The Effect of Excipients on the Molecular Mobility of Lyophilized Formulations, as Measured by Glass Transition Temperature and NMR Relaxation-Based Critical Mobility Temperature

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Purpose. The dependence of the molecular mobility of lyophilized formulations on pharmaceutical polymer excipients was studied. Molecular mobility as determined by NMR relaxation-based critical temperature of molecular mobility (T_{mc}) and glass transition temperature (T_g) is discussed in relation to the plasticizing effect of water in formulations.

Methods. The T_{mc} and T_g of lyophilized γ-globulin formulations containing 6 different polymer excipients such as dextran, polyvinylpyrrolidone (PVP) and methylcellulose (MC) was determined by NMR and DSC. The molecular mobility of water in the formulations was determined by proton NMR and dielectric relaxation spectrometry (DRS).

Results. T_{mc} varied with polymer excipients. T_{mc} increased as the ratio of bound water to mobile water increased and as the molecular mobility of mobile water decreased. The formulation containing MC exhibited a lower T_{mc} than the formulation containing dextran because of the smaller ratio of bound water and the higher molecular mobility of mobile water. The T_{mc} of the formulation containing PVP was higher than that expected from the higher T_2 values of water because of the lower molecular mobility of mobile water regardless of the higher ratio of mobile water. The T_{mc} of these lyophilized formulations was higher than their T_g by 23°C to 34°C, indicating that the formulations became a NMR-detected microscopically liquidized state below their T_g.

Conclusions. The quantity and the molecular mobility of mobile water in lyophilized formulations can be considered to affect the T_{mc} of lyophilized formulations, which in turn governs their stability.

KEY WORDS: lyophilized formulation; NMR relaxation; dielectric relaxation; molecular mobility; excipient.

INTRODUCTION

Glass transition temperature (T_g) of amorphous pharmaceuticals governs their physical stability (1,2). Diffusion-controlled degradation such as crystallization of amorphous pharmaceuticals (3–6) and diffusion-controlled aggregation of proteins in lyophilized formulations (7–10) have been related to T_g. T_g is the critical temperature at which molecular mobility changes; as for polymers, T_g is the critical temperature of α-relaxation. Recently, the critical temperature at which lyophilized protein formulations begin to exhibit a Lorentzian relaxation process due to liquid protons of a protein or excipient (T_{mc}) has been proposed as another critical temperature of molecular mobility of formulations (11–13). Protein aggregation in lyophilized bovine serum γ-globulin formulations containing poly(vinyl alcohol) and dextran at temperatures above the T_{mc} conformed to the Williams-Landel-Ferry equation in which T_g was replaced by T_{mc}, indicating that protein aggregation is related to T_{mc} more closely than T_g (13). T_{mc} as well as T_g appear to be very important characteristics of pharmaceutical formulations, and they can be used as a parameter for screening the most suitable excipients in preformulaton processes.

The present paper describes the dependence of the T_{mc} of lyophilized formulations on the pharmaceutical polymers used as the excipient. The T_{mc} of lyophilized γ-globulin formulations containing water soluble polymers, dextran, α,β-poly(N-hydroxyethyl)-L-aspartamide (PHEA), polyvinylpyrrolidone (PVP), carboxymethylcellulose sodium salt (CMC-Na), hydroxypropylmethylcellulose (HPMC) and methylcellulose (MC) was determined by NMR relaxation measurements as a function of water content. The molecular mobility of water in these formulations was determined by NMR and dielectric relaxation spectrometry (DRS). The plasticising effect of water in these formulations is discussed in relation to T_{mc}. The relationship between the T_{mc} and T_g of these formulations is also discussed.

MATERIALS AND METHODS

Preparation of Lyophilized BGG Formulations

Dextran (D-4133, average molecular weight of 42,000) and HPMC (29441-1) were purchased from Sigma Chemical Co., Inc. (St. Louis, MO) and Aldrich Chemical Co., Inc. (Milwaukee, WI), respectively. PVP (161-03105), CMC-Na (039-01335) and MC (136-07172) were obtained from Wako Pure Chemical Industries Ltd. (Osaka). PHEA was prepared via polysuccinimide by polycondensation of aspartic acid as reported (14). Bovine serum γ-globulin (BGG) (G5009) was purchased from Sigma Chemical Co., Inc. (St. Louis, MO).

Ninety microliters of 10% w/v BGG solution was added to 40 g of a 2.5% w/w polymer solution. Four hundred microliters of the solution was frozen in a polypropylene sample tube (10 mm diameter) by immersion in liquid nitrogen for 10 min, and then dried at a vacuum level below 5 Pa for 23.5 h in a lyophilizer (Freezevac C-1, Tozai Tsusho Co., Tokyo), as previously described (13). The shelf temperature was between −35 and −30°C for the first 1 h, 20°C for the subsequent 19 h, and 30°C for the last 3.5 h.

The water content of the lyophilized BGG formulations containing various polymers was adjusted by storing at 15°C for 24 h in a desiccator with a saturated solution of LiCl H_2O (12% relative humidity (RH)), potassium acetate (23.4% RH), K_2CO_3 $2H_2O$ (43% RH), NaBr $2H_2O$ (60.2% RH), or NaCl (75% RH). The Karl Fisher method (684 KF Coulometer, Switzerland) was used for water content determination.

^{1}H NMR MEASUREMENT

The free induction decay (FID) of protons in lyophilized BGG formulations containing various polymer excipients was obtained at temperatures ranging from 5°C to 85°C, using a pulse NMR spectrometer (25 MHz, JNM-MU25, JEOL, Tokyo), as previously described (13).

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The spin-spin relaxation time (T_2) of water protons in the lyophilized formulations was calculated according to the Lorentzian equation from the FID signals between 200 and 1000 μs (signals with intensity of less than 1% of the initial intensity were excluded for the calculation). Signals between 100 and 200 μs were also used for the formulations with relatively small amounts of water.

The T_2 of polymer protons was calculated from FID signals between 2.6 μs and 100 μs after FID signals due to water protons were subtracted using the T_2 values of water protons calculated above. The FID of polymer protons was analyzed by assuming two relaxation processes; a Gaussian-type relaxation process due to solid polymer protons and a Lorentzian relaxation process due to liquid polymer protons. The T_2 of solid polymer protons ($T_{2(lm)}$) and the proportion of liquid polymer protons (P_{hm}) were estimated according to equation 1 representing the sum of the Abragam (15) and Lorentzian equations, by assuming the T_2 of liquid polymer protons ($T_{2(hm)}$) of 20 μs, as described previously (12).

$$F(t) = (1 - P_{hm}) \exp(-t^2/2T_{2(lm)}^2) \sin(ct)/ct + P_{hm} (\exp(-t/T_{2(hm)})) \quad (1)$$

The constant c of the Abragam equation was estimated to be 0.1 for CMC-Na, 0.12 for dextran and 0.15 for PVP. For PHEA, HPMC and MC, the relaxation of solid polymer protons conformed to the Gaussian equation (namely, the term of sin(ct)/ct in the Abragam equation was unity).

Differential Scanning Calorimetry (DSC)

Thermograms of lyophilized BGG formulations containing various polymer were obtained at a scan rate of 5°C/min by calorimetry (DSC 2920, TA Instruments, New Castle, DE), as previously described (13).

Dielectric Relaxation Spectrometry (DRS)

For DRS measurements, lyophilized BGG formulations containing dextran, MC and PVP were stored at 15°C for 24 h under high humidity conditions (75% RH (NaCl saturated solution) for PVP, 86% RH (KCl) for dextran, and 86% RH and 98% RH (K_2SO_4) for MC). Dielectric relaxation of water in these lyophilized formulations was measured at frequencies between 10^8 and 10^{10} Hz at 25 °C by the time domain reflectometry method developed by Mashimo *et al.* (16). A digitalizing oscilloscope (54120B Hewlett Packard) was used. Dielectric dispersion ε′ and dielectric absorption ε″ curves were analyzed according to equation 2.

$$\varepsilon^* = \varepsilon' - i\varepsilon'' = \varepsilon_\infty + \frac{\Delta\varepsilon_l}{1 + (i\omega\tau_l)\,\beta_l} + \frac{\Delta\varepsilon_h}{1 + (i\omega\tau_h)\,\beta_h} \quad (2)$$

where e* is complex permittivity; $\Delta\varepsilon_l$, τ_l and β_l are the relaxation strength, the relaxation time, and the Cole-Cole parameter representing the distribution of relaxation time of water with low mobility; $\Delta\varepsilon_h$, τ_h and β_h are those of water with high mobility; ε_∞ is limiting high frequency permittivity. Dielectric relaxation parameters were estimated by non-linear regression analysis. β_l and β_h were assumed not to vary with water content, and estimated to be 0.84 and 0.73, respectively. The ratio of the amount of water with high mobility to that of water with low mobility ($[H_2O]_h/[H_2O]_l$) was estimated from the ratio of $\Delta\varepsilon_h$ to $\Delta\varepsilon_l$.

RESULTS

NMR Relaxation-Based Critical Mobility Temperature (T_{mc}) of Lyophilized Formulations

Polymer protons in lyophilized BGG formulations containing various polymer excipients exhibited Lorentzian relaxation due to liquid protons with higher mobility in addition to Gaussian-type relaxation due to solid protons with lower mobility at temperatures above the critical temperature of molecular mobility defined as T_{mc} (12). Figures 1 and 2 show the proportion of Lorentzian relaxation (P_{hm}) and the T_2 of the Gaussian-type relaxation ($T_{2(lm)}$), respectively, that were observed with lyophilized formulations of various water content as a function of temperature. The T_{mc} determined from the temperature-dependence of P_{hm} (Fig. 1) depended on water content and varied among polymer excipients used even for the formulations stored under the same humidity conditions.

As shown in Fig. 2, $T_{2(lm)}$ of the formulations containing PHEA, HPMC and MC was relatively low, and the dependence of their $T_{2(lm)}$ on temperature was unclear. This may be due to the conformity of the relaxation of solid polymer protons to the Gaussian equation instead of the Abragam equation. In contrast, an increase in $T_{2(lm)}$ with temperature is apparent for the formulations containing dextran, PVP and CMC-Na. A marked increase in $T_{2(lm)}$ of these formulations was observed at a temperature higher than the T_{mc}(indicated by arrow).

Figure 3 shows the T_{mc} of lyophilized formulations containing various polymer excipients as a function of water content. As the water content increased, T_{mc} for each formulation decreased. The formulations containing PHEA, MC and HPMC exhibited comparable T_{mc} values at lower water contents than the formulations with dextran and CMC-Na. This indicates that the formulations containing PHEA, MC and HPMC become microscopically liquidized (i.e., contain liquid protons detected by NMR) at a lower temperature than the formulations containing dextran and CMC-Na at the same water content.

Grass Transition Temperature of Lyophilized Formulations

Figure 4 shows the DSC thermograms of the lyophilized formulations containing various polymer excipients with various water contents. Heat capacity change was not very apparent for the formulations containing CMC-Na, HPMC and MC. T_g could be determined from the observed change in heat capacity only for the formulations containing PHEA, dextran and PVP. The T_g of these formulations are compared with their T_{mc} in Fig. 5. T_g was higher than T_{mc} by 23°C to 34°C. This indicates that NMR-detected microscopic liquidization occurs in these formulations at a temperature much lower than their T_g.

As shown in Fig. 2, a marked increase in $T_{2(lm)}$ of the formulations containing dextran, PVP and CMC-Na was observed at around T_g, i.e., at a temperature higher than T_{mc}. This increase in $T_{2(lm)}$ appears to be due to a marked increase in molecular mobility upon reaching the rubbery state at T_g.

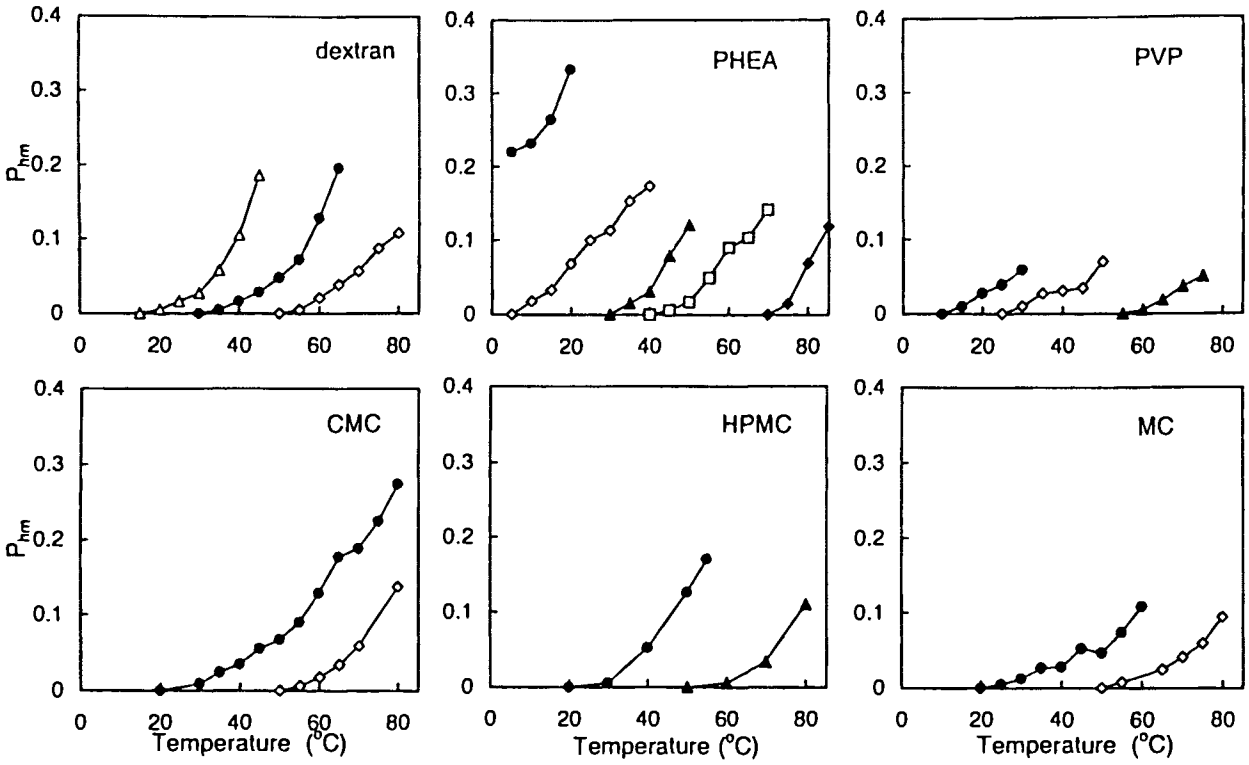

Fig. 1. Proportion of polymer protons exhibiting the higher mobility (Lorentzian) relaxation process in lyophilized formulations containing various polymer excipients as a function of temperature. Water content of the formulations was adjusted by storing under relative humidities of 12% (□), 23.4% (▲), 43% (◇), 60.2% (●), or 75% (△) and without storage after lyophilization (◆).

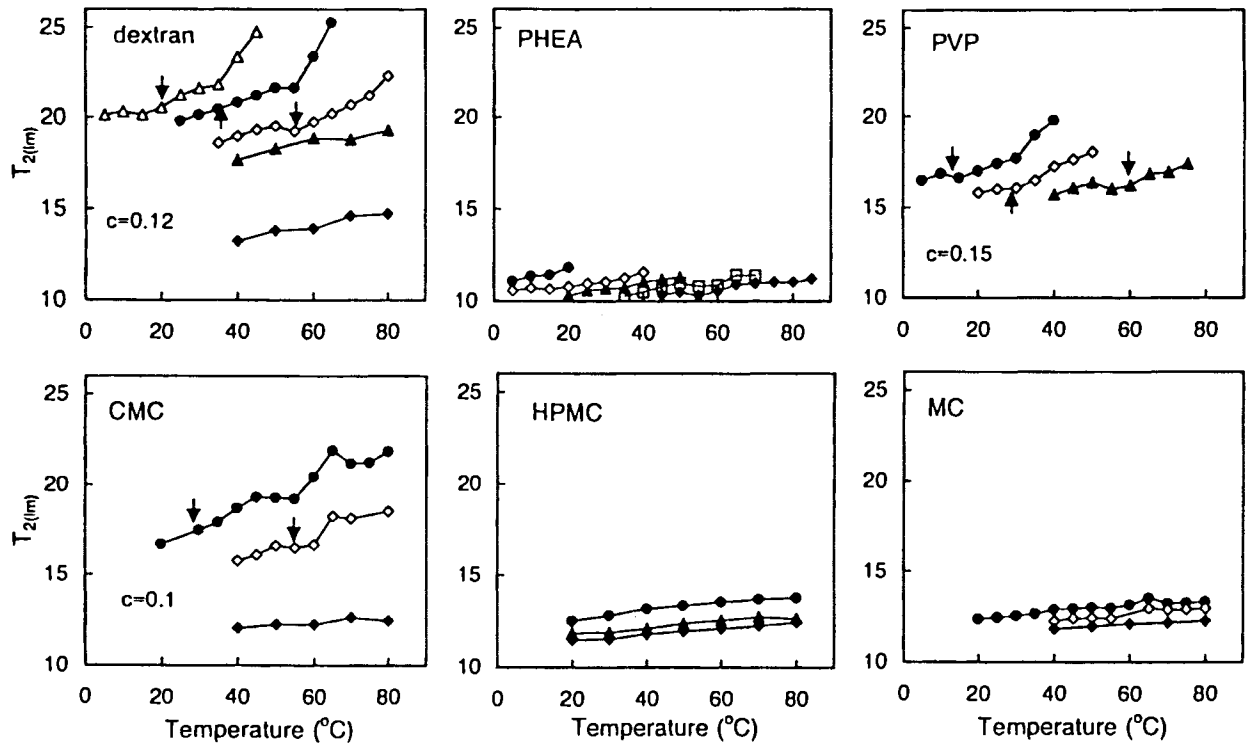

Fig. 2. Spin-spin relaxation time of polymer protons exhibiting Gaussian-type relaxation ($T_{2(lm)}$). Symbols are the same as in Fig. 1.

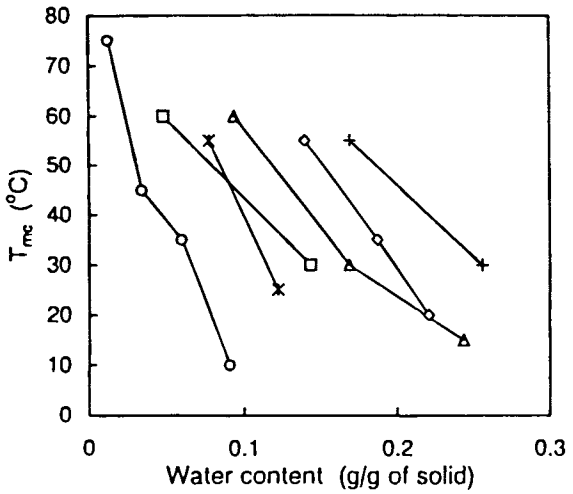

Fig. 3. T_{mc} of lyophilized formulations containing PHEA(○), MC (*), HPMC (□), PVP (△), dextran (◇), and CMC-Na (+) as a function of water content.

Molecular Mobility of Water in Lyophilized Formulations Determined by NMR and DRS

The molecular mobility of water in the lyophilized formulations containing various polymer excipients was determined by NMR relaxation measurements and DRS. Figure 6 shows the T_2 of water in these formulations as a function of water content. T_2 increased with increasing water content. Formulations containing MC, PHEA, and HPMC exhibited a significant increase in T_2 of water at lower water contents compared with the formulations containing dextran and CMC-Na. This suggests that the molecular mobility of water in the former formulations are higher than that in the latter formulations.

Figure 7 shows the dielectric dispersion and absorption curves obtained with the formulations containing dextran, MC and PVP. Each formulation exhibited two relaxation processes due to water with low mobility (bound water) and water with high mobility (mobile water) at frequencies of 10^8–10^9 Hz and 10^9–10^{10} Hz, respectively. The dielectric relaxation time of bound water and mobile water, τ_l and τ_h, respectively, was estimated by curve-fitting according to equation 2, the data of which is shown in Table I. The ratio of mobile water to bound water ($[H_2O]_h/[H_2O]_l$) is also shown in Table I. A rough comparison of the formulations with comparable water content indicates that τ_h increased in the order, MC < dextran < PVP, and $[H_2O]_h/[H_2O]_l$ increases in the order, dextran < MC < PVP. The formulation containing MC exhibited a smaller τ_h and a higher $[H_2O]_h/[H_2O]_l$ than the formulation containing dextran, indicating that the formulation with MC contains a larger amount of mobile water with higher mobility than the formulation containing dextran. On the other hand, the formulation containing PVP exhibited a larger τ_h and a higher $[H_2O]_h/[H_2O]_l$ than the formulations containing dextran and MC. This finding indicates that the formulation with PVP contains a larger amount of mobile water than the formulations with dextran and MC, although the molecular mobility of the mobile water is lower as indicated by the larger τ_h.

DISCUSSION

The T_{mc} of the lyophilized formulations varied with polymer excipients. The formulations containing PHEA, MC and

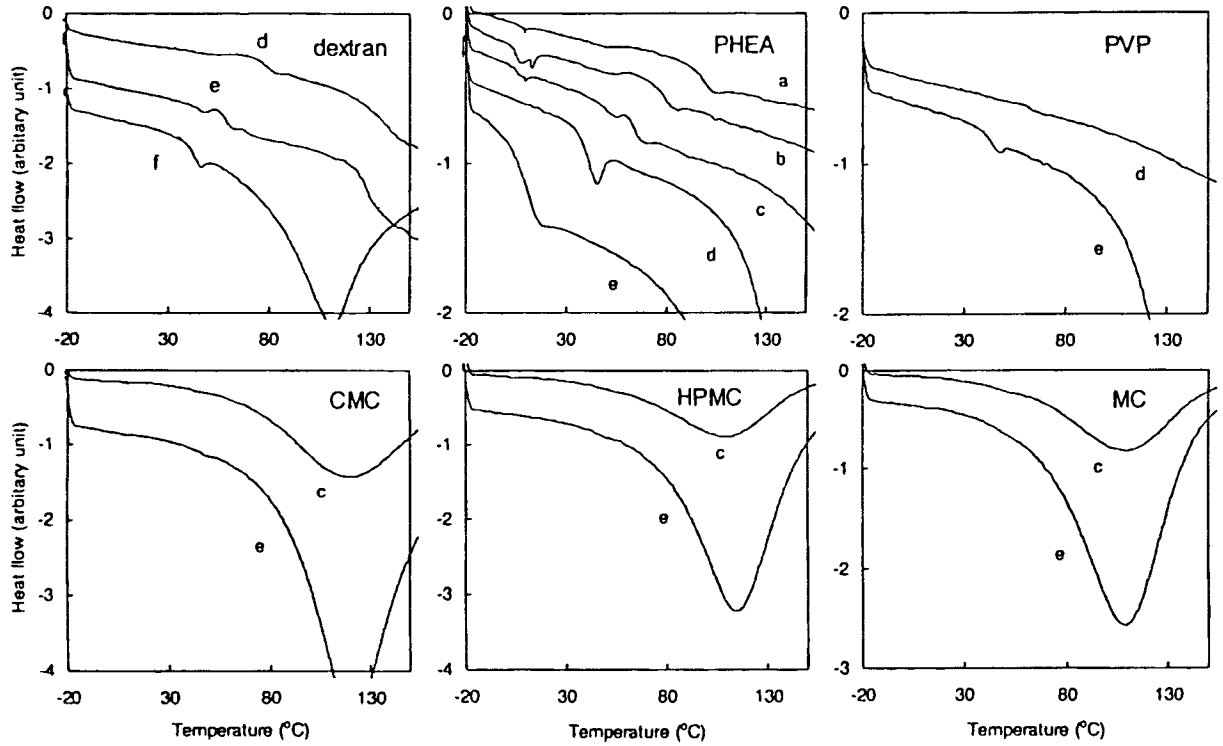

Fig. 4. DSC thermograms of the lyophilized formulations containing various polymer excipients with various water content. Water content of the formulations was adjusted by storing under relative humidity of 12% (b), 23.4% (c), 43% (d), 60.2% (e), or 75% (f). Or without storage after lyophilization (a).

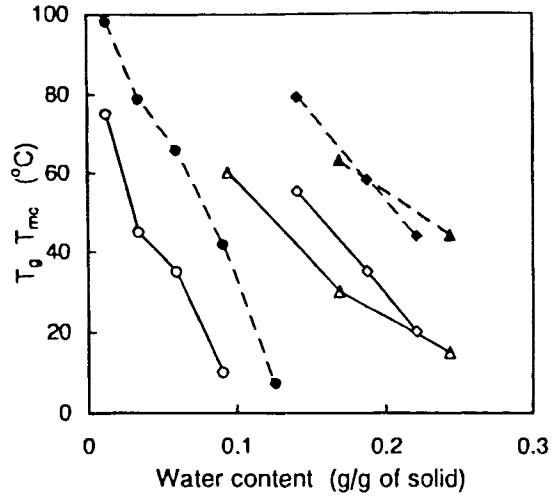

Fig. 5. T_{mc} (○△◇) and T_g(●▲◆)of lyophilized formulations containing PHEA(○●), PVP (△▲), and dextran (◇ ◆).

HPMC exhibited lower T_{mc} values than the formulations with dextran and CMC-Na of comparable water content (Fig. 3). This difference in T_{mc} may result from difference in the T_g and density of dry excipient, as represented by the Gordon-Taylor equation (17). Furthermore, this may also be ascribed to the difference in the nature of interaction between excipient and water. For example, using a polymer excipient that binds water molecules strongly may decrease the plasticising effect of water, resulting in a smaller degree of water absorption-induced decrease in the T_{mc} of lyophilized formulations.

The molecular mobility of water in lyophilized formulations was determined by NMR relaxation measurements and DRS. The water content-dependence of T_2 suggested that the average molecular mobility of water in formulations containing MC, PHEA, and HPMC are higher than the water mobility in formulations containing dextran and CMC-Na, under the assumption that molecular mobility can be represented by the apparent T_2 (Fig. 6). As shown in Fig. 3, the T_{mc} of the formulation containing MC was lower than that of the formulation

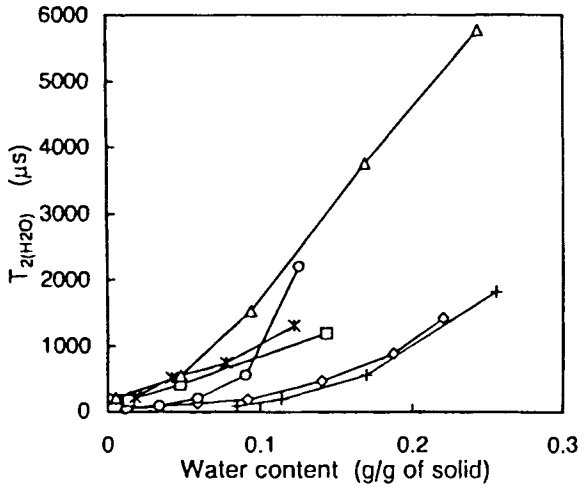

Fig. 6. T_2 of water in lyophilized formulations containing PHEA(○), MC (*), HPMC (□), PVP (△), dextran (◇), and CMC-Na (+) as a function of water content. 40°C.

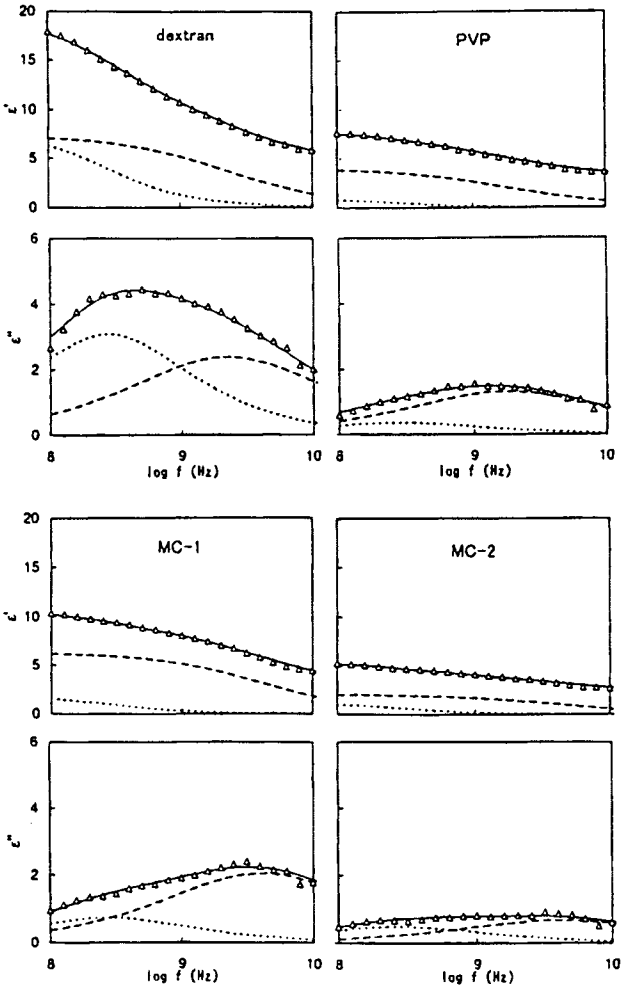

Fig. 7. Dielectric dispersion and absorption curves of lyophilized formulations containing dextran, PVP, and MC. Water content is 0.288 g/g of solid (dextran), 0.345 g/g of solid (PVP), and 0.377 and 0.225 g/g of solid (MC-1 and MC-2, respectively). 25°C. Key: (△) experimental data; ε′ and ε″ calculated for each dielectric relaxation are represented by (.....) and ((----)); summation of ε′ and ε″ for both dielectric relaxation is represented by (————).

containing dextran of a comparable water content. This may be explained by the higher average molecular mobility of water in the formulation containing MC compared to the formulation containing dextran. This consideration is also supported by the DRS data which indicates that the formulation with MC contains a larger amount of mobile water with higher mobility than the formulation containing dextran (Table I). However, higher average molecular mobility of water as indicated by a larger

Table I. Dielectric Relaxation Parameters of Lyophilized Formulations with MC, Dextran, and PVP

Polymer	Water content (g/g of solid)	$\log(\tau_l)$	$\log(\tau_h)$	$[H2O]_h/[H2O]_l$
MC	0.38	−9.24	−10.41	3.3
	0.23	−9.24	−10.41	1.7
Dextran	0.29	−9.24	−10.15	0.93
PVP	0.34	−9.24	−10.04	4.4

T_2 may not necessarily result in a greater degree of the decrease in T_{mc}.

The formulation containing PVP exhibited median T_{mc} values (i.e., among the polymer excipients shown in Fig. 3) that are lower than expected from the highest average molecular mobility of water as indicated by the largest T_2 (Fig. 6). DRS studies indicated that the formulation with PVP contains a larger amount of mobile water than the formulations with dextran and MC, although the molecular mobility of the mobile water is lower as represented by a larger τ_h. Since T_2 represents the average molecular mobility of bound water and mobile water, the formulation containing PVP having a large ratio of mobile water may exhibit a large T_2. However, the molecular mobility of mobile water in the formulation with PVP is lower than that in the formulations with dextran and MC. This lower mobility of mobile water may lead to a smaller degree of the decrease in T_{mc} than expected from the large T_2. Thus, the formulation with PVP may exhibit median T_{mc} values among the polymer excipients studied.

Zografi *et al.* reported that the presence of bound water in mixtures of absorbed water with solid amorphous PVP as suggested from water absorption isotherms, is considered to be unlikely from the high degree of mobility as determined by the translational diffusion coefficients of water (18). This appears to be consistent with the findings of the present study that the formulation with PVP contains a relatively large amount of mobile water with a relatively low molecular mobility.

The T_{mc} values of the lyophilized formulations studied during the present investigation were lower than their respective T_g values by 23°C to 34°C, indicating that the formulations become a NMR-detected microscopically liquidized states below their T_g. The previous study showed that the stability of lyophilized formulations is related to T_{mc} more closely than to T_g (13). Thus, T_{mc} determined by NMR relaxation measurements can be considered as a useful parameter for the characterization of lyophilized formulations. Furthermore, as exemplified by the formulations containing CMC-Na, HPMC and MC studied in the present investigation, lyophilized formulations often exhibit unclear changes in heat capacity so that their T_g cannot be determined easily by standard calorimetric techniques (5). Therefore, T_{mc} determination may also be useful as an alternative to T_g in such cases.

CONCLUSIONS

T_{mc}, that is the NMR relaxation-based critical temperature of molecular mobility, of lyophilized formulations varied with the polymer excipients, dextran, PHEA, PVP, CMC-Na, HPMC and MC. The determined T_{mc} values were lower than their corresponding T_g values by 23°C to 34°C, indicating that each formulation becomes a NMR-detected microscopically liquidized state below its T_g. The results from DRS and NMR studies suggest that the T_{mc} of lyophilized formulations increases as the ratio of bound water to mobile water increases and as the molecular mobility of mobile water decreases. The formulation with MC exhibited a lower T_{mc} than the formulation with dextran because of the smaller ratio of bound water and higher molecular mobility of mobile water. The T_{mc} of the formulation with PVP was higher than that expected from the large T_2 values of water because of the lower molecular mobility of mobile water regardless of the higher ratio of mobile water. Thus, the quantity and the molecular mobility of mobile water can be considered to affect the T_{mc} of lyophilized formulations, which in turn governs their stability.

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