Effect of Water on the Molecular Mobility of Sucrose and Poly(vinylpyrrolidone) in a Colyophilized Formulation as Measured by ^{13}C-NMR Relaxation Time

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Individual molecular mobility of sucrose and poly(vinylpyrrolidone) (PVP) in a colyophilized mixture of 1 : 1 by weight has been determined by ^{13}C spin–lattice relaxation times in the laboratory frame (T_1) and in the rotating frame ($T_{1\rho}$) for systems containing absorbed water at various levels. The T_1 of the PVP pyrrolidone ring carbon increased with storage relative humidity (RH) in lyophilized PVP alone, indicating that the MHz-order motions of PVP side chain increased with storage RH. However, in the colyophilized mixture, the side chain motions of PVP did not change with storage RH, and showed similar mobility to sucrose. This may be caused by hydrogen bonding between the PVP ring carbonyl group and hydroxyl group of sucrose, as suggested by a previous FT-Raman study. The mid-kHz-order motions of sucrose in the sucrose–PVP mixture as determined by $T_{1\rho}$ did not increase with storage RH as much as in lyophilized sucrose alone. This suggests that the molecular mobility of sucrose decreases in the presence of PVP due to hydrogen bonding between the hydroxyl group of sucrose and the carbonyl group of PVP. Inhibition of sucrose crystallization by PVP in the presence of water appears to be linked to the effect of PVP on the molecular mobility of sucrose.

Key words mobility; NMR; sucrose; PVP; crystallization; lyophilization

Sucrose is widely used as a cryoprotectant of lyophilized protein formulations. However, the crystallization of sucrose during storage is an issue of some concern. Recent studies of colyophilized amorphous molecular dispersions of sucrose and poly(vinylpyrrolidone) (PVP) have demonstrated the ability of PVP to inhibit sucrose crystallization under increased temperature and relative humidity conditions, down to fairly low levels of PVP.[1—4] Other studies with sucrose–PVP molecular dispersions, using FT-Raman spectroscopy have demonstrated intermolecular hydrogen bonding between sucrose and PVP, not observed with the corresponding physical mixtures.[5,6] Interestingly, in both physical mixtures and the colyophilized systems, the amount of water absorbed by either type of mixture could be predicted as a function of relative humidity from the weighted average of the individual components, but only in the case of the molecular dispersions was crystallization inhibited.[2,4] To explain these effects on crystallization, it has been suggested that PVP, with a much higher T_g than sucrose, reduces the molecular mobility of sucrose in preventing nucleation and crystal growth through hydrogen bonding.[1,4] In dry systems containing amorphous sucrose and PVP, as measured by enthalpy relaxation, molecular mobility of the molecular dispersions was indeed decreased relative to that of sucrose alone, but not in the case of physical mixtures.[7] However, the molecular mobility of sucrose in such mixtures could not be determined directly by such enthalpy relaxation measurements.

NMR relaxation measurements provide information on molecular motions of specific atoms in a molecule on a MHz time scale through measurements of the spin–lattice relaxation time in the laboratory frame, T_1, and on a mid kHz time scale through measurements of spin–lattice relaxation time in the rotating frame, $T_{1\rho}$. Solid-state ^{13}C-NMR, in particular, is very useful to determine the individual molecular motions of various components in a mixture.[8—12] In a previous paper, molecular mobility of PVP in mixtures of PVP and absorbed water was determined from ^{13}C-NMR relaxation time measurements.[10]

In the present study, therefore, the molecular mobility of sucrose and PVP in a colyophilized mixture of 1 : 1 by weight has been determined by ^{13}C-NMR relaxation time measurements in systems containing absorbed water at various levels. The major question to be addressed is to what extent sucrose and PVP in an amorphous molecular dispersion impact on one another's molecular mobility as a function of water content.

Experimental

Materials PVP (K-90) with a nominal average molecular weight of 360000, given by the supplier, and sucrose were received from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and used as received.

Preparation of Amorphous PVP, Sucrose and a PVP–Sucrose Mixture Amorphous PVP, sucrose and a sucrose–PVP mixture at a 1 : 1 weight ratio were prepared by freeze-drying from a 5% w/v aqueous solution. Fifty microliters of each solution were frozen in glass tubes (5 mm internal diameter) by immersion into liquid nitrogen, followed by drying under a vacuum of approximately 5 Pa. The sample temperature was maintained at −20 °C for 48 h, 0 °C for 48 h, and then 20 °C for 24 h. Lyophilized samples were stored at 5 °C for 24 h with P_2O_5 under vacuum, or in the presence of an appropriate saturated salt solution ($LiBr \cdot 2H_2O$, 6% relative humidity (RH); $LiCl \cdot H_2O$, 11% RH; potassium acetate, 22% RH). The water content of the lyophilized samples was determined by the Karl–Fisher method (Model 684 KF Coulometer, Metrohm) after being dissolved in methanol in a glove bag (RH <5%). The resulting water absorption isotherms at 5 °C are shown in Fig. 1. All samples were found to be amorphous by differential scanning calorimetry (20 °C/min), using a model 2920 DSC (TA Instrument, New Castle, DE, U.S.A.).

^{13}C Spin–Lattice Relaxation Times, T_1 and $T_{1\rho}$ High-resolution ^{13}C solid-state NMR measurements were conducted using a Unity plus spectrometer (Varian Inc. Palo Alto, CA, U.S.A.) operating at a proton resonance frequency of 400 MHz. Figure 2 shows typical high-resolution ^{13}C-NMR spectra of PVP, sucrose and the 1 : 1 colyophilized mixture.

The peak at 20 ppm was assigned to the C-2 carbon of the PVP ring,[10,13] and used for T_1 and $T_{1\rho}$ measurement to determine the molecular mobility of PVP. As a measure of molecular mobility of sucrose, the peak at 75 ppm was used for T_1 and $T_{1\rho}$ measurement because of its high signal to noise ratio, although this peak is an overlap of several methyne carbons of sucrose.

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T_1 was determined using a pulse sequence reported by Torchia.[15] $T_{1\rho}$ was determined from the signal intensity measured at various durations of the carbon spin-locking field (1—8 ms). Ninety degree pulse length for carbon and proton was 4.4 μs. The sample rotor was spun at 4 kHz, and 50—200 scans were accumulated for each spectrum. The delay time between each scan was 5 s. Spin–lattice relaxation processes (T_1 and $T_{1\rho}$) were analyzed by a single exponential function, assuming that heterogeneity of the relaxation process was negligible.

Results and Discussion

Figure 3 shows a plot of log T_1 *vs.* $1/T$ for the C-2 carbon in the PVP pyrrolidone ring and methyne carbons in sucrose, both in the amorphous state, for samples stored at 0 and 22% RH and 5 °C before NMR measurement. It should be pointed out that the absolute values of T_1 for sucrose and PVP do not reflect the relative molecular mobility of their respective carbons, so that only the extent of change in T_1 with temperature for each species can be analyzed. It is important to note also that the T_1 values reported in all cases reflect the motion of those carbons indicated by an NMR peak at 20 ppm for PVP and 75 ppm for sucrose. Here we note that the T_1 values of both PVP and sucrose decrease as the temperature is increased at both relative humidity storage conditions, with what appears to be a greater rate of change for the sucrose carbons. Such a change in T_1 indicates that the temperature range studied represents a "slow motional regime" and that such a decrease in T_1 represents an increase in the molecular mobility of the respective PVP and sucrose carbons with an increase in temperature.

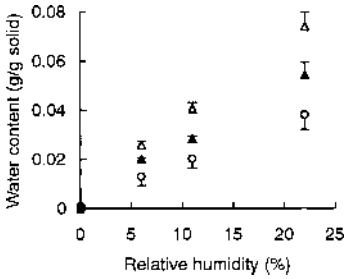

Fig. 1. Water Content of Lyophilized Formulations Stored at 5 °C and Various Relative Humidity for 1 d

△ PVP; ○ sucrose; ▲ sucrose–PVP mixture (1 : 1). Error bars in the figure represent the standard deviation of three or four measurements.

In Figs. 4 and 5 we present T_1 values for PVP and sucrose, respectively, as pure components and as 1 : 1 colyophilized mixtures, stored at 5 °C and a range of relative humidity from 0 to 22% RH. This range of relative humidity assured that no crystallization of sucrose would occur at this temperature over the timeframe of the experiments. In Fig. 4 we see that the T_1 values of PVP alone decrease as water content increases, reflecting an increase in mobility or a change in the mode of motion of the PVP side chain, as reported previously.[10] In contrast, the T_1 for PVP in the lyophilized mixture exhibited no significant change with water content. In Fig. 5 we observe that for both sucrose alone and in molecular dispersion with PVP there is no apparent change in T_1 for the sucrose carbons with increasing water content. It is possible that the lack of any change in the T_1 values of sucrose arises from recrystallization of sucrose during the NMR measurement at 5 °C. Such effect, however, seems unlikely at such low temperature. Especially for sucrose–PVP mixture, no crystallization of sucrose was observed even at 80% RH and room temperature for 3 months.[2,4] However we still checked

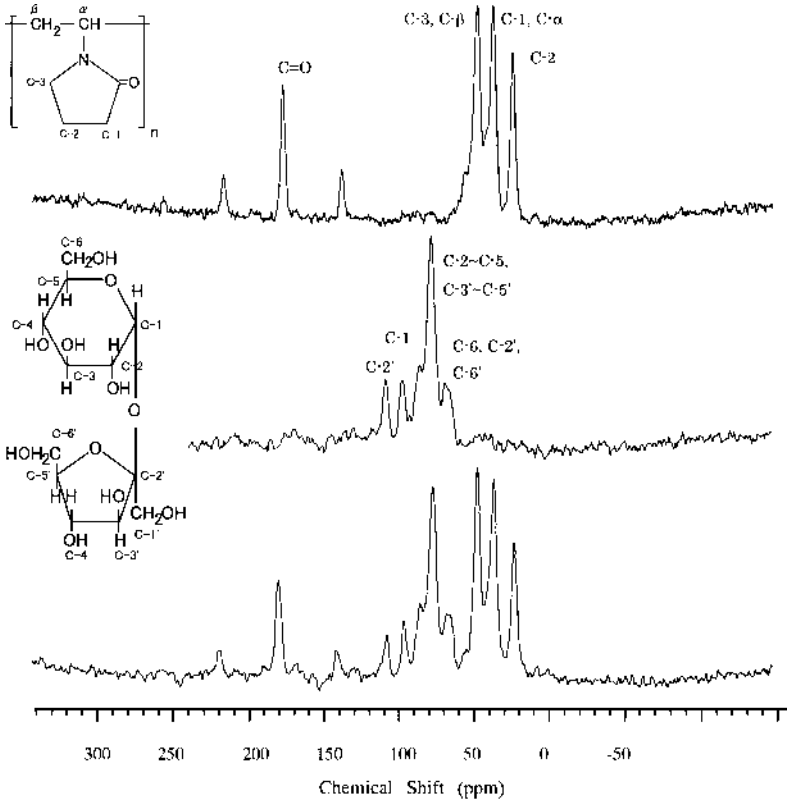

Fig. 2. Typical High-Resolution ^{13}C-Solid State NMR Spectra

(A) PVP, (B) sucrose, (C) sucrose–PVP mixture (1 : 1).

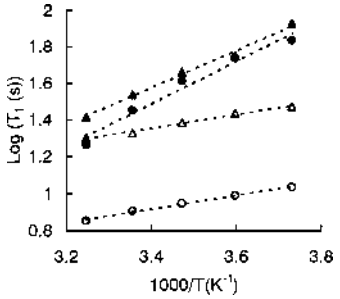

Fig. 3. Temperature Dependence of the T_1 of PVP Carbon (Open Symbols) and Sucrose Carbon (Closed Symbols) in Lyophilized PVP and Sucrose

Storage RH: △▲ 0, ○● 0.22%.

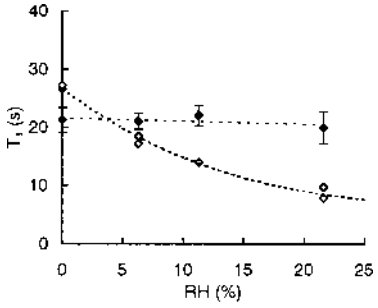

Fig. 4. Effect of Storage RH on the T_1 of PVP Carbon in Lyophilized PVP (Open Symbols) and Sucrose–PVP Mixture (Closed Symbols)

T_1 was determined at 5 °C. Error bars in the figure represent the standard deviation of three or four measurements.

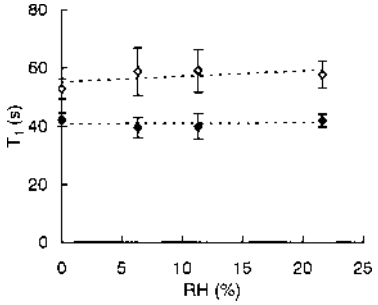

Fig. 5. Effect of Storage RH on the T_1 of Sucrose Carbon in Lyophilized Sucrose (Open Symbols) and Sucrose–PVP Mixture (Closed Symbols)

T_1 was determined at 5 °C. Error bars in the figure represent the standard deviation of three or four measurements.

for such potential problems by measuring the heats of crystallization for all samples prior to and after the NMR measurement. In all cases there was no evidence of crystallization of sucrose. The loss of significant amounts of water from the sample during the NMR measurement may also cause no change in the T_1 values of sucrose with water content, but this possibility was excluded by the fact that the glass transition temperature of samples after the NMR measurement was not different from that prior to the NMR measurement. The finding that T_1 for sucrose alone, and in the mixture, did not change with increasing water content would appear to indicate that the carbon being probed in response to excitation on MHz timescales is likely associated with internal motions of the sugar ring that are not affected by the presence of water.

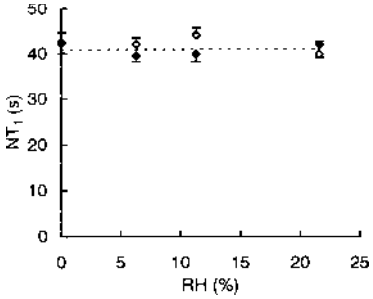

Fig. 6. Normalized T_1 of PVP Carbon (Open Symbols) and Sucrose Carbon (Closed Symbols) in Sucrose–PVP Mixture as a Function of Storage RH

T_1 was determined at 5 °C. Error bars in the figure represent the standard deviation of three or four measurements.

This still leaves us with the results observed in Fig. 4 where the PVP carbon motion is enhanced with increased water content for PVP alone, but not when it is in a molecular dispersion. This suggests the possibility that in the 1 : 1 molecular dispersion, the PVP pyrrolidone ring being hydrogen bonded to a hydroxyl group of sucrose, can only undergo local rotational motion similar to that associated with sucrose, as discussed above, and therefore it also is not affected by the presence of water when excitation is in the MHz timeframe. This hypothesis is supported by the normalized T_1 values shown in Fig. 6, where N equals the number of proton atoms bound to the carbon atom of interest. The T_1 of the carbon that is bound to hydrogen can be described by the intensity of the spin–lattice interaction and molecular mobility according to Eq. 1, assuming the single-correlation time model.[8)]

$$\frac{1}{NT_1}=\frac{h^2\gamma_H^2\gamma_C^2}{10(2\pi)^2 r_{C-H}^2}\left\{\frac{\tau_c}{1+(\omega_H-\omega_C)^2\tau_c^2}+\frac{3\tau_c}{1+\omega_C^2\tau_c^2}+\frac{6\tau_c}{1+(\omega_H+\omega_C)^2\tau_c^2}\right\} \quad (1)$$

where h is Plank's constant, r_{C-H} is the distance between the proton and carbon atoms, and γ_H and γ_C, represent the gyromagnetic ratios of the proton and carbon atoms, respectively. ω_H and ω_C are the resonance frequency of the proton and carbon atom, respectively. For our purposes, we assume that the r_{C-H} of the PVP carbon is the same as that of the sucrose carbons. As seen in Fig. 6, the normalized T_1 values for the PVP carbon are quite similar to those of the sucrose carbons, indicating that the PVP carbons in the presence of sucrose have a motion that is similar to the motion of sucrose carbons. The values of normalized T_1 are plotted against temperature in Fig. 7. The temperature dependence of sucrose was similar to that of PVP. This finding supports the conclusion that PVP motion is correlated with sucrose motion in the colyophilized mixture.

It is well known that T_1 reflects molecular motions on a MHz time scale, whereas $T_{1\rho}$ reflects molecular motions on a mid-kHz time scale.[8)] The correlation of the molecular mobility between PVP and sucrose on a mid-kHz time scale is suggested from the temperature dependences of $T_{1\rho}$ of the PVP and sucrose carbons. Figure 8 shows the temperature dependence of $T_{1\rho}$ of the PVP carbon at 20 ppm and the sucrose carbons at 75 ppm in colyophilized samples. $T_{1\rho}$ of the

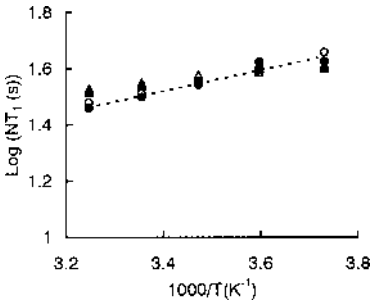

Fig. 7. Temperature Dependence of the Normalized T_1 of PVP Carbon (Open Symbols) and Sucrose Carbon (Closed Symbols) in Sucrose–PVP Mixture

Storage RH: △▲ 0, ○● 0.22%.

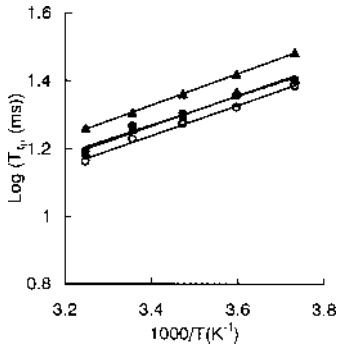

Fig. 8. Temperature Dependence of the $T_{1\rho}$ of PVP Carbon (Open Symbols) and Sucrose Carbon (Closed Symbols) in Sucrose–PVP Mixture

Storage RH: △▲ 0, ○● 0.22%.

PVP carbon increased as temperature decreased for both dry samples and samples stored at 22% RH. The slope of the Arrhenius plot for the $T_{1\rho}$ of the PVP carbon was similar to that of $T_{1\rho}$ of the sucrose carbons. The previous FT-Raman studies suggest the observed correlation of molecular mobility between PVP and sucrose in a colyophilized mixture can be attributed to hydrogen bonding between the carbonyl group of PVP and the hydroxyl groups of sucrose.

Although the MHz-order motion of sucrose carbon reflected in T_1 was not affected by water (Fig. 5), the effect of storage RH was observed for the mid-kHz-order motion of sucrose carbon that is reflected in $T_{1\rho}$, as shown in Fig. 9, which shows the effect of storage RH on the $T_{1\rho}$ of the sucrose carbons in lyophilized sucrose and the sucrose–PVP mixture. The $T_{1\rho}$ of the sucrose carbons in lyophilized sucrose decreased as storage RH increased, suggesting that water molecules enhance the mid-kHz-order motion of the sucrose carbons by a plasticization effect of water. The motion of larger segments such as a motion of the whole sugar ring may be affected by water to a greater degree than the local motion of the sucrose carbons that is reflected in T_1.

The change in the $T_{1\rho}$ with storage RH was not significant for the sucrose carbons in the sucrose–PVP mixture. This can be explained by assuming that PVP inhibits water absorption to sucrose. However, this explanation is excluded by the finding that the amount of water absorbed by the colyophilized mixture is the weight average of pure PVP and sucrose (Fig. 1).[4] Thus, the presence of PVP in the molecular dispersion appears to reduce the effect of water to increase sucrose mobility. This may be ascribed to the high T_g

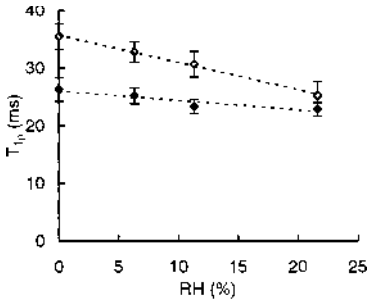

Fig. 9. Effect of Storage RH on the $T_{1\rho}$ of Sucrose Carbon in Lyophilized Sucrose (Open Symbols) and in Sucrose–PVP Mixture (Closed Symbols)

$T_{1\rho}$ was determined at 5 °C. Error bars in the figure represent the standard deviation of three or four measurements.

of PVP and possible interaction between sucrose and PVP *via* hydrogen bonding; the latter is supported by the effect of sucrose on the mobility of PVP as measured by T_1 (Figs. 5—7). Hydrogen bonding occurs between the carbonyl group of PVP and hydroxyl groups of sucrose.[5,6] Likewise, water interacts with the PVP carbonyl group[16] and hydroxyl groups on sucrose. The nitrogen in pyrrolidone ring is too sterically hindered to be the site.[5] Hydrogen bonding between PVP and sucrose, coupled with the antiplasticizing effects of PVP, would be expected to reduce the overall free volume of the system reducing overall molecular mobility including that of sucrose. Since it has been shown that water is able to access sucrose and PVP in a manner equivalent to the weighted contributions of individual components,[4] the observed reduction of $T_{1\rho}$ change (Fig. 9) indicates that despite full access to water the antiplasticizing effect of PVP on water–sucrose–PVP system offsets any plasticizing effects of water to give a net reduction in free volume and hence molecular mobility.

The previously observed inhibition of sucrose crystallization in PVP the dispersion[1—4] may be largely ascribed to this reduced molecular mobility. Reduced rotational relaxation as seen with NMR $T_{1\rho}$ arises from a local viscosity that is increased primarily by the presence of PVP, which should slow down any tendencies for nucleation and growth of sucrose to occur.

Conclusions

Individual molecular mobility of PVP and sucrose in colyophilized formulation was determined by ^{13}C-NMR relaxation measurements. The MHz-order motions of the PVP side chain as determined by T_1 increased with storage RH in lyophilized PVP alone. However, in the colyophilized mixture, the side chain motions of PVP did not change with storage RH, and showed similar mobility to sucrose. The change in molecular mobility of the PVP side chain in the presence of sucrose may be attributed to hydrogen bonding between the PVP ring carbonyl group and hydroxyl group of sucrose. The mid-kHz-order motions of sucrose in sucrose-PVP mixture as determined by $T_{1\rho}$ did not increase with storage RH as much as in lyophilized sucrose alone. This suggests that the molecular mobility of sucrose decreases in the presence of PVP due to hydrogen bonding between the hydroxyl group of sucrose and the carbonyl group of PVP. The effect of PVP on the molecular mobility of sucrose appears to link to inhibition of sucrose crystallization by PVP in the presence of water.

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