The Influence of Water on the Stability of Lyophilized Formulations with Inositol and Mannitol as Excipients

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The stability of a moisture-sensitive drug in lyophilized products was investigated under conditions with varying water content and temperature using two model formulations: a formulation containing inositol (IF) as the excipient and a formulation containing mannitol (MF) as the excipient. IF showed better chemical stability (a lower hydrolysis rate) than MF when both formulations contained 2% water. However, in the case of formulations with 8% water, MF showed similar or better stability than IF. From the results of hygroscopicity and phase transition experiments for both formulations, it was assumed that this stability profile was exhibited because 1) more water was taken up into the amorphous inositol in IF than into the crystalline Form-III mannitol in MF at a low water content, so that drug hydrolysis in IF was suppressed compared with MF and 2) when the water content increased, the amorphous inositol crystallized to anhydrate in IF causing expulsion of absorbed water from the excipient, meaning that IF lost its superior chemical stability due to the highly mobile water generated by the crystallization. This assumption was supported by the results of the ^{2}H-NMR measurement, which estimated water mobility from the signal shape and the spin–lattice relaxation time (T_1) of deuterium oxide.

Key words lyophilization; stability; phase transition; water mobility; hygroscopicity; ^{2}H-NMR

Lyophilization is widely used in manufacturing pharmaceutical dosage forms, especially in the case of drug substances that are susceptible to chemical degradation in liquid formulations. Although both chemical and physical stability problems in lyophilized formulations have been known for many years, in some cases less attention has been paid to the solid-state properties of active pharmaceutical ingredients and excipients in such formulations than to those in tablet and capsule formulations. For example, conversion between crystalline and amorphous forms can greatly affect the stability and solubility of drugs in lyophilized formulations.[1—3)]

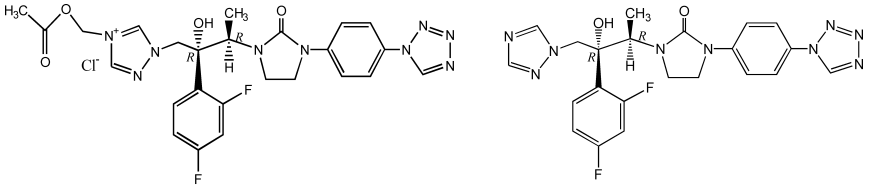
Residual moisture may have a significant influence on the stability and quality of formulations because water can act not only as a chemical reactant but also as a plasticizer to facilitate the crystallization of amorphous materials.[2)] Of more significance for drug stability, as some researchers have demonstrated, is the location of water molecules in the solid matrix and the nature of water–solid interactions rather than the amount of water present.[2—4)] When water acts as a plasticizer, it increases the mobility of the system, leading to enhanced reactivity. NMR is a powerful method for determining mobility in solids. Aso *et al.* estimated water mobility in solid samples by solid-state ^{2}H-NMR and investigated the influence of mobility on drug stability.[4,5)]

In this study the stability of freeze-dried products containing the water-soluble prodrug, 4-acetoxymethyl-1-[(2*R*,3*R*)-2-(2,4-difluorophenyl)-2-hydroxy-3-[2-oxo-3-[4-(1*H*-1-tetrazolyl)phenyl]-1-imidazolidinyl]butyl]-1*H*-1,2,4-triazolium chloride (TAK-457),[6)] a proprietary active ingredient, and commonly used bulking agents, was measured at 25 °C and 40 °C. TAK-457 was developed as an injectable prodrug of 1-[(1*R*,2*R*)-2-(2,4-difluorophenyl)-2-hydroxy-1-methyl-3-(1*H*-1,2,4-triazol-1-yl)propyl]-3-[4-(1*H*-1-tetrazolyl)phenyl]-2-imidazolidinone (TAK-456)[6)] with improved water solubility against fungal infections (Fig. 1).[6)] This study is unique and helps us reveal the influence of residual water, temperature, and excipients on stability, because TAK-457 degrades to TAK-456 predominantly *via* hydrolysis in its solid state and the amounts of other degradation products are negligible. Two model formulations of TAK-457 containing inositol or mannitol as the excipient were used. Hygroscopicity, phase transition, and water mobility in these formulations were estimated in order to investigate the differences in the chemical stability of the drug. Water mobility was determined by ^{2}H-NMR.

Experimental

Materials TAK-457 was manufactured at Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). D(−)-Mannitol of JIS special grade was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Inositol was purchased from Shiratori Pharmaceutical Co., Ltd. (Chiba, Japan).

Sample Preparation Lyophilized formulations were obtained by placing vials containing 30.6 mg of TAK-457 with 12.5 mg of inositol or mannitol in 2.5 ml of aqueous solution onto the shelf of a freeze dryer. This was



TAK-457 TAK-456

Fig. 1. Chemical Structures of TAK-457 and TAK-456

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followed by freezing at −50 °C for 4 h and then primary drying at a shelf temperature of −10 °C and a chamber pressure of 60 mTorr for 22 h. Secondary drying was then carried out at a shelf temperature of 45 °C and a chamber pressure of 60 mTorr for 6 h. Lyophilized samples with 2% and 8% water were prepared for the stability study by storing the formulations under a constant humidity of about 53% relative humidity (RH) or 75% RH at ambient temperature (about 25 °C) and monitoring their weight increase.

HPLC Analysis A liquid chromatograph was equipped with a 275-nm detector and a 4.6×150 mm YMC-Pack Pro C18 column (YMC Co., Ltd., Kyoto, Japan) and was operated at a constant temperature of 25 °C. The mobile phase was a mixture of 0.05 mol/l sodium dihydrogenphosphate solution and acetonitrile (13 : 7). The flow rate was 1.0 ml/min. The sample solution was prepared by dissolving the contents of a vial in the mobile phase. The concentration of TAK-457 in the vial was determined by the calibration method using a standard solution of TAK-457.

Karl Fischer Titrimetry (KFT) The initial water content was determined using an Aquacounter AQ-7 (Hiranuma Sangyo Co., Ltd., Ibaraki, Japan). The contents of a vial were dissolved in 2 ml of methanol. Precise 1-ml portions of this methanol solution were transferred into a titration cell with Aqualyte RS as the anolyte and Aqualyte CN as the catholyte (Hiranuma Sangyo Co., Ltd., Ibaraki, Japan).

Sorption and Desorption Isotherm Isothermal sorption and desorption profiles were measured using the SGA-100 Symmetric Vapor Sorption Analyzer, a humidity-controlled microbalance system (VTI Corporation, Hialeah, FL, U.S.A.). Sorption and desorption data were collected over a range of 5 to 95% RH at 5% RH intervals under a nitrogen purge. Samples were dried at 25 °C for up to 2 h prior to analysis with the equilibrium criteria defined as a weight change ≤0.01% in the 2 min. The weight change was monitored at each RH for a maximum time of 4 h allowed for the samples to reach equilibration. A time-course measurement of water uptake and phase transition during sorption was performed for samples stored in desiccators containing saturated solutions of sodium chloride (75% RH) at a constant temperature of 25 °C.[7]

X-Ray Powder Diffraction (XRPD) X-Ray powder diffraction (XRPD) patterns were measured using a Rint Ultima+ 2100 (Rigaku Corporation, Tokyo, Japan) with Cu$K\alpha$ radiation at 40 kV and 50 mA. Samples were measured in steps of 0.02° with a scan speed of 6° per minute from 3° to 40°, 2θ.

NMR Measurement A CMX-300 infinity spectrometer (Chemagnetics Inc., CO, U.S.A.) was operated at a resonance frequency of 46.1 MHz. The spin–lattice relaxation time (T_1) of deuterium oxide absorbed in the formulation was determined by an inversion recovery pulse sequence with 4096 pulse repetitions and a recycling time of 2.0 s.

Results and Discussion

Stability The stability of TAK-457 in lyophilized products was investigated under conditions of varying water content and temperature using two kinds of formulations: an inositol formulation (IF) containing inositol as the excipient and a mannitol formulation (MF) containing mannitol as the excipient. Samples containing 2% and 8% water were prepared by exposing the intact lyophilized formulations in glass vials to moisture. The vials were sealed and then stored at 25 °C and 40 °C. The drug contents in the initial and stored samples were measured by HPLC. The content decreased during storage predominantly *via* hydrolysis. The main degradation product of TAK-457 was TAK-456, as shown in the representative chromatograms (Fig. 2). Even when the drug content decreased to about 20% after 14-d storage of the samples with 8% water at 25 °C, the amounts of other degradation products were negligible (below 0.5%).

In the comparison between IF and MF samples containing 2% water, as shown in Fig. 3A, IF showed better stability at both 25 °C and 40 °C. However, in the case of samples with 8% water, MF showed similar or slightly better stability (Fig. 3B). Interestingly, not only was the hydrolysis reaction of the drug significantly affected by the excipients, but the stability between the formulations also changed due to the water content, even though both formulations were manufactured under the same lyophilization conditions using solutions containing the same amounts of the drug and the excipients (almost the same molar concentrations of excipient). Some reports have indicated that drug degradation rates could be in-

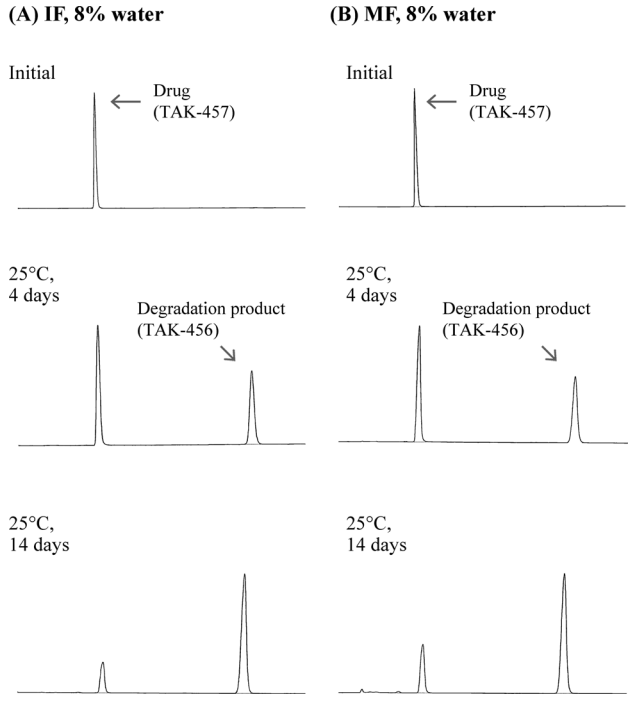

Fig. 2. HPLC Chromatograms Obtained from IF (A) and MF (B) Containing 8% Water Stored at 25 °C

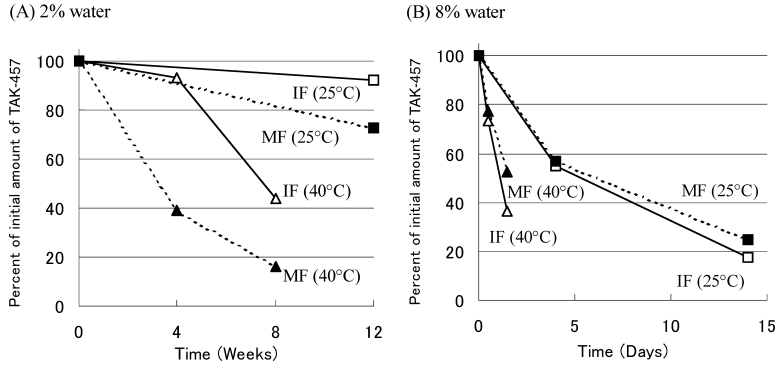

Fig. 3. The Chemical Stability of TAK-457 in Formulations Containing 2% Water (A) and 8% Water (B)

The percent of the initial amount (=content at each time point/content at initial, %) of TAK-457 is shown by: □ (IF, 25 °C), △ (IF, 40 °C), ■ (MF, 25 °C), ▲ (MF, 40 °C).

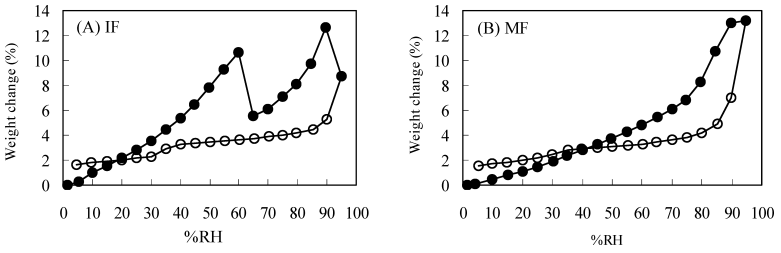

Fig. 4. Isothermal Water-Sorption and -Desorption Profiles of IF (A) and MF (B) at 25 °C

Closed and open circles indicate the profiles under the conditions of increasing and decreasing humidity, respectively.

fluenced by water mobility rather than the amount of total water.[2—4] Although other parameters that could affect the formulation stability should also be considered including the molecular mobility of the drug itself, this report is focusing on the association of water with the formulations and water mobility in the two formulations, both of which could have considerable influence on drug stability, as well as how variations in water contents result in changes to stability superiority.

Hygroscopicity The sorption and desorption isotherms between 5% and 95% relative humidity (RH) at 25 °C were measured using a humidity-controlled microbalance system. As shown in Fig. 4, although both formulations took up moisture up to a maximum of about 13%, IF showed a weight decrease in the course of the sorption process between 60% RH and 65% RH, whereas MF showed a stable increase with humidity. A similar result was also reported for a mixture of amorphous and crystalline lactose monohydrate by Buckton and Darcy,[8] in which the amorphous region recrystallized during moisture uptake due to a plasticizer effect that reduced the glass transition temperature of the amorphous materials.[2] It was presumed that when moisture uptake reached about 10% in IF, the glass transition temperature of the amorphous material was reduced to allow crystallization at 25 °C and that this was accompanied with expulsion of the absorbed water from the material, resulting in weight loss.[1,8] The desorption profile of IF showed the continuous removal of adsorbed water as did that of MF.

Phase Transition in Formulations with Moisture Uptake The polymorphism of components in the formulations was determined during the course of moisture uptake. Intact lyophilized formulations were stored in open glass vials under a constant humidity of 75% RH at 25 °C using a desiccator with a saturated solution of sodium chloride. The weight change and XRPD patterns were measured over time for 24 h.

The moisture-uptake profile of IF (Fig. 5) was indicative of the crystallization of amorphous components as discussed above. From the XRPD pattern, it was shown that both the drug and inositol in the intact IF were of an amorphous state (Fig. 6A). From initiation to 4 h with a stable weight increase (Fig. 5), no change was observed in the XRPD patterns of the IF samples, which showed no diffraction peaks. After the weight started to decrease at 4 h, crystalline peaks appeared (Fig. 6B). It has been found that the drug substance (TAK-457) can be crystalline as an anhydrate or a hydrate, and that inositol can also be as an anhydrate[9] or a dihydrate.[10] From a comparison of the XRPD patterns with all possible forms for the drug and inositol (Figs. 6C—F) it was found that all

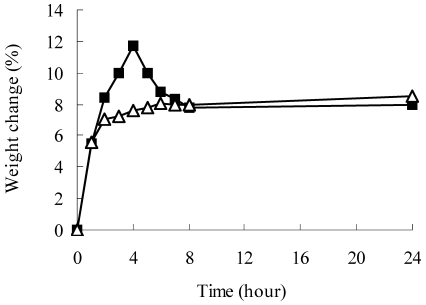

Fig. 5. Moisture Uptake Profiles of IF (■) and MF (△) under 75% RH at 25 °C

of the peaks observed in the stored IF samples (Fig. 6B) were due to anhydrous inositol (Fig. 6C). Once the weight fell to 8%, there was no further change in its weight or the diffraction pattern up to 24 h, meaning that the stored formulation was composed of amorphous drug and anhydrous inositol. The intact MF sample showed some diffraction peaks (Fig. 7E). These peaks were consistent with those of Form-III mannitol, as assigned by Burger *et al.*,[11] which is known to be generated by freeze-drying.[11,12] The stored MF samples had no phase transition and an 8.5% moisture uptake for 24 h (Fig. 7D). When the vials of the stored IF and MF were sealed and placed at 50 °C for 12 h, the crystallization of amorphous drug to hydrate was observed in MF (Fig. 7C), but no further phase transition was observed in IF.

The results show that amorphous drug hydrated with Form-III mannitol whereas no hydration occurred with anhydrous inositol even though the total water contents were similar in both formulations. This is probably caused by differences in the water affinity of the excipients. It was presumed that amorphous inositol associated more strongly with water than Form-III mannitol, leading to the superior chemical stability of IF to MF in the presence of a low water content. However, if the phase transition of inositol to the anhydrate occurs by water absorption, it should decrease the water affinity of the excipient. Additionally the released water might have significant influence on the chemical stability of drug (discussed in detail later). These influences by the crystallization of inositol were thought to lead to IF losing its superiority in the presence of a high water content.

Molecular Mobility of Water Estimated by NMR It has been demonstrated in some reports that the stability of moisture-sensitive drugs correlates well with the amount of highly mobile water, a reactant of hydrolysis, rather than total water.[4,5,13] In a comparison of cephalothin stability among mixtures of cephalothin and various excipients, it was sug-

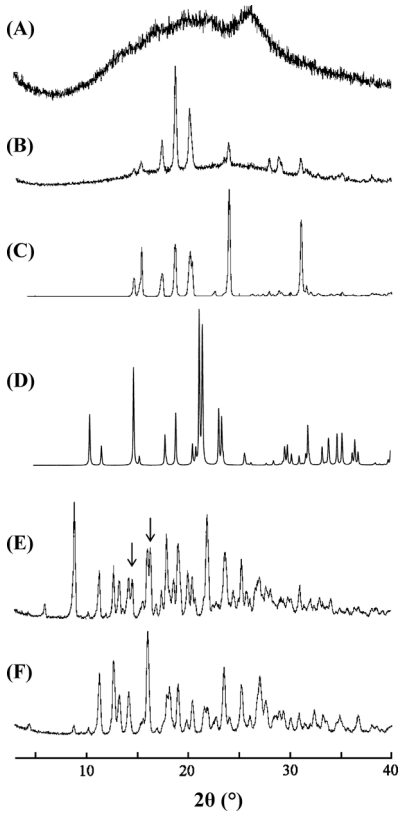

Fig. 6. XRPD Patterns of IF and Its Components

(A) IF, intact; (B) IF, unsealed and stored at 25 °C/75% RH for 5 h; (C) inositol, anhydrate; (D) inositol, dihydrate*; (E) TAK-457, anhydrate; and (F) TAK-457, hydrate. The peaks indicated by arrows represent characteristic peaks of the anhydrous drug (E). * Calculated using crystal data[10] by Mercury.[17]

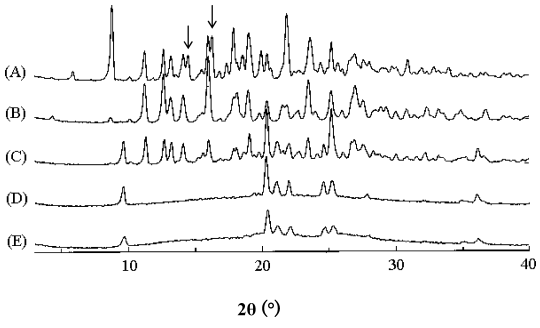

Fig. 7. XRPD Patterns of Stored MF and TAK-457

(A) TAK-457, anhydrate; (B) TAK-457, hydrate; (C) MF, sealed and stored at 50 °C for 12 h following the moisture-uptake experiment; (D) MF, unsealed and stored at 25 °C/75% RH for 24 h; and (E) MF, intact. The peaks indicated by arrows represent characteristic peaks of the anhydrous drug (A). The XRPD pattern (E) was consistent with that of Form-III mannitol.[11]

gested by Aso *et al.*[4,5] that water mobility estimated by the ^{2}H-NMR technique could be used as a measure to investigate differences in drug stability. Water mobility in the two model formulations of TAK-457 was measured by ^{2}H-NMR in order to estimate the association of water in the formulations. IF and MF with 7—8% deuterium oxide were prepared by placing intact formulations under 51% RH in a desiccator containing a saturated deuterium oxide solution of calcium nitrate. In this preparation, care was taken not to cause the crystallization of inositol by moisture uptake. The ^{2}H-NMR spectra and the spin–lattice relaxation times (T_1) of deuterium oxide in the formulations were measured at 20 °C, 40 °C, and 55 °C (Fig. 8, Table 1).

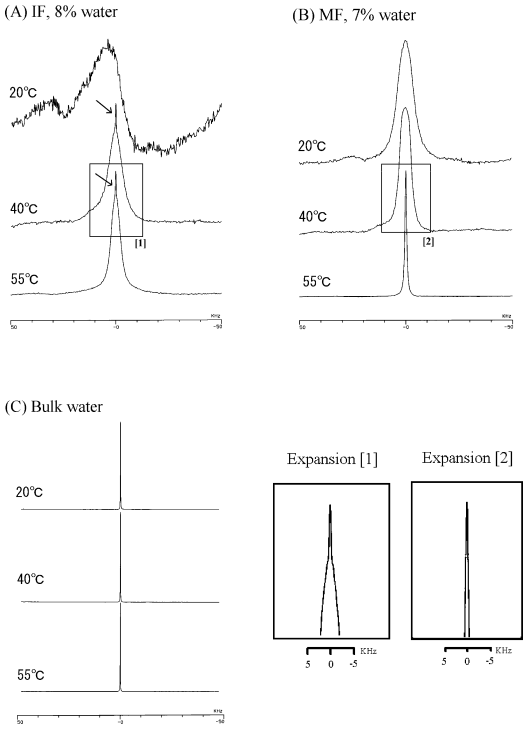

Fig. 8. ^{2}H-NMR Spectra of D_2O in IF (A) and MF (B) Along with Bulk D_2O (C) at 20 °C, 40 °C, and 55 °C

As pointed out by arrows in (A), sharp signals were observed at 40 °C and 55 °C, which were superimposed on top of broad signals.

Table 1. ^{2}H Spin–Lattice Relaxation Time (T_1) of Deuterium Oxide in the Formulations with 7—8% Water and Bulk Water

Temperature	T_1 (ms)		
	IF[a]	MF[b]	Bulk water
20 °C	5.3	5.3	270.2
40 °C	6.3,[c] 27.6[d]	7.0	410.5
55 °C	7.7,[c] 114.6[d]	8.5	516.6

a) With 8% water, *b*) with 7% water, *c*) calculated from a broader peak, *d*) calculated from a narrower peak.

It is recognized that a broader signal may be derived from water molecules with lower mobility, although the contribution of the chemical exchange of ^{2}H between deuterium oxide and a hydroxyl group of the excipients to the signal broadening cannot be excluded in this study.[5] In Fig. 8 broad signals were observed from IF and MF compared with bulk water indicating that water mobility was restricted in each of the formulations. MF showed a single broad peak at each temperature and the peak became narrower due to the increase of water mobility as the temperature was elevated. On the other hand, in IF, there was an extremely broad single peak at 20 °C and a sharp peak superimposed on the broad peak was observed at 40 °C and 55 °C (Fig. 8A), indicating that there were at least two water populations with distinctly different mobilities above 40 °C. It is thought that the broad and the sharp signals in IF were derived from water associating with inositol and released water generated by the crystallization of inositol during the temperature increase. Even

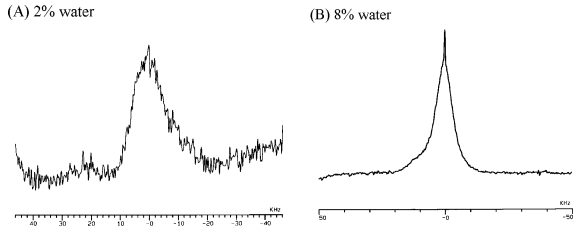

Fig. 9. ^{2}H-NMR Spectra of D_2O in IF in the Presence of 2% Water (A) and 8% Water (B) at 40 °C

though signal was not detected from IF at 20 °C in the presence of a low water content (2%) due to insufficient peak intensity, a broad single peak was observed by increasing temperature up to 40 °C, indicating that only associated water existed in the formulation (Fig. 9). Because the signal of restricted water in IF was broader than that in MF at each temperature, it was suggested that IF had a water population with a lower mobility than that of MF due to the higher water affinity of inositol than Form-III mannitol.

The spin–lattice relaxation time (T_1) presented more quantitative information.[5] At 40 °C and 55 °C, two T_1s corresponding to the broad signals (the associated water) and the sharp signals (the released water) were calculated for IF. As shown in Table 1, the T_1 value for each water population increased with temperature due to the increase of mobility.[14—16] The much shorter values of T_1 for the broad signals of the associated water in IF and MF than the value of bulk water showed that the water mobility was largely restricted in each of the formulations. Although the longer T_1 values for the sharp signals observed above 40 °C in IF than the values corresponding to the associated water represented the released water with high mobility, these values also indicated obvious interactions between the released water and the formulation from comparison with still longer values of bulk water.

Conclusions

It was concluded that amorphous inositol associated more strongly with water, a chemical reactant of drug hydrolysis, in a lyophilized formulation than Form-III mannitol and that water would be less reactive with the drug in IF than in MF. The NMR measurement suggested that water motion was more restricted in IF than in MF, being consistent with the better chemical stability of the drug in IF. This shows that utilization of suitable amorphous materials as excipients could be a promising approach to stabilizing lyophilized formulations of moisture-sensitive drugs. It should be noted, however, that such amorphous materials might carry a risk of causing crystallization by water absorption as exhibited in this study. Therefore when such amorphous excipients are used for lyophilized formulations, it is crucial to evaluate the amount of water which formulations can hold without risk of crystallization. It is also important to protect the formulations from moisture as much as possible and to monitor water content adequately during the manufacturing process and storage. This study also indicated that ^{2}H-NMR analysis of water mobility is a useful tool for investigating the chemical and physical stability of drugs in formulations.

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