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Effect of pH, Counter Ion, and Phosphate Concentration on the Glass Transition Temperature of Freeze-Dried Sugar-Phosphate Mixtures

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Purpose. The aim of the present work is to study the interaction of phosphate salts with trehalose and sucrose in freeze-dried matrices, particularly the effect of the salts on the glass transition temperature (T_g) of the sugars.

Methods. Freeze-dried trehalose and sucrose systems containing different amounts of sodium or potassium phosphate were analyzed by differential scanning calorimetry to determine the T_g and by Fourier-transform infrared spectroscopy (FTIR) analysis to evaluate the strength of the interaction between sugars and phosphate ions.

Results. Sucrose-phosphate mixtures show an increase in T_g up to 40°C in a broad pH range (4–9) compared to that of pure sucrose. Sucrose-phosphate mixtures exhibit a higher T_g than pure sucrose while retaining higher water contents. Trehalose-phosphate mixtures (having a T_g of 135°C at a pH of 8.8) are a better option than pure trehalose for preservation of labile materials. The –OH stretching of the sugars in the presence of phosphates decreases with increase in pH, indicating an increase in the sugar-phosphate interaction.

Conclusions. Sugar-phosphate mixtures exhibit several interesting features that make them useful for lyophilization of labile molecules; T_g values much higher than those observed for the pure sugars can be obtained upon the addition of phosphate.

KEY WORDS: freeze-drying; glass transition, sucrose; phosphate; trehalose.

INTRODUCTION

The stabilization of labile biomaterials is of interest for both industrial and research applications in various fields. Research efforts in the last decade have shown that disaccharides can provide significant protection for labile structures during dehydration and ensuing storage (1–4). Kinetic and specific interactions are believed to be involved in the protection of labile materials by lyoprotectants. At the kinetic level, molecular mobility and chemical processes are retarded due to the formation of a glassy matrix created during dehydration. Specific interactions involving hydrogen bonding of the protectants and the labile molecules help preserve the structure of the latter in the absence of water (5–8). Although the stabilizing effects of sugars are significant, it is of interest to improve their performance as protectants even further. It has been shown that combinations of sugars with various ions are superior to the sugars alone as protein stabilizing agents (9). There is evidence for the existence of sugar-metal complexes in solution, and several complexes of sugars and sugar derivatives with inorganic salts have been isolated in solid form (10–11). There is also evidence for the modification of the thermophysical properties of concentrated aqueous trehalose solutions in the presence of sodium chloride (12) or sodium tetraborate (13). Miller *et al.* demonstrated that the stabilizing effect of trehalose could be enhanced by the addition of borate ions to the system (13). Aqueous mixtures of trehalose with borax showed great increase in the glass transition temperature, T_g, compared to that of trehalose alone; this observation was explained on the basis of the formation of a reversible network between trehalose and borate (14). The ability of this system to protect enzymes (13) and bacteria (15) during freezing and drying processes has been documented in the literature. Unfortunately, even though sugar-borate mixtures provide effective protection, they cannot be used for consumer-oriented applications as they can be toxic in high concentrations and are not regulated by the FDA. Phosphate, having similar chemical structure to borate and being biologically benign, could provide an effective alternative to borate. Previous studies have shown that the T_g of a frozen sugar-phosphate solution can be markedly higher than that of the pure sugar solution (16). Recent reports have shown that upon the addition of phosphate ions to a sugar lyoprotectant formulation, the stability of freeze-dried *L. acidophilus* can be improved considerably (15,17); furthermore, these matrices exhibit an increased ability to maintain a depressed melting transition temperature of lipid bilayers in the presence of small amounts of water (18). Although many physical properties of sugar-phosphate mixtures have been determined, they have only been studied in the frozen state. It is of interest to determine the effect of phosphate salt addition on the sugar T_g in the dehydrated state, as well as under low moisture conditions, in order to asses its applicability as an alternative to pure sugars for lyoprotection of pharmaceutical products.

Previous reports have shown that, for lyophilization of biologic systems, it can be highly advantageous to retain a small but non-negligible amount of water in the lyophilized samples (17). In the particular case of disaccharide glasses, a small amount of water can decrease the glass transition temperature considerably and render such systems ineffective for room-temperature storage of biologic products. The use of phosphate-sugar mixtures raises the interesting prospect of using disaccharide matrices (with all of their attributes), while retaining significant amounts of water and maintaining relatively high glass transition temperatures. The aim of the present work is to study the vitrification of dehydrated trehalose and sucrose systems in the presence of phosphate salts and controlled amounts of residual moisture, and to explore the range of usefulness of disaccharide-phosphate mixtures for lyophilization of pharmaceutical products.

MATERIALS AND METHODS

Chemicals

Trehalose and sucrose were purchased from Pfanstiehl Laboratories (Waukegan, IL). The phosphate salts used were

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KH_2PO_4, K_2HPO_4, NaH_2PO_4 and Na_2HPO_4 (Fisher Scientific, NJ). All solutions were prepared in nanopure water.

Sugar-Phosphate Sample Preparation

Amorphous systems were prepared by freeze-drying solutions containing 20% (wt/wt) sugar. Various ratios of KH_2PO_4 and K_2HPO_4 (or NaH_2PO_4 and Na_2HPO_4) were mixed to give the specified pH. When the salts were completely dissolved, the sugars were added in order to obtain the desired phosphate/sugar molar ratios (0.1–1.0). Aliquots of 0.5 ml of the sugar-phosphate solution were placed in 5 ml glass vials, and then immediately quenched with liquid nitrogen. The samples were then freeze-dried for 48 h in a Virtis Genesis 12EL (New York) freeze-dryer at a pressure of 30 mTorr and a condenser temperature of -80°C. After freeze-drying, the samples were placed in a vacuum oven to further remove the residual water. The samples, under vacuum, were transferred to a glove box (VAC, NEXUS ONE, Hawthorne, CA), and then loaded into preweighed differential scanning calorimetry (DSC) pans and sealed for calorimetric analysis, and into preweighed glass vials for water content analysis.

A series of sugar and sugar-phosphate samples that varied in pH or counter ion concentration (potassium or sodium) independently were also prepared. For sugar and sugar-phosphate samples at the same pH, the various counter ion concentrations were obtained by adding either NaCl or KCl (depending on the phosphate counter ion) to the sugar and sugar-phosphate solution. For sugar and sugar-phosphate samples at the same counter ion concentration, the various pH values were obtained by the addition of nominal amounts of base or acid.

For systems involving titration, sugar-phosphate solutions were prepared as described previously, then a very small volume of 12 N NaOH or 12N HCl were added 30 min after the mixtures have completely dissolved. The solutions were then left to equilibrate for at least 30 min prior to being quenched with liquid nitrogen. The samples were subsequently freeze-dried and prepared for analysis as described above.

To obtain the desired range of sample hydrations, freeze-dried samples were loaded into DSC pans and glass vials, and then placed in desiccators over saturated salt solutions that provided constant relative humidities between 11 and 33% (19) at 20°C for 1 week.

pH Measurement

pH measurements were made using an Accumet Research AR15 pH meter (Fisher Scientific, NJ). Triplicate measurements were made for each sample and the average value reported.

Differential Scanning Calorimetry

Differential scanning calorimetry was used to determine the glass transition temperature, T_g. T_g was recorded as the onset temperature of the discontinuity in the curve of heat flow vs. temperature. The instrument used was a TA Q100 DSC (New Castle, DE). Two scans were carried out for each sample. All measurements were made at 10°C/min, using sealed aluminum pans (crimped pans, TA), and an empty pan was used as a reference. The average value of three replicate samples is reported. Data were analyzed using Universal Analysis.

Water Content Analysis

Residual water measurements for both dehydrated and humidified samples were made using a Karl Fisher Coulometer Metrohm, Model 737 (Herisau, Switzerland).

Fourier-Transform Infrared Spectroscopy Analysis

The pellets for Fourier-transform infrared spectroscopy (FTIR) analysis were prepared by mixing the freeze-dried sugar or sugar-phosphate samples with KBr (90 wt%). Care was taken to minimize the sample exposure to moisture.

FTIR absorption measurements were performed with a Nicolet Magna-IR 860 Fourier transform infrared spectrometer (Madison, WI). The optical bench was purged with dry CO_2 free air during analysis. An average of 1024 scans was recorded at a resolution of 4 cm^{-1} in the transmission mode.

Spectral analysis was performed using the Omnic software version 6.0a. The spectral region between 3000 and 3600 cm^{-1}, corresponding to the –OH stretching vibration, was selected for analysis. The –OH stretching peak is very broad, hence the wavenumber was calculated as the average of the spectral positions at 80% of the maximum peak height (20).

X-ray Powder Diffraction

A PadV Scintag scanning X-ray powder diffractometer (Scintag, Inc., Santa Clara, CA) was used to establish the amorphous/crystalline nature of the freeze-dried samples. The radiation used was generated by a Cu Kα filter with a wavelength of 1.5418 Å at 45 kV and 40 mA. Samples were scanned from 10 to 45° 2θ at a scanning rate of 1° 2θ/min.

Optical Microscopy

Polarized light microscopy was performed using an Olympus-UMA optical microscope with a Sony CCD-IRIS video camera to analyze the amorphous/crystalline nature of the freeze-dried sugar and sugar-phosphate samples.

RESULTS AND DISCUSSION

Figure 1 shows typical DSC thermograms obtained for sugar and sugar-phosphate mixtures exhibiting glass transition. The effect of phosphate salt addition on the glass transition temperature, T_g, and water sorption behavior (at 20°C) is shown in Fig. 2 for freeze-dried trehalose (a) and sucrose (b) systems equilibrated at different relative humidities. Equimolar amounts of KH_2PO_4 and K_2HPO_4 were used to arrive at systems having a pH of 6.6. The glass transition temperature of sucrose-phosphate systems increases with increasing phosphate content (Fig. 2b). For a phosphate/sucrose molar ratio of 1, the T_g of dehydrated sucrose-phosphate mixture is 90.3°C at a residual water content of 0.6%, while that of dehydrated sucrose alone is 63.1°C at a residual water content of 0.4%. The T_g of sucrose-phosphate mixtures is higher than that of sucrose even at higher water contents. In contrast, the addition of phosphate does not increase the T_g of the trehalose system; rather, the addition of phosphate salts decreases T_g (Fig. 2a). For dehydrated trehalose samples equilibrated at 33% relative humidity, the T_g is 43.1°C at a

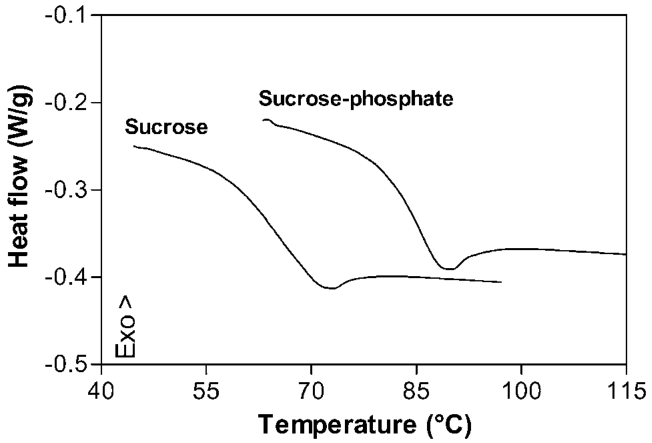

Fig. 1. DSC thermograms of freeze-dried sucrose and sucrose-phosphate mixture (containing potassium phosphate at phosphate/sucrose molar ratio of 0.5 at pH of 6.6). Both samples contained 0.6% (wt) water. The heating rate was 10°C/min.

residual water content of 7.0%, while the addition of phosphate at a phosphate/trehalose molar ratio of 1 decreases the T_g to 41.9°C at a residual water content of 6.2%. Both sugar–phosphate systems show increased water content with increasing relative humidity. At a relative humidity of 33%, the addition of phosphate at a phosphate/sugar molar ratio of 1 decreases the residual water content by 0.8% for trehalose and increases it by 2.2% for sucrose. For sucrose-phosphate samples, the water uptake increases gradually with increasing phosphate content (Fig. 2b). Mazzobre *et al.*, however, have observed an increase in water uptake by trehalose in the presence of various chloride salts (21).

Some of our previous work has revealed a marked effect of pH on T_g for aqueous solutions of trehalose with either borate or phosphate ions (16). To better understand the effect of pH on T_g, we performed a systematic study of the effect of initial solution pH on the T_g of dehydrated sugar-phosphate systems containing different phosphate counterions (potassium and sodium). Two different phosphate salts were considered to study the effects that counterion type and concentration may have on the sugar-phosphate interaction. Figure 3 shows the glass transition temperature as a function of pH for trehalose (Figs. 3a and 3c) and sucrose (Fig. 3b and 3d) samples containing different phosphate/sugar molar ratios. For both of the pure-sugar systems, trehalose and sucrose, different pH values were obtained by the addition of base or acid. Between pH values of 4 and 9, the T_g remains unchanged at 117°C and 58°C for trehalose and sucrose systems, respectively. For the sugar-phosphate mixtures at a given phosphate/sugar molar ratio, the different pH values were obtained by mixing various ratios of KH_2PO_4 and K_2HPO_4 (Figs. 3a and 3b) or NaH_2PO_4 and Na_2HPO_4 (Figs. 3c and 3d). At any phosphate/sugar molar ratio, the T_g increases with pH for both sugars. Using the potassium phosphate salts at a phosphate/sugar molar ratio of 1, the T_g increases from 95°C to 135°C for trehalose-phosphate and from 61°C to 101°C for sucrose-phosphate as the pH is increased from 4 to 9. However, the behavior of the two sugars is different in several respects. For trehalose, the addition of phosphate increases the T_g only at pH values greater than 6.6 (Figs. 3a and 3c). At a pH of 4.5, the addition of phosphate at a phosphate/trehalose molar ratio of 0.5 can reduce the T_g from 117°C to 95°C at similar residual water contents. However, for sucrose, T_g is increased at any pH, even at a nominal phosphate addition of 0.1 phosphate/sucrose molar ratio (Figs. 3b and 3d). The pH has an effect on the T_g, or the interaction strength between the sugars and the phosphates, but it appears that sucrose has a higher affinity for phosphate than trehalose. In aqueous solution, at high pH (pH = 9), the phosphates are predominantly in the HPO_4^{2-} form, and interact strongly with the sugars giving rise to the formation of a network that upon dehydration exhibits a higher T_g than that of the pure sugar. In the low pH regime (pH = 4), the majority of the phosphates are in the $H_2PO_4^{1-}$ form, which does not interact strongly with the sugars. Results suggest that the difference in the T_g – pH trend observed for the two sugars is due to the difference in the intermolecular hydrogen bonding network of the two sugars (22). The largest T_g increase for both of the sugar-phosphate mixtures is observed at the highest phosphate/sugar molar ratio (y = 1) and at the highest solution pH (pH

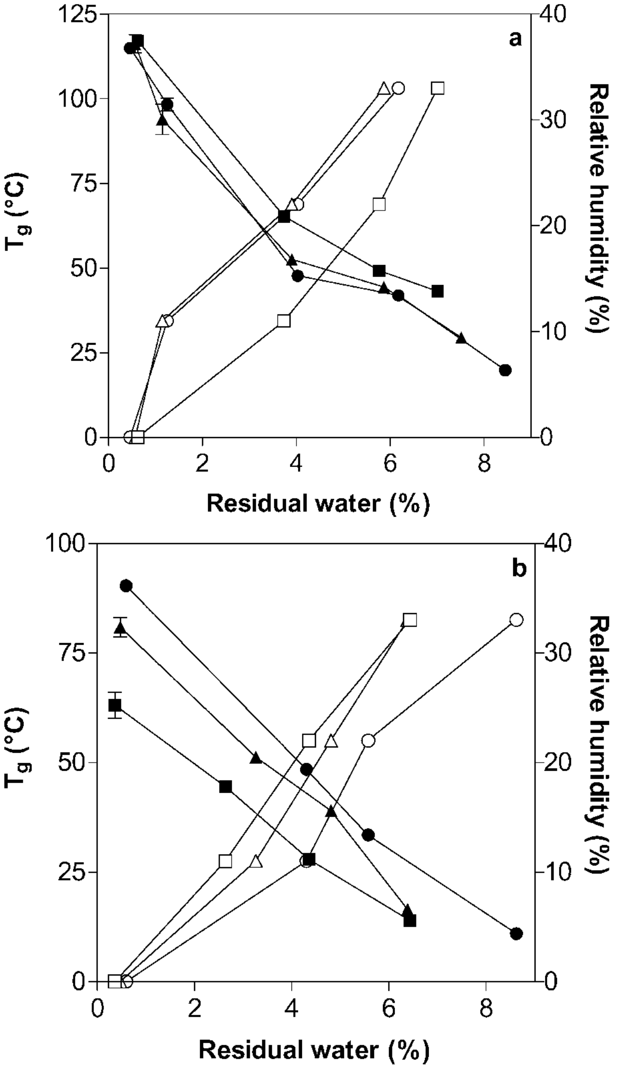

Fig. 2. Effect of potassium phosphate salt addition on the glass transition temperatures (filled symbols, left axis) and water sorption behavior at 20°C (open symbols, right axis) of freeze-dried trehalose (a) and sucrose (b) systems containing different phosphate/sugar molar ratios: 0.0 (■, □); 0.5 (▲, △); and 1.0 (●, ○).

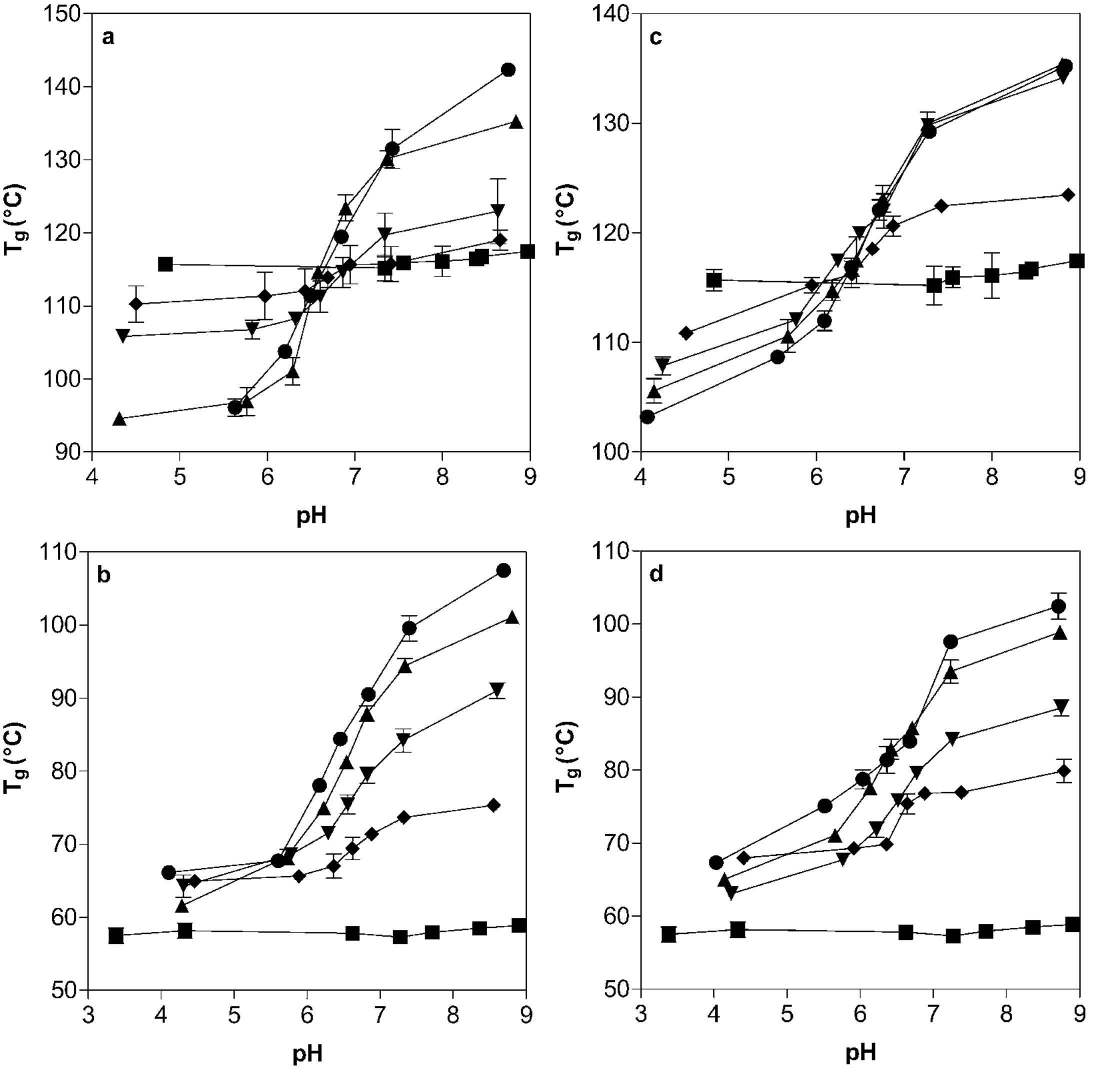

Fig. 3. Glass transition temperature as a function of pH for freeze-dried trehalose (a and c) and sucrose (b and d) systems containing potassium (a and b) or sodium (c and d) phosphate salts at the following phosphate/sugar molar ratios: 0.0 (■); 0.1 (◆); 0.3 (▼); 0.5 (▲) and 1.0 (●).

= 9) studied here. For potassium phosphate addition at a phosphate/sugar molar ratio of 1 at a solution pH of 9, T_g is increased by 25°C and by 50°C for trehalose and sucrose, respectively. For sucrose-phosphate systems, similar T_g pH relation was observed irrespective of the type of counterion used (Figs. 3b and 3d). We do not report results for phosphate/sugar molar ratios greater than 1 because phase separation occurred for freeze-dried trehalose- and sucrose-phosphate mixtures. We have shown that sugar-phosphate interactions in the solution state have an impact on the solid state properties. However, it is difficult to establish a clear and unambiguous relation between solution properties and solid state behavior because the distribution of the phosphate species change as water is removed from the system (23).

Figure 4 shows X-ray diffraction spectra for sucrose-phosphate samples at different pH values. Sucrose-phosphate samples remain amorphous at 0.5 phosphate/sugar molar ratio, regardless of the pH. However, incipient crystallization of sugar and potassium monobasic phosphate salt was detected at pH = 4 for samples having a phosphate/sugar molar ratio of 1. Crystallization was confirmed by polarized light microscopy analysis (data not shown). Phase separation was also observed by DSC analysis of sugar-phosphate samples having phosphate/sugar molar ratios greater than 1, which exhibited 2 T_g's (data not shown). Similar results were observed for trehalose-phosphate samples. The precipitation of the potassium monobasic phosphate salt upon freezing at low pH has been discussed in the literature (23,24). This is consistent with our observation for the freeze-dried sugar-phosphate mixtures, which show crystallization only at low pH. Murase *et al.*, however, report that phosphate salt precipitation can be inhibited by the presence of additional solutes such as sugars or polymers (24). This hindrance of salt precipitation was only observed for sugar-phosphate mixtures at pH = 9, thereby providing additional evidence for the strong interactions between sugar and phosphate molecules at high pH.

For both potassium and sodium, phosphate addition to sucrose increases T_g monotonically with pH; an increase of 50°C is observed at a phosphate/sucrose molar ratio of 1 and at an initial solution pH of 9. However for trehalose-phosphate systems, the effect of phosphate addition on T_g is different for these two counterions. For sodium, the increase in T_g ceases at a phosphate/trehalose molar ratio of 0.3 at pH values above 6.6 (Fig. 3c). For potassium, T_g continues to

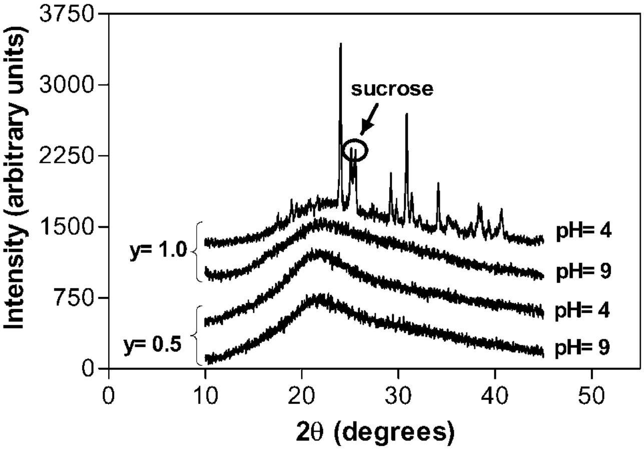

Fig. 4. XRD spectra for sucrose-phosphate mixtures (potassium phosphate at phosphate/sucrose molar ratios (y) of 0.5 and 1 at pH = 4 and 9) containing less than 1% (wt) water scanned at 1° 2θ/min. Except for the sucrose crystallization peaks (indicated in the figure), all of the peaks observed in the spectrum for the sample at phosphate/sucrose molar ratio of 1 at pH = 4 correspond to crystallization peaks for pure potassium monobasic phosphate.

increase with phosphate addition (Fig. 3a). Also at a phosphate/trehalose molar ratio of 0.3, the T_g for the trehalose-phosphate mixtures containing the sodium counterion is higher than that for the potassium systems by 10°C at pH values above 7. Depending on the type of sugar chosen, the choice of counterions can have an effect on the T_g of the sugar-phosphate mixtures.

It is unclear whether the change in T_g discussed above can be attributed solely to the effect of pH, since for systems containing phosphate salts, the pH was varied by changing the counterion concentration. In order to isolate the effects of pH and counterion concentration on T_g, we changed these two variables independently. We prepared a set of samples at the same pH containing different concentrations of potassium counterions (Table I) and another set of samples at various pH values that contained the same potassium ion concentration (Table II). For the pure sugar samples, T_g values do not change when the pH or the potassium ion concentration were changed (Tables I and II). By increasing the potassium ion concentration by 0.11 at a constant pH of 7, the T_g of trehalose remains at 94°C while that for sucrose remains at 59°C. The phosphate ion concentrations used in experiments shown in Tables 1 and 2 are the same as previously shown in the samples in which both the pH and the counter ion concentration were changed (Fig. 3). Increasing the pH from 3.5 to 9 at a constant potassium ion concentration of 0.28 does not change the T_g of the sugars; the T_g for trehalose remains at 93°C while that for sucrose remains at 58°C. For both of the sugar-phosphate mixtures, the glass transition temperature increases with rising pH, but not with increasing counterion concentration. By changing the pH from 4.5 to 7.5, the T_g increases from 97°C to 119°C and from 60°C to 81°C for trehalose-phosphate and sucrose-phopshate mixtures, respectively (Table II). However, the T_g's for both of the sugar-phosphate mixtures remain constant with changing counterion concentration at a constant pH of 7 (Table I). The increase in glass transition temperature by addition of phosphate can therefore be attributed to the change in pH. Similar results were obtained for the sugar-phosphate mixtures containing sodium ions (data not shown).

Table I. Glass Transition Temperature for Sugar and Sugar-Phosphate Samples Containing Different Amounts of Potassium Counter Ion at a Constant pH of 7

Sample	Potassium/sugar (molar ratio)	T_g (°C)*
Trehalose	0.28	93.5
	0.37	94.9
Trehalose-phosphate	0.28	109.7
	0.37	108.8
Sucrose	0.28	59.0
	0.37	59.8
Sucrose-phosphate	0.28	70.1
	0.37	70.7

The phosphate/sugar molar ratio employed in these samples is 0.5. All samples contain less than 1% (wt) residual water. Samples for DCS analysis typically weighed 3 mg and were scanned twice at 10°C/min.

* The standard deviation for the glass transition temperature is 1°C.

Several authors have also analyzed the effect of cations on the glass transition temperatures of polyhydroxy compounds. The results have been contradictory. Vacuum-dried mixtures of glycerol with $MgCl_2$ show a pronounced increase in T_g with increase in the salt/glycerol molar ratio (25). However, freeze-dried mixtures of trehalose or sucrose with $MgCl_2$ at a sugar/salt molar ratio of 5:1 do not show changes in T_g compared to the pure sugars (26,27). In the absence of phosphate, we do not observe any changes in T_g upon addition of potassium or sodium.

Results suggest that specific interactions between the sugars and the phosphates give rise to mixtures with higher T_g values than those observed for the pure sugars (Fig. 3). The resulting sugar-phosphate "complexes" are very sensitive to pH changes due to the different protonation states of the phosphate ions. In order to analyze the strength of the sugar-phosphate interaction (due to hydrogen bonding), we per-

Table II. Glass Transition Temperatures for Sugar and Sugar-Phosphate Samples Having Different pH, at a Constant Potassium/Sugar Molar Ratio of 0.28

Sample	pH	T_g (°C)*
Trehalose	3.5	92.9
	7.6	93.6
	9.3	94.6
Trehalose-phosphate	4.6	96.6
	6.5	99.2
	7.5	118.8
Sucrose	3.4	57.5
	7.3	57.3
	8.9	58.9
Sucrose-phosphate	4.5	59.6
	6.5	72.5
	7.5	80.6

The phosphate/sugar molar ratio employed in these samples is 0.5. All samples contain less than 1% (wt) residual water. Samples for DSC analysis typically weighed 3 mg and were scanned twice at 10°C/min.

* The standard deviation for the glass transition temperature is 1°C.

formed FTIR measurements on the sugar-phosphate samples. We focus our attention on the -OH stretching. Figure 5a shows a typical FTIR spectrum for a sugar or sugar-phosphate mixture, and Fig. 5b shows the shift in the wavenumber of –OH stretching modes for dehydrated trehalose and sucrose samples in the presence and in the absence of phosphate (0.5 phosphate/sugar molar ratio) at various pH values. Pure dehydrated trehalose and sucrose show the –OH stretching wavenumber at 3600cm^{-1}, and this wavenumber is reduced by the addition of phosphate. As the pH of the sugar-phosphate mixtures is increased from 4.5 to 9, the wavenumber for –OH stretching decreases by 50cm^{-1} and 60cm^{-1} for the dehydrated trehalose- and sucrose-phosphate mixtures, respectively. This down-shift in the wavenumber of –OH stretching indicates further stretching of the sugar –OH groups, which can be viewed as a manifestation of a weaker interaction between the oxygen and the hydrogen. This may be caused by the increased interaction between the sugars and the phosphates at a higher pH, an interpretation which is consistent with the increased T_g discussed above (Fig. 3).

The T_g increases with increase in pH at any phosphate/sugar molar ratio; the preservation of biologic materials, however, is more favorable at neutral pH values. We have therefore explored the possibility of retaining the high T_g values (attained at high pH values) at lower pH values. Solutions of sugar-phosphate mixtures (0.5 phosphate/sugar molar ratio) were prepared having a pH of 8.8, and acid was added to lower the pH to 6.8. Figure 6 shows the T_g values obtained for the freeze-dried trehalose-phosphate and sucrose-phosphate samples having a pH of 8.8 and 6.8 (in solution). For the samples with addition of acid, the T_g of the freeze-dried sugar-phosphate mixtures drops to the value expected at the lowered pH. Similarly, addition of base to trehalose- or sucrose-phosphate solution prepared at a pH of 4.0 resulted in an increase in T_g of the freeze-dried samples, but not any higher than the values expected at the raised pH (data not shown). From the above results, we conclude that the sugar-phosphate interaction in solution is completely reversible within the time scale of our experiments, and it is sensitive to pH changes.

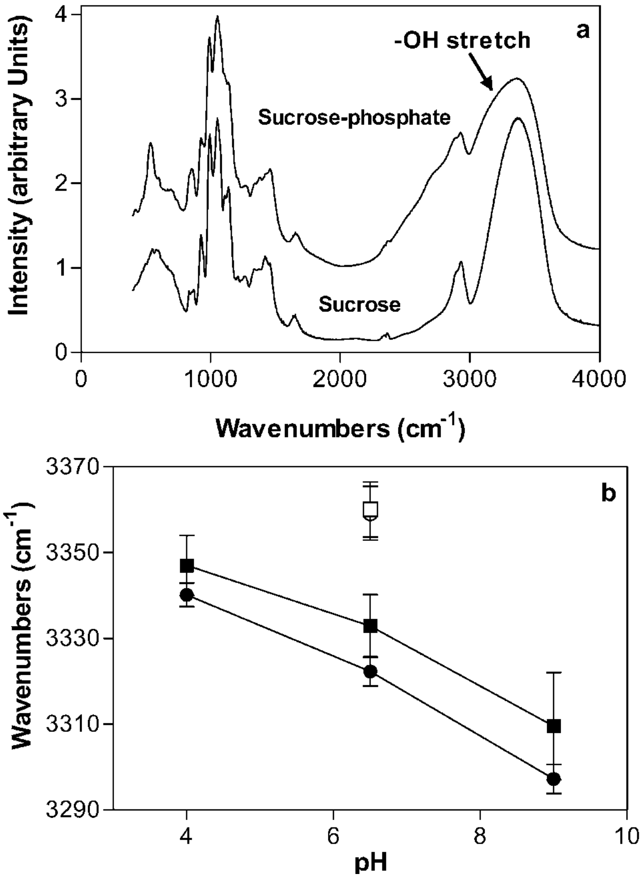

Fig. 5. (a) FTIR spectra for freeze-dried sucrose and sucrose-phosphate (containing potassium phosphate at phosphate/sucrose molar ratios of 0.5 at pH = 9). (b) FTIR analysis of the –OH stretching for freeze-dried sugar systems as a function pH in the presence of phosphate (0.5 phosphate/sugar molar ratio), filled symbols; and in the absence of phosphate, empty symbols: trehalose (■,□), sucrose (●,○).

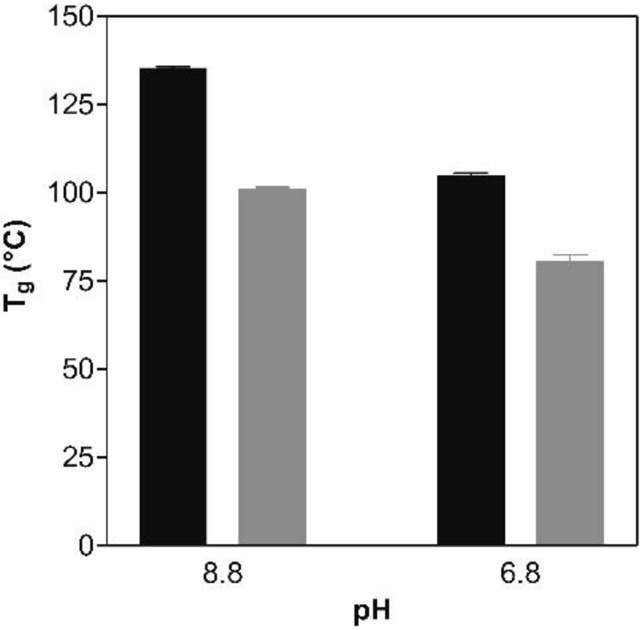

Fig. 6. Effect of pH changes (in the solution prior to freeze-drying) on the glass transition temperatures for freeze-dried trehalose (black bar) and sucrose (grey bar) systems containing potassium phosphate at a phosphate/sugar molar ratio of 0.5. The samples having a pH of 6.8 were obtained by the addition of HCl to the solution at pH 8.8 (prior to dehydration).

The collection of data gathered in this work indicates that sugar-phosphate mixtures exhibit a number of interesting features for use in lyoprotectant formulations for labile molecules. T_g values much higher than those observed for pure sugars can be obtained upon the addition of phosphate. In the case of sucrose-phosphate mixtures, the T_g can be increased in a broad pH range (4–9), and T_g's 40°C higher than those for pure sucrose can be obtained at physiologic pH values. Furthermore, it is possible for the sucrose-phosphate mixtures to exhibit a T_g higher than that of pure sucrose while retaining higher water contents, a feature that is advantageous for lyophilization of complex biologic structures such as cells. Trehalose-phosphate mixtures (having a T_g of 135°C at a pH of 8.8) are a better option compared to trehalose to preserve molecules that can withstand conditions of high pH.

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