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Stabilization mechanism of limaprost in solid dosage form

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Abstract

The effect of polymeric pharmaceutical excipients on the degradation of limaprost by hydrolysis was assessed by near infrared (NIR) spectroscopy and spin–spin relaxation time (T_2) measurements of proton NMR. Freeze-dried limaprost-alfadex formulated with various polymeric pharmaceutical excipients was exposed under humidified condition at 25 °C and 75% relative humidity. The freeze-dried limaprost-alfadex formulated with cellulose derivatives, hydroxypropylmethylcellulose (HPMC) and hydroxypropylcellulose (HPC-L), degraded easily. However, degradation was suppressed in samples formulated with polysaccharides, dextran40, dextrin, and pullulan, although the water sorption was more than 10% (w/w). A second-derivative NIR study showed the changes in the water mobility in the mixtures. The absorption peak near 1900 nm, which was assigned to water with high mobility, was observed in the humidified HPMC and HPC-L. The proton NMR spin–spin relaxation time measurements indicated that the structural relaxation of a polymeric excipient changed upon humidification. The polysaccharides showed only Gaussian relaxations, but the cellulose derivatives showed Lorentzian relaxations and Gaussian relaxations. The T_2 values of the Gaussian relaxation in HPMC and HPC-L were higher than those in dextran40, dextrin, and pullulan throughout the humidifying period. The higher molecular mobility of HPMC and HPC-L is related to the mobility of water, which may accelerate limaprost degradation.

Keywords: Limaprost; Stability; Water mobility; Near infrared spectroscopy; Spin–spin relaxation time (T_2)

1. Introduction

Pharmaceutical excipients used for solid formulation of moisture-sensitive drugs influence the degradation rate of active pharmaceutical ingredients (APIs, Du and Hoag, 2001). Heideman and Jarosz have reported that the mobility of water is different among excipients in the solid dosage forms (Heidemann and Jarosz, 1991). A higher mobility of water molecules had a greater effect on the degradation rate. Hence, investigating the state of water in solid dosage forms is essential to clarify the stabilization mechanism.

Near infra-red (NIR) spectroscopy is a powerful tool to explicate the interaction between excipients and water, which affects the mobility of water in the solid dosage form (Hogan and Buckton, 2001; Columbano et al., 2002; Derbyshire et al., 2002; Airaksinen et al., 2005). Buckton et al. have reported the state of water in spray-dried lactose using NIR spectroscopy (Buckton et al., 1998). They found that as the amount of moisture sorption increased, the intensity and position of the peak assigned to water in lactose changed. These results indicated that the molecular mobility of adsorbed water in the lactose became high. Suzuki et al. have reported the interaction between microcrystalline cellulose and water during granulation by measuring the NIR intensity of absorption bands at 1898 nm and 1920 nm, which are assigned to the O–H stretching vibration of bulk water molecules and the combination of O–H stretching and deformation vibration of water molecules, respectively (Suzuki et al., 2001).

Another promising method to investigate the molecular mobility in the solid dosage form is NMR relaxation time

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measurements (Yoshioka et al., 2003; Koga et al., 2004; Masuda et al., 2005). The difference of water mobility among excipients has been reported to affect the degradation rates of cephalothin (Aso et al., 1994). A study on the relationship between the molecular mobility-changing temperature (T_{mc}) where the Lorentzian relaxation decay, which is due to higher mobility, and the Gaussian relaxation decay are observed, and the stability of freeze-dried γ globulin with an excipient have shown that the ratio of water with a higher mobility in the freeze-dried dosage form generated the difference of the T_{mc} among excipients and consequently, the difference in the γ globulin stability (Yoshioka et al., 1997, 1998, 1999).

Opalmon® tablets contain limaprost-alfadex, which is an α-cyclodextrin inclusion compound of limaprost, a PGE_1 (prostaglandin E_1) derivative (Fig. 1). Under high humidity conditions, limaprost degrades mainly into 17*S*,20-dimethyl-*trans*-Δ^2-PGA_1(11-deoxy-Δ^{10}). A stability study of freeze-dried limaprost-alfadex formulated with various pharmaceutical excipients has reported that the freeze-dried formulations with lactose or hydroxypropylmethylcellulose (HPMC) generated 4.9% of 11-deoxy-Δ^{10} after 1 week storage at 25 °C and 75% RH (Sekiya et al., 2006). On the other hand, the freeze-dried formulation with dextran40 generated only 0.7% of 11-deoxy-Δ^{10} under the same conditions. These results indicate that the excipients affect the degradation rate of limaprost in the solid dosage form.

To explicate the mechanism on how the stability of limaprost was affected by excipients in formulations, we evaluated the factors affecting the degradation, such as water content, pH and water mobility. The mobility of water in humidified excipients was investigated using NIR spectroscopy and the structural relaxation of polymeric excipients using spin–spin relaxation time (T_2) measurements of proton NMR. Both polysaccharides (dextran40, dextrin and pullulan) and cellulose derivatives (HPMC and hydroxypropylcellulose (HPC-L)) were used as excipients because they have differing water mobilities and structural relaxations. The degradation behavior of limaprost in the solid formulation was well explained in terms of the mobility of water and the excipients.

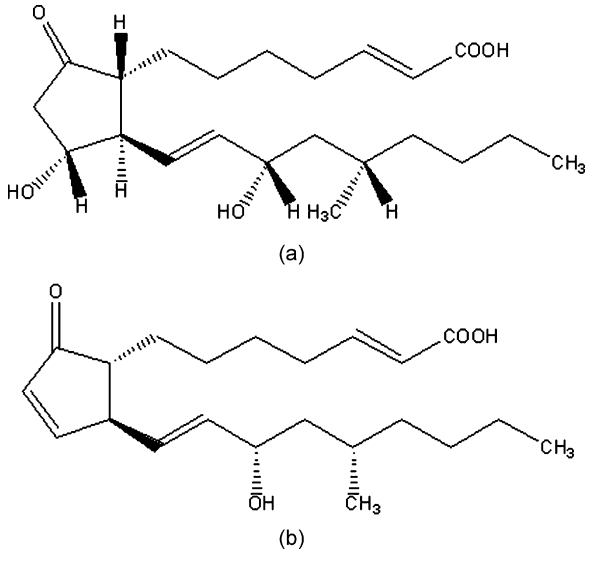

Fig. 1. Chemical structure of (a) limaprost and (b) its major degradation compound, 17*S*,20-dimethyl-*trans*-Δ^2-PGA_1(11-deoxy-Δ^{10}).

2. Materials and methods

2.1. Materials

Limaprost-alfadex (weight ratio of limaprost to α-cyclodextrin, 1:32.3) was supplied by ONO Pharmaceutical Co., Ltd., Japan. Dextran40k (molecular weight of 35,000) was purchased from Sigma (USA). Dextrin (Pinedex #1, molecular weight of 2300) and Pullulan (PI-20, molecular weight of 200,000) were obtained from Matsutani Chemical Industry Co., Ltd. (Hyogo, Japan) and Hayashibara Co., Ltd. (Okayama, Japan), respectively. Hydroxypropylmethylcellulose (HPMC 2910) and hydroxypropylcellulose (HPC-L) were supplied from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and Nippon Soda Co., Ltd. (Tokyo, Japan), respectively. Other reagents were of analytical reagent grade.

2.2. Preparation of lyophilized limaprost formulations

Limaprost-alfadex and excipients were dissolved in distilled water at 1:7 weight:weight ratio (w/w). The solution was filled into glass vials and lyophilized with Triomaster (Kyowa Vacuum Engineering Co., Ltd., Japan). For the stability study, the lyophilized samples were stored at 25 °C and 75% relative humidity (RH).

2.3. Water content and pH measurement

The water content of the initial samples was determined by the Karl Fisher method (Moisture meter CA100, Mitsubishi Chemical Co., Ltd., Japan). Each sample was weighed after 1, 2, or 4 weeks of storage. The water content was determined by following equation:

$$\frac{\text{sample weight after storage} - \text{sample weight before storage}}{\text{sample weight before storage}} \times 100\% \quad (1)$$

The pH of each solution was determined with a pH Meter F22 (HORIBA, Ltd. Japan).

2.4. Purity and related substances assay

Each freeze-dried sample was dissolved in 3 mL of purified water in a glass vial. An internal standard solution (propylparaben/ethanol solution (1 → 4000)) was added and mixed with a vortex mixer. Two hundred microliters of the solution was analyzed by HPLC (LC2010CHT, Shimadzu Corporation, Japan). The content of limaprost and its related substances, 11-deoxy-Δ^{10}, were assayed. A chromatographic system and the conditions were as follows: detector, UV (215 nm); column, Ø4.6 mm, 15 cm length, ODS column; column temperature,

35 °C; mobile phase, 0.02 mol/L potassium dihydrogenphosphate/acetonitrile/isopropyl alcohol (9:5:2, volume ratio); flow rate, 0.8 mL/min.

2.5. NIR measurements

Lyophilized samples were placed into clear glass vials and stored in a desiccator at 25 °C and 97.3% relative humidity (RH) (adjusted with a KCl-saturated solution). Periodically the vials were placed on the lens of a Rapid Content™ analyzer module attached to a NIR Systems 6500 spectrophotometer (FOSS, UK). For each sample, the NIR spectrophotometer recorded the mean spectrum of 32 scans over the wavelength region 1100–2500 nm. Replicate determinations of new samples did not significantly differ from the original data sets.

2.6. ^1H NMR measurements

Lyophilized samples were placed into an NMR sample tube (10 mm diameter) and stored in a desiccator at 25 °C and 75.0% RH and were adjusted by NaCl-saturated solution. Measurements were carried out using a pulse NMR spectrometer (25 MHz, JNM-MU25, JEOL, Japan). T_2 was measured by 90° pulses with widths of 2 μs. Free induction decay (FID) signals of protons in the samples were obtained at each sampling time. The "solid echo", which had an echo delay of 10 μs, was used in the detection stage of all measurements. The measurement was repeated 128 times with a recycling delay time of 3 s.

T_2 of water protons in the lyophilized polymer was calculated according to the Lorentzian equation from the FID signals between 200 μs and 1000 μs. The T_2 of polymer protons was calculated from the FID signals between 0 μs and 100 μs. After the FID signals due to water protons were subtracted, the obtained polymer FID signal was analyzed to calculate the T_2 values and the proportions of two relaxation processes: a Gaussian type relaxation process (2) and a Lorentzian relaxation process (3).

$$F(t) = \exp\left(-\frac{t^2}{2T_2^2}\right)\frac{\sin(ct)}{ct} \quad (2)$$

$$F(t) = \exp\left(-\frac{t}{T_2}\right) \quad (3)$$

where c is the constant.

When two types of protons, which have varying mobility like water with a high mobility and a polymer with a low one, exist in a formulation, the relaxation pattern is the sum of the (2) and (3) curves.

$$F(t) = P_{AG}\exp\left(-\frac{t^2}{2T_2^2}\right)\frac{\sin(ct)}{ct} + P_{AL}\exp\left(-\frac{t}{T_2}\right) \quad (4)$$

where P_{AG} and P_{AL} are the amplitude of Gaussian and Lorentzian type components, respectively. All the calculations were carried out by nonlinear least-square regression analysis.

3. Results and discussion

3.1. Effect of additives on the water content and degradation of limaprost

Table 1 summarizes the increases in the water content and the 11-deoxy-Δ^{10} yield after storing the lyophilized limaprost formulations at 25 °C and 75.0% RH. Just prior to the humidifying experiment, the 11-deoxy-Δ^{10} yield in limaprost-alfadex was 0.4%, but increased to 5.6% after storing 2 weeks. Because limaprost formulations with freeze-dried lactose degrades more during storage, limaprost formulations lyophilized with various polymeric excipients have been investigated. The 11-deoxy-Δ^{10} yield in the lyophilized limaprost formulations depends on the type of polymer used for the experiment. Among the hydrophilic polymers, polysaccharides (dextran40, dextrin, and pullulan) had lower yields than limaprost-alfadex and limaprost formulations with freeze-dried lactose. Although the water content after storage was extremely high, it is concluded that hydrophilic pharmaceutical excipients contribute to the stabilization of limaprost. On the contrary, cellulose derivatives (HPMC, HPC-L) gave high yields of 11-deoxy-Δ^{10}. These excipients remarkably destabilized limaprost even if the increased water content was lower than the polysaccharides. Hence, the difference in the stability upon adding an excipient to the limaprost formulation is not due to the difference in the amount of water uptake upon humidification.

Effect of pH of the sample solution, which may affect the stability of limaprost during the storage, was investigated. Stability experiments performed using buffer solutions have demonstrated that a limaprost aqueous solution is most stable at a solution pH of 3–4 (Sekiya et al., 2006). The pH of the aqueous solution of lyophilized limaprost with HPC-L was 3.0, which is within the stable pH region of limaprost. The stability of limaprost and the pH values of the sample solutions are not correlated as shown in Table 1.

Table 2 shows the effect of α-cyclodextrin, which was used as a solubilizing agent in the formulations, on the stability of limaprost. To prepare the lyophilized product in this experiment, limaprost and an excipient were dissolved in a 10% ethanol solution. The 11-deoxy-Δ^{10} yield in the sample lyophilized with lactose decreased from 37.9% to 9.7% by the addition of α-cyclodextrin. Lyophilization with dextran alone improved the

Table 1
Effect of additives on degradation of limaprost

Investigated formulation	11-Deoxy-Δ^{10} yield (%)			Water content (%)	pH[a]
	Initial	1 week	2 weeks	2 weeks	
Limaprost-alfadex	0.4	1.9	5.6	8.3	5.0
+Lactose	0.3	4.2	7.0	4.9	4.7
+Dextran 40	0.3	0.7	1.2	23.3	4.2
+Dextrin	0.3	0.9	1.4	16.1	4.1
+Pullulan	0.3	0.9	1.6	12.8	4.6
+HPMC	1.1	4.9	11.0	12.7	5.5
+HPC-L	1.3	14.4	25.5	11.3	3.0

All samples were stored at 25 °C and 75% RH.
[a] Samples were dissolved in 3 mL of water to measure pH at 25 °C.

Table 2
Effect of α-cyclodextrin on degradation of limaprost

Investigated formulation	11-Deoxy-Δ^{10} yield (%)	
	Initial	1 month
Limaprost	–	–
+Lactose[a]	1.2	37.9
+α-Cyclodextrin + Lactose	0.5	9.7
+Dextran[a]	0.8	8.7
+α-Cyclodextrin + Dextran	0.2	4.2

All the samples were stored at 25 °C and 75% RH.
[a] Samples were dissolved in 10% ethanol to prepare the lyophilized product.

stability of limaprost to 8.7%, but the limaprost was more stabilized in the presence of both dextran and α-cyclodextrin (4.2%). Hence, the polymeric excipient might play a key role in stabilizing limaprost.

In addition to the influence of α-cyclodextrin, the mobility of water adsorbed in the humidification process and subsequent mobilization of the polymer may greatly influence the stability of limaprost. Thus, we investigated the water mobility in humidified excipients using NIR spectroscopy and the structural relaxation of the excipient by spin–spin relaxation time (T_2) measurements of proton NMR.

3.2. Water mobility in the lyophilized polymer evaluated by NIR spectra

The NIR band around 1880–1980 nm represents the molecular states of water due to the combination of the OH stretching vibration band and the OH deformation band, which are in the IR region. The peak position reflects the molecular mobility of water adsorbed by humidification (Ohtake et al., 2006). When the mobility of water is highly restricted through intermolecular hydrogen bond formation, the NIR band appears at a higher wavelength. For bulk or free water, the NIR band appears near 1900 nm. For the NIR experiments, the water content of each sample was adjusted to 3.5% to compare the mobility of water in the excipients at the same water content. When the water content was greater than 3.5%, it was difficult to differentiate the NIR peak of bound water, which is observed around 1930 nm, from the other peaks.

Fig. 2 shows the second derivative NIR spectra of the lyophilized limaprost formulations. The second derivative transformation was performed to correct the baseline shifts. The NIR band around 1930 nm, which was regarded as weakly interacted water, is observed in all of the samples. However, humidified samples with HPC-L and HPMC demonstrated a shoulder and a tail, respectively, along with a broad band from 1880 nm to 1980 nm. The specific bands of the higher mobility were not observed for the humidified samples with dextran40, dextrin, and pullulan. These results indicate that there are two types of water with different mobilities exist in the samples with HPC-L and HPMC. However, the molecular mobility of water in the samples with dextran40, dextrin, and pullulan should be reduced even in the presence of a lot of water due to the stronger intermolecular interaction.

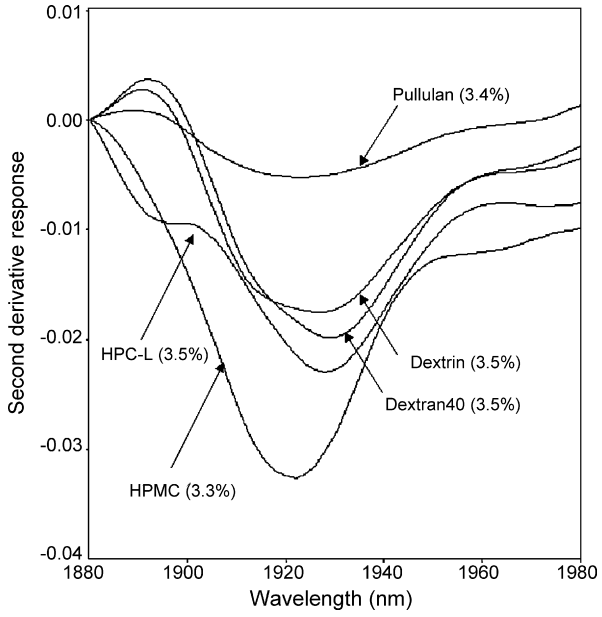

Fig. 2. Second derivative NIR spectra of lyophilized excipients after storage at 25 °C and 75% RH. Water content is shown in the parenthesis.

3.3. Water mobility in the lyophilized polymer evaluated by proton NMR

Fig. 3 shows the changes in the spin–spin relaxation time (T_2) of (a) Gaussian type and (b) Lorentzian type components of lyophilized polymer protons. When two types of protons, one with a high mobility like water and the other with a low mobility similar to a solid polymer, exist in a lyophilized polymer, the relaxation pattern should be expressed as the sum of Lorentzian and Gaussian type components by fitting with Eq. (4). As shown in Fig. 3, dextran40, dextrin, and pullulan show T_2 of the Gaussian type component. HPMC and HPC-L both have two types of protons with different mobilities.

The T_2 of all the polymer samples increased as the humidifying period increased. Among the Gaussian type components, the T_2 values of HPMC and HPC-L were higher than those of dextran40, dextrin, and pullulan. These results indicate that the mobility of the protons of HPMC and HPC-L, which destabilize the limaprost formulation in humidifying conditions, is higher than that of dextran40, dextrin, and pullulan, which stabilize the formulation. Humidification should increase the mobility, which should in turn influence the acceleration of hydrolysis of the limaprost.

Tables 3 and 4 summarize the amplitude of the Gaussian type (P_{AG}) and Lorentzian type (P_{AL}) components and the water increase of the humidified excipients. The amplitude of P_{AG} decreased as the water adsorption increased in all excipients. On the other hand, humidification slightly increased the amplitude of P_{AL} in HPMC and HPC-L. After humidifying for 2 weeks (336 h), the extent of water increase of limaprost formulation with HPMC and HPC-L was lower than that with dextran40, dextrin and pullulan as shown in Table 1. These results suggest that the spin–spin relaxation time measurement of the polymer should be applicable to estimate the

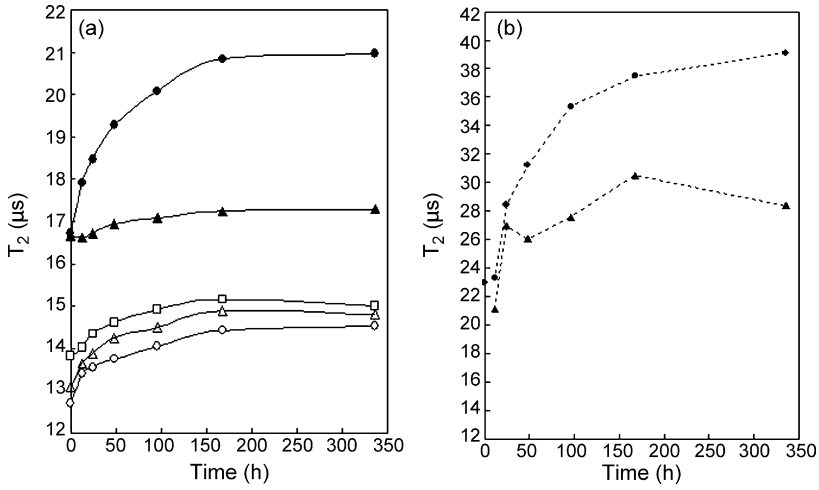

Fig. 3. Spin–spin relaxation time (T_2) of lyophilized dextran40 (○), dextrin (△), pullulan (□), HPC-L (●), and HPMC (▲) protons. (a) Gaussian type and (b) Lorentzian type relaxations as a function of storage time under a 75% RH.

Table 3
Variation of Gaussian type component (P_{AG}) and water content as a function of storage time

Storage period (h)	Dextran 40		Dextrin		Pullulan	
	P_{AG}	Water content (%)	P_{AG}	Water content (%)	P_{AG}	Water content (%)
0	1.00	–	1.00	–	0.94	–
12	1.00	3.5	0.97	3.2	0.87	3.0
24	0.93	6.5	0.90	5.8	0.82	5.4
48	0.83	10.3	0.82	9.2	0.77	7.9
96	0.74	13.2	0.76	12.8	0.73	11.6
168	0.71	18.6	0.74	14.3	0.71	13.3
336	0.68	20.7	0.73	14.8	0.70	14.8

All the samples were stored at 25 °C, 75% RH.

changes in the mobility of the limaprost formulation by water adsorption.

It has been reported that when the temperature is above the molecular mobility-changing temperature (T_{mc}), which is where the Lorentzian relaxation begins to decay, formulation becomes microscopically liquidized due to the liquid protons (Yoshioka et al., 1998). In the cases of HPMC and HPC-L, which have both Lorentzian and Gaussian relaxations, the temperature used was above the T_{mc} of each polymer. Thus, a liquid-like high molecular mobility of the polymer protons seems to appear due to the water adsorption. The higher mobility of the polymer protons accelerates the access of water to the limaprost molecules, which leads to hydrolysis.

Table 4
Amplitude of Gaussian and Lorentzian type components (P_{AG} and P_{AL}) and water content as a function of storage time

Storage period (h)	HPMC			HPC-L		
	P_{AG}	P_{AL}	Water content (%)	P_{AG}	P_{AL}	Water content (%)
0	0.92	–	–	0.72	0.27	–
12	0.81	0.10	1.1	0.61	0.34	1.9
24	0.82	0.07	2.2	0.59	0.35	3.5
48	0.77	0.10	3.5	0.50	0.42	5.6
96	0.74	0.11	5.3	0.45	0.44	8.2
168	0.73	0.11	6.3	0.41	0.46	9.4
336	0.70	0.13	7.3	0.38	0.48	10.7

All samples were stored at 25 °C and 75% RH.

4. Conclusion

The stabilization mechanism of freeze-dried limaprost-alfadex formulated with polymeric pharmaceutical excipients under humidification was explained by the increased mobility of water and the polymer protons. Degradation of limaprost in the lyophilized formulation with a polymer was not related to the pH, the increase in the amount of water, or the presence of α-cyclodextrin in the limaprost formulation. Limaprost-alfadex formulated with polysaccharides (dextran40, dextrin, and pullulan) stabilized limaprost due to the restricted water mobility in the presence of the polymer. For the formulations with cellulose derivatives (HPMC and HPC-L), limaprost was destabilized due to the presence of highly mobile water. Because the storage conditions were above T_{mc} of HPMC and HPC-L, the liquid-like polymer protons appeared to accelerate the water access to the limaprost molecules, which lead to hydrolysis. The NIR spectroscopy and T_2 measurements using proton NMR should be promising tools to evaluate the mobility of water and polymer protons.

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