Chem. Pharm. Bull. **44**(3) 582—586 (1996) Vol. 44, No. 3

Possible Enhancing Mechanism of the Cutaneous Permeation of 4-Biphenylylacetic Acid by β-Cyclodextrin Derivatives in Hydrophilic Ointment

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Received October 19, 1995; accepted December 1, 1995

The enhancing effects of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) on the percutaneous absorption of 4-biphenylylacetic acid (BPAA), a nonsteroidal anti-inflammatory drug, in hydrophilic ointment were studied and compared with the parent β-cyclodextrin (β-CyD). ^{13}C-NMR measurements suggested that the biphenyl group of BPAA is preferably included within the cavity of three β-CyDs. The three β-CyDs remarkably enhanced the release of BPAA from the hydrophilic ointment base and the *in vitro* cutaneous permeation, depending on the increase in solubility of BPAA in the ointment base. Pretreatment of the ointment containing DM-β-CyD or HP-β-CyD onto the isolated skin of hairless mice, however, provided no effects on the skin permeation of BPAA. When propylene glycol was used as a vehicle, both the release rate and cutaneous permeation parameters showed no appreciable difference between BPAA alone and its HP-β-CyD complex, because the solubilities of BPAA and its HP-β-CyD complex were almost comparable in the vehicle. The present results suggested that the enhancing effect of β-CyDs on the percutaneous absorption of BPAA can be mainly ascribed to an increase in the solubility of BPAA in the hydrophilic ointment.

Key words hydrophilic β-cyclodextrin derivative; 4-biphenylylacetic acid; cutaneous permeation; drug release; solubility in ointment

We have recently demonstrated that heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CyD) and 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) significantly improved the anti-inflammatory effect of a hydrophilic ointment containing 4-biphenylylacetic acid (BPAA), a nonsteroidal anti-inflammatory agent in rats.[2)] The *in vitro* drug release and the *in vivo* percutaneous absorption studies revealed that the release of BPAA from the hydrophilic ointment and the permeability of the drug into the dorsal skin of rats *in vivo* were increased by complexation with these β-CyD derivatives (β-CyDs). In addition, the two β-CyDs are reported to enhance the cutaneous permeation of drugs in a different manner; DM-β-CyD enhanced the penetration of sulfanilic acid by reducing the barrier function after application of the drug in solution onto the isolated skin of guinea pigs,[3)] whereas HP-β-CyD enhanced the cutaneous penetration of liarozole by influencing the distribution and partitioning of the drug in the skin of rats.[4,5)] Therefore, the present study deals with the elucidation of the enhancing mechanism of β-CyDs on the percutaneous absorption of BPAA. At first, the inclusion mode of BPAA within β-CyDs cavities was examined, and changes in the dissolution rate of the drug by inclusion complexations were compared. Then, the *in vitro* release of BPAA from the hydrophilic ointment base and the *in vitro* cutaneous permeability of BPAA after application of the ointments containing the drug and its β-CyD complexes onto the isolated dorsal skin of hairless mice were investigated. Furthermore, the effects of β-CyDs on the physicochemical properties of BPAA were compared between the hydrophilic ointment base and the propylene glycol vehicle.

Experimental

Materials BPAA was donated by Nippon Lederle, Ltd. (Saitama, Japan), and flurbiprofen was obtained from Mitsubishi Chemical Industries, Ltd. (Tokyo, Japan). β-CyD and HP-β-CyD were donated by Nihon Shokuhin Kako, Ltd. (Tokyo, Japan). The average degree of substitution of 2-hydroxypropyl groups in HP-β-CyD was confirmed to be 5.8 by ^{1}H-NMR.[5,6)] DM-β-CyD and propylene glycol were purchased from Toshin Chemical, Ltd. (Tokyo, Japan) and Wako Pure Chemical Industry, Ltd. (Osaka, Japan), respectively. The hydrophilic ointment base (Japanese Pharmacopoeia XII) was obtained from Iwaki Seiyaku, Ltd. (Tokyo, Japan), consisting of white petrolatum (25% (w/w)), stearyl alcohol (20% (w/w)), propylene glycol (12% (w/w)), polyethylene hydrogenated caster oil (4% (w/w)), glycerol monostearate (1% (w/w)), methyl 4-hydroxybenzoate (0.1% (w/w)), propyl 4-hydroxybenzoate (0.1% (w/w)), and purified water (an appropriate amount). All other chemicals and solvents used were of analytical regeant grade.

^{13}C-NMR Studies ^{13}C-NMR experiments were run using a JEOL JNM-GX 270 spectrometer, operating at 67.9 MHz for ^{13}C carbon. Chemical shifts were given relative to external tetramethylsilane (TMS) with an accuracy of ±0.015 ppm. The concentration of sample solutions for both BPAA and β-CyDs was 0.02 M in 0.05 M NaOD.

Preparation of Solid Complexes The solid complexes of BPAA with β-CyDs in a molar ratio of 1 : 1 were prepared by the kneading method,[7)] and were characterized by differential thermal analysis and powder X-ray diffraction measurements, as reported previously.[2)]

Dissolution Studies The dissolution rate of the β-CyD complexes was measured according to the dispersed amount method.[8)] The experimental conditions were as follows: sample, BPAA (40 mg, <100 mesh) or an equivalent amount of the β-CyD complexes (<100 mesh); dissolution medium, 100 ml of water; temperature, 37 °C; stirring speed, 91 rpm. At appropriate intervals, an aliquot (0.5 ml) was filtered, diluted with the dissolution medium, and determined at 254 nm using a UV spectrophotometer (Hitachi 650-10 LC, Tokyo, Japan).

Solubility Measurements The solubility of BPAA or its β-CyD complexes in the hydrophilic ointment base at 37 °C was determined as the sum of solubility in the solution phase (propylene glycol and purified water) and semi-solid phase (stearyl alcohol and white petrolatum).[9)] In addition, the solubility of BPAA and its β-CyD complexes in propylene glycol at 37 °C was measured using the method of Higuchi and Connors.[10)] The concentration of BPAA in the hydrophilic ointment base and in propylene glycol was determined by high performance liquid

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chromatography (HPLC) as follows: a Hitachi L-6000 machine equipped with an ERC-ODS-1161 column (3 μm, 6 × 100 mm, Erma Optical Works, Tokyo, Japan) was used. The mobile phase was methanol–0.1 M acetic acid (7:3, v/v), the flow rate was 1.0 ml/min, and BPAA was detected by a UV monitor at 255 nm.

***In Vitro* Release Studies** The ointments containing BPAA or its β-CyD complexes (equivalent to 1.0% (w/w) BPAA) were prepared by mixing the substances with the hydrophilic ointment base. The release of BPAA from the hydrophilic ointment (600 mg) into normal saline (9 ml) was determined at 25 °C using a Loveday type horizontal diffusion cell.[11] A silicon membrane (0.85 cm^2, Dow Corning Co., U.S.A.) was used as a barrier for the diffusion of the vehicle. At appropriate intervals, the sample solutions (0.5 ml) were withdrawn from the receiver phase, and the concentration of BPAA was determined by HPLC. To analyze the release behavior of BPAA or its β-CyD complexes from the vehicle, equations 1 and 2 were used when BPAA was fully dissolved, and when BPAA was only partly dissolved in the vehicles, respectively[12,13]:

$$Q = 2C_0(D \cdot t/\pi)^{1/2} \quad (1)$$

$$Q = [(2C_0 - C_s)C_s \cdot D \cdot t]^{1/2} \quad (2)$$

where, Q is the amount of BPAA released per unit area at time t; C_0 is the initial concentration of BPAA, free or in β-CyD complexes; D is the apparent diffusion coefficient; and C_s is the solubility of BPAA or its β-CyD complexes in the ointment base.

***In Vitro* Cutaneous Permeation Studies** Female hairless mouse (8 weeks old) was killed by cervical dislocation, and the abdominal skin was excised; adhering fat and other visceral tissue were removed. The skin was then attached to the diffusion cell as described above. The donor phase was charged with the hydrophilic ointment (600 mg) or propylene glycol (0.5 ml) containing BPAA or its β-CyD complexes (equivalent to 1.0% (w/w) and 0.2% (w/v) BPAA for the ointment and propylene glycol preparations, respectively). The receptor phase containing de-aerated saline (5.0 ml) was stirred continuously at 25 °C. Sample solution (0.5 ml) was taken from the receiver phase at determined intervals, and an internal standard (flurbiprofen) was added to the aliquots. BPAA was extracted by the mixed organic solvents (cyclohexane : diethyl ether = 3 : 1, v/v). After the solvent was evaporated, the residue was dissolved with methanol (100 μl), and was injected into HPLC.

Results and Discussion

Mode of Inclusion We have previously reported the inclusion complex formations of BPAA with three β-CyDs in aqueous solution by means of the solubility method and UV and circular dichroism (CD) spectroscopies.[2] In this paper, ^{13}C-NMR was employed for elucidation of the inclusion mode of BPAA-β-CyD systems to discuss the enhancing mechanism of the cutaneous permeation of BPAA. Table 1 summarizes the effects of β-CyDs on ^{13}C-NMR chemical shifts of BPAA (see Fig. 1 for chemical structures of BPAA and β-CyD and their carbon numbering). The C2, C3, C6, and C7 carbons of the biphenyl group of BPAA showed upfield shifts in all β-CyD systems, whereas the C4, C5 and C8 carbons in the long axis of the biphenyl group showed rather smaller displacements. The C2, C3, C6 and C7 carbons seem to be in close contact with the interior wall of CyDs, thus leading to upfield shifts owing to a hydrophobic effect.[14] Similar ^{13}C displacements were observed for the flurbiprofen–CyD systems.[15] The C10 carboxyl carbon shifted upfield, which may be due to the suppression of protolytic dissociation through the inclusion complexation with CyDs. In contrast, the C1 and C9 carbons in the neighborhood of the carboxyl group shifted downfield, which was in agreement with the deshielding phenomenon reported by Inoue *et al.*[16] in which the carbons located outside of the cavity shift downfield. In addition, the electron-withdrawing effect of the carboxyl group may be enhanced by the suppression of the protolytic dissociation in the hydrophobic environment of CyDs. The chemical shift change of carbons, except for C2, C3 and C4, was in the order of HP-β-CyD ~ β-CyD < DM-β-CyD, coinciding with the magnitude of the stability constant of the complexes (β-CyD; 2250 M^{-1}, DM-β-CyD; 4710 M^{-1}, HP-β-CyD; 1780 M^{-1}).[2] No correlation of the displacement of the C2, C3 and C4 carbons with the stability constant may be ascribed to the different steric effect of substituents (methyl and hydroxypropyl groups), which are located in the rim of the cavity, on the chemical shifts. This may be pronounced in the HP-β-CyD system, because it is a mixture of multi-components with different degrees of substitution. The above results indicate that the biphenyl group, particularly the terminal benzene (C5–C8), of BPAA is preferably included in the cavity of β-CyDs, while the carboxyl group and its neighbor are located outside or on the rim of the cavity. This inclusion mode was in good agreement with that determined by X-ray analysis of BPAA–trimethyl-β-CyD complex.[17] Table 2 lists the displacement of ^{13}C-NMR chemical shifts of β-CyDs by the addition of BPAA, where HP-β-CyD was omitted due to the difficulty of signal assignments

Table 1. Effects of β-CyDs on ^{13}C-NMR Chemical Shifts of BPAA

Carbon	Without β-CyD, δ_0	With β-CyDs, $\Delta\delta$[a]		
		β-CyD	DM-β-CyD	HP-β-CyD
1	137.07	0.24	0.52	0.19
2	129.90	−0.22	−0.01	−0.20
3	129.24	−0.32	−0.14	−0.03
4	140.53	−0.07	0.00	0.15
5	138.60	0.12	−0.31	0.14
6	126.73	−0.39	−0.74	−0.34
7	127.07	−0.54	−0.81	−0.57
8	127.66	−0.05	0.14	0.20
9	44.27	0.22	0.25	0.19
10	180.83	−0.68	−1.24	−0.74

a) Chemical shift changes (ppm) are expressed as $\Delta\delta = \delta_{complex} - \delta_0$. Negative sign indicates upfield displacement.

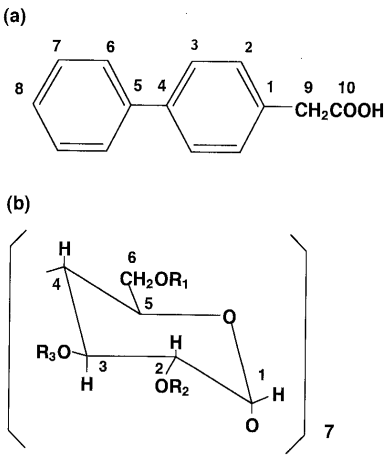

Fig. 1. Structures of (a) BPAA and (b) β-CyDs

Table 2. Effects of BPAA on ^{13}C-NMR Chemical Shifts of β-CyDs

Carbon	β-CyD		DM-β-CyD	
	Without BPAA, δ_0	$\Delta\delta$ [a]	Without BPAA, δ_0	$\Delta\delta$ [a]
1	102.34	0.02	99.51	0.27
2	72.68	−0.10	81.44	−0.03
3	73.52	0.04	72.37	0.04
4	81.48	−0.31	81.84	0.26
5	71.92	0.11	70.01	0.26
6	60.47	−0.39	70.71	−0.55
R1	—	—	58.33	0.07
R2	—	—	59.44	0.06

a) Chemical shift changes (ppm) are expressed as $\Delta\delta=\delta_{complex}-\delta_0$. Negative sign indicates upfield displacement.

because HP-β-CyD is a mixture of multiple components. The effect of DM-β-CyD on the ^{13}C-signals was generally greater than that of β-CyD, coinciding with the magnitude of the stability constant of the complexes. Relatively larger displacement was observed in carbons having higher molecular motions, *i.e.*, neighbors of the C5–C6 primary hydroxyl group and the C1–O–C4 glycosidic bond, suggesting changes in the conformations of the primary hydroxyl groups and the macrocyclic CyD ring, respectively. The conformational change in the macrocycle through the inclusion of BPAA seemed to be different between β-CyD and DM-β-CyD because of the different ^{13}C displacements. In ^{1}H-NMR spectra, the signals of H3 and H5 (located inside of the cavity) shifted upfield, probably due to the ring current effect of the benzene ring,[18] although data were not shown here, suggesting that the biphenyl group of BPAA is included in the cavity.

Solubility Table 3 lists the apparent solubility of BPAA and its β-CyD complexes in the hydrophilic ointment base and in propylene glycol at 37 °C. The solubility of BPAA in the hydrophilic ointment base was increased by the complexation with β-CyDs, in the order of β-CyD < HP-β-CyD < DM-β-CyD, whereas that in propylene glycol was comparable to that of the HP-β-CyD complex. The increasing effect of β-CyDs in the hydrophilic ointment base was attributable to the increase in solubility of BPAA in the water phase of the ointment base, because the ointment is an o/w emulsion base. Therefore, it was obvious that BPAA is only partly dissolved, whereas it is fully dissolved in the hydrophilic ointment base and propylene glycol, respectively.

***In Vitro* Release Behavior** As shown in Fig. 2, the dissolution rate of BPAA was increased by the complexation with β-CyDs, in the order of HP-β-CyD < β-CyD < DM-β-CyD, corresponding to the magnitude of the stability constants. Then, the release profiles of BPAA from the hydrophilic ointments containing BPAA and its β-CyD complexes as a function of the square root of time were examined. Under the present experimental conditions, only the free fraction of BPAA was available for release, because β-CyDs could not permeate the silicone membrane. As shown in Fig. 3, all plots exhibited linearity apart from an initial delay, indicating that the release is controlled by diffusion. The apparent release rate constants and diffusion coefficients were calculated using the solubility data in the ointment bases and Eq. 2 (Table 3). The results are summarized in Table 4. The release rate constants for the β-CyD complex, DM-β-CyD complex and HP-β-CyD complex were 1.2, 1.5, and 1.6 times higher than that of BPAA alone, respectively, and the diffusion coefficients were 7.1, 23.7, and 7.9-fold lower than that of BPAA alone, respectively. Consequently, it is evident that the enhancing effects of β-CyDs on BPAA

Table 3. Apparent Solubility of BPAA or Its β-CyD Complexes in the Hydrophilic Ointment Base and Propylene Glycol at 37 °C

System	Hydrophilic ointment base (μg/cm^3)	Propylene glycol (mg/cm^3)
BPAA alone	42.4	11.8
β-CyD complex	428.5	—[a]
DM-β-CyD complex	2579	—[a]
HP-β-CyD complex	931	11.0

a) Not determined.

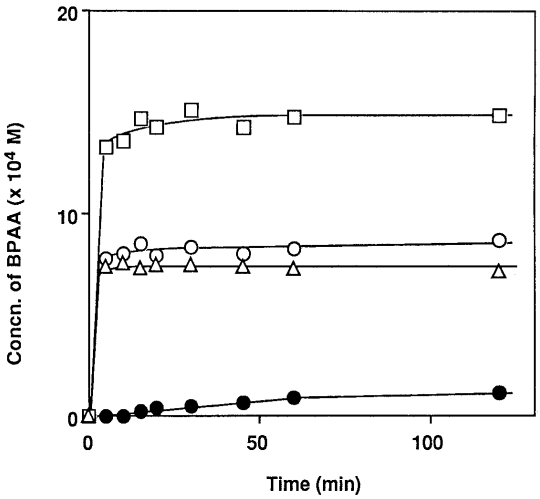

Fig. 2. Dissolution Profiles of BPAA and Its β-CyD Complexes in Water at 37 °C

●, BPAA alone; ○, β-CyD complex; □, DM-β-CyD complex; △, HP-β-CyD complex.

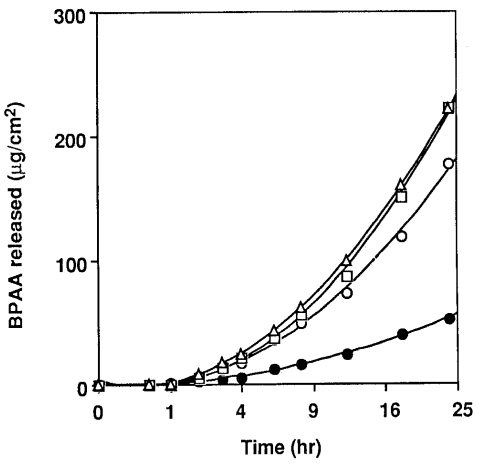

Fig. 3. Release Profiles of BPAA from Hydrophilic Ointments Containing BPAA or Its β-CyD Complexes through Silicone Membrane in Saline at 25 °C

●, BPAA alone; ○, β-CyD complex; □, DM-β-CyD complex; △, HP-β-CyD complex.

release were in good agreement with those of the solubility of the complexes in the ointment base, rather than the dissolution rate of the complexes.

***In Vitro* Cutaneous Permeation Behavior** Figure 4 shows the cutaneous permeation profiles of BPAA and its β-CyD complexes in a molar ratio of 1 : 1. The permeation parameters were calculated from Fick's second low,[19] assuming that the skin is a homogeneous single membrane and an infinite condition. The data are listed in Table 5. The complexations of BPAA with DM-β-CyD and HP-β-CyD resulted in 1.6 and 1.3-fold enhancement of the flux as compared with BPAA alone, respectively. No significant enhancing effect was observed for the parent β-CyD system. The apparent diffusion coefficients of DM-β-CyD complex and HP-β-CyD complex were identically increased about thrice compared to that of BPAA alone, whereas the apparent partition coefficient, permeation constant and lag time of BPAA were decreased by complexation with β-CyDs. The order of the flux values corresponded to that of the solubilizing effect of BPAA in the vehicle. Vollmer *et al.*[20] have recently reported that DM-β-CyD at higher concentrations may reduce the skin barrier function through the extraction of lipid constituents. Under the present experimental conditions, however, the pretreatment of skin by an ointment containing DM-β-CyD or HP-β-CyD provided no effects on the penetration of BPAA. The insufficient pretreatment effect observed for DM-β-CyD may be due to the low concentration employed. The results obtained here suggested that the enhancing effects of β-CyDs on the flux of BPAA can be ascribed to an increase in the solubility of BPAA in the vehicle.

Table 4. Apparent Release Parameters of BPAA from Hydrophilic Ointments Containing BPAA or Its β-CyD Complexes (Equivalent to 1% w/w BPAA) at 25 °C

System	k'[a] ($\mu g/cm^2/h^{1/2}$)	D[b] ($\times 10^4 cm^2/h$)
BPAA alone	20.25	4.97
β-CyD complex	24.19	0.70
DM-β-CyD complex	30.56	0.21
HP-β-CyD complex	33.12	0.63

a) Apparent release rate constant of BPAA from hydrophilic ointment base. *b*) Apparent diffusion coefficient of BPAA in hydrophiic ointment base.

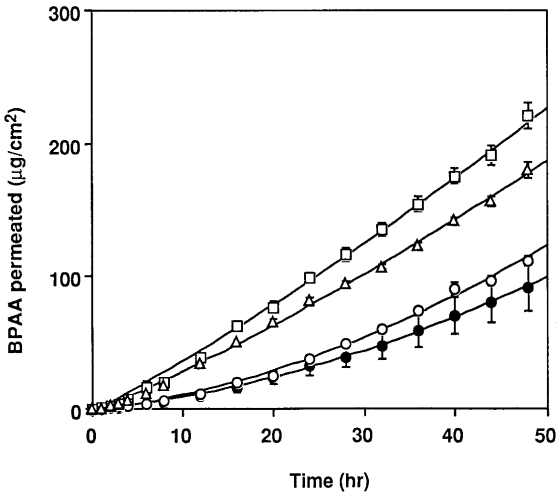

Fig. 4. Permeation Profiles of BPAA from Hydrophilic Ointments Containing BPAA or Its β-CyD Complexes through Isolated Dorsal Skin of Hairless Mice at 25 °C

●, BPAA alone; ○, β-CyD complex; □, DM-β-CyD complex; △, HP-β-CyD complex. Each point represents the mean±S.E. of 4—7 experiments.

In order to confirm whether the enhancing effects of β-CyDs on the release and the cutaneous permeability of BPAA could be attributed to an incremental increase in the solubility of BPAA in the hydrophilic ointment base, we further studied the *in vitro* release and cutaneous permeability of BPAA using propylene glycol as a vehicle. The solubilities of BPAA and its HP-β-CyD complex in propylene glycol were 11.8 and 11.0 mg/ml, respectively (Table 3). In addition, the viscosities of propylene glycol containing BPAA and HP-β-CyD complex (equivalent to 1.0% (w/v) BPAA) were 40.7 and 42.6 CP, respectively. Thus, no appreciable difference between BPAA and HP-β-CyD complex was observed for these physicochemical data. Table 6 summarizes the apparent release parameters of BPAA from propylene glycol solution containing BPAA or HP-β-CyD complex using Eq. 1. In addition, Fig. 5 shows the *in vitro* permeation profiles of BPAA after the application of propylene glycol containing BPAA or HP-β-CyD complex onto the skins of hairless mice. Interestingly, the release patterns and parameters showed no significant difference between BPAA alone and the HP-β-CyD complex. Therefore, the enhancing effect of HP-β-CyD on the release and cutaneous permeation of the drug from the hydrophilic ointment can be ascribed to the increase in solubility of BPAA in the vehicle.[21] Under the present condition, the enhancing mechanism of DM-β-CyD seems to be similar to that of HP-β-CyD, as judged from the following facts: 1) no significant difference was observed for the inclusion mode of three BPAA-β-CyDs complexes, 2) the order of the enhancing effects of release and permeation of BPAA by β-CyDs were in good agreement with the solubilizing abilities, and 3) pretreatment of β-CyDs to the skin area provided no effects on the cutaneous permeation of BPAA. Furthermore, the

Table 5. Parameters for Cutaneous Permeation of BPAA through Dorsal Skin of Hairless Mice[a]

System	Flux ($\mu g/cm^2/h$)	D[b] ($\times 10^5/cm^2/h$)	K[c]	Kp[d] ($\times 10^2$ cm/h)	LT[e] (h)
BPAA alone	3.49±1.02	7.3±0.4	63.0±8.4	6.65±1.20	11.3±0.6
β-CyD complex	3.62±0.23	7.0±0.6	8.8±1.3*	0.62±0.17**	12.0±1.0
DM-β-CyD complex	5.67±0.20	24.8±4.5**	0.7±0.1*	0.22±0.01**	3.6±0.5***
HP-β-CyD complex	4.55±0.11	23.7±1.2***	1.5±0.1*	0.49±0.01**	3.5±0.2***

a) Each value represents the mean±S.E. of 4—7 hairless mice. *b*) Apparent diffusion coefficient. *c*) Apparent partition coefficient. *d*) Apparent permeability constant. *e*) Apparent lag time. * $p<0.05$, ** $p<0.01$, *** $p<0.0001$, compared to BPAA alone.

Table 6. Apparent Release Parameters of BPAA from Propylene Glycol Containing BPAA or Its HP-β-CyD Complex (Equivalent to 0.2% (w/v) BPAA) at 25 °C

System	$k'^{a)}$ ($\mu g/cm^2/h^{1/2}$)	$D^{b)}$ ($\times 10^4 cm^2/h$)
BPAA alone	5.62	67.1
HP-β-CyD complex	5.67	68.5

a) Apparent release rate constant of BPAA from propylene glycol. *b*) Apparent diffusion coefficient of BPAA in propylene glycol.

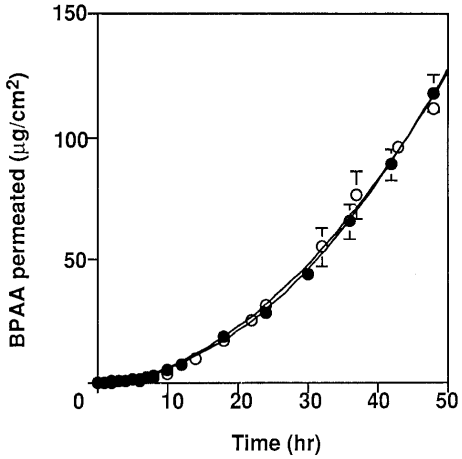

Fig. 5. Permeation Profiles of BPAA from Propylene Glycol Containing BPAA or Its HP-β-CyD Complex through Isolated Dorsal Skin of Hairless Mice at 25 °C

●, BPAA alone; ○, HP-β-CyD complex. Each point represents the mean ± S.E. of 4 experiments.

relationship between the solubility (Table 3), the release rate (Table 4) and the flux (Table 5) of BPAA was examined using multiple regression analysis (all possible regression). Consequently, the following equation was obtained:

$$\text{flux} = 7.57 \times 10^{-4}(\pm 1.57 \times 10^{-4}) \cdot \text{solubility} + 3.61 \times 10^{-2}(\pm 2.78 \times 10^{-2}) \cdot \text{release rate} + 2.60 \quad (r = 0.991,\ F = 29.87)$$

The partial correlation coefficients for solubility and release terms in the above equation were 0.979 and 0.771, respectively. Therefore, the release of BPAA from the vehicle is probably the rate-limiting step in the cutaneous permeation of the drug after application of the ointment onto the skin.

In conclusion, the results presented here suggest that the enhancing effects of both DM- and HP-β-CyDs are mainly attributed to an increase in the solubility of poorly water-soluble BPAA in the hydrophilic ointment base, which consequently provided the greater drug release from the vehicle and hence the enhancement of the percutaneous drug absorption.

Acknowledgments The authors would like to thank Miss Y. Nagamatsu and H. Kido for their technical assistance. We also thank Lederle (Japan), Ltd. (Saitama, Japan) for the donation of BPAA.

References and Notes

1) Present address: *School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432–1, Hachioji, Tokyo 192–03, Japan.*
2) Arima H., Adachi H., Irie T., Uekama K., *Drug Invest.*, **2**, 155 (1990).
3) Okamoto H., Komatsu H., Hashida M., Sezaki H., *Int. J. Pharm.*, **30**, 35 (1986).
4) Vollmer U., Müller B. W., Mesens J., Wilffert B., Peters T., *Int. J. Pharm.*, **99**, 51 (1993).
5) Loftsson T., Bodor N., *Acta Pharm. Nord.*, **1**, 185 (1989).
6) Rao C. T., Fales H. M., Pitha J., *J. Pharm. Sci.*, **7**, 612 (1990).
7) Tsuruoka M., Hashimoto T., Seo H., Ichimasa S., Ueno O., Fujinaga T., Otagiri M., Uekama K., *Yakugaku Zasshi*, **101**, 360 (1981).
8) Nogami H., Nagai T., Yotsuyanagi Y., *Chem. Pharm. Bull.*, **17**, 499 (1969).
9) Uekama K., Masaki K., Arimori T., Irie T., Hirayama F., *Yakugaku Zasshi*, **107**, 449 (1987).
10) Higuchi T., Connors K. A., *Adv. Anal. Chem. Instr.*, **4**, 117 (1965).
11) Loveday D. E., *J. Soc. Cosm. Chem.*, **12**, 224 (1961).
12) Higuchi T., *J. Pharm. Sci.*, **50**, 874 (1961).
13) Higuchi W. I., *J. Pharm. Sci.*, **51**, 802 (1962).
14) Conover W. W., Fried J., *Proc. Natl. Acad. Sci.*, **71**, 2157 (1974).
15) Otagiri M., Imai T., Hirayama F., Uekama K., *Acta Pharm. Suec.*, **20**, 11 (1983).
16) Inoue Y., Hoshi H., Sakurai M., Chujo R., *J. Am. Chem. Soc.*, **107**, 2319 (1985).
17) Harata K., Hirayama F., Arima H., Uekama K., Miyaji T., *J. Chem. Soc., Perkin Trans. 2*, **1992**, 1159.
18) Demarco P. V., Thakkar A. L., *Chem. Commun.*, **1970**, 2.
19) Crank J. (ed)., "The Mathematics of Diffusion," Oxford University Press, London, 1975.
20) Vollmer U., Müller B. W., Peeters J., Mesens J., Wilffert B., Peters T., *J. Pharm. Pharmacol.*, **46**, 19 (1994).
21) Loftsson T., Friðriksdottir H., Ingvarsdottir G., Sigurðardottir A. M., *Drug Devel. Indust. Pharm.*, **20**, 1699 (1994).