Stabilization of Prostaglandin E_1 in Fatty Alcohol Propylene Glycol Ointment by Acidic Cyclodextrin Derivative, *O*-Carboxymethyl-*O*-ethyl-β-cyclodextrin

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To improve the instability of prostaglandin E_1 (PGE_1) in ointments, potential use of *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin (CME-β-CyD) was examined, comparing with parent β-CyD. Inclusion complexation of PGE_1 in aqueous solution and in solid state was investigated by circular dichroism and carbon-13 nuclear magnetic resonance spectroscopies, kinetic method, powder X-ray diffractometry and thermal analysis. The inclusion ability of CME-β-CyD against PGE_1 was much higher than that of β-CyD, *i.e.*, stability constants determined by the kinetic method were 880 and 290 M^{-1} for the CME-β-CyD and β-CyD complexes, respectively. The chemical instability of PGE_1 in fatty alcohol propylene glycol (FAPG) ointment and in aqueous solution was significantly improved by the complexation with CME-β-CyD, while parent β-CyD accelerated the degradation in neutral and alkaline solutions. The stabilizing effect of CME-β-CyD seemed to come from 1) the adjustment of microscopic and/or macroscopic pH to about 4 where PGE_1 was most stable, 2) the low hygroscopicity of CME-β-CyD preventing access of water molecules to PGE_1 and 3) the inclusion of the reactive site, where the first effect contributed most significantly to the stabilization. The *in vitro* release of PGE_1 from FAPG ointments was enhanced by complexation with CME-β-CyD, and its superior release characteristics were retained even after aging. The limited data obtained here suggest that CME-β-CyD is useful for improvements of not only the chemical instability of PGE_1 in ointments as well as in solution, but also the release rate from the ointment.

Keywords prostaglandin E_1; *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin; β-cyclodextrin; inclusion complex formation; FAPG ointment; stabilization; drug release

The chemical stabilization of E-type prostaglandins (PGEs) is a very important issue for the development of new dosage forms and formulation changes.[1] In the currently available PGE formulations, inclusion complex formations with natural cyclodextrins (CyDs) such as α- and β-CyDs are successfully utilized for the solubilization and stabilization of PGEs.[2,3] Although these CyDs markedly improve the instability of PGEs in the solid state, they rather accelerate the dehydration rate of PGEs in neutral and alkaline solutions, where hydroxyl groups of CyDs function as a general base.[4] This suggests that CyD derivatives where the catalytic groups are blocked may be preferable from the viewpoint of the stabilization of guest molecules.[5]

In a preliminary study,[6] we found that an acidic CyD derivative, *O*-carboxymethyl-*O*-ethyl-β-CyD (CME-β-CyD), improves the percutaneous absorption of prostaglandin E_1 (PGE_1), particularly in combination with lipophilic absorption-enhancers such as 1-[2-(decylthio)-ethyl]azacyclopentane-2-one (HPE-101) and Azone®, and the therapeutic efficacy of PGE_1 in the skin of hairless mice is markedly imporoved. CME-β-CyD would be suited for the stabilization of PGE_1, since hydroxyl groups of β-CyD are substituted by carboxymethyl and ethyl groups and the carboxylic acid gives a weak acidic environment[7] favorable for the stability of PGEs.[4,8] In this paper, we report on improvements of the chemical instability of PGE_1 in ointments and in water as well as its release property from ointments, by using CME-β-CyD.

Experimental

Materials PGE_1 was donated from Hisamitsu Pharmaceutical Co. (Saga, Japan). β-CyD, 2-hydroxypropyl-β-CyD (degree of substitution (D.S.) = 5.8) and heptakis(2,6-di-*O*-methyl)-β-CyD (DM-β-CyD) were supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan), and β-CyD and DM-β-CyD were recrystallized from water and methanol, respectively. CME-β-CyD was supplied by Wako Pure Chemical Co. (Osaka, Japan) and the D.S. of carboxymethyl and ethyl groups were 1.83 and 10.7, respectively, which were determined by nuclear magnetic resonance (NMR) and fast atom bombardment mass spectrometries (FAB-MS), non-aqueous titration and idometry of JP XI.[7] Heptakis(2,6-di-*O*-ethyl)-β-CyD (DE-β-CyD) and heptakis(2,3,6-tri-*O*-ethyl)-β-CyD (TE-β-CyD)[9] were prepared using diethylsulfate, whose characterizations in detail will be reported elsewhere. Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Apparatus The circular dichroism (CD) spectra were obtained by a Jasco J-50A recording spectropolarimeter (Tokyo, Japan), and expressed in terms of molar ellipticity [θ]. The ^{13}C-NMR spectra were taken on a JNM-FX270 (JEOL, Tokyo, Japan) operating at 67.94 MHz. The ^{13}C-NMR spectra were recorded for degassed solutions of PGE_1 (0.02 M) in the absence and presence of CME-β-CyD (0.02 M) in 0.1 M sodium borate buffer (pH meter reading of 9.3) in 5 mm spinning tubes at an ambient temperature (about 25 °C) using D_2O solvent. ^{13}C-Chemical shifts were referenced to external tetramethylsilane with an accuracy of ±0.014 ppm. No degradation of PGE_1 during NMR measurements was confirmed. Powder X-ray diffraction patterns were taken on a Rigaku Denki Geiger Flex 2012 diffractometer (Tokyo, Japan) operating under the same condition as those reported.[7] Differential thermal analysis (DTA) was accomplished with a Rigaku Denki TAS 100 (Tokyo, Japan) operating at a scanning rate of 10 °C/min.

Preparation of PGE_1–β-CyD Complexes The CyD complexes were prepared according to the kneading method[10] using methanol as a solvent. Methanol was chosen by considering the stability and solubility of both substrates. For example, PGE_1 (10 mg) and β-CyD (160.1 mg) or CME-β-CyD (217.4 mg) in a molar ratio of 1 : 5 (PGE_1 : CyDs) were triturated in a small amount (1.0 ml) of methanol, and the slurry was further kneaded thoroughly. The paste thus obtained was dried under reduced pressure at room temperature for 12 h. The molar ratio of 1 : 5 (PGE_1 : CyDs) was chosen, since this ratio was reported to be a suitable formulation for the practical application of PGE_1[11] and to give the superior stabilization and percutaneous absorption of PGE_1.[6]

Preparation of Ointments Ointments of various bases such as white petrolatum, hydrophilic ointment, hydrophilic petrolatum, absorptive ointment and macrogol were prepared according to the method of JP XI. The fatty alcohol propylene glycol (FAPG) ointment was prepared by the reported method[12] with slight modification, *i.e.*, compositions were stearyl alcohol (9.5 g), cetyl alcohol (8.0 g), 1-docosanol (12.0 g) and propylene glycol (70.5 g). Gel ointment was prepared using 1 w/v% Hiviswako® No. 104 (Wako Pure Chemical Co.) and an aliquot of 10 N NaOH solution. PGE_1 or its β-CyD complexes (equivalent to 0.01 w/w%

of PGE_1) was added to the ointment bases and kneaded thoroughly.

Measurements of pH and Water Content in Ointments pH: The ointment (400 mg) containing PGE_1 or its β-CyD complexes was suspended in a 2.0 ml water, and pH of the suspension was measured using a Horiba F-7 pH meter (Tokyo, Japan) at 25 °C.

Water Content: The test sample (1 g) was placed in an incubator adjusted at a 75% relative humidity (R.H.) and 40 °C, and 40 d after preparation the water content in ointments was measured by the Karl–Fischer method using a MKA-3P moisture meter (Kyoto Electronics Co., Kyoto, Japan).

Stability Tests of PGE_1 Ointments The ointment containing PGE_1 or its β-CyD complexes was placed in an aluminum tube whose inner wall was coated with phenol resin in order to prevent adsorption of PGE_1, and the tube was stored in an incubator at a constant R.H. and temperature. At appropriate time intervals, a weighed sample (200 mg) was shaken with the mobile phase (6 ml) of high performance liquid chromatography (HPLC) described below, in order to extract PGE_1. After centrifugation (3000 rpm, 5 min) and filtration (DISMIC 25JP filter, Advantec Toyo Co., Tokyo, Japan), an aliquot of the filtrate was analyzed for PGE_1 by HPLC. The HPLC conditions were as follows: pump and detector, Hitachi L-6000 and L-4000, respectively (Tokyo, Japan); column, Tosoh TSK-gel ODS-120T (5 μm, 4.6 mm diameter × 150 mm, Tokyo, Japan); mobile phase, 0.01 M potassium dihydrogenphosphate/acetonitrile (3 : 2); flow rate, 1.0 ml/min; detection, 201 nm; internal standard, cortisone 21-acetate.

Stability Tests of PGE_1 in Aqueous Solution The dehydration rate of PGE_1 in the absence and presence of β-CyDs were spectrophotometrically monitored by measuring the appearances of PGA_1 and PGB_1 at 220 and 284 nm, respectively, as reported by Monkhouse.[13] The reaction was initiated by the addtion of a stock solution of PGE_1 in ethanol to sodium phosphate buffers of various pHs (μ=0.2) at 60 °C. The final concentrations of PGE_1, β-CyDs and ethanol were 5.0×10^{-5} M, 5.0×10^{-3} M and 2.0 v/v%, respectively. The graphically calculated rate constants were refined to obtain the best fit by using a nonlinear least-squares method,[14] as reported previously.[4]

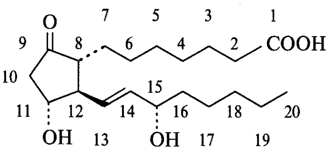
***In Vitro* Release Studies** The release of PGE_1 from ointments (500 mg) into normal saline (9 ml) was determined at 25 °C, using a horizontal diffusion cell[15] and a cellophane membrane (0.85 cm^2, pore size 2.4 nm) as a barrier for the diffusion of the vehicle. The concentration of PGE_1 was measured by HPLC under the same conditions as those described in stability tests. No degradation of PGE_1 during the *in vitro* release experiments was confirmed. Aging studies of the ointments containing PGE_1 or its β-CyD complexes placed in aluminum tubes were carried out under the condition of 75% R.H. and 40 °C.

Results and Discussion

Inclusion Complexation of PGE_1 with CME-β-CyD Figure 1 shows CD spectra of PGE_1 in the absence and presence of β-CyD or CME-β-CyD in phosphate buffers. PGE_1 exhibited a negative CD band around 292 nm due to $n \rightarrow \pi^*$ transition of C9 carbonyl group.[16] This optical activity was decreased by the addition of β-CyDs, where the effect was much larger with CME-β-CyD than with β-CyD, suggesting a higher affinity of PGE_1 to the CME-CyD cavity (see stability constants described later). The perturbation of CD spectrum by CME-β-CyD was greater at pH 2.0 than at pHs 4.0 and 6.0, indicating a favorable interaction between the unionized guest and host molecules (pK_a of the carboxyl groups of PGE_1 and CME-β-CyD = 5.02 and 3.75, respectively).[7,17] The chromophore of PGE_1 (C9 carbonyl group of the five-membered ring) may be located in the hydrophobic cavity of CyDs, since PGE_1 exhibited a similar change in the CD spectrum when it was dissolved in less polar solvents such as ethanol or dioxane.[16]

The favorable inclusion of the five-membered ring of

TABLE I. Effects of β-CyDs on the ^{13}C-NMR Chemical Shift of PGE_1 in Sodium Borate Buffer[a]



Carbon	PGE_1 alone[b] δ (ppm)	With β-CyDs,[c] $\Delta\delta$[d] (ppm)	
		β-CyD	CME-β-CyD
1	184.068	−0.561	−0.374
2	36.039	0.057	0.259
3	24.420	0.144	0.187
5	26.507	−0.590	−0.360
8	53.173	−0.302	−0.417
9	170.360	−1.167	−1.325
11	72.755	−0.043	−0.317
12	54.267	0.116	0.029
13	131.874	0.057	−0.058
14	135.891	0.532	0.360
15	71.286	0.620	−0.014
16	37.680	0.068	0.058
18	30.942	0.043	0.216
19	22.058	0.187	0.187
20	13.390	0.087	0.216

a) D_2O as solvent (pH meter readings of 9.3). *b*) The concentration of PGE_1 was 2×10^{-2} M. *c*) The concentrations of β-CyDs were 2×10^{-2} M. *d*) $\Delta\delta = \delta_{complex} - \delta_0$. Negative signs indicate upfield displacement.

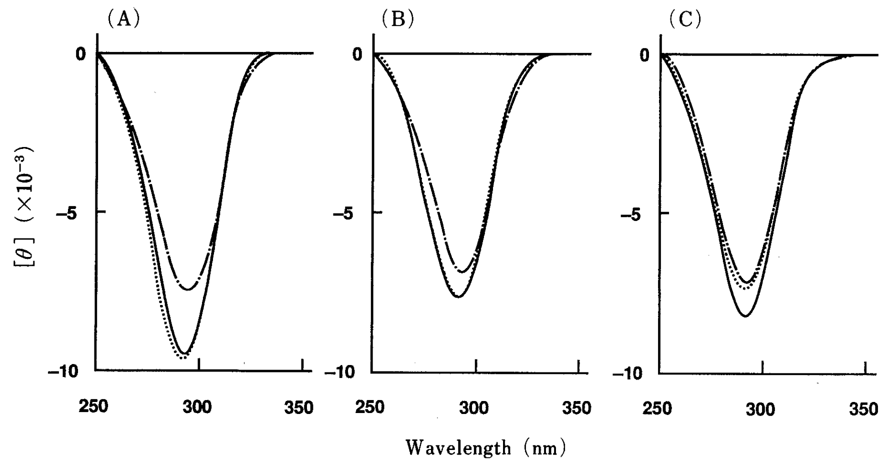


Fig. 1. CD Spectra of PGE_1 (1×10^{-4} M) in the Absence and Presence of β-CyDs (1×10^{-2} M) in Phosphate Buffer at Various pH

(A) pH 2.0; (B) pH 4.0; (C) pH 6.0. ——, PGE_1 alone; ------, with β-CyD; —·—, with CME-β-CyD.

PGE_1 was also supported by ^{13}C-NMR spectroscopic studies. Table I shows ^{13}C-chemical shift displacements of PGE_1 by the addition of CME-β-CyD and β-CyD. The displacements for certain carbons of PGE_1 could not be quantitatively monitored, since the signals overlapped each other (C4 and C7, C6 and C17) and C10 carbon gave a very broadening peak in the presence of β-CyD, probably due to the enolization and chemical exchange between H and D. Furthermore, the displacements for CME-β-CyD were not monitored because CME-β-CyD used is a chemically related mixture with different degrees of substitution and each carbon gave several ^{13}C-signals which made it difficult to estimate the change in chemical shifts. The upfield shifts were observed for the five-membered ring carbons, particularly the C9 carbonyl carbon, and the terminal carboxyl carbon of PGE_1, while other carbons showed downfield shifts. Since ^{13}C-NMR peaks of prostaglandins are known to shift upfield when they are located in a hydrophobic environment,[18,19] the above results suggested that the five-membered ring of PGE_1 is embedded in the hydrophobic CyD cavities of β-CyD and CME-β-CyD, and the terminal carboxylate group may be involved in the interaction with CME-β-CyD.[20] The C15 carbon, a kink point at which the alkyl chain (C16—C20) of PGE_1 protrudes from the relatively rigid moiety (C12—C15), showed the large downfield shift in the β-CyD system, while the slight upfield shift in the CME-β-CyD system, suggesting different conformations of the terminal alkyl chain between both complexes.

PGE_1 is known to be extremely susceptible to dehydration under high acidic and alkaline conditions, giving PGA_1 which is then isomerized consecutively to PGB_1 under alkaline conditions, with loss of the pharmacological activity.[21] Therefore, the interaction of PGE_1 with β-CyDs was investigated by the kinetic method,[22] which also provides useful information on the stabilizing effect of CyDs. Figure 2 shows the effects of the concentration of β-CyD and CME-β-CyD on the dehydration rate of PGE_1 to PGA_1 at pH 11.0 and 60 °C. This reaction condition was chosen for the convenience of kinetic measurement due to the moderate reaction rate. The dependencies of the apparent rate constant (k_{obs}) on CyD concentration were quantitatively analyzed in terms of Eq. 1,[22] to obtain the stability constants (K_c) and rate constants (k_c) of a complexes on the basis of 1 : 1 complexation scheme as reported previously,[4] where k_0 and $(CyD)_t$ are the rate constant in the absence of CyDs and the total concentration of CyDs, respectively. The plots according to Eq. 1 gave a good straight line with a correlation coefficient of 0.999 as shown in Fig. 3, and the K_c values and kinetic parameters are listed in Table II. The dehydration of PGE_1 was accelerated about 11 times by complexation with parent

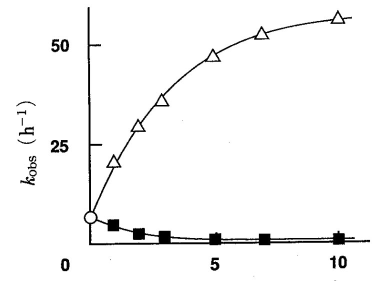


Fig. 2. Observed Rate Constants for the Dehydration of PGE_1 as a Function of Concentration of β-CyDs in Phosphate Buffer (pH 11.0, μ=0.2) at 60 °C

○, PGE_1 alone; △, with β-CyD; ■, with CME-β-CyD. Average of the value for duplicate measurements, which coincide with each other within ±2%.

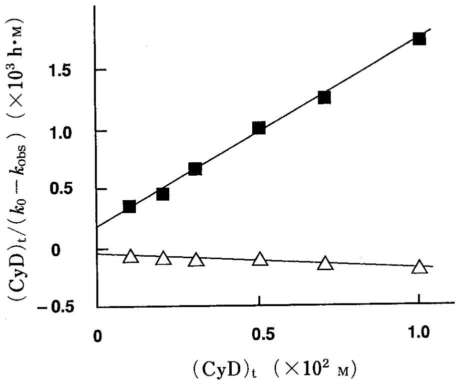


Fig. 3. Determination of K_c and k_c for PGE_1–β-CyDs Complexes by Plotting the Kinetic Data (Fig. 2) According to Eq. 1

△, with β-CyD; ■, with CME-β-CyD.

Table II. Rate Constants (k_c) and Stability Constants (K_c) of PGE_1–β-CyDs Complexes in Phosphate Buffer (pH 11.0, μ=0.2) at 60 °C

System	k_0 or k_c (h^{-1})	k_c/k_0	K_c (M^{-1})
PGE_1 alone	6.45	—	—
With β-CyD	71.93	11.15	290
With CME-β-CyD	0.22	0.03	880

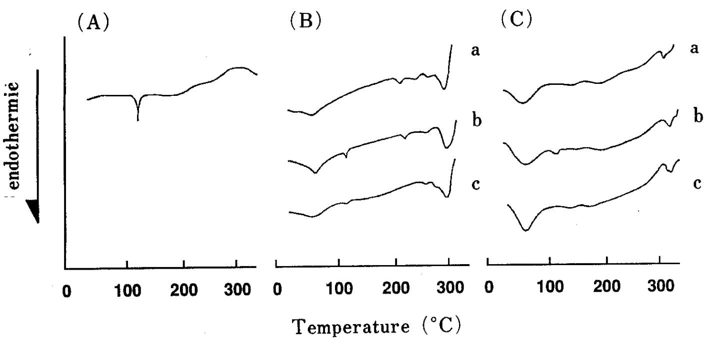


Fig. 4. DTA Thermograms of PGE_1–β-CyDs Systems

(A) PGE_1 alone; (B) β-CyD system; (C) CME-β-CyD system. a, β-CyDs alone; b, physical mixture of PGE_1 and β-CyDs; c, PGE_1–β-CyDs complexes.

β-CyD, which may be due to the catalytic action of hydroxyl groups of β-CyD participating as a general base in the reaction, as reported.[4] In sharp contrast, CME-β-CyD decelerated the dehydration (decelerating ratio $k_0/k_c=30$), probably due to the blocking of the catalytic action by the substitution and the adjustment of a microscopic pH[23,24] around the reactive site to about 4 where PGE_1 was most stable, as described later. The K_C value of the CME-β-CyD complex was 3 times larger than that of the β-CyD complex, indicating a higher affinity of PGE_1 to the CME-β-CyD cavity.

$$\frac{(\mathrm{CyD})_t}{k_0-k_{\mathrm{obs}}}=\frac{1}{k_0-k_c}(\mathrm{CyD})_t+\frac{1}{K_c(k_0-k_c)} \qquad (1)$$

The solid complexes of PGE_1 with β-CyD and CME-β-CyD were prepared by the kneading method[10] and their interaction was studied by thermal analysis and X-ray diffractometry. Figure 4 shows DTA thermograms of PGE_1–β-CyD complexes. The physical mixture of PGE_1 and β-CyD gave an endothermic peak at 116 °C due to a melting of PGE_1, whereas this peak completely disappeared in the complexes. Figure 5 shows powder X-ray diffractograms of PGE_1–β-CyD complexes. The diffractogram of the complexes was apparently different from that of the corresponding physical mixture, for example, the diffraction peak at 7° of PGE_1 disappeared in the amorphous CME-β-CyD complex. These results indicated clearly that PGE_1 interacts with both β-CyDs in the solid state.

Stabilization of PGE_1 in FAPG Ointments by CME-β-CyD An attempt was made to evaluate the effect of CME-β-CyD on the stability of PGE_1 in ointments.

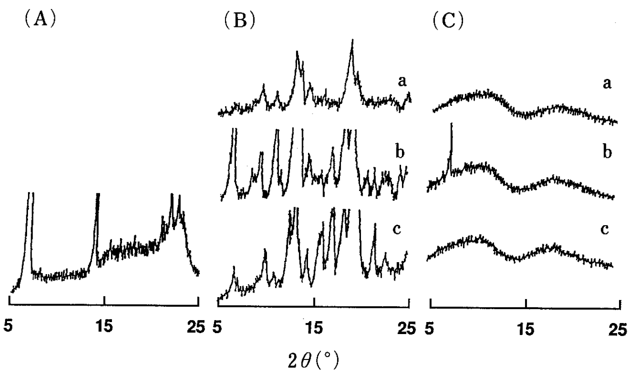


Fig. 5. Powder X-Ray Diffraction Patterns of PGE_1–β-CyDs Systems

(A) PGE_1 alone; (B) β-CyD system; (C) CME-β-CyD system. a, β-CyDs alone; b, physical mixture of PGE_1 and β-CyDs; c, PGE_1–β-CyDs complexes.

Table III. Effects of Various Ointment Bases on the Stability of PGE_1 at 25 °C

Ointment base	Remaining PGE_1 (%)
White petrolatum	49.48[a]
Hydrophilic petrolatum	90.30[b]
Hydrophilic ointment	71.68[b]
Absorptive ointment	44.42[a]
Macrogol ointment	92.50[b]
FAPG ointment	88.70[b]
Gel (Hiviswako® No. 104)	Not detected[a]

a) Fifteen days after preparation. *b*) Thirty days after preparation. Average of the value for triplicate measurements, which coincide with each other within ±2%.

Table III summarizes the survey on the stability of PGE_1 in various ointment bases, where the main degradation pathway was the dehydration of PGE_1 to PGA_1. PGE_1 was more stable in hydrophilic petrolatum, macrogol and FAPG bases, which may be due to the lower water content of ointments responsible for the dehydration of PGE_1. Therefore, FAPG was chosen as an ointment base for PGE_1–β-CyD complexes, by taking into account the above stability test, together with the results on the percutaneous absorption of PGE_1 that some absorption-enhancers such as HPE-101 worked most effectively in FAPG ointment.[6] Figure 6 shows the degradation rates of PGE_1 in FAPG ointments containing various β-CyD complexes at 40 °C and 75% R.H. β-CyD derivatives decelerated the degradation of PGE_1 in the ointments, except for parent β-CyD which showed no stabilization. Among β-CyDs employed, CME-β-CyD exhibited the highest stabilization, for example, after 60 d about 70% of the initial PGE_1 content remained intact, while only 30% for PGE_1 alone.

In order to gain insight into the stabilizing mechanism of CME-β-CyD, pH and water content of the FAPG ointments were measured, and some environmental effects on the stability of PGE_1 were investigated. Table IV shows pH and water content of FAPG ointments containing PGE_1 or its β-CyDs complexes. The pH (about 7.5) of FAPG ointment was slightly decreased by the addition of PGE_1 or PGE_1–β-CyD complex (about 6.5), whereas

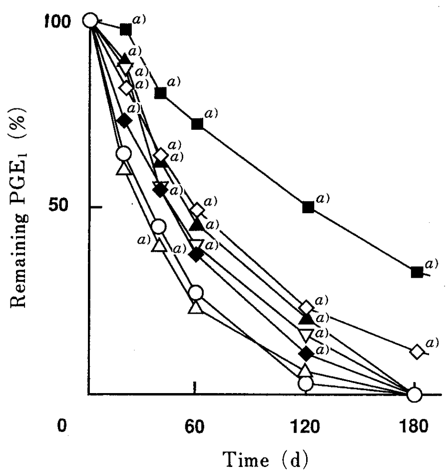


Fig. 6. Effects of β-CyDs on the Chemical Stability of PGE_1 in FAPG Ointments Containing PGE_1 and Its β-CyDs Complexes (0.01 w/w% as PGE_1) Stored at 40 °C, 75% R.H.

○, PGE_1 alone; △, β-CyD complex; ▲, DM-β-CyD complex; ▽, HP-β-CyD complex; ■, CME-β-CyD complex; ◆, DE-β-CyD complex; ◇, TE-β-CyD complex. Average of the value for triplicate measurements, which coincide with each other within ±2%. *a*) $p<0.05$ *versus* PGE_1 alone.

Table IV. Some Physicochemical Properties of FAPG Ointments Containing PGE_1 and Its β-CyDs Complexes (0.01 w/w% as PGE_1)

System	pH[a]	Water content[b] (%)
PGE_1 alone	6.57	3.17
β-CyD complex	6.46	3.82
CME-β-CyD complex	4.05	3.61

a) Determined as 20% aqueous suspension. *b*) Measured immediately after preparation. Average of the values for duplicate measurements, which coincide with each other within ±3%.

the CME-β-CyD complex markedly lowered the pH to about 4. Water contents of the FAPG ointments were not significantly different between the FAPG ointments containing PGE_1 and its complexes (about 3.2—3.8%). Figure 7 shows the results on the stability of PGE_1 in FAPG ointments with different pHs (about 3—7) which were adjusted by adding lactic acid. PGE_1 was most stable in the ointment of pH about 4, and CME-β-CyD exhibited the stabilizing effect even at these pH regions: for example, the residual PGE_1 in the ointment of pH 3.6 were 81.2 and 70.1% for the CME-β-CyD complex and PGE_1 alone, respectively, after the storage of 40 d at 40 °C and 75% R.H. Figure 8 shows the effect of the water content in the ointment on the stability of PGE_1. The degradation of PGE_1 in the CME-β-CyD complex was least susceptible to the influence of water content, while that in the β-CyD complex was accelerated with an increase in water content. The less hygroscopic nature of CME-β-CyD owing to the presence of hydrophobic substituents (ethyl groups, D.S. = 10.7)[7] seems to prevent access of the water molecules responsible for the degradation to the PGE_1 molecule.

The stabilizing effect of CME-β-CyD on PGE_1 in an aqueous solution was also investigated. Figure 9 shows the pH-profiles for the degradation rate of PGE_1 in the absence and presence of β-CyD or CME-β-CyD over the pH range of 2—8 at 60 °C. The shape of this pH-profile was similar to that of PGE_2 as reported previously,[4] although the rate of PGE_1 was slower than that of PGE_2 in neutral and alkaline regions.[13] Parent β-CyD decelerated the degradation of PGE_1 below pH about 5, while it accelerated the degradation above pH 5. As described above, this acceleration is ascribable to the catalytic effect of hydroxyl groups of β-CyD. On the other hand, CME-β-CyD decelerated the degradation of PGE_1 in all pH regions studied. PGE_1 was most stable at pH 3.5—4.0 both in the absence and presence of β-CyDs. The above results indicate that the stabilizing effect of CME-β-CyD on PGE_1 in FAPG ointment may come from 1) the adjustment of microscopic and/or macroscopic pH around the reactive site of PGE_1 to about 4 where PGE_1 was most stable, 2) the low hygroscopicity of CME-β-CyD inhibiting access of water molecules responsible for the degradation and 3) inclusion of the reactive site, where the first effect contributed most significantly to the stabilization.

Effect of Aging on Release of PGE_1 from FAPG Ointment From the viewpoint of quality assurance, the effect of aging on the release behavior of PGE_1 from FAPG ointments containing the CME-β-CyD complex was examined and compared with that of the β-CyD complex. Figure 10 shows a typical example of the release profile of

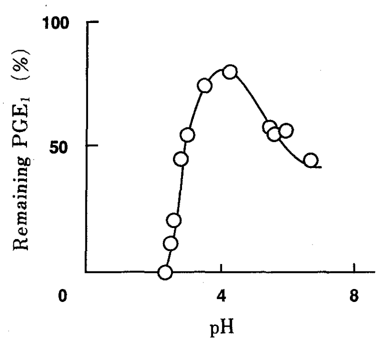


Fig. 7. Chemical Stability of PGE_1 in FAPG Ointments Containing PGE_1 (0.01 w/w% as PGE_1) Stored at 40 °C, 75% R.H. for 40 d as a Function of the pH of Ointments

Average of the value for triplicate measurements, which coincide with each other within ±2%.

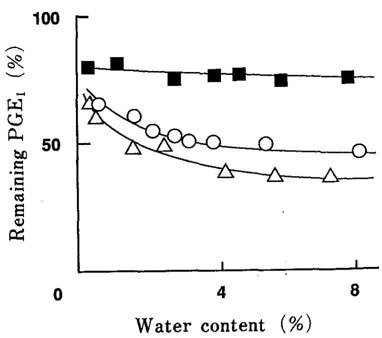


Fig. 8. Chemical Stability of PGE_1 in FAPG Ointments Containing PGE_1 and Its β-CyDs Complexes (0.01 w/w% as PGE_1) Stored at 40 °C, 75% R.H. for 40 d as a Function of Water Content in Ointments

○, PGE_1 alone; △, with β-CyD; ■, with CME-β-CyD. Average of the value for triplicate measurements, which coincide with each other within ±2%.

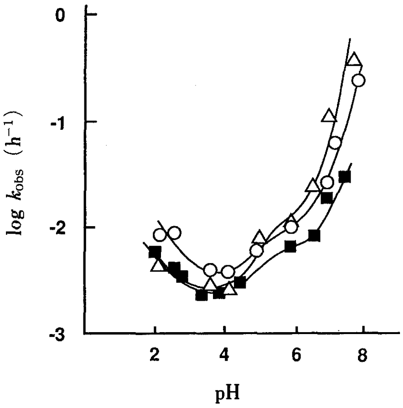


Fig. 9. pH-Profiles for the Dehydration Rates of PGE_1[a] in the Absence and Presence of β-CyDs (5×10^{-3} M) in Phosphate Buffer at 60 °C

○, PGE_1 alone; △, with β-CyD; ■, with CME-β-CyD. *a*) The initial concentration was 5×10^{-5} M. Average of the value for duplicate measurements, which coincide with each other within ±2%.

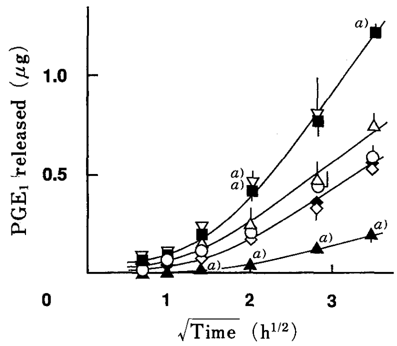


Fig. 10. Release Profiles of PGE_1 from FAPG Ointments Containing PGE_1 and Its β-CyDs Complexes (0.01 w/w% as PGE_1) through the Cellophane Membrane at 25 °C

○, PGE_1 alone; △, β-CyD complex; ▲, DM-β-CyD complex; ▽, HP-β-CyD complex; ■, CME-β-CyD complex; ◆, DE-β-CyD complex; ◇, TE-β-CyD complex. Each value represents the mean±S.E. of 3 experiments. *a*) $p<0.05$ *versus* PGE_1 alone.

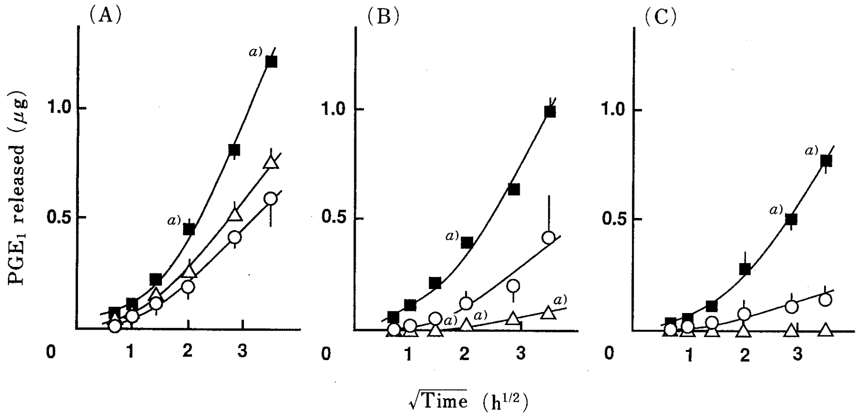


Fig. 11. Release Profiles of PGE_1 from FAPG Ointments Containing PGE_1 and Its β-CyDs Complexes (0.01 w/w% as PGE_1) through the Cellophane Membrane at 25 °C Stored at 40 °C, 75% R.H.

(A) Immediately after preparation; (B) 20 d after preparation; (C) 40 d after preparation. ○, PGE_1 alone; △, β-CyD complex; ■, CME-β-CyD complex. Each value represents the mean ± S.E. of 3 experiments. *a)* $p<0.05$ *versus* PGE_1 alone.

PGE_1 from FAPG ointments containing PGE_1 or its various β-CyD complexes, where the CME-β-CyD and HP-β-CyD complexes showed superior release of PGE_1, while DM-β-CyD decelerated the release. The enhanced release of PGE_1 may be due to the increase in thermodynamic activity such as solubility and diffusibility of the drug in the ointment, by means of water soluble complex formulation, as reported previously.[25] Then, the changes in the release profile of PGE_1 from the ointment stored at 40 °C and 75% R.H. were surveyed. As shown in Fig. 11, the release rate of the β-CyD complex was found to decrease significantly during storage, as expected from the stability of PGE_1 in the complex. On the other hand, the relatively higher release rate was maintained in the CME-β-CyD complex even 40 d after storage. The present data suggest that CME-β-CyD is useful not only for stabilization of PGE_1 in FAPG ointment and in aqueous solution but also for improvement of the release property of PGE_1 from the ointment. Furthermore, the percutaneous absorption of PGE_1 is significantly improved when it is formulated as the CME-β-CyD complex in combination with absorption-enhancers in FAPG ointment, as reported previously.[6]

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