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The stabilization mechanism of latanoprost

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ABSTRACT

The content of latanoprost is likely to decrease in solution because of the adsorption to eye drop containers and hydrolysis. We reduced these problems and established a formulation of latanoprost eye drops which is stable at room temperature. We assume that the additive surfactants form micelles and stabilize latanoprost in this formulation. In this study, we elucidated the latanoprost stabilization mechanism.

It was revealed by Arrhenius analysis that the adsorption to eye drop containers and hydrolysis of latanoprost were temperature-dependent. In addition, polyethylene glycol monostearates inhibited the adsorption and hydrolysis of latanoprost at 1 mg/mL, which exceeded the critical micelle concentration. By the fluorescent probe method, it was suggested that the surfactants were associated with benzalkonium chloride and formed complex micelles consisting of about 10 molecules, and latanoprost interacted with the micelles at 1:1. By ^{1}H NMR, it was revealed that adsorption was inhibited by arranging the hydrophobic group toward the center of complex micelles and that hydrolysis was inhibited by interaction between the ester group and the complex micelles.

It was shown that the latanoprost is stabilized by the interaction with complex micelles. It was effective for the inhibition of both adsorption and degradation.

1. Introduction

Prostaglandin derivatives are likely to degrade in aqueous solutions and adsorb to containers (Morishima et al., 2002; Sakai and Ohtori, 2005). Temperature-dependent reduction of the latanoprost content, a therapeutic drug for glaucoma, has also been reported (Morgan et al., 2001), but, to our knowledge, there has been no report on its mechanism. In our previous report, we confirmed that the latanoprost content reduction was due to adsorption to the eye drop container and hydrolysis accompanying the production of latanoprost acid (Ochiai et al., 2010). To overcome these problems, we established a latanoprost eye drop formulation which can be stored at room temperature by adding the surfactants polyethylene glycol monostearate 25 (MYS-25) and 40 (polyoxyl 40 stearate, MYS-40). In this formulation, the surfactants used as additives may have formed micelles and stabilized latanoprost. In the present study, we elucidated the mechanism of latanoprost stabilization in solution by these additives. The adsorption and degradation of latanoprost were investigated employing Arrhenius analysis (Yoshioka, 1995), and temperature dependence was confirmed. In addition, we investigated the influences of the concentration and molecular weight (number of added moles) of the 2 surfactants as well as the cause of latanoprost degradation observed when the additives were added at a high concentration in the previous report (Ochiai et al., 2010). Furthermore, the latanoprost stabilization mechanism was elucidated by analyzing the micelle structure and state of interaction between the micelles and the latanoprost employing the fluorescent probe method (Ueno et al., 1988; Wolszczak and Miller, 2002) and nuclear magnetic resonance (NMR) spectroscopy (Ueno et al., 1992; Bernardez, 2008).

2. Materials and methods

2.1. Materials

Latanoprost (Everlight Chemical Industrial Corporation, Taiwan), benzalkonium chloride (BZC, Maruishi Pharmaceutical Co., Ltd., Osaka), MYS-25, MYS-40 (Nihon Surfactant Kogyo K.K., Tokyo), sodium chloride, disodium hydrogen phosphate hydrate, sodium dihydrogen phosphate dihydrate, polyethylene glycol 1540 (PEG1540), pyrene, 1-dodecylpyridinium chloride (DPCI), sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), and D_2O (Wako Pure Chemical Industries Ltd., Osaka) were used. For the container, an eye drop container made of low-density polyethylene (LDPE) (Taisei Kako Co., Ltd.) and a glass ampule were used.

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2.2. Model formulations

Model formulations shown in Table 1 were prepared.

2.3. Latanoprost content

The model formulations were used as sample solutions. The latanoprost contents of the sample solutions were measured by liquid chromatography under the following conditions (number of measurements: 3): The liquid chromatograph used was Alliance (Nihon Waters K.K., Tokyo). For the column, mobile phase, and detector, a reverse-phase silica column (octadecyl silanized silica gel, particle size: 5 μm, inner diameter: 4.6 mm, length: 25 cm), mixture of sodium 1-hexanesulfonate aqueous solution and acetonitrile, and ultraviolet absorption spectrophotometer were used, respectively.

2.4. Latanoprost acid content

Hydrochloric acid was added to the model preparations to prepare sample solutions. Under the analytical conditions of latanoprost content measurement described above, the main degradation product of latanoprost, latanoprost acid, in the sample solutions was measured (number of measurements: 3).

2.5. Adsorption to the eye drop container

Latanoprost adsorbed to the eye drop container was measured referring to the reported method (Wong et al., 2006). The eye drop container (bottle and nozzle) was washed with water and dried, cut into about 2 mm × 2 mm pieces, and extracted with acetonitrile. Using the extract as a sample solution, measurement was performed under the analytical conditions described above (number of measurements: 3).

2.6. Water loss

The mass of the eye drops was measured at the initial and measurement points of the stability test using an electronic balance (XP205, Mettler-Toledo Inc., Tokyo), and the water loss was determined from the change in the mass (number of measurements: 3).

2.7. Free polyethylene glycol content

The model preparations were used as sample solutions. The free polyethylene glycol (PEG) content was measured in the sample solutions using liquid chromatography/mass spectrometry (HP1100, Agilent Technologies, Tokyo/LCQ DECA XP, Thermo Fisher Scientific K.K., Kanagawa) (number of measurements: 3). For the column, mobile phase, ionization method, and detection, a reverse-phase silica column (octadecyl silanized silica gel, particle size: 5 μm, inner diameter: 4.6 mm, length: 25 cm), mixture of water and acetonitrile, electrospray ionization, and positive ion mode were employed, respectively.

2.8. Fluorescence spectrum measurement

Fluorescence spectra of the sample solutions were measured using a fluorometer (F-4500, Hitachi Ltd., Tokyo). The excitation wavelength was 341 nm, and fluorescence spectrum was measured within a range of 350–500 nm (number of measurements: 3).

2.9. ^{1}H NMR spectrum measurement

Using an NMR spectrum measurement device (JNM-A500, JEOL Ltd., Tokyo), ^{1}H NMR spectra of the sample solutions were measured. The conditions were: resonance frequency, 500 MHz; measurement temperature, 23 °C; and number of scans, 10,000. For the standard substance, DSS was used (number of measurements: 1).

2.10. Stability test of model preparations

Formulation No. 1 was stored at 30, 40, 50, and 60 ± 2 °C in a dark place and the stability was evaluated. Each preparation was sampled at 0, 1, 2, and 4 weeks, and the latanoprost and latanoprost acid contents, adsorption to the eye drop container, and water loss were measured. The latanoprost content was presented as the mean rate of remained drug (%) regarding the initial content as 100%. Formulation No. 2–16 and 22–24 were stored at 60 ± 2 °C for 4 weeks in a dark, and the influences of the concentrations and molecular weights of the additives on stability were investigated. In addition, the free PEG content was measured in Formulation No. 2 stored for 4 weeks. For the container, an eye drop container made of LDPE was used, and the fill volume was 2.5 mL.

2.11. Stability test of PEG-added model preparations

Formulation No. 1 and 17–21 were stored at 60 ± 2 °C for 8 weeks in a dark place and the influence of free PEG on stability was investigated. Each preparation was sampled at 0, 2, 4, and 8 weeks, and the latanoprost and latanoprost acid contents were measured. For the container, glass ampules were used, and the fill volume was 2.5 mL.

2.12. Structural analysis of micelles

Sample solutions were prepared by adding 0.1 μg/mL of pyrene to the control formulation and Formulation No. 1 and 2, and the fluorescence spectrum was measured. As an index of micelle formation, the fluorescence intensity ratio of the first to third peak (I_1/I_3) of the fluorescence spectrum of pyrene was determined (Ueno et al., 1988; Wolszczak and Miller, 2002). In addition, 0.1 μg/mL of pyrene was added to Formulation No. 25–33, followed by the addition of 10–50 μmol/L of DPCI as a quencher to prepare sample solutions, and the fluorescence spectrum was measured. The fluorescence intensity ratio of the first peak of pyrene (I) in the fluorescence spectrum to that in the absence of DPCI (I_0) was determined, and the association number was calculated using Eq. (1) (Ueno et al., 1988; Suzuki, 1993):

$$\ln\left(\frac{I_0}{I}\right) = \frac{[Q]\cdot n}{[Ct] - [\text{momoner}]} \quad (1)$$

where n is the association number, [Q] is the quencher (DPCI) concentration (mol/L), [Ct] is the surfactant concentration (mol/L) and [momoner] is the monomer concentration (mol/L).

2.13. Micelle–latanoprost interaction (Ueno et al., 1992; Bernardez, 2008)

Using D_2O as a solvent, sample solutions with the same compositions as those of the control formulation and Formulation No. 1 and 2 were prepared, and the ^{1}H NMR spectrum was measured. Sample solutions with the same compositions as those of Formulation No. 25–33 were also prepared using D_2O and the ^{1}H NMR spectrum was measured (number of scans: 16).

Table 1
Formulations for latanoprost eye drops used in this study.

Ingredients	Formulation No. (mg/mL)																					
	Control	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Latanoprost	0.05[a]																					
Sodium chloride	Osmolar ratio 0.9–1.0																					
Dibasic sodium phosphate Sodium dihydrogen phosphate dihydrate	pH 6.5–6.9																					
Benzalkonium chloride	–											0.2[b]										
MYS-25	–	–	1.0[c]	0.1	0.3	0.5	1.0	1.8	3.0	5.0	–	–	–	–	–	–	–	–	–	–	–	–
MYS-40	–	–	0.8[d]	–	–	–	–	–	–	–	0.1[e]	0.3	0.5	1.0[f]	1.8	3.0	5.0[g]	–	–	–	–	–
PEG1540	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1	0.5	1.0	5.0	10.0

Ingredients	Formulation No. (mmol/L)											
	22	23	24	25	26	27	28	29	30	31	32	33
Latanoprost	0.12											
Sodium chloride	Osmolar ratio 0.9–1.0											
Dibasic sodium phosphate Sodium dihydrogen phosphate dihydrate	pH 6.5–6.9											
Benzalkonium chloride		0.56		1.0	0.75	0.5	0.25	–	0.75	0.5	0.25	–
MYS-25	0.05	0.5	2.5	–	0.25	0.5	0.75	1.0	–	–	–	–
MYS-40	–	–	–	–	–	–	–	–	0.25	0.5	0.75	1.0

[a] 0.12 mmol/L.
[b] 0.56 mmol/L.
[c] 0.72 mmol/L.
[d] 0.39 mmol/L.
[e] 0.05 mmol/L.
[f] 0.5 mmol/L.
[g] 2.5 mmol/L.

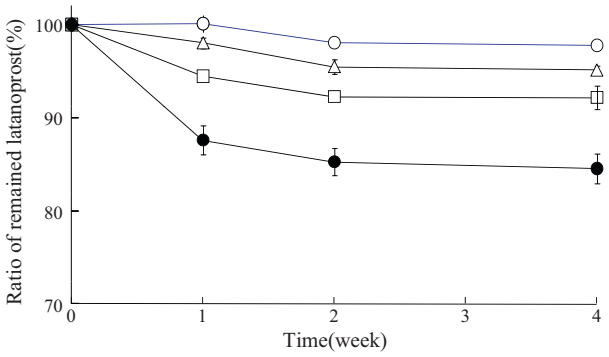

Fig. 1. Stability of Formulation No. 1 at 30 °C (○), 40 °C (△), 50 °C (□) and 60 °C (●). Each point represents the mean ± S.D. (n = 3).

3. Results and discussion

3.1. Stability of latanoprost in solution

Based on Fig. 1, the latanoprost content of Formulation No. 1 decreased over time on storage at 30, 40, 50, and 60 °C in a temperature-dependent manner. The latanoprost acid content, adsorption to the eye drop container, and water loss were also evaluated, and all these increased over time in a temperature-dependent manner. Based on Table 2, at all temperatures, the latanoprost content reduction was consistent with the total reductions due to latanoprost acid production and adsorption to the eye drop container and the content increase due to water loss, confirming that the latanoprost content reduction was caused by latanoprost acid production and adsorption to the eye drop container. The reaction rate constant k of the latanoprost acid production and adsorption to the eye drop container were calculated as the pseudo-first-order reaction, and also investigated employing Arrhenius analysis (Yoshioka, 1995). According to adsorption to the eye drop container, the investigation was conducted with the data for two weeks. As shown in Fig. 2, the Arrhenius plot showed a favorable straight line for both items, revealing that latanoprost acid production and adsorption to the container were temperature-dependent. The activation energy E_a of the production of the latanoprost acid and adsorption to the eye drop container were 81.3 kJ/mol and 84.1 kJ/mol, respectively. These findings clarified that the stability of latanoprost in solution is temperature-dependent.

Fig. 3 reveals that both MYS-25 and MYS-40 inhibited latanoprost acid production and adsorption to the container in a manner dependent on the additive concentration, and a sufficient inhibitory effect was obtained at 1 mg/mL (Formulation No. 6 and 13). In our previous report (Ochiai et al., 2010), we identified the critical micelle concentrations (cmc) of MYS-25 and MYS-40 as approximately 0.5 mg/mL. Therefore, these additives were considered to form micelles and simultaneously inhibit the adsorption to the container and degradation of latanoprost, but both additives showed an insufficient inhibitory effect at 0.5 mg/mL (Formulation No. 5 and 12), suggesting that the micelle concentration was insufficient to stabilize latanoprost when the additive concentration was 0.5 mg/mL, and 1 mg/mL was necessary to exhibit a sufficient stabilizing effect.

Fig. 4 shows that when the additives were added at an identical molar concentration, MYS-40, with its greater molecular weight (number of added PEG molars), more inhibited latanoprost content reduction, latanoprost acid production, and adsorption to the container (Formulation No. 10, 13 and 16). Latanoprost may have been stabilized by interaction with micelles. When the number of added PEG molars increased, adsorption to the container and hydrolysis of latanoprost that had interacted with micelles may have been reduced through steric hindrance by the outer PEG chain of micelles.

3.2. Influence of free PEG contained in polyethylene glycol monostearate

In our previous study, the latanoprost content decreased due to its degradation when polyethylene glycol monostearate was added at a high concentration (5 mg/mL or higher) (Ochiai et al., 2010), and the influence of a trace amount of free PEG contained in polyoxyethylene surfactants as an impurity (International Pharmaceutical Excipients Council Japan, 2007) was considered. It was also observed in the previous study that latanoprost degraded in PEG4000- and PEG6000-added formulations. Thus, we assumed that PEG is involved in the degradation of latanoprost in the presence of polyethylene glycol monostearate at a high level, and used PEG1540 (number of added molars: 35) as a model of free PEG contained in MYS-25 and MYS-40. Based on Fig. 5, latanoprost content reduction was promoted when PEG1540 was added at 5 mg/mL or higher (Formulation No. 20 and 21). This content reduction was due to the production of degradation products other than latanoprost acid, revealing that free PEG present at a high level in the addi-

Table 2
Stability of Formulation No. 1 at 30 °C, 40 °C, 50 °C and 60 °C.

Storage temp.	Item	Initial	1 week	2 weeks	4 weeks
30 °C	Ratio of remained latanoprost (%)	100	100.1 ± 0.9	98.1 ± 0.5	97.8 ± 0.4
	Latanoprost acid (%)	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
	Adsorbed latanoprost to container (%)	0.8 ± 0.2	2.3 ± 1.1	1.8 ± 0.2	3.9 ± 0.6
	Water loss (%)	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.0
40 °C	Ratio of remained latanoprost (%)	100	98.0 ± 0.6	95.5 ± 0.8	95.1 ± 0.4
	Latanoprost acid (%)	0.0 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	1.1 ± 0.0
	Adsorbed latanoprost to container (%)	0.8 ± 0.2	3.3 ± 1.3	5.1 ± 0.1	5.4 ± 0.3
	Water loss (%)	0.0 ± 0.0	0.2 ± 0.1	0.4 ± 0.3	0.5 ± 0.0
50 °C	Ratio of remained latanoprost (%)	100	94.5 ± 0.5	92.3 ± 0.5	92.2 ± 1.3
	Latanoprost acid (%)	0.0 ± 0.0	0.6 ± 0.0	1.2 ± 0.0	2.3 ± 0.1
	Adsorbed latanoprost to container (%)	0.8 ± 0.2	9.7 ± 0.6	11.2 ± 0.8	10.5 ± 2.0
	Water loss (%)	0.0 ± 0.0	0.8 ± 0.0	1.5 ± 0.0	3.1 ± 0.0
60 °C	Ratio of remained latanoprost (%)	100	87.6 ± 1.6	85.3 ± 1.4	84.5 ± 1.6
	Latanoprost acid (%)	0.0 ± 0.0	1.7 ± 0.0	3.3 ± 0.1	6.2 ± 0.4
	Adsorbed latanoprost to container (%)	0.8 ± 0.2	18.0 ± 1.6	21.8 ± 0.6	20.9 ± 1.3
	Water loss (%)	0.0 ± 0.0	1.7 ± 0.1	3.4 ± 0.0	6.7 ± 0.3

Each value represents the mean ± S.D. (n = 3).

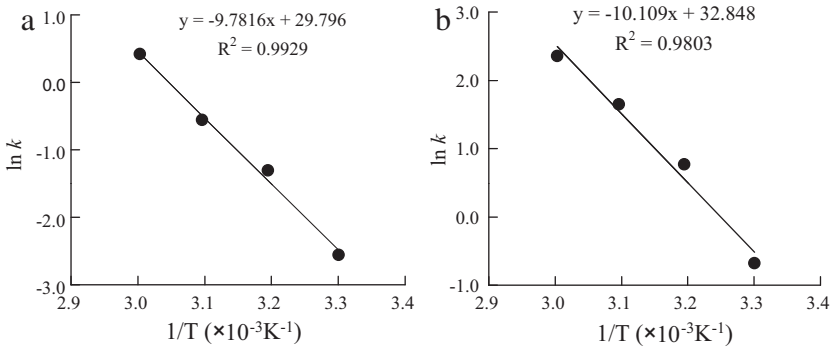

Fig. 2. Arrhenius plot of Formulation No. 1. (a) Latanoprost acid, (b) Adsorbed latanoprost to container.

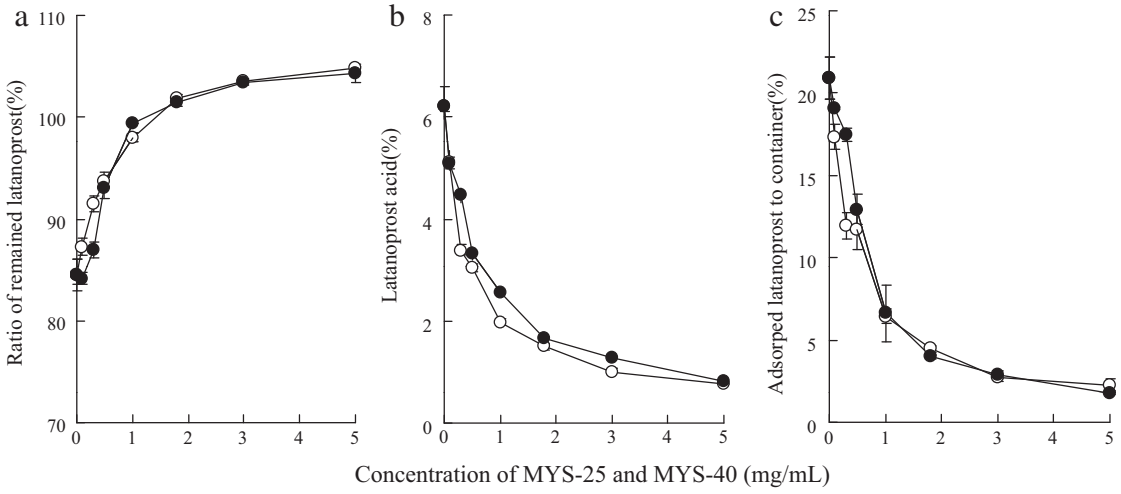

Fig. 3. Influences of concentration of MYS-25 and MYS-40 for stability of latanoprost. (a) Ratio of remained latanoprost, (b) Latanoprost acid, (c) Adsorbed latanoprost to container. MYS-25 (○), Formulation No. 1 (0 mg/mL), No. 3 (0.1 mg/mL), No. 4 (0.3 mg/mL), No. 5 (0.5 mg/mL), No. 6 (1.0 mg/mL), No. 7 (1.8 mg/mL), No. 8 (3.0 mg/mL), No. 9 (5.0 mg/mL). MYS-40 (●), Formulation No. 1 (0 mg/mL), No. 10 (0.1 mg/mL), No. 11 (0.3 mg/mL), No. 12 (0.5 mg/mL), No. 13 (1.0 mg/mL), No. 14 (1.8 mg/mL), No. 15 (3.0 mg/mL), No. 16 (5.0 mg/mL). Each point represents the mean ± S.D. at 60 °C for 4 weeks ($n = 3$).

tive induced latanoprost degradation through a process other than hydrolysis. On the other hand, free PEG was not increased in Formulation No. 2 after storage at 60 °C for 4 weeks. Therefore, latanoprost degradation observed in the previous study was caused by free PEG contained in MYS-25 when MYS-25 was excessively added. Based on Fig. 5, when the free PEG content of the preparation is 1 mg/mL or lower, it may not influence the stability of latanoprost, and, accordingly, it may have no influence at the concentration in the

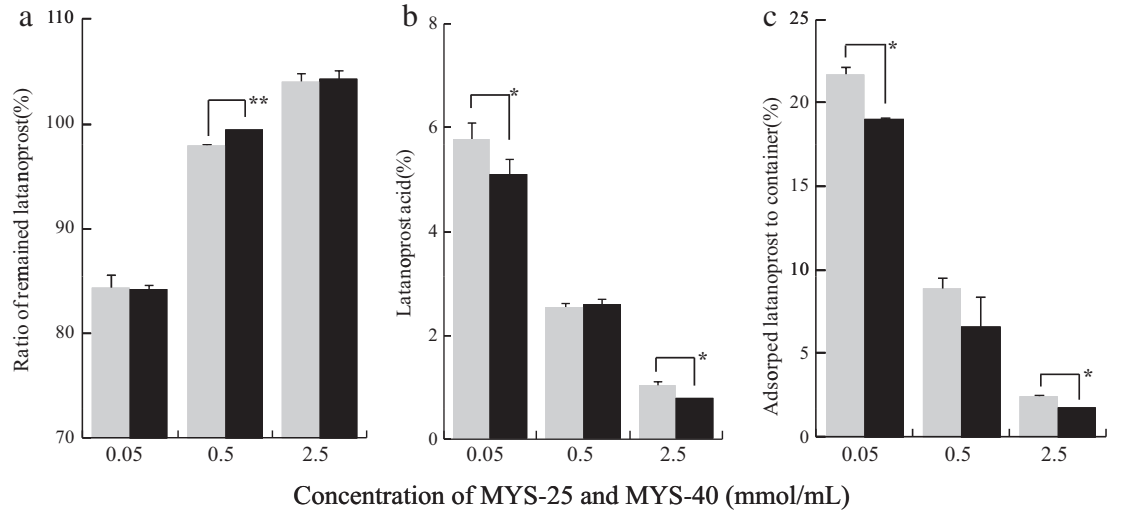

Fig. 4. Influences of length of PEG chains for stability of latanoprost. (a) Ratio of remained latanoprost, (b) Latanoprost acid, (c) Adsorbed latanoprost to container. MYS-25 (▒), Formulation No. 22 (0.05 mmol/L), No. 23 (0.5 mmol/L), No. 24 (2.5 mmol/L). MYS-40 (■), Formulation No. 10 (0.05 mmol/L), No. 13 (0.5 mmol/L), No. 16 (2.5 mmol/L). Each point represents the mean ± S.D. at 60 °C for 4 weeks ($n = 3$). *$p < 0.05$, **$p < 0.01$ (t-test).

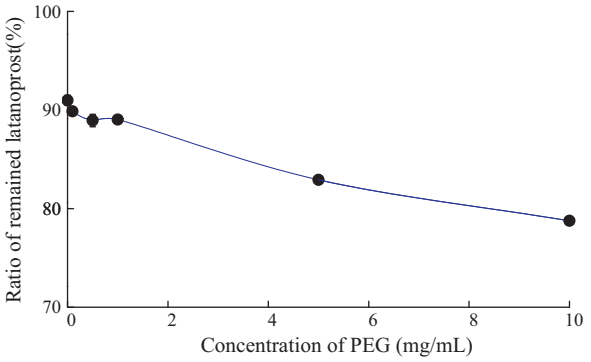

Fig. 5. Influences of concentration of PEG 1540 for stability of latanoprost. PEG1540 (●), Formulation No. 1 (0 mg/mL), No. 17 (0.1 mg/mL), No. 18 (0.5 mg/mL), No. 19 (1.0 mg/mL), No. 20 (5.0 mg/mL), No. 21 (10.0 mg/mL). Each point represents the mean ± S.D. at 60 °C for 8 weeks ($n = 3$).

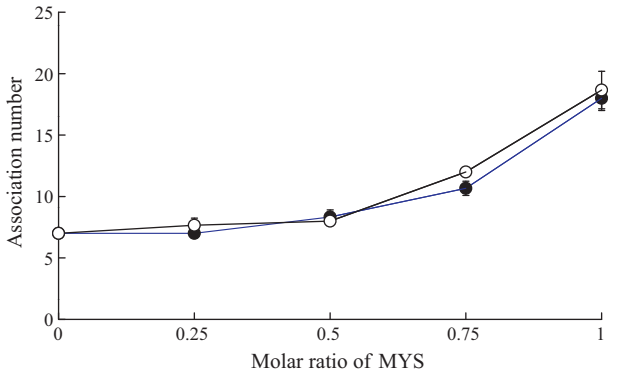

Fig. 6. Relationship between the association number for complex micelles and molar fraction of MYS. MYS-25/BZC complex micelles (●), Formulation No. 25 (0), No. 26 (0.25), No. 27 (0.5), No. 28 (0.75), No. 29 (1.0). MYS-40/BZC complex micelles (○), Formulation No. 25 (0), No. 30 (0.25), No. 31 (0.5), No. 32 (0.75), No. 33 (1.0). Each point represents the mean ± S.D. ($n = 3$).

latanoprost eye drop formulation established by us (Formulation No. 2).

3.3. Structural analysis of micelles

The fluorescence spectrum of pyrene shows 5 peaks. The intensity ratio of the first to third peak (I_1/I_3) varies depending on the environmental polarity of solubilized pyrene, and a reduction of I_1/I_3 indicates reduction of the polarity. When micelles are present in an aqueous solution, pyrene is dissolved in the hydrophobic inner region of micelles, reducing I_1/I_3. Accordingly, the presence or absence of micelle formation can be detected using I_1/I_3 as an index (Ueno et al., 1988; Wolszczak and Miller, 2002). As shown in Table 3, I_1/I_3 in the fluorescence spectrum of pyrene was 1.43 in the absence of the additives (control), whereas it decreased to 1.08 in Formulation No. 1 containing only BZC and 1.05 in Formulation No. 2 containing MYS-25 and MYS-40, confirming that BZC formed micelles in Formulation No. 1 containing no polyethylene glycol monostearate, while MYS-25, MYS-40, and BZC formed complex micelles in polyethylene glycol monostearate-added Formulation No. 2.

As shown in Fig. 6, the association number for complex micelles comprised of 2 components: MYS-25 and BZC, increased as the molar fraction of MYS-25 (Formulation No. 28 and 29). A similar tendency was noted in MYS-40/BZC complex micelles (Formulation No. 32 and 33). Accordingly, a similar relationship may have been present between the total molar fraction of MYS-25 and MYS-40 and association number in formulations containing the 3 components: MYS-25, MYS-40, and BZC. The molar concentrations of MYS-25, MYS-40, and BZC in Formulation No. 2 were 0.72, 0.39, and 0.56 mol/L, respectively, and the total molar fraction of MYS-25 and MYS-40 was 0.66. Based on Fig. 6, it was assumed that complex micelles composed of about 10 molecules of BZC, MYS-25, and MYS-40 were formed in Formulation No. 2. Since the total molar concentration of MYS-25, MYS-40, and BZC in Formulation No. 2 was 1.67 mmol/L, the complex micelle concentration was calculated to be about 0.17 mmol/L. On the other hand, in Formulation No. 1 to which no polyethylene glycol monostearate was added, micelles may have been formed by the association of 7 BZC molecules based on Fig. 6, and the micelle concentration was calculated to be about 0.08 mmol/L, showing that the micelle concentration in Formulation No. 1 was insufficient and latanoprost that had not interacted with micelles may have been adsorbed or hydrolyzed, whereas it was sufficient to stabilize latanoprost in Formulation No. 2. In Table 2, the total of latanoprost acid production and adsorption to the eye drop container accounted for about 27% in Formulation No. 1 after storage at 60 °C for 4 weeks. Assuming that latanoprost and micelles interacted at 1:1, since the latanoprost content of the formulation was 0.12 mmol/L, latanoprost which could not interact with micelles in Formulation No. 1 accounted for about 33%. This value was consistent with the total of the latanoprost acid content and amount adsorbed to the container in Formulation No. 1 after storage at 60 °C for 4 weeks. Based on these findings, latanoprost may be stabilized on interaction with micelles at 1:1, and this was the optimum ratio for the concentration of the additive.

Table 3
Changes of I_1/I_3 in the fluorescence spectrum of pylene in Formulation No. 1 and 2.

Formulation No.	Concentration of additives (mg/mL)			I_1/I_3
	MYS-25	MYS-40	BZC	
Control	–	–	–	1.43 ± 0.02
1	–	–	0.2	1.08 ± 0.00
2	1.0	0.8	0.2	1.05 ± 0.01

Each value represents the mean ± S.D. ($n = 3$).

3.4. Interaction between micelles and latanoprost

In ^{1}H NMR, signals shift toward the low magnetic field when protons are restricted by hydrogen bonding and intermolecular interaction, and toward the high magnetic field when protons are released. Therefore, the presence or absence of interaction with micelles can be identified based on the chemical shifts of signals

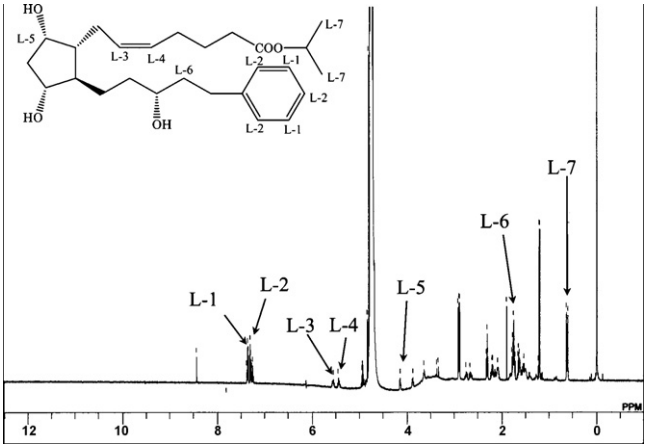

Fig. 7. ^{1}H NMR spectrum of latanoprost.

Table 4
Changes in proton chemical shifts of latanoprost by ^{1}H NMR.

Formulation No.	Concentration of additives (mg/mL)			Chemical shift (ppm)						
	MYS-25	MYS-40	BZC	L-1	L-2	L-3	L-4	L-5	L-6	L-7
Control	–	–	–	7.37	7.31	5.56	5.45	4.15	1.76	1.22
1	–	–	0.2	7.20	7.20	5.46	5.30	4.00	1.76	1.17
2	1.0	0.8	0.2	7.20	7.20	5.49	5.31	4.04	1.76	1.19

(Ueno et al., 1992; Bernardez, 2008). The ^{1}H NMR spectrum of latanoprost was shown in Fig. 7. In Table 4, the signals of the latanoprost structural formula shifted toward the high magnetic field in both Formulation No. 1 and 2, showing that latanoprost interacted with micelles composed of BZC alone in Formulation No. 1 and complex micelles in Formulation No. 2. The structural formula of latanoprost is shown in Fig. 7. In Formulation No. 2, signals of the hydrophobic phenyl groups (L-1 and L-2) and the double-bonded methylene groups (L-3 and L-4) showed marked chemical shift changes, suggesting that latanoprost interacted in the hydrophobic central region of the complex micelles. The signal of the 5-ring hydroxyl group (L-5) also showed a marked chemical shift changes, indicating interaction by hydrogen bonding with the hydrophilic region of complex micelles. Accordingly, it was assumed that latanoprost is present in the middle between the hydrophobic and the hydrophilic regions of complex micelles in an arrangement positioning the hydrophobic group toward the center of these complex micelles. Generally, the partition coefficient of a drug and its adsorption velocity to a plastic container are correlated (Ichibangase et al., 1990), and adsorption is enhanced as hydrophobicity increases. Accordingly, the hydrophobic group may have influenced the adsorption of latanoprost to the eye drop container. Since latanoprost interacts with micelles by positioning the hydrophobic group toward the center of complex micelles, it was assumed that the adsorption region was protected, resulting in the inhibition of adsorption to the eye drop container. On the other hand, latanoprost acid is produced by the hydrolysis of ester sites. In Table 4, a proton derived from an ester site, the isopropyl group (L-7), showed a slight chemical shift toward the low magnetic field, suggesting its interaction with complex micelles. Therefore, hydrolysis was also assumed to be inhibited by protection by complex micelles. As shown in Table 2, the level of latanoprost adsorbed to the eye drop container reached a plateau after 2 weeks, whereas the latanoprost acid level increased with time. This phenomenon was assumed to be due to a relatively weak interaction between the ester site of latanoprost and complex micelles. Latanoprost may have interacted with BZC micelles in Formulation No. 1, but, as described in the section of structural analysis of micelles, latanoprost content reduction occurred because the micelle concentration may have been insufficient to stabilize latanoprost.

The structural formula of BZC, MYS-25 and MYS-40 were shown in Fig. 8. In Fig. 9, the proton signals derived from the methylene groups (M-3 and M-4) adjacent to the ester site in the MYS-25 structure shifted toward the low magnetic field as the molar fraction of MYS-25 decreased in MYS-25/BZC complex micelles (Formulation

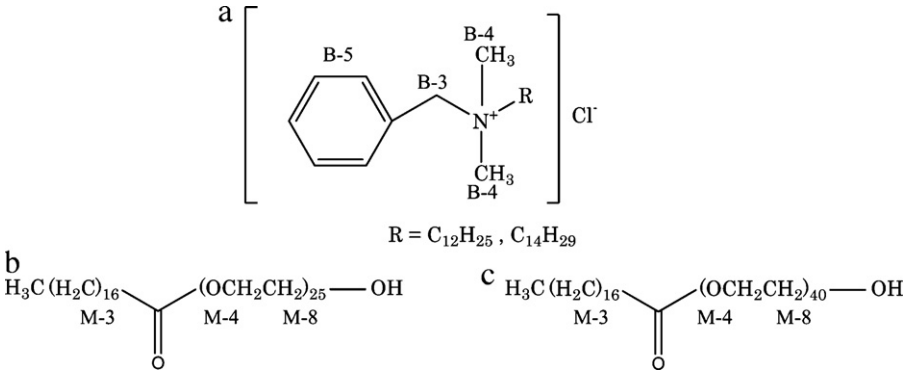

Fig. 8. The structural formula of BZC (a), MYS-25 (b) and MYS-40 (c).

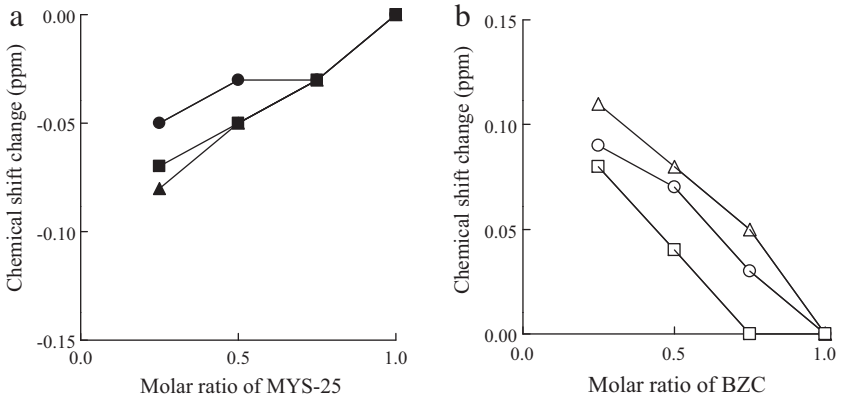

Fig. 9. Relationship between the chemical shift change of signals for MYS-25/BZC and molar fraction of each surfactants. (a) Signals for MYS-25, Formulation No. 26 (0.25), No. 27 (0.5), No. 28 (0.75), No. 29 (1.0), ●: M-3, ▲: M-4, ■: M-8. (b) Signals for BZC, Formulation No. 28 (0.25) No. 27 (0.5), No. 26 (0.75), No. 25 (1.0), ○: B-3, △: B-4, □: B-5.

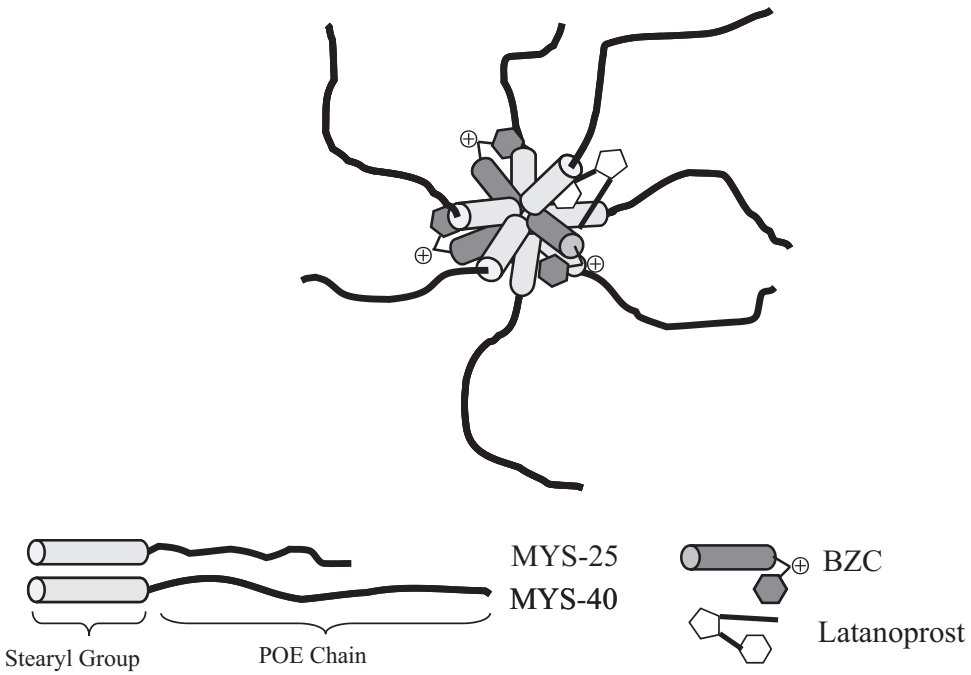

Fig. 10. Estimated schematic representation of the complex micelle and latanoprost in Formulation No. 2.

No. 26–29), whereas the proton signals derived from the phenyl group and near-by methyl and methylene groups (B-3, B-4, and B-5) in the BZC structure shifted toward the high magnetic field as the molar fraction of BZC decreased (Formulation No. 25–28). Similar tendencies were also noted in MYS-40/BZC complex micelles. Thus, as shown in Fig. 10, in the complex micelle structure of Formulation No. 2, BZC molecules may have been present near the boundary between the hydrophobic and the hydrophilic groups. In addition, as described above, latanoprost may have been similarly arranged positioning the hydrophobic group toward the center of complex micelles, through which latanoprost may have been protected and stabilized by the micelles.

4. Conclusions

It was revealed that the latanoprost stabilization mechanism involves interaction with complex micelles of surfactants, in which the interaction is effective for the inhibition of both adsorption and degradation. The complex micelle-based stabilization technique established by us may be applicable to other drugs. We hope that this technique will be applied in other studies on drug stabilization.

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