International Journal of Pharmaceutics, 30 (1986) 35–45
Elsevier

IJP 00992

Effects of β-cyclodextrin and di-O-methyl-β-cyclodextrin on the percutaneous absorption of butylparaben, indomethacin and sulfanilic acid

Hirokazu Okamoto, Hideo Komatsu *, Mitsuru Hashida and Hitoshi Sezaki

Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606 (Japan)

(Received July 9th, 1985)
(Modified version received November 20th, 1985)
(Accepted December 2nd, 1985)

Key words: percutaneous absorption—cyclodextrins—complex formation—penetration enhancement—diffusion model—diffusion constant—partitioning to skin

Summary

The effects of β-cyclodextrin (β-CD) and di-O-methyl-β-cyclodextrin (DM-β-CD) on the percutaneous absorption of butylparaben (BP), indomethacin (IM), and sulfanilic acid (SA) were investigated. The flow-through type diffusion cell was used for in vitro penetration experiments. All of the recorded data were fitted to the diffusion equation describing the drug penetration through a homogeneous plane membrane by the non-linear least-squares computer program, and two parameters corresponding to diffusion constant of drug and partition coefficient of that between skin and vehicle were obtained. Both cyclodextrins (CDs) decreased the penetration of BP depending on their concentrations. The decrease in BP penetration was explained by that in calculated apparent partition coefficient of BP and good correlation was observed between the partition coefficient and free BP fraction estimated based on the complex formation stoichiometry. IM penetration was also decreased by complex formation but some additional effects, somewhat different between β-CD and DM-β-CD, were observed. On the contrary, the penetration of SA which scarcely formed complex with either CD was significantly enhanced by DM-β-CD. This was attributed to the effect of DM-β-CD on the skin to reduce its barrier function.

Introduction

Skin has become a matter of interest in the pharmaceutical field because of its potency as the route of systemic administration (Shaw, 1982). But the difficulty to employ the transdermal route for systemic administration is that skin is essentially a barrier against penetration and permeation of external substances, including drugs for therapeutic uses (Scheuplein and Blank, 1971). One of the available methods to improve the transdermal transport is to reduce this barrier function of skin by the aid of penetration enhancers or accelerants (Hadgraft, 1984).

Cyclodextrins (CDs) which can include many kinds of molecules in their cavities are now widely applied to pharmaceutical formulations. An example of successful application of CDs in the pharmaceutical field is the improvement of bioavailability. The bioavailability of slightly soluble drugs administered in the form of freshly prepared oral

* *Present address:* Pola Laboratories, 27-1, Takashimadai, Kanagawa-ku, Yokohama 221, Japan.
Correspondence: H. Sezaki, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

suspensions or suppositories with CDs was improved owing to the enhancement of their dissolution rates (Nambu et al., 1978; Iwaoku et al., 1982). On the other hand, the membrane penetration constant and in situ absorption rate of acetohexamide or phenobarbital in solution were found to be decreased by the addition of β-cyclodextrin (β-CD; Uekama et al., 1980; Iwaoku et al., 1982). Though various studies of the effects of CDs on the drug absorption from the gastrointestinal tract have been carried out, there has been little information on the utilities of CDs as the additives for topical preparations. In the pharmaceutical field, β-CD has been extensively used but its relatively low aqueous solubility has limited its application. Recently, the methylated CDs have received considerable attention because of their high aqueous solubility and potency of complex formation (Uekama, 1985).

In the present study, three model drugs, butylparaben (BP), indomethacin (IM) and sulfanilic acid (SA), with different aqueous solubilities, lipophilicities and skin permeabilities were chosen as penetrants, and the effects of β-CD and di-O-methyl-β-cyclodextrin (DM-β-CD) on the percutaneous penetration of dissolved drug were investigated.

Materials and Methods

Materials

Butylparaben (Tokyo Chemical Industry), indomethacin (Sigma Chemicals), sulfanilic acid, β-cyclodextrin (Nakarai Chemicals), and di-O-methyl-β-cyclodextrin (Toshin Chemicals) were purchased commercially and used without further purification. Radiolabelled compounds used in this study were [^{14}C]butylparaben (1.94 mCi/mmol; Daiichi Pure Chemicals), [^{14}C]indomethacin (22.0 mCi/mmol; New England Nuclear), and [^{14}C]-sulfanilic acid (3.90 mCi/mmol; Daiichi Pure Chemicals).

Determination of physicochemical properties of drugs and complexes

The aqueous solubility of each drug was determined in the following manner. Excess amount of drug was dissolved in water and shaken at 37°C for 24–48 h. After equilibration, the sample was filtrated with Millipore Filter (pore size, 0.22 μm; Millipore) preheated at 37°C in an incubator. The filtrate was adequately diluted with water and analyzed spectrophotometrically.

BP (0.0515 μmol: 0.1 μCi), IM (0.00227 μmol: 0.05 μCi), or SA (0.0256 μmol: 0.1 μCi) was equilibrated between 2 ml of water (saturated with chloroform) and 2 ml of chloroform (saturated with water) at 37°C for 24–48 h. The radioactivities in the both layers were determined by scintillation counting, and the partition coefficient was calculated as their ratio.

According to the solubility method of Higuchi and Connors (1965), the stability constant of drug/CD complex at 37°C was determined. Excess amount of drug and successively increasing amount of CD were dissolved in water. The sample was shaken at 37°C for 24–48 h until the system reached equilibrium. After equilibration, the sample was filtrated with Millipore Filter preheated at 37°C. The filtrate was adequately diluted with water and analyzed spectrophotometrically. The total concentration of the dissolved drug was plotted against that of CD, and the stability constant was calculated from the initial linear region of this diagram.

In vitro percutaneous penetration experiment

Through all the experiments, the full thickness of skin obtained from the dorsal surface of a male guinea pig (Hartley strain) immediately prior to the experiment was used. The weights of the animals are listed in Table 2. After the removal of the dorsal hair with electric clippers, the skin was excised from sacrificed animal and the adipose tissue was removed carefully. The skin was punched out into a 3 cm diameter disk and mounted on the flow-through type diffusion cell shown in Fig. 1 (exposed area, 3.14 cm^2). Two to 4 pieces of skin were obtained from each animal. The test solution had been prepared to contain 5–10 μCi of radiolabelled compound per 10 ml of aqueous system and left at 37°C for about 24 h to reach equilibrium. At zero time, 2 ml of aliquots of the test solution were applied to the donor cell preheated at 37°C. The apparatus was thermo-

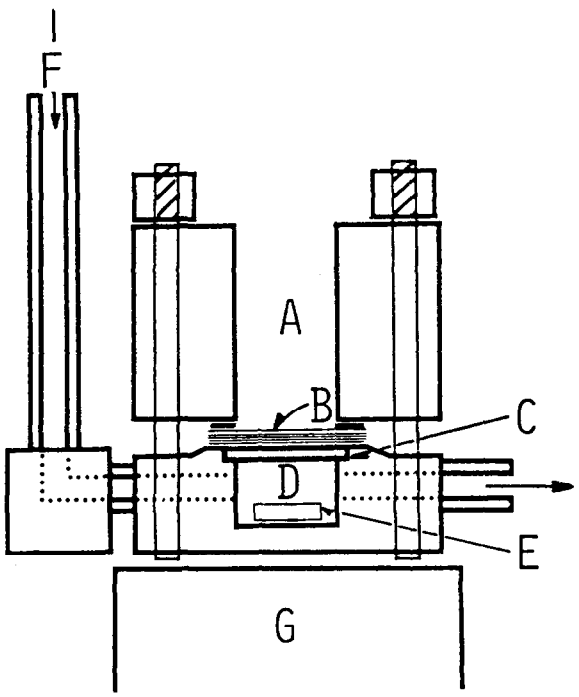

Fig. 1. Schematic view of the flow-through type diffusion cell used for the measurement of percutaneous penetration in vitro. A, donor compartment; B, skin; C, screen support; D, receptor compartment; E, stirring bar; F, flow of the receptor fluid; G, magnetic stirrer.

stated at 37°C in water baths throughout the experiment. To prevent the test solution from evaporation during the experiment, the donor cell was sealed with Parafilm "M" (American Can). The dermal side of the skin was continuously washed with saline which flowed at 5–6 ml/h. The receptor fluid was collected at appropriate intervals, and 1 or 2 ml aliquots of that were withdrawn into a vial for the determination of radioactivity.

In the pretreatment experiment, water or DM-β-CD solution had applied on the epidermal side of the skin for 24 h. After the removal of the solution and washing of the donor cell with water, the test solution without CD was applied as described above.

After 30 h of the experiment, the test solution in the donor cell was removed and analyzed for the determination of the amount of unabsorbed drug. The skin was taken off after washing its surface with water or ethanol and punched out into a 1 cm diameter disk. This was dissolved in 1 ml of Soluene-350 (Packard Instrument) and analyzed for determining the drug accumulation in the skin.

All the determination of drug was carried out by scintillation counting (Model LSC-903, Aloka). Bray's solution was used for the determination of the drug in aqueous medium and toluene scintillator was used for the skin sample.

Mathematical model

For analyzing the drug transfer in a simple manner, the skin was assumed to be a homogeneous plane barrier sheet. The total amount of penetrant Q_t which penetrated through a homogeneous membrane in time t from the donor solution at constant concentration (C_0) to the receptor phase at sink condition is given by:

$$Q_t = A\ell KC_0\left[\frac{Dt}{\ell^2} - \frac{1}{6} - \frac{2}{\pi^2}\sum_1^\infty \frac{(-1)^n}{n^2} \times \exp(-Dn^2\pi^2 t/\ell^2)\right] \quad (1)$$

where A = area for application; ℓ = thickness of membrane; K = partition coefficient of penetrant between membrane and donor solution; and D = diffusion constant (Crank, 1975; Foreman and Kelly, 1976). Since there is some difficulty in determining the thickness of the real diffusion barrier correctly, two parameters involving the skin thickness, that is, diffusion parameter D′ and partition parameter K′, were defined by:

$$D' = D/\ell^2 \quad (2)$$

$$K' = K \cdot \ell \quad (3)$$

Using these parameters, the Eqn. 1 may be rewritten as:

$$Q_t = AK'C_0\left[D't - \frac{1}{6} - \frac{2}{\pi^2}\sum_1^\infty \frac{(-1)^n}{n^2} \times \exp(-D'n^2\pi^2 t)\right] \quad (4)$$

To determine the parameters D′ and K′, each set of data was fitted to Eqn. 4 by non-linear least-squares computer program (MULTI; Yamaoka et al., 1981). Permeability constant K_p and lag time LT were calculated by the following:

$$K_p = D' \cdot K' \quad (5)$$

$$LT = 1/6D' \quad (6)$$

Results

The phase solubility diagrams of BP/β-CD system and BP/DM-β-CD system (Fig. 2a) had the feature of type B_s according to Higuchi and Connors (1965). The stoichiometry of a complex can be determined from the plateau region of such a diagram. Analysis of this region indicated that a 1 : 1 complex was formed in each system. The stability constants were calculated from the initial linear region on the basis of a 1 : 1 complex formation (Table 1). For the IM/CD or SA/CD systems, the diagrams had no plateau region (Fig. 2b, c) and the stoichiometries were not able to be determined. The tentative stability constants of these complexes, calculated based on the assumption of a 1 : 1 interaction, are also shown in Table 1. Table 1 shows that the stability constant increases depending on the partition coefficient of the guest molecule between chloroform and water. SA, rather a hydrophilic molecule, was scarcely included by either CD. BP and IM, the hydrophobic molecules, formed complexes more effectively with DM-β-CD than with β-CD.

The systems employed as the test solutions of the diffusion experiments are listed in Table 2. Only the system "BP-β-H" had become a suspension and all the others were clear solutions. For each penetrant, a series of experiments was carried out with five systems; that is, systems with no CD (control), with β-CD (two concentrations), and with DM-β-CD (two concentrations). The free drug concentration was calculated from the concentrations of CD and drug and the stability constant (Table 2).

In Figs. 3–6, the total amount of penetrant that appeared in the receptor fluid is plotted as a function of time. Except for the curves representing SA penetration from solutions with CDs, the penetration profile showed the linear region following the curved reagion, which means that steady-state had been reached.

The recovery percentages of drug from donor solution, receptor solution, and skin are shown in Table 3. The fact that the total recovery percentage is about 100 gives assurance for the experiments. Diffusion parameter D′, partition parameter K′, permeability constant K_p, and lag

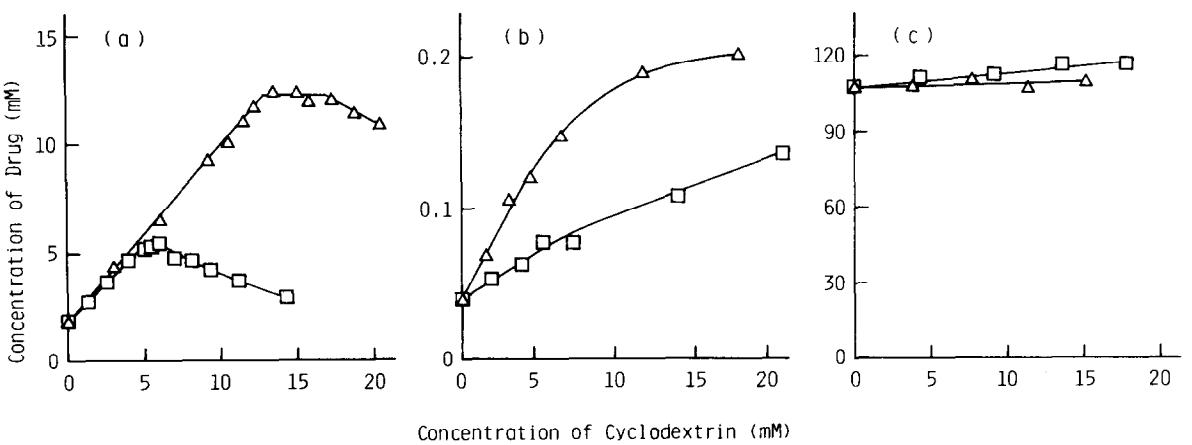

Fig. 2. Phase solubility diagrams of BP/β-CD system (a, □), BP/DM-β-CD system (a, △), IM/β-CD system (b, □), IM/DM-β-CD system (b, △), SA/β-CD system (c, □), and SA/DM-β-CD system (c, △), in water at 37°C.

TABLE 1
PHYSICOCHEMICAL PROPERTIES OF BUTYLPARABEN (BP), SULFANILIC ACID (SA) AND INDOMETHACIN (IM) AT 37°C

Drug	Solubility in water (mM)	Partition coefficient [a]	Stability constant of complex (M^{-1})	
			β-CD	DM-β-CD
BP	1.8	293	1669 [b]	2257 [b]
IM	0.04	82.6	168 [b]	406 [b]
SA	107	3.52×10^{-4}	6.7 [b]	1.2 [b]

[a] Partition coefficient between chloroform and water.
[b] Calculated based on the assumption of 1 : 1 stoichiometry.

TABLE 2
EXPERIMENTAL CONDITIONS: SYSTEM COMPOSITIONS OF TEST SOLUTIONS AND WEIGHTS OF USED GUINEA PIGS

System [a]	Test solution				Weight of guinea pig [c] (g)
	Concentration (mM)			Free drug fraction (%)	
	$[CD]_{total}$	$[Drug]_{total}$	$[Drug]_{free}$ [b]		
(1) BP-W	–	1.00	–	100.00	408.8 ± 13.4 (4)
(2) BP-β-L	6.00	5.15	1.18	22.83	456.3 ± 23.8 (4)
(3) BP-β-H [d]	14.22	5.15	0.220	4.27	456.3 ± 23.8 (4)
(4) BP-D-L	5.89	5.15	1.04	20.14	398.3 ± 14.3 (3)
(5) BP-D-H	13.92	5.15	0.241	4.68	401.7 ± 6.2 (3)
(6) IM-W	–	2.27×10^{-4}	–	100.00	268.8 ± 8.9 (4)
(7) IM-β-L	5.87	2.27×10^{-4}	1.14×10^{-4}	50.35	278.6 ± 8.7 (7)
(8) IM-β-H	17.61	2.27×10^{-4}	5.74×10^{-5}	25.27	280.0 ± 17.7 (4)
(9) IM-D-L	5.87	2.27×10^{-4}	6.71×10^{-5}	29.56	280.0 ± 4.1 (3)
(10) IM-D-H	17.61	2.27×10^{-4}	2.78×10^{-5}	12.26	275.8 ± 13.7 (6)
(11) SA-W	–	5.77	–	100.00	410.0 ± 25.7 (6)
(12) SA-β-L	5.77	5.77	5.56	96.41	390.0 ± 14.1 (3)
(13) SA-β-H	17.31	5.77	5.19	89.92	399.3 ± 14.5 (7)
(14) SA-D-L	5.77	5.77	5.73	99.32	410.0 ± 11.4 (5)
(15) SA-D-H	17.31	5.77	5.65	97.98	413.0 ± 17.2 (5)
(16) SA-W-P	–	5.77	5.77	100.00	328.8 ± 16.3 (4)
(17) SA-D-P	17.31	5.77	5.77	100.00	325.0 ± 18.4 (4)

[a] The first term stands for the penetrant. The second term stands for control (W), β-CD (β), or DM-β-CD (D). The third term stands for the concentration of CD, i.e. lower concentration (L) or higher concentration (H). P means the pretreatment experiment.
[b] Calculated value according to the following relationship on the basis of a 1 : 1 complex formation ($K_{1:1}$ = stability constant of a 1 : 1 complex).

$$K_{1:1} = \frac{[\text{Complex}]}{[CD]_{free} \cdot [Drug]_{free}} = \frac{[Drug]_{total} - [Drug]_{free}}{([CD]_{total} - [Drug]_{total} + [Drug]_{free}) \cdot [Drug]_{free}}$$

[c] Means ± standard deviations. Numbers are given in parentheses.
[d] This system had become a suspension. The concentration of *dissolved* BP determined by filtration of the suspension was 2.65 mM. On the calculation of $[Drug]_{free}$ of this system, this value was used as $[Drug]_{total}$ and 9.07 mM (14.22 – (5.15 – 2.65)) was used as $[CD]_{total}$.

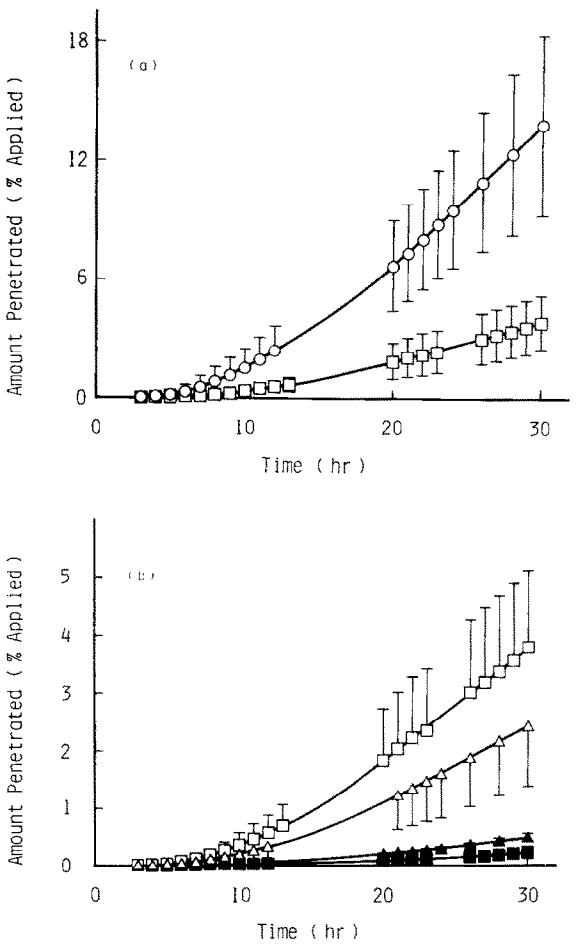

Fig. 3. Effect of cyclodextrins on the percutaneous penetration of butylparaben in water at 37°C. ○, system "BP-W" (4); □, system "BP-β-L" (4); ■, system "BP-β-H" (4); △, system "BP-D-L" (3); ▲, system "BP-D-H" (3). Each point represents the mean with standard deviation. Numbers of experiments are given in parentheses.

time LT calculated according to Eqns. 4–6 are summarized in Table 4.

Because of the restricted solubility, the BP concentration of system "BP-W" was set at 1 mM, approximately the same as the free BP concentration of systems "BP-β-L" and "BP-D-L" (Table 2). BP itself penetrated through the skin rather well (14% at 30 h). Both CDs significantly decreased the penetration of BP depending on their concentrations (Table 3 and Fig. 7a). The linear relationship between the total amount of penetrated BP and the calculated permeability

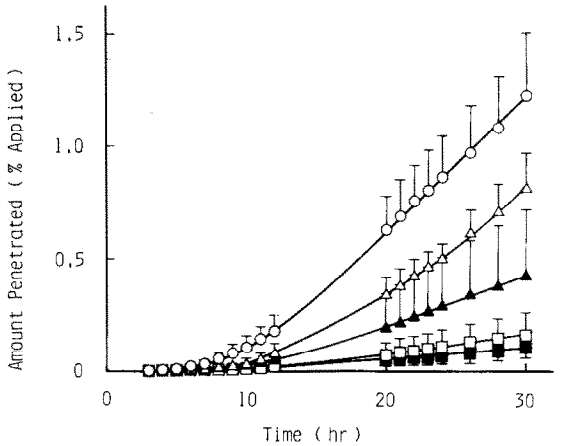

Fig. 4. Effect of cyclodextrins on the percutaneous penetration of indomethacin in water at 37°C. ○, system "IM-W" (4); □, system "IM-β-L" (7); ■, system "IM-β-H" (4); △, system "IM-D-L" (3); ▲, system "IM-D-H" (6). Each point represents the mean with standard deviation. Numbers of experiments are given in parentheses.

constant K_p is obvious as shown in Fig. 7b. This means that the amount of penetrated drug depends on the steady-state flux. Fig. 7c, d reveal that the K_p value, the product of D′ and K′ as shown in Eqn. 5, varied in proportion to the K′ value, the calculated partition coefficient of BP between the skin and the test solution, but not the

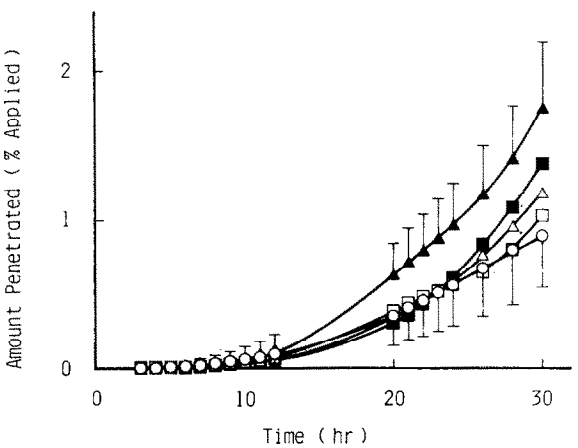

Fig. 5. Effect of cyclodextrins on the percutaneous penetration of sulfanilic acid in water at 37°C. ○, system "SA-W" (6); □, system "SA-β-L" (3); ■, system "SA-β-H" (7); △, system "SA-D-L" (5); ▲, system "SA-D-H" (5). Each point of systems "SA-W" and "SA-D-H" represents the mean with standard deviation, and that of the other systems represents only the mean value. Numbers of experiments are given in parentheses.

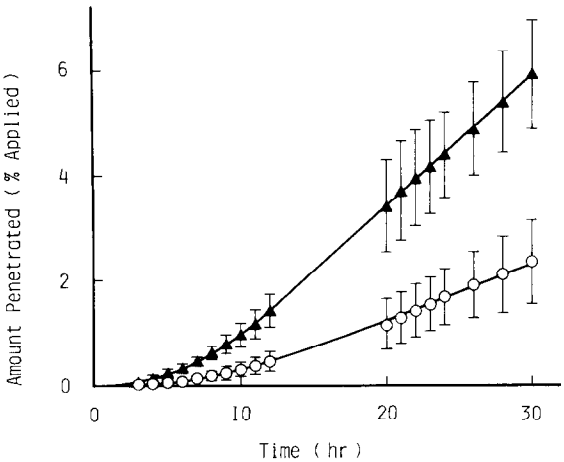

Fig. 6. Effect of pretreatment of skin with di-O-methyl-β-cyclodextrin solution (17.31 mM) on the percutaneous penetration of sulfanilic acid in water at 37°C. ○, system "SA-W-P" pretreated with water; ▲, system "SA-D-P" pretreated with DM-β-CD solution. Each point represents the mean of 4 experiments with standard deviation.

D′ value. Fig. 7e, f show that the decrease in the K_p and K′ values were in proportion to that in the free BP fraction by complex formation with each

TABLE 4
PARAMETERS FOR PERCUTANEOUS PENETRATION OF BUTYLPARABEN (BP), INDOMETHACIN (IM) AND SULFANILIC ACID (SA) WITH AND WITHOUT CYCLODEXTRINS [a]

System	D′ $\times 10^2$ (h^{-1})	K′ $\times 10$ (cm)	K_p $\times 10^3$ (cm/h)	LT (h)
(1) BP-W	1.525	2.960	4.514	10.93
(2) BP-β-L	1.374	0.966	1.327	12.13
(3) BP-β-H	0.926	0.112	0.104	18.00
(4) BP-D-L	1.203	0.769	0.925	13.85
(5) BP-D-H	0.983	0.218	0.214	16.96
(6) IM-W	1.451	0.290	0.421	11.49
(7) IM-β-L	1.290	0.045	0.058	12.92
(8) IM-β-H	3.489	0.007	0.023	4.78
(9) IM-D-L	0.924	0.421	0.389	18.04
(10) IM-D-H	1.183	0.141	0.167	14.09
(11) SA-W	0.967	0.419	0.405	17.24
(12) SA-β-L	0.767	0.741	0.568	21.73
(13) SA-β-H	0.388	6.492	2.519	42.96
(14) SA-D-L	0.538	2.070	1.114	30.98
(15) SA-D-H	0.736	1.402	1.032	22.64
(16) SA-W-P	1.729	0.417	0.721	9.64
(17) SA-D-P	2.459	0.660	1.623	6.78

[a] D′, diffusion parameter; K′, partition parameter; K_p, permeability constant; LT, lag time.

TABLE 3
DRUG RECOVERY PERCENTAGES FROM DONOR, SKIN AND RECEPTOR AFTER 30 HOURS EXPERIMENT [a]

System	n	Drug recovery (%)			
		Total	Donor	Skin	Receptor
(1) BP-W	4	85.67 ± 9.07	29.28 ± 4.64	42.61 ± 12.54	13.77 ± 5.43
(2) BP-β-L	4	99.89 ± 4.38	77.51 ± 4.82 **	18.59 ± 1.59 **	3.79 ± 1.38 **
(3) BP-β-H	4	104.22 ± 16.62	94.08 ± 17.16 **	9.91 ± 2.10 **	0.23 ± 0.10 **
(4) BP-D-L	3	102.60 ± 1.17	90.88 ± 2.06 **	9.28 ± 1.28 **	2.44 ± 1.04 **
(5) BP-D-H	3	103.89 ± 0.67	100.73 ± 1.01 **	2.66 ± 0.36 **	0.49 ± 0.07 **
(6) IM-W	4	97.39 ± 4.78	66.16 ± 5.44	30.00 ± 8.71	1.23 ± 0.28
(7) IM-β-L	7	105.37 ± 1.73	95.67 ± 1.95 **	9.54 ± 3.41 **	0.16 ± 0.10 **
(8) IM-β-H	4	105.25 ± 2.84	101.89 ± 2.83 **	3.26 ± 0.68 **	0.10 ± 0.04 **
(9) IM-D-L	3	100.01 ± 2.23	88.96 ± 1.22 **	10.24 ± 2.24 **	0.82 ± 0.15 *
(10) IM-D-H	6	104.79 ± 1.48	97.83 ± 2.74 **	6.54 ± 2.01 **	0.43 ± 0.29 **
(11) SA-W	6	102.68 ± 2.43	89.59 ± 3.92	12.20 ± 1.67	0.89 ± 0.35
(12) SA-β-L	3	104.16 ± 1.51	92.33 ± 1.45	10.80 ± 3.06	1.04 ± 0.28
(13) SA-β-H	7	103.63 ± 1.46	91.50 ± 0.75	10.74 ± 0.91	1.39 ± 0.58
(14) SA-D-L	5	103.97 ± 1.79	86.84 ± 1.49	15.95 ± 1.06 **	1.18 ± 0.59
(15) SA-D-H	5	103.54 ± 7.50	87.67 ± 6.23	14.12 ± 1.68	1.76 ± 0.44 **
(16) SA-W-P	4	99.47 ± 3.03	85.57 ± 2.77	11.56 ± 1.04	2.34 ± 0.80
(17) SA-D-P	4	97.36 ± 2.66	81.70 ± 2.68 *	9.74 ± 3.01	5.91 ± 1.04 **

[a] Means ± standard deviations.
* $P < 0.05$, ** $P < 0.001$ (Student's t-test), when compared to each control value.

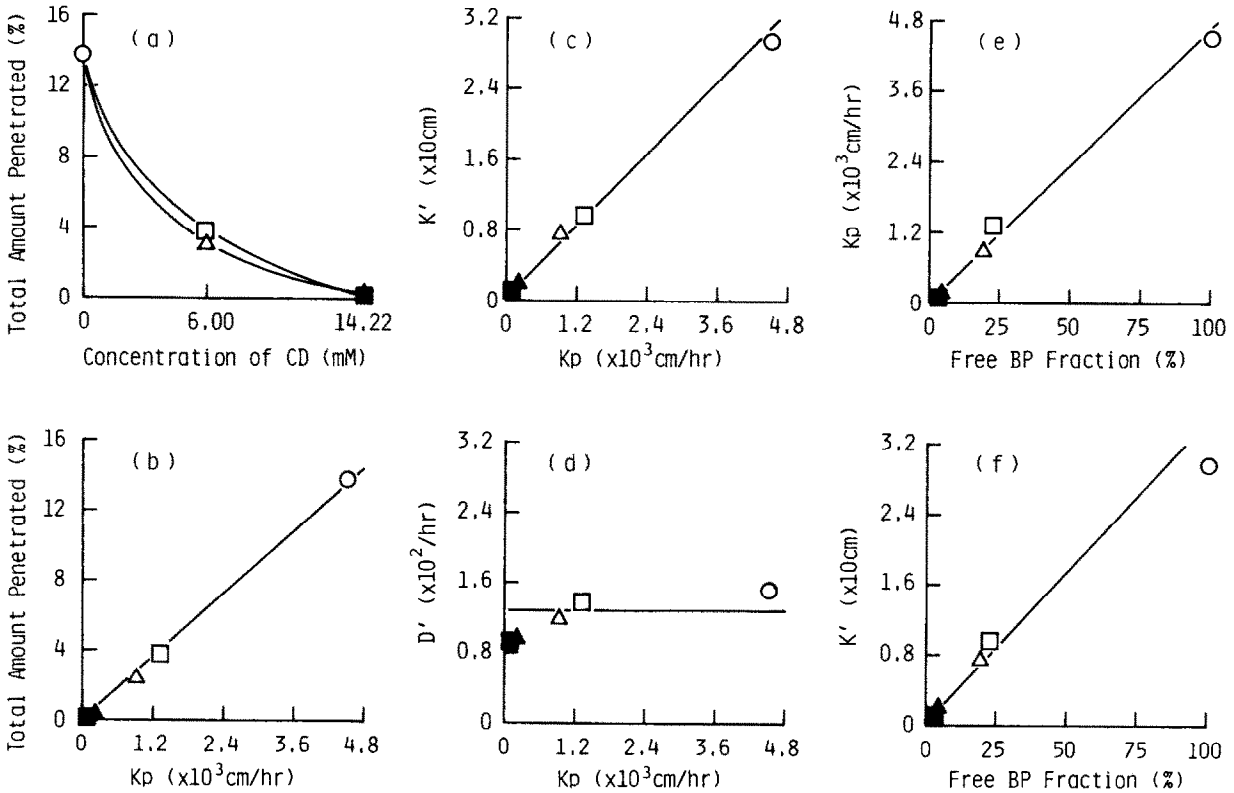

Fig. 7. Relationships between the parameters of the percutaneous penetration of butylparaben. ○, system "BP-W"; □, system "BP-β-L"; ■, system "BP-β-H"; △, system "BP-D-L"; ▲, system "BP-D-H".

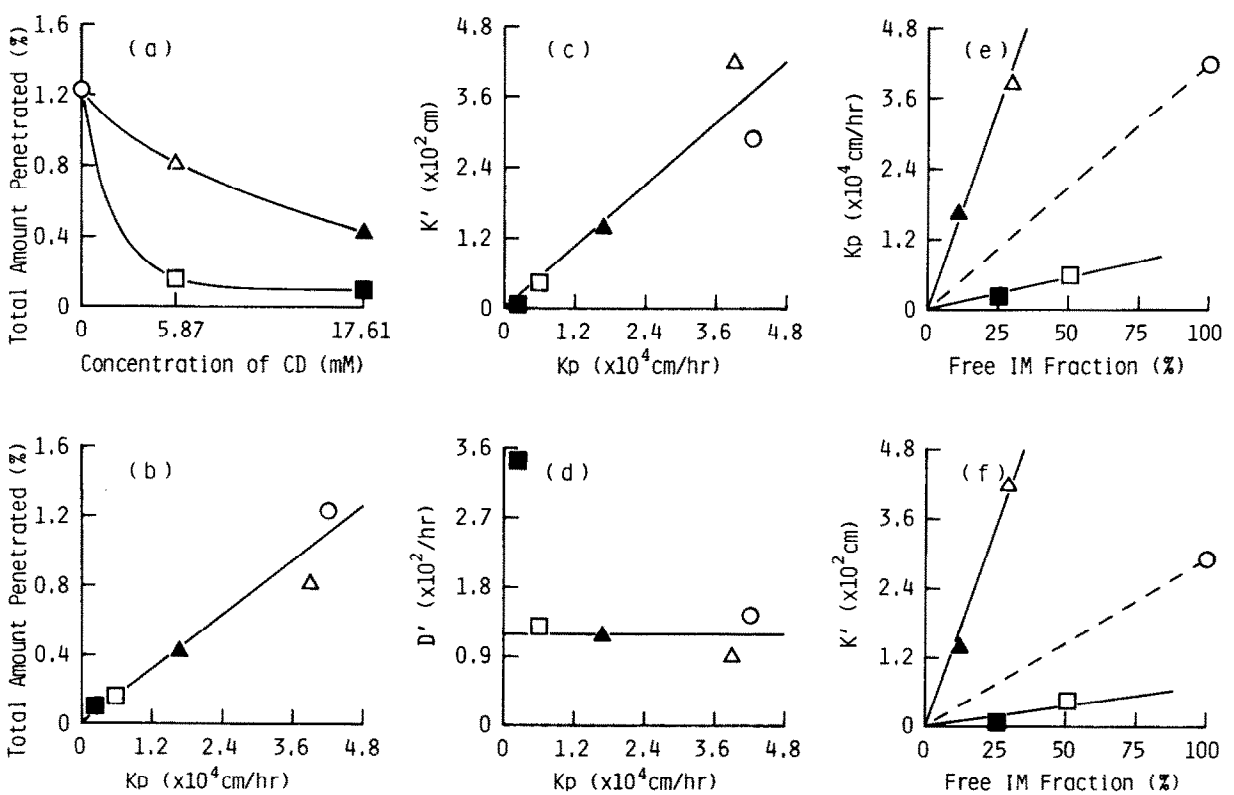

Fig. 8. Relationships between the parameters of the percutaneous penetration of indomethacin. ○, system "IM-W"; □, system

CD. The amount of BP accumulated in the skin was significantly decreased by the addition of CDs and in proportion to the K′ value (Table 3).

Fig. 8a shows the decreasing effect of both CDs on the IM penetration. β-CD decreased the IM penetration more effectively than DM-β-CD ($P < 0.001$ at low concentration; $P < 0.01$ at high concentration). The relationship between the total amount of penetrated IM and the K_p value was linear, and the change of the K_p value occurred depending on the K′ value rather than the D′ value (Figs. 8b–d). In Fig. 8e, f, fairly complicated effects of the complex formation on the IM penetration was demonstrated. Both CDs decreased the amount of IM in the skin significantly, and the complicated effects of complex formation were also seen in the relationship between that and the K′ value (Tables 3 and 4). System "IM-β-H" gave somewhat different parameters in comparison with the other IM systems (Table 4). To elucidate the cause of this, further study will be needed.

Though SA had scarcely formed a complex with either CD, its penetration showed the linear relationship with CD concentrations (Fig. 9a), and the significant enhancement was observed when 17.31 mM of DM-β-CD was added as shown in Table 3. The total amount of penetrated SA and the K_p value did not show the linear relationship (Fig. 9b). K_p varied depending both on K′ and D′, and the systems which have high K′ value have low D′ value (Fig. 9c, d).

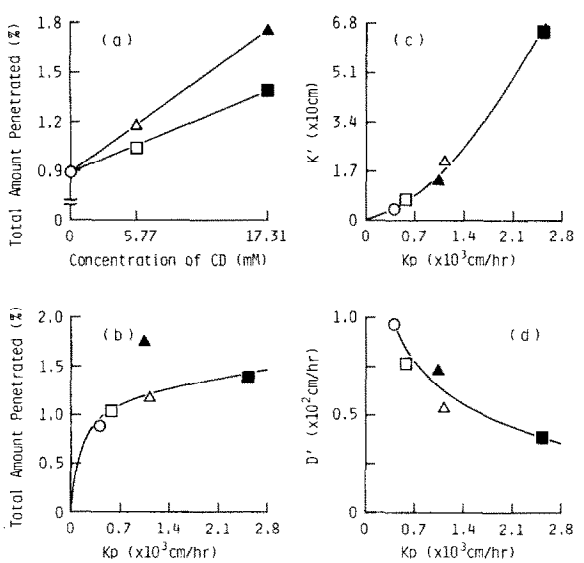

Fig. 9. Relationships between the parameters of the percutaneous penetration of sulfanilic acid. ○, system "SA-W"; □, system "SA-β-L"; ■, system "SA-β-H"; △, system "SA-D-L"; ▲, system "SA-D-H".

To obtain information on the mechanisms of the enhancing effect of DM-β-CD, the penetration of SA after pretreatment of the skin with water or DM-β-CD solution (17.31 mM) was investigated (Fig. 6). The SA penetration was significantly increased by DM-β-CD pretreatment (Table 3). Contrary to the result of the CD-coexisting experiments, the diffusion parameter D′ was increased by the pretreatment of DM-β-CD and the increase in both D′ and K′ resulted in the increase in SA penetration (Table 4).

Discussion

There have recently been some approaches to elucidate the mechanisms of the action of percutaneous penetration enhancers such as 2-pyrrolidone, dimethylformamide and 1-dodecylazacycloheptan-2-one in terms of diffusion constant and partition coefficient (Southwell and Barry, 1983; Chow et al., 1984). In these studies, however, parameters were calculated from the linear region of the total amount of penetrated drug–time curve corresponding to steady-state. In the present study, all recorded data were made to be usable for fitting to the diffusion equation and estimation of useful parameters, D′ and K′. This method may be simpler and more objective in that D′ and K′ are determined simultaneously without any subjective judgement on the linearity of set of data.

In the present results, the penetration of BP was decreased by CDs, and this effect was well correlated with the decrease in the apparent partition coefficient of BP (Fig. 7a–d). The non-ionic surfactant, polysorbate 80, had also been reported to reduce the apparent partition coefficient and percutaneous penetration of BP (Komatsu and Suzuki, 1979; Komatsu, 1984). However, the reducing effect was not correlated directly with the decrease in BP concentration in the outer phase;

that is, the BP penetration through the skin was greater than that expected assuming that free BP would be absorbed in the same manner as when BP solution without polysorbate 80 was applied. In the case of CDs, the BP penetration occurred in proportion to the free BP fraction (Fig. 7e, f). This finding suggests the following two points: (1) free BP is capable of penetrating the skin but its CD complex does not effectively partition into or penetrate through the skin; and (2) CDs have little influence either on the skin barrier function against BP penetration or on the affinity of BP for the skin unlike polysorbate 80.

It has been reported that ^{14}C-labelled β-CD was not absorbed in its intact form either from the stomach or the small intestine of rat (Szejtli et al., 1980). This can lend support for the consideration that CDs or their complexes are impermeable through skin, the stronger barrier.

IM is also a hydrophobic molecule and the complex formation is fundamentally assumed to result in the decrease in the IM penetration. However, the different tendency of the action between β-CD and DM-β-CD shown in Fig. 8e, f requires further examination. One problem is about the accuracy of the stability constant determination. Kurozumi et al. (1975) reported the 1 : 1 stoichiometry of powdered IM/β-CD complex prepared by freeze-drying method whereas Szejtli and Szente (1981) reported the 1 : 2 complex formation. But the stoichiometries of IM/β-CD and IM/DM-β-CD complexes in aqueous solution have not been reported. In this study, no information on this problem was obtained from the phase solubility diagrams of these systems. Apart from the problem of the accuracy of the determination of the stoichiometry and the stability constant, it would seem that some fundamental difference exists between the effect of β-CD and DM-β-CD on the percutaneous absorption of IM. The steeper slope of the phase solubility diagram of IM/DM-β-CD system than that of IM/β-CD system (Fig. 2b) implies that DM-β-CD includes IM more effectively than β-CD. In other words, less free IM fraction may exist when DM-β-CD is added than when β-CD is added at the same concentration. This consideration leads to the idea that if both CDs affect the skin to the same extent and reduce the IM penetration depending on the degree of the complex formation, less IM penetration would be observed in the systems with DM-β-CD than with β-CD. But the opposite tendency was observed (Fig. 8a). Though this study did not elucidate the mechanism, β-CD and DM-β-CD may have somewhat different effects on the percutaneous penetration of IM.

It is clear from the pretreatment experiments (Fig. 6) that the significant enhancement of SA penetration caused by coexisting DM-β-CD is due to the direct effect of DM-β-CD on the skin barrier function. Since SA is scarcely included in CDs, the contribution of SA/CD complexes to the percutaneous penetration seemed to be negligible. The rather prolonged lag time by the addition of CD, comparing with that of system "SA-W" (Table 4), in spite of the enhancement of the penetration suggests that CDs mildly affect and gradually decrease the skin barrier function. This results in the prolongation of the time necessary to attain the steady-state condition. The increase in the diffusion constant and the decrease in lag time of SA penetration caused by DM-β-CD are obvious from the pretreatment experiments (Table 4). The prolongation of lag time was also observed for the BP and IM penetration from the systems with CDs (Table 4). This suggests that CDs always affect the skin barrier function to some extent resulting in the significant acceleration of the penetration of SA (System SA-D-H) but not of BP, which shows good absorbability by itself.

Some mechanisms of the action of percutaneous penetration enhancers have been reported, one of which is the hydration of skin and another is the disturbance of the lipids constituting the skin barrier (Hadgraft, 1984). It has been reported that CDs cause the release of some membrane components such as cholesterol, phospholipids and proteins from erythrocytes (Irie et al., 1982). The intrinsic irritancy of the rather high concentration of CDs on skin was also reported and similar mechanisms as in the case of erythrocytes were suggested (Uekama et al., 1982). These observations imply the action of CDs on the skin barrier to extract its components by complex formation. Our preliminary study has shown the extraction of

lipid components such as cholesterol and triglycerides from powdered skin (Hide powder; Wako Pure Chemical Industries) by DM-β-CD (unpublished data). One of the mechanisms of the significant enhancement of the SA penetration is thought to be the lipid extraction by DM-β-CD.

Through the present investigation, the following effects of CD and drug/CD complex on the percutaneous absorption have been elucidated. (1) Drug/CD complex does slightly penetrate the skin. (2) The drug penetration is decreased as a consequence of the decrease of the free drug fraction by the complex formation with CD. (3) CD enhances the percutaneous penetration of hydrophilic molecule, such as SA, by varying the skin barrier function. (4) The effect of CD on the percutaneous drug absorption can be regulated to some extent by means of its chemical modification such as methylation.

References

Chow, D.S.-L., Kaka, I. and Wang, T.I., Concentration-dependent enhancement of 1-dodecylazacycloheptan-2-one on the percutaneous penetration kinetics of triamcinolone acetonide. J. Pharm. Sci., 73 (1984) 1794–1799.

Crank, J., The Mathematics of Diffusion, Oxford University Press, London, 1975.

Foreman, M.I. and Kelly, I., The diffusion of nandrolone through hydrated human cadaver skin. Br. J. Dermatol., 95 (1976) 265–270.

Hadgraft, J., Penetration enhancers in percutaneous absorption. Pharm. Int., 5 (1984) 252–254.

Higuchi, T. and Connors, K.A., Phase-solubility techniques. Adv. Anal. Chem. Instr., 4 (1965) 117–212.

Irie, T., Otagiri, M., Sunada, M., Uekama, K., Ohtani, Y., Yamada, Y. and Sugiyama, Y., Cyclodextrin-induced hemolysis and shape changes of human erythrocytes in vitro. J. Pharm. Dyn., 5 (1982) 741–744.

Iwaoku, R., Arimori, K., Nakano, M. and Uekama, K., Enhanced absorption of phenobarbital from suppositories containing phenobarbital–β-cyclodextrin inclusion complex. Chem. Pharm. Bull., 30 (1982) 1416–1421.

Kurozumi, M., Nambu, N. and Nagai, T., Inclusion compounds of non-steroidal antiinflammatory and other slightly water soluble drugs with α- and β-cyclodextrins in powdered form. Chem. Pharm. Bull., 23 (1975) 3062–3068.

Komatsu, H. and Suzuki, M., Percutaneous absorption of butylparaben through guinea pig skin in vitro. J. Pharm. Sci., 68 (1979) 596–598.

Komatsu, H., Percutaneous absorption of butylparaben in vitro. II. Effects of micellar trapping of the drug and percutaneous absorption of nonionic surfactants. Chem. Pharm. Bull., 32 (1984) 3739–3743.

Nambu, N., Shimoda, M., Takahashi, Y., Ueda, H. and Nagai, T., Bioavailability of powdered inclusion compounds of nonsteroidal antiinflammatory drugs with β-cyclodextrin in rabbits and dogs. Chem. Pharm. Bull., 26 (1978) 2952–2956.

Scheuplein, R.J. and Blank, I.H., Permeability of the skin. Physiol. Rev., 51 (1971) 702–747.

Shaw, J.E., Transdermal therapeutic systems. In Brandau, R. and Lippold, B.H. (Eds.), Dermal and Transdermal Absorption, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, 1982, pp. 171–187.

Southwell, D. and Barry, B.W., Penetration enhancers for human skin: mode of action of 2-pyrrolidone and dimethylformamide on partition and diffusion of model compounds water, *n*-alconols, and caffeine. J. Invest. Dermatol., 80 (1983) 507–514.

Szejtli, J., Gerlóczy, A. and Fónagy, A., Intestinal absorption of ^{14}C-labelled β-cyclodextrin in rats. Arzneim.-Forsch., 30 (1980) 808–810.

Szejtli, J. and Szente, L., Interaction between indomethacin and β-cyclodextrin. Pharmazie, 36 (1981) 694–698.

Uekama, K., Matsuo, N., Hirayama, F., Ichibagase, H., Arimori, K., Tsubaki, K. and Satake, K., Enhanced bioavailability of acetohexamide by β-cyclodextrin complexation. Yakugaku Zasshi, 100 (1980) 903–909.

Uekama, K., Irie, T., Sunada, M., Otagiri, M., Arimatsu, Y. and Nomura, S., Alleviation of prochlorperazine-induced primary irritation of skin by cyclodextrin complexation. Chem. Pharm. Bull., 30 (1982) 3860–3862.

Uekama, K., Pharmaceutical applications of methylated cyclodextrins. Pharm. Int., 6 (1985) 61–65.

Yamaoka, K., Tanigawara, Y., Nakagawa, T. and Uno, T., A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharm. Dyn., 4 (1981) 879–885.