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Effects of molecular weight of polyvinylpyrrolidone on the glass transition and crystallization of co-lyophilized sucrose

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Abstract

The purpose of the present study was to investigate the effects of molecular weight (MW) of polyvinylpyrrolidone (PVP) on glass transition and crystallization of sucrose. Thus, sucrose was co-lyophilized with 2.5 and 5.0% w/w PVP of different molecular weights, which were characterized using gel permeation chromatography. Freeze drying was carried out for 48 h at a shelf temperature of -40°C and a pressure of about 36 Pa. The samples were then dried in a vacuum oven at 24°C for 12 h before drying for a further 12 h at 40°C. Differential scanning calorimetry (DSC) was employed to measure the glass transition temperature (T_g), dynamic crystallization temperature (T_c) and isothermal crystallization induction time (t_c) at 85°C of sucrose. Isothermal water vapour sorption of each sample was also measured at different relative humidities. T_g values of sucrose varied from 48.3 ± 0.8°C for freeze-dried (FD) sucrose alone to 58.8 ± 0.8°C for the mixture containing 5.0% PVP of nominal MW 300 K. PVP increased sucrose T_g significantly (ANOVA $P < 0.05$). Although there was no significant difference ($P > 0.05$) in T_g of the mixtures containing 2.5% w/w PVP of different MW, samples with 5.0% PVP of MW 300 K produced a significantly higher ($P < 0.05$) T_g than the other mixtures. All mixtures were shown to possess higher ($P < 0.01$) T_c than FD sucrose alone, which exhibited a T_c of approximately 85°C. PVP of MW 300 K consistently induced a significantly ($P < 0.05$) higher T_c of sucrose than PVP of smaller MW. Increasing PVP concentration from 2.5 to 5.0% also resulted in a substantial increase in sucrose T_c. Using isothermal water vapour absorption, sucrose t_c was found to increase up to over 10 times when it was co-lyophilized with 2.5% PVP, the actual value of t_c being dependent upon the MW of the PVP. For example, PVP of MW 300 K resulted in a sucrose t_c at 85°C (89.1–95.6 min), which was approximately seven times higher than that of 2.5% PVP of MW 24 or 40 K. A longer t_c of sucrose was also observed for mixtures containing PVP of MW 300 K than when sucrose was mixed with PVP of smaller MW. Thus the effect of PVP on sucrose T_g, T_c and t_c was found to be dependent upon MW. PVP of higher MW was more efficient in inhibiting sucrose crystallization and by stabilizing glassy structures of the sugar, these polymers may improve the stability of co-lyophilized proteins and peptides. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Freeze drying; Sucrose; Polyvinylpyrrolidone; Molecular weight; Glass transition; Crystallization

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1. Introduction

Formulation of proteins and peptides still presents a challenge since many of these agents are susceptible to chemical (e.g. deamidation or oxidation) and/or physical degradation (e.g. aggregation and precipitation) in liquid formulations (Manning et al., 1989; Arakawa et al., 1993), leading to deactivation of these compounds. Such a problem may theoretically be avoided or minimised if the formulation comprises a dried solid, where degradative reactions are slowed down (Carpenter and Chang, 1996). Spray and/or freeze drying are the most commonly employed techniques to prepare solid proteins and stabilising excipient(s) are often added to protect proteins from denaturation by the freezing and dehydration stress encountered during lyophilization (Carpenter and Chang, 1996). These stabilising agents are mostly sugars and polyols such as trehalose, sucrose and mannitol. Rapid freezing, combined with subsequent drying of these excipients, will maintain the excipients in a glassy state (amorphous form), which is considered crucial in stabilising proteins. The glassy structure of these sugars is believed to protect the conformational structure of the proteins and inhibit the unfolding and aggregation of such molecules (Chang et al., 1996). However, a sugar glass is thermodynamically unstable and it will transform to a more stable, crystalline form under ambient conditions. The transformation (crystallization) is found to exclude protein molecules from the stabilised structure and consequently leads to destabilization of the proteins (Izutsu et al., 1993). Therefore, it is of a great importance to stabilise the glassy structure of the protectants so as to improve the physico-chemical and biological stabilities of the proteins and peptides. A sugar glass may be stabilised by adding polymers such as polyvinylpyrrolidone (PVP) which has been shown to inhibit the crystallization of amorphous sugar (Shamblin et al., 1996).

PVP is a synthetic polymer consisting essentially of linear 1-vinyl-2-pyrrolidinone groups, the degree of polymerization of which results in polymers of various molecular weights (Wade and Weller, 1994). The glass transition temperature (T_g) of amorphous PVP was shown to be dependent upon the MW, a higher MW leading to a higher T_g (Maria del Pilar et al., 1992). Since the aqueous viscosity of a solution of PVP is dependent upon the MW of the polymer (Wade and Weller, 1994), it may be reasonable to assume that PVP of different MW will have different effects on the crystallization of co-lyophilised sugar glass. Therefore, it was the aim of this study to investigate the effects of the molecular weight of PVP, as a model polymer on the glass transition and crystallization of co-lyophilised sucrose, employed as a model sugar glass.

2. Materials and methods

Sucrose was purchased from Tate and Lyle, London, UK; Polyvinylpyrrolidone (PVP) of a nominal MW of 10 000 (10 K); 24 000 (24 K); 40 000 (40 K) and 300 000 (300 K) were obtained from Sigma-Aldrich Co. Ltd., Dorset, UK. All materials were labelled as having purity over 99% and were used without further purification. Lithium chloride, magnesium chloride and potassium chloride were all of reagent grade and were purchased from Sigma-Aldrich Co. Ltd., Dorset, UK. Dimethylformamide of HPLC grade was also obtained from Sigma-Aldrich Co. Ltd., Dorset, UK.

2.1. Analysis of PVP molecular weight by gel permeation chromatography (GPC)

Sample solutions were prepared by adding 10 ml of dimethylformamide to 20 mg of sample before warming at 85°C in a water bath for 30 min to dissolve the polymer. After thorough mixing and prior to the chromatography, the solutions were filtered through a 0.2 μm PTFE membrane (Aldrich-Sigma Co. Ltd., Dorset, UK).

The molecular weight of PVP was measured by GPC using a GPC-210 system (Polymer Laboratories Ltd., Shropshire, UK) fitted with a PLgel 2 × mixed bed-B column (30 cm, 10 μm, Polymer Laboratories Ltd., Shropshire, UK). Dimethylformamide with 0.1% (w/v) ammonium acetate was employed as mobile phase running at a flow rate of 1.0 ml min^{-1} at a temperature of 80°C and a refractive index detector. The GPC system was calibrated with poly(methylmethacrylate) (PMMA) (Polymer Standards Calibration Kits, Polymer Laboratories Ltd., Shropshire, UK). The data were collected and analysed using Polymer Laboratories 'Caliber' software (Polymer Laboratories Ltd., Shropshire, UK). Each solution was run in duplicate and the results were expressed as the 'PMMA equivalent' molecular weights.

2.2. Freeze-drying of sucrose with different grades of PVP

Solutions containing PVP and sucrose were prepared by dissolving 2 g of PVP–sucrose mixture in 5 ml deionised water. The ratios of sucrose to PVP were chosen in order for PVP to contribute either 2.5 or 5.0%, w/w, to the total solutes. Sucrose and PVP were placed in a 100 ml round bottom, wide-necked flask. Water (5 ml) was then carefully added to the flask such that all the solutes were submerged in the solvent. The mixture was then allowed to stand overnight to ensure complete dissolution of the PVP and sucrose. The flask was submerged in liquid nitrogen for at least 1 min under constant manual swirling such that a frozen thin layer was formed on the glass wall. Freeze drying was then carried out at −40°C at a pressure of approximately 36 Pa for 48 h in a Laboratory Freeze Drier Model S.B.3 (ChemLab Instruments Ltd., Essex, UK). The dried samples were then transferred to a vacuum oven and dried at 24°C for 12 h before drying for a further 12 h at 40°C. Finally, the samples were transferred to a 20 ml sample vial, sealed and placed in a desiccator over silica gel until required for further experimentation.

2.3. Measurements of T_g and T_c of sucrose in the presence of co-lyophilized PVP

Differential scanning calorimetry (DSC) was employed to determine the glass transition temperature (T_g) and non-isothermal crystallization temperature (T_c) of sucrose samples. The calorimeter used was a Mettler TA 4000 (Mettler Instrumente AG, CH-8608 Greifensee, Switzerland) thermal analysis system, with a DSC20 furnace. Thermograms were analyzed using Mettler GraphWare TA72PS.1 software. An empty aluminium pan (40 μl) was used as the reference for all measurements. The instrument was calibrated using tin, indium and gallium as standard materials. Weighed samples (5–10 mg) were measured in hermetically sealed aluminium pans. The pans after sealing were placed into the pre-equilibrated DSC furnace (25°C). Before each measurement, the sample was heated to 75°C at 20°C min^{-1} and then allowed to cool to 25°C in order to expose the samples to a similar history of heat treatment. After the pre-treatment, the sample was heated to 250°C at a heating rate of 20°C min^{-1}. Additional experiments were carried out for the same mixtures using a heating rate of 10°C min^{-1}. T_g was taken as the midpoint between onset and end of the change in specific heat observed as a shift in the baseline on the thermogram of a dynamic scan. The non-isothermal T_c was recorded as the temperature corresponding to the maximum of the exothermic peak associated with the crystallization of amorphous sucrose. Samples of each mixture were analysed in triplicate.

2.4. Isothermal crystallization of sucrose

The isothermal crystallization of sucrose was measured as a function of temperature using DSC as described by Roos and Karel (1991). Samples in a sealed aluminium pan were heated to a pre-determined temperature at a heating rate of 10°C min^{-1}. The sample was then held at that temperature until an exothermic peak, corresponding to the crystallization of amorphous lactose, was obtained. The peak time was recorded as the induction time for the crystallization of sucrose. Each sample was measured in duplicate.

2.5. Isothermal water vapour uptake

Accurately weighed samples (100 mg) were each placed into pre-weighed 10 ml glass vials, which were then placed into three separate desiccators containing saturated salt solutions which determined the relative humidity (RH). Also placed into the desiccator were three pre-weighed empty glass vials, used as controls. Three different salts, i.e. lithium chloride, magnesium chloride and potassium chloride were employed to obtain an RH of 11, 33 and 43%, respectively, which was monitored by a hygrometer to ensure that equilibrium had been reached. The temperature in the desiccator was monitored by a digital temperature probe and meter and this was found to range between 19 and 23°C throughout the experiments. At predetermined intervals, the weight gain of the sample was measured gravimetrically, after subtraction of the average weight gain of the three blank vials. The weight gain was then converted to grams of water vapour uptake per 100 g of the powder sample. All mixtures were analysed in triplicate.

Additional experiments were carried out to determine the isothermal water vapour uptake at higher RH using a Mettler TA 4000 thermal analysis system with a TG 50 thermobalance. The relative humidity within the chamber of the balance was controlled between 62 and 65%, monitored with a hygrometer, using saturated sodium chloride solution. The balance was tared with an aluminium crucible (70 μl). The sample (about 50 mg) was placed into the crucible, which was in turn placed on the tared balance. The change in the sample weight with time was continuously monitored using the thermal analysis system and the water vapour uptake isotherms were analysed using a Mettler GraphWare system.

3. Results

3.1. MW of different grades of PVP

Fig. 1 shows the MW distribution of the different grades of PVP employed in this study. The average MW (Table 1) was found to be different from that assigned by the manufacturer on the basis of viscometry measurement. Nevertheless the MW of the samples increased in the same order suggested by the sample nomenclature. For example, PVP of a nominal MW 10 K was shown to possess a weight-average MW of approximately 6800 and a number-average MW of ap-

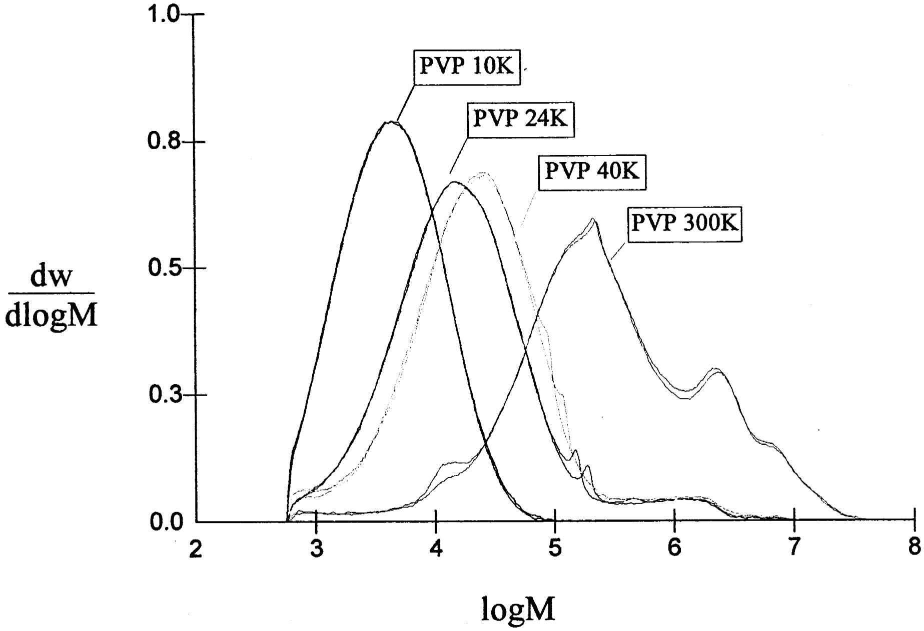


Fig. 1. The calculated molecular weight distributions for duplicate runs for each samploe of PVP. The plot is normalised with respe to area, the y axis being a function of weight fraction.

Table 1
The weight-average molecular weight (Mw), number-average molecular weight (Mn) and polydispersibility of different grades of PVP

Samples	Mw	Mn	Polydispersibility
PVP 10 K	6730	2970	2.3
	6950	3000	2.3
PVP 24 K	82 900	7780	11.0
	87 000	7860	11.0
PVP 40 K	107 000	9500	11.0
	98 200	10 000	9.8
PVP 300 K	1 250 000	46 000	27.0
	1 240 000	46 500	27.0

proximately 3000. However PVP 24 K was shown to have a similar average MW and MW distribution to those of PVP 40 K.

3.2. DSC thermograms

Typical power-time curves of freeze-dried sucrose with or without PVP are shown in Fig. 2. It can be seen that the freeze-dried sucrose exhibits a glass transition between 50 and 70°C, an exothermic peak due to crystallization of amorphous sucrose between 80 and 160°C and a melting endotherm at about 180°C.

Table 2
Glass transition temperatures of different sucrose powder as measured by DSC at a heating rate of 20 and 10°C/min[a]

Sample compositions	20°C min^{-1}	10°C min^{-1}
Sucrose	49.1 ± 1.2	48.3 ± 0.8
Sucrose/2.5% PVP 10 K	55.0 ± 1.4	53.6 ± 1.3
Sucrose/2.5% PVP 24 K	54.9 ± 1.6	53.4 ± 0.9
Sucrose/2.5% PVP 40 K	54.6 ± 1.3	53.2 ± 0.8
Sucrose/2.5% PVP 300 K	56.0 ± 1.7	55.3 ± 1.0
Sucrose/5% PVP 10 K	56.6 ± 1.5	54.5 ± 1.2
Sucrose/5% PVP 24 K	54.0 ± 0.8	53.3 ± 1.3
Sucrose/5% PVP 40 K	55.1 ± 1.2	52.4 ± 0.5
Sucrose/5% PVP 300 K	60.2 ± 1.4	58.8 ± 0.8

[a] Mean ± SD, $n = 3$.

3.3. Glass transition temperature (T_g)

T_g values of these samples are summarized in Table 2. The T_g value measured at a heating rate of 10°C min^{-1} was slightly but statistically insignificantly (ANOVA, $P > 0.05$) lower than that of the same sample but measured at a heating rate of 20°C min^{-1}. Addition of PVP to sucrose re-

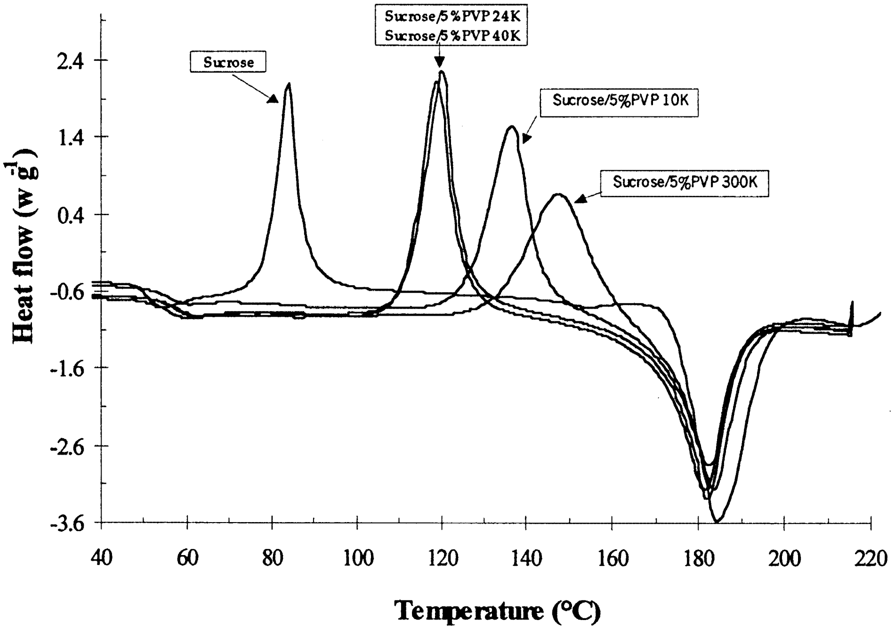


Fig. 2. DSC traces of sucrose with or without PVP after measured at a heating rate of 10°C min^{-1}.

sulted in a slight but nevertheless significant ($P < 0.05$) increase in T_g of co-lyophilised sucrose. There was no significant difference ($P > 0.05$) observed in the T_g values of sucrose containing 2.5% w/w PVP of different MW. However samples composed of 5.0% PVP of MW 300 K with sugar produced a T_g of sucrose, which was significantly higher ($P < 0.05$) than those of the samples containing 5.0% PVP of MW between 10 and 40 K.

3.4. Crystallization temperature

The temperature of crystallization (T_c) of each sample is summarized in Table 3. All sucrose–PVP mixtures were shown to possess higher ($P < 0.01$) T_c of sucrose than the freeze-dried sucrose.

PVP of MW 300 K consistently resulted in T_c of sucrose, which was significantly ($P < 0.05$) higher than those of the mixtures containing PVP of smaller MW, when present at the same weight concentration and compared at the same heating rate. However, the mixtures containing PVP of MW 10 K produced significantly ($P < 0.05$) higher T_c than the mixtures containing PVP of MW of either 24 or 40 K (Table 3). Increasing the PVP concentration from 2.5 to 5.0% also resulted in a significant ($P < 0.05$) increase in the T_c of sucrose from mixtures containing the corresponding PVP. T_c measured at a heating rate of 10°C min^{-1} was slightly but insignificantly ($P > 0.05$) lower than T_c measured at 20°C min^{-1}.

Table 3
Crystallization temperature T_c of sucrose from freeze-dried powders containing PVP of different MW[a]

Sample compositions	20°C min^{-1}	10°C min^{-1}
Sucrose	86.9 ± 2.1	85.3 ± 1.9
Sucrose/2.5% PVP 10 K	119.7 ± 1.8	115.5 ± 1.8
Sucrose/2.5% PVP 24 K	105.5 ± 2.3	105.1 ± 2.2
Sucrose/2.5% PVP 40 K	105.3 ± 2.4	104.8 ± 3.2
Sucrose/2.5% PVP 300 K	125.8 ± 2.2	120.2 ± 1.4
Sucrose/5% PVP 10 K	137.4 ± 1.4	134.5 ± 1.0
Sucrose/5% PVP 24 K	126.5 ± 2.3	122.6 ± 3.2
Sucrose/5% PVP 40 K	126.2 ± 2.5	120.2 ± 1.6
Sucrose/5% PVP 300 K	156.4 ± 3.6	151.5 ± 3.2

[a] Mean ± SD, $n = 3$.

Table 4
Crystallization induction time and heat of crystallization of sucrose measured by isothermal conditions at 85°C ($n = 2$)

Sample compositions	Crystallization induction time (min)	Heat of crystallization (J/g)
Sucrose	1.3, 1.6	40.5, 44.4
Sucrose/2.5% PVP 10 K	24.2, 26.9	60.2, 64.6
Sucrose/2.5% PVP 24 K	13.5, 15.3	59.4, 63.5
Sucrose/2.5% PVP 40 K	11.7, 12.8	58.5, 57.8
Sucrose/2.5% PVP 300 K	89.1, 95.6	64.9, 68.5
Sucrose/5% PVP 10 K	128.9, 130.5	72.0, 76.9
Sucrose/5% PVP 24 K	86.2, 87.8	67.5, 72.3
Sucrose/5% PVP 40 K	92.3, 103.2	70.3, 72.8
Sucrose/5% PVP 300 K	181.5, 192.3	75.2, 78.9

3.5. Crystallization induction time

The isothermal crystallization induction time (t_c) of sucrose from different samples is shown in Table 4. Freeze-dried sucrose showed a t_c of 1–2 min at 85°C. An approximately 10-fold increase in the induction time was observed for sucrose mixtures with 2.5% PVP of MW 24 and 40 K. The mixture containing 2.5% PVP of MW 300 K was found to have a t_c of sucrose, which was approximately 7 times higher than those of sucrose mixtures with 2.5% PVP of MW 24–40 K, but only 3–4 times higher than mixtures containing the same weight concentration of PVP of MW 10 K. Increasing the concentrations of PVP of the smaller MW from 2.5 to 5.0% increased t_c of sucrose by approximately a further 6–7 times. The mixtures containing 2.5% PVP of MW 300 K showed an induction time for sucrose similar to those of the mixtures with 5.0% PVP of MW 24–40 K. The highest t_c (~ 3 h) was achieved by 5.0% PVP of MW 300 K. These results further confirm that whilst addition of small amounts of PVP was able to inhibit the crystallization of sucrose, PVP of MW 300 K was the most efficient at inhibiting crystallization. Table 4 also shows the heat of crystallization of sucrose (ΔH_c) from the various samples. All sucrose–PVP mixtures

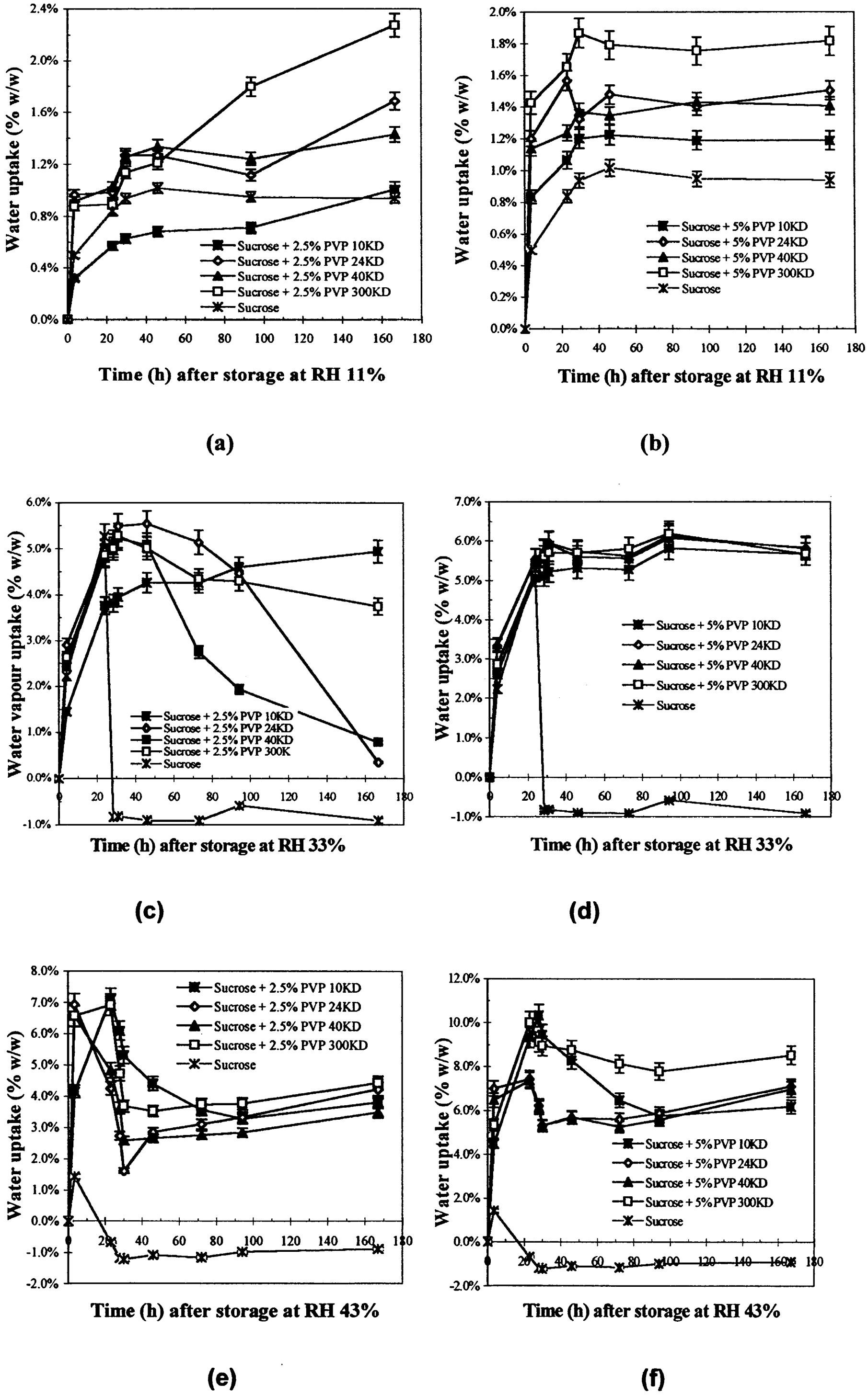
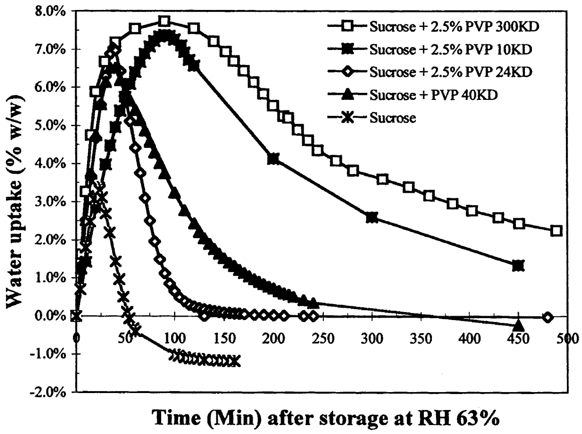


Fig. 3. Water uptake isotherms of each sample at different RH (error bars denote SD, $n = 3$).



(g)

Fig. 3. (*Continued*)

produced a ΔH_c, which was higher than that of freeze-dried sucrose on its own, with the mixture containing PVP of MW 300 K producing the highest ΔH_c. Increasing the concentrations of PVP from 2.5 to 5.0% generally resulted in an increase in the ΔH_c of sucrose from the mixtures.

3.6. Water vapour sorption

The water sorption isotherms of each sample under different RH are shown in Fig. 3. It can be seen that different mixtures produced different water sorption isotherms. At RH 11%, mixtures containing PVP of MW 300 K absorbed the most water whilst mixtures containing PVP of MW 10 K took up the least water. Mixtures containing PVP of 24 and 40 K displayed similar water sorption profiles. At RH 33%, all mixtures containing the same concentration of PVP showed similar maximal water uptake, approximately 5.5% w/w for mixtures containing 2.5% PVP and 6% w/w for those with 5.0% PVP. Freeze-dried sucrose without PVP had a maximal water uptake of about 5.0% water at RH 33%. At RH 43%, all mixtures containing 2.5% PVP showed similar maximal water adsorption of around 7% whilst mixtures containing 5.0% PVP of MW 10 or 300 K exhibited a peak water sorption of about 10%, which was significantly ($P < 0.01$) higher than that (~ 7%) of mixtures containing PVP 24 or 40 K. Higher water uptake was also observed for mixtures containing 2.5% PVP of 10 or 300 K at RH 63%. Furthermore, sucrose–PVP mixtures generally took up more water than freeze-dried sucrose without PVP at all RHs investigated.

At higher RH, e.g. 43 or 63%, all samples were shown to possess two-stage water sorption profiles. Water sorption increased with time until it reached a maximum after which, it decreased with time. Such a phenomenon is believed to be primarily due to sucrose crystallization within the powder once sufficient water is present (Carstensen and Scoik, 1990). Once crystallization occurs, the sucrose cannot retain the moisture of the hydrated amorphate, and the loss of moisture is indicative, both qualitatively and quantitatively, of the conversion from amorphous to the crystalline state. The time period over which the sample takes up water and remains constant is generally taken as the overall induction time, required for nucleation to occur, while the initiation of a weight loss is assumed to be governed primarily by crystal growth (Saleki-Gerhardt and Zografi, 1994). In the present work, the crystallization induction time of sucrose from different mixtures was estimated as the time period over

Table 5
The ranges of crystallization induction time (h) of sucrose from different mixtures at varying RH under room temperature (19–23°C, $n = 3$)[a]

Sample compositions	11%	33%	43%	63%
Sucrose	>168	24–29	<3.5	<0.5
Sucrose/2.5% PVP 10 K	>168	>168	23–28	2–2.5
Sucrose/2.5% PVP 24 K	>168	73–94	4–23	1
Sucrose/2.5% PVP 40 K	>168	46–73	4–23	1
Sucrose/2.5% PVP 300 K	>168	>168	28–30	2.5–3
Sucrose/5% PVP 10 K	>168	>168	28–30	ND
Sucrose/5% PVP 24 K	>168	>168	23–28	ND
Sucrose/5% PVP 40 K	>168	>168	23–28	ND
Sucrose/5% PVP 300 K	>168	>168	30–46	ND

[a] ND, not determined.

which the sample took up water, remained constant and a 10% decrease in the water content from the peak value occurred. The estimated value of the induction for each sample at different RHs is tabulated in Table 5. It can be seen that the induction was over 168 h at RH 11%, suggesting that crystallization did not occur after storage at this RH for a week. Increasing the RH to 33% was shown to greatly reduce the induction time of freeze-dried sucrose without PVP and also markedly decrease the induction time of the mixtures containing 2.5% PVP of MW 24 or 40 K. A sharp loss in water content from 5.0 to -1% between 24 and 49 h, observed for freeze-dried sucrose without PVP, was indicative of a complete crystallization of sucrose from this sample. After crystallization, not only the sorbed water but also some previously existing water was excluded from the powder, leading to a negative water content in the water sorption isotherms of the sample (Fig. 3). Sucrose crystallization from mixtures containing 2.5% PVP of MW 24 and 40 K occurred less readily than that from freeze-dried sucrose without PVP but more easily than that from 2.5% PVP of MW of either 10 or 300 K. Crystallization was found to proceed much more quickly when the RH was increased to RH 43% such that noticeable crystallization of sucrose occurred from all the samples investigated. Crystallization was also found to proceed to completion from freeze-dried sucrose without PVP whilst crystallization from sucrose–PVP mixtures was inhibited to such an extent that complete crystallization of these samples were not achieved after 168 h even at RH 43%. Of the sucrose–PVP mixtures, crystallization was also shown to proceed the most rapidly from mixtures containing PVP of MW 24 and 40 K, followed by mixtures containing PVP of MW 10 K with the mixtures containing PVP of MW 300 K the slowest. Similarly at RH 63%, crystallization from sucrose powder without PVP proceeded the most rapidly, followed by sucrose–PVP mixtures in the order PVP 24 K and 40 K > PVP 10 K > PVP 300 K. All the results obtained with water sorption isotherms concurred with the findings obtained by thermoanalysis in that PVP of MW 300 K proved to stabilise the amorphous structure of freeze-dried sucrose most effectively.

4. Discussion

The sample nomenclature implies a molecular weight, which is generated by the manufacturers on the basis of dilute solution viscometry. In the present study, the MW of PVP determined by GPC was expressed as the PMMA equivalent molecular weight, and the use of the different techniques accounts, in part, for the differences in the assigned and measured MWs of PVP.

The freeze-dried sucrose exhibits a glass thermal transition, followed by a crystallization exotherm, suggesting that the sucrose was in an amorphous form, since crystalline sucrose only has a melting endotherm in its DSC thermogram. Molecules have higher mobility in the rubbery state than in the glassy state then, after the glassy transition temperature, the heat capacity of the amorphous material will be increased. Further increasing the temperature will induce such a high mobility in the molecules that crystallization will occur, resulting in an exothermic peak. Such a crystallization often proceeds to completion, after which the amorphous material is effectively converted entirely to a crystalline material.

All these samples possessed approximately 2–3% w/w of residual water (data not shown), suggesting that the drying procedure described above was unable to completely dehydrate the powder samples. Samples containing sucrose alone were shown to have a T_g of around 49°C, which was lower than the reported 74°C of amorphous sucrose (Shamblin et al., 1996). Such a discrepancy may largely be due to the fact that the sucrose sample in this study had a residual water content of 2.6% (w/w) whilst the previously reported T_g was for amorphous sucrose with 'zero' residual water. Water is known to be a plasticiser that reduces substantially glass transition temperature of amorphous materials.

Increasing MW of PVP was shown to increase the T_g of the polymer (Maria del Pilar et al., 1992), which would theoretically be expected to result in different T_g values of the sucrose–PVP mixtures. However, the relatively high residual water content in these mixtures, together with small amounts (2.5% w/w) of the PVP employed, may have disguised the effects of MW of PVP on the T_g of sucrose–PVP mixtures such that similar T_g values were measured for the mixtures containing PVP of MW between 10 and 300 K. Increasing the PVP concentration to 5.0% should enhance the role of PVP MW and indeed, the sucrose–PVP mixtures containing PVP of MW 300 K exhibited higher T_g than the mixtures containing PVP of smaller MW.

Sucrose crystallization was inhibited in the presence of PVP and this is in agreement with some previous reports (Shamblin et al., 1996). Inhibition of sucrose crystallization may, at least in part, be attributed to the change in mobility of molecules by means of increasing T_g of the mixture as shown in Table 2. Molecular mobility in an amorphous state is a function of the difference between the temperature of the sample (T) and T_g, i.e. differential temperature, $T-T_g$. The larger the differential temperature, the higher is the molecular mobility. Increasing T_g would increase the crystallization temperature if other conditions are kept constant. Therefore, the higher T_g of sucrose–PVP mixtures may contribute to their higher T_c values as compared with those of the sucrose without added PVP. The mixtures containing PVP of MW 300 K produced a higher T_g in comparison to other mixtures containing PVP of smaller MW, at a similar moisture content and temperatures. Sucrose molecules in the former mixtures would be expected to have a lower mobility than in the case of the latter and consequently a higher T_c of sucrose, as compared with those in the presence of PVP of lower MW.

The crystallization induction time is also a function of the difference between the sample temperature T and T_g. Larger differences are associated with greater molecular mobility and hence a higher tendency for crystallization to occur (Ferry, 1980). Since the mixtures with PVP of MW 300 K had a higher T_g than those containing PVP of smaller MW, when analysed at the same temperature, the former samples should have lower values of $T-T_g$, leading to longer crystallization induction times.

ΔH_c is proportional to the amorphous content (Saleki-Gerhardt and Zografi, 1994), with a higher ΔH_c indicating a greater amorphous content, i.e. lower crystallinity. Thus, PVP could have reduced the crystallinity of freeze-dried sucrose by increasing amorphous sucrose content. The more PVP that was added, the higher the amorphicity of the freeze-dried sucrose and a PVP of MW 300 K was more efficient in producing amorphous sucrose than other grades of PVP of smaller MW. Further, the maximal water uptake has been reported to be proportional to the amorphous content of sucrose (Saleki-Gerhardt and Zografi, 1994). Therefore, higher water uptake for sucrose–PVP mixtures may be considered indicative of a higher amorphous content than the freeze-dried sucrose alone. Increasing water sorption by increasing the PVP concentration in the sucrose–PVP was also suggestive of an increased amorphous content. All these results further confirmed the findings by DSC that PVP increased the amorphous content of sucrose and the higher the PVP concentration, the more amorphous sucrose was present in the mixtures.

From Fig. 3, it can also be seen that mixtures containing PVP of MW 10 K, regardless of concentration, consistently showed a slower rate of water sorption than mixtures containing other grades of PVP. For example, mixtures containing PVP of MW 10 K took up significantly less ($P < 0.05$) water than mixtures of other grades of PVP throughout the test at RH 11%. A slower rate of water sorption from mixtures of PVP MW 10 K was also observed at RH 33, 43 and 63%. The slow water sorption may be attributable to the exceptionally slow sucrose crystallization from these mixtures and this may explain why mixtures containing PVP of MW 10 K had higher T_c and t_c than mixtures containing PVP 24 or 40 K although they possessed similar values of T_g. Water is widely known to be a catalyst for crystallization. Slower water uptake will hinder both nucleation and growth rate of crystallization, leading to a slower crystallization of sucrose from the mixtures.

5. Conclusions

Regardless of its molecular weight, PVP inhibits crystallization of amorphous sucrose by means of increasing the glass transition temperature of the mixtures. The effect of PVP on both the non-isothermal and isothermal crystallization of sucrose would appear to be dependent upon MW. PVP of higher molecular weight may be more efficient in inhibiting sucrose crystallization from the sucrose–PVP mixtures and by stabilising glassy structures of sugars, these polymers might be expected to maintain the physicochemical properties of co-lyophilised proteins and peptides.

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