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Stabilisation and delivery of labile materials by amorphous carbohydrates and their derivatives

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Abstract

The effective stabilisation of labile biological materials, such as proteins and peptides, requires that stabilising excipients should be glass-forming with high glass transition temperatures (T_g) and processing should be performed at low temperatures (i.e., freeze-drying). Below T_g, any pharmaceutically active material in the amorphous matrix is claimed to be stable. Repeatedly, however, degradation has been shown to occur below T_g. A better predictor of stability has been proposed to be the zero mobility temperature (T_0) which lies below T_g. Thus, to work most effectively a glass-forming excipient must have not only a high T_g but also a high T_0. The importance of T_g, T_0 and other properties of carbohydrates that are advantageous for use as excipients in protein stabilisation are discussed. The perceived advantage of the freeze-drying process is that the rate of degradation of a pharmaceutically active material is reduced. This perceived advantage may not be as great as would be expected due freeze-concentration which accelerates many chemical reactions. The paper describes how a consideration of the relationship between temperature, time and degradation of the pharmaceutically active material has allowed a faster, more effective drying process (Q-T4*sys*®) to be developed. A limitation of all amorphous carbohydrate based systems is that to a greater or lesser degree they are hygroscopic and unable to give controlled release. The paper describes how the SoliDose® system, which utilises non-hygroscopic chemically modified carbohydrates, can offer the potential to stabilise labile biologicals in the glassy state (as in conventional amorphous carbohydrate systems) while allowing for improved bioavailability and controlled release.

Keywords: Stability; Glass; Bioavailability; Controlled release; Freeze-drying

1. Stabilisation

1.1. Use of the glassy state for protein stabilisation

Ideally, it is desirable that a formulation of a drug substance is in the liquid state, since this offers the most convenient and cost effective method of manufacture. However, this is often not possible as the liquid state can give rise to stability problems and the dry state is therefore the necessary condition for the stabilisation of many drug substances, particularly parenterals [1]. The most stable dry state condition is the crystalline state [1], but many potentially useful pharmaceutically active materials, e.g., proteins, do not easily crystallise. For these materials, the formation of the amorphous (glassy) state is a necessity [2]. The glassy preparation derives its stabilising properties from its high viscosity. A considerable body of literature now exists on the formulation of pharmaceuticals for drying to

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the glassy state [3–16]. In essence, the principle is to add a glass-forming excipient (typically a carbohydrate, polymer, protein or amino acid) to a solution of the pharmaceutically active material and to dry this solution under conditions that result in the formation of an amorphous glassy matrix in which the pharmaceutically active material is protected from degradation.

1.2. Crystallisation and glass formation

Dry solids can exist in the crystalline or amorphous (glassy) state. In the crystalline state, the structure is defined and all free water is excluded. In the amorphous state, there is no defined long-range order. An example of an amorphous solid is window glass. This displays all the characteristics associated with a glass including transparency and brittleness. Other materials can exist as glasses including many polymers, metal alloys [17], carbohydrates and carbohydrate polymers [18].

1.3. Excipient selection

To be effective as a glass-forming excipient in a formulation it is desirable that the excipient has a number of properties. These include

- Non-reactivity,
- Good glass former,
- High glass transition temperature (T_g),
- Fragility,
- High zero mobility temperature (T_0), and
- Resistance to crystallisation.

1.4. Non-reactivity

It is important that the glass-forming excipient does not undergo any reaction with the pharmaceutically active protein or peptide. The potential for reaction in systems that involve drying from solution is high. Reactions can take place during preparation of the solution for drying but by far the most important stage of the process is the water removal step. During this step solutes are concentrated, this concentration accelerates intermolecular reactions [2,19]. This concentration step has been shown to not only increase reaction rates but also promote reactions that do not occur in dilute solutions [20].

The Maillard reaction between proteins and carbohydrates is potentially one of the most important factors to consider when selecting excipients. This involves a reaction between the amino groups of proteins and reducing sugars. The result is loss of functionality of proteins and discolouration (browning) of dried preparations. O'Brien [21] has compared sucrose, trehalose and glucose in model freeze-dried systems containing lysine. Not surprisingly the use of glucose (a reducing sugar) caused a browning reaction. The same browning reaction was also observed with sucrose. It was proposed that although sucrose is non-reducing (and thus browning would not be expected) the lability of sucrose results in the formation of glucose. Indeed at low pH the rate of browning was no different to that for glucose [22]. Although sucrose is generally only labile in acidic conditions (below pH 5) it should be remembered that pH can change during drying. Even if a solution is prepared at neutral pH the concentration of a solution during drying can cause the crystallisation of one of the buffer salts which in turn results in drastic pH changes to below pH 2 [23]. O'Brien found no evidence of browning with trehalose, even at temperatures of 90°C. This he ascribed to the greater stability of the trehalose molecule with the disaccharide bond being more resistant to hydrolysis and thus glucose formation.

1.5. Good glass former

Stabilisation of proteins in the glassy state involves concentration and supersaturation of the glass former as the solution is dried. A good glass former must therefore be able to form supersaturated solutions easily rather than crystallise as the solubility limit is reached. Chang

and Randell [24] have studied a range of carbohydrates and salts commonly used in protein formulations and observed three types of behaviour; glass-forming, eutectic and doubly unstable. Glass-forming materials easily form glasses on concentration. Eutectic materials always crystallise as they are concentrated. Doubly unstable materials form glasses on concentration but crystallise when the T_g is exceeded during processing or storage. For protein stabilisation, where a stable glassy state is needed, glass-forming excipients are required (such as sucrose and trehalose). Doubly unstable excipients, such as mannitol can be used, but usually require the addition of other stabilising agents, such as amino acids, to limit devitrification. This adds further complication to the formulation process.

1.6. High T_g

The stability of the final product depends on the T_g of the dried preparation. Many excipients, such as buffers or salts, are added for specific purposes not related to processing and these often depress the T_g of the final product. An excipient added to aid glass formation must therefore have a high T_g to compensate for this depression. Consequently, the glass-forming excipient should be selected on the basis of its compatibility with the end-use application and on its glass transition temperature (T_g). The values of T_g for commonly used glass-forming excipients reported in the literature do vary. In part this is due to differences in interpreting DSC thermograms and also the purity of the excipients used. For instance a small amount of water can significantly depress T_g. Table 1 shows the T_gs for a range of anhydrous carbohydrates. There is a general trend for T_g to increase with molecular weight.

As discussed earlier, the excipient must have a high T_g to provide for stability in the final formulation. As the T_g of anhydrous trehalose is 120°C, it is the preferred excipient for protein stabilisation in dried products [6,7,11,14,25–31] and for haemoglobin-based blood products [32],. Even in the presence of 1–2% residual moisture the trehalose glass will have a T_g of above 90°C offering potential to protect a protein even under extreme conditions.

Table 1
Consensus values of T_g for glass-forming excipients

Glass former	T_g (°C)
Trehalose	120
Ficoll	110
Dextran	83
Sucrose	77
Maltotriose	76
Lactose	70
Maltose	44
Glucose	30
Fructose	13
Galactose	10
Sorbitol	−2
Xylose	−10
Ribose	−10
Glycerol	−93

1.7. Fragility and high T_0

To be effective as a stabilising system, the pharmaceutically active material must be dried into the glassy state such that the amorphous product is physically stable and the pharmaceutically active material within it must be chemically stable. It is widely believed that chemical stability of an pharmaceutically active material is a direct function of the T_g of the dried product [33]. Only if T_g is exceeded during storage can the product physically collapse, individual components crystallise or the pharmaceutically active material degrade [34].

Although the relationship between the *physical* stability of the amorphous formulation and T_g is established, it has now been shown that T_g is not the upper limit of *chemical* stability—some reactions still proceed below this temperature [35]. The amount of degradation varies depending on the glass former [4]. Examples include the stability of catalase which cannot be related to the T_g of the stabilising excipient [36] and the poor storage stability of human growth

hormone (hGH) in a dextran formulation, even though it has a high T_g [34].

Angell [37] has recently proposed the concept of 'strong' and 'fragile' glasses. Strong glasses show a broad glass transition by differential scanning calorimetry indicating a gradual loss of rigidity as configurational entropy increases with increasing temperature. By contrast, fragile glasses show a sharp glass transition, indicating a sudden change from the glassy to a more fluid rubbery state. As temperature is reduced below T_g, there is a continuing loss of configurational entropy. At some temperature, T_0, configuration entropy reaches zero. This temperature is the zero mobility temperature since, in the absence of entropy, molecular motion effectively stops.

Hancock et al. [38] have studied T_0 in carbohydrate based pharmaceutical systems and proposed that this, rather than T_g, defines stability. Using a different technique to determine T_0, a study of sucrose and trehalose has shown that the T_0 value for sucrose is 3.5°C [39]. The $T_g - T_0$ difference is 61.5°C this is in good agreement with the value of 60°C found by Hancock et al. [38]. It is proposed that to ensure the long-term stability of a product stabilised with sucrose, it is advisable to store the product below its T_0 temperature of 3.5°C. The T_0 value of trehalose at 44°C is sufficiently high so that room temperature storage, with guaranteed stability, is possible. It is interesting to note that Pikal [40] rates the stabilising properties of trehalose as greater than dextran. Dextran, although not analysed here in detail, has a broad glass transition indicating a strong glass. Thus, the ranking of Pikal appears to correspond to fragility—the more fragile the glass, the better it is as a stabilising agent.

Employing the concepts of T_g and T_0 we can categorise the occurrence of chemical reactions into three regions. Above T_g, all chemical reactions can potentially proceed. Reactions such as aggregation that require significant molecular motion will stop at T_g. Between T_0 and T_g reactions that involve small chemical species such as oxidation and hydrolysis may potentially proceed although at very low rates. Below T_0, all reactions stop.

1.8. Resistance to crystallisation

Differential scanning calorimetry studies performed on sucrose [41] show that sucrose crystallises 30 to 40°C above the T_g. Trehalose does not crystallise under the same conditions. Indeed, we have found that samples of trehalose glass can be held at 180°C, well in excess of T_g, for periods in excess of 48 h with no crystallisation.

In the DSC, where heating rates of 5 or 10°/min are used, crystallisation of sucrose takes place 30 to 40°C above T_g . However, crystallisation above T_g is a function of both $T - T_g$ and time. Thus, if a sucrose glass is heated more slowly, then crystallisation will occur much closer to T_g. The implications for stability during storage are significant. Many products are subject to temperature abuse during transport and storage [42] and under such conditions T_g may be exceeded. Thus, if a sucrose based product is subjected to even 1 h at a temperature 5°C above the glass transition, there is a high risk that crystallisation will take place. As a result, the protective effect of the amorphous matrix that the sucrose glass provides will be permanently lost, even if the product is subsequently returned to lower temperatures. It could be argued that this is not significant because a sucrose-based product could be formulated to have a T_g much in excess of the temperatures it will experience during storage. Of course, this is possible, but relies either on formulation with additional excipients with higher T_gs (e.g., HSA) or very thorough drying. It should be noted that a water content of 2 to 3% is typical of many products after 1–2 months storage, as moisture ingress from the stopper into the product takes place [43]. Moisture content of this magnitude reduces the T_g of sucrose to between 28 and 40°C [41]—temperatures that are commonly experienced during storage. As trehalose does not easily crystallise above T_g,

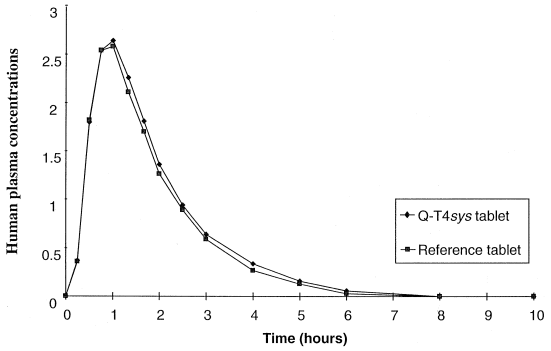

Fig. 1. Plasma concentration of an antibiotic stabilised by the Q-T4*sys*®, in a tablet presentation, following storage at 40°C for 3 months relative to a reference tablet stored at 4°C.

trehalose based formulations are much more resistant to temperature abuse than might otherwise be expected.

1.9. Process methods and stability

The perceived advantage of the freeze-drying process is that low temperatures are employed and, thus, the rate of degradation of an pharmaceutically active material is reduced. This perceived advantage of low temperatures may not be as great as would be expected. Firstly, freeze-drying involves freezing and this in turn leads to freeze-concentration which accelerates many chemical reactions. Secondly, even if rates of reaction are reduced, freeze-drying is a comparatively long process often taking a number of days.

The result of an analysis of the physico-chemical interactions between glass-forming excipients and processing is the development of the controlled evaporative drying system Q-T4*sys*®. The system utilises conventional processing equipment and provides the following advantages for the stabilisation of labile biologicals:

- Enhanced storage stability,
- Enhanced thermal stability,
- Rapid processing,
- Freeze-avoidance,
- Simple formulation, and
- Extended range of labile materials that can be stabilised.

1.10. Applications of Q-T4sys® drying

The Q-T4*sys*® formulation and ambient temperature drying process have been used for the stabilisation of a number of labile materials.

1.10.1. Stabilisation of an antibiotic

A labile antibiotic was dried using trehalose and the Q-T4*sys*® process. Following 3 months storage at 40°C, an in vivo bioequivalence study was performed against reference material that had been stored at 4°C. As can be seen in Fig. 1, the Q-T4*sys*® formulation is bioequivalent to the reference material indicating that the antibiotic has been stabilised against storage at 40°C.

1.10.2. Stabilisation of a monoclonal antibody

Fig. 2 shows the residual activity of a monoclonal antibody measured by agglutination via specific cell surface antigen following formulation with trehalose and processing by Q-T4*sys*® technology. After 24 months, 100% activity remains after storage at 52°C.

1.10.3. Stabilisation of hGH

Fig. 3 demonstrates the stability of a Q-T4*sys*® stabilised preparation of hGH when stored at 25°C for over a year. A commercial

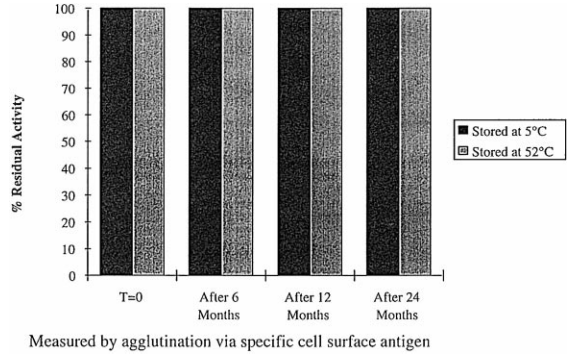

Fig. 2. Residual activity of a monoclonal antibody following stabilisation by the Q-T*sys*® and storage at 52°C for 2 years.

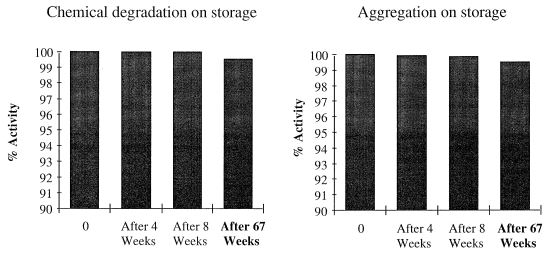

Fig. 3. Degradation (measured by reverse phase HPLC) and aggregation (measured by gel filtration HPLC) of Q-T4*sys*® stabilised hGH following storage for 67 weeks at 25°C.

formulation used as a comparator showed an increase in aggregates (measured by gel filtration HPLC) of 5% and a reduction in purity (by reverse phase HPLC) of 12% over a shorter 5-month storage period.

1.10.4. Stabilisation of cellular systems

The above has demonstrated that trehalose and the Q-T4*sys*® technology can be used to stabilise isolated labile materials. The stabilisation of cellular materials is complicated by the presence of cell walls and membranes. However, as shown in Fig. 4, when trehalose is able to form a glass within the cell and stabilise the cellular contents in the glassy state it is possible to stabilise intact cells.

1.10.5. Viral inactivation

Many proteins are extracted from plasma. The potential for viral contamination requires that viral inactivation procedures are applied. For selective viral inactivation by heat, the soluble proteins in plasma need to be protected whilst the virus remains susceptible. Protection of soluble proteins from these degradation reactions can be achieved by formulating the plasma in an amorphous carbohydrate glass. Heat inactivation is carried out at temperatures greater than 60°C, thus, the T_g of the formulation must be high. Trehalose with a glass transition of 120°C allows viral inactivation at temperatures up to 90°C—even in the presence of residual moisture. Formulations based on sucrose (which has a T_g of 77°C when anhydrous) generally have lower T_gs and are not therefore stable at such elevated temperatures.

The formation of the glassy state can be used selectively to stabilise plasma proteins and inactivate viruses when a dried product is exposed to dry heat. Trehalose protects soluble proteins but as trehalose does not, unless special conditions are used, penetrate inside the virus, selective inactivation is possible. To be effective a balance must be obtained between residual moisture content (this enables effective viral inactivation) and T_g (the glass protects the activity of the plasma proteins). It is generally perceived a residual moisture content of $> 1.0\%$ is required [44].

When containing sufficient residual moisture to allow viral inactivation, the majority of glass-forming excipients have a T_g that is insufficient to protect the plasma proteins. An exception is trehalose, which when dried with plasma results in a product which can be heated for viral inactivation without damage of soluble proteins. As shown in Fig. 5, when formulated with trehalose and dried by the Q-T4*sys*® process an enzyme is stabilised against the high

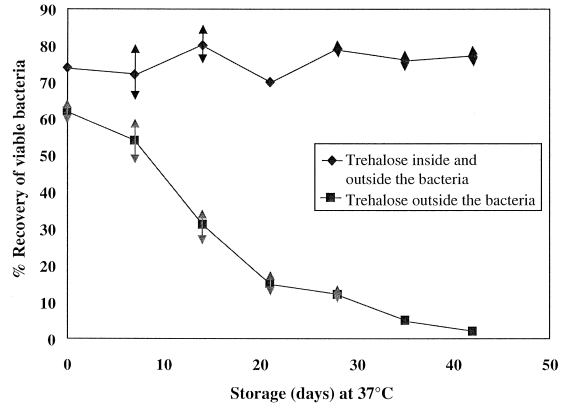

Fig. 4. Stability of *E. coli* 9484 stabilised by the Q-T4*sys*® process (resulting in glass formation both inside and outside the cell) compared to a control with glass formation only outside the cell.

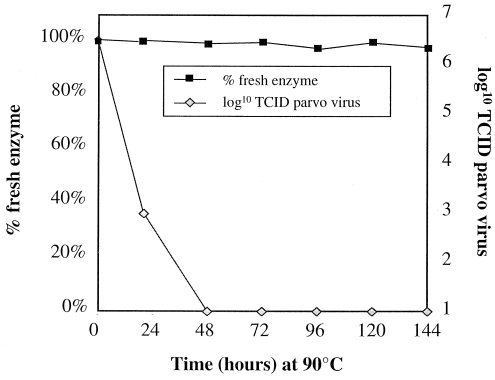

Fig. 5. Viral inactivation of parvovirus at 90°C occurs while the Q-T4*sys*® stabilised alkaline phophatase remains fully active.

temperatures (up to 90°C) while the virus is inactivated.

2. Bioavailability and controlled release

2.1. Overcoming the limitations of carbohydrate glasses

A limitation of all amorphous carbohydrate based systems is that to a greater or lesser degree they are hygroscopic and unable to give controlled release. On the other hand, current controlled release systems and processing techniques, often developed with robust classical pharmaceutically active materials, are unable to provide sufficient stability to a labile biological material to allow its commercial exploitation in a controlled release form. With the growth in the development of clinically effective biopharmaceuticals, this problem of stability combined with delivery is becoming ever more important. The SoliDose® system utilises non-hygroscopic chemically modified carbohydrates to stabilise labile biological materials, particularly proteins, in the glassy state. The SoliDose® system can be used to provide a range of final presentation formats from conventional tablets through to small particles. Such modified carbohydrates offer the potential to stabilise and pharmaceutically active material in the glassy and provide for the following additional benefits:

- Controlled release rates from hours to months even from small particles,
- Improved bioavailability,
- Minimal hygroscopicity.

A number of different processing methods for the SoliDose® system can be used. These are simple currently used commercial processes for example, co-melting, solvent evaporation, solvent sublimation, spray drying and extrusion.

2.2. Controlled release

Controlled release can be obtained from small particles (< 5 μm) such as required for pulmonary delivery. Alternatively, the particles can be larger. Fig. 6 shows the in vivo plasma level of a small hydrophilic molecule when administered, as particles of circa 20 μm, orally to dogs. As can be seen, the effective therapeutic blood level can be maintained for in excess of 12 h.

2.3. Enhanced bioavailability

In addition, to be able to provide for controlled release, the SoliDose® system can when formulated correctly provide for enhanced bioavailability. Fig. 7 shows the results from an in vivo study of a small poorly soluble, hy-

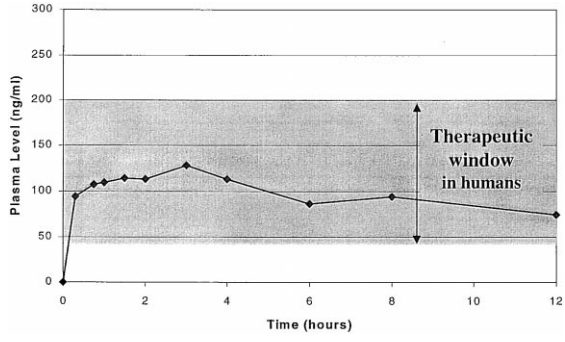

Fig. 6. Controlled release of a small hydrophillic molecule administered to dogs in a SoliDose® formulation.

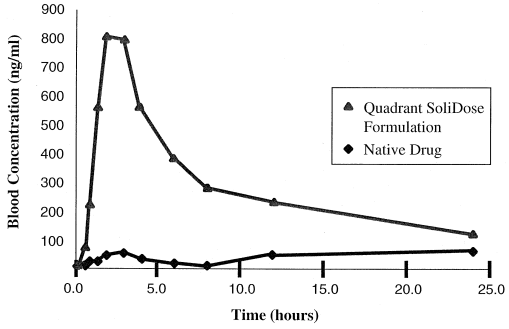

Fig. 7. Enhanced bioavailability of a hydrophobic peptide in dogs following SoliDose® formulation. The control is the native drug substance.

drophobic peptide. The incorporation of the peptide into the amorphous SoliDose® formulation substantially increases the bioavailability as indicated by the increase in the area under the curve.

3. Conclusion

The glassy state is important in the stabilisation of labile peptides and proteins but factors such as non-reactivity, glass-forming ability, T_0 and resistance to crystallisation in addition to the T_g must be considered when selecting an excipient. The properties of trehalose are favourable against all these factors and this may account for the often superior stabilising performance of trehalose when compared with other excipients. Modification of the trehalose molecule can allow the potential for the stabilising properties of glasses to be exploited while permitting enhanced bioavailability and controlled release.

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