FROZEN STATE TRANSITIONS IN RELATION TO FREEZE DRYING

Y. H. Roos

Department of Food Technology, PO Box 27 (Viikki B), FIN-00014 University of Helsinki, Helsinki, Finland

Abstract

Freeze-drying is used as a gentle dehydration method for heat sensitive materials especially in food and pharmaceutical industries. Most materials, including dissolved sugars in water, do not crystallise during freezing prior to freeze-drying. Supersaturated, freeze-concentrated solutions are amorphous materials and they solidify into a glassy state when their temperature is depressed to below the glass transition temperature, T_g. Differential scanning calorimetry has been used to show that maximally freeze-concentrated sugar solutions, when properly frozen, show during heating a glass transition, T_g', which is followed by ice melting endotherm with onset at T_m'. Low molecular weight materials are difficult to freeze-dry as they have low T_g', and T_m' slightly above T_g'. High molecular weight materials, such as carbohydrate polymers, exhibit improved dehydration characteristics and they have T_g' and T_m' at about the same temperature close to the melting point of pure water. The amorphous, glassy structure typical of freeze-dried materials is formed during prefreezing and retained after removal of ice and the unfrozen water from the freeze-concentrated material. Dehydration temperatures below T_g' allow removal of ice within the solid, glassy solutes, but temperatures above T_m' result in collapse. The frozen state transitions and properties of freeze-dried materials can be shown in state diagrams which are used to derive proper freeze-drying conditions and storage requirements for various materials.

Keywords: collapse, freeze-drying, glass transition, ice melting, water plasticisation

Introduction

Freeze-drying is an important drying method providing a gentle dehydration of heat sensitive materials especially in food and pharmaceutical industries. A successful freeze-drying process retains the volume of the material which, in the dry state, is usually highly porous, brittle, and hygroscopic, but has excellent rehydration properties. However, the structure of a number of materials, in particular, that of those containing sugars may collapse during dehydration if the temperature of ice within the material is higher than the collapse temperature, T_c, of the material. The collapsed materials exhibit poor dehydration properties and loss of desired quality characteristics [1].

It is well known that materials which form eutectic solutions with water, such as salts, collapse if the freeze-drying temperature is higher than the eutectic melting temperature, T_e, e.g., −22°C for NaCl [2]. Such collapse results from the presence

of liquid water that is removed by evaporation. Obviously, eutectic materials exist as crystalline solids after freeze-drying. However, most organic and biological materials, including dissolved sugars in water, do not crystallise during freezing prior to freeze-drying. During the prefreezing step water is removed from solution as ice, and the solute phase becomes freeze-concentrated. Freeze-concentration increases with decreasing temperature and at temperatures below T_e the viscosity of the freeze-concentrated, unfrozen solute phase also increases, as, in addition to the lowering temperature, water is removed from the supersaturated solution as ice. At low temperatures the high viscosity also delays ice formation and the glassy state is formed before all water is removed from the solution as ice [3]. Ice formation ceases due to glass formation and, therefore, frozen materials at low temperatures often contain maximally freeze-concentrated, amorphous solutes [4, 5]. Differential scanning calorimetry (DSC) has been used to show that sugar solutions, when properly frozen, show during heating a glass transition which is followed by the ice melting endotherm [5]. The glass transition is observed at a material specific, initial concentration independent temperature which is usually referred to as the glass transition temperature of the maximally freeze-concentrated solute matrix, T_g' [4, 5]. The T_g' is followed by ice melting which has its onset at T_m' [5]. Low molecular weight materials with low T_g' have T_m' slightly above T_g' while high molecular weight materials, such as carbohydrate polymers, have T_g' and T_m' at about the same temperature close to the melting point of pure water [6]. Most maximally freeze-concentrated carbohydrate matrices contain about 80% w/w solids and 20% w/w unfrozen water and have T_g' well above the T_g of pure water of $-135°C$ [7, 8].

    The frozen state transitions of various materials have proved to affect various material properties in cryopreservation and freeze-drying. The main emphasis of the present article will be on establishing relationships between the observed transitions of freeze-concentrated solids and water, and on the effects of the transitions on freeze-drying behaviour and physicochemical properties of the freeze-dried materials. Our premise is that the control of the prefreezing and freeze-drying processes, on the basis of knowledge of frozen state transitions, can be used to decrease quality changes and predict the drying behaviour of various materials.

Phase and state behaviour of solutes and water

Freezing of pure water

    The principle of freeze-drying is based on the phase behaviour of pure water. It is well known that at atmospheric pressure the physical state of water is dependent on temperature, and water may exist as ice, liquid water, and water vapour. A reduction in pressure results in a decrease in boiling temperature until the triple point pressure of 6.104 mbar, at which all three phases are at equilibrium, is reached. The triple point temperature is 0.0099°C below which water may exist only as ice or water vapour. The main requirement for freeze-drying, i.e., removal of water by sublimation directly from the solid to the vapour state is that the temperature is below that of the triple point. This can be achieved by lowering temperature or pres-

sure. In common freeze-drying methods the pressure is decreased to well below that of the triple point, and used to control temperature of the sublimating ice.

Effects of solutes on ice formation

Solutes in water reduce vapour pressure, which in dilute solutions occurs according to the Raoult's law. The Raoult's law states that the vapour pressure of water in a solution, p, is equal to the product of vapour pressure of pure water, p_0, at the same temperature multiplied by its mole fraction, x_w, i.e., $p = p_0 x_w$. The requirement for ice formation is that the vapour pressure of water is higher than the vapour pressure of ice, p_i, at the same temperature. The equilibrium melting temperature is the temperature where $p = p_i$. Therefore, the equilibrium melting temperature of water in solution is lower than that of pure ice, as the vapour pressure of water in solution equals that of ice at a lower temperature. However, ice formation in solutions results in freeze-concentration of solutes and there is a dynamic decrease in ice melting temperature with increasing ice formation due to the decreasing vapour pressure of unfrozen water. The various effects of solutes on ice formation and melting temperature are shown schematically in Fig. 1. It is also important to notice that ice formation above the eutectic temperature can be considered as equilibrium ice formation while nonequilibrium ice formation occurs at temperatures below T_m' where ice formation becomes kinetically restricted [9].

Glass transition

Supercooled liquids may exist as solid, glassy materials or in a viscous liquid state. The transition between the glassy and supercooled liquid states occurs over a glass transition temperature range. The transition exhibits properties of a second-order phase transition, and it can be detected from changes in a number of thermal, mechanical, and dielectric properties [9]. The transition is often determined by DSC which detects a change in heat capacity, ΔC_p, that occurs over the glass transition temperature range. In water–solute systems the transition occurs over a temperature range located between the glass transition temperatures of the pure components. The glass transition temperature is often given as the onset or midpoint temperature of the calorimetric glass transition temperature range. The glass transition temperature of pure water in various studies has been found to occur between –144 and –134°C [9], but the values found for ΔC_p over the transition have varied from 0.1 [10] to 1.94 J $(g\ °C)^{-1}$ [11]. However, the values often used as T_g and ΔC_p for water have been –135°C [8] and 1.94 J $(g\ °C)^{-1}$ [11], respectively. Most freeze-dried materials consists of amorphous solids in which water acts as a plasticizer. The T_g of stable, low-moisture, freeze-dried materials must be well above storage temperature, as the materials cannot support their own weight and collapse due to liquid flow when stored at temperatures above T_g [9, 12]. The plasticizing effect of water is observed from a dramatic decrease of T_g with increasing water content of the material. Depression of T_g to below storage temperature, as a result of water plasticization, may cause stickiness, caking, collapse, crystallization of amorphous sugars, loss of encapsulated compounds, and other quality defects [9, 12].

State diagrams

State diagrams are used to characterize the physical state of amorphous, biological materials as a function of temperature and water content [13]. They are used to show the T_g as a function of water content and they often contain information of solubility limits and effects of freeze-concentration on transition temperatures.

State diagrams can be based on experimental measurements of T_g, solubility, and equilibrium melting temperature at various water contents [5–7, 9]. Completely amorphous materials with low water contents can be obtained, e.g., by freeze-drying or rapid cooling from melt. The water content of the material can be
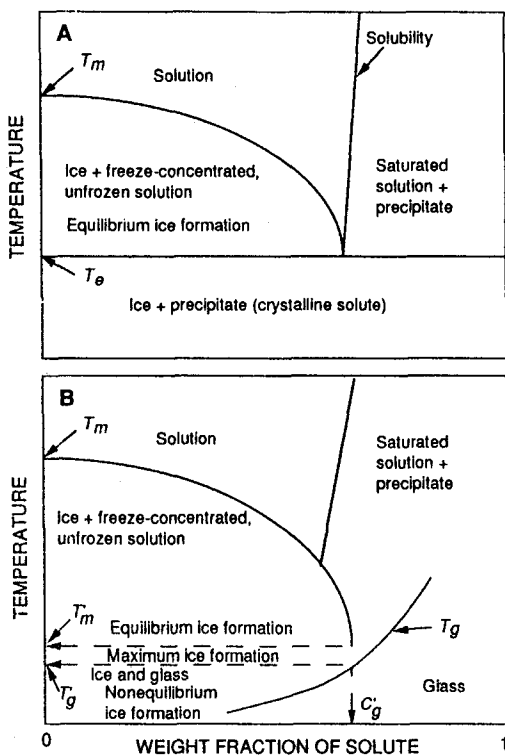

Fig. 1 Ice formation in solutions. A. Ice formation in an eutectic solution occurs at equilibrium according to the melting temperature, T_m, curve until both water and solute crystallize at the eutectic temperature, T_e. B. Ice formation in biological materials occurs often according to the T_m curve at equilibrium, but as the solutes do not crystallize maximum ice formation occurs between the onset temperature of ice melting within the maximally freeze-concentrated solute phase, T'_m, and glass transition temperature of the maximally freeze-concentrated solute phase, T'_g. The T_g curve indicates the glass transition temperature at various solute concentrations, and the solute concentration for the maximally freeze-concentrated solute matrix is given by C'_g. Nonequilibrium ice formation, controlled by the viscosity of the freeze-concentrated unfrozen matrix and T_g, occurs in less than maximally freeze-concentrated solutions at temperatures below T'_g

adjusted by rehumidification or addition of water. However, the transition tempera-
tures are extremely sensitive to water and a homogeneous distribution of an exactly
known water content is required. Determination of the frozen state transitions re-
quires water contents higher than 20% w/w which is often the unfrozen water con-
tent of maximally freeze-concentrated solutes [9]. The glass transition curve can be
predicted using the Gordon-Taylor [14] or Couchman-Karasz [15] equations which
can be fitted to experimental T_g data. The equations define the T_g of the mixture of
solids and water in terms of component T_g (T_{g1} and T_{g2} for solids and water, respec-
tively), weight fractions (w_1 and w_2 for solids and water, respectively), and a con-
stant, k, in the Gordon-Taylor Eq. (1) or the change in heat capacities, ΔC_{p1} and
ΔC_{p2}, over the glass transitions of solids and water, respectively, in the Couchman-
Karasz Eq. (2). The use of the Gordon-Taylor equation is often the most conven-
ient, as the determination of ΔC_{p1} can be difficult and the existing differences be-
tween the reported ΔC_{p2} values.

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \qquad (1)$$

$$\ln T_g = \frac{w_1 \Delta C_{p1} \ln T_{g1} + w_2 \Delta C_{p2} \ln T_{g2}}{w_1 \Delta C_{p1} + w_2 \Delta C_{p2}} \qquad (2)$$

State diagrams have been reported for various sugars [7], polysaccharides [16],
cereal proteins [17], and sugar-protein mixtures [18]. A typical example of state
diagrams is that of sucrose (Fig. 2) showing the glass transition curve, T_g', T_m', and
equilibrium melting temperature, T_m. The solute concentration in a maximally
freeze-concentrated matrix is given by C_g'. T_g, T_g', T_m', C_g', and constant k of the Gor-
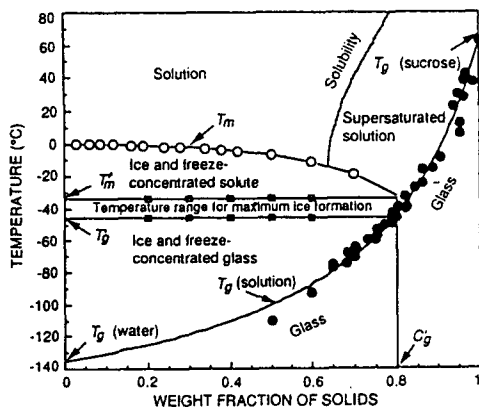don-Taylor equation are given for various sugars in Table 1.

Fig. 2 State diagram for sucrose showing experimental and predicted data for equilibrium
ice melting temperature, T_m, onset temperature for ice melting within the maximally
freeze-concentrated solute matrix, T_m', glass transition temperature of the maximally
freeze-concentrated solute matrix, T_g', with concentration, C_g', and glass transition
temperature, T_g, as a function of solute concentration. After Roos [9]

Table 1 Glass transition temperatures, T_g, for various sugars and sugar alcohols. Maximum freeze-concentration occurs between T_g' and T_m' which are the glass transition temperature of the maximally freeze-concentrated solutes with solute concentration of C_g' and onset of ice melting wthin the maximally freeze-concentrated solute matrix, respectively. The constant, k, is that used in the Gordon-Taylor equation to obtain C_g' [7, 9].

Compound	$T_g/°C^a$	$T_g'/°C^a$	$T_m'/°C$	k	$C_g'/(\%~w/w)$
Pentoses					
Arabinose	–2	–66	–53	3.55	79.3
Ribose	–20	–67	–53	3.02	81.4
Xylose	6	–65	–53	3.78	78.9
Hexoses					
Fructose	5	–57	–46	3.76	82.5
Fucose	26	–62	–48	4.37	78.4
Galactose	30	–56	–45	4.49	80.5
Glucose	31	–57	–46	4.52	80.0
Mannose	25	–58	–45	4.34	80.1
Rhamnose	–7	–60	–47	3.40	82.8
Sorbose	19	–57	–44	4.17	81.0
Disaccharides					
Lactose	101	–41	–30	6.56	81.3
Lactulose	79	–42	–32	5.92	82.0
Maltose	87	–42	–32	6.15	81.6
Melibiose	85	–42	–32	6.10	81.7
Sucrose	62	–46	–34	5.42	81.7
Trehalose	100	–40	–30	6.54	81.6
Oligosaccharides					
Raffinose	70	–36	–28	5.66	84.1
Sugar alcohols					
Maltitol	39	–47	–37	4.75	82.9
Sorbitol	–9	–63	–49	3.35	81.7
Xylitol	–29	–72	–57	2.76	80.2

[a]Onset of the glass transition temperature range

Structure formation

Ice formation

At freezing temperatures ice formation occurs according to the equilibrium melting temperature curve until the freezing temperature is sufficiently low to allow maximum ice formation. As shown by the state diagram for sucrose (Fig. 2) maximum ice formation occurs over the temperature range of T_g' to T_m'. Separation of

water as ice and the increase in solute concentration in the unfrozen phase results in an increase in viscosity. At temperatures below T'_m but above T'_g the viscosity of the unfrozen phase increases significantly and ice formation becomes extremely slow, as the viscosity increases to above 10^7 Pa s [5]. At temperatures below T'_g the viscosity of the unfrozen phase reaches the typical value of 10^{12} Pa s for glassy materials and ice formation ceases.

Time-dependent ice formation is often observed in DSC scans of rapidly cooled solutions. Heating scans of rapidly cooled sugar solutions exhibit a glass transition at a relatively low temperature and a devitrification exotherm at a higher temperature which is followed by the ice melting endotherm. As shown schematically in Fig. 3, the exotherm occurs due to ice formation during reheating. No exotherm appears after proper annealing and maximum ice formation at $T'_g < T < T'_m$. Therefore, heating curves of annealed and maximally freeze-concentrated solutions show glass transition of the maximally freeze-concentrated solute phase and onset of ice melting at initial concentration-independent T'_g and T'_m, respectively. T'_g and T'_m values for various sugars are given in Table 1. It should be noticed that monosaccharides have low T'_m values and they are extremely difficult to freeze-dry. The T'_g and T'_m increase with increasing molecular weight and those of polysaccharides occur at about the same temperature slightly below the melting temperature of pure ice [6].

In freeze-drying the amorphous structure of freeze-concentrated solids formed in prefreezing must be retained during removal of ice and unfrozen water. The material temperature during sublimation is critical for a successful freeze-drying process. Temperatures below T'_g allow removal of ice within the solid, glassy solutes and there are practically no changes in volume. At temperatures above T'_m the unfrozen water content increases which results in water plasticization of the freeze-concentrated solutes. Therefore, the freeze-concentrated solute matrix is transformed into the liquid state. The plasticized solute matrix may flow above T'_m and
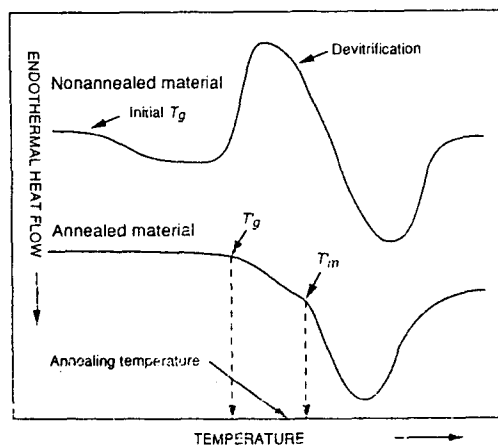

Fig. 3 Schematic representation of thermograms typical of rapidly cooled, nonannealed sugar solutions and annealed sugar solutions with maximum ice formation after proper annealing at $T'_g < T < T'_m$

most materials are likely to foam and collapse in freeze-drying when dehydration temperatures higher than the respective T'_m are used. The state diagrams which show the T_g and frozen state transition temperatures as a function of concentration are extremely useful in evaluation of proper freeze-drying conditions.

Importance of vitrification

Maximally freeze-concentrated materials can be considered as solids which contain the unfrozen solute phase in the high viscosity solid state within the glass transition temperature range or in the glassy state and a separate ice phase. In freeze-drying the unfrozen solids can support their own weight when the sublimation temperature of ice is kept below T'_m and no foaming or collapse are observed (Fig. 4). After removal of ice and unfrozen water the glassy state of solids in the dehydrated part of the material can be maintained when the T_g of the material is higher than material temperature. The same requirement applies after dehydration, as collapse of the dehydrated solids occurs if the material is stored above its T_g (Fig. 4). The dehydrated materials may also collapse due to water sorption from the surrounding atmosphere, which results in water plasticization and a possible decrease of the T_g to below ambient temperature. Several monosaccharides cannot be freeze-dried because their T'_m values are lower than practically achievable sublimation temperatures or low anhydrous T_g (Table 1). The low material T_g and high hygroscopicity are the main causes of collapse during dehydration or collapse occurring after suc-

Maximally freeze-concentrated solids
Ice

$T < T_m$

Dehydration by sublimation

Pores left by ice crystals
Glassy solids

$T > T_g$

Freeze-concentrated solids
Ice

$T > T_m$

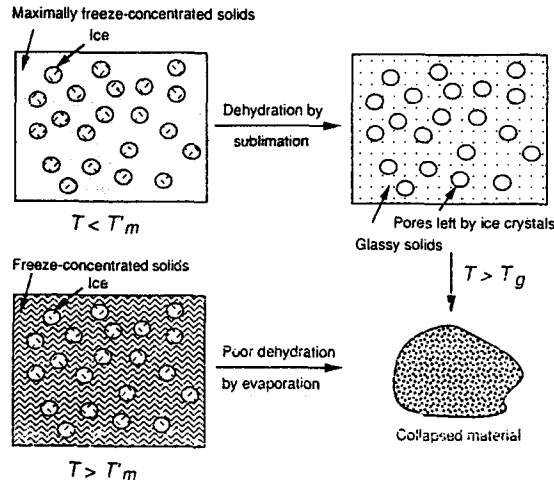Poor dehydration by evaporation

Collapsed material

Fig. 4 Effects of vitrification on the structure of freeze-dried materials. Sublimation temperatures lower than the onset temperature of ice melting after maximum ice formation, T'_m, allow removal of ice without collapse. Frozen layer temperatures higher than T'_m during freeze-drying cause plasticization of freeze-concentrated solutes which cannot support their own weight and collapse. Successfully freeze-dried materials may collapse when stored at temperatures higher than the glass transition temperature, T_g, of the material or as a result of water sorption and plasticization which may depress the T_g to below ambient temperature

cessful freeze-drying. However, it should be noticed that collapse processes are time-dependent with relaxation times decreasing with increasing temperature above T_g [9, 12]. Sufficient flow for collapse during freeze-drying has been observed, as the viscosity of the unfrozen solute phase decreases to below 10^4–10^7 Pa s [19], corresponding to that of the isoviscous state at 20 to 40°C above T_g [9].

Effects of molecular weight

The T_g of homopolymers is highly dependent on molecular weight and it increases almost linearly with the reciprocal of the molecular weight. The same applies to both T_g' and T_m' [6, 9] which, however, merge for high molecular weight carbohydrates close to 0°C [6]. The increase of the transition temperatures with molecular weight can be used in product development to improve freeze-drying behaviour and stability of the dehydrated materials. Generally addition of higher molecular weight solids increases T_g, T_g', and T_m'. Therefore, such materials as maltodextrins may be added in biological materials to avoid collapse and loss of quality in freeze-drying and subsequent storage.

Conclusions

Freezing of solutions and biological materials causes freeze-concentration of solutes into an amorphous, unfrozen phase. The freeze-concentrated unfrozen solute phase solidifies into the glassy state at temperatures below the T_g' when maximum ice formation has occurred at a temperature below the onset temperature of ice melting within the maximally freeze-concentrated solute phase, T_m'. Plasticization of the unfrozen phase occurs above T_m' due to ice melting, which results in significant decrease in viscosity, foaming, collapse, poor dehydration, and loss of quality. At temperatures below T_m' the freeze-concentrated solute phase can support its own weight and collapse is avoided. Therefore, the materials can be dehydrated into glassy materials which may collapse in the dehydrated state, as a result of thermal or water plasticization. State diagrams with information of conditions for equilibrium and nonequilibrium ice formation and frozen state transition temperatures can be used in selection of proper prefreezing, drying, and storage conditions. The solids composition may also be modified according to available processing and storage conditions to improve dehydration characteristics and material stability.

References

1 R. J. Bellows and C. J. King, Cryobiology, 9 (1972) 559.
2 K. Ito, Chem. Pharm. Bull., 18 (1970) 1509.
3 D. Rasmussen and B. Luyet, Biodynamica, 10 (1969) 319.
4 L. Slade and H. Levine, Pure Appl. Chem., 60 (1988) 1841.
5 Y. Roos and M. Karel, Int. J. Food Sci. Technol., 26 (1991) 553.
6 Y. Roos and M. Karel, J. Food Sci., 56 (1991) 1676.
7 Y. Roos, Carbohydr. Res., 238 (1993) 39.
8 G. P. Johari, A. Hallbrucker and E. Mayer, Nature, 330 (1987) 552.

 9 Y. Roos, Phase Transitions in Foods, Academic Press, San Diego, 1995.
10 G. P. Johari, A. Hallbrucker and E. Mayer, J. Phys. Chem., 94 (1990) 1212.
11 M. Sugisaki, H. Suga and S. Seki, Bull. Chem. Soc. Jpn., 41 (1968) 2591.
12 H. Levine and L. Slade, in J.M.V. Blanshard and J. R. Mitchell (ed.), Food Structure - Its Creation and Evaluation, Butterworths, London 1988, p. 149.
13 F. Franks, M. H. Asquith, C. C. Hammond, H. B. Skaer and P. Echlin, J. Microsc., 110 (1977) 223.
14 M. Gordon and J. S. Taylor, J. Appl. Chem., 2 (1952) 493.
15 P. R. Couchman and F. E. Karasz, Macromol., 11 (1978) 117.
16 M. T. Kalichevsky, E. M. Jaroszkiewicz, S. Ablett, J. M. V. Blanshard and P. J. Lillford, Carbohydr. Polym., 18 (1992) 77.
17 J. L. Kokini, A. M. Cocero, H. Madeka and E. de Graaf, Trends Food Sci. Technol., 5 (1994) 281.
18 M. T. Kalichevsky, J. M. V. Blanshard and P. F. Tokarczuk, Int. J. Food Sci. Technol., 28 (1993) 139.