Effect of the Binding of Water to Excipients as Measured by ^{2}H-NMR Relaxation Time on Cephalothin Decomposition Rate

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^{2}H-NMR spectra and the relaxation time of deuterium oxide adsorbed on some pharmaceutical excipients were measured using a high resolution NMR spectrometer in order to clarify the effect of the binding of water to some pharmaceutical excipients on drug decomposition. The decomposition rate of cephalothin was determined in the presence of corn starch and MCC as model excipients under various humidity conditions. The decomposition rate increased with the increasing water content of the excipients. The spin-lattice relaxation time increased with increasing water content similarly to the decomposition rate. This suggests that the ^{2}H-NMR spin-lattice relaxation time can be used as a measure of the water mobility which affects the decomposition rate of drugs in the solid state.

Keywords stability; relaxation time; water; solid state; ^{2}H-NMR

The chemical stability of a drug in solid dosage form is affected by the interaction between the drug and excipients which are added to the dosage form. The interaction is classified in three different ways: (1) reaction between the drug and the excipients, (2) change in pH of the environment and (3) hygroscopicity of the dosage form.[1] Sorption of water can take place with any excipient during the formulation process and storage. The amount of water in the dosage form is important for degradation rate of the active ingredient. It has been proposed that the decomposition rate correlates with the amount of mobile water present in the solid state system.[1,2] Information of the mobility of water molecules in the dosage may form the basis for the assessment of its stability.

Spin-lattice relaxation time (T_1) and spin–spin relaxation time (T_2) measure the relative molecular mobility of water in the system compared to free water; as the mobility of the water in the system increases, T_1 and T_2 increase. We have reported that both kanamycin catalyzed degradation of flomoxef in gelatin gels and the inactivation of β-galactosidase in phosphate buffer correlated with water mobility in the systems, as measured by the ^{17}O-NMR spin-lattice relaxation time of water.[3,4]

^{17}O is often the nucleus chosen to investigate water mobility in liquid systems where the quantity of ^{17}O is sufficient for measurement. In dry systems, however, the quantity of ^{17}O is too low. For low water content samples, ^{2}H-NMR has been used since ^{2}H enriched water is easily available. This provides a sufficient signal-to-noise ratio in the dry system as reported in various food materials such as lactose, maltodextrine, casein and potato starch,[5–8] though the contribution of chemical exchange to relaxation cannot be excluded.

In this study the decomposition rate of cephalothin, a model drug, was measured in the presence of corn starch and crystalline cellulose. The water content and spin-lattice relaxation time of deuterium oxide were also determined to study the effect of water mobility on the drug decomposition rate. In addition, this paper describes the ^{2}H-NMR spectrum of deuterium oxide adsorbed on starch and some cellulose derivatives, crystalline cellulose, hydroxypropyl methyl cellulose and hydroxypropyl cellulose.

Experimental

Materials Deuterium oxide (99.75%), corn starch, crystalline cellulose (MCC) and sodium cephalothin were purchased from Wako Pure Chemical Industries Ltd. (Osaka), Kozakai Seiyaku Co. (Tokyo), Merck (Rahway, NJ) and Sigma (St. Louis, MO), respectively. Hydroxypropyl cellulose (HPC) and hydroxypropyl methyl cellulose (HPMC) were obtained from Nippon Soda Co. (Tokyo) and Shin-etsu Kagaku Co. (Tokyo), respectively. Other chemical used were of reagent grade.

Cephalothin mixtures with corn starch and MCC were obtained by freeze-drying from a suspension of the excipients. One gram of corn starch or MCC was suspended in 50 ml of water containing 5 mg of sodium cephalothin. A half milliliter aliquots of the suspension were transferred to glass vessels, allowed to stand for 1 h at −40 °C, and freeze-dried at *ca.* 5 Pa for 16 h using a Freezvac-1CFS vacuum freeze dryer (Tozai Tsusho Co., Tokyo).

Adsorption of Deuterium Oxide on Pharmaceutical Excipients Excipients were stored at 25 °C in the presence of a saturated deuterium oxide solution of lithium chloride, magnesium chloride, sodium bromide and sodium chloride. After 3 and 7 d, ^{2}H-NMR measurement and water content determination were carried out. Water content was determined by the Karl Fisher method using a model 684 KF Coulometer (Metrohm, Swiss).

NMR Measurement A Varian VXR400s spectrometer was operated at 61.4 MHz and 25 °C for ^{2}H-NMR measurements. The sweep width was 20000 Hz. T_1 was measured using an inversion recovery pulse sequence (180–τ–90° pulse sequence). Magnetization of the spin in z direction was inversed by applying a 180° pulse. After a delay time, τ, the partially relaxed spectrum was measured by applying a 90° pulse. As the delay time τ increased, the height of the signal changed from negative to positive, as shown in the Fig. 1, and reached a constant value. T_1 was calculated from the signal intensity change according to Eq. 1.

$$H_\tau = H_0\{1-2\exp(-\tau/T_1)\} \quad (1)$$

where H_τ and H_0 are signal intensity at delay time τ and at $\tau = 5T_1$, respectively. All NMR data except for the HPMC samples could be described by Eq. 1 according to single exponential decay.

Determination of the Cephalothin Decomposition Rate in the Presence of Corn Starch and MCC Cephalothin mixtures with corn starch and MCC were stored at 25 °C under various relative humidity conditions. Relative humidity was controlled using a saturated aqueous solution of lithium chloride, magnesium chloride, sodium bromide and sodium chloride. At appropriate time intervals, two cephalothin mixture samples were withdrawn and the remaining cephalothin was assayed by HPLC. Water content, determined by the Karl Fisher method, and the spin-lattice relaxation time of ^2H_2O were also measured after 3 and 7 d of storage as described above. The water content and the spin-lattice relaxa-

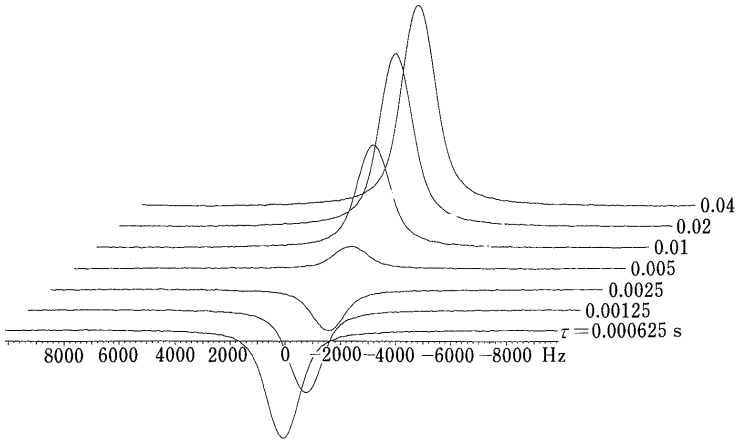

Fig. 1. Partially Relaxed Fourier Transform Spectra of Deuterium Oxide Adsorbed on Corn Starch

tion time of the samples stored for 3 d were similar to those of the samples stored for 7 d.

Assay of Cephalothin Two milliliters of a methanol solution of acetanilide (25 μg/ml, as an internal standard) and 8 ml of phosphate buffer (pH 2.5, 50 mM) were added to the cephalothin mixtures. After centrifugation, the supernatant was injected into a Hitachi model 655A chromatograph (Tokyo) equipped with an Inertsil ODS-II column (150 × 4.6 mm, GL Science, Tokyo) maintained at 35 °C. The column eluate was monitored by UV absorbance at 230 nm.

Results and Discussion

^{2}H-NMR of Deuterium Oxide Adsorbed on Pharmaceutical Excipients Deuterium oxide adsorbed on various pharmaceutical excipients provided different ^{2}H-NMR signal patterns. A broad signal was observed with corn starch (Fig. 2a) and MCC (data not shown). The line width at half-height of the signals was much larger than that of pure water (about 1 Hz). The broad signal may be caused by mainly a decrease in water mobility, though the contribution of a chemical exchange of ^{2}H between deuterium oxide and a hydroxy group of the excipient to the line broadening of the signal cannot be excluded. This indicates that the mobility of water molecules adsorbed on corn starch or MCC is much smaller than that of pure water.

On the other hand, a sharp signal was observed with HPMC, which was superimposed by a broad signal as shown in Fig. 2b. The intensity of the sharp signal decreased with a decreasing water content to a larger extent than that of the broad signal, and a sharp signal was not observed for the HPMC sample stored at a humidity below 33% relative humidily (RH). This suggests that there are two or more water molecule populations, which cannot exchange between each other within NMR measurements. The broad signal relaxed rapidly compared with the sharp one. At a delay time of 0.01 s, the intensity of the broad signal was a positive value, but that of the sharp signal was still negative, indicating that the two signals relaxed independently with different relaxation times. This supports the speculation described above.

A doublet signal was observed with the HPC sample, as shown in Fig. 2c. The doublet signal is characteristic for solid state ^{2}H-NMR, indicating anisotropic motion of water molecules with a symmetrical axis of the molecule.[8] This may be ascribed to the strong binding of water to

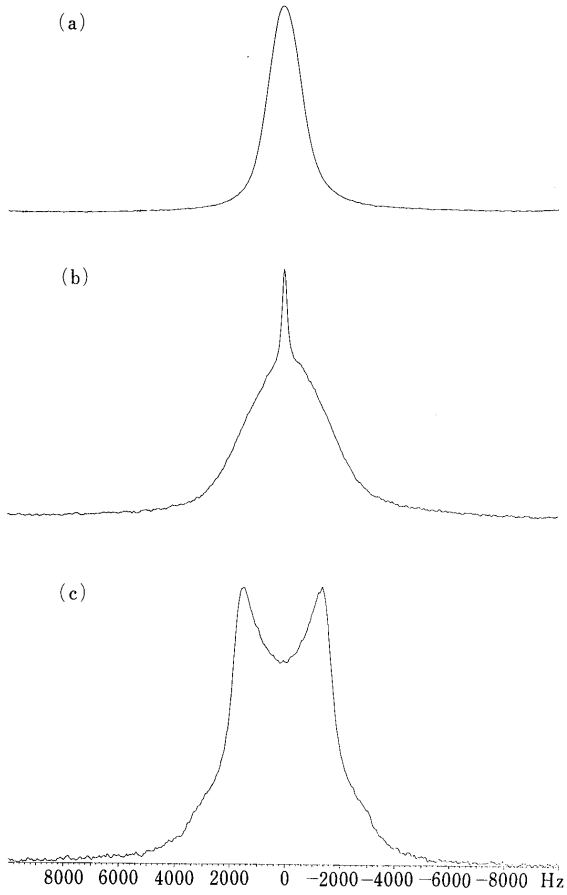

Fig. 2. ^{2}H-NMR Spectra of Deuterium Oxide Adsorbed on Corn Starch (a), HPMC (b) and HPC (c)

HPC, in comparison to the other excipients studied. These different ^{2}H-NMR signal patterns of deuterium oxide suggest different binding strengths of water molecules to the excipients.

Cephalothin Decomposition Rate in the Presence of Corn Starch and MCC Cephalothin decomposition was followed in the presence of corn starch and MCC, the model pharmaceutical excipients, to study the effects of water mobility in the excipients on the decomposition rate. It has been reported that cephalothin decomposition involves hydrolysis of the β-lactam ring and an acetoxy-

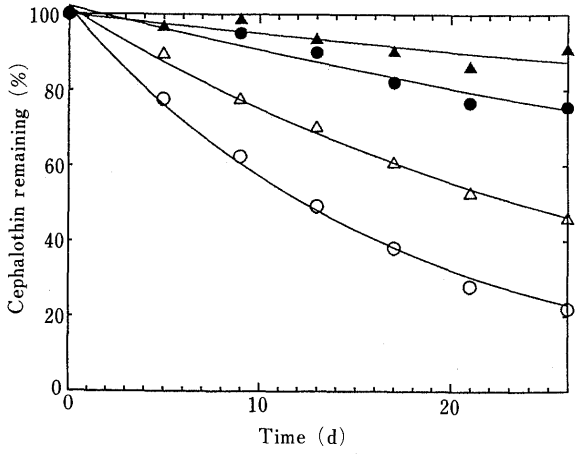

Fig. 3. Typical Time-Courses of Cephalothin Decomposition in the Presence of MCC at 25 °C

(▲), 11% RH; (●), 33% RH; (△), 50% RH; (○), 75% RH.

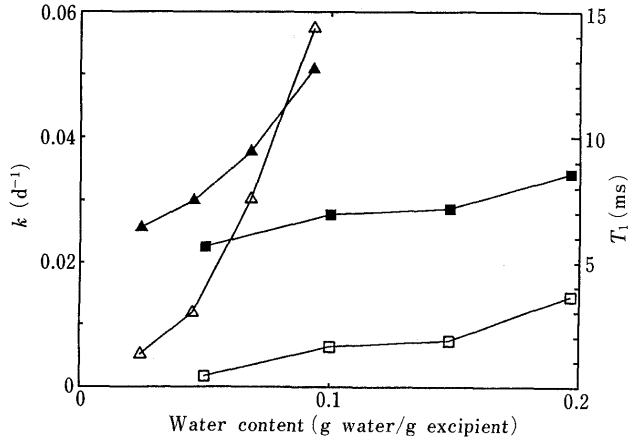

Fig. 4. Cephalothin Decomposition Rate Constant (△, □) and Spin-Lattice Relaxation Time of Deuterium Oxide (▲, ■) in the Presence of MCC (△, ▲) and Corn Starch (□, ■) at 25 °C

methyl group in aqueous solution, and that decomposition rate is pH independent at a pH range of 3 to 8.[9] The chromatogram of the decomposition products of cephalothin in the presence of corn starch and MCC was similar to that of the decomposition products in water. It appeared that the decomposition mechanism of cephalothin in the presence of corn starch and MCC was the same as that in water, and that the decomposition rate was not affected by the change in pH caused by cephalothin decomposition. Figure 3 shows the time profiles of cephalothin decomposition in the presence of MCC under various humidity conditions. The decomposition rate increased with increasing relative humidity. The cephalothin decomposition rate constant was calculated according to a first-order rate equation. Figure 4 shows the cephalothin decomposition rate constant and apparent spin-lattice relaxation time of deuterium oxide in mixtures of cephalothin and the excipients as a function of the water content of the mixtures. The apparent T_1 increased with water content in both mixtures, indicating that the average water mobility in the mixtures increased with water content. The T_1 of deuterium oxide in the MCC mixture was larger than that of deuterium oxide in the corn starch mixture: for example, about 13 and 7 ms for MCC and corn starch, respectively, at a water content level of 0.1. This suggests that the average water mobility in the MCC mixture is larger than that in the corn starch mixture.

The cephalothin decomposition rate constant in both excipients increased as the water content increased. The cephalothin decomposition rate constant in the MCC mixture was larger than that in the corn starch mixture when compared at same water content. The decomposition rate increased similarly to T_1, suggesting that T_1 may be used as one measure of a drug's decomposition rate.

The T_1 of deuterium oxide is represented by Eq. 2.[10]

$$\frac{1}{T_1}=\frac{3}{40}\left(1+\frac{\eta^2}{3}\right)\left(\frac{e^2qQ}{h}\right)^2\left[\frac{\tau_c}{1+\omega^2\tau_c^2}+\frac{4\tau_c}{1+4\omega^4\tau_c^4}\right] \quad (2)$$

where η is an asymmetric parameter of the electrical field gradient, e^2qQ/h is the quadrupole coupling constant, τ_c is the correlation time of molecular reorientation, and ω is radio frequency of the spectrometer. The quadrupole constant of deuterium oxide molecules bound to pharmaceutical excipients is affected by the binding strength of water to the excipients. We assumed that the quadrupole coupling constant of deuterium oxide bound to corn starch or MCC was not changed between samples with different water contents. In the cephalothin mixtures with corn starch and MCC, the ^{2}H nuclei are distributed between two or more motional states with different intrinsic relaxation times. If we assume that two motional states, bound and free water, exist in the mixtures, and that the exchange of water molecules between the motional states is fast, then the apparent T_1 is the weighted average of the intrinsic relaxation time of water present in the system as expressed by Eq. 3.[10]

$$\frac{1}{T_1}=\frac{p_f}{T_f}+\frac{p_b}{T_b} \quad (3)$$

where P_f and P_b are mole fractions of free and bound water, and T_f and T_b are the intrinsic relaxation time of free and bound ater, respectively. Equation 3 can be rewritten as below,

$$\frac{1}{T_1}=\frac{1}{T_b}+p_f\left(\frac{1}{T_f}-\frac{1}{T_b}\right) \quad (4)$$

The increase in apparent T_1 with water content observed for the corn starch and MCC mixtures indicated an increase in the mole fraction of free water, which caused an increase in the cephalothin decomposition rate. The difference in T_1 between MCC and corn starch with same water content suggests a different mole fraction of mobile water and/or a different intrinsic relaxation time of bound water.

In conclusion, the ^{2}H-NMR technique could provide information on water mobility in solid state pharmaceutical excipients. The cephalothin decomposition rates in corn starch and MCC mixtures could be related to ^{2}H-NMR relaxation time. Different ^{2}H-NMR signal patterns of deuterium oxide adsorbed on corn starch, MCC, HPMC and HPC suggested different binding strengths of water molecules to the excipients.

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