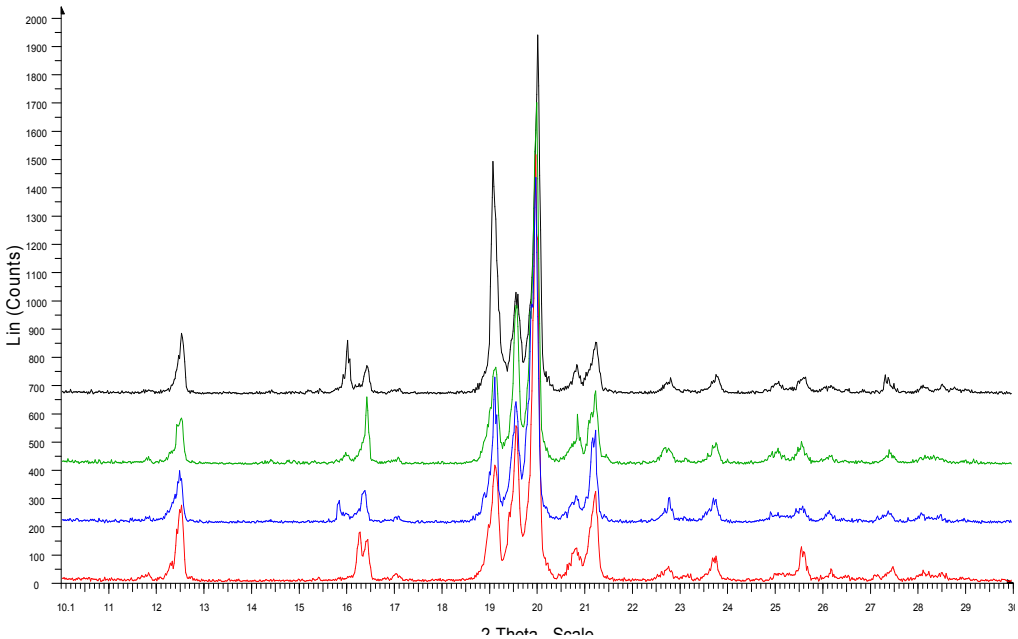


Fig 64. DSC Traces of Physical Blends of Lactose and Saccharin

Due to the similarity in the melting of the α-form of lactose and saccharin, the calculation of enthalpy values for these two phases is difficult. However, as an endotherm can be clearly discerned when the two materials are together the above data will serve well as an indication of co-crystallinity.

PXRD analysis (Fig 65) of the two sugars showed that the majority of peaks corresponded to the α-lactose monohydrate. However, characteristic peaks for saccharin can be observed at the 2θ positions of ~ 15.4°, 15.9° and 16.2°; these reflections were observed to increase in intensity with increasing amounts of saccharin.



Red: Saccharin 5% w/w, Blue: Saccharin 10% w/w, Green: Saccharin 15% w/w, Black: Saccharin 20% w/w

Fig 65. PXRD Diffractogram for Physical Blends of Lactose and Saccharin

PXRD data allowed the calculation of the unit cell volumes (Fig 66) of the physically blended materials to be calculated, the materials with saccharin present appeared to exhibit larger unit cells compared to solely lactose.

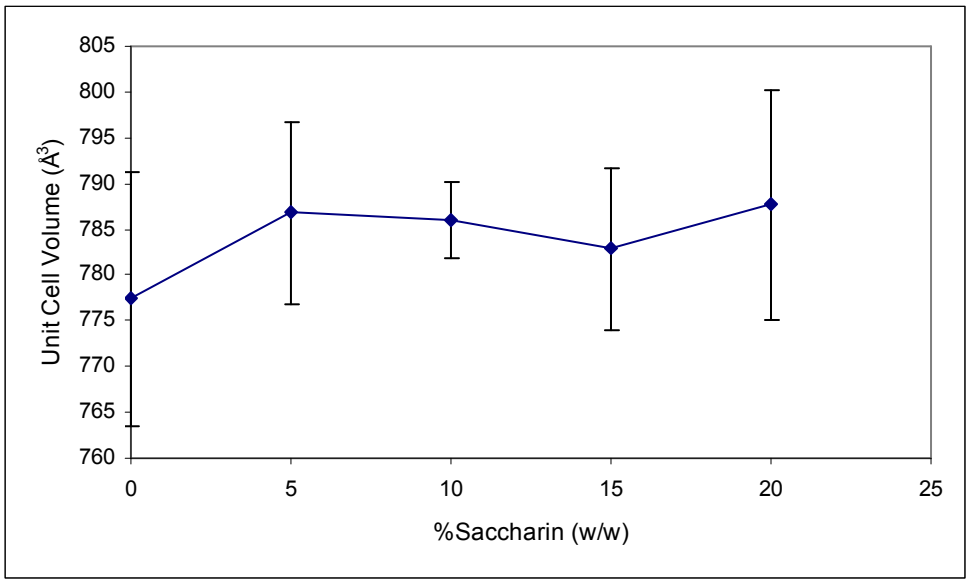


Fig 66. Unit Cell Volume vs. % Saccharin

5.2.9.2. Co-Crystallisation of Lactose and Saccharin

DSC analysis of the materials (Fig 67) showed that all of the materials displayed melting behaviour indicative of α-lactose monohydrate. A dehydration endotherm is observed at ~ 145°C and the melting of α-lactose is observed at ~ 190°C. The values of ΔH for these endotherms were found to decrease with increasing amounts of saccharin up to a quantity of 20% w/w of saccharin. With this quantity of saccharin present, the values for ΔH increase compared to the material containing 15% w/w of saccharin.

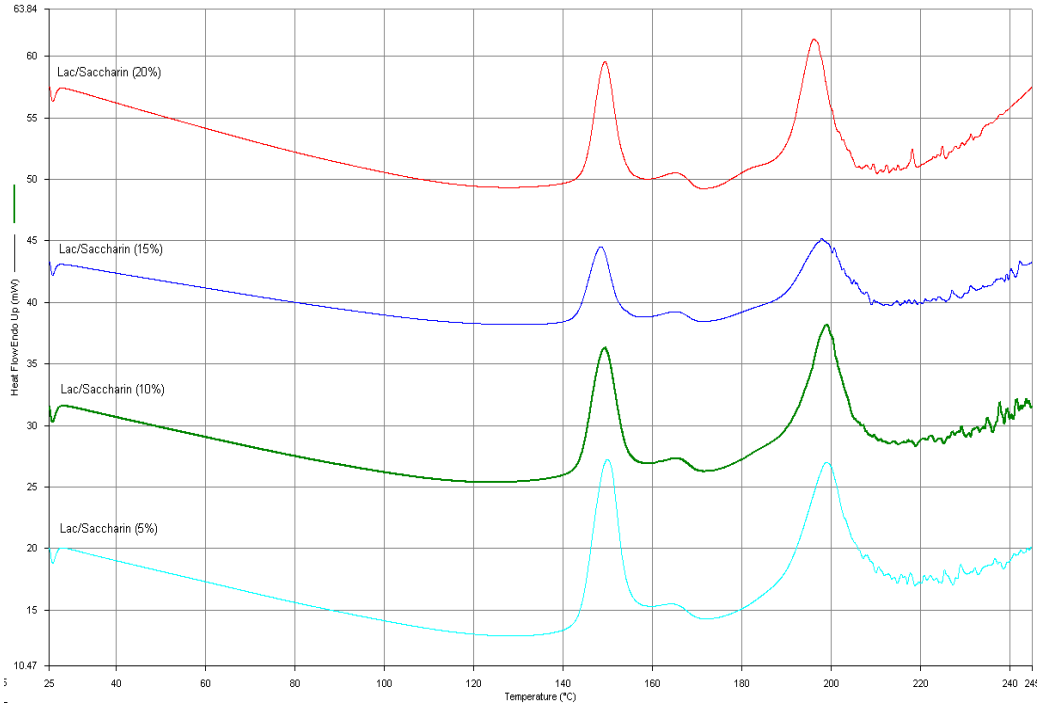
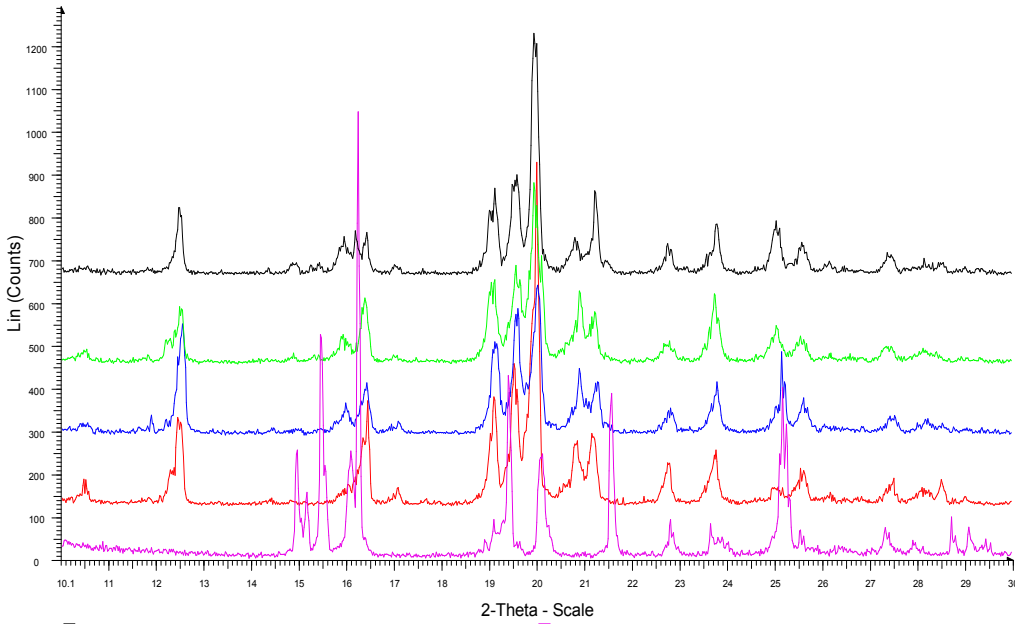


Fig 67. DSC Traces for Co-Crystalline Lactose and Saccharin Materials

	Onset 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	ΔH 2 (Jg^{-1})
Lac/Sacc (5% w/w)	143.99	81.107	190.795	81.303
Lac/Sacc (10% w/w)	143.975	68.374	192.988	63.268
Lac/Sacc (15% w/w)	143.433	36.934	192.508	53.029
Lac/Sacc (20% w/w)	144.899	56.207	190.595	83.093

Table 18. DSC Data for Co-Crystalline Lactose and Saccharin Materials

PXRD analysis (Fig 68) gave results that disagreed with DSC analysis (Fig 67); solely lactose related materials are formed up to 15% w/w of added saccharin. Above this level, peaks emerge at the 2θ positions of ~ 15.2°, 15.85° and 16.12° that suggest the presence of saccharin as a separate phase.



Red: Saccharin 5% w/w, Blue: Saccharin 10% w/w, Green: Saccharin 15% w/w, Black: Saccharin 20% w/w

Fig 68. PXRD Diffractogram for Co-Crystalline Lactose and Saccharin

PXRD data allow calculation of the unit cell volumes (Fig 69) for the co-crystallised materials and the data show that they all exhibit larger unit cells compared to solely lactose. However, this elevation for this series of materials cannot be construed as a significant increase. All the data points associated with the unit cell volume fall outside of a 95% confidence interval.

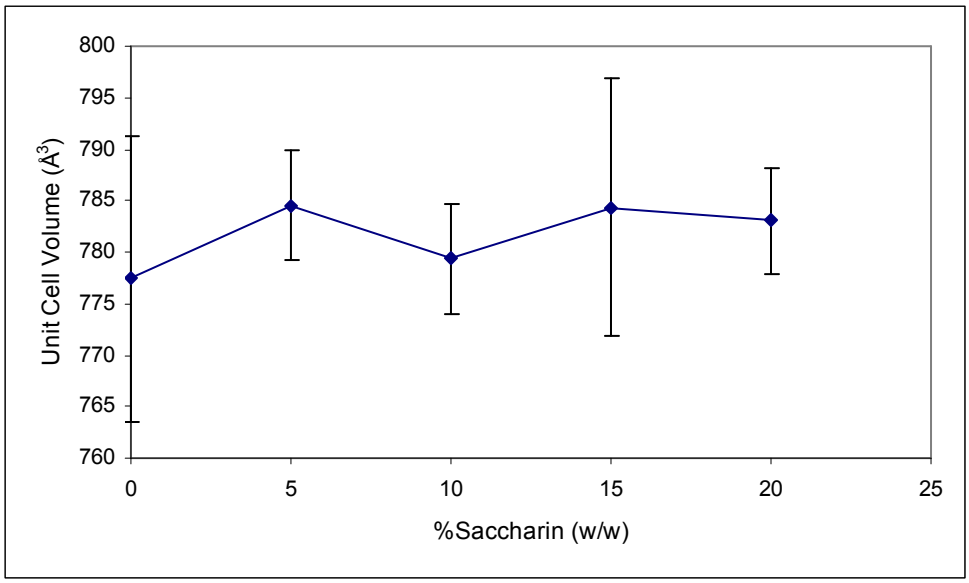


Fig 69. Unit Cell Volume vs. % Saccharin for Co-Crystalline Lactose and Saccharin Materials

5.3. Discussion

5.3.1. Re-crystallisation of Lactose

Lactose re-crystallised from water showed thermal behaviour that was indicative of the presence of α-lactose monohydrate. Dehydration of the material was observed at ~ 145°C which was then followed by the melting of the α-form at ~ 205°C. The thermal behaviour of the dehydration endotherm matched well as did the melting temperature for the α-form with the temperatures reported in literature.[1, 5] However, PXRD analysis (Fig 2) showed the presence of another phase other than α-lactose monohydrate.

The diffractogram (Fig 2) for re-crystallised lactose shows reflections that indicate lactose to be in its α-monohydrate state. Using PowderCel (a program designed for constructing model diffractograms of materials), this diffractogram pattern was compared with a model for α-lactose monohydrate reported by Beevers.[6] This comparison showed a good correlation between the observed

peaks and the expected peaks (see Appendix 1) accompanied by a shift to higher 2θ values. However, the reflection noted at ~ 10° that was not attributable to the α-monohydrate form of lactose, which suggests the presence of another anomer of lactose. As such, the diffractograms for re-crystallised lactose was compared with a model built from the structure reported for a 1:1 α:β lactose crystal reported by Lefebrve *et al*.[7] This comparison showed that the majority of reflections were accounted for but the reflection at ~ 10° was still un-accounted for. Further comparisons with the two anhydrous lactose forms reported yielded similar results.[8, 9] Comparison of re-crystallised lactose with a model reported for β-lactose revealed a correlation with a portion of the reflections matching but some strong reflections are not accounted for; however, the reflection at ~ 10° was accounted for by the model for β-lactose.[10] It appears that lactose re-crystallised from water yields a mixture of the α- and β-anomers of lactose using the methodology employed herein. In an effort to conclusively elucidate the identity of the phase responsible for the reflection at ~ 10°, a portion of α-lactose monohydrate was converted to solely the β-anomer *via* the method outlined by Yoshino *et al*[11] and subsequently analysed by DSC and PXRD (see Appendix 1).[11] DSC analysis (Fig 1, Appendix 1) showed the melting of solely β-lactose at a temperature of ~ 225°C close to the one reported in literature, comparison of the diffractograms (Fig 2, Appendix 1) for the converted lactose with a model reported for β-lactose showed an exact match.[1, 5]

From the evidence found from PXRD analysis it can be suggested that lactose re-crystallised using this methodology (see Section 8.3.1.) yields a material that contains both the α- and β-anomers of lactose. The material can be called a mixture of the two anomers rather than a mixed crystal containing both the α- and β-forms from consideration of the melting point of the re-crystallised lactose. Work by Drapier-Beche *et al.* on the thermal characterisation of lactose crystals containing the two anomers in ratios of 5:3 or 3:2 (α:β) showed that these materials exhibited lower melting points compared to α-lactose monohydrate.[12] The melting point of the material with the α:β ratio of 5:3 was ~ 209°C and a crystal with the ratio of 3:2 (α:β) was reported to melt at 291°C. Neither of these values corresponds to the observed melting point of the re-crystallised lactose (~200°C). As such, it can be suggested that the material yielded from re-crystallisation does not give crystals containing a mixed ratio of

the two anomers of lactose; rather the material is predominantly α-lactose monohydrate with a small quantity of the β-anomer being present. This is logical as crystals containing mixed ratios of α- and β-anomers are formed *via* re-crystallisation from alcohol or by the addition of salts or concentrated acids and alkalis.[12] This is not the case here as water is the re-crystallisation solvent and there are no added salts. As the material appears to be predominantly the α-lactose monohydrate form (with a small amount of the β-form), this does fit with the notion that all lactose supersaturated solutions crystallised below 93.5°C contain predominantly the α-lactose monohydrate anomer.[13] Conclusive evidence for the above postulations can be gained by fitting of the pattern with model diffractions for both the α- and β-anomer of lactose. This enabled a ratio of the two anomers to be determined; the Le Bail extraction showed that α-lactose was present at the level of 97%. So, it can be said that all materials re-crystallised in the manner outline in Section 8.3.1. will be predominantly the α-lactose monohydrate form with a small quantity of β-lactose present. As such, the melting of the lactose form at ~ 210°C will be referred to as the melting of the α-lactose monohydrate form. Therefore, for the purposes of this discussion, all of the co-crystalline materials will be likened to re-crystallised α-lactose monohydrate for calculation of unit cell parameters, unless specified otherwise.

5.3.2. Co-crystalline Materials formed from Lactose and Maltose

The addition of maltose to a supersaturated lactose solution via the co-crystallisation protocol (see Section 8.3.2) showed material that exhibits solely single phases up to a level of 20% w/w of added maltose. DSC analysis (Fig 2) shows, up to the level of 20% w/w, two endothermic peaks can be observed that correspond to lactose. The dehydration endotherm can be seen at ~ 145°C and the peak at ~ 200°C corresponds to the alpha form of lactose.[5, 12]

The enthalpy value of the endotherm relating to the α-form of lactose at ~ 200°C will be considered solely for this discussion with regard to all materials formed. The dehydration endotherm at ~ 145°C is related to water of crystallisation and not to the actual melting of the co-crystalline material.[5] Consideration of these values (see section 5.2.2., table 2) shows that a general reduction is observed that is proportional to the quantity of added maltose in the range of 7-17% w/w. Higher levels of maltose induce a lower value calculated

for ΔH. Such a depression suggests a doping of the lactose structure with maltose. DSC theory shows that upon the inclusion of an impurity into a phase induces a reduction in ΔH coupled with a lowering of the onset melting temperature.[14, 15] This thermal behaviour is indicative of disruption within the crystal lattice and thus increasing lattice strain arising from crystal imperfections.[16] As this behaviour is noted from DSC analysis (see section 5.2.2., fig 3) of co-crystallised lactose and maltose materials, it strongly suggests the inclusion of maltose into the lactose crystalline matrix. Further evidence for this is garnered from comparison with the physical blends of the two disaccharides. The enthalpy associated with the α-lactose peak in these materials is higher compared to the value observed for the same peak observed in materials formed from the co-crystallisation process.[2]

Upon elevation of the level of included maltose to 20% w/w, the DSC analysis (see section 5.2.2., fig 3) showed the presence of an endotherm at ~ 120°C. This suggests the presence of a separate maltose monohydrate phase. It is worth noting that the value of ΔH obtained (11.933 Jg^{-1}) for maltose melting is lower compared to the value obtained (18.4 Jg^{-1}) for maltose melting in the analogous physical blend.[2] This suggests that even when the limit of addition is reached, the co-crystallisation process may still include a proportion of the added component.

PXRD analysis (see section 5.2.2. fig 4) of the co-crystallised lactose and maltose materials gave results that supported those obtained from DSC analysis. Solely lactose related materials were formed up to a level of 20% w/w of added maltose, at this level; peaks appeared at the 2θ positions of ~ 14.6° and 21.8°. These peaks could not be attributed to any form of lactose and subsequent comparisons found them to coincide with peaks expected for the monohydrate form of maltose. From the analysis of the yielded diffractograms (see section 5.2.2. fig 4), the peak at the 2θ position of ~ 10° was observable and thus further suggests the presence of a lactose material that consists of a mixed anomer ratio. The unit cells for the co-crystallised lactose and maltose materials were calculated and can be seen in figure five.

The calculated unit cell volumes of the co-crystallised materials (see section 5.2.2. fig 5) all have a larger volume compared to lactose re-crystallised from a supersaturated solution. This suggests that the added maltose potentially

becomes included within the crystalline structure of lactose and as a result; maltose may become a part of the lactose crystal structure. However, this theory cannot be conclusively proved due to the lack of significance in the unit cell volume increases. All of the data points fall outside of a 95% confidence interval and therefore cannot be construed as significant. Whilst the hypothesis that any added maltose becomes integrated within a lactose lattice, it is a conceivable notion. The position of the maltose within the lactose crystal structure can not be ascertained from these results. However it is not beyond the realms of possibility that it could reside either in the periodic bond chain of which the layers in a crystal consist, or sandwiched between the periodic bond chains and held in place by hydrogen bonding between hydroxyl groups.

As the structural differences between α-lactose monohydrate and maltose monohydrate are small, the notion that they are interchangeable during crystal growth is not impossible to conceive of. Work by Garnier *et al.* on the effects of structurally related additives (SRA) on the growth of α-lactose monohydrate from a supersaturated solution showed the above hypothesis is possible.[17] The work performed by Garnier *et al.* showed that SRA that were closely related to α-lactose monohydrate could become included within the structure and additives behaving in this manner could be indicated by either elongation along the *b* or *a* axis of the final crystal.[17] Typically, evidence for the inclusion of an impurity within a host lattice is drawn from significant changes in the volume of the unit cell of the host material. Any change in unit cell volume is accompanied by alteration of the axis of the unit cell. It has been shown that biogenic calcite can exhibit fluctuations in its unit cell as a function of organic impurities, the presence of such impurities was shown to induce elongation along the *a* axis of the unit cell.[18] Furthermore, the increase was shown to be linear depending on the level of the organic species resent; higher levels induced longer elongations and higher unit cell volumes.[18] It has also been shown that the unit cell volume (and its axis) of mixed metal alloys can be elevated, or reduced, by altering the ratio of metals in the final alloy.[19, 20] This effect is induced by the varying size of the ionic radii of the metals been interchanged.[20] Whilst these examples are predominantly inorganic in nature, it is not un-feasible that such phenomenon could be observed in co-crystalline sugars. The addition of maltose to supersaturated lactose does induce an elevation in the unit cell volume.

Unfortunately these are not significant increases, though it still does suggest inclusion. In accordance of examples in literature, that show variation in *both* unit cell volume and axis length as a function of inclusion or substitution, analysis of the axis lengths of co-crystalline lactose and maltose materials may provide some evidence toward their being co-crystalline. Calculation of the unit cell volumes (see section 5.2.2., fig 5) for the co-crystalline material allowed the unit cell axis lengths to be obtained. A plot of the *a*, *b* and *c* axis lengths against the quantity of added maltose can be seen below (Fig 70a, b and c respectively).

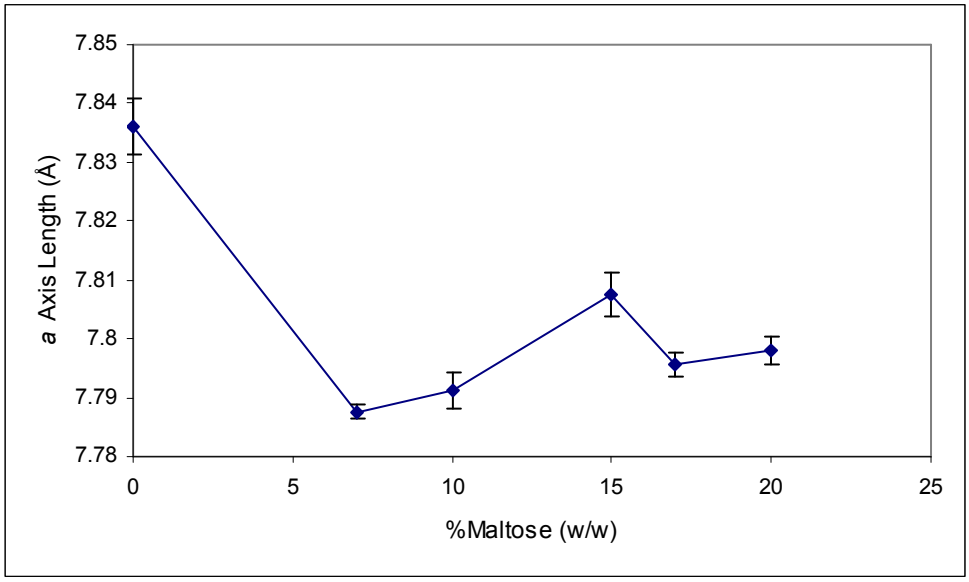


Fig 70a. Plot of *a* axis length *vs*. % Maltose

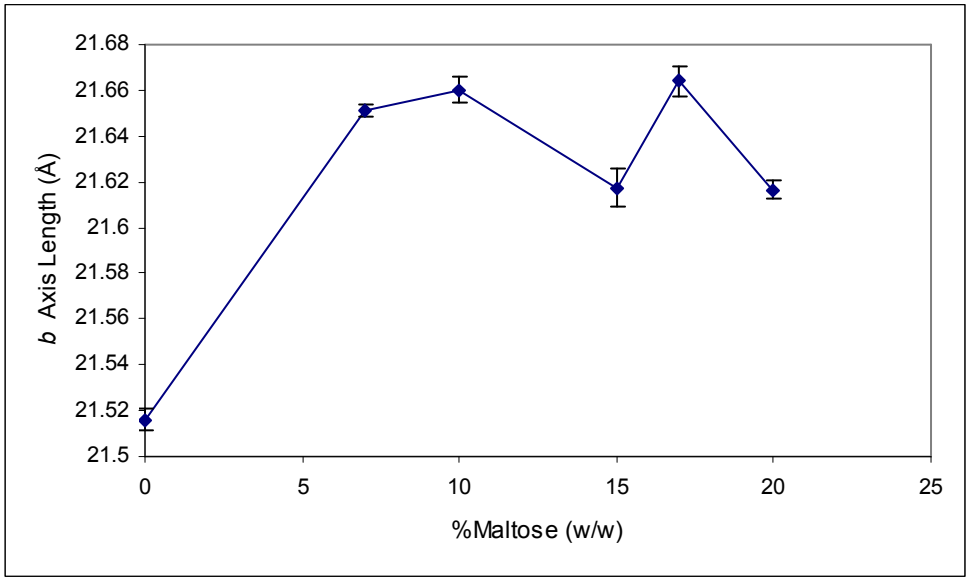


Fig 70b. Plot of *b* axis length *vs*. % Maltose

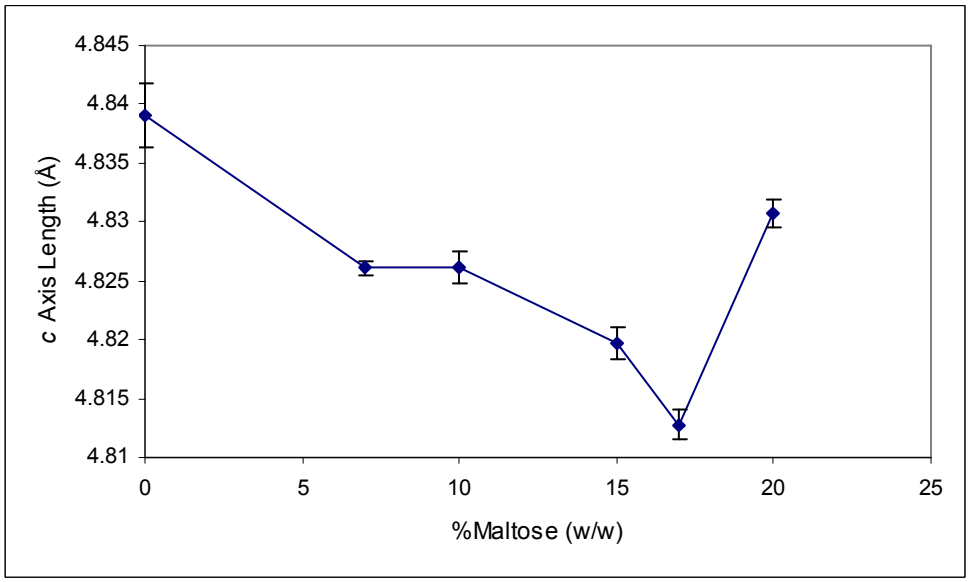


Fig 70c. Plot of *c* axis length *vs*. % Maltose

It can be clearly seen that the added maltose does not induce any elongation along the *a* or *c* axis of the unit cell. In both cases, a marked decrease can be observed (Fig 70a and 70c) compared to the values obtained for re-crystallised lactose. In comparison, the *b* axis of the unit cell undergoes significant elongation as function of added maltose. All of the data points for this elongation fall within a 95% confidence interval. It appears that adding maltose to a supersaturated solution induces an elongation along the *b* axis, coupled with a contraction of both the *a* and *c* axis.

It has been shown that bio-mineralisation, the term used for the growth of both bio-genic aragonite and calcite yields materials that differ from the pure geological materials.[18, 21] These differences were attributed to the presence of organic species altering the structure of the inorganic material. Interestingly, in an effort to reproduce these findings, Pokroy *et al.* grew calcite in the presence of caspartin (a protein extracted from mollusc shells). These experiments showed a proportional elongation of the *c* axis that was related to the presence of added caspartin.[18] Pokroy *et al.* goes on to further say that any anisotropic distortion of the unit cell, and its parameters, is induced by the presence of intra-crystalline speices.[18] It must be stated that the observed elongations of the unit cell axis were accompanied by an elevation of the unit cell volume.[18, 21]

The data garnered for materials formed *via* the addition of maltose to a supersaturated lactose solution suggests the possible inclusion of the additional maltose within the host lactose structure. The contraction, and elongation, of the unit cell axis is suggestive of the added maltose affecting the structure of the lactose lattice. Furthermore, DSC data is symptomatic of a doping of a host phase by an added impurity. Unfortunately, whilst there is evidence to provide weight to the hypothesis of added maltose becoming part of the lactose structure, it cannot be conclusively proven due to the unit cell data. The unit cells do show elevation compared to re-crystallised lactose, however, these are not significant increases. As such, a crucial piece of evidence required to underpin this theory is lacking. It can be confidently suggested that added maltose becomes integrated within the host lactose lattice, however, further work on reducing experimental error is necessary to provide data robust enough to prove this theory.

Interestingly, the limit of addition found here (17% w/w of maltose) is higher than that reported by Maillet (15% w/w).[2] A possible hypothesis for this is available by considering the effects of the β-lactose (found in the co-crystalline materials formed) on the growth of crystals from solution. It has been shown that β-lactose is a poison for the growth of α-lactose monohydrate from solution since it selectively blocks certain faces on the growing crystal and from this promotes growth along the *b* axis.[23]

Interestingly, the formation of solely lactose phases stops at a certain level of added maltose. At a level of 20% w/w of added maltose, a portion of the disaccharide may include partially within the lactose structure and a portion

crystallises on its own. An explanation for this occurrence may be obtained from consideration of the events that constitute the process of the lactose crystal structure growing from the supersaturated solution and the effects of the added maltose upon this process.

In a pure supersaturated lactose solution, induction of crystallisation results in growth units from solution becoming integrated onto the growing nucleus and this process continues until crystallisation halts. Upon the introduction of an impurity into the supersaturated solution, there is the possibility of the impurity becoming included within the final crystal structure *via* its adsorption onto the growing nucleus. The successful integration of an impurity is dependent on the strength at which it adsorbs onto the growing crystal. If a strong adsorption is apparent then any subsequent desorption is more difficult compared to when an impurity adsorbs weakly. A reluctance to depart the growing crystal *via* desorption by the impurity increases the chances of it becoming irrevocably bound within the final crystal lattice. A slow desorption will result in another layer of growth units becoming deposited which traps the impurity. Kubota *et al*. hypothesised that this process of adsorption and desorption of growth units and impurities within supersaturated solutions is not instantaneous and final which has implications for the inclusion of impurities.[23] Kubota suggested that high levels of supersaturation result in the adsorption of growth units preferentially over impurities, this trend becomes reversed at low levels of supersaturation.[24] These ideas were built on data obtained from crystal growth studies of paraffin, sucrose and potassium di-hydrogen phosphate in the presence of impurities. In all three cases studied, the growth of the crystals was slower compared to a pure solution.[25-27] Furthermore, this hypothesis has also been reported for the effects of additives on lactose crystallisation from supersaturated solutions.[28]

The application of these theories to the co-crystallisation of sugars and the appearance of a secondary phase when co-crystallising lactose with maltose at a level of 20% w/w allows for the forwarding of a possible explanation.

Firstly, the supersaturation level within the lactose solution has to be considered; using the methodology outlined in Section 8.3.1., at 110°C the lactose solution has a *S* value of ~ 0.598. Upon reaching the *S* value obtained by heating the solution to 110°C, the impurity (maltose) is added Using the model

proposed by Kubota[24], at this level of supersaturation the adsorption of the maltose may be extremely rapid and as such, the maltose may become included within the lactose matrix. As the solution cools, the supersaturation ratio will increase, (from ~ 0.598 to 1.10) and as such the inclusion of lactose growth units will be preferred over the inclusion of impurities. Application of this to the data (DSC and PXRD data) garnered from co-crystallising lactose with maltose allows the supposition that in the levels of 7-17% w/w of maltose, the time between the addition of the disaccharide and the ceasing of crystal growth is sufficient to suggest a possibly complete inclusion of the added maltose. Elevation of the level of added maltose to 20% w/w results in a quantity of maltose that is too large to completely incorporate into the lactose structure. It is possible that a proportion is adsorbed and trapped but a portion will remain un-incorporated and will crystallise. This postulation fits well with evidence obtained from DSC (Fig 3) and PXRD (Fig 4) analyses. Furthermore, it is possible that upon addition to the supersaturated lactose solution, not all of the maltose is taken up into solution. As such, the un-dissolved maltose triggers crystallisation (due to the elevation in local concentration being sufficient to promote nucleation) and serves as a template for nuclei present in solution to build upon.[29] As crystallisation has been prompted (rather than nucleation occurring via nuclei collision forming a stable nucleus for further crystal growth) the time between low *S* values and high *S* values will be lower compared to the supersaturated solutions containing quantities of maltose below 20% w/w. Thus, this makes the possibility of complete inclusion of maltose more unlikely.

5.3.3.1. Co-crystallisation of Lactose with Maltose and Saccharin

The investigation of producing co-crystalline materials that would be suitable for diabetic consumption led to the notion of adding a sweetener, in this case, saccharin, to lactose along with maltose.

Co-crystallising supersaturated lactose solutions with varying amounts of maltose (5-25% w/w) and a constant quantity of saccharin (2% w/w) yielded solely lactose phases up to a level of 18% w/w of added maltose. Above this level, a second phase was observable that can be attributed to the presence of maltose.

Evidence for the possible co-crystalline nature of materials containing 5-18% w/w of maltose and 2% w/w of saccharin can be seen from the data obtained from DSC (see section 5.2.3.2., fig 8) and PXRD (see section 5.2.3.2, fig 9) analyses. The enthalpy values for the endotherm at ~ 200°C drop dramatically when compared to the same peak observed within physical blends of the materials. This decrease, accompanied by a broadening of the endotherm as well as a decreasing onset temperature, is suggestive of a doping of the lactose phase by the added maltose and saccharin.[14-16] This trend appeared to be linked to the quantity of the included sugars. Generally, higher quantities of added sugars gave rise to lower values for ΔH and onset temperatures. From this, it can be hypothesised that these lower values suggest higher levels of inclusion for the added maltose and saccharin. Further evidence for the suggested inclusion can be drawn from comparisons with physical blends of lactose, maltose and saccharin. The co-crystalline material exhibits dramatically lower values of ΔH for the melting of lactose monohydrate.

Elevation of the level of added maltose to 20 and 25% w/w (with 2% w/w of saccharin) yielded material that displayed two phases. At a level of 20% w/w of maltose the DSC scans (see section 5.2.3.2, fig 11) showed characteristic melting of maltose at ~ 120°C. Even though the material obtained from the co-crystallisation of lactose with maltose (20-25% w/w) and saccharin (2% w/w) is not indicative of the presence of solely lactose, the values of ΔH for the α-lactose endotherm are still depressed when compared to physical blends of the materials in analogous ratios. This suggests that a proportion of the added maltose and saccharin may become included within the lactose structure. With the breaching of the limit of inclusion within this system, which is evident from the observable melting of maltose, no melting of saccharin is observable. This may be attributable to the similarity in the melting point of saccharin and the alpha anomer of lactose. Any saccharin melting may be lost within the melting of the α-lactose.

A curiosity arises when adding saccharin along with maltose. The comparison of the potentially co-crystalline material containing only 20% w/w maltose with the material containing an identical quantity of maltose along with 2% w/w of saccharin reveals that the latter material exhibits a much lower enthalpy value (0.067 Jg^{-1} *versus* 11.933 Jg^{-1}) for maltose melting. This

observation suggests that when 20% w/w of maltose is added with saccharin, a lower quantity of maltose is present as a separate phase. It appears from these data that the addition of saccharin along with maltose may result in a higher quantity of included maltose.

These higher levels of possible inclusion may be attributable to the interaction of the added components when they adsorb onto the growing crystal structure. The added components may adsorb more tenaciously (*via* H-bonding) to the growing crystal compared to when maltose is added alone. Due to this possible increase in adsorption strength, the added maltose and saccharin have a higher chance of becoming bound within the final lactose crystal structure. As such, this gives a possible explanation for why a higher proportion of maltose becomes included (though not fully included) when added with saccharin. Therefore, it appears that saccharin possibly facilitates higher limits of inclusion for maltose and such behaviour may be attributed to an aspect of the saccharin structure shown in Fig 71.

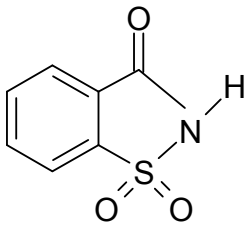


Fig 71. The Structure of Saccharin

The structure possesses carboxamide and sulphonamide functional groups, both of which can be involved in recognition and H-bonding within pharmaceutical co-crystals.[30] As such, it has the ability to adsorb and consequently, saccharin may form a more favourable adsorption site on the growing crystal for further adsorption of maltose thus raising the limit of inclusion.

Evidence for the possible inclusion of the two components can again be garnered from diffractograms yielded from PXRD analysis (see section 5.2.3.2., fig 9). As noted when lactose is co-crystallised with maltose, it appears that some β-lactose is formed as evidenced from the reflection at ~ 10°. Fitting of the pattern revealed that the material is predominantly α-lactose. The ratio of the two

anomers was shown to be approximately 60:40 in favour of the alpha anomer. As such, any comparison will be made against the unit cell for re-crystallised lactose. Calculation of the unit cells, using both models for the α- and β-anomer of lactose showed that an identical trend in the fluctuations of the unit cell volumes arise when using both the α- or the β-anomer as the base for comparison. Consequently, utilisation of the α-lactose monohydrate unit cell dimensions found for re-crystallised lactose will be used as the basis for which all comparisons will be made.

Calculation of the unit cell dimensions shows that the higher levels of added sugars (to re-crystallised lactose) yield higher unit cell volumes (Fig 11) for the levels of maltose of 5-18% w/w. However, these elevations were found to be not significant; all of the data points fall outside of a 95% confidence interval. As such, the hypothesis of any added maltose, along with saccharin, becoming included into a lactose lattice cannot be conclusively drawn from this. In an effort to provide evidence toward the hypothesis that adding saccharides to a supersaturated solution results in their potential inclusion within the host lattice, plots (Fig 72a, b, and c) of the *a*, *b* and *c* axis were performed.

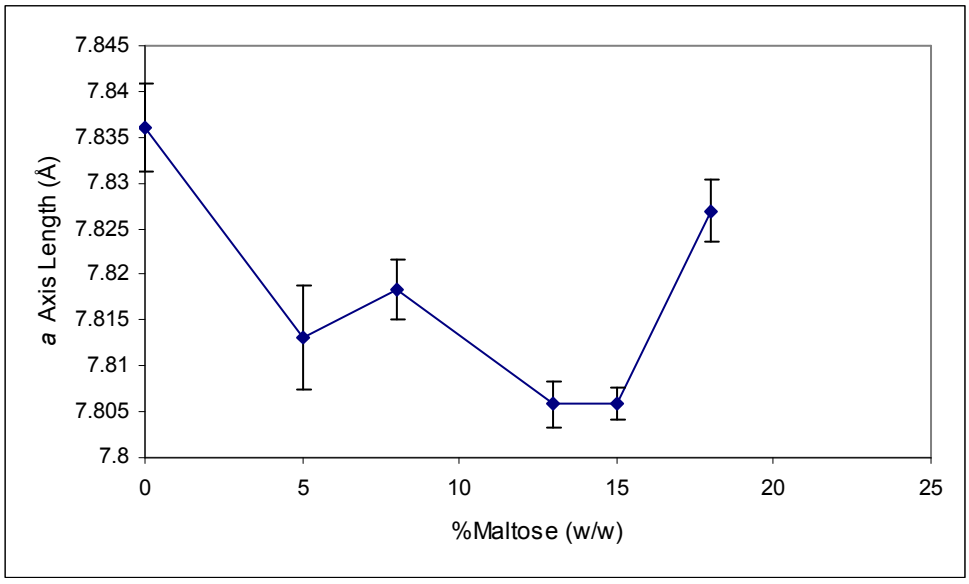


Fig 72a. Plot of *a* axis length *vs*. % Maltose

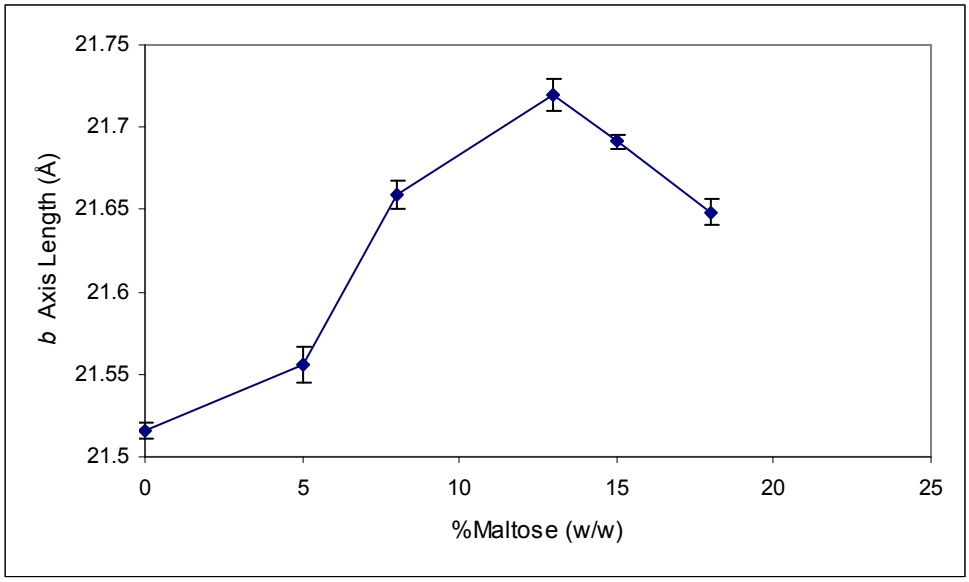


Fig 72b. Plot of *b* axis length *vs*. % Maltose

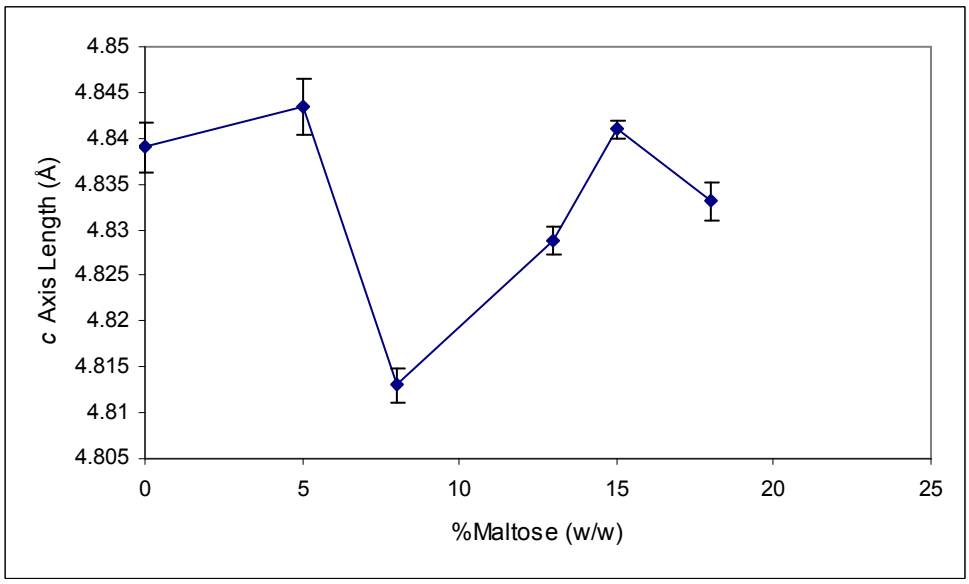


Fig 72c. Plot of *c* axis length *vs*. % Maltose

The above plots show that there is a general decrease in the length of both the *a* and *c* axis. However, there is elongation along the *b* axis, furthermore, these elongations are significant. All of the data points in figure 72b fall within a 95% confidence interval. As such, these elongations appear to be as a function of any added maltose and saccharin. As noted in the materials with solely maltose added, this elongation along the *b* axis is suggestive of the presence of the added saccharides as intra-crystalline species. Whilst this cannot be conclusively proved, due to the lack of significance in the unit cell volume elevations, it is

possible that addition of maltose, along with saccharin, results in them becoming included within the lactose lattice. Such an inclusion possibly induces an increase in the unit cell volume and an elongation of the *b* axis. What is needed to further prove this hypothesis is continued work on reducing the experimental error. Reduction of this factor may yield data that has statistical significance for the unit cell volumes, and thus, provide more substantial evidence for the notion of inclusion.

The data from PXRD shows that the final material formed from adding maltose/saccharin to a supersaturated lactose solution is a mix of both anomers of lactose. Fitting of the pattern shows that this ratio was in favour of the alpha-anomer of lactose. This fits in well with the data obtained from DSC analysis (see section 5.2.3.2, fig 8) as the dehydration endotherm is reduced significantly. As such, if there was a lesser presence of the α-lactose monohydrate form in the final material, it is logical to suggest that this could be signified by a smaller enthalpy for the dehydration as there is not as much present. The formation of a higher proportion of the β-anomer is unusual. The addition of maltose to a supersaturated lactose solution does not yield a material containing such a high proportion of the β-anomer.

It has been previously noted (see Section 5.3.1) that even lactose re-crystallised from water yields a mixture of the two anomers with the majority being in the α-form. It appears that when maltose, along with saccharin, are added in the ratios studied herein, they promote the crystallisation of lactose in a more significant (i.e. more of the β-form present than expected) ratio from solution. This is unusual when considered in the light of lactose behaviour in water and its subsequent crystallisation. Lactose establishes an equilibrium in water with the equilibrium position for the α:β ratio lying predominantly on the side of the β-anomer. Crystallisation of lactose from solution starts with the formation of the solid α-lactose monohydrate and as such, the equilibrium between the two is shifted. Due to this shift, equilibrium needs to be re-established between the two anomers. Therefore, the β-anomer mutarotates to the α-form which supersaturates the solution with respect to the α-form and crystallisation can continue.[31]

However, the formation of predominantly the β-anomer has been shown to be achievable by dissolving α-lactose monohydrate in di-methyl sulfoxide

(DMSO) followed by precipitation by the addition of ethanol.[32] A possible explanation may be that since DMSO is an aprotic solvent and that is has been shown to slow down the rate of mutarotation of the β-anomer to the α-form. Upon crystallisation the β-form will result, thus the slower the rate of mutarotation, the higher the β-anomer content of the final crystal.[32] It appears that in a lactose re-crystallisation solution, the presence of maltose at the levels of 5-18% w/w along with saccharin at the level of 2% w/w may induce this same reduction in the mutarotation rate and thus a final material with a higher proportion of β-lactose content. This influence does not arise from solvent action (as is the case with DMSO) therefore it must relate to the action of the added sugars. Work has been performed on the effect of added sucrose on the rate of mutarotation, from β to α anomeric forms, by Patel *et al*. This work showed that the addition of sucrose slowed the rate of mutarotation and the higher the level, the slower the rate. This reduction in the mutarotation rate was postulated to arise from either the added sugar directly interfering with mutarotation or from an increased solution viscosity reducing the rate.[33] The addition of maltose, along with saccharin may have a similar effect and as such, more β-lactose crystallises out from solution rather than expected.

Upon elevating of the level of added maltose to 20 and 25% w/w (with 2% w/w of saccharin), respectively, in the co-crystallisation process solely the α-lactose monohydrate form was obtained on the basis PXRD analysis. Unlike previous materials, no reflections were discernable for the β-anomer of lactose and the pattern corresponded solely to the α-anomer of lactose. It appears that increasing the level of maltose results in conditions that do not slow down the rate of mutarotation to a sufficient degree to allow the β-anomer to form in higher levels. The PXRD data (see section 5.2.3.2. fig 12) does not conclusively suggest the total inclusion of the added sugars. Calculation of the unit cells for these materials (see section 5.2.3.2. fig 13) showed a slight increase in volume for material containing 25% w/w of maltose. The material containing 20% w/w of maltose exhibited a slightly smaller unit cell volume compared to re-crystallised lactose. The unit cell elevation however, is not significant, as the data point does not fall into a 95% confidence interval. In an effort to see if any added material may have become included, plots of all three axis (Fig 74a, 74b and 74c) against the values for re-crystallised lactose was performed.

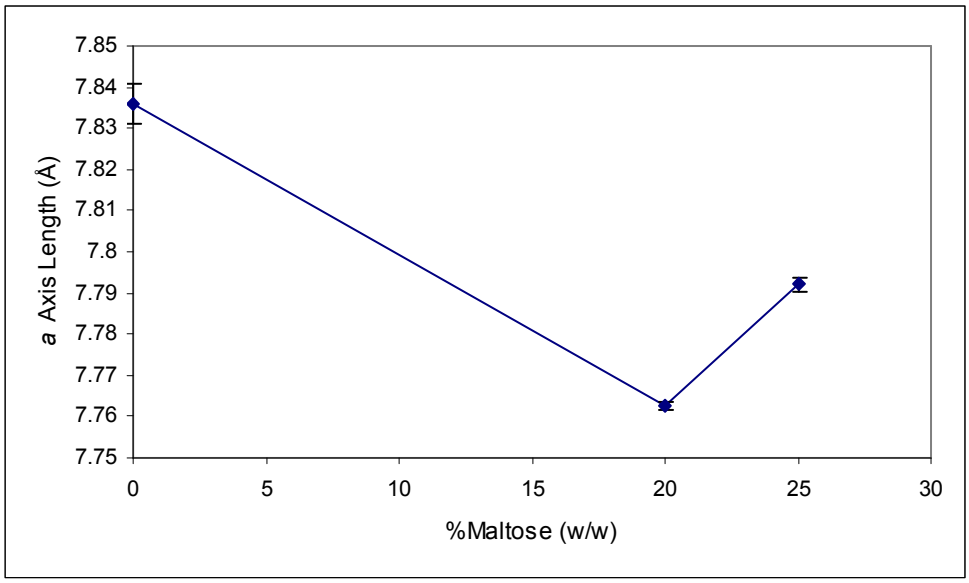


Fig 74a. Plot of *a* axis length vs. % Maltose

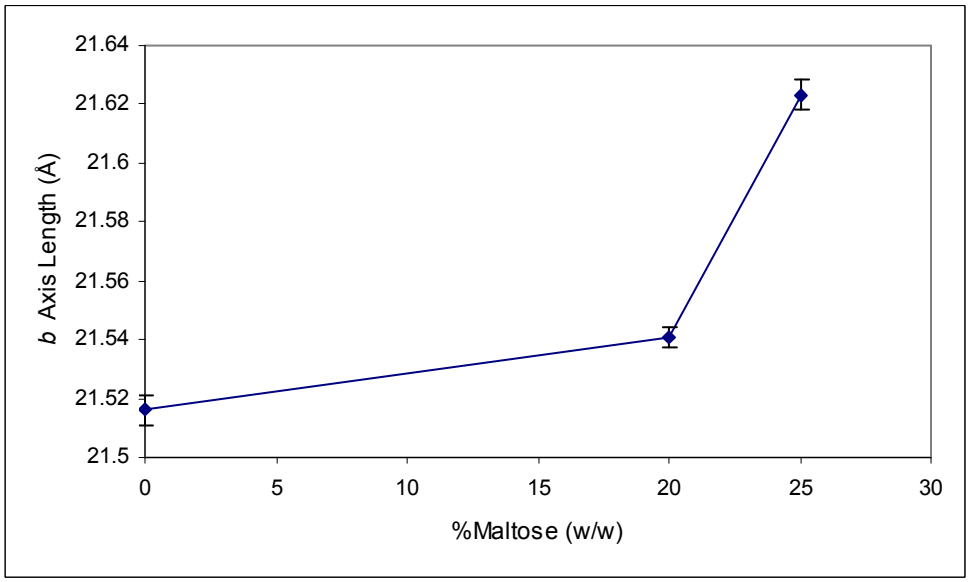


Fig 74b. Plot of *b* axis length vs. % Maltose

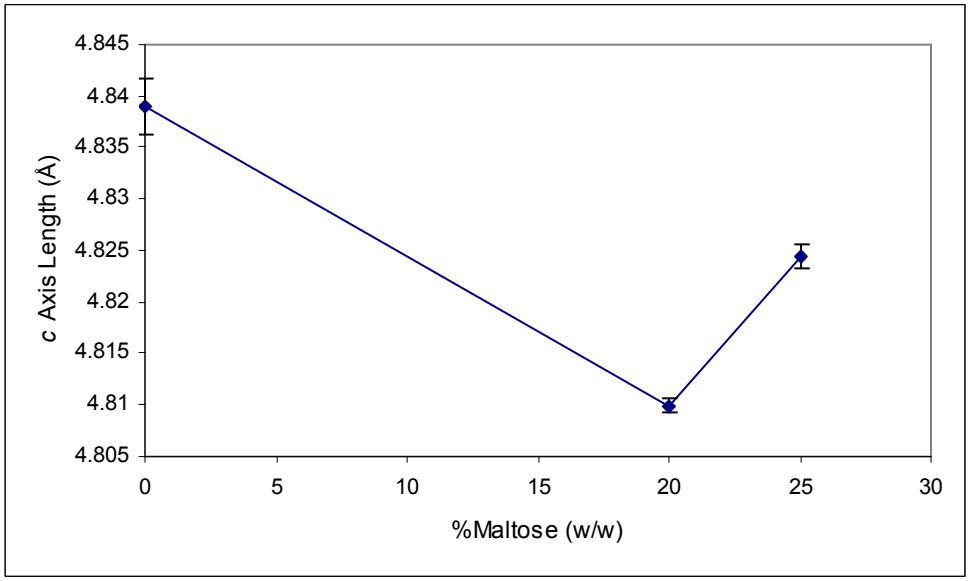


Fig 74c. Plot of *c* axis length vs. % Maltose

The above plots show a similar trend to the behaviour of the axis lengths for materials that exhibit solely lactose behaviour in DSC and PXRD analysis. There is a declination of both the *a* and *c* axis length, and an elongation of the *b* axis. The elongations along the *b* axis can be construed as significant as both data points fall within a 95% confidence interval. As noted for materials where only lactose can be observed in both forms of analysis (DSC and PXRD) this isn't conclusive evidence for the inclusion of maltose and saccharin. However, it is suggestive and further work in reducing the experimental error associated with the unit cell volumes may help provide more robust data. Production of this should help further the argument that any added materials may become included within the lattice of the host material.

Again, the formation of a separate phase is interesting when a certain point of maltose is added. This rational for this may arise from similar reasoning as outlined in Section 5.3.2. The adsorption of maltose (and saccharin) is potentially rapid at the low value of *S* formed at the temperature of addition (~110°C). This rate decreases with increasing values of *S* (lowering temperature, i.e. crystallisation) until the preference is for lactose growth units. For the series of maltose (5-18% w/w) and saccharin (2% w/w) the time between these two points is sufficient to allow possible entrapment of the added sugars into the lactose structure. Above these levels, the quantity is too great for complete inclusion of the added materials in a similar manner to when maltose is added to

a lactose supersaturated solution in the level of 20% w/w. A quantity of undissolved maltose and saccharin may induce nucleation (rather than nucleation occurring *via* collisions) by changing the local concentration; thus serving as a template for the subsequent crystallisation.[29] From this, it can be inferred that when crystallisation in a supersaturated lactose solution is induced by undissolved sugars, the yielded material exhibits solely the α-lactose monohydrate structure. A possible explanation for this can be found from the behaviour of lactose in water and its subsequent crystallisation.[31] The induction of crystallisation (when 20% w/w of maltose and 2% w/w of saccharin are added) results in the solution forming solid α-lactose monohydrate suggesting that the β-anomer mutarotates to satisfy the equilibrium between the two anomers. Consequently, as crystallisation proceeds, α-lactose monohydrate will continue to be formed and the β-anomer will mutarotate to give the α-anomer of lactose rather than the β-anomer. Another point that is worth noting is that when the maltose and saccharin act as a possible template for nucleation, the structure detected is the α-anomer. This suggests that both of the sugars added together, appear to mimic the structure of α-lactose. As such, this provides further evidence for why this combination of sugars includes well into a supersaturated lactose solution and allows for a greater quantity of maltose to be incorporated compared to adding maltose alone.

Although solely co-crystalline materials were not formed (with 20% w/w of maltose and 2% w/w of saccharin) it must be noted that the added saccharin may facilitate a greater quantity of maltose to be included compared to when no saccharin is present. This may be due to maltose, when used in conjunction with saccharin, adsorbs more tenaciously compared to when maltose is added on its own and thus has a higher chance of becoming bound within the lactose structure. As the maltose adsorbs more strongly, the chances of it desorbing are reduced, hence a higher chance of it becoming trapped in the lactose structure.

5.3.3.2. Elevated Levels of Saccharin

Due to the possible effects of higher levels of addable maltose when used in conjunction with saccharin, the effects of increasing the quantity of added saccharin were studied. Co-crystallisation of lactose with maltose (25% w/w) and saccharin (4-10% w/w) did not result in the formation of solely lactose material.

PXRD analysis (see section 5.2.3.3., fig 15) revealed the presence of three phases within the material; however. DSC analysis (see section 5.2.3.3., fig 14) only showed a maltose endotherm at ~ 117°C for the materials containing 4% w/w of saccharin. No endotherm could be detected for the material containing 10% w/w of saccharin.

DSC data (see section 5.2.3.3., fig 14) showed the two endotherms corresponding to α-lactose monohydrate with the dehydration endotherm emerging at ~ 145°C and the α-form was observed at ~ 200°C. Analysis of the ΔH values for the lactose endotherms shows that incorporation of maltose. The presence of saccharin results in a depression of ΔH, a lowering of the onset temperature and a broadening of the endotherms, which is indicative of the incorporation of an impurity into a phase.[14-16] The material containing 4% w/w of saccharin also exhibited an endotherm at ~ 117°C that corresponds to maltose. Interestingly, the ΔH for this peak is lower (by 2.266 Jg^{-1}) compared to material containing 25% w/w of maltose and 2% w/w of saccharin. This further suggests that saccharin potentially facilitates the incorporation of maltose into lactose. Comparison with the material containing 10% w/w of saccharin appears to confirm this. DSC analysis (Fig 14) of this material does not display an endotherm, thus indicating the presence of maltose. It appears that the elevated levels of saccharin may potentially facilitate the incorporation of maltose into a lactose crystal structure. For both sets of materials, DSC analysis (Fig 14) did not show the presence of a saccharin phase. This could be due to the similarity in the melting points of the α-form of lactose and saccharin. The saccharin peak may be lost in the α- lactose melting.

PXRD data (Fig 15) did not fully agree with DSC data (Fig 14) for materials containing 4-10% w/w of saccharin. A separate maltose phase is observable at the 2θ positions of ~ 14.5° and ~21.9° and a peak emerging at the 2θ position of ~ 16.7° indicates the presence of a saccharin phase for both sets of materials. This suggests that maltose and saccharin are present as separate phases within the material. It may be that DSC analysis is not sensitive enough to detect this low a level of a separate phase. Interestingly, the co-crystalline material formed displays solely the α-lactose monohydrate structure, rather than a material containing a higher than expected proportion of β-lactose. Again, the quantity of added sugars employed in these co-crystallisations does not appear to

slow down the rate of mutarotation sufficiently to allow formation of the β-anomer of lactose.

The unit cell volumes for these materials did not show any elevation, they appeared to be slightly larger (10% w/w of saccharin) or slightly lower (5% w/w of saccharin). There is no evidence from the unit cell volume calculation to indicate that the added sugars have become included in the lactose lattice. There are no significant elongations along any axis, nor any significant increase in the unit cell volume. It is possible, from the DSC data, to suggest that the added sugars in this ratio may become included. Unfortunately, PXRD data cannot support this.

Whilst the above data suggest a possible crystalline inclusion within the lactose structure, the possibility of the added materials being amorphous was considered and as such, the materials were subjected to autosorb analysis.

The above material behaved in a manner indicative of a material displaying a crystalline morphology. With each elevation in the level of humidity, a small increase in mass is observed. This is expected as the sample will adsorb an amount of water due to hydrogen bonding at the surface of the sugar. The percentage weight increase in mass rises sharply upon the initial increase in humidity. This weight increase drops shortly after the initial increase in humidity and tapers out to a steady value of percentage weight gain.

The above observation can be explained by consideration of the water activity (a_w) of the sugars and how this relates to the environment to which the samples are subjected.

Under static conditions (e.g. 25°C and 25% humidity) a static equilibrium exists between a_w of the sugar and the environment, a_w is a function of the relationship (See equation 5.1) between the partial pressure of the water vapour at the surface of the product *(p)* and the partial pressure of the water vapour in the environment *(p_s)*. Under static conditions, a_w is a measure of water that can be exchanged between the product and the environment. As a_w is defined under static conditions, the values of p and p_s are equal. Any exchange of moisture between the product and the environment is driven by a difference in value of the two pressures.

$$a_w = p / p_s \qquad (5.1)$$

A hygroscopic product, by definition, undergoes changes in moisture content by alteration of one of two variables:

i) a change in the partial pressure of water vapour in the product environment;

ii) alteration of the temperature of the product.

We can safely surmise that any changes in the moisture content (therefore, any changes in mass of the sample) are related to a change in p and not as a function of temperature as this remains constant throughout the analysis.

Therefore, the sharp increase in weight upon elevation of the humidity is due to uptake of moisture (via adsorption at the surface of the sugar) by the sample, this would yield an increase in p of the sample resulting in a difference between p and p_s. As the conditions (humidity, temperature) are retained at constant levels for a period of time, the value of p will logically try and become in equilibrium with the environmental (p_s) conditions. As the value of p_s will remain constant, the equilibrium is achieved by a change in p. For a change in p to occur, water vapour must be lost from the sample to the environment and, as such, a decrease in mass would (and is) observed. This re-establishment of the equilibrium between p and p_s is reflected by a steady decline in the values of percentage weight gain for the samples. It is logical to suggest that attainment of equilibrium conditions will result in no exchange of moisture between the sample and the environment, hence no change in the value of the percentage weight gain would be observed.

Another factor to consider when explaining the 'spiking' of the percentage weight gain values upon increasing the humidity levels is the physical nature of the sample. An increase in the humidity may result in a proportion of the sample becoming 'syrup' like. If this occurs, it is likely that a proportion of water that is bound within the crystal lattice (water of crystallisation) will become free. A further increase in the value of p (coupled with the effect of

moisture adsorbed from the environment on p) will occur; hence a change will occur in an effort to re-establish the equilibrium.

The data (see section 5.2.3.3., fig 17) from this analysis suggested that any possible inclusions within the material are in the crystalline state and not in the amorphous state. Material formed from the co-crystallisation methodology can be though of as crystalline in nature. Elevation of RH results (Fig 17) in an increase in the percentage weight gain of the sample, as the samples are held at each level of RH. The percentage weight gain decreases (Fig 17) steadily as the equilibrium between p and p_o is re-established, which is indicative of a crystalline material. To confirm this, the co-crystalline material was compared with an analogous physical blend. The physical blend behaved in an identical manner to material yielded from the co-crystallisation process. Elevation of the RH level is accompanied by an increase in the value of percentage weight gain data (Fig 17) which decreases as the samples are held at each level of RH. The re-attainment of the equilibrium p and p_o is there being achieved.

There was a difference between the two sets of materials and how much moisture was adsorbed. Materials formed in the co-crystallisation process adsorbed moisture more readily than the physical blends. This implies a physical difference between the materials. A possible explanation is that the physical blends contain more soluble components. More soluble components would result in facile formation of syrup type components, resulting in elevation of the p value, therefore reducing water adsorption from the environment. The material formed from the co-crystallisation process could be the opposite of the above, which is why adsorption occurs more readily.

Like other materials (e.g. see section 5.3.2), the formation of a separate maltose phase may be a result of insufficient time between low values of S (temperature at which supersaturated solution is formed) and higher values of S (dropping temperature and crystallisation). However, it appears that the potential incorporation of maltose and saccharin simultaneously facilitates the incorporation of higher amounts of maltose within a lactose lattice. No single phases were yielded with higher levels of added saccharin; DSC data (Table 6) suggests that more maltose is potentially incorporated when compared to lower levels of added saccharin. This possibility of saccharin to facilitating the inclusion of maltose may again be as a result of an increased tenacity in the

adsorption of maltose. Thus reducing the chance of desorption and increasing the likelihood of it becoming a part of the final lactose structure. More importantly, the higher levels of included maltose are not observed as a function of the amorphous state. Autosorb analysis suggests this notion as subjection of co-crystalline lactose, maltose and saccharin material (see Section 5.3.3.1) to this analysis strongly suggested that the material has no amorphous content. It can therefore be implied that the co-crystalline lactose and maltose materials with higher levels of saccharin have no amorphous content either.

5.3.3.3. Staggered Addition of Maltose and Saccharin to Lactose

The staggered addition of maltose (18% w/w) and saccharin (2% w/w) to a supersaturated lactose solution does not result in the formation of solely lactose related material (see Section 5.3.3.1). DSC analysis (see section 5.2.3.4., fig 18) showed an endotherm corresponding to maltose emerged at 117°C and PXRD diffractograms (see section 5.2.3.4., fig 19) showed characteristic peaks at the 2θ position ~ 14.5° and ~ 21.9° indicating the presence of maltose.

DSC traces (Fig 18) for the materials showed two endotherms, the dehydration endotherm emerges at ~ 145°C and the α-form of lactose emerges at 200°C. The ΔH values (see section 5.2.3.4, table 7) for the α-lactose endotherm decrease compared to the physical blends of the materials. This is accompanied by depression of the onset temperature and a broadening of the endotherms, which is indicative of inclusion of an impurity into the lactose phase.[14-16] The staggered addition appears to influence the quantity of maltose incorporated within the lactose structure; the addition of maltose first results in a lower level of inclusion compared to when saccharin is added first. This is evident from comparison of ΔH values for the endotherm at ~ 117°C, that when maltose is added first (followed by saccharin) the enthalpy value for maltose melting is higher by 3.604 Jg^{-1} suggesting a higher quantity of un-incorporated maltose.

Determination of the unit cell parameters showed that whilst the materials do not exhibit single phases (i.e. melting of lactose), a degree of inclusion may have occurred. Both sets of materials exhibited larger unit cell volumes (see section 5.2.3.4., fig 20) compared to solely re-crystallised lactose. The unit cell volumes showed that a higher level of inclusion is matched by a larger unit cell volume. However, these elevations are not significant as the data points

associated with them fall outside of a 95% confidence interval. In an effort to provide some further evidence toward the notion of maltose and saccharin becoming integrated within the lactose lattice, the axis of the unit cells were also analysed. With saccharin added first, the *b* axis became elongated (with respect to re-crystallised lactose) to 21.6619 Å ± 0.0072. Addition of maltose first resulted in elongation to 21.5601 Å ± 0.0032, both of these increases are significant as they fall into a 95% confidence interval. As such, this does suggest that a possible inclusion may have occurred. Further work on reducing experimental error is necessary in order to produce data robust enough to support the elevation of the unit cell volume.

Interestingly, when identical proportions of the two sugars were added simultaneously, an elevated proportion of the lactose β-anomer (with total inclusion of maltose and saccharin) crystallised from solution. Staggered addition gives a material that potentially includes a proportion of the added sugars (with a proportion present as a separate phase, see section 5.2.3.4, figs 18 and 19) and yields a final lactose material that displays solely the α-lactose monohydrate structure when analysed by PXRD (Fig 19). A possible reason for this observation may be similar to those outlined in Section 5.3.2. The supersaturated lactose solution has had the added sugars introduced in a manner that does not yield solely co-crystalline material; as such this suggests that nucleation has been induced and consequently (in a similar manner as outlined in Section 5.3.2.) the α-monohydrate anomer of lactose crystallises from solution.

As both methods of adding the sugars result in the formation of a possible co-crystalline phase and a separate maltose phase, the limit of inclusion has been breached. As the breaching of this limit may be attributed to a proportion of the added sugar being not being taken up into solution, the differences in the level of inclusion when staggering the addition of maltose and saccharin may stem from how much the local concentration is changed by which sugar is added first. As maltose is added in the level of 18% w/w (followed by 2% w/w of saccharin) the initial addition appears to be sufficient to promote crystallisation. The degree to how much the local concentration is affected appears to be greater than when saccharin is added first. The implications of this mean that the time between low *S* levels (temperature at which supersaturated solution is formed) and higher values of *S* (dropping temperature and crystallisation) may be lower when

maltose is added first than when saccharin is added first. As such, the differences in the observed levels of inclusion may arise from this. A higher change in local concentration will give a lower period of time between low *S* and high *S* and a lower level of inclusion.

5.3.4.1. Co-Crystalline Lactose and Galactose Materials

The synthesis of possible co-crystalline material *via* the incorporation of galactose into a supersaturated lactose solution showed that no co-crystalline material was formed. The results obtained from the DSC analysis show (see section 5.2.5.2., fig 28) that lactose with 4% w/w of galactose added shows solely lactose melting. Above this level, two phases are observable in DSC analysis; a phase attributable to the melting of lactose and other related to galactose. From DSC analysis, it appears that the addition of 4% w/w of galactose yields a potentially co-crystalline lactose-galactose material. However, this is not true as PXRD analysis (see section 5.2.5.2, fig 29) clearly shows reflections representative of galactose.

It appears that co-crystallising lactose with galactose (in the range of 4-15% w/w) does not yield any single phase materials. Whilst this is unexpected, analysis of the data obtained from DSC and PXRD analysis may reveal some insights into the final material.

The notion that a proportion of galactose incorporates into the lactose crystal structure can be suggested by consideration of the enthalpy values for the endotherm corresponding to lactose monohydrate. As already observed (see Sections 5.2.2.2 and 5.2.3 for examples) for other possible co-crystalline materials, the inclusion of an added impurity is noticeable by a depression of ΔH for the lactose monohydrate endotherm accompanied by said endotherm broadening and exhibiting a lower onset temperature (see section 5.2.5.2 table 12). These changes are characteristic of a doping of a phase and as such, evidence is put forward for the idea of a portion of the added galactose becoming possibly included within the final lactose crystal structure.[14-16]

At the level of 7% w/w of added galactose and above, an endotherm (see Fig 28 and Table 12) relating to galactose appears. This increases in ΔH with increasing levels of added galactose. As such, this suggests that higher amounts of galactose are not being included into the lactose lattice. However, a proportion

of the added galactose could always be possibly included within the lactose structure. Comparison of the ΔH values for the galactose endotherm in physical blends and materials formed from the co-crystallisation method (Fig 75) show that the ΔH values in these materials are lower compared to the physical blends. This provides further evidence for the hypothesis of a proportion of galactose becoming potentially incorporated into the lactose lattice.

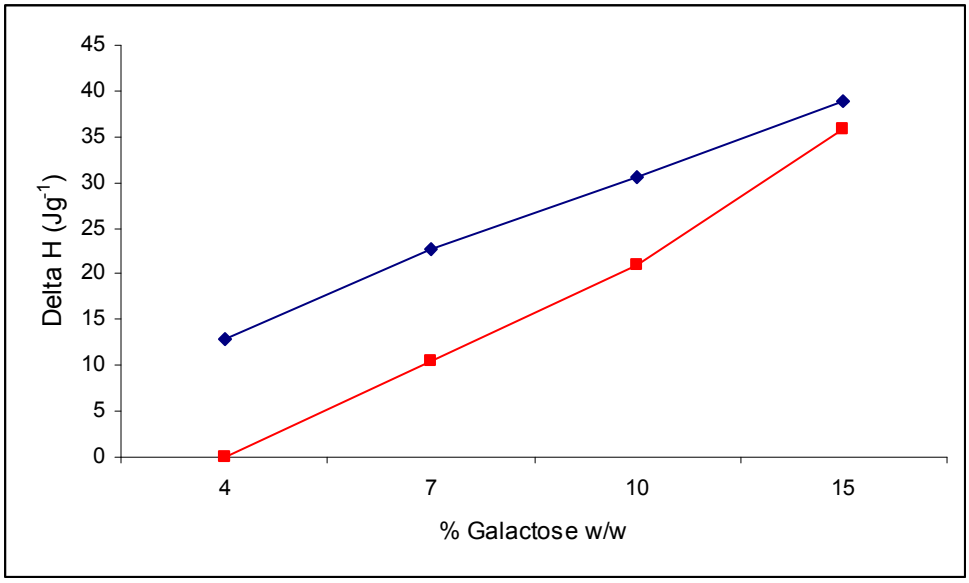


Fig 75. ΔH for the Galactose Endotherm for Physical Blend (blue) & Co-Crystalline Material (red)

PXRD diffractograms (Fig 29) did not agree with DSC data (Fig 28 and Table 12) as already stated. All materials yielded from co-crystallising lactose with galactose showed two phases. The majority of reflections can be attributed to α-lactose monohydrate, however reflections at the positions of 18.6° and 21.6° 2θ is highly suggestive toward the presence of galactose. This can be conclusively proved by comparison of physical blends and materials formed in the co-crystallisation process (both containing 7% w/w of galactose). Juxtaposition of the two patterns shows a clear match between the expected peaks for lactose and the characteristic ones for galactose. The diffractograms (see section 5.2.5.2, fig 29) allowed the determination of the unit cells. There was no clear trend in the unit cell volumes, there was a slight increase (which does point toward a potential inclusion) compared to re-crystallised lactose. However, this cannot be seen as a significant rise in unit cell volume as all of the data

points fall outside of a 95% confidence interval. Though the notion of any added monosaccharides becoming intra-crystalline species cannot be conclusively drawn from the unit cell data, analysis of the unit cell axis may prove further evidence toward this idea.

As already stated, it has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from the addition of galactose to a supersaturated lactose solution may provide suggestive data toward any proportion of galactose becoming included. A plot of the *a*, *b* and *c* axis (Fig 76a, 76b and 76c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

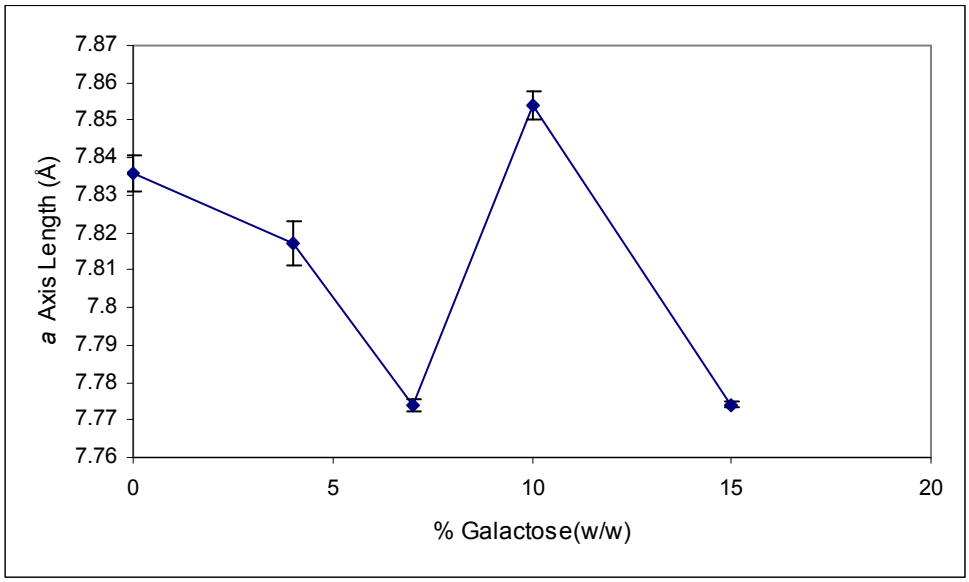


Fig 76a. *a* Axis Length *vs*. % Galactose for Co-Crystalline Lactose and Galactose Materials

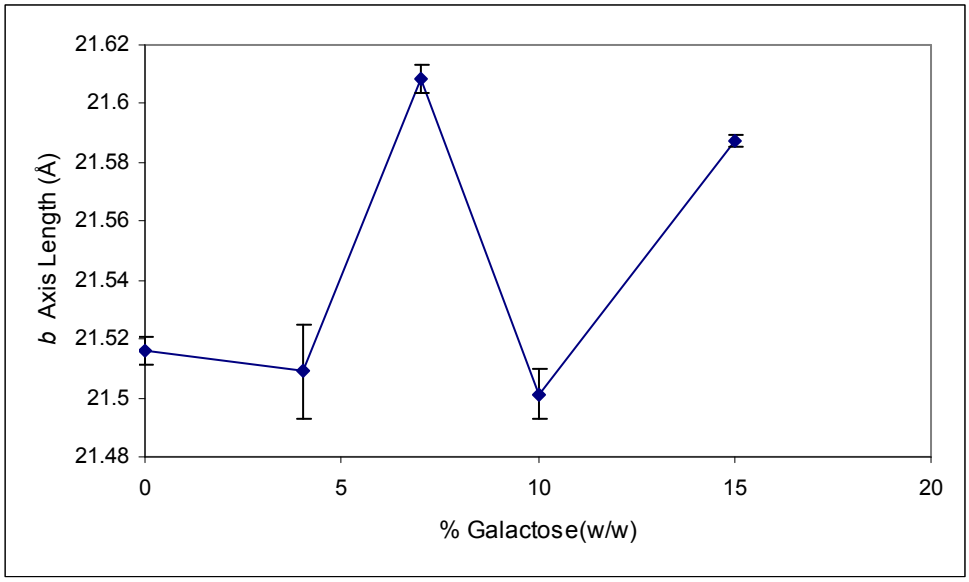


Fig 76b. *b* Axis Length *vs*. % Galactose for Co-Crystalline Lactose and Galactose Materials

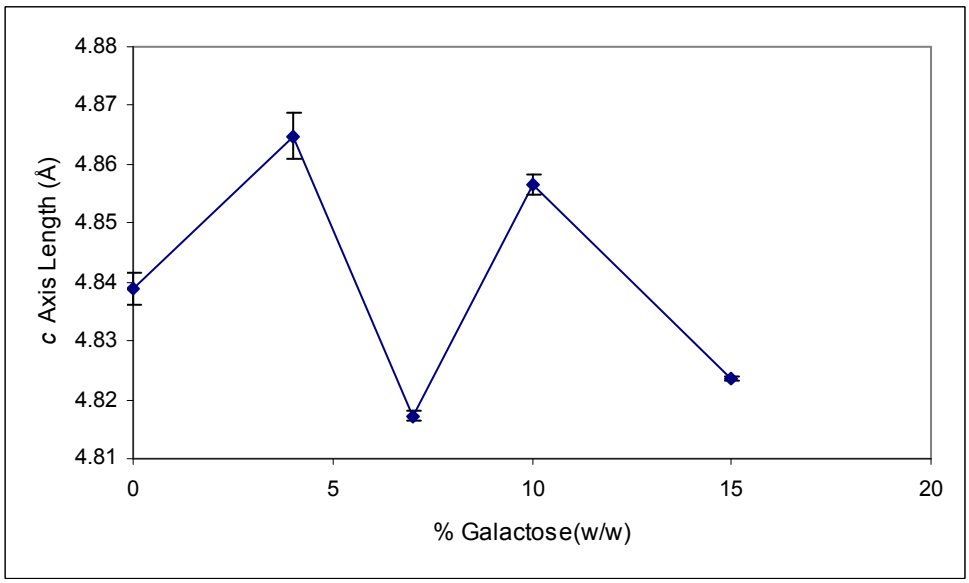


Fig 76c. *c* Axis Length *vs*. % Galactose for Co-Crystalline Lactose and Galactose Materials

As can be seen from the above plots, there is no real trend in characteristics of the axis lengths. There is some potential evidence toward the idea of a proportion of galactose becoming included. Some data points show elongation compared to re-crystallised lactose that is significant in nature. But, the lack of a trend within the actual data series points toward experimental error. In order to draw a valid idea on the nature of materials formed from this process, a repeated formation of lactose-galactose materials would be necessary.

When crystallising any sugar, there is the possibility of forming the sugar in its amorphous state. In order to ascertain if material formed from the co-crystallisation process are crystalline in nature, they were subjected to autosorb analysis.

Materials formed from co-crystallising lactose and galactose (4-15% w/w) materials were subjected to autosorb analysis to ascertain the physical nature of the two sugars. The data collected (Fig 58) revealed that materials yielded from co-crystallisation of lactose and galactose have no amorphous content and are predominantly crystalline.

The above materials all behaved in a manner indicative of materials displaying a crystalline morphology. Such is supported by analytical data and the evidence provided in Section 5.3.3.2. With each elevation in the level of humidity, a small increase in mass is observed; this is expected as the sample will adsorb an amount of water *via* hydrogen bonding at the surface of the carbohydrate. The percentage weight increase in mass rises sharply upon the initial increase in humidity (see section 5.2.5.3., fig 31). This weight increase drops shortly after the initial increase in humidity and tapers out to a steady value of percentage weight gain.

There appears to be no relationship between the amount of included galactose and the amount of moisture adsorbed by the co-crystalline material. All values for percentage weight gain are of a similar magnitude upon each increase in humidity. Further confirmation of the crystalline nature of co-crystalline material was achieved by comparison with physical blends of crystalline lactose and either crystalline or amorphous galactose.

Material containing crystalline galactose behaved in an analogous manner to the co-crystalline material. Thus, elevation of the RH level resulted in an elevation of the *p* value for the material. This value decreased slowly (reflected by decreasing values for the percentage weight gain) as the samples were held at each level of RH.

Whilst behaving in an identical manner to co-crystallised material with regard to adsorption behaviour, physical blends do not adsorb as much moisture upon each elevation of RH. This implies a physical difference between the materials; a possible explanation is that the physical blends contain more soluble components. More soluble components would result in facile formation of syrup

type components; resulting in elevation of the *p* value, therefore reducing water adsorption from the environment. The co-crystallised material could be the opposite of the above, hence explaining why adsorption occurs more readily.

The materials containing amorphous galactose behaved in the manner expected of amorphous material when exposed to humidity. All samples adsorbed moisture readily (see section 5.2.5.3. fig 31) from the atmosphere and, unlike crystalline material; this was not accompanied by re-attainment of the equilibrium between *p* and p_o. There was a direct relationship between the amount of amorphous material and the amount of adsorption, the higher the quantity of amorphous galactose, the greater the amount of adsorbed moisture. Elevation of the RH from 25% too 50% results in a percentage weight gain of ~ 0.2% for the material containing 7% w/w of amorphous galactose. For the material containing 15% w/w of amorphous galactose the percentage weight gain is ~ 0.5% for this change in humidity. This trend in the uptake of moisture by the samples continues throughout each step up in the level of humidity.

The combination of DSC, PXRD and autosorb data (Figs 28, 29 and 31 respectively) for co-crystallised lactose and galactose materials suggests that a small portion may become an intra-crystalline species. This cannot be conclusively proven, due to the lack of any coherent data from PXRD analysis. The behaviour noted in the melting of lactose in DSC is suggestive of a doping of a host phase. However, further work is needed to provide sufficient data so that the notion of added galactose becoming an intra-crystalline species can be confidently proposed.

5.3.4.2. Co-Crystalline Lactose and Galactose Materials (Post Autosorb)

The co-crystallised materials subjected to the autosorb run were retained in order to ascertain if any physical changes had occurred within the material over time.

DSC analysis revealed (see section 5.2.5.4., fig 32) that material that exhibited solely lactose melting in DSC analysis had now clearly become a combination of lactose and galactose. Lactose and galactose (4% w/w) initially displayed two endotherms that relate to the presence of solely lactose. The same material, after being subjected to humidity (90% RH, see Fig 31), displayed a

new endotherm at ~ 160°C (see Fig 32) that was indicative to the presence of galactose. Analysis of co-crystallised lactose and galactose (7-15% w/w) revealed (Fig 32, Table 13) no change. All materials still displayed a mixture of the two phases, lactose and galactose. However, DSC data (see section 5.2.5.4., fig 32, Table 13) showed that the enthalpic value for galactose melting increased. Comparison of ΔH values for the galactose endotherm for materials prior and post autosorb (se section 5.2.5.4, fig 33) shows that the enthalpy values have increased in the post autosorb materials. This suggests elevated levels of the galactose phase are present. There is only one combination of materials that bucks this trend which is co-crystallised lactose with 10% galactose w/w. This material displays a decrease in the enthalpy of the galactose endotherm (by 0.834 Jg^{-1}). Such a decrease suggests that subjection to humidity has caused either a potential incorporation of galactose into the lactose lattice or a proportion has been turned amorphous. The first consideration appears to be invalid; a further inclusion of galactose within the lactose structure would be suggested by a reduction in ΔH for the lactose monohydrate endotherm compared to the same endotherm prior to autosorb analysis. This is not observed; rather an increase in ΔH for the lactose endotherm of 4.346 Jg^{-1} is seen for the lactose monohydrate endotherm. This increase in enthalpy suggests an increase in purity of the lactose phase. If the incorporation of an impurity (i.e. galactose) into a phase is accompanied by a decrease in ΔH then an increase in ΔH suggests a migration of the impurity. Therefore, it appears that autosorb analysis has rendered some of the un-incorporated galactose into an amorphous state which is difficult to detect by DSC as the quantity may be to low for the sensitivity of the equipment.

The migration of galactose from within the lactose crystalline structure has been noted previously by Maillet in other co-crystalline systems.[2] It has been shown that co-crystalline materials degrade after a period of storage. Materials initially exhibiting co-crystallinity separate into the original sugar components that were added together.[2] A possible suggestion for this is that the separation of the sugars in the co-crystalline material may result from a function of humidity. The moisture may be the factor causing the co-crystallised material to separate. However, this would logically suggest that the inclusion was in the amorphous state. The re-crystallisation of amorphous sugars by exposure to humidity has been reported within literature and as such, this phenomenon may have occurred

with the samples in question.[33, 34] This statement could be true; however, it is completely contradictory to the data garnered from the autosorb analysis (Fig 31) of the co-crystallised materials. The data strongly suggests a distinct lack of any amorphous content as all the samples adsorbed and desorbed moisture in a manner that is indicative of crystalline material. The separation of galactose from the lactose matrix may occur as a function of what physical state the co-crystalline material is in at the end of the autosorb run. Lactose deliquescence has been shown to occur at ~ 99% RH. This deliquescence of a carbohydrate results in a saturated solution to form as a result of physical changes that occur in the material.[35] As such, the possible migration of the initially included galactose from the lactose structure is an imaginable concept. The co-crystalline lactose and galactose materials were subjected to ~ 100% RH which may induce the observed changes. A loss of co-crystallinity has been noted by Maillet,[2] over a prolonged period of time and the application of significantly more intense conditions of humidity appears to accelerate this process. Migration of the included component may be related to a function of atmospheric water.[2] This process may occur *via* dissolution of a portion of the co-crystalline lactose and galactose materials at high RH levels (~ 99% RH). This would result in the crystal constituents (i.e. lactose molecules and galactose molecules) becoming released into solution. When this portion re-crystallises, the galactose does not become re-incorporated into the lactose crystal structure and forms as a separate phase.

PXRD data (see section 5.2.5.4., fig 33) is in good agreement with the DSC data (Fig 32) for these samples. All materials exhibit two phases, one relating to lactose and another related to galactose.

5.3.4.3. Co-Crystallisation of Lactose and Galactose Starting from a Physical Blend

Alteration of the co-crystallisation methodology *via* starting from a physical blend of the materials rather than adding galactose to a supersaturated lactose solution gave single phases at a higher level compared to the initial method of co-crystallising. DSC analysis (see section 5.2.5.5., fig 36, table 14) showed that when starting from a physical blend, single phased material was formed up to 10% w/w of added galactose, which is higher than the limit

reported in Section 5.3.4.2. Inclusion of galactose is suggested by the reduced value for ΔH calculated for the melting of α-lactose at ~ 210°C.[14-16] Further indication of a possible inclusion of the galactose is obtained from comparison of the enthalpy values for physical blends of the two materials with those calculated from the co-crystallised materials. The value for ΔH (Fig 36, Table 14) in the co-crystallised material is lower than the calculated value in the physical blend of the materials thus suggesting a doping of the lactose structure. Elevation of the level of galactose present in the material to 10% w/w resulted in an endotherm emerging at ~ 160°C that corresponds to galactose. Interestingly, the enthalpy associated with this melting is lower (Fig 77) when compared to the same endotherm in an analogous ratio of the two materials co-crystallised *via* addition of the monosaccharide added to a supersaturated solution at 110°C.

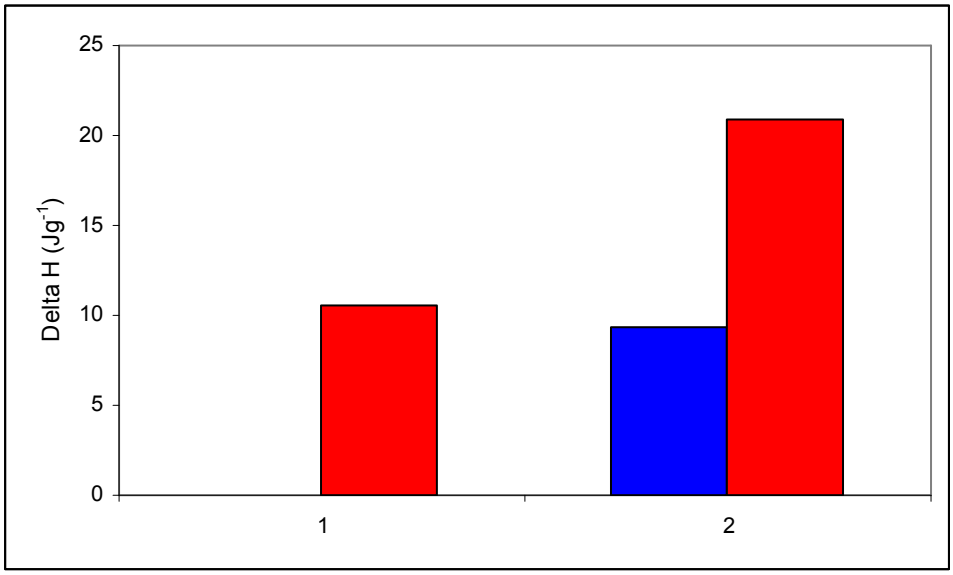


Fig 77. Comparison of ΔH Values for the Melting of Galactose in Co-Crystalline Material Formed From a Physical Blend (Blue) and with Addition of Galactose at 110°C (Red)

This observation shows that altering the methodology may facilitate higher amounts of galactose within the lactose structure even when the limit of inclusion has been breached.

PXRD data (see section 5.2.5.5., fig 37) was in agreement with DSC data (Fig 36) in that material with 7% w/w of galactose present shows no characteristic reflections for this material. Increasing the level to 10% w/w results in characteristic peaks emerging that are indicative of galactose.

Interestingly, there was observable peak splitting within the pattern for both materials. The identity of this phase cannot be elucidated from the data obtained herein, however, these peaks may relate to the presence of a co-crystalline species. They could not be related to any form of lactose, nor galactose. Further work is necessary in order to ascertain if these peaks can be reproduced and if so, what their identity is.

Determination of the unit cell parameters showed that the co-crystallised materials formed from a physical blend have larger unit cell volumes compared to solely lactose. Again, this increase cannot be construed as significant however, due to the data points falling outside of a 95% confidence interval.

The data obtained from PXRD analysis is complex for these materials, in order to understand what has occurred further work is necessary. The appearance of a potential separate phase is very interesting, as such, investigation on what these reflections relate to is essential. It does appear from DSC analysis that galactose potentially becomes integrated within the host lactose lattice. However, due to the strange data found from PXRD analysis, this cannot be conclusively proven or refuted at the present time.

5.3.5. Co-Crystalline Lactose, Maltose and Aspartame Materials

The addition of another sweetener suitable for diabetic consumption, aspartame, along with maltose to a supersaturated lactose solution showed material indicative of lactose forming for up to 18% w/w of added maltose and 2% w/w of aspartame. The single phased nature of DSC suggests possible inclusion of the added components. This can be proposed on the basis of decreasing enthalpy values (see section 5.2.4.2., fig 22, table 10) calculated for the endotherm corresponding to the α-form of lactose that emerges at ~ 200°C. Higher amounts of added maltose were observed to produce lower values (Table 10) of ΔH for the endotherm corresponding to the α-anomer of lactose. This fits well with the notion that decreasing enthalpy values are synonymous with a doping of a phase; in this case, lactose may have become doped with maltose and aspartame.[14-16]

The PXRD data (see section 5.2.4.2., fig 23) supported DSC data (Fig 22) to show that solely lactose materials were formed up to a level of 18% w/w of maltose and 2% w/w aspartame. At these levels, a separate maltose phase

was also detectable. Interestingly, all materials exhibited solely the α-lactose monohydrate structure in PXRD analysis (Fig 23). The diffraction patterns allowed the calculation of the unit cell volumes (Fig 24) and this showed no real trend.

It has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from addition of maltose/aspartame to a supersaturated lactose solution may provide data in a similar vein. A plot of the *a*, *b* and *c* axis (Fig 79a, 79b and 79c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

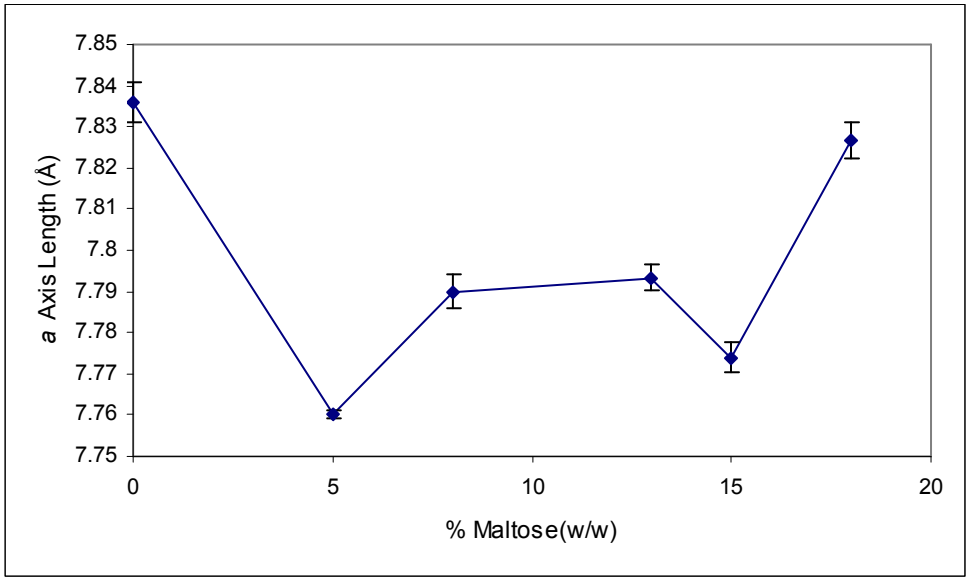


Fig 79a. *a* Axis Length for Lactose, Maltose and Aspartame Materials

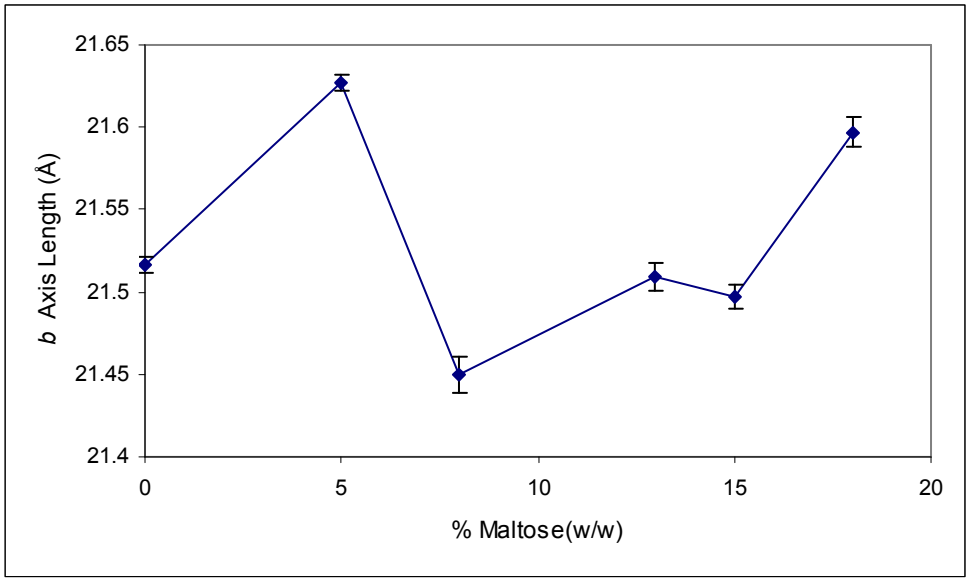


Fig 79b. *b* Axis Length for Lactose, Maltose and Aspartame Materials

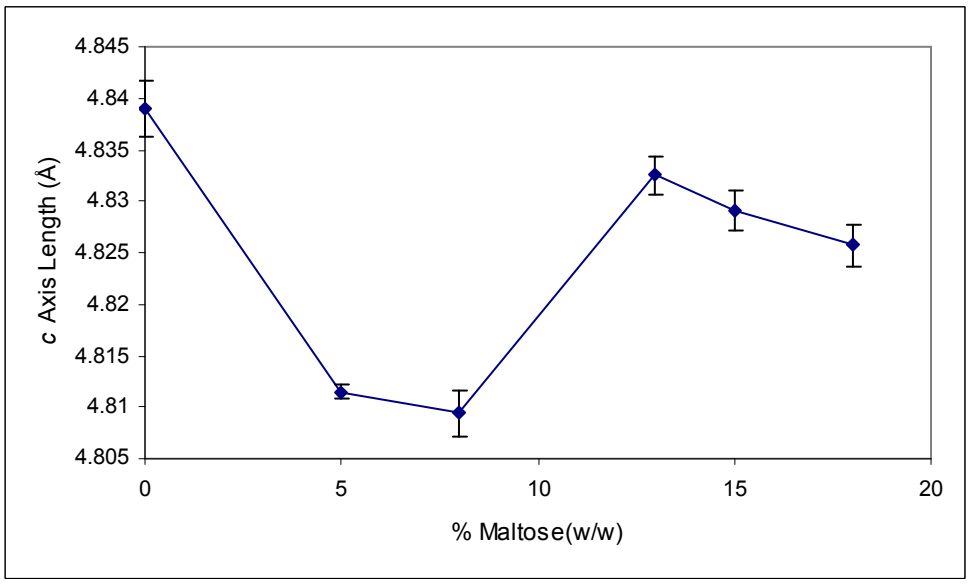


Fig 79c. *c* Axis Length for Lactose, Maltose and Aspartame Materials

As can be seen from the above plots, all of the materials yielded from this series exhibit smaller *a* and *c* axis compared to re-crystallised lactose. It is worth noting that materials containing 5% and 18% w/w of maltose (along with 2% w/w of aspartame) exhibit elongated *b* axis. It is not clear what this actually means from a possible inclusion perspective. The elongation is suggestive of the added sugars becoming intra-crystalline species, however, the lack of any significant unit cell volume inflation hinders this theory. The absence of a trend in the PXRD data points toward inherent experimental error. There is suggestive

data from DSC analysis that points toward the possibility of lactose phase becoming doped. It can be strongly suggested that the added materials become included as DSC analysis also shows solely lactose melting, and PXRD shows diffraction patterns indicative of lactose. However, without robust data from PXRD to support this, the notion of inclusion of added sugars cannot be conclusively proven.

It is interesting to note that when saccharin is added along with maltose, (18% w/w and 2% w/w of saccharin) solely phases attributable to lactose are observed in DSC and PXRD analysis. This drop in the level of addable (whilst retaining single phases) maltose from 18% w/w to 15% w/w when it is included with aspartame must be related to an aspect of aspartame. As already noted in section 5.3.3.1, saccharin may facilitate the potential inclusion of maltose. This was suggested to be attributed to a structural aspect of the molecule, namely the presence of sulphonamide and carboxamide functionalities. From the structure of aspartame (Fig 80) it can be postulated that this difference arises due to structural differences between aspartame and saccharin.

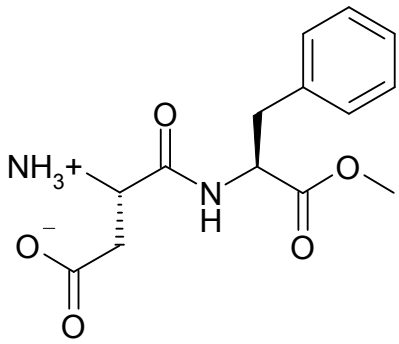


Fig 80. Structure of Aspartame

Aspartame is a larger molecule than saccharin and thus can be conceived to be of no help when facilitating the inclusion of maltose. Aspartame may adsorb onto the growing crystal and due to its structure, interfere with the adsorption of maltose. From this, it can be inferred that there is a reduced chance of maltose becoming irrevocably bound in the final structure and forming as a separate phase. The reduction in the level of included maltose is not related to a function of the supersaturation conditions in the solution. It has already been

shown (see Section 5.2.2.2) that maltose can be present in the level of 17% w/w when added on its own, whilst showing solely phase related to lactose.

5.3.6.1. Co-Crystalline Lactose, Galactose and Glucose

Co-crystallisation of galactose (5-20% w/w) and glucose (5-20% w/w) into a supersaturated lactose solution showed (Section 5.2.5.2) that up to the level of 15% w/w of each monosaccharide stated, solely lactose related materials are yielded. Upon the elevation to 20% w/w of each monosaccharide, an endotherm emerges at ~ 155°C (see section 5.2.6.2., fig 42) that corresponds to galactose. No melting for glucose could be observed. All materials exhibited two peaks (Fig 42), one for the dehydration of lactose monohydrate (~ 145°C) and the other corresponding to the α-form of lactose (~ 200°C). Analysis (Table 16) of the behaviour with regard to the enthalpy values of the α -lactose endotherm indicated that the added galactose and glucose possibly incorporate directly within the lactose lattice. This is signified by decreasing ΔH values accompanied by depression of the onset temperatures and a broadening of said peaks. This behaviour is indicative of an impurity present within a phase. Increasing amounts of added galactose and glucose have a more profound effect on this phenomenon; indicating a higher possible degree of inclusion.[14-16]

PXRD data (see section 5.2.6.2., fig 43) did not support the DSC data (Fig 42) completely. Solely single phases were yielded for the addition of galactose and glucose into a supersaturated lactose solution up to a certain level. Diffractograms (Fig 43) suggested the presence of solely lactose monohydrate materials up to 15% w/w of each monosaccharide. Upon elevation to 20% w/w resulted in characteristic peaks emerging that correspond to galactose and glucose. The diffractograms produced enabled the calculation of the unit cells of the materials. These did show a slight elevation in unit cell volume. However these were not significant increases (see section 5.2.6.2. fig 44) as the data points fall outside of a 95% confidence interval.

It has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from addition of glucose/galactose to a supersaturated lactose solution may provide data in a similar vein. A plot of the *a*, *b* and *c* axis (Fig 81a, 81b and

81c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

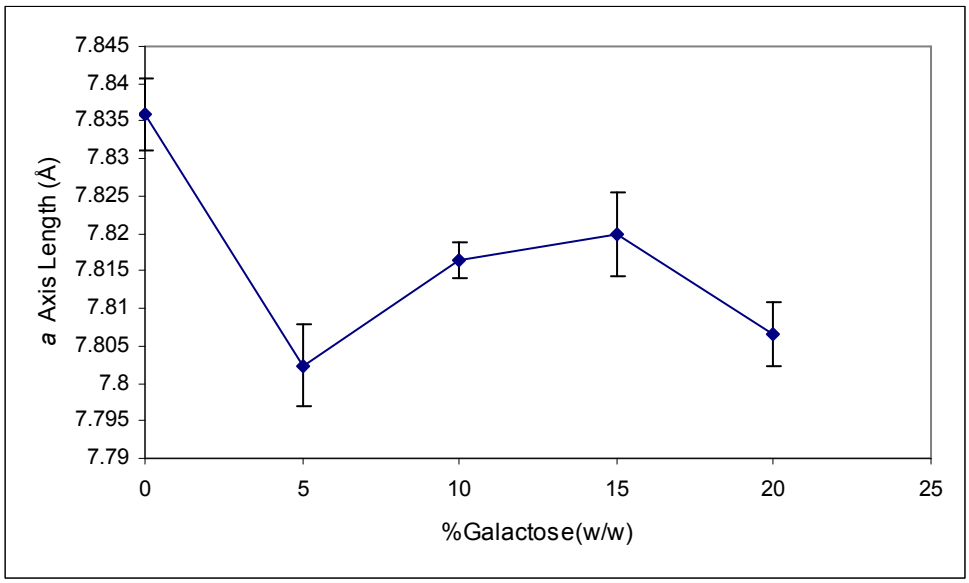


Fig 81a. Plot of *a* axis length vs. % Galactose

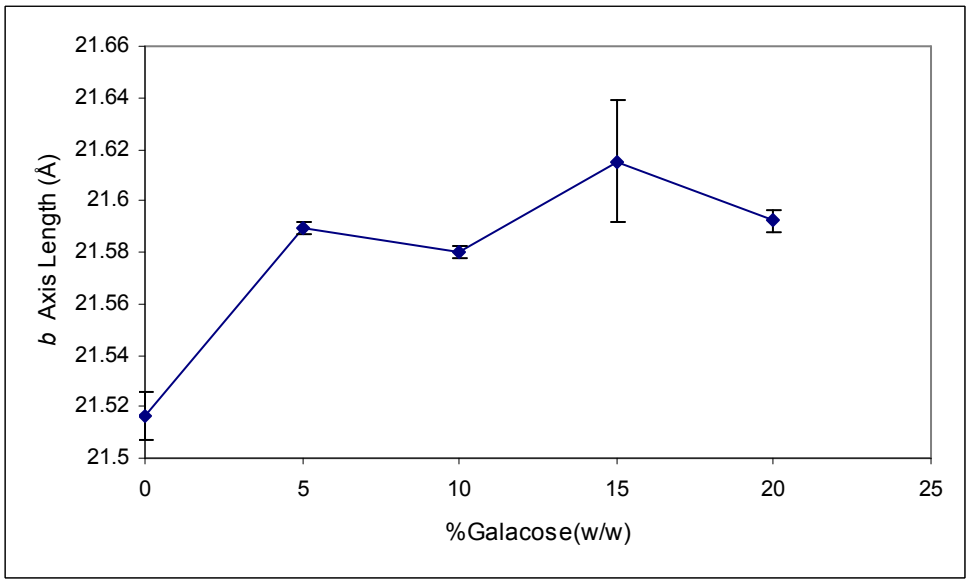


Fig 81b. Plot of *b* axis length vs. % Galactose

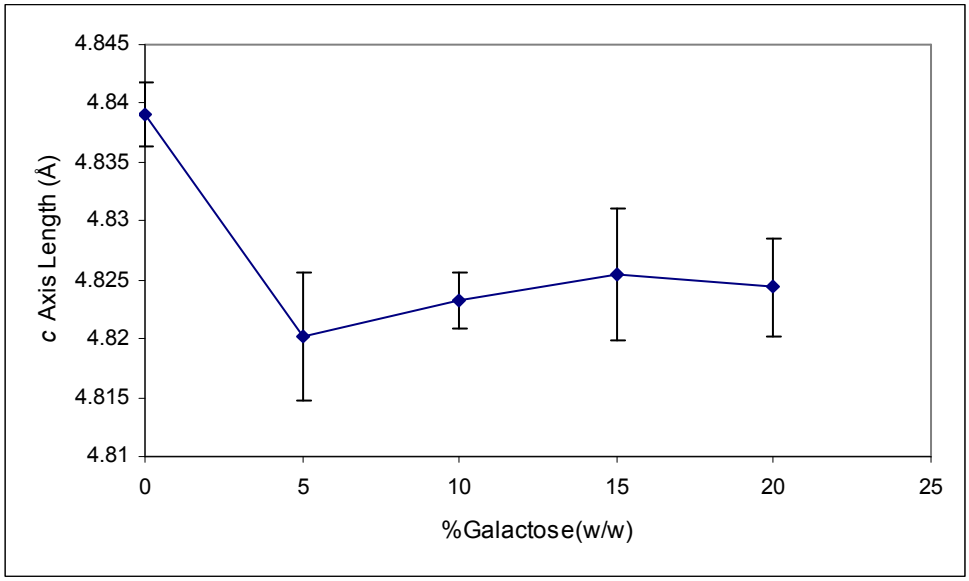


Fig 81c. Plot of *c* axis length vs. % Galactose

The above plots show that there is a decrease of both the *a* and *c* axis, and an elongation along the *b* axis. This trend appears to relate to the addition of both glucose and galactose to a supersaturated lactose solution. The elongation along the *b* axis suggests the added monosaccharides become possibly intra-crystalline species within the host lactose lattice. This hypothesis cannot be conclusively proven due to the lack of significance in the unit cell volume data though. However, the data obtained from DSC, coupled with the appearance of a solely lactose phase in PXRD (for materials with 5-15% w/w of each monosaccharide) is suggestive of inclusion. These two points, combined with the trend noted in the unit cell axis is suggestive of a possible inclusion of glucose and galactose into a lactose lattice. Further work on reducing the experimental error associated with the unit cell volumes may provide data that can tie all these points together and yield robust evidence for this hypothesis.

The results, based on both PXRD and DSC data (Figs 42 and 43 respectively), suggest that the simultaneous addition of galactose and glucose yields a potentially stronger adsorption for both moieties compared to the addition of solely one of them. Glucose alone, includes up to a level of 10% w/w as shown by Maillet whilst galactose does not include well at all.[2] A stronger adsorption (and thus, a slower desorption) may occur as both the added components constitute a lactose molecule. As such, the adsorption of the included mono-saccharides onto the growing crystal may occur with them

adsorbing in a manner that is akin to a lactose molecule. This hypothesis would allow for the higher limit of inclusion as adsorption in this manner could result in a higher number of H-bonds being retained (thus, a stronger adsorption) within the growing face compared to addition of solely galactose or glucose. A greater number of retained H-bonds may result as the two added mono-saccharides mimic a lactose molecule and therefore may also imitate the position of the H-bonds within a lactose crystalline structure. The appearance of separate phases relating to the added monosaccharides at the level of 20% w/w may be due to similar reasons outlined in Section 5.3.2. The time for preferential adsorption of galactose and glucose is not sufficient for the quantity added; therefore, the two may form their own crystalline phases as they have not become adsorbed and included within the lactose structure. Furthermore, the level of added sugar is high. The possibility of seeding of the solution is conceivable. The occurrence of this will shorten the time for preferential inclusion of the added sugars. This can be proposed as nucleation of the solution has been induced rather than occurring in a more homogeneous fashion; hence, crystallisation will be faster and this imparts a smaller time span for preferential inclusion of any added monosaccharides.

Bipartite materials (e.g. co-crystalline lactose and galactose) have been proven to include the added component in its crystalline state. Co-crystalline lactose, galactose and glucose were subjected to an autosorb analysis (see section 5.2.6.2., fig 45) in order to ascertain if the included components are crystalline or amorphous.

The autosorb analysis (Fig 45) revealed that the materials formed from co-crystallising galactose and glucose with lactose results in the final material being in the crystalline state. All materials exhibited behaviour indicative of crystalline material. All the samples increased in mass with each elevation of RH. This is followed by declining values of percentage mass as the samples are held at each level of RH. This indicates re-establishment of the equilibrium between p and p_o *via* moisture loss from the material (see Section 5.3.3.2.). There is no relationship between the amount of the included monosaccharides and the amount of moisture adsorbed, all samples adsorb moisture relating to an increase in mass between ~ 0.01 and 0.05% for each rise in the level of RH (Fig 45).

Comparison of co-crystallised materials (lactose along with 5-15% w/w of added glucose and galactose) with physical blends (lactose along with 5-15% w/w of added glucose and galactose) supported the crystalline nature of the co-crystallised material. The physical blends behaved in an analogous manner to the co-crystallised materials. Elevation of the RH (Fig 45) results in an increase in the percentage mass of the samples followed by a steady decline in the value of percentage mass increase as the re-establishment between p and p_o is achieved.

Whilst behaving in an identical manner to co-crystallised materials with regard to adsorption behaviour, physical blends do not adsorb as much moisture upon each elevation of RH. This implies a physical difference between the materials. A possible explanation is that the physical blends contain more soluble components. More soluble components would result in facile formation of syrup resulting in elevation of the p value, therefore reducing water adsorption from the environment. The co-crystallised material could be the opposite of the above, hence why adsorption occurs more readily.

5.3.6.2. Co-Crystalline Lactose, Galactose and Glucose Materials Formed with Addition at 90°C

Modification of the co-crystallisation methodology from adding the materials to be incorporated at 110°C (see Section 8.3.9) to adding them at 90°C followed by continuing to heat to 110°C (see Section 8.3.10) resulted in the formation of some potentially co-crystalline materials. DSC data (see section 5.2.6.3. fig 46) showed the materials display only a dehydration endotherm at ~ 145°C and the α-anomer melting at ~ 200°C. The values of ΔH for the melting of the α-anomer are observed to decrease with increasing amounts of the added monosaccharides. The values for this endotherm are significantly lower for co-crystalline materials when compared to analogous physical blends. This reduction in ΔH is accompanied by a decreasing onset temperature and a broadening of the endotherm. These phenomena are indicative of a phase becoming doped by an additive.[14-16] As can be clearly seen from the DSC data (Fig 46), higher amounts of added galactose and glucose are synonymous with lower enthalpy values. This suggests that lactose is becoming increasingly doped by the added monosaccharides.

PXRD data (see section 5.2.6.3., fig 47) did not completely support DSC data (Fig 46). Diffraction data showed that solely lactose phases are only formed up to a level to 10% w/w of the two monosaccharides. Upon elevation of the level of added galactose to 15% w/w, a reflection at the position of ~ 18.6° 2θ is observable. This is indicative of the presence of galactose as a separate phase. The discrepancies between the two sets of data may arise from a difference in sensitivity of the two methods. It appears that PXRD can detect galactose at much lower levels compared to DSC. The addition of galactose and glucose at 90°C, followed by heating to 110°C yields materials that potentially includes galactose in a higher proportion compared to when galactose is co-crystallised using the normal methodology.

The plot of unit cell volumes showed (see section 5.2.6.3., fig 48) that all material are larger than solely lactose. However, these elevations are not significant as all of the data points for this value fall outside of a 95% confidence interval. Whilst the data from DSC analysis, and the observance of single phases in PXRD analysis (for 5-10% w/w of added monosaccharides) along with the unit cell volume data, there is evidence toward the idea that the added sugars may become intra-crystalline species. However, as the unit cell volume increases are not significant, this cannot be conclusively proven.

As noted above for co-crystallisation of the two monosaccharides using the initial method of co-crystallisation, analysis of the unit cell axis may prove further evidence toward the idea of added sugars becoming intra-crystalline species. As stated, it has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from addition of glucose/galactose to a supersaturated lactose solution at 90°C (followed by continued heating) may provide data in a similar vein. A plot of the *a*, *b* and *c* axis (Fig 82a, 82b and 82c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

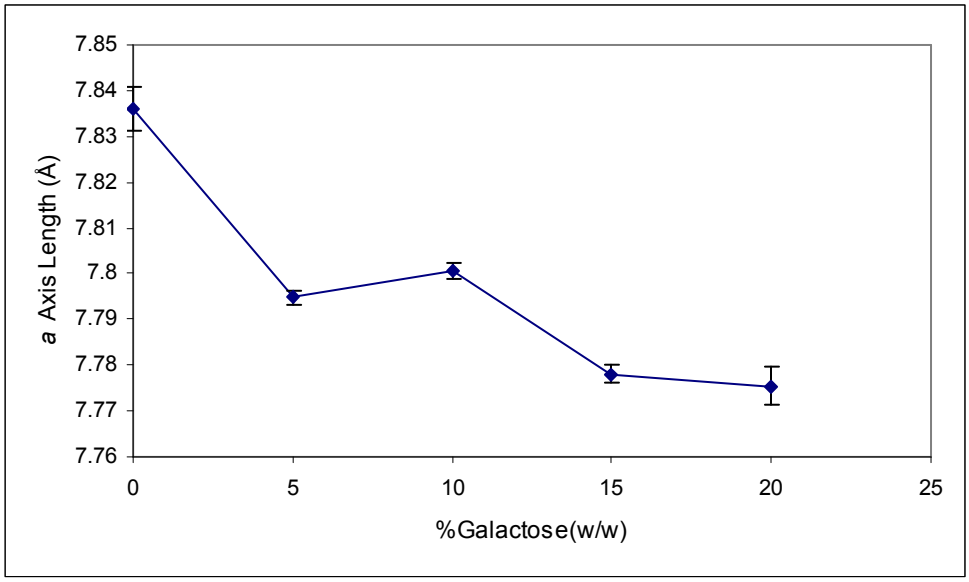


Fig 82a. *a* Axis Length for Co-Crystalline Lactose, Galactose and Glucose Materials formed with Addition at 90°C

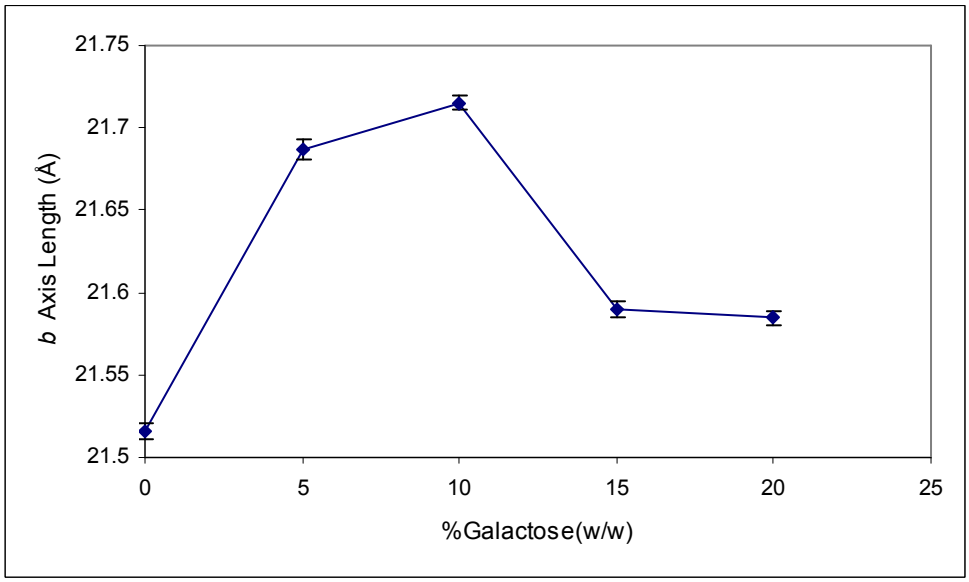


Fig 82b. *b* Axis Length for Co-Crystalline Lactose, Galactose and Glucose Materials formed with Addition at 90°C

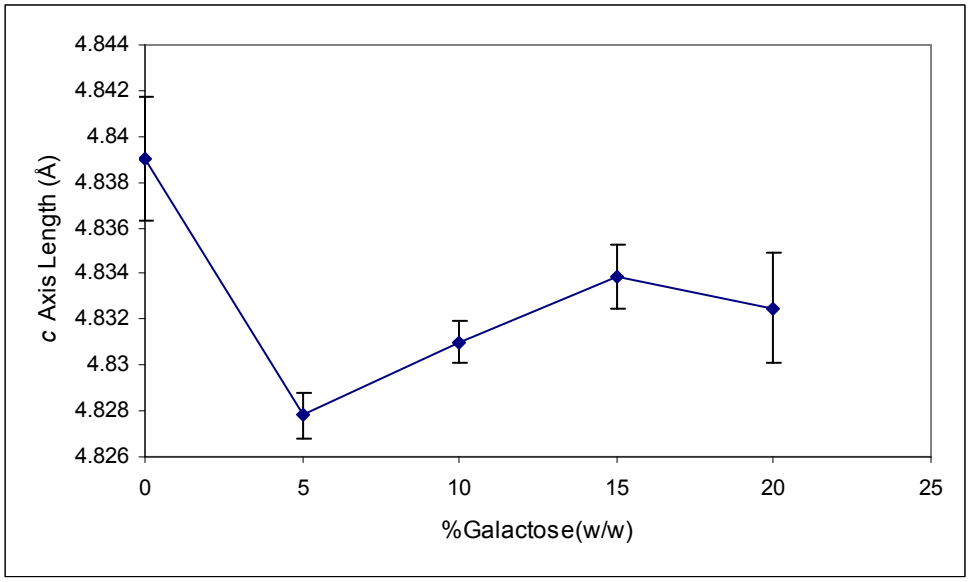


Fig 82c. *c* Axis Length for Co-Crystalline Lactose, Galactose and Glucose Materials formed with Addition at 90°C

The above plots show that there is a decrease of both the *a* and *c* axis, and an elongation along the *b* axis. This trend appears to relate to the addition of both glucose and galactose to a supersaturated lactose solution. The elongation along the *b* axis suggests the added monosaccharides become possibly intra-crystalline species within the host lactose lattice. Unfortunately, this hypothesis cannot be conclusively proven due to the lack of significance in the unit cell volume data though. However, the data obtained from DSC, coupled with the appearance of a solely lactose phase in PXRD (for materials with 5-10% w/w of each monosaccharide) is suggestive of a potential inclusion. These two points, combined with the trend noted in the unit cell axis is suggestive of a possible inclusion of glucose and galactose into a lactose lattice. Further work on reducing the experimental error associated with the unit cell volumes is required in order to prove the above outlined hypothesis.

The addition of equimolar quantities of galactose and glucose to a supersaturated lactose solution yields potentially co-crystalline material up to a level of 15% w/w of each sugar. When the two sugars are added at 90°C, followed by heating to 110°C, this limit drops to 10% w/w of the two monosaccharides. The addition of 15-20% w/w of galactose and glucose yields a potentially co-crystalline material and galactose. The amount of galactose present as a separate phase is potentially small. It is only picked up in PXRD analysis

and not DSC. The reasons for observing a declination in the quantity of the two monosaccharides potentially incorporable into a lactose lattice is unclear. It may be due to experimental error, a repeat formation of lactose with 15% w/w of each monosaccharide may yield material that is suggestive of solely lactose upon a repeated attempt. For this difference to be completely understood, repeat experiments are necessary in order to determine if alteration of the methodology alters the amount of galactose/glucose potentially included as intra-crystalline species.

As has been postulated in Section 5.3.6.1, the addition of 20% w/w of galactose and glucose to a supersaturated lactose solution may result in the solution becoming seeded. This will increases the rate of crystallisation and consequently, the time available for the inclusion of the added materials is reduced. Ergo, a separate phase of the two materials becomes evident from DSC (Fig 46) and PXRD (Fig 47) analysis. With the addition of the two sugars at 90°C, followed by continued heating to 110°C, it can be put forward that there should be a higher chance of the added components becoming taken up into solution. Using this scenario, the effects of seeding would be circumvented and the chance of complete inclusion of the added components would be raised as the time for preferential inclusion will be elevated. Relating this idea to the model for inclusion proposed by Kubota[24], the negation of a seeding factor may result in a protracted period between the initial level of *S* (at 110°C) and the level of *S* at which the material is allowed to crystallise.[24] It can be postulated from these data that a kinetic factor is relevant to the inclusion of additives within a host structure. However, the above outlined idea does not appear to happen. It is plausible to suggest that addition of saccharides at a lower temperature, followed by continued heating, should allow for negation of any seeding effect. The data obtained from this series of materials does not reflect this. Repeating the forming of these materials, using this methodology, would be essential in order to elucidate exactly what occurs when altering the initial method of co-crystallisation.

5.3.6.3. Co-Crystalline Lactose, Galactose and Glucose formed from a Physical Blend

Modification of the co-crystallisation methodology to start from a physical blend of the materials resulted (see Section 8.3.11) in the formation of materials related to lactose at all levels of added monosaccharides. DSC data (Fig 49) show that the material displays melting behaviour indicative of α-lactose monohydrate: a dehydration endotherm is observed at ~ 145°C and the α-lactose is seen to melt at ~ 210°C. The values of ΔH for the α-lactose endotherm decrease (see section 5.2.6.4, table 13) compared to the values obtained for ΔH for the same endotherm in a physical blend of the sugars. The reduction in enthalpy for this endotherm is also accompanied by a depression of the onset melting temperature and a broadening of the endotherm itself. This is suggestive of an inclusion of the galactose and glucose within the lactose crystal structure.[14-16] As can be seen from the DSC data (Table 13) the reduction in ΔH is proportional to the quantity of the monosaccharides. Higher amounts of glucose and galactose give rise to lower enthalpy values. This trend points towards a higher degree of potential inclusion within the lactose structure.

PXRD data (see section 5.2.6.4., fig 50) correlated with DSC data (Fig 49) in that all materials prepared in this manner displayed solely lactose phases. Determination of the unit cell volumes showed (see section 5.2.6.4., fig 51) that all materials have a larger volume compared to solely lactose, which suggests the inclusion of the added components as intra-crystalline species within the lactose structure. However, these increases cannot be construed as significant due to the fact that the data associated with the elevations falls outside of a 95% confidence interval. Though the notion of any added monosaccharides becoming intra-crystalline species cannot be conclusively drawn from the unit cell data, analysis of the unit cell axis may prove further evidence toward this idea.

As already stated, it has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from a physical blend of lactose with 5-20% w/w of glucose/galactose may provide data in a similar vein. A plot of the *a*, *b* and *c* axis (Fig 83a, 83b and 83c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

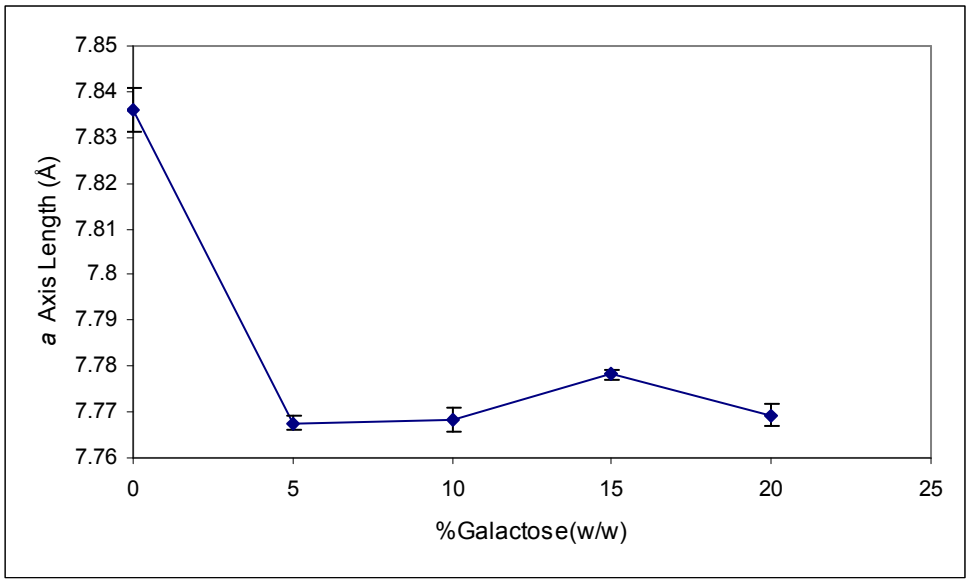


Fig 83a. *a* Axis Length *vs*. % Galactose for Co-Crystalline Lactose, Galactose and Glucose Materials Formed from a Physical Blend

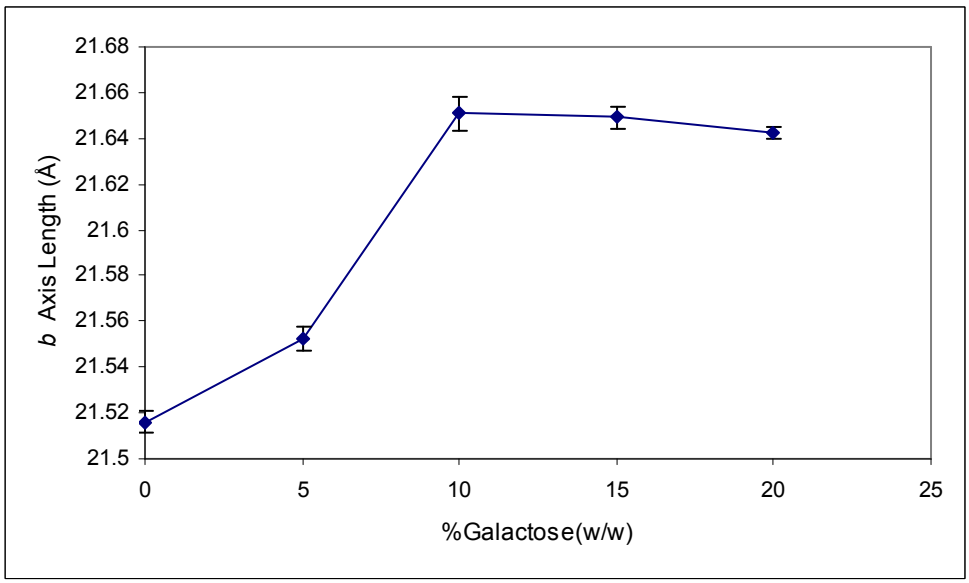


Fig 83b. *b* Axis Length *vs*. % Galactose for Co-Crystalline Lactose, Galactose and Glucose Materials Formed from a Physical Blend

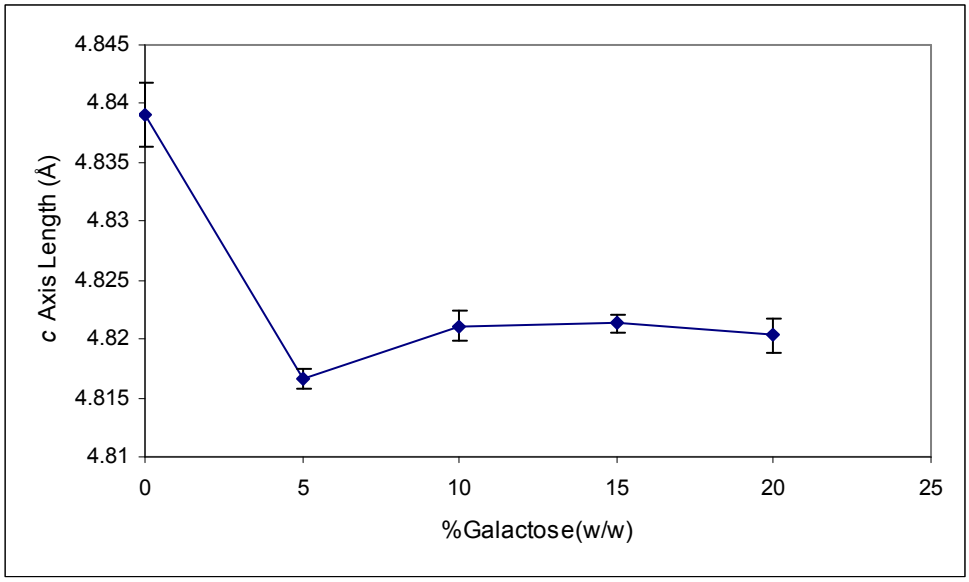


Fig 83c. *c* Axis Length *vs*. % Galactose for Co-Crystalline Lactose, Galactose and Glucose Materials Formed from a Physical Blend

The above plots show that there is a decrease of both the *a* and *c* axis, and an elongation along the *b* axis. This trend appears to relate to the addition of both glucose and galactose to a supersaturated lactose solution. The elongation along the *b* axis suggests the added monosaccharides become possibly intra-crystalline species within the host lactose lattice. Unfortunately, this hypothesis cannot be conclusively proven due to the lack of significance in the unit cell volume data though. However, the data obtained from DSC, coupled with the appearance of a solely lactose phases in PXRD is suggestive of a potential inclusion. These two points, combined with the trend noted in the unit cell axis is suggestive of a possible inclusion of glucose and galactose into a lactose lattice. Further work on reducing the experimental error associated with the unit cell volumes is required in order to prove the above outlined hypothesis.

Starting from a physical blend of lactose, galactose and glucose may facilitate the potential inclusion of the two monosaccharides into a lactose structure. As has already been seen, adding 20% w/w of galactose, along with glucose, to a supersaturated lactose solution results in an identifiable galactose phase emerging. Proceeding from a physical blend yields material that is indicative of solely lactose in its monohydrate form. A reason for this can be found in Section 5.3.6.2. and its validity may actually hold for this series of materials.

Starting from a physical blend may remove the effect that seeding induces. Consequently, a longer period for inclusion will result as the rate of crystallisation that will not be raised. It is interesting to note that testing of this theory by addition of the two monosaccharides at 90°C, followed by heating to 110°C does not display the same results. Proceeding from a physical blend of the sugars may remove any seeding effects, so, it appears further work on where (i.e. at what temperature) this phenomenon is removed is necessary.

5.3.7. Co-Crystalline Lactose with Galactose and Fructose

Co-crystallisation of lactose with galactose (5-15% w/w) and fructose (5-15% w/w) resulted in the formation of solely lactose related materials up to 10% w/w of galactose and fructose as shown in Section 5.2.6.2. Above this level of potentially included monosaccharides, DSC (see section 5.2.7.2, fig 55) and PXRD (see section 5.2.7.2., fig 56) analyses reveal phases relating to the presence of galactose and fructose.

DSC analysis (Fig 55) showed for solely the co-crystallised material, two endotherms corresponding to lactose, α-lactose monohydrate emerging at ~ 145°C and the α-anhydrous form of lactose emerging at ~ 200°C. The potential inclusion of galactose (up to 10% w/w) and fructose (up to 10% w/w) results in a decrease in the enthalpy of the lactose endotherms accompanied by a depression of the onset temperature and broadening of the lactose endotherm (Table 15). The ΔH values for the α-lactose monohydrate peak observed in physical blends of the sugars are higher in value compared to the values obtained for the peak seen in the co-crystalline material. This reduction in ΔH has been imparted by the co-crystallisation process and is suggestive of inclusion of the added monosaccharides within the lactose structure.[14-16] This behaviour is more pronounced with higher levels of the included monosaccharides, which suggests that a higher level of inclusion has been achieved. Elevation of the level of included monosaccharides to 15% w/w results in an endotherm appearing at ~ 160°C that signifies the presence of a separate galactose phase. No melting could be observed for fructose at the level of 15% w/w possibly due to the similarity in the melting points of fructose and lactose.

The possible elevation of the level of incorporable galactose, when included alongside fructose, is possible when taking into account the nature of

fructose. Within the solid-state, fructose will always crystallise in its β-fructopyranose form even though in solution, it will mutarotate through five possible anomers.[37] The structure of β-fructopyranose is markedly similar to glucose (Fig 84) and as such, it is feasible to imagine that fructose will facilitate the incorporation of galactose in a similar manner to when glucose is added alongside.

Fig 84. Structure of D-fructopyranose (left) and D-glucose (right)

What should be commented on is the fact that a higher degree of potential inclusion (for galactose) is observable when using fructose along with galactose. This has also been observed when adding glucose, along with galactose to a supersaturated lactose solution. From this it can be inferred that β-fructopyranose may continue the H-bond network as successfully as glucose. Thus, fructose may be regarded as a potentially successful intra-crystalline and is comparable with glucose. This point serves to further illustrate the idea that inclusion of any added sugars may depend on the similarity between the sugar that forms the dominant crystal structure and the additives that are to be included.

PXRD data (Fig 56) supports the DSC data (Fig 55, Table 16), such that a potentially co-crystalline material is formed up to a level of 10% w/w of galactose and fructose. These diffraction patterns (Fig 57) are suggestive of crystalline lactose. Elevation of the level of galactose and fructose to 15% w/w results in peaks appearing at the 2θ positions ~ 18.4° and 21.6° corresponding to a galactose phase and at the 2θ positions ~ 14° and ~ 28.2° corresponding to fructose. Determination of the unit cell volumes (see section 5.2.7.2., fig 57) suggests that the added galactose and fructose may become intra-crystalline species. However, these elevations in unit cell volume are not significant as all of the data points associated with this fall outside of a 95% confidence interval.

Though the notion of any added monosaccharides becoming intra-crystalline species cannot be conclusively drawn from the unit cell data, analysis of the unit cell axis may prove further evidence toward this idea.

As already stated, it has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from the addition of fructose/galactose to a supersaturated lactose solution may provide suggestive data. A plot of the *a*, *b* and *c* axis (Fig 85a, 85b and 85c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

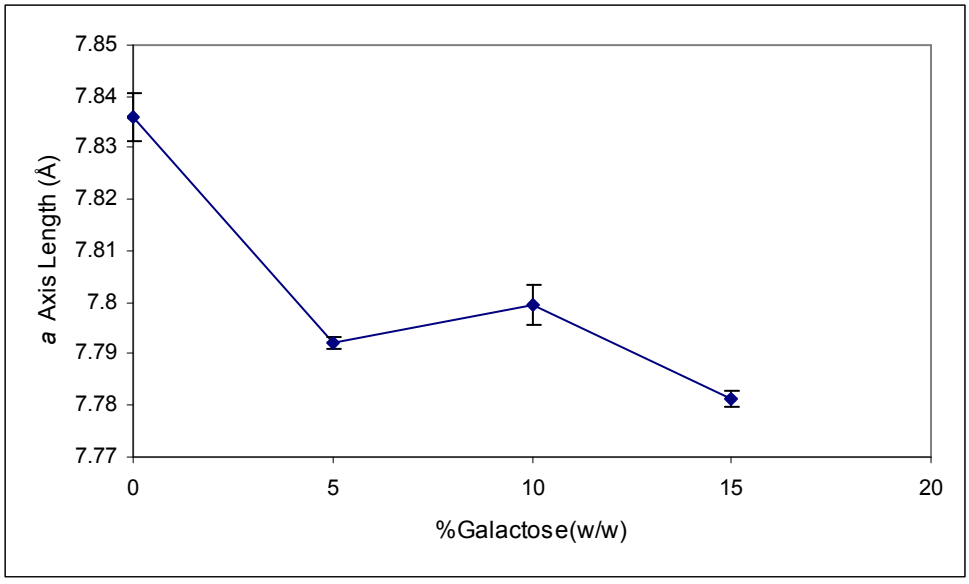


Fig 85a. *a* Axis Length vs. % Galactose for Co-Crystalline Lactose, Galactose and Fructose Materials

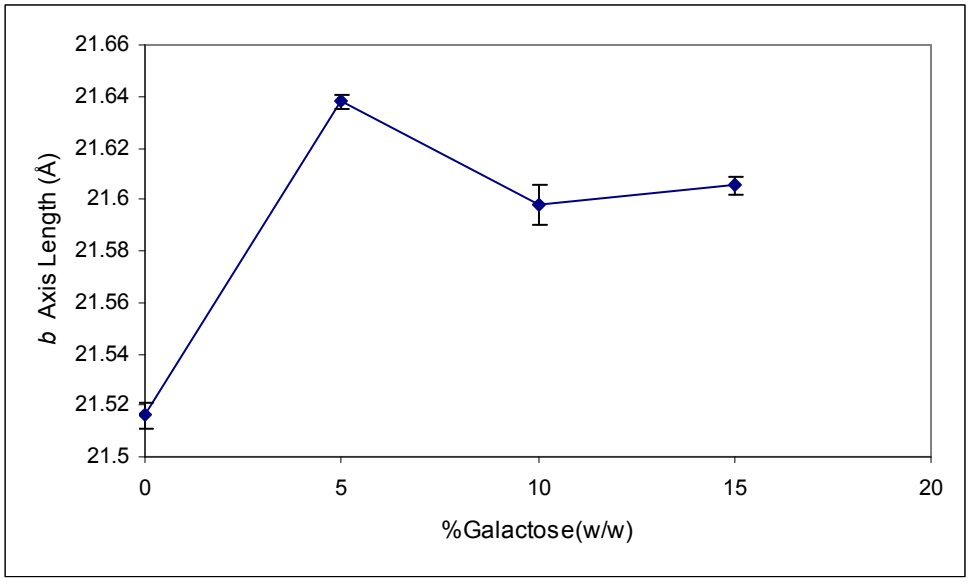


Fig 85b. *b* Axis Length vs. % Galactose for Co-Crystalline Lactose, Galactose and Fructose Materials

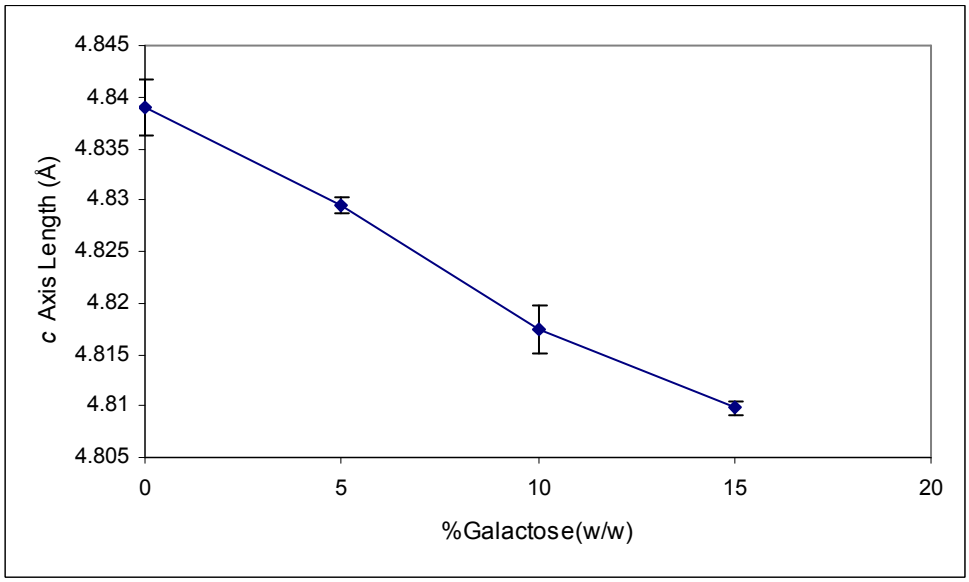


Fig 85c. *c* Axis Length vs. % Galactose for Co-Crystalline Lactose, Galactose and Fructose Materials

The above plots show that there is a decrease of both the *a* and *c* axis, and an elongation along the *b* axis. This trend appears to relate to the addition of both glucose and galactose to a supersaturated lactose solution. The elongation along the *b* axis suggests the added monosaccharides become possibly intra-crystalline species within the host lactose lattice. Unfortunately, this hypothesis cannot be conclusively proven due to the lack of significance in the unit cell volume data

though. However, the data obtained from DSC, coupled with the appearance of a solely lactose phases in PXRD is suggestive of a potential inclusion. These two points, combined with the trend noted in the unit cell axis is suggestive of a possible inclusion of glucose and galactose into a lactose lattice. Further work on reducing the experimental error associated with the unit cell volumes is required in order to prove the above outlined hypothesis.

Materials formed in this series were subjected to an autosorb analysis (see section 5.2.7.2., fig 58) in order to ascertain the physical nature of the material. Autosorb analysis should show if there is any amorphous content to materials yielded from the co-crystallisation methodology.

The autosorb analysis suggests that the co-crystallisation of lactose with galactose and fructose yields crystalline material. With each subsequent increase in the RH level, an increase in the percentage mass for each sample is observed, this increase in mass declines as the samples are held at each level of RH. This observation suggests that the re-attainment of the equilibrium between p and p_o is in progress and this behaviour is indicative of crystalline material. To support this, physical blends in analogous ratios to the co-crystallised material were also subjected to autosorb analysis. The physical blends behaved in an identical manner to the co-crystalline material. Elevation of the RH results in an increase in the percentage mass for each sample followed by a steady decline as the equilibrium between p and p_o is established.

There was no relationship between the amount of included monosaccharides in the two sets of materials and the amount of adsorption based on the relative weight gains of the materials. However, there was a difference between the two sets of materials with respect to how much moisture was adsorbed, such that co-crystallised materials adsorbed water (as signified by higher levels of weight gain) more readily than the physical blends. This implies a physical difference between the materials. A possible explanation is that the physical blends contain more soluble components. More soluble components would result in facile formation of syrup type components. Such would result in elevation of the p value, due to reducing water adsorption from the environment. The co-crystalline material could be the opposite of the above, hence would explain why water adsorption occurs more readily.

5.3.8. Co-Crystallisation of Lactose and Polydextrose

Co-crystallisation of lactose with polydextrose gave materials that exhibited supposed possible co-crystallinity by both DSC (see section 5.2.8.2., fig 61) and PXRD (see section 5.2.8.2., fig 62) analyses. DSC analysis (Fig 61) showed that the endothermic peaks ΔH values decreased when polydextrose was present (see section 5.2.8.2., table 17). This was also accompanied by a decrease in the onset temperature and together, these two observations are suggestive of a doping of a material.[14-16] Previously, this has been linked to a possible crystalline inclusion of an added material within the crystal structure of lactose. However, it is difficult to conceive that polydextrose has changed from an amorphous state to that of a crystalline. A problem arises due to the lack of an identifiable T_g for polydextrose within the scan, though this is to be expected. Work by Saklatavla *et al*. on low amorphous content within crystalline materials showed that DSC is not suited to detection of amorphous materials in the quantity of 10% w/w or less.[38] However, it is worth noting that disorder within a crystal can also be induced by amorphous compounds as well as crystalline materials.[39] Therefore, it may be the case here that the amorphous polydextrose may have possibly integrated itself within the lactose crystal structure. This fits with the notion that the co-crystallisation methodology used (e.g. see section 5.3.2) can potentially include added materials within a crystal structure whether it be amorphous or crystalline in nature.

PXRD data (Fig 62) shows diffractograms that are consistent with the reflections expected for α-lactose monohydrate. In agreement with DSC analysis, no indications of a phase related to polydextrose are observable. This is logical as polydextrose is amorphous by nature and therefore will not be detected by PXRD analysis. The determination of the unit cell volumes for these materials showed (see section 5.2.8.2., fig 63) there to be a proportional increase in size corresponding to polydextrose. It must be stated that these increases are not significant in nature as all of the data pints fall outside of a 95% confidence interval. However, it does suggest that the added polydextrose could include within the lactose crystal structure *via* a possible solid-liquid inclusion.

5.3.9. Co-Crystallisation of Lactose and Saccharin

Co-crystallisation of saccharin (5-20% w/w) with lactose yielded materials that are indicative of solely lactose could be formed up to 15% w/w of added saccharin as seen in section 5.2.8.2. This limit could only be observed by PXRD analysis (see section 5.2.9.2., fig 68). DSC data (see section 5.2.9.2, fig 67) suggests that potential co-crystalline phases are formed for all levels of added saccharin.

DSC traces (Fig 67) of the materials showed the expected melting behaviour for α-lactose monohydrate. In addition, a dehydration endotherm is apparent at ~ 145°C and the melting of the α-form can be seen at ~ 200°C. The calculated (Table 18) values of ΔH were observed to drop with increasing amounts of added saccharin until a level of 20% w/w is reached; at this point the value for ΔH rises. The decline in ΔH for the materials containing 5-15% w/w of saccharin is suggestive of a doping of the lactose structure by saccharin.[14-16] As this decline is proportional to the quantity of the added saccharin it can be suggested that lower enthalpy values may result from a more severe doping of the lactose structure. With the inclusion of 20% w/w of saccharin, the ΔH value rises compared to the value calculated for material containing 15% w/w of saccharin, which suggests a relaxation of the strain upon the lactose crystal lattice.

PXRD analysis (Fig 68) was not in agreement with the data obtained from DSC analysis as already stated above. At a level of 20% w/w of added saccharin, characteristic reflections are observable in the PXRD diffractogram (Fig 68) that are suggestive toward the presence of a separate, crystalline saccharin phase. Observation of a separate saccharin phase allows an explanation to be proposed for the sudden rise in enthalpy for this material. As noted above, the appearance of this phase coincides with a sudden increase in enthalpy. This increase indicated a relieving of the lattice strain induced by the saccharin and is possibly caused by interactions between the separate saccharin phase and the co-crystalline lactose phase.

Data from PXRD analysis (Fig 68) allowed the unit cell volumes to be determined. All of the calculated unit cell volumes (see section 5.2.9.2., fig 69) were larger compared to re-crystallised lactose. However, these increases cannot be construed as significant as the data associated with it falls outside of a 95%

confidence interval. Though the notion of any added saccharin becoming an intra-crystalline species cannot be conclusively drawn from the unit cell data, analysis of the unit cell axis may prove further evidence toward this idea.

As already stated, it has been observed that alteration of unit cell axis lengths, as a function of added impurities, is an indicator to the presence of an intra-crystalline species.[18, 20-21] Consequently, analysis of the unit cell parameters for the materials formed from the addition of saccharin to a supersaturated lactose solution may provide suggestive data. A plot of the *a*, *b* and *c* axis (Fig 86a, 86b and 86c) compared to their respective lengths calculated for re-crystallised lactose can be seen below.

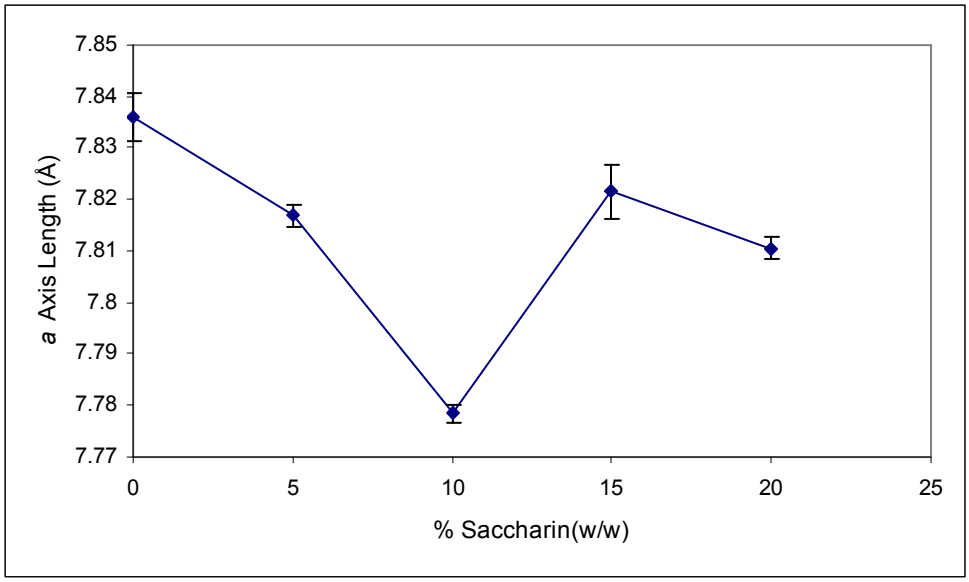


Fig 86a. *a* Axis Length vs. % Saccharin for Co-Crystalline Lactose and Saccharin Materials

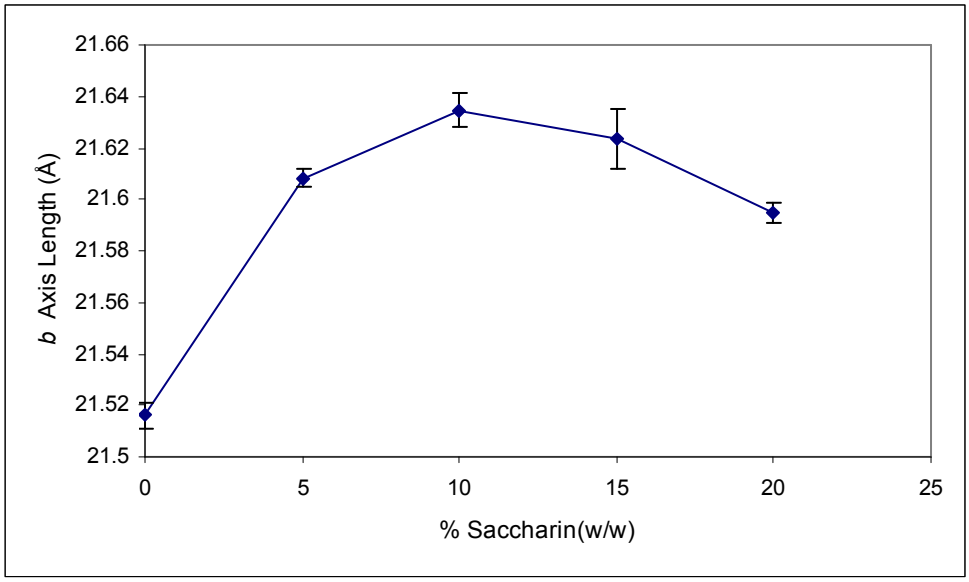


Fig 86b. *b* Axis Length vs. % Saccharin for Co-Crystalline Lactose and Saccharin Materials

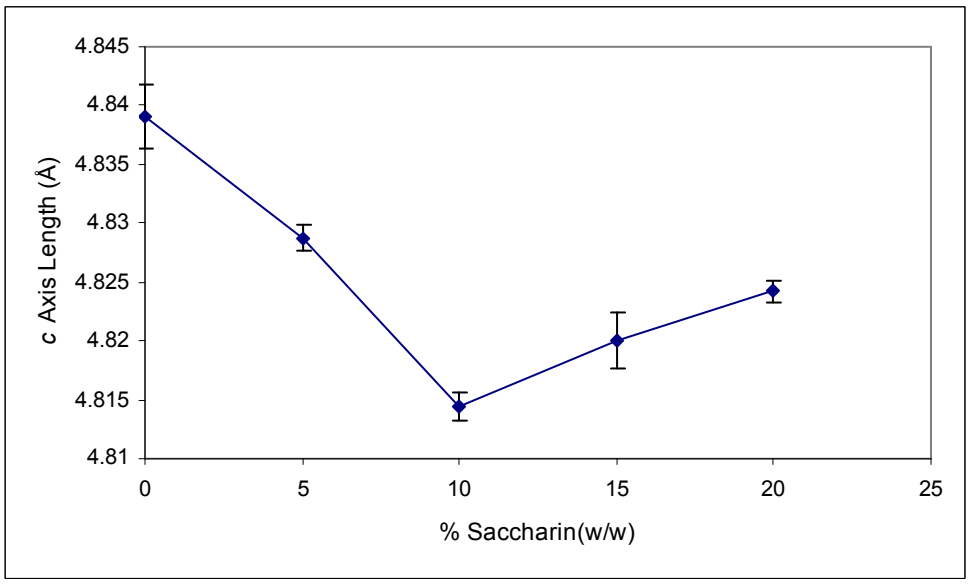


Fig 86c. *c* Axis Length vs. % Saccharin for Co-Crystalline Lactose and Saccharin Materials

The above plots show that there is a decrease of both the *a* and *c* axis, and an elongation along the *b* axis. This trend appears to relate to the addition of saccharin to a supersaturated lactose solution. The elongation along the *b* axis suggests the added saccharin becomes a possibly intra-crystalline species within the host lactose lattice. Unfortunately, this hypothesis cannot be conclusively proven due to the lack of significance in the unit cell volume data though. However, the data obtained from DSC, coupled with the appearance of a solely lactose phases in PXRD is suggestive of a potential inclusion. These two points,

combined with the trend noted in the unit cell axis is suggestive of a possible inclusion of glucose and galactose into a lactose lattice. Further work on reducing the experimental error associated with the unit cell volumes is required in order to prove the above outlined hypothesis

Interestingly, it takes a high level of added saccharin to yield a material displaying both a phase related to lactose and another to saccharin. This is unusual as it has been shown (e.g. see section 5.2.5.2, galactose and glucose can be suggested to include for up to 15% w/w each into lactose) that generally, high levels of added sugars (whilst the final material is indicative of solely lactose) is synonymous with materials that are similar in structure to lactose. Saccharin appears to display unique characteristics when introduced into supersaturated lactose solutions. When used in conjunction with maltose, it appears to increase the amount of the disaccharide potentially incorporable into the structure. The exhibition of these properties may result from the structural features of the saccharin molecule and from this a reason for the high level of inclusion can be proposed.

As already suggested (e.g. see section 5.3.6.1), if a material is to potentially incorporate well into a lactose crystal structure it has to be able to replicate the H-bond network within the three-dimensional crystal lattice. If a material is able to behave in this manner then it could exhibit elevated levels of inclusion (e.g. saccharin helps increase the amount of maltose that can be incorporated into a lactose structure, see section 5.3.3.1). As such, it can be postulated that saccharin can display these necessary qualities to enable a high level of inclusion. The functional groups present in the saccharin molecule could be orientated in such a way that they correlate well with H-bond receptor and donor sites within the lactose structure. Consequently, the molecule will adsorb well onto the growing structure and ergo, will have a higher chance of irrevocable entrapment within the final lactose crystal lattice.

However, even though it appears that saccharin is an excellent additive when trying to form co-crystalline lactose materials, a separate phase related to saccharin does appear as shown by PXRD analysis (Fig 68). As outlined in previous sections (e.g. section 5.3.2), the formation of a phase related to the material added to lactose occurs due to insufficient time between the generated *S*

level (*i.e.* the lactose solution formed at 110°C) and the final level of *S* when the solution is crystallised for complete inclusion.

5.4. Conclusions

The co-crystallisation of a saturated lactose solution with different saccharides showed that up to a certain limit of added components, solely phases related to lactose are formed. This limit appears to depend on the nature of the added sugar. Once the limit is breached, a separate crystalline phase relating to the added saccharide is observable by DSC and PXRD analysis. The appearance of this separate phase may relate to the adsorption equilibrium that exists in the crystallisation solution and the effect this has on the adsorption of the added components. Hypothetically, at low values of *S*, which occur when the lactose solution is heated, the adsorption of the added component is preferred over adsorption of lactose growth units onto the growing crystal structure. Increasing values of *S*, which occur as the solution cools and crystallises, shift this preference to the adsorption of lactose growth units onto the growing crystal. At this point, the added component may not be readily adsorbed onto the growing crystal. As such, the formation of a separate crystalline phase relating to the added component occurs. The period of time between preference for impurity adsorption (*i.e.* the added component) and preference for lactose adsorption is the window for which inclusion can occur. How well the added components utilise this time is indicated by the limits of inclusion that are observed for the various mono- and di-saccharides that are added to a supersaturated lactose solution.

Successful inclusion by an added component is dependent on the structural similarity between itself and lactose. If the added component, upon adsorption, can successfully replicate the H-bond network then a high degree of inclusion will occur. A high degree of inclusion may occur if a successful replication transpires as the tenacity of the adsorption will be greater when compared to an un-successful replication of the H-bond network. A high amount of H-bonding will infer a reduced chance of desorption of the adsorbed component away from the growing crystal, consequently the odds of an irrevocable entrapment are increased compared to a component that does not mimic the H-bond network well. This can be clearly seen when comparing the

limits of inclusion for maltose (20% w/w) and galactose (4% w/w). From this it can be inferred that maltose is an excellent mimic of lactose. As such, the H-bond network is preserved well and the added maltose includes at a high level of 17% w/w. The opposite for this appears to be true for galactose. The H-bond network is not replicated well and consequently the monosaccharide cannot include at a high level, galactose can only include up to 4% w/w. Further evidence for this theory can be shown from the data garnered from the co-crystallisation of lactose with galactose and glucose (see Section 5.2.5.). Using this combination elevates the level of incorporable galactose from 7% w/w to 10% w/w. This is logical as lactose consists of the two added monosaccharides, ergo it can be postulated that together, galactose and glucose form a pseudo lactose molecule which could include well into a lactose crystal structure.

The elevation in quantity of added saccharides (with retention of single phases) has also been shown to be achievable. This has been achieved by the simultaneous inclusion of some components un-related to lactose, namely fructose and saccharin. Fructose raised the limit of addable galactose from 7% to 10% w/w. This is plausible when considering that fructose can mutarotate between a furan- and pyranose forms. It is possible that the presence of fructose in its pyranose form might help facilitate the inclusion of galactose. Saccharin has been shown to help with the incorporation of maltose. This behaviour may be due to the presence of sulphonamide and carboxamide groups present on the saccharin molecule.

Modification of the co-crystallisation methodology has also been shown (Section 8.3.8) to help improve the possible incorporation of glucose and galactose within a lactose crystal structure. Co-crystallisation starting from a physical blend (see section 5.2.5) was found to raise the limit to 20% w/w of added galactose. This observation may stem from crystallisation occurring *via* molecular collisions rather than inducement by seeding. Application of this methodology to co-crystallising galactose with lactose appeared to have a similar effect. However, PXRD analysis of these materials showed that an additional phase (un-related to lactose or galactose, in any of its anomeric forms) was observable. These unexpected peaks could not be identified, and it is possible that they are the result of a co-crystalline phase. In order to elucidate the nature of this phase, repeated formation of these materials is necessary.

It can also be shown that an increase in the amount of incorporated material need not stem from a method proceeding from a physical blend. Addition of galactose and glucose at 90°C, followed by heating to 110°C induces similar, if not as notable, results. Using these ideas, the hypothetical window for inclusion is widened and consequently the amount of addable galactose is increased.

The notion of inclusion within the lactose crystal structure by the added components can be suggested by both forms of analysis. DSC provides the strongest support for this idea. All materials exhibiting solely melting of lactose show a reduction in enthalpy for the α-lactose endotherm accompanied by a depression of the onset melting point. Both these are classic indicators of a doping of a dominant phase by an impurity and the data show this effect to be amplified by higher quantities of added saccharides. This reduction in enthalpy is postulated to arise from strain imparted upon the lattice structure of lactose by the introduction of impurities.

PXRD analysis, whilst not providing conclusive evidence, did yield suggestive data. Typically, co-crystallising of lactose with mono- and disaccharides yields materials that exhibit larger unit cell volumes ca. re-crystallised lactose. Unfortunately, these elevations could not be construed as significant as all of the data points fall outside of a 95% confidence interval. However, analysis of the axis shows that there does appear to be a trend related to the presence of added sugars. It has been shown that generally, both the *a* and *c* axis (for co-crystallised materials) shrink with added sugars whilst the *b* axis has been shown to elongate. Interestingly, all three of these changes can be shown to be significant as the data points all reside within a 95% confidence level. Such changes in unit cell axis have been noted in literature, within predominantly inorganic systems, as a result of added impurities. Whilst sugars are completely organic in nature, it is not inconceivable that such fluctuations in unit cell parameters could be observed when co-crystallising sugars. The major limiting factor hindering the theory of added sugars becoming intra-crystalline species is the lack of significance in the unit cell volume data. Ergo, it can be suggested that reduction of experimental error may provide robust enough data in order to provide substantial evidence toward this theory.

The notion that the data shows potential inclusion can be explained by the idea that the added saccharides are present in the amorphous state. This has been shown to be false. The autosorb analyses of various co-crystallised materials showed that the materials did not contain any amorphous content; all materials gave behaviour that is indicative of a crystalline material.

5.5. References

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6. Hydrated Form of Sucrose

6.1. Introduction

The melting point of sucrose has been established to reside in the range of 186-192°C, furthermore, sucrose has been shown to exist solely in the anhydrous state.[1] Generally, the melting of sucrose produces only one peak observable in DSC analysis; work by Maulny showed that a second peak was observable at ~ 150°C for the melting of high purity sucrose.[2] Work from this laboratory showed the peak at ~ 150°C was not a consequence of salt- or thermal catalysed degradation of sucrose and that the appearance of this peak was related to the purity of the sucrose used.[3] Further work by Maulny allowed for isolation of the phase responsible for the emergence of this peak during DSC analysis, the work described herein is related to replication of this phase and a more complete understanding of the factors that influence its formation.

6.2. Results

6.2.1. DSC Analysis of the Hydrated Form of Sucrose isolated by Maulny[2]

A small sample of the hydrated form of sucrose remained from the work undertaken by Maulny, a fraction of this was subjected to DSC analysis in order to establish a reference point for comparison.[2]

DSC analysis (Fig 1) showed the presence of a single endothermic transition starting at ~ 149°C that is completely un-related to the expected melting of sucrose.

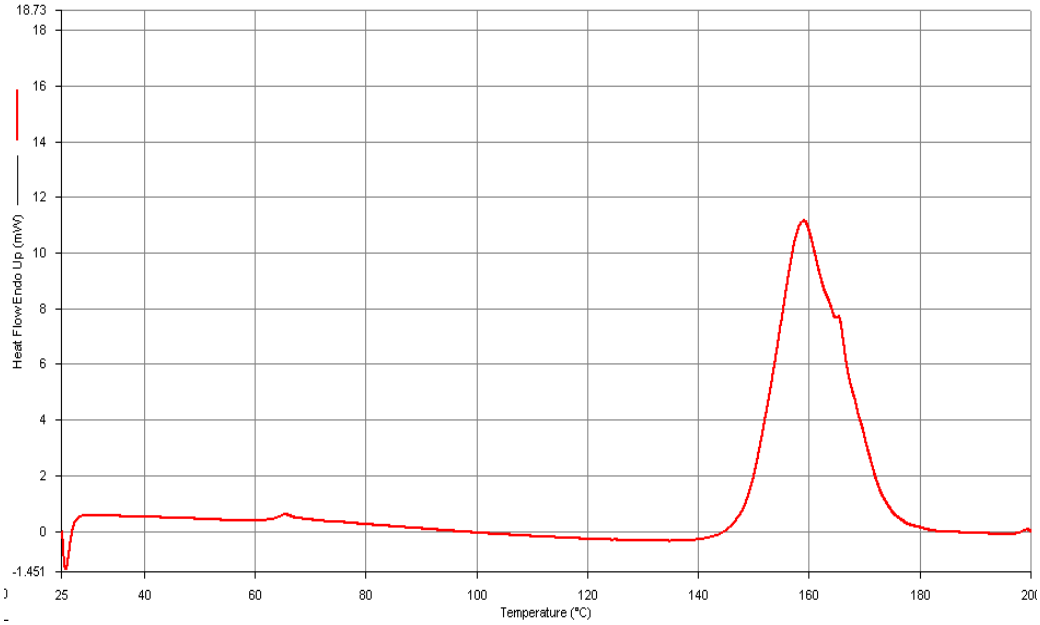


Fig 1. DSC Analysis of a Hydrated Form of Sucrose

	Onset (°C)	Peak (°C)	ΔH (Jg^{-1})
Hydrated Sucrose	149.12	159.0	114.872

Table 1. DSC Data for a Hydrate form of Sucrose

Due to the extremely small amount of the sample remaining, PXRD analysis was not performed as a portion was required to be retained in case replication was not achieved.

6.2.2. Isolation and Formation of a Hydrated Sucrose Phase

Using the methodology outlined in Section 8.4.1., several sucrose solutions were re-crystallised. DSC analysis (Fig 2) shows that all of the solutions exhibited two phases; one that relates to sucrose appearing at ~ 180°C and one emerging at ~ 150°C that relates to the hydrated form of sucrose.

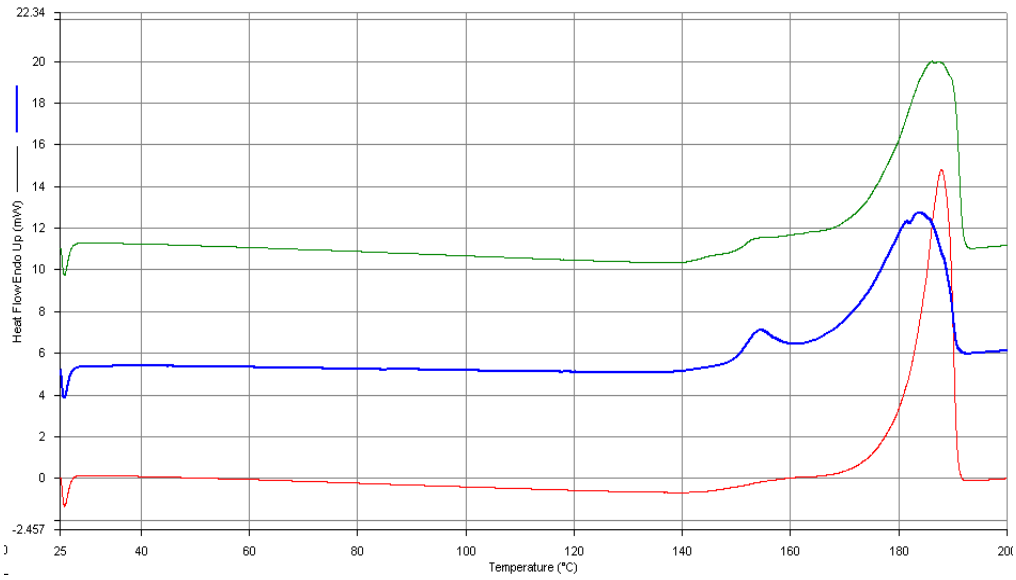


Fig 2. DSC Analysis of Sucrose Re-Crystallised in Humid Conditions (50% RH)

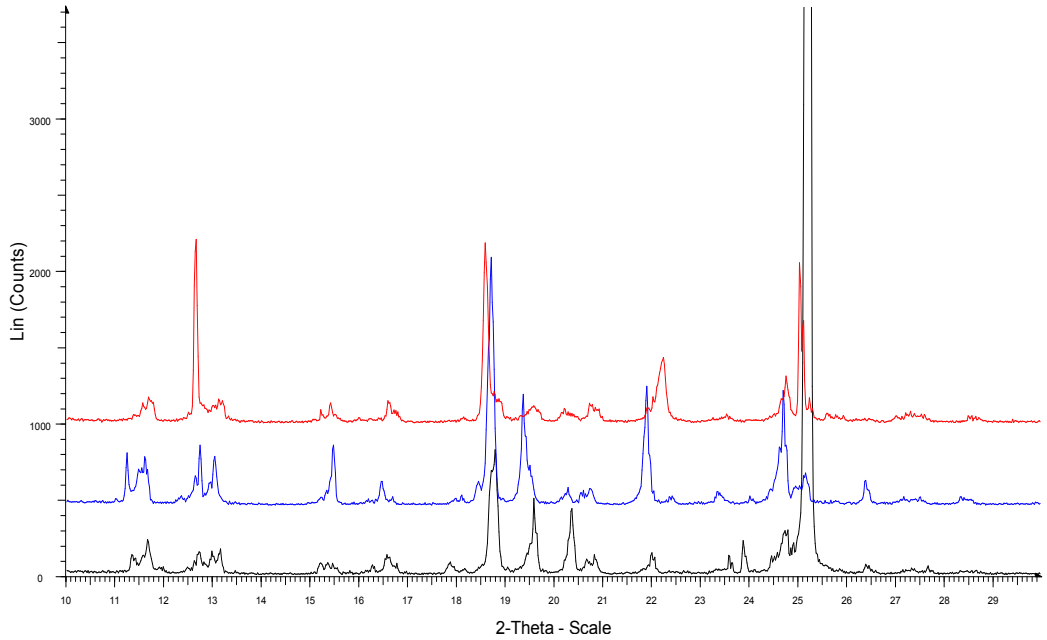
	Onset 1 (°C)	Peak 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	Peak 2 (°C)	ΔH 2 (Jg^{-1})
Sample 1	151.139	156.2	0.979	181.417	187.866	107.822
Sample 2	150.050	154.166	6.212	171.201	184.133	87.390
Sample 3	150.657	153.566	2.352	174.851	186.2	96.157

Table 2. DSC Data for Attempted Formation of an isolated form of Sucrose

From the DSC data (Table 2) it can be seen that both forms of sucrose are present; however, higher amounts of the hydrated phase present are signified by larger enthalpy values. What can also be seen from the data is that lower values of enthalpy calculated for sucrose melting at ~170-180°C are coincident with higher values of ΔH for the melting of the sucrose phase emerging at ~ 150°C. It can be state with a reasonable degree of certainty that the phase emerging at ~ 150°C is related to the hydrated sucrose phase, it exhibits marked similarities in the melting temperatures to those observed for the isolated phase analysed by DSC in Section 6.2.1.

PXRD analysis (Fig 3) of the materials formed gave diffractogram patterns that looked markedly dissimilar to those expected for sucrose. Comparison with a model pattern (see appendix 1) for sucrose, shows that

unexpected peaks emerge at the approximate 2θ positions of 11.35°, 15.2°, 17.86° and 20.68° for all three samples.



Black: Sample 1, Blue: Sample 2, Red: Sample 3

Fig 3. PXRD Diffractograms for Sucrose Materials Attempted to be transformed into a Hydrated Phase

The presence of these extra reflections tallies with the data garnered from DSC analysis. Two phases are observable in the DSC analysis of all three samples; this is mirrored in the PXRD data for all three samples as well. It appears re-crystallisation of sucrose under these conditions yields a material that may contain potentially two different forms of sucrose. Comparison of the yielded diffractograms (see appendix 1) with model patterns using powder cell software clearly showed this difference. The patterns expected for sucrose and those yielded using this re-crystallisation technique were clearly different. Materials displaying this unique diffractogram were reproduced on three separate occasions.

6.2.3. Formation of a Hydrated Sucrose Phase *via* Glucose Addition

With the notion that the co-crystallisation of a physical blend of sucrose and galactose produced a hydrated phase (as seen in Chapter 4, Section 4.2.8.2.), an attempt to re-produce a hydrated sucrose was undertaken by using glucose and altering the methodology as outlined in Section 8.4.2. The temperature of the sucrose solution was dropped from 128°C to 100°C: at this temperature a proportion of glucose was added in the range of 4-10% w/w.

DSC analysis of the materials formed from this methodology (Fig 4) showed that materials containing 4-10% w/w of added glucose all exhibited melting behaviour indicative of the hydrated form of sucrose; no melting of glucose could be observed.

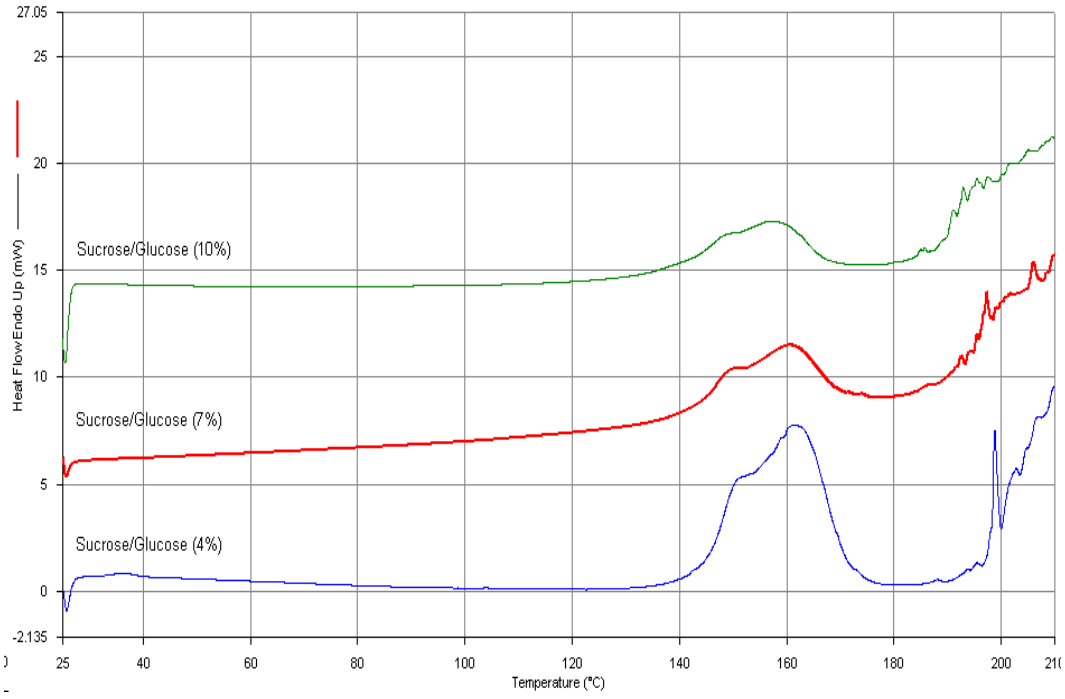


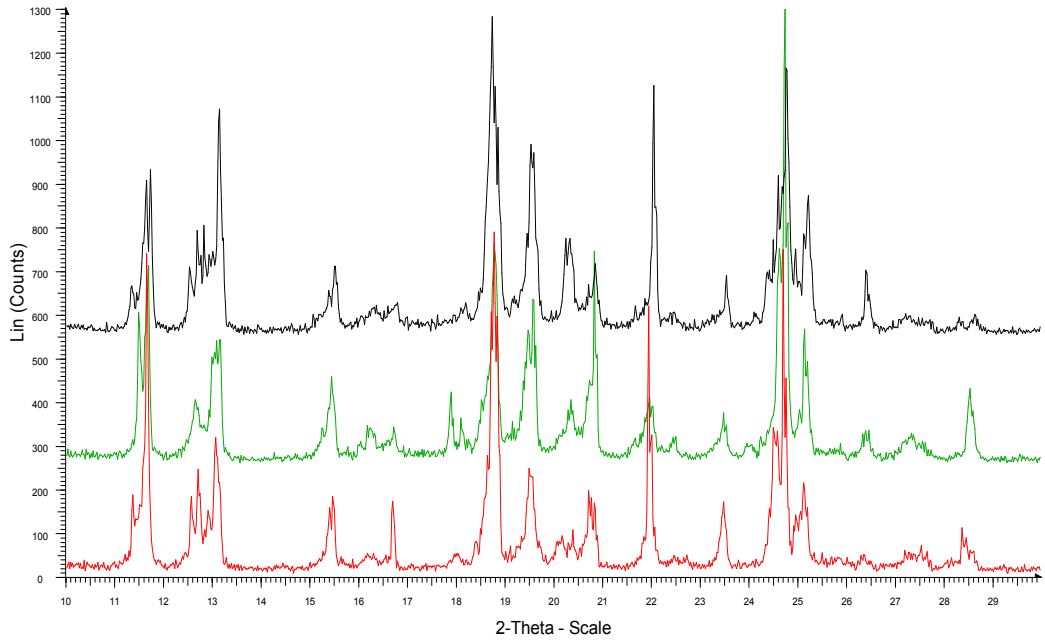
Fig 4. DSC Traces of Sucrose and Glucose Materials Formed at 100°C

	Onset (°C)	Peak (°C)	ΔH (Jg^{-1})
Suc/Glu (4%)	152.543	161.4	88.977
Suc/Glu (7%)	143.420	160.433	74.429
Suc/Glu (10%)	142.302	157.406	52.534

Table 3. DSC Data for Sucrose and Glucose Materials Formed at 100°C

From the DSC data (Table 3) it can be seen that all the materials display melting behaviour that matched well with those expected for the hydrated form of sucrose. Calculation of the enthalpy values for the melting of this phase shows that, with increasing amounts of glucose present, lower values of ΔH are observed. This is also accompanied by a drop in the onset and peak melting points.

Interestingly, PXRD analysis of these materials (Fig 5) showed that all of the materials appeared to exhibit two phases. Unexpected peaks can be observed at the 2θ positions of 11.3°, 12.5° and in the case of material containing 4% w/w of glucose, an unexpected peak can be observed at the 2θ position of 20°.



Red: 4% Glucose w/w, Green: 7% Glucose w/w, Black: 10% w/w Glucose w/w

Fig 5. PXRD Diffractograms for Sucrose and Glucose Materials Formed at 100°C

These unexpected peaks do not relate to the presence of a separate glucose phase, this tallies with the observations noted in DSC analysis. From DSC analysis, only a single phase can be observed. It appears that the data obtained from PXRD analysis suggests the presence of two phases within the material formed form the co-crystallisation method. Again, like materials re-

crystallised in section 6.2.2., these materials appear to display a phase that is completely unexpected.

From these results, it appears that modification of the crystallisation methodology allows for the formation of a hydrated form of sucrose. What is also apparent is that with higher amounts of glucose added to the solution, the value of ΔH for its melting becomes less. This suggests that the glucose may impede the formation of this phase; to this end, sucrose was re-crystallised at 100°C and dried in two different environments (one being an oven at 55°C, one being an oven at 55°C and 50% RH) in order to ascertain if a pure, isolated hydrate form would be yielded.

DSC analysis (Fig 6) of sucrose re-crystallised at 100°C, followed by a portion dried in a conventional oven and a humidity controlled oven (50% RH) showed that a mixture of phases is obtained.

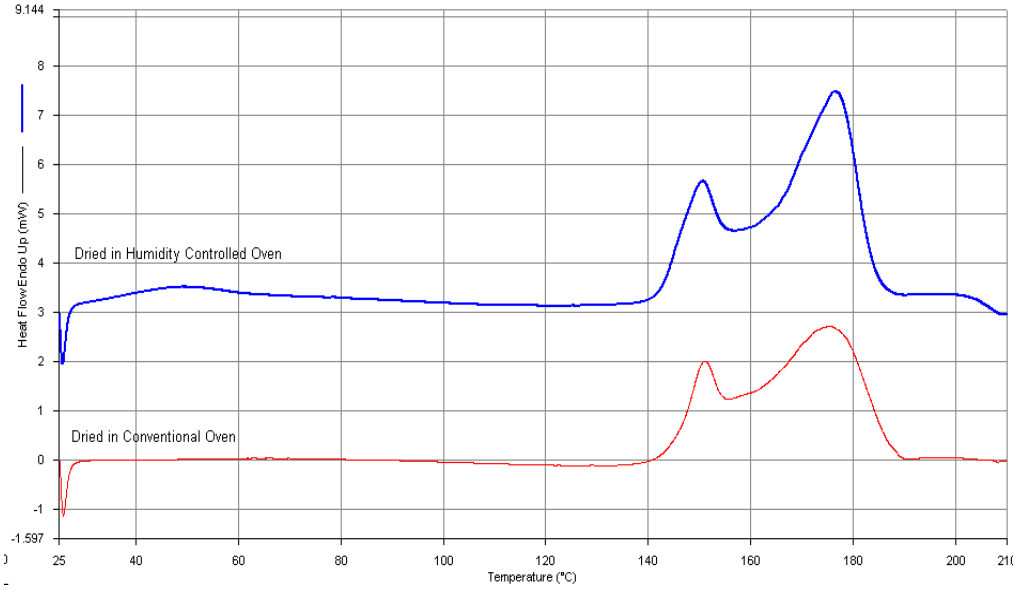


Fig 6. DSC Analysis of Sucrose Re-Crystallised at 100°C and Dried in a Conventional Oven and a Humidity Controlled Oven (RH set at 50%)

	Onset 1 (°C)	Peak 1 (°C)	ΔH 1 (Jg^{-1})	Onset 2 (°C)	Peak 2 (°C)	ΔH 2 (Jg^{-1})
Conventional Oven Dried Sucrose	149.491	150.766	8.207	162.963	176.166	42.235
Humidity Controlled Oven Dried	143.108	150.266	12.715	164.502	176.8	47.498

Table 4. DSC Data for Sucrose Re-Crystallised at 100°C and Dried in Two Different Ways

Form the DSC data (Table 4) it can be noted that, depending on the drying conditions employed, a variation in the enthalpy value can be seen for the peak emerging at ~ 150°C. Drying a sucrose solution, in a humidity controlled oven, set at 50°C and 50% RH, results in a larger enthalpy value for the peak at ~ 150°C.

PXRD analysis was unavailable due to exposure of the respective materials to a solvent source by another member of the lab.

6.3. Discussion

6.3.1. DSC Analysis of an Isolated Form of Hydrated Sucrose

The sample analysed was obtained from the work by Maulny, the phase analysed here was formed at 50°C and 50% RH from a 61.5% concentrated sucrose solution.[2]

As can be seen from the DSC trace (see section 6.3.1., fig 1), the melting of the phase is completely unrelated to the expected melting of sucrose typically found at ~ 180°C. The DSC trace shows absolutely no indication of the presence of sucrose in its expected phase. This reinforces the idea that a new form of sucrose, i.e. a hydrated form of sucrose was formed. Sucrose normally exists in its anhydrous phase at room temperature and a hydrate, under these conditions, is not known. However, it remains to discuss whether the phase formed here is a hydrate akin to e.g. trehalose dihydrate, or is it a sucrose structure with water bound in another manner, such as interstitial.

In solution, sugar displays an affinity for water; the strength of this affinity relates, principally, to the interaction of the sugar hydroxyl groups and ring oxygens with water and sugar-sugar interaction. It can be said that crystalline sugar hydrates result from a strong interaction of the molecule with water. These hydrated sugar molecules are resistant to crystallisation due to this and consequently hydration water does not leave readily.[4] Sugars that behave in this manner include glucose, fructose and trehalose. Within these three sugars, there are differences in the hydration number with trehalose exhibiting the highest and glucose the lowest.[4] Whilst these sugars retain a portion of the water that is hydrating the sugar is solution, the amount of water hydrating a sugar molecule decreases as its concentration increases (i.e. crystallisation) with the above sugars. A stoichiometric amount remains after crystallisation stops.[5] The removal of water hydrating a sugar molecule in solution is related to its nucleation and crystallisation. The formation of stable nuclei occurs by removal of hydrating water; this allows sugar-sugar bonding to occur which the water would otherwise interfere with.

Sucrose, like all other sugars, becomes hydrated in solution. Namely, the hydroxyl groups and ring oxygens hydrogen bond to water molecules. The number of water molecules hydrating a single sucrose molecule varies depending on the technique used to determine it.[4] What has been shown from molecular modelling is that, in concentrated aqueous solutions of sucrose, a water bridge between the glucose and fructose moieties exists.[6, 7] This water bridge is believed to be in high population; the water molecule is thought to be the last to be removed before incorporation of a sucrose molecule into the crystal structure. Previously, these hydrates have not been observed in the crystalline state, but it does suggest an affinity for water by sucrose. It may be the case reported herein that this bridging molecule is not removed upon incorporation of sucrose into the final crystal lattice. The methodology employed appears to overt this step. This is not expected since the removal of hydration water is considered to be the rate limiting step in sucrose crystallisation. Non-removal of this water results in a hydrated sugar form as is the case noted with various hydrated sugars.[5]

What is important to note is the work performed on amorphous sucrose exposed to varying levels of humidity and the subsequent analysis of these materials by DSC.[8] It was shown that exposure of amorphous sucrose led to three

notable events (Fig 7): i) the glass transition (T_g) of sucrose occurring, ii) the re-crystallisation of sucrose (T_c) and finally, iii) the melting of the crystalline sucrose (T_m).

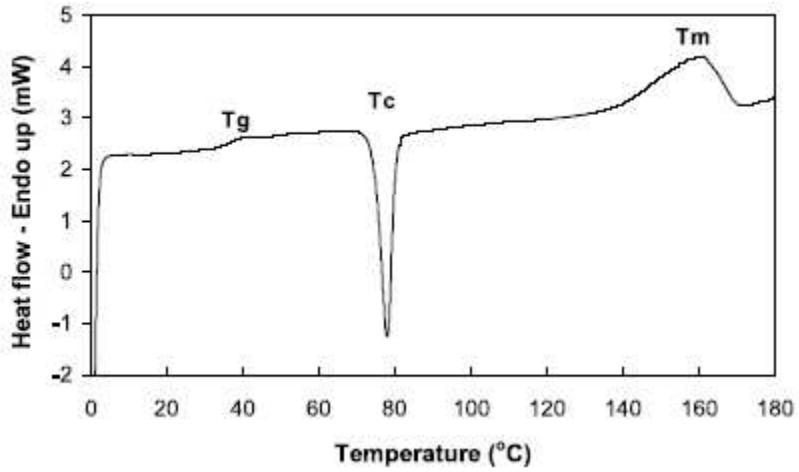


Fig 7. DSC Analysis of Amorphous Sucrose Exposed to Humid Conditions[8]

The T_m point for sucrose is remarkably similar to that of the hydrated form isolated using the methodology employed by Maulny.[2] However, the DSC analysis of the hydrated material reported here does not exhibit either a T_g nor a T_c transition; it can be said that the material discussed is not formed *via* transformation form an amorphous state. Furthermore, the sucrose phase being discussed is highly likely to be a form containing water, as evidenced by the vastly reduced T_m temperature; high water content has been shown to drastically reduce this value.[9]

What can be concluded from this point in the discussion is that the form found is strongly suggested to be a hydrated form of sucrose; it appears that the methodology employed in the crystallisation process allows for some water of hydration to remain within the final sucrose structure. The mechanism for this is not obvious, consequently, the conditions used to form the hydrated phase need to be considered with respect to their ramifications on how sucrose will crystallise from solution.

For crystallisation to occur; a driving force is necessary which results from a difference in concentration, known as supersaturation (*S*). The magnitude of this difference thus affects the crystallisation of sucrose. If this difference puts

sucrose concentration within the labile zone (S = 1.3) of concentrations, spontaneous nucleation results; if in the metastable (S = 1.2), only existing seed crystals will continue to grow but nucleation can occur, and if the solution is under-saturated, no growth will occur.[10]

The crystallisation methodology used to isolate the hydrated form of sucrose utilises a solution having a value of S equal to 0.845 at 50°C, which puts this solution firmly into the under-saturated section of the sucrose supersolubility diagram (see Chapter 1, Section 1.2.2.1, Fig 1). Crystallisation of this solution occurs at 50°C and 50% RH and in essence, the value of S will remain the same. Furthermore, the initial solution is under-saturated since the crystallisation method used employs a 61.5% concentrated solution, which equates to a value of S equal to 0.922 at 20°C. All these points tend toward the notion that the solution used should not yield sucrose crystals. However, this was not found to be the case as crystalline material was formed as shown from the PXRD data in the work by Maulny on this phase and the DSC data shown here (6.2.1. Fig 1).[2]

A possible explanation for the formation of crystalline material from this solution can be found from the work by Mathlouthi and Genotelle on the role of water in sucrose crystallisation.[11] The work showed that sucrose solutions in the range of 44%-67% at 20°C allows the formation of a sucrose nucleus that is stable enough to remain in solution, as such, it can be considered that if one such nucleus can form others will. As such, the solution will eventually crystallise as the concentration will eventually rise to a level which will be tantamount to conditions allowing for crystal growth. What can be inferred from this is that the sucrose solution under these conditions will eventually become supersaturated. Once a solution is supersaturated, crystallisation will eventually occur regardless of what conditions are imposed upon the solution.[10] Therefore the sucrose solution used to form the isolated hydrate form will become crystalline under the crystallisation conditions employed. This crystallisation will take place over a prolonged period of time, as was found.

Therefore, there is evidence for the formation of a crystalline sucrose phase from this (i.e. a 61.5% concentrated solution) solution at an extremely slow rate of crystal growth. This slow rate of growth will be further retarded by the humid atmosphere present in the crystallisation environment. Yoseph *et al*. showed that RH levels of 40-50% reduce the concentration of the sucrose film

that develops on solutions crystallising, and this has the effect of reducing concentration and thus, slowing crystal growth.[12] It can therefore be suggested that the formation of a hydrated form of sucrose may be influenced by both the solution concentration, and the atmospheric conditions under which it is crystallised.

As already stated, all sugars become hydrated in solution; this degree of hydration drops as concentration increases as sugar-sugar interactions start to occur.[4] At the concentration used herein, it has been shown that there are two types of interactions occurring in solution; sugar-sugar and sugar-water interactions.[11] What may occur during crystallisation is that sucrose-sucrose clusters are formed, but, due to the low rate of growth there may be areas between these clusters where there are still sucrose-water molecular interactions. Therefore, if the concentration does not change drastically, such interactions may still exist. As crystallisation proceeds, these sucrose-water molecules may become trapped within a sucrose lattice. As such, these molecules might be the last to become incorporated into the final lattice. Integration of sucrose into a lattice occurs *via* the removal of any water of hydration. which is usually lost to the surroundings. However, in this scenario, the water cannot escape due to the high humidity. Consequently, it can be suggested that this water remains within the lattice by interacting with the surrounding sucrose hydroxyl groups; it becomes shared between sucrose molecules rather than associating with just one. Such retention of water would fit well with the melting behaviour noted via DSC analysis (fig 1) and also coincide with the lack of a noticeable T_g and T_c transitions for the material. Tthe water present in the sucrose structure is not a result of exposing amorphous sucrose to humidity.

6.3.2. Isolation and Formation of a Hydrated Form of Sucrose

As seen from DSC analysis (Fig 2), efforts to re-create solely the isolated hydrated form of sucrose were unsuccessful. All traces showed a mixture of the hydrate and the expected form of sucrose. The reasons for this are not clear. The method outlined by Maulny was followed exactly and the equipment used was the same.[2] It can be suggested that the conditions used this time could not be identical to the conditions experienced previously, which could be attributable differences in the source of sucrose used to variations in the water quality used to

form the solutions. As has been shown, the presence of minerals severely effects the formation of this hydrated phase.[3] The appearance of two separate phases in DSC analysis suggests that the conditions present do have the potential to form this phase; however, there is an unknown factor interfering with its formation.

The appearance of two phases within DSC analysis was also mirrored by analysis obtained from PXRD. All the samples re-crystallised in this manner showed un-expected peaks at the 2θ positions of 11.35°, 15.2°, 17.86° and 20.68°. This strongly suggests the presence of another phase within the material, other than sucrose. The identity of this phase cannot be conclusively elucidated from the data obtained from PXRD. However, it can be suggested that these unexpected peaks relate to the structure of the proposed hydrated form of sucrose. Whilst the previous work by Maulny was suggestive of a potentially hydrated form of sucrose, the evidence herein can be construed to suggest a potential polymorph.

At this stage it recommended that a more rigorous understanding of each of the parameters involved in this method be established to better explain the formation of this phase. Upon successful isolation, an in-depth study of its structure would be necessary in order to ascertain if the isolated form is indeed a polymorph of sucrose, or not. Unfortunately, this lies outside the time limits of the present study.

6.3.3. Formation of a Hydrated Sucrose Phase via Glucose Addition

As can be seen from DSC analysis (Section 6.2.3., Fig 4), the utilisation of a sucrose solution formed at 100°C, followed by the addition of 4-10% w/w of glucose, yielded a material that appears to relate to the hydrated form of sucrose. This is highly unusual, as a typical co-crystallisation methodology and crystallisation conditions were employed. What is evident is that it is the addition of glucose to the sucrose solution that prompts the formation of a hydrated phase.

The heating of the sucrose solution to 100°C followed by crystallisation results in the appearance of two phases, both the hydrated form and the anhydrous form are observable by DSC analysis. What is interesting to note is the difference that an alteration in the crystallisation environment induces on the amount of the hydrated form present. Table 4 shows that crystallisation of this solution at 50% RH results in a larger quantity of the hydrate phase being present

compared to the crystallisation of the solution in a conventional (dry) oven. This is indicated by the higher enthalpy values associated with the melting of the hydrate phase when the solution is crystallised in humid conditions; this suggests a higher amount of this phase being present. Utilisation of a humid crystallisation environment has been shown to slow down the rate of crystal growth, thus it is proposed that, a slow rate of crystal growth is necessary for the formation of solely a hydrated phase.[12]

From this observation, it becomes more apparent what the impact of glucose addition is. It has been shown that the addition of additives to supersaturated sucrose solutions imparts a reduced growth rate; additives stop the inclusion of sucrose onto the growing lattice by blocking appropriate adsorption sites.[13, 14] The glucose added appears to become incorporated within the final sucrose structure. No characteristic reflections are noticeable in PXRD analysis nor can the melting of glucose be observed in DSC analysis. Whilst no characteristic reflections could be observed for glucose, it must be noted that several unexpected peaks un-related to those expected of sucrose, were observed. All the materials in this series showed peaks at the 2θ positions of 11.35°, 15.2°, 17.86° and 20.68°. Similarly, peaks at these positions were also noted in sucrose materials re-crystallised in the absence of glucose. Materials formed in this manner showed two observable phases in DSC and PXRD analysis, one relating to the expected for of sucrose whilst the other may relate to a hydrated form. It can be drawn from this that the presence of a peak at ~ 150°C, along with peaks at the 2θ positions of 11.35°, 15.2°, 17.86° and 20.68° are strongly suggestive of the presence of a different form of sucrose. So, the formation of a potentially new form of sucrose can be induced by the addition of glucose to a re-crystallisation solution.

This reduction in growth rate appears to be sufficient enough to allow the formation of a hydrated phase containing glucose. Further elucidations cannot be found at this time, as already stated, a much clearer understanding of how this unique form of sucrose is obtained from solution is required. It can be confidently proposed that a key factor is the rate of growth of sucrose from solution.

6.4. Conclusions

The formation of an isolated hydrated form of sucrose appears to rely on the rate of crystallisation being slow. This is observable from the crystallisation of a sucrose solution formed at 100°C in a humid atmosphere. The material yielded is a mixture of the hydrated and anhydrous forms of sucrose, drying in a humid atmosphere results in a greater quantity of the hydrate form being present compared to drying in a dry oven. This notion of a slow crystallisation rate is also evident from the addition of glucose to a sucrose solution at 100°C, the inclusion of 4-10% w/w of glucose results in the formation of the hydrated phase. This observation can be partly explained by the reduction in crystal growth rate induced by the addition of glucose.

The mechanism for the formation of solely a hydrated sucrose phase is un-clear but it can be suggested that it relates to the entrapment of water of hydration within the sucrose lattice. Entrapment of water in this manner may arise from the idea that, at the concentrations used, both sucrose-sucrose and sucrose-water interactions are occurring. Formation of the sucrose lattice may result in the trapping of sucrose-water molecules, and the sucrose within this combination, when integrated into the crystal lattice will lose its water of hydration which cannot migrate from the lattice due to its position. Consequently, the final hydrated sucrose material has bound water, which fits well with reports of the melting characteristics of its crystalline form and the amount of water determined to be present.

A clear understanding of the exact parameters that influence the formation of a hydrated phase is lacking, hence a rigorous investigation needs to be undertaken to reliably reproduce this unique phase.

6.5. References

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7. General Conclusions

The co-crystallisation of sugars, by the addition of various saccharides to either a supersaturated sucrose or lactose solution, has allowed information to be obtained on the nature of the materials obtained relative to the processes used. What has become clear is that any added saccharide, to a host, potentially becomes an intra-crystalline species within the host material. This hypothesis is based on evidence from DSC and PXRD data that is strongly suggestive toward this theory. However, the notion of adding to a crystal structure is typically synonymous with elevated unit cell volumes for the host phase. Whilst this has been observed for may systems studied, these elevations have, crucially, been insignificant. Due to this lack of conclusive evidence, the notion of added saccharides becoming included with a host cannot be completely proven. There is a strong argument for this postulation to be garnered from DSC. The enthalpy of melting (for the host phase) is observed to decline, which is also accompanied by depression of the melting point and the onset temperature. This behaviour is strongly suggestive of a host phase becoming doped with an added material. Further evidence for the argument that added sugars become intra-crystalline species can also be drawn from data obtained for the unit cell axis of the co-crystallised materials. With the addition of sugars to a sucrose supersaturated solution, it has been shown that all three axis become significantly elongated as a function of added saccharides. Within lactose co-crystallised materials, it has been shown that the *b* axis becomes elongated; this is twinned with a declination in length of both the *a* and *c* axis. It has been noted that this behaviour *can* be linked with examples of intra-crystalline inclusions reported in literature. But, whilst there is supportive data toward the notion of added sugars becoming intra-crystalline species, it cannot be conclusively proven due to the lack of significance in the unit cell volume data. What this provokes is a need for work on reducing experimental error. A reduction in this may yield robust data that can support, more strongly, the idea that the co-crystallisation of sugars results in the inclusion of the added sugar within the host material.

Whilst there is a substantial volume of data to support the notion of a direct inclusion, there is the possibility that added an added saccharide becomes amorphous. This can be used to explain the formation of co-crystalline materials.

This thesis is in opposition to this assumption since autosorb analysis of co-crystallised materials has shown that they behave in a manner (i.e. they adsorb and desorb atmospheric moisture, amorphous materials only adsorb moisture) that is indicative of crystalline material.

It was observed that, depending on the sugar to be added, variation in the level of addition was found. Some systems showed solely single phases (i.e. only sucrose or lactose) for high levels of added sugars, whilst others did not. The rational behind this can be thought to arise from the behaviour of sugars crystallising from solution, and the effects of foreign species upon this process.

When a supersaturated sugar solution is formed, followed by the addition of a chosen sugar e.g. glucose, equilibrium exists for the adsorption of either the host or impurity onto the growing lattice. At low levels of supersaturation, the adsorption of impurities is preferred over host growth units. This trend is reversed at higher levels of supersaturation and the host growth units are preferentially adsorbed over impurities. For an additive to adsorb and include within the host structure to a high level, it must be able to adsorb rapidly during the time it is preferentially adsorbed, i.e. the level of supersaturation in solution at which an additive is adsorbed preferentially over growth units of the host solution. A good adsorption is probably due to the ability of the additive being able to replicate the H-bond network holding the molecules together in the host material's crystalline structure. This implies that a high degree of structural similarity between the additive and the host is required to enable a high level of additive to be accepted in a co-crystalline mixture. Additives adsorbing in this manner will form a high number of H-bonds with the growing host structure. Consequently, it will bind tightly and therefore not desorb easily. Any sugar adsorbing in this manner will exhibit a high level of inclusion. For example, there is an inclusion of 17% w/w of maltose within a lactose structure (see Section 5.2.2.2) since the only dissimilarity in structure between lactose and maltose is the orientation of one hydroxyl group. Hence, they are similar in structure and consequently include within the lactose lattice. However, this is not always the case, as illustrated by galactose not being included well into a lactose structure. It can be inferred from this that galactose does not adsorb as tenaciously as maltose, and therefore will desorb more easily and will not adsorb as well in the time for its preferential inclusion. What can be concluded from

these examples is that a kinetic factor is relevant in co-crystallisation. Furthermore, the structural similarity between the additive and the host structure is crucial. A good tessellation between the two ensures a high amount of H-bonding that, in turn, allows for the additives to be better entrapment within the host lattice.

Whilst it has been shown that differences exist for the level of inclusion between sugars, it has also been shown that this level can be influenced by modification of the methodologies. This can also be achieved also by including structurally relevant components along with the saccharide.

Starting from a physical blend of the saccharides to be co-crystallised allows for higher limits of sugars to be included within a host lattice. Starting from a physical blend of sucrose and glucose allows for a 100% increase in the amount of glucose incorporable when compared to adding glucose at 128°C. Further increases were also noted when co-crystallising lactose with galactose as well as co-crystallising lactose, galactose and glucose from physical blends (see Sections 5.2.4.5 and 5.2.5.4). The negation of a seeding of a supersaturated lactose solution by galactose and glucose has been shown to be achievable by adding these sugars at 90°C, followed by heating to 110°C. Such increases might be due to the removal of seeding of the supersaturated host solution by the saccharide added (see Section 5.2.5.3). In effect, this will result in a slower rate of crystal growth. Consequently, the period of time for the preferential inclusion of the chosen sugars will be increased. This is reflected in the higher levels of sugars being incorporated (e.g. see Sections 4.2.6. and 5.2.5.3) when using this methodology.

Utilisation of a protocol by adding, a saccharide that does not include well, to a saccharide structurally related to the host, has been shown to be effective in heightening the amount of sugars addable whilst retaining single phases. For example, the simultaneous addition of fructose, along with glucose, to a supersaturated sucrose solution raises the limit of included glucose (see Section 4.2.7). A similar phenomenon was also noted (see Section 5.2.5.2) when glucose was included along with galactose into a supersaturated lactose solution. The amount of galactose incorporated was elevated using this approach. These increases have been attributed to the formation of a pseudo host molecule by the added saccharides. As such, they will replicate the H-bond structure of the host

material and adsorb well. This may lead to higher chances of their irrevocable entrapment in the host crystal lattice.

Interestingly, it has also been shown (see Section 5.2.3) that the utilisation of structurally unrelated additives can also influence, to a small degree, the higher incorporation of saccharides. The simultaneous inclusion of maltose aided by saccharin has been shown to allow 20% w/w of maltose to be included.

Whilst the above summary has been directed toward the effects that added saccharides impart on co-crystallisation, the level of supersaturation can also be a factor. Modifying the level of supersaturation of a sucrose solution, by changing the solution temperature, has been shown (see Section 4.2.3.1 and 4.2.3.2) to influence the amount of incorporable glucose. The lower the temperature, the larger the amount of un-included glucose appears to be the trend. Lower temperatures mean higher values of *S*, consequently, the time between preferential inclusion of additives and a preference for host growth unit adsorption is reduced. As such, there is less time for adsorption and inclusion of glucose. This is reflected in the data garnered (see Sections 4.2.3.1 and 4.2.3.2) from these investigations.

Finally, the formation of a unique form of sucrose has been identified. Modification of the co-crystallisation methodology has allowed for the isolation of a hydrated form of sucrose. Whilst the reasons for its formation have not been conclusively elucidated, it has been shown that a slow rate of crystal growth is needed. This potentially allows for the retention of sucrose-water molecules that become trapped within the sucrose structure. Consequently, the water of hydration cannot readily escape, thus imparting unique characteristics on the resulting material. This is reflected by a unique melting point observed in DSC analysis (see Section 6.2.1).

In summary, the co-crystallisation of sugars appears to rely, primarily, on two factors:

i. The structural similarity between the host structure and the component added. It is proposed that a high degree of tessellation results in a strong adsorption. Consequently, the sugar becomes trapped in the final host structure

ii. The time allowed for the inclusion of the added sugars. The prolonging of time for the preferential adsorption of added sugars appears to be crucial when trying to achieve co-crystallinity.

It is the interplay between these two factors that determines the extent of how well an additive includes into a host structure.

8. Experimental

8.1. Physical Blends of Sugars

8.1.1. Physical Blends of Sucrose, Glucose and Fructose

Sucrose (30g), α-D-glucose monohydrate (7% w/w) and fructose (2-6% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.2. Physical Blends of Sucrose and Galactose

Sucrose (30g) and galactose (4-7% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.3. Physical Blends of Lactose, Maltose and Saccharin

α-D-lactose monohydrate (30g), maltose monohydrate (5-25% w/w) and saccharin (2% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.3. Physical Blends of Lactose, Maltose and Higher Amounts of Saccharin

α-D-lactose monohydrate (30g), maltose monohydrate (25% w/w) and saccharin (4-10% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.4. Physical Blends of Lactose, Maltose and Aspartame

α-D-lactose monohydrate (30g), maltose monohydrate (5-18% w/w) and aspartame (2% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.5. Physical Blends of Lactose and Galactose

α-D-lactose monohydrate (30g) and galactose (4-15% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.6. Physical Blends of Lactose, Glucose and Galactose

α-D-lactose monohydrate (30g), α-D-glucose monohydrate (5-20% w/w) and galactose (5-20% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.7. Physical Blends of Lactose, Galactose and Fructose

α-D-lactose monohydrate (30g), galactose (5-15% w/w) and fructose (5-15% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.8. Physical Blends of Lactose and Polydextrose

α-D-lactose monohydrate (30g) and polydextrose (5-10% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.1.9. Physical Blends of Lactose and Saccharin

α-D-lactose monohydrate (30g) and saccharin (5-20% w/w) were ground together using a pestle and mortar. Ground samples were kept in sample tubes at ambient laboratory conditions of ~ 20°C and 20% RH.

8.2. Co-Crystallisation of Sucrose

8.2.1. Re-Crystallisation of Sucrose

Sucrose (30g) and distilled water (6ml) were heated to 128°C, the viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.2. Co-Crystallisation of Sucrose and Glucose.

Sucrose (30g) and distilled water (6ml) were heated to 128°C, at this temperature, α-D-glucose monohydrate (1-10% w/w) was added and the solution re-heated to 128°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.3. Co-Crystallisation of Sucrose and Glucose at 110°C

Sucrose (30g) and distilled water (6ml) were heated to 110°C, at this temperature, α-D-glucose monohydrate (1-10% w/w) was added and the solution re-heated to 110°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.4. Co-Crystallisation of Sucrose and Glucose at 120°C

Sucrose (30g) and distilled water (6ml) were heated to 120°C, at this temperature, α-D-glucose monohydrate (1-10% w/w) was added and the solution re-heated to 120°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.5. Co-Crystallisation of Sucrose and Glucose on a Smaller Scale

Sucrose (10g) and distilled water (2ml) were heated to 128°C, at this temperature, α-D-glucose monohydrate (1-10% w/w) was added and the solution re-heated to 128°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.6. Co-Crystallisation of Sucrose and Glucose from a Physical Blend

Sucrose (30g) and α-D-glucose monohydrate (4-15% w/w) were ground together using a pestle and mortar, distilled water (6ml) was added and the mixture was heated to 128°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.7. Co-Crystallisation of Sucrose and Glucose at 110°C followed by Continued heating to 128°C

Sucrose (30g) and distilled water (6ml) were heated to 110°C, at this temperature, α-D-glucose monohydrate (4-15% w/w) was added and the mixture was heated to 128°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.8. Co-Crystallisation of Sucrose, Glucose and Fructose

Sucrose (30g) and distilled water (6ml) were heated to 128°C, at this temperature, α-D-glucose monohydrate (7% w/w) and fructose (2-6% w/w) were added and the mixture was re-heated to 128°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.2.9. Co-Crystallisation of Sucrose and Galactose

Sucrose (30g) and distilled water (6ml) were heated to 128°C, at this temperature, galactose (4-7% w/w) was added and the mixture was re-heated to 128°C. The viscous solution was then removed from the heat, agitated vigorously for 30 sec with a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3. Co-Crystallisation of Lactose

8.3.1. Re-Crystallisation of Lactose

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, the viscous solution was then removed from the heat. The solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.2. Co-Crystallisation of Lactose with Maltose

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, maltose monohydrate (7-20% w/w) was added, the solution was then re-heated to 110°C. The solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.3. Co-Crystallisation of Lactose, Maltose with Added Saccharin

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, maltose monohydrate (5-25% w/w) and saccharin (2% w/w) were added simultaneously, the solution was then re-heated to 110°C. The solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.4. Co-Crystallisation of Lactose, Maltose with Higher amounts of Saccharin

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, maltose monohydrate (25% w/w) and saccharin (4-10% w/w) were added simultaneously, the solution was then re-heated to 110°C. The solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.5. Co-Crystallisation of Lactose with Staggered Addition of Maltose and Saccharin

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, maltose monohydrate (18% w/w) was added followed by the addition of saccharin (2% w/w), the solution was then re-heated to 110°C. This process was then reversed with saccharin (2% w/w) being added followed by maltose (18% w/w). For both variations, the solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.6. Co-Crystallisation of Lactose, Maltose and Aspartame

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, maltose monohydrate (5-18% w/w) and aspartame (2% w/w) were added, the solution was then re-heated to 110°C. The solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.7. Co-Crystallisation of Lactose and Galactose

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, galactose (4-15% w/w) was added, the solution was then re-heated to 110°C. The solution was vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.8. Co-Crystallisation of Lactose and Galactose from a Physical Blend

α-D-lactose monohydrate (30g) and galactose (7-10% w/w) were ground together using a pestle and mortar, distilled water (12ml) was then added and the solution was heated to 110°C. At this temperature, the solution was removed from the heat vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.9. Co-Crystallisation of Lactose, Glucose and Galactose

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, α-D-glucose monohydrate (5-20% w/w) and galactose (5-20% w/w) were added, the solution was then re-heated to 110°C. The solution was then remove from the heat and vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.10. Co-Crystallisation of Lactose with Glucose and Galactose Addition at 90°C followed by continued heating to 110°C

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 90°C, at this temperature, α-D-glucose monohydrate (5-20% w/w) and galactose (5-20% w/w) were added, the solution was then heated to 110°C. The solution was removed from the heat and vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.11. Co-Crystallisation of Lactose with Glucose and Galactose Starting from a Physical Blend

α-D-lactose monohydrate (30g), α-D-glucose monohydrate (5-20% w/w) and galactose (5-20% w/w) were ground together using a pestle and mortar, to this, distilled water (12ml) was added and the solution was heated to 110°C. The solution was removed from the heat and vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.12. Co-Crystallisation of Lactose, Fructose and Galactose

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, fructose (5-15% w/w) and galactose (5-15% w/w) were added, the solution was then re-heated to 110°C. The solution was then remove from the heat and vigorously agitated for 30 seconds using a spatula.

Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.13. Co-Crystallisation of Lactose and Polydextrose

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, polydextrose (5-10% w/w) was added, the solution was then re-heated to 110°C. The solution was then remove from the heat and vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.3.14. Co-Crystallisation of Lactose and Saccharin

α-D-lactose monohydrate (30g) and distilled water (12ml) were heated to 110°C, at this temperature, saccharin (5-20% w/w) was added, the solution was then re-heated to 110°C. The solution was then remove from the heat and vigorously agitated for 30 seconds using a spatula. Samples were then transferred to foil dishes and crystallised in an oven at 55°C; samples were allowed to dry until no further loss of moisture could be detected.

8.4. Formation of an Isolated Hydrated Form of Sucrose

8.4.1. Isolation and Formation of a Hydrated Form of Sucrose

Sucrose (15.375g) and distilled water (9.6 ml) were heated to 50°C and held there for 10 min. The resulting solution was filtered through a porosity 4 glass sinter and transferred to a petri dish. The solution was allowed to crystallise at 50°C and 50% RH until completely crystalline.

8.4.2. Formation of a Hydrated Phase via Glucose Addition to a 100°C Sucrose Solution

Sucrose (30g) and distilled water (6 ml) were heated to 100°C, at this temperature; α-D-glucose monohydrate (4-10% w/w) was added. The solution was then transferred to a foil dish and allowed to crystallise in an oven at 55°C; samples were allowed to dry until no further loss in moisture could be detected.

8.5. Methods of Analysis

8.5.1. Powder X-Ray Diffraction

Powder X-Ray diffraction (PXRD) was used to identify all products synthesised, the purity of compounds was verified by use of PXRD. This was done with the aid of the JCPDS (Joint Committee on Powder Diffraction Standards) [1] database, which contains the powder patterns of all the compounds known to date. The Powder Cell program [2] was used to check if the desired phase was formed by comparison with model diffractograms from the JCPDS database. The unit cell dimensions of any materials prepared were calculated using the Checkcell program. [3]

X-rays, being electromagnetic waves, are susceptible to diffraction. Having wavelengths similar to the inter atomic distances in crystals they interact with a crystal lattice in the same way that light interacts with a conventional diffraction grating. In other words, they are dispersed in different directions according to wavelength. An important difference is that X-rays penetrate below the surface of the crystal and rays reflected from successive atomic layers may or may not be in phase. The condition for a maximum of reflected intensity is that the contribution from successive planes should be in phase. If the interplanar spacing is *d*, this condition is expressed by Braggs law.

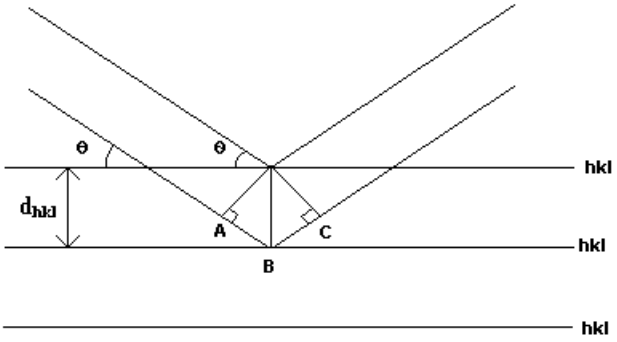

Fig 1. Schematic of In Phase Reflections & Bragg Equation.

$$n\lambda = d\sin\theta \quad (8.1)$$

Where λ is the wavelength of the incoming X-rays, θ is the diffraction angle which is equal to the angle of incidence of the incoming X-rays and *n* is an integer. If the X-ray beam is monochromatic and λ is known, the beam will undergo diffraction by the crystals to form a diffraction pattern of sharp reflections. Measurements of the various θ angles can be used to determine the interplanar spacing *d* characteristic of the diffraction crystal. X-ray powder diffraction provides a powerful technique for identification of polycrystallite compounds.

8.5.1.1. Sample Preparation

The sample was crushed to a fine powder by grinding. The powder was mounted on a flat sample holder and flattened with a glass slide. This ensures that the reflections are due to the crystals rather than irregularities on the surface of the powder. Furthermore, it also helps orientate the crystals in a random manner, this is essential for collection of all possible reflections.

Powder diffraction data were collected using a Siemens D5000 diffractometer. The radiation source was copper Kα run at 30 KV, 40 mA. Traces were collected using a step scan of size 0.02˚ 2θ, over a 10 - 30˚ 2θ range with a time per step of 1 second. The divergence slit used was 1˚ and the receiving slit 0.1˚.

8.5.2. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) is the measurement of heat absorption or heat loss from a sample as a function of temperature. Melting points and phase transitions of various phases can be determined, as well as glass transition (T_g) temperature of compounds. This data provides information about the identity, quality, purity and stability of a wide range of materials.

The use of DSC coupled with other analytical techniques is a powerful method for the characterisation of compounds.

A Pyris series 1 DSC 7 Perkin Elmer connected to a thermal analysis controller Perkin Elmer TAC7/DX was utilised in the analysis of all phases formed. Dry nitrogen was used to purge the sample head and the dry box. The instrument was calibrated using indium at the same temperature scanning rate of the sample. Approximately, 5 – 10mg of the sample was weighed in hermetically sealed aluminium sample pans. The heat of fusion (ΔH) was obtained by integration of the melting endotherm. Samples containing lactose were heated from 25 - 230°C with the scanning rate set at 10°C min^{-1}.

8.5.3. Autosorb Analysis

An autosorb by Biosystems was used to collect data on weight gain of samples when subjected to varying levels of humidity. All the samples were subjected to RH levels of 25%, 50%, 75% and 85%, all the samples are held at each level of RH for three days.

8.5.4. Statistical Significance

There are, regularly, differences between two data points recorded from scientific investigations. For this change to be meaningful, there has to significance in the elevation, or declination of said value. If a value is massively different from another, this cannot be construed as a signficant increase (or decrease) if the calculated level of confidence falls outside of a 95% confidence interval. A difference can fall within two levels that are normally used in statistics; 95% (2σ) and 99% (3σ). How a difference can fall into one of these three levels relates to experimental error, namely, the standard deviation attributed to a measured data point. Clegg has shown a simple method for

calculating the estimated standard difference (e.s.d) for the value of variance between two points.[4] The equation Clegg uses can be seen below:

$$\sigma^2(A-B) = \sigma^2(A) + \sigma^2(B)$$

Where *A* and *B* are the two values being compared. This equation gives the e.s.d. of the difference between two values. If the calculated difference is, for example, 1σ, then the difference falls outside of a 95% (2σ) interval and is insignificant. If the calculated difference is, for example, (3σ) then the difference falls into a 99% confidence interval is definitely significant. Values of σ between 2σ and 3σ can be construed as possibly significant. This method has been utilised for calculated if any difference between values obtained from PXRD analysis are significant.

8.6. References

1. JCPDS-International Centre for Diffraction Data, Swarthermore, P. A.
2. Kraus W. and Nolze G., Federal Institute for Materials Research and Testing
3. Laugier J. and Bernard B., Laboratoire des Materiaux et du Genie Physique Ecole Nationale Superieure de Physique de Grenuble
4. W. Clegg, *The Interpretation of Results*, in *Crystal Strucure Analysis: Principles and Practice*, IUCr Texts on Crystallography 6, 2001, Oxford

Appendix 1

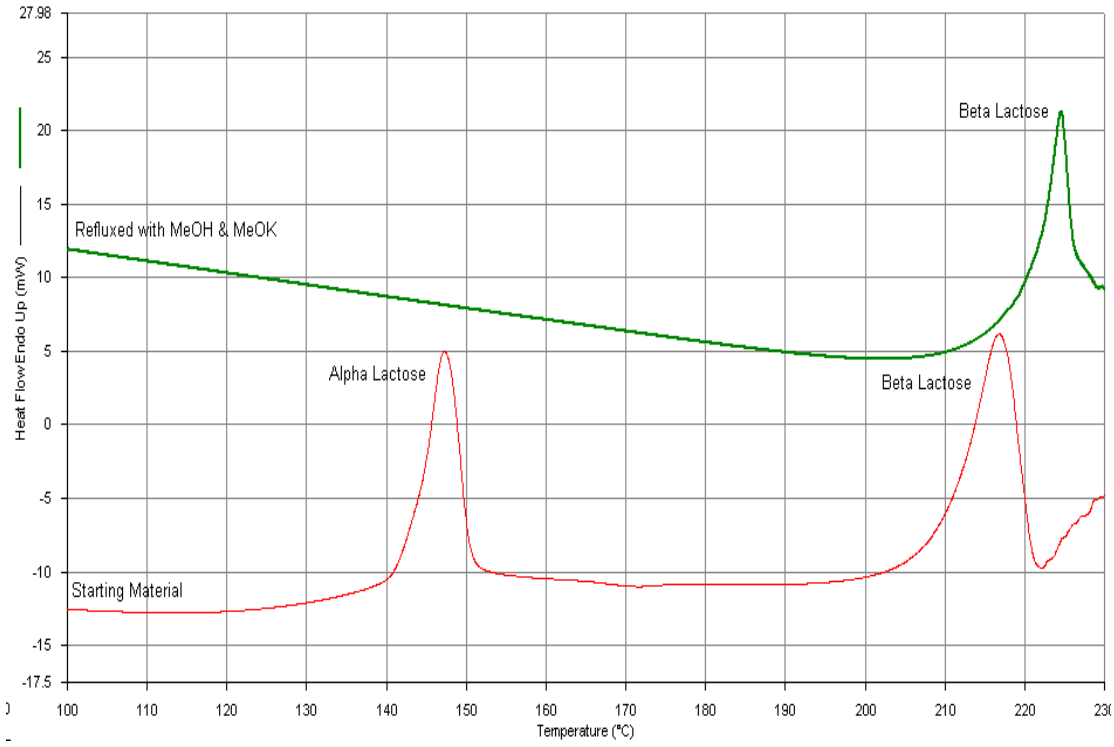

Fig 1. DSC Analysis of α-Lactose Monohydrate and β-Lactose formed by Conversion of α-Lactose Monohydrate

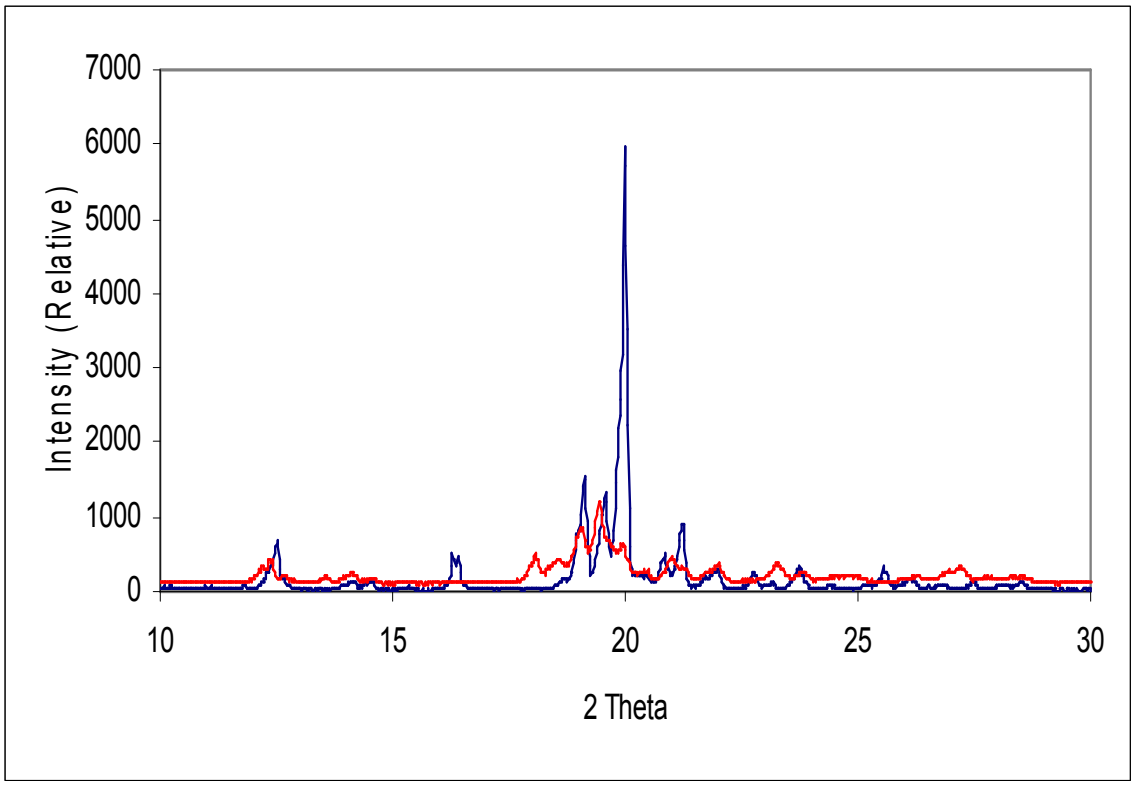

Fig 2. PXRD Diffractograms for α-Lactose Monohydrate (Blue) & β-Lactose formed from Conversion of α-Lactose Monohydrate

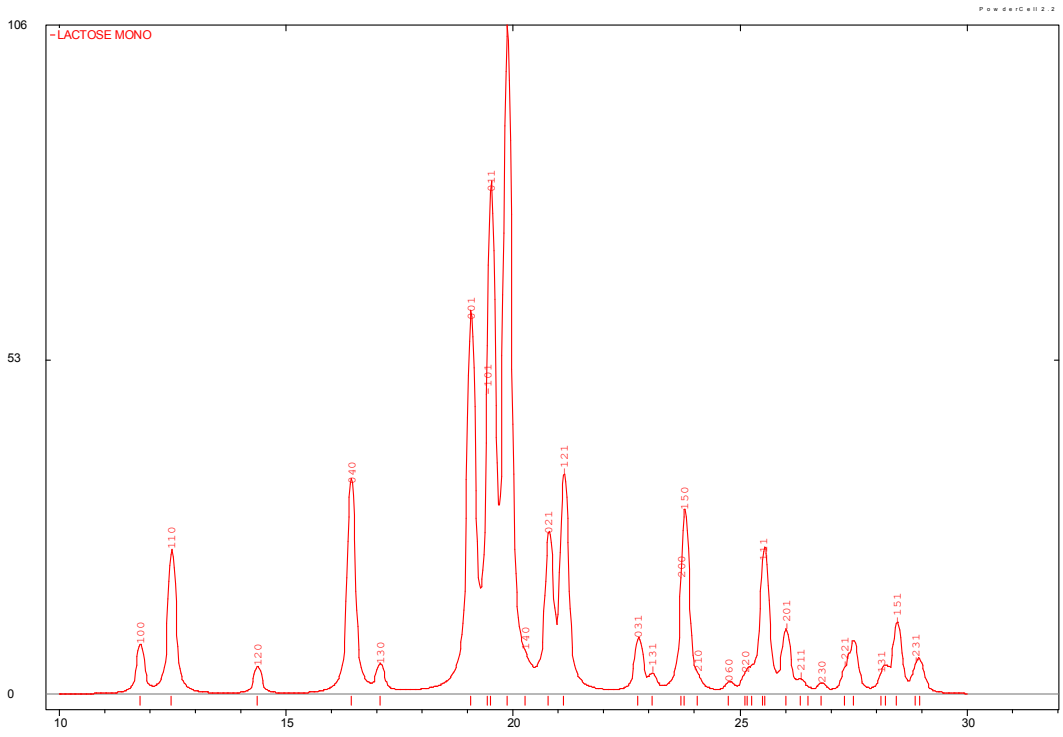

Fig 3. Model Diffractogram for α-Lactose Monohydrate

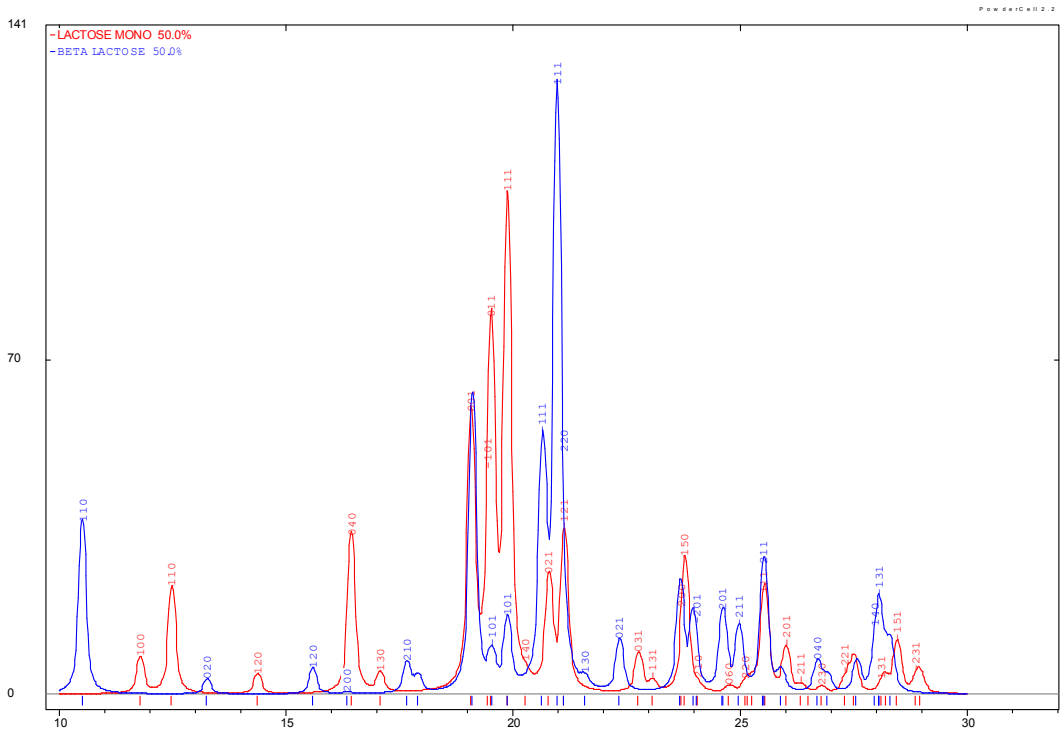

Fig 4. Overlap of Model Diffractograms for α-Lactose Monohydrate (red) and β-Lactose (blue)

Solubility of Crystalline Sucrose in Water

Temp (°C)	**g sucrose in 100g solution**
0	64.45
5	64.90
10	65.43
15	66.04
20	66.72
25	67.47
30	68.29
35	69.17
40	70.10
45	71.90
50	72.12
55	73.18
60	74.26
65	75.37
70	76.48
75	77.59
80	78.68
85	79.74
90	80.77
95	81.74
100	82.65

Solubility of α-D-Lactose Monohydrate in Aqueous Solution

Temp (°C)	**Solubility (g/100 g H_20)**
0	11.9
10	14.9
15	16.9
20	19.1
25	21.8
30	24.8
40	32.8
50	43.5
60	58.4
64	65.8
70	78.3
74	86.2
80	104.6
87.2	122.5
90	138.5
100	157.6
107	177.0
121.5	227.0
133.6	273.0
138.8	306.0
158.8	429.1
178.8	651.9
200	1233.3