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Molecular Mobility of Lyophilized Poly(vinylpyrrolidone) and Methylcellulose as Determined by the Laboratory and Rotating Frame Spin-Lattice Relaxation Times of ^{1}H and ^{13}C

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Laboratory- and rotating- frame spin-lattice relaxation times (T_1 and $T_{1\rho}$) of ^{1}H and ^{13}C in lyophilized poly(vinylpyrrolidone) (PVP) and methylcellulose (MC) are determined to examine feasibility of using T_1 and $T_{1\rho}$ as a measure of molecular motions on large time scales related to the storage stability of lyophilized formulations. The $T_{1\rho}$ of proton and carbon was found to reflect the mobility of PVP and MC backbones, indicating that it is useful as a measure of large-time-scale molecular motions. In contrast to the $T_{1\rho}$, the T_1 of proton measured in the same temperature range reflected the mobility of PVP and MC side chains. The T_1 of proton may be useful as a measure of local molecular motions on a smaller-time-scale, although the measurement is interfered by moisture under some conditions. The temperature dependence of T_1 and $T_{1\rho}$ indicated that methylene in the MC molecule had much higher mobility than that in the dextran molecule, also indicated that methylene in the PVP side chain had a higher mobility than that in the MC side chain.

Key words molecular mobility; NMR; glass transition; poly(vinylpyrrolidone); methylcellulose; spin-lattice relaxation time

Close relationships between the storage stability and molecular mobility of lyophilized pharmaceutical formulations, as demonstrated by recent studies,[1—6] suggest that molecular mobility may represent a useful measure of storage stability.

Molecular motions that induce degradation of lyophilized glassy formulations containing polymer excipients are expected not to be localized motions of polymer side chains on small time scales, but rather molecular motions on larger scales involving global segmental motions of polymer backbones. Large-time-scale molecular motions have been determined by thermomechanical analysis and dynamic mechanical analysis at temperatures above glass transition temperature (T_g),[7] but such analyses are unsuitable for evaluation of motions at temperatures below T_g. In contrast, NMR relaxation measurements, which have been used as a parameter of molecular mobility in food systems[8—11] and pharmaceutical formulations,[5,12—14] can provide information regarding large-time-scale molecular motions that occur even in glassy polymer materials at temperatures below T_g. Although NMR relaxation times reflect molecular motions on smaller time scales at temperatures substantially lower than T_g, large-time-scale molecular motions that begin to emerge with increasing temperature can be sensitively detected by NMR relaxation times. For lyophilized dextran, spin-lattice relaxation times of methine proton and carbon in the glucose ring were found to be indicative of the increased large-time-scale molecular motion associated with the beginning of glass transition.[13,14]

The purpose of the present study is to examine whether NMR relaxation times can be used as a general indicator of large-time-scale molecular motions related to the storage stability of lyophilized formulations. Laboratory- and rotating-frame spin-lattice relaxation times (T_1 and $T_{1\rho}$) of ^{1}H and ^{13}C in lyophilized poly(vinylpyrrolidone) (PVP) and methylcellulose (MC) are determined to discuss the feasibility of using T_1 and $T_{1\rho}$ as a measure of large-time-scale molecular motions.

Experimental

Preparation of Lyophilized PVP and MC PVP (K-30, 161-03105) was purchased from Wako Pure Chemical Ind. Ltd. (Osaka). MC was synthesized by methylation of cellulose (Merck) with ^{13}C-labeled methyl iodide (Aldrich) and purified with dialysis tube. Lyophilized PVP and MC were prepared from a D_2O (Wako Pure Chemical Ind. Ltd.) solution of 2.5% w/w polymer. Freeze drying was carried out at a vacuum level below 5 Pa for 23.5 h in a lyophilizer (Freezevac C-1, Tozai Tsusho Co., Tokyo). 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 PVP and MC was adjusted by storing at 15 °C for 24 h in a desiccator with a saturated D_2O solution of LiCl D_2O (12% relative humidity (RH)), potassium acetate (23% RH), K_2CO_3 $2D_2O$ (43% RH), NaBr $2D_2O$ (60% RH) or NaCl (75% RH).

Determination of T_1 and $T_{1\rho}$ by ^{1}H Pulsed NMR The T_1 of proton in lyophilized PVP and MC at temperatures ranging from −90 to 150 °C was determined by the inversion recovery method using a pulsed NMR spectrometer (25 MHz, JNM-MU25, JEOL, Tokyo). $T_{1\rho}$ was also determined at temperatures ranging from −90 to 150 °C. A spin locking field equivalent to 42 kHz was applied. Signal decay was describable with a mono-exponential equation, and T_1 and $T_{1\rho}$ were estimated by nonlinear least-squares regression analysis.

Determination of $T_{1\rho}$ by High-Resolution ^{13}C Solid-State NMR The $T_{1\rho}$ of methine carbon in the PVP backbone (Fig. 1) was determined for the peak at 43 ppm at temperatures ranging from 5 to 65 °C with lyophilized PVP at 23% RH and 60% RH, using a UNITY plus spectrometer operating at a proton resonance frequency of 400 MHz (Varian Inc., CA, U.S.A.), as previously described.[13] The peak due to carbon in the pyrrolidone ring may be included in the peak at 43 ppm, but its contribution to the $T_{1\rho}$ of methine carbon was assumed to be negligible. The $T_{1\rho}$ of methine and methyl carbons in the MC molecule (Fig. 1) were determined for the peak at 75 ppm and 60 ppm, respectively, with lyophilized MC at 75% RH. Spin-locking field was equivalent to 57 kHz. Signal decay was describable with a mono-exponential equation.

Results and Discussion

Molecular Motion of Lyophilized PVP Figure 2 shows the temperature dependence of the $T_{1\rho}$ and T_1 of proton in lyophilized PVP in the absence of moisture. The effect of humidity on the $T_{1\rho}$ is shown in Fig. 3. Figure 3 also shows the $T_{1\rho}$ of methine carbon determined at 23% RH and 60% RH. In the absence of moisture, $T_{1\rho}$ decreased with increasing temperature in the temperature range above −50 °C. These changes in $T_{1\rho}$ can be attributed to the relaxation of methine

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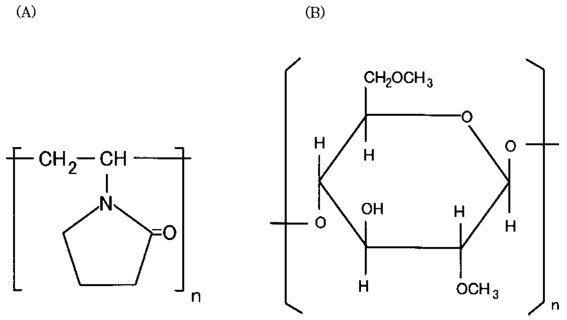

Fig. 1. Diagrams of PVP (A) and MC (B) Units

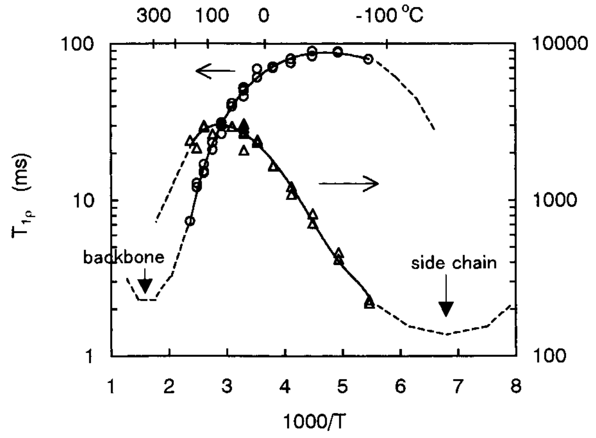

Fig. 2. Temperature Dependence of $T_{1\rho}$ (○) and T_1 (△) of PVP Proton in the Dry State

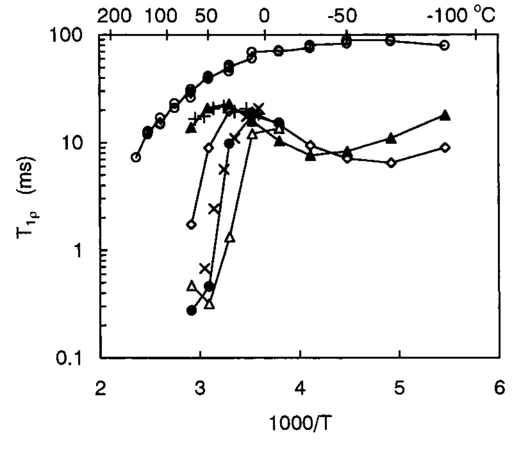

Fig. 3. Temperature Dependence of $T_{1\rho}$ of PVP Proton at 75% RH (△), 60% RH (●), 42% RH (◇), 23% RH (▲), and Dry State (○) and That of $T_{1\rho}$ of PVP Carbon (43 ppm) at 60% RH (×) and 23% RH (+)

and methylene protons in the PVP backbone, because the temperature dependence of the $T_{1\rho}$ observed at 23% RH and 60% RH was almost consistent with that of the $T_{1\rho}$ value of methine carbon (Fig. 3) and the mobility of the methylene proton should be similar to that of the methine proton.

The changes in $T_{1\rho}$ due to the relaxation of methine and methylene protons in the PVP backbone was shifted to lower temperatures as humidity increased (Fig. 3), indicating that the mobility of the PVP backbone increased. The minimum value of $T_{1\rho}$ due to the PVP backbone protons was observed at approximately 50 °C at 75% RH (the correlation time was calculated to be approximately 1.2×10^{-5} s), but not observed within the temperature range examined at lower humidities. The minimum value of $T_{1\rho}$ in the absence of moisture is expected at a higher temperature, as sketched by a dotted line in Fig. 2.

The relaxation of methine and methylene protons in the PVP backbone was not detected by T_1, which efficiently reflects molecular motions on smaller time scales than $T_{1\rho}$. T_1 exhibited a maximum value around 90 °C and decreased with decreasing temperature in the absence of humidity (Fig. 2). These changes in T_1 may be attributed to the relaxation of proton in the PVP pyrrolidone ring (PVP side chain), which exhibit motions on a smaller time scale than the PVP backbone protons (the minimum of this relaxation is expected at a lower temperature, as sketched by a dotted line). This relaxation was not reflected by $T_{1\rho}$ in the temperature range examined.

The mobility of the PVP backbone should be affected by the glass transition. The $T_{1\rho}$ of methine and methylene protons in the PVP backbone varied largely around the T_g that was 174 °C in the dry state, and 83, 63 and 44 °C at 23% RH, 43% RH and 60% RH, respectively (Fig. 3). The decreases in $T_{1\rho}$ with increasing temperature appeared to begin around the previously reported T_{mc}, at which glass transition begun to be detected by NMR relaxation measurements (60, 30 and 15 °C at 23% RH, 43% RH and 60% RH, respectively).[15] The $T_{1\rho}$ of methine carbon also exhibited a rapid decrease even at temperatures below the T_g. These findings indicate that the mobility of the PVP backbone begins to increase when temperature increased beyond the T_{mc}.

Any significant changes in the $T_{1\rho}$ of proton other than that due to the relaxation of the PVP backbone protons were not observed in the temperature range above −90 °C in the dry state. At 23% RH and 43% RH, however, changes in $T_{1\rho}$ over a wide range of temperature were observed at lower temperatures (Fig. 3). At 60% RH and 75% RH, T_1 and $T_{1\rho}$ could not be determined in the lower temperature range because of the rapid increase in T_1 that may be attributed to freezing of water. The changes in $T_{1\rho}$ at lower temperatures observed at 23% RH and 43% RH can be considered due to the relaxation of water molecules bounded to PVP molecules, because the minimum of $T_{1\rho}$ shifted to lower temperature with increasing humidity, and the dry sample did not exhibit such changes. There is, indeed, a question whether this relaxation can be brought about by a small amount of H_2O contaminant in D_2O.

Molecular Motion of Lyophilized MC Figure 4 shows the temperature dependence of the $T_{1\rho}$ and T_1 of proton in lyophilized MC without moisture. The $T_{1\rho}$ exhibited a maximum around 70 °C and a minimum around −50 °C, indicating two relaxation processes; one observed at higher temperatures may be due to methine proton in the MC backbone ($T_{1\rho}$ values at higher temperatures are required to see the whole relaxation behavior as sketched by a dotted line), and the other at lower temperatures due to methylene proton in the MC side chain. The relaxation due to methylene proton was reflected on the T_1 at higher temperatures around 100 °C. The T_1 of proton exhibited another relaxation at lower temperatures (T_1 values at lower temperatures are required to see the whole relaxation behavior as sketched by a dotted line), which may be attributed to methyl proton in the MC side

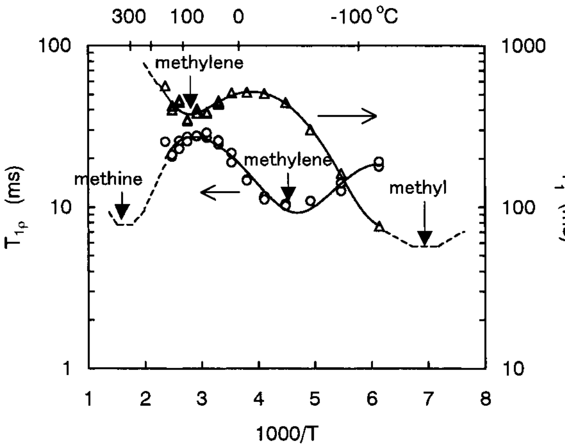

Fig. 4. Temperature Dependence of $T_{1\rho}$ (○) and T_1 (△) of MC Proton in the Dry State

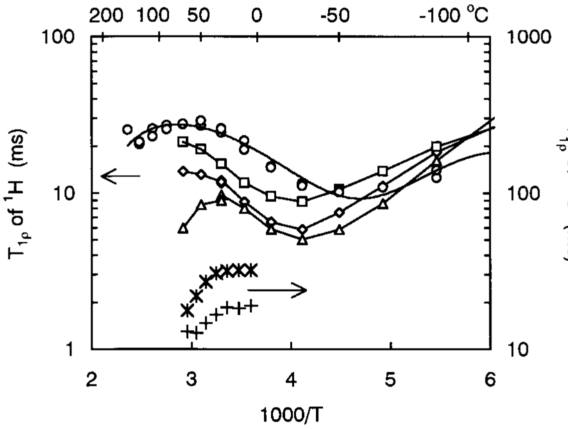

Fig. 5. Temperature Dependence of $T_{1\rho}$ of MC Proton at 75% RH (△), 43% RH (◇), 12% RH (□), and Dry State (○), and That of $T_{1\rho}$ of MC Methyl Carbon (✳) and Methine Carbon (+) at 75% RH

chain.

Figure 5 shows the effect of humidity on the $T_{1\rho}$ of proton in lyophilized MC. Although the $T_{1\rho}$ of methine proton in the presence of moisture was not obtained at higher temperatures, decreases in $T_{1\rho}$ with increasing temperature were observed in the temperature range above 30 °C at 75% RH. These decreases in $T_{1\rho}$ may indicate increases in the mobility of the MC backbone. Figure 5 also shows the temperature dependence of the $T_{1\rho}$ of methine and methyl carbons in the lyophilized MC observed at 75% RH. Both methine and methyl carbons exhibited a discontinuity in the temperature dependence of $T_{1\rho}$ at approximately 30 °C. These findings suggest that the molecular mobility of the MC backbone and side chain increases due to glass transition around this temperature, although the T_g of the lyophilized MC could not be determined by DSC because of unclear changes in the heat capacity.

In the presence of moisture, another change in $T_{1\rho}$ other than that due to the relaxation of methine proton was observed over a wide range of temperatures (with a minimum around −30 °C) (Fig. 5). This change in $T_{1\rho}$ cannot be attributed to the relaxation of methylene proton, which was observed in the dry state, because the minimum of $T_{1\rho}$ was observed at a higher temperature than that in the dry state. Therefore, this change in $T_{1\rho}$ may be attributed to the relaxation of water molecules.

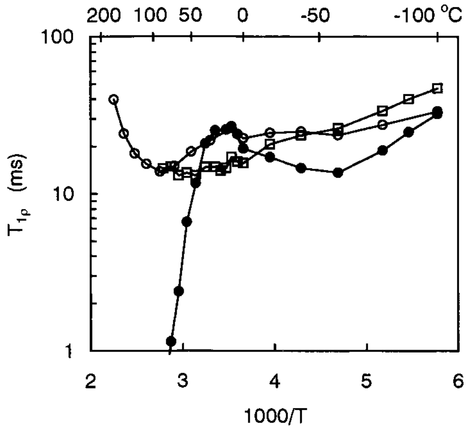

Fig. 6. Temperature Dependence of $T_{1\rho}$ of Dextran Proton at 60% RH (●), 12% RH (□), and Dry State (○)

Comparison of Molecular Mobility between Lyophilized Dextran, MC and PVP As shown in Fig. 4, the minimum of $T_{1\rho}$ due to methylene proton in the MC side chain was observed at approximately −50 °C in the dry state. In contrast to lyophilized MC, lyophilized dextran exhibited a minimum of $T_{1\rho}$ due to methylene proton at approximately 90 °C in the dry state, as shown in Fig. 6 that has been previously reported.[14] The minimum of $T_{1\rho}$ due to methylene proton shifted to a lower temperature, as humidity increased, and observed at approximately −50 °C at 60% RH. These findings suggest that methylene proton in the lyophilized MC has much higher mobility than that in the lyophilized dextran. This seems to be rational because methylene proton is located in the side chain for MC, and in the backbone for dextran.

Compared with methylene proton in the MC side chain that exhibited a temperature dependence of $T_{1\rho}$ with a minimum around −50 °C (Fig. 4), methylene proton in the PVP side chain exhibited a temperature dependence without a minimum in the temperature range examined, suggesting a minimum occurring at a temperature lower than −100 °C (Fig. 2). This finding suggests that metylene in the PVP side chain has a higher mobility than that in the MC side chain.

In conclusion, the $T_{1\rho}$ of proton and carbon was found to reflect the mobility of PVP and MC backbones, indicating that it is useful as a measure of large-time-scale molecular motions in lyophilized formulations. In contrast to the $T_{1\rho}$, the T_1 of proton reflected the mobility of PVP and MC side chains. The T_1 of proton may be useful as a measure of local molecular motions on a smaller-time-scale, although the measurement is interfered by moisture under some conditions.

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