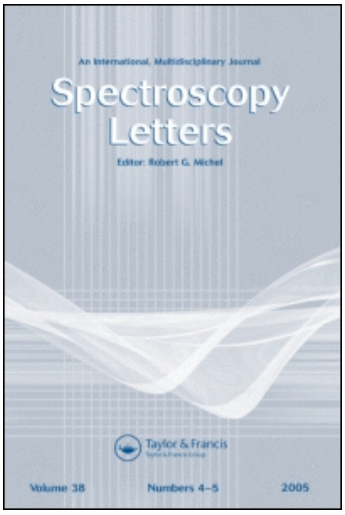

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

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Online publication date: 22 June 2010

To cite this Article Imamura, Koreyoshi , Ohyama, Ken-ichi , Tani, Kazuko , Yokoyama, Toru , Maruyama, Yoshinobu , Imanaka, Hiroyuki and Nakanishi, Kazuhiro(2008) 'Fourier Self-Deconvolution Analysis of Hydrogen Bonding States of Polyvinylpyrrolidone in an Amorphous Sugar Matrix Below and Above the Glass Transition Temperature', Spectroscopy Letters, 41: 6, 305 — 312
To link to this Article: DOI: 10.1080/00387010802370959
URL: http://dx.doi.org/10.1080/00387010802370959

Spectroscopy Letters, 41:305–312, 2008

ISSN: 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010802370959

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Regular Article

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ABSTRACT In an amorphous mixture of sugar and polyvinylpyrrolidone (PVP), PVP carbonyl groups form hydrogen bonds with sugar hydroxyl groups, thereby improving the physical stability of the amorphous matrix against a glass-to-rubber transition. Herein, Fourier self-deconvolved IR bands due to the C=O stretching vibration of PVP in sugar–PVP mixtures were analyzed. The C=O groups in sugar–PVP mixtures generally had four vibrational states, corresponding with free and hydrogen-bonded C=O in three different modes. Changes in these vibrational states induced by increasing the temperature were compared among various sugar–PVP mixtures. Formation and thermal disruption characteristics of different modes of sugar–PVP hydrogen bondings are discussed.

KEYWORDS amorphous sugar, carbonyl stretching vibration, fourier self-deconvolution, glass transition temperature, polyvinylpyrrolidone

INTRODUCTION

Binary systems, composed of a sugar and polymer additive in the amorphous states, are commonly used for disintegration, dilution, and stabilization in the drug and food manufacturing industries. In amorphous sugar–polymer mixtures, the polymer additive generally serves to improve the physical stability of the amorphous sugar matrix, thereby inhibiting the glass-to-rubber transition. Consequently the glass transition temperature, Tg, of the matrix is increased.

In an amorphous mixture of a sugar and polymer additive, the interactions between sugar and polymer additive molecules play an important role in increasing the Tg of the amorphous matrix that results from addition of the polymer. Characteristics of the sugar–polymer interactions have been

Received 28 September 2007; accepted 2 April 2008.

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investigated by examining the vibrational states of functional groups participating in the interactions: Shamblin et al.[1] and Taylor and Zografi[2] conducted FT-RAMAN analyses of freeze-dried disaccharides and oligosaccharides containing polyvinylpyrrolidone (PVP) as the polymer additive and, based on the negative shift in the absorption band due to the C=O stretching vibration of PVP, concluded that sugar–PVP hydrogen bond had formed. The extent to which sugar–PVP interactions formed also was compared among different sugar–PVP mixtures by examining the magnitude of the negative shift in the C=O stretching vibration band of PVP.[1,2]

However, it is assumed that the functional group responsible for the sugar–polymer interaction has multiple vibrational states. For example, in the sugar–PVP mixed system, the band due to C=O stretching vibration is composed of multiple component bands with different vibration frequencies, which correspond with the various degrees of restriction of C=O stretching vibration due to the interactions with the sugar molecules. It is expected that the composition of the component bands would change the peak position of the apparent single C=O band, as well as altering the half-width. In actuality, previous FTIR analysis of a freeze-dried trehalose–PVP mixture by our group revealed the presence of five component bands in the apparent single C=O stretching band of PVP: two were assigned to free C=O groups and the other three were assigned to C=O groups restricted by hydrogen bonds with sugar molecules.[3] IR component band analysis allows quantitative estimation of the degree of sugar–PVP hydrogen bond formation.[3] Based on these results, analysis of the component bands contained in the apparent single band corresponding with the functional group responsible for the sugar–polymer interaction is essential to better understand the formation characteristics of sugar–polymer interactions.

The current study analyzed the component bands of the C=O stretching vibration of PVP in IR spectra under various conditions, including varied temperature and sugar type and content. The IR spectra for the sugar–PVP mixture at different temperatures were obtained by temperature scanning–Fourier transform IR spectroscopy (TS-FTIR) in our previous study.[4] The IR bands corresponding with C=O stretching vibration were deconvolved into individual component bands, and the composition of the component bands for different temperatures and sugar type and contents were determined. Based on the results, the formation and thermal disruption characteristics of sugar–PVP hydrogen bonds and their possible relationship to the physical stability of the amorphous sugar matrix and to the glass-to-rubber transition are discussed.

MATERIALS AND METHODS

α-D-Glucose, trehalose (1-O-α-D-glucopyranosyl-α-D-glucopyranoside), α-maltose (4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside), isomaltose (6-*O*-α-D-glucopyranosyl-α-D-glucopyranoside), and sucrose (β-D-fructofuranosyl-α-D-glucopyranoside) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Maltotriose and maltoheptaose were products of Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Polyvinylpyrrolidone, with a mean MW of 360,000 (PVP 360k; Wako Pure Chemical Industries), served as the primary polymer additive. Alternatively, PVP with MWs of 24,500 and 1,000,000 were used, but the results for these PVPs did not differ markedly from the results using PVP 360k. All chemicals used in this study were of reagent grade and were used without further purification.

Preparation of amorphous sugar–PVP mixtures and TS-FTIR analysis was conducted as previously described.[5] In brief, a 10-μL droplet of an aqueous solution containing 10 mg/mL sugar was dried on a BaF_2 disk in a stream of nitrogen gas (10 L/min) for at least 10 min, followed by preheating to approximately 120°C using a SpectraTech Heated Cell (Shelton, CT). After cooling the sample to less than 25°C with a stream of nitrogen gas, the thoroughly dehydrated amorphous sample was then heated at a rate of 1°C/min to 150–200°C. During heating, IR spectra of the sample were collected at appropriate intervals.

The IR absorption band due to the C=O stretching vibration of PVP was analyzed because the interaction between the sugar and PVP is mostly hydrogen bonding between the hydroxyl groups of the sugar and the carbonyl groups of PVP.[1,6–8] The band due to C=O at 1700–1600 cm^{-1} was deconvolved by Fourier self-deconvolution using OMNIC version 4.1a software (Nicolet, Madison, WI) at a resolution factor K of 2.0 and an unresolved band half-width

of 30 cm^{-1}. These settings were optimal to maintain a flat baseline on both sides of the C=O band and to show as many band components as likely.[3,9] The overlapping bands in the deconvolved band were resolved using the PeakFit version 4.12 software program (SeaSolve Software Inc., Framingham, MA). The wavenumbers and areas of the individual component bands were determined to estimate the proportions of free C=O groups and those hydrogen-bonded in different modes.

To check whether or not the relative intensity of IR absorption due to the C=O stretching vibration changes upon the formation of hydrogen bonds,[10] we determined the peak areas of IR band due to the C=O stretching vibration (1600–1700 cm^{-1}) for PVP dried in the absence of sugar and fully hydrated in an aqueous solution as well as the sum of peak areas in the wavenumber regions of 1350–1500 cm^{-1}. The ratio of the peak areas in the two wavenumber regions was calculated, and the difference in the area ratio between dehydrated and fully hydrated PVP samples was found to be within 5%. Hence, it was concluded that the change in the relative absorption intensity for the C=O stretching band due to formation of hydrogen bonds was negligible, and the fractions of the C=O groups in the individual interaction states could be calculated directly as the ratios of each component band area to the total.

At least two TS-FTIR measurements and analyses were performed on each sample, and the deviations of the component bands were within ±5% of the average values.

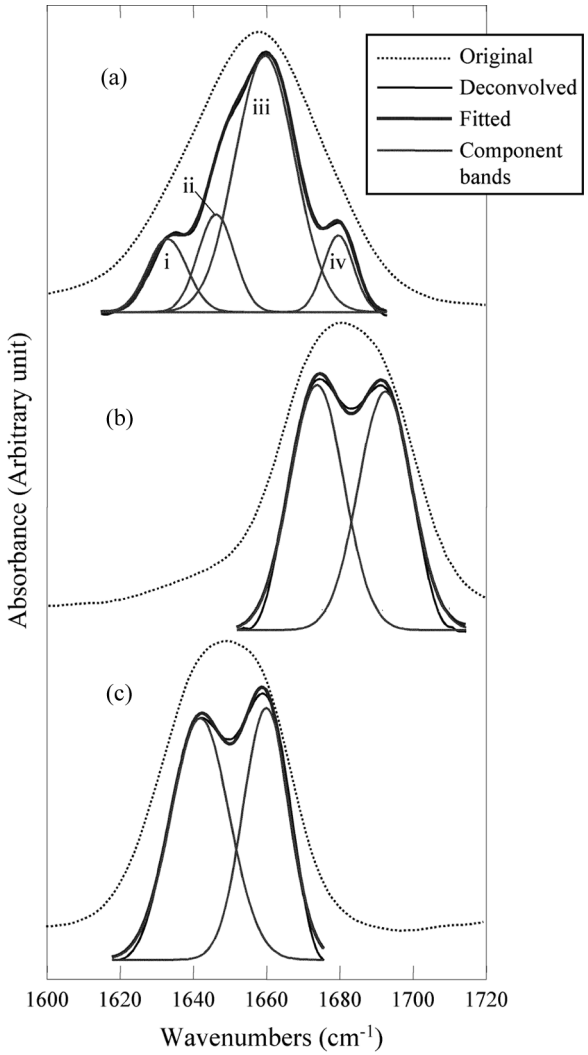

FIGURE 1 **Fourier self-deconvolved IR bands for C=O stretching of PVP 360k and the fitted individual component bands (at room temperature). (a) PVP dehydrated with 4 g/g PVP of maltose; (b) PVP dehydrated alone; (c) 50 mg/mL PVP aqueous solution.[3]**

RESULTS

Figure 1 shows the Fourier self-deconvolved IR bands corresponding with the C=O stretching vibration of PVP dried in the (a) presence and (b) absence of sugar (4 g/g PVP of maltose) and (c) fully hydrated in an aqueous solution. As shown in Fig. 1, the C=O stretching band for the sugar–PVP mixture [spectrum (a)] is composed of four component bands (i–iv), whereas the sugar-free sample [spectrum (b)] shows two component bands at approximately 1695 cm^{-1} and 1675 cm^{-1}, which are assigned to the free C=O groups of PVP. The peak position of the component band at approximately 1695 cm^{-1} negatively shifted with increasing sugar content and coincided with that of the component band (i) for the sugar-PVP mixture at a sugar content of 1.0 g sugar/g PVP. Component band (i) for the sugar–PVP mixture is thus assigned to free C=O groups. The other component band (~1675 cm^{-1}) for the sugar-free sample was diminished in the presence of ≥1 g sugar/g PVP. In contrast, (c) the IR band for fully hydrated PVP is composed of two component bands, the peak positions of which are completely consistent with those of component bands (ii) and (iii) for the sugar–PVP mixture. For the sugar–PVP, component band (iv) is positioned at a lower wavenumber than are the other four component bands, indicating the greatest restriction of C=O stretching vibration, probably due to embedding in the solid-phase matrix.[3] Therefore, the three

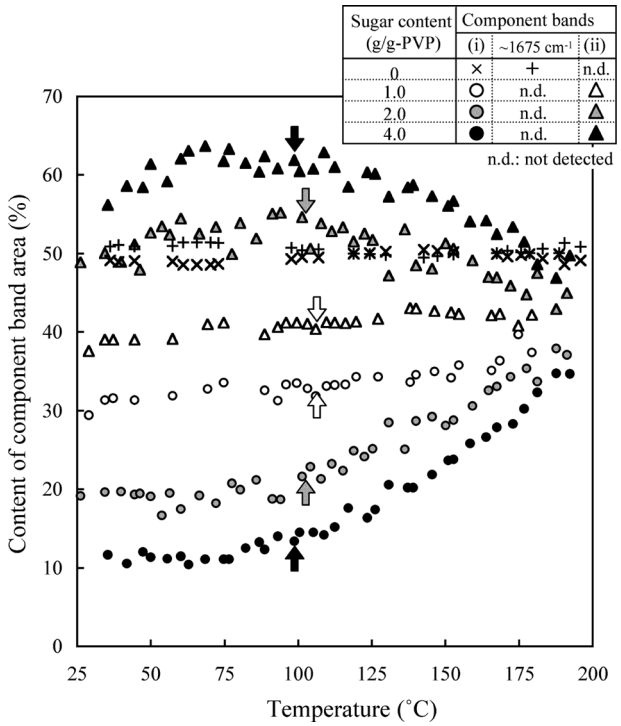

FIGURE 2 **Change in the contents of the individual component band (i and ii at ~ 1675 cm^{-1}) areas for the C=O stretching of PVP 360k dried in the presence of 0–4.0 g maltose due to increasing temperature. Arrows indicate Tg values.**

component bands (ii–iv) are assigned to hydrogen-bonded C=O groups. The peak positions of the four component bands (i–iv) for PVP dried in the presence of sugar are consistent with those for the sugar (trehalose)–PVP sample obtained by freeze-drying in our previous study.[3] Furthermore, the deviations of the peak positions of the four detected four component bands (i–iv) were within $\pm 2\,cm^{-1}$ irrespective of temperature, the sugar content (>1 g/g-PVP), and type of sugar. Based on the above-mentioned assignments of the component bands for the C=O stretching vibration, the formation and thermal disruption characteristics of sugar–PVP hydrogen bonds in various sugar–PVP mixtures were investigated.

The contents of component bands (i) and (ii) were plotted against temperatures for sugar–PVP mixtures, as shown in Fig. 2. The content of component band (i) increases slowly with increasing temperature, up to Tg (Table 1), and the increase in the content of band (i) is significant above Tg. In contrast, the content of component band (ii) significantly decreases at temperatures in excess of Tg. These results directly evidence that the glass-to-rubber transition of the amorphous matrix disrupts the sugar–PVP hydrogen bonds. On the other hand, the contents of component bands (iii) and (iv) at 25°C were respectively ca. 24% and 8% and continuously, but gradually, decreased to ca. 15% and 3% with increasing temperature up to 190°C (data not shown), suggesting that the thermal disruption characteristics of sugar–PVP hydrogen bonds differ by the interaction mode, corresponding with each component band.

It should be noted that the content of component band (ii) increases by approximately 10% at temperatures below ~75°C. Because, in the temperature

TABLE 1 **Glass Transition and peak positions of undeconvolved IR bands due to PVP C=O stretching vibration for sugar–PVP (360k) mixtures at 25°C and 150°C[a]**

Sugar	Sugar content (g/g PVP)	Tg (°C) (Sugar alone)	R_{Tg} (–)	Peak position (cm^{-1})[b] at 25°C	Peak position (cm^{-1})[b] at 150°C
Glucose	4.0	40 (37)	0.021	1655	1659
Trehalose	4.0	115 (105)	0.13	1659	1660
	1.0	106 (90)	0.18	1661	1665
Maltose	2.0	104	0.16	1658	1660
	4.0	99	0.10	1658	1660
Isomaltose	4.0	96 (81)	0.15	1657	1660
Sucrose	4.0	67 (62)	0.042	1657	1660
Maltotriose	4.0	132 (113)	0.28	1660	1663
Maltoheptaose	4.0	161 (153)	0.30	1661	1663

[a]The increase in Tg [R_{Tg} (–)] was normalized as follows[4]:

$$R_{Tg} = (Tg^{mix} - Tg^{sugar})/(Tg^{PVP} - Tg^{sugar}),$$

where Tg^{mix}, Tg^{sugar}, and Tg^{PVP} are the Tg values for the sugar–PVP mixture, sugar alone, and PVP alone, respectively. $Tg^{PVP} = 180°C$.[4]
[b]Deviations of the peak position were within $\pm 1\,cm^{-1}$.

range below ~75°C the content of component band (i) does not decrease, but increases slightly, and the contents of component bands (iii) and (iv) tended to decline as described above, the increase in component band (ii) content in the low-temperature range indicates that the conversion of sugar–PVP hydrogen bonds from one mode to another can occur: The maltose–PVP hydrogen bonds responsible for component bands (iii) and (iv) are partially converted to those of component band (ii).

Figure 2 shows the results for different sugar (maltose) contents. In the temperature range examined, the contents of bands (i) and (ii) decrease and increase, respectively, with increasing sugar content, indicating that the number of free C=O groups of PVP is reduced. These changes emphasize the increase in the content of component band (i) and decrease in the content of component band (ii) with temperatures in excess of Tg. The increase in component band (ii) content in the low-temperature range, due to the conversion from component bands (iii) and (iv), also becomes more significant with increasing sugar content, whereas the contents of component bands (iii) and (iv) appear constant at a sugar content of $\geq$1 g sugar/g PVP, indicating that they are saturated at sugar content of $\leq$1 g/g PVP. Similar tendencies were observed for the sugar–PVP mixture obtained by freeze-drying in our previous study.[3]

Figure 3 shows the relationships between the contents of component bands and temperature for different types of sugars. A larger oligomer matrix tends to have more free C=O groups at room

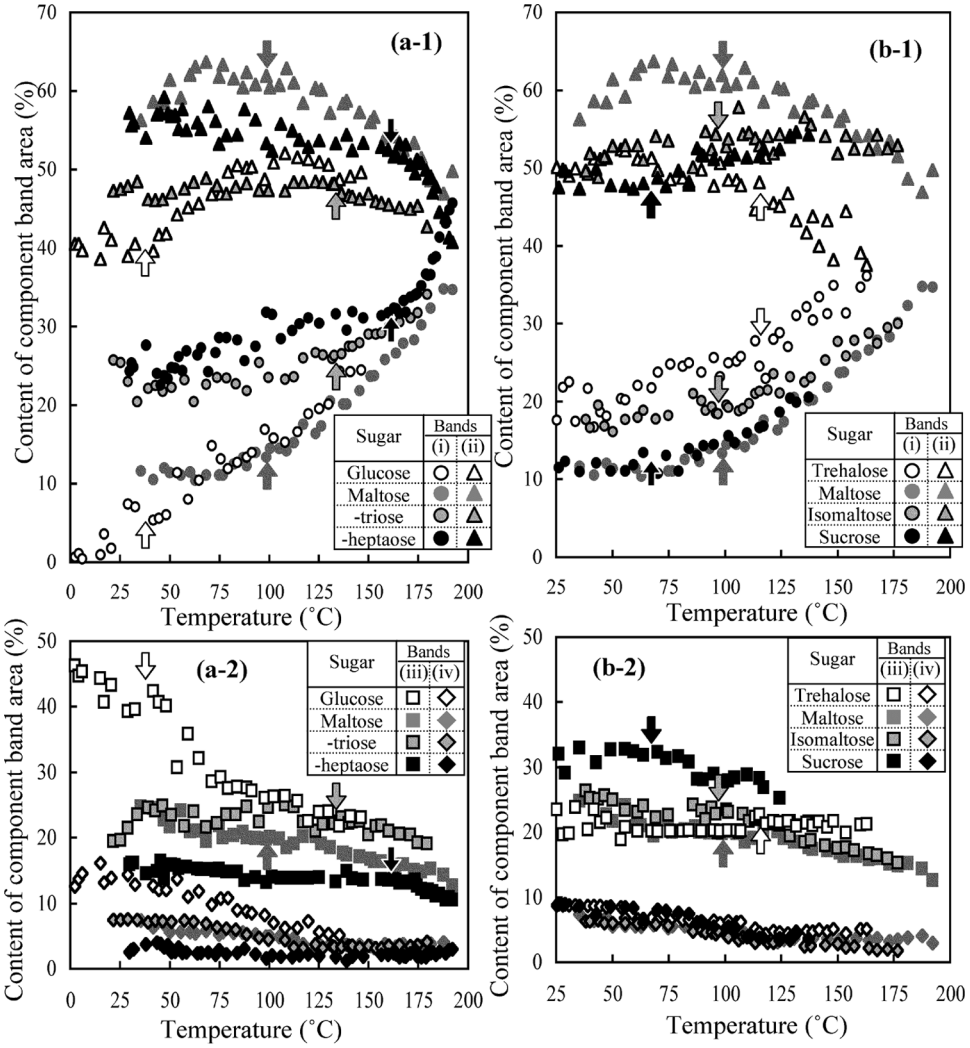

FIGURE 3 **Change in the contents of the individual component band (i and ii, top; iii and iv, bottom) areas for the C=O stretching of PVP 360k dried in the presence of 0.25 g of various sugars due to increasing temperature. (a-1, 2) Glucose, maltose, maltotriose, and maltoheptaose; (b-1, 2) trehalose, maltose, isomaltose, and sucrose. Arrows indicate Tg values.**

temperature: The content of component band (i) for glucose at 25°C is only a few percent, indicating that more than 95% of the C=O groups form hydrogen bonds with glucose molecules. The content of component band (i) at room temperature increases up to 25% (maltoheptaose) as the size of the sugar molecule becomes larger. The component bands (iii) and (iv) at temperatures below 100°C are more significant for smaller size of sugar, whereas there appears no clear correlation between molecular size of sugar and the content of component band (ii). Sucrose shows markedly lower content of component band (i) and higher content of component band (iii) than the other disaccharides in the low-temperature range below 100°C, indicating its peculiar characteristics of hydrogen bond formation.[11]

As for the effect of temperature change on the content of each component band, isomaltose and trehalose show patterns similar to that of maltose (Fig. 2). The effect of temperature change on component bands (i), (iii), and (iv) for maltotriose and multoheptaose also is similar to that observed for maltose. However, an increase in component band (ii) in the low-temperature range is not observed, indicating that the conversion among different modes of sugar–PVP hydrogen bonds does not occur in these amorphous oligosaccharides. In contrast, glucose and sucrose show different patterns as follows: The content of component band (ii) continues to increase in the temperature range examined, which includes Tg, and the decrease in the contents of component bands (iii) and (iv) are significant at temperatures in excess of Tg.

DISCUSSION

PVP present in an amorphous sugar matrix forms three different modes of hydrogen bonds with sugar molecules, as indicated by the three component bands (ii–iv) (Fig. 1). Judging from the fact that the three modes of sugar–PVP hydrogen bonding differ in their thermal disruption characteristics (Fig. 2), it is assumed that these three modes of sugar–PVP hydrogen bonding may have different effects on the Tg of sugar–PVP mixtures. The effect of temperature change on the content of component band (iii) for different types of sugar appears to coincide with the relative magnitude of increase in Tg resulting from addition of PVP (Table 1) as follows: As shown in Fig. 3, the thermal disruption of sugar–PVP hydrogen bonds responsible for component band (iii) is significant for amorphous glucose and sucrose. In contrast, the hydrogen bonds between maltotriose and mattoheptaose and PVP, responsible for component band (iii), are preserved as long as the glass-to-rubber transition does not occur. On the other hand, our previous study revealed that hydrogen bonding among sugar molecules, which serves to hold the matrices, is reduced in amorphous glucose and sucrose by the formation of sugar–PVP hydrogen bonds.[4] This property of amorphous glucose and sucrose likely impaired the contribution of sugar–PVP hydrogen bonds to the increase in Tg,[4] as indicated by the small relative increase in Tg (Table 1), whereas the presence of PVP in amorphous maltotriose and maltoheptaose did not lower the degree of sugar–sugar hydrogen bonding; thus, the increase in Tg is not inhibited, as shown in Table 1.

In contrast, component band (ii) for glucose and sucrose continues to increase above Tg, whereas the increase in Tg for these sugars induced by PVP is minimal (Table 1), suggesting the absence of any correlation between the increase in Tg and the content of component band (ii). The sugar–PVP hydrogen bonding responsible for component band (iv) may contribute less to the increase in Tg than does that for component band (iii) because the sugar–PVP hydrogen bonds in component band (iv) are few in number.

As shown in Fig. 2, the contents of component bands (iii) and (iv) are saturated by 1 g sugar/g PVP, whereas the content of component band (ii) appears to increase continuously with increasing sugar content up to 4 g sugar/g PVP. This result suggests that the C=O groups responsible for component bands (iii) and (iv) are easier for sugar molecules to access than are the C=O groups of component band (ii). One possible explanation for the difference in the accessibility of sugar molecules is that the sugar–PVP hydrogen bonds corresponding with component bands (iii) and (iv) form between exterior regions of loose intramolecular aggregates of PVP molecules and sugar molecules, while the C=O groups present inside the aggregates form sugar–PVP hydrogen bonds for component band (ii). Thermal expansion of PVP molecules that results

from heating may facilitate the access of sugar molecules inside the PVP molecules, thereby increasing the content of component band (ii), as observed for amorphous monosaccharides and disaccharides at temperatures below ~75°C (Fig. 3). However, access of the larger oligomers, maltotriose and maltoheptaose, may be prevented because of their larger molecular size.

As described above, the peak positions of component bands (ii–iv) are common within $\pm 2\,cm^{-1}$ for all the examined sugar–PVP mixtures. Thus, it can be deduced that the modes of sugar–PVP hydrogen bondings are common independent of types of sugar. On the other hand, the fractions of each mode of sugar–PVP hydrogen bonds markedly vary depending on the type of sugar as shown in Fig. 3. Smaller sugars are more likely to form hydrogen bonds with C=O groups of PVP, as indicated by the lower content of component band (i) and the higher contents of component bands (iii) and (iv) (Fig. 3). This relationship is consistent with that observed by Taylor and Zografi[2] in the comparisons of peak positions of unresolved IR bands for C=O stretching vibration for several sugar–PVP mixtures. The ability of sugars to form hydrogen bonds with proteins also is greater for smaller sugars compared with large oligosaccharides.[12–14] The relationship between sugar size and hydrogen-bonding ability may be due to the difference in packing states of the sugar molecules in amorphous matrices as follows: Smaller sugars tend to form amorphous matrices of higher density[15] and thus more tightly enclose polymer additives or protein molecules.

In addition to polymer additives including PVP, the presence of certain types of salts, borate,[16,17] citrate,[18] and phosphate,[19,20] also has been reported to increase Tg of amorphous sugars. The increase in Tg by these salts has been considered to occur through the interactions between sugar OH and these salts.[16–20] This might be similar to the mechanism of the increase in Tg by PVP, in which hydrogen bondings between sugar O–H and PVP C=O groups serve to hold the amorphous sugar matrix in place.[2,4] However, borate[16,17] and citrate[18] (0.2 g/g sugar) increased Tg of amorphous sugars (trehalose and sucrose) by approximately 25°C, which are markedly greater than the magnitude of increased Tg by PVP, as shown in Table 1. Furthermore, the magnitude of increased Tg for trehalose by phosphate varied from minus a few tens to plus several tens of degrees Celsius, depending on the pH of the sugar–phosphate solution prior to dehydration,[20] because phosphate has four different ionization forms.[19] These findings may demonstrate that the interactions of these salts with sugar molecules are considerably different in mode and strength from the sugar–PVP hydrogen bonds.

To date, the formation characteristics of sugar–PVP hydrogen bonds have been described based on analyses of the peak position of the IR band of C=O stretching vibration,[1,2] which is composed of multiple overlapping component bands. The reported results successfully demonstrated that the degree of sugar–PVP hydrogen bond formation varies with the sugar type and content. Also, in our previous study,[4] the peak position of the undeconvolved C=O stretching band appeared to shift depending on the degree of hydrogen bondings between sugar and PVP C=O groups. However, as shown in Table 1, the difference in the peak positions for the herein examined sugars are below $6\,cm^{-1}$, and the shift in the peak position of the PVP C=O stretching band, as a result of increasing the temperature from 25°C to 150°C, was at most $4\,cm^{-1}$. The small deviations of peak positions within several wavenumbers provide only qualitative insights into the magnitude correlation among the degrees of sugar–PVP hydrogen bonding for different types of sugar and temperatures. On the other hand, the decrease in sugar–PVP hydrogen bonding in this temperature range is apparent from the temperature dependency of component band (i) (Figs. 2 and 3). In our previous study,[3] it was demonstrated that the degree of sugar–PVP hydrogen bonding estimated using Fourier self-deconvolution analysis of the C=O stretching vibration of PVP coincided with that calculated based on the water sorption behavior of sugar–PVP mixtures. Furthermore, this study indicates that the analyzing method using Fourier self-deconvolution may allow the investigation of the formation and disruption characteristics of different modes of sugar–polymer interactions that have differing effects on the physical properties of sugar–polymer mixtures. Hence, vibrational state analysis using Fourier self-deconvolution is promising for quantitative analysis of the interactions in a sugar–additive binary system.

ACKNOWLEDGMENT

This work was supported by grant-in-aid for the Encouragement of Young Scientists (no. 18760594) from the Ministry of Education, Science, Sport and Culture of Japan and by the Okayama Foundation for Science and Technology.

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