2-Hydroxypropylated Cyclodextrins as a Sustained-Release Carrier for Fragrance Materials

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The potential use of three kinds of 2-hydroxypropylated cyclodextrins [HP-CyDs: 2-hydroxypropyl-α-cyclodextrin (HP-α-CyD), 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) and 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CyD)] in the solubilizing of fragrance materials and the retarding of their release rates was examined. Of the HP-CyDs, HP-β-CyD significantly increased the solubility of six fragrance materials in water, and the apparent 1:1 stability constant of the HP-β-CyD complexes was higher than those of the HP-α-CyD and HP-γ-CyD complexes. Ultraviolet, circular dichroism and nuclear magnetic resonance spectroscopic studies suggested that the inclusion mode of eugenol is different between the HP-β-CyD complex and the HP-α- or HP-γ-CyD complexes. The release rates of *d*-camphor and 3-*l*-methoxypropane 1,2-diol from water were remarkably decreased by the addition of HP-CyDs, where the rate of decrease was in agreement with that of the stability constant ranking of the complexes, *i.e.*, fragrance materials alone < HP-α-CyD < HP-γ-CyD < HP-β-CyD. In order to retard the transport of fragrance materials to skin, the suppressive effect of HP-CyDs was examined *in vitro*. The mean transit time for eugenol through the skin barrier increased in the order of eugenol alone < HP-γ-CyD < HP-α-CyD < HP-β-CyD, and the suppressive effects of HP-α-CyD and HP-β-CyD were remarkably greater than that of the nonionic surfactant, HCO-60. The present data suggest that HP-β-CyD has a significant advantage over the other HP-CyDs and HCO-60 with respect to providing high aqueous solubility and the sustained release of fragrance materials while maintaining a lack of toxicity in topical liquid preparations of fragrance materials.

Key words 2-hydroxypropylated cyclodextrin; fragrance material; solubilization; sustained release; cutaneous permeation

Cyclodextrins (CyDs) are known to form inclusion complexes with various guest molecules.[1] The usefulness of chemically modified CyDs for improving the pharmaceutical properties of drugs, cosmetics and foods is now well demonstrated.[2,3] Of the modified CyDs, 2-hydroxypropylated CyDs (HP-CyDs) are expected to be useful as parenteral drug carriers, because of their higher aqueous solubility (>50% in water), excellent solubilizing ability for poorly water-soluble chemicals and low toxicity.[4,5] Fragrance materials have desirable effects on our life and health, *e.g.*, aroma therapy and controlled-release studies of fragrances have been widely examined. Until now, surfactants such as polyoxyethylene hydrogenated castor oil 60EO (HCO-60) have been used to solubilize fragrance materials in liquid preparations. However, skin has a high sensitivity to fragrance materials and surfactants.[6,7] Therefore, a lessened transport of fragrance materials and surfactants to skin is preferable from a viewpoint of safety. In our previous paper, the interaction of various fragrance materials with 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) was examined.[8] The present study deals with the complex formation of six typical fragrance materials with three kinds of HP-CyDs [2-hydroxypropyl-α-cyclodextrin (HP-α-CyD), HP-β-CyD and 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CyD)] using ultraviolet (UV), circular dichroism (CD), and proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopic methods. In addition, the effects of HP-CyDs on the solubility of fragrance materials, on the release of *d*-camphor and 3-*l*-methoxypropane 1,2-diol from water, and on the *in vitro* cutaneous permeation of eugenol were investigated, and compared with HCO-60.

Experimental

Materials Benzyl acetate, eugenol, linalol, *l*-menthol, 3-*l*-methoxypropane 1,2-diol and *d*-camphor were purchased from V. Mane Fils (Paris, France). HP-α-CyD, HP-β-CyD and HP-γ-CyD were donated by Nihon Shokuhin Kako, Ltd. (Shizuoka, Japan); the average substitution degrees of 2-hydroxypropyl groups per α-CyD, β-CyD and γ-CyD molecules were determined to be 4.3, 4.8 and 4.8, respectively, by means of ^{1}H-NMR.[9] All other chemicals and solvents were of analytical reagent grade.

Spectroscopies UV and CD spectra were recorded with a Hitachi U-3200 spectrophotometer (Tokyo, Japan) and a Jasco J-700 recording polarimeter (Tokyo, Japan), respectively. ^{1}H-NMR spectra were recorded at 25 °C on a Jeol JNM-A500 spectrometer (Tokyo, Japan) operating at 500.00 MHz. The concentrations of eugenol and CyDs were 5.0×10^{-3} M in deuterium oxide (D_2O), and ^{1}H chemical shifts were measured, using tetramethyl silane (TMS) as an external reference, with an accuracy of ±0.0012 ppm.

Solubility Measurements A constant but excess amount of the fragrance materials was added to aqueous solutions containing a given concentration of HP-CyDs. These were mixed by a magnetic stirrer at 25 °C, and after equilibrium was attained the mixture was centrifuged (3000 rpm × 5 min) and the supernatant was filtered through a pipette with a cotton plug. After diluting the filtrate, benzyl acetate, linalol and eugenol, and *l*-menthol, 3-*l*-methoxypropane 1,2-diol and *d*-camphor were assayed by high performance liquid chromatography (HPLC) or gas chromatography (GC) under the following conditions. HPLC apparatus: a Jasco 860-CO HPLC machine (Nihon Bunko, Tokyo, Japan); a Capcell-Pak C18 SG column 4.6 × 250 mm (Shiseido, Tokyo, Japan); detection, UV at 256 nm (benzyl acetate), 215 nm (linalol), 235 nm (eugenol); mobile phase, water : methanol (v/v) = 2 : 8 (benzyl acetate), 3 : 7 (linalol, eugenol); amounts of injection, 4.0 μl; flow rate, 1.0 ml/min. GC apparatus: a GC-380 machine (GL Science, Tokyo, Japan) with a flame ionization detector (FID); a Ulbon HR-1 column 0.53 mm × 50 m (Sinwa Kagaku Kogyo, Tokyo, Japan); mobile phase, helium gas; flow rate, 50 ml/min; detector temperature, 280 °C; injection

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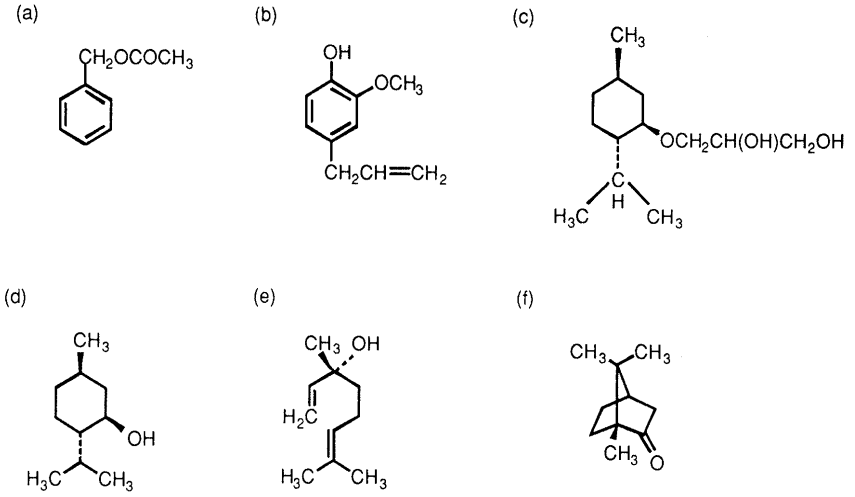


Fig. 1. Chemical Structures of Fragrance Materials

(a) Benzyl acetate, (b) eugenol, (c) 3-*l*-methoxypropane 1,2-diol, (d) *l*-menthol, (e) linalol, (f) *d*-camphor.

temperature, 260 °C; column temperature linearly increased from 110 °C to 230 °C at 20 °C/min. The apparent stability constant (K_c) of the complex was calculated from the initial straight line portion of the solubility diagrams, according to the method of Higuchi and Connors.[10]

Release of Fragrance The release test of *d*-camphor and 3-*l*-methoxypropane 1,2-diol was performed in water at 60 °C. Aqueous solutions (5 ml) containing fragrance materials (0.1% w/v) and HP-CyDs (2.0% w/v) or HCO-60 (0.2%) were placed in 30 ml vials and allowed to stand in a chamber at 60 °C. At appropriate intervals, the vial was weighted, an aliquot (0.1 ml) was withdrawn from the vial, and the concentration of fragrance materials was determined by GC as described above. The weight change caused by the evaporation of water was corrected in all experiments.

***In Vitro* Cutaneous Permeation** The dorsal epidermis of a female hairless mouse (8 weeks old) was attached to a perpendicular diffusion cell[11] and the test was performed at 25 °C. An aqueous solution (3.6 ml) of eugenol was placed in the donor side and physiological saline (3.6 ml) was placed in the receiver side. The initial concentrations of eugenol, HP-CyDs and HCO-60 in the donor cell were 0.1, 2.0 and 0.2% w/v, respectively. At appropriate intervals, an aliquot (0.8 ml) was withdrawn from the receiver phase and analyzed for eugenol by HPLC as described above.

Results and Discussion

Solubilization Figure 1 shows the structures of six typical fragrance materials employed in the present study. The inclusion complexation of the fragrance materials with HP-CyDs was studied using the solubility method.[10] The solubility of the fragrance materials increased linearly as a function of HP-CyD concentration, showing a typical A_L type phase solubility diagram. Since the above results suggested the 1 : 1 complexation of guest/host molecules,[10] the 1 : 1 stability constants (K_c) of the complexes were calculated from the initial linear portion of the solubility diagrams (Table 1). The stability constant increased in the order of HP-γ-CyD ≦ HP-α-CyD < HP-β-CyD. HP-β-CyD had a superior ability to solubilize the fragrance materials.

Confirmation of Inclusion Complexation by UV, CD and NMR Spectroscopies The inclusion complexation of eugenol, a representative of fragrance materials, with HP-CyDs was confirmed by UV, CD and ^{1}H-NMR spectroscopies. Figure 2a shows the effect of HP-CyDs on the UV absorption spectrum of eugenol around 280 nm, where the UV peak was shifted to a longer wavelength by the addition of HP-CyDs, especially by HP-β-CyD. In the CD spectra (Fig. 2b), the optical activity of eugenol was induced at about 230 and 280 nm by the addition of HP-CyDs, in which the magnitude was in the order of HP-α-CyD ≦ HP-γ-CyD < HP-β-CyD. Since intrinsic Cotton effects of HP-CyDs are observed only below 220 nm and eugenol has no assymmetric carbon in a molecule,

Table 1. Apparent Stability Constants (K_c) for the Complexation of Fragrance Materials with HP-CyDs at 25 °C

Fragrance material	HP-α-CyD (M^{-1})	HP-β-CyD (M^{-1})	HP-γ-CyD (M^{-1})
Benzyl acetate	110	310	100
Eugenol	500	4600	200
3-*l*-Methoxypropane-1,2-diol	130	320	155
l-Menthol	290	4000	160
Linalol	80	1610	65
d-Camphor	40	1530	610

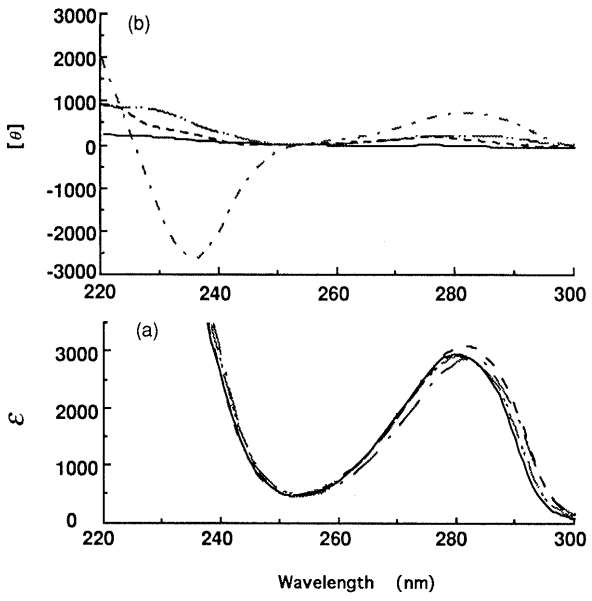
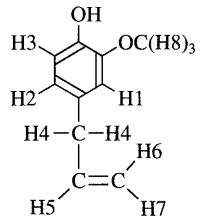


Fig. 2. UV (a) and CD (b) Spectra of Eugenol (3.0×10^{-4} M) in the Absence and Presence of HP-CyDs (1.5×10^{-2} M) in Water at 25 °C

——, without HP-CyDs; – – –, with HP-α-CyD; —·—, with HP-β-CyD; —··—, with HP-γ-CyD.

Table 2. Effects of HP-CyDs and Natural CyDs on ^{1}H-NMR Chemical Shifts of Eugenol[a]



Proton	Without CyDs (δ_o, ppm)[c]	Chemical shift displacement ($\Delta\delta$, ppm)[b] With HP-CyDs (with natural CyDs)					
		α-CyDs		β-CyDs		γ-CyDs	
H1	6.851	0.00	(0.00)[d]	−0.123	(−0.125)	−0.031	(−0.013)
H2	6.691	0.049	(0.027)	−0.035	(−0.030)	−0.015	(0.00)
H3	6.789	0.053	(0.045)	0.033	(0.022)	0.00	(0.00)
H4	3.247	0.117	(0.071)	−0.014	(−0.010)	0.00	(0.00)
H5	5.939	0.044	(0.030)	−0.014	(0.00)	0.00	(0.00)
H6	5.020	0.010	(0.00)	−0.103	(−0.098)	—	(—)[e]
H7	4.980	0.016	(—)	0.011	(0.025)	0.015	(0.014)
H8	3.764	—	(—)	—	(—)	—	(—)

a) Concentrations of eugenol and CyDs were 5.0×10^{-3} M in D_2O. *b*) Chemical shift displacements were expressed as $\Delta\delta = \delta_{\text{with CyDs}} - \delta_o$. *c*) Downfield from external TMS. *d*) Changes less than 0.005 ppm were expressed as 0.00. *e*) Could not be determined accurately because of the overlap with CyD signals.

these CD bands above 220 nm are attributable to the induced optical activity of eugenol through the inclusion complexation with HP-CyDs. The sign of induced CD bands is known to depend upon a spatial relationship between the asymmetric center and the perturbed chromophore. For example, Harata reported that the electronic transition of an included guest molecule with a transition dipole moment, parallel to the *z*-axis of the CyD cavity, gives a positive CD, and that with a transition dipole moment perpendicular to the *z*-axis gives a negative CD.[12,13] It is apparent that the CyD-induced CD band of eugenol was different between the β-CyD system and α- or γ-CyD systems, *i.e.*, there was a large negative sign of HP-β-CyD and small positive signs of HP-α-CyD and HP-γ-CyD around 230 nm. These results suggested that the direction of the transition moment of eugenol in the HP-β-CyD complex differs from those in the HP-α-CyD and γ-CyD complexes. To gain insight into the different inclusion modes of the eugenol–HP-CyD complexes, ^{1}H-NMR spectroscopic studies were carried out. Table 2 shows the displacement of ^{1}H-NMR chemical shifts of eugenol by the addition of HP-CyDs, together with those by the addition of natural CyDs. All signals, except for the H1, of eugenol moved downfield through the inclusion complexation with α-CyDs, probably due to the steric perturbation caused by the small cavity size of α-CyD.[14–16] These results suggest that the phenolic hydroxyl and methoxyl groups and the H1 proton may be located outside of the α-CyD cavity. On the other hand, all signals, except for the H3 and H7 protons, moved upfield through the inclusion complexation with β-CyDs, which may be due to the typical hydrophobic effect on NMR chemical shifts.[17] The environment of the terminal protons, H3 and H7, seems to be different from that of other protons in the β-CyD cavity, and these protons may be located in the hydrophilic sites of β-CyDs. In the case

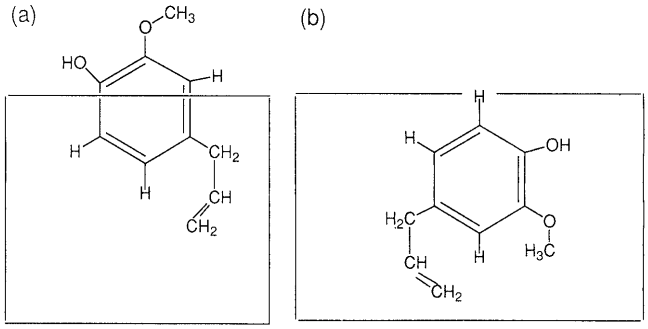


Fig. 3. Proposed Inclusion Modes of Eugenol in HP-α-CyD (a) and HP-β-CyD (b) Complexes

of γ-CyD complexes, the shift changes were very small, probably because the guest molecule is included loosely in the large cavity. Figure 3 shows the inclusion mode of eugenol in the HP-α-CyD and HP-β-CyD complexes estimated on the basis of CD and NMR studies, *i.e.*, the eugenol molecule is shallowly included in the α-CyD cavity where the allyl group and the H2 and H3 protons of the guest molecule are preferably included, whereas the eugenol molecule is deeply included in the β-CyD cavity where the methoxyl and allyl groups are preferably inserted. These inclusion modes are supported by Corey–Pauling–Koltun (CPK) molecular model constructions.

Release of Fragrance Figure 4 shows the time courses of the release of residual amounts of *d*-camphor and 3-*l*-methoxypropane 1,2-diol in water at 60 °C. The release rate of *d*-camphor was faster than that of 3-*l*-methoxypropane because of the lower vapor pressure of the former compared with that of the latter. The release rate of both fragrance materials decreased in the order of fragrance material alone > HP-α-CyD > HP-γ-CyD > HP-β-CyD systems, indicating that HP-CyDs retard the

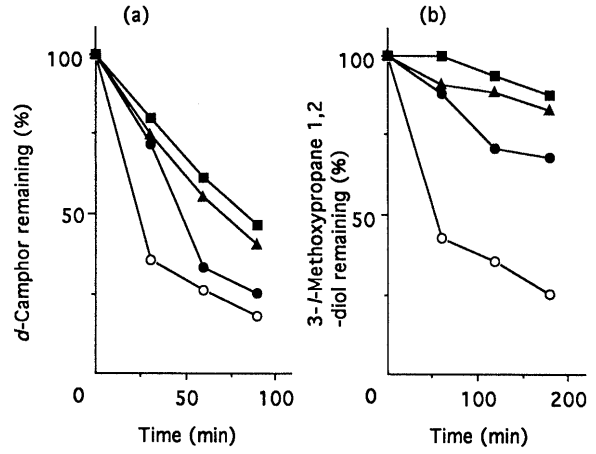


Fig. 4. Release Profiles of *d*-Camphor (a) and 3-*l*-Methoxypropane 1,2-Diol (b) in the Absence and Presence of HP-CyDs at 60 °C

○, without HP-CyDs; ●, with HP-α-CyD; ■, with HP-β-CyD; ▲, with HP-γ-CyD.

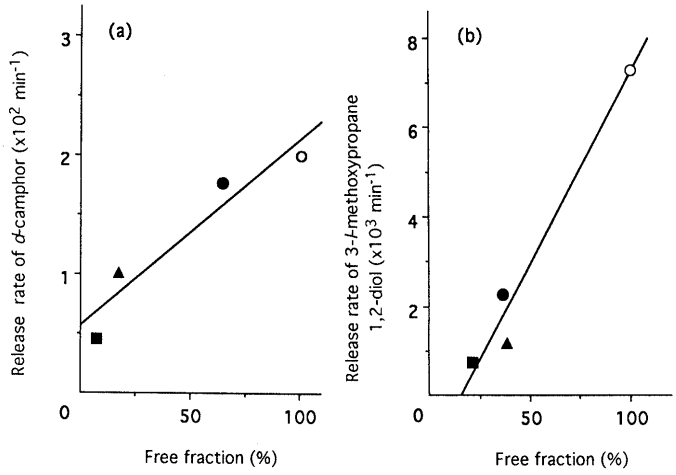


Fig. 5. Relationship between Free Fraction of *d*-Camphor (a) and 3-*l*-Methoxypropane 1,2-Diol (b) in Donor Solution and Release Rate

○, without HP-CyDs; ●, with HP-α-CyD; ■, with HP-β-CyD; ▲, with HP-γ-CyD.

release of the perfume. As shown in Fig. 5, a positive correlation between the suppressive effect of HP-CyDs and the free fraction of fragrances was observed (correlation coefficients in Fig. 5a and 5b: 0.954 and 0.916, respectively), where free fractions of the guest molecules were calculated using their stability constants. These results suggest that only the free fraction of the fragrance materials, which is in equilibrium with the complexed fraction, is released from water. Furthermore, the release of eugenol was also markedly decreased by the addition of HP-CyDs, especially HP-β-CyD, and its suppressive effect was greater than that of a nonionic surfactant, HCO-60 (0.2% w/v). This result was in close agreement with the results obtained by sensory tests of the fragrance materials.

***In Vitro* Permeation Study** The effects of HP-CyDs on *in vitro* permeation of eugenol through hairless mouse skin were examined and compared with those of HCO-60. Figure 6 shows the time courses of amounts of eugenol which permeated into the receiver phase. It is clear that the flux of the cutaneous permeation of eugenol was significantly decreased by the addition of HP-CyDs, the

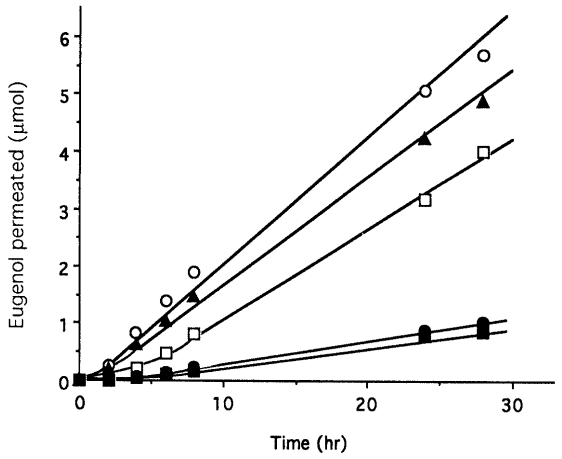


Fig. 6. *In Vitro* Permeation Profiles of Eugenol through Isolated Dorsal Skin of Hairless Mice at 25 °C

○, without additives; □, HCO-60; ●, with HP-α-CyD, ■, with HP-β-CyD; ▲, with HP-γ-CyD.

Table 3. Distribution Profiles[a] of Eugenol 28 h after Application onto Isolated Dorsal Skin of Hairless Mice in the Absence and Presence of HP-CyDs and HCO-60

System	Donor phase (% of dose)	Skin (% of dose)	Receiver cell (% of dose)
Without additives	59.7	12.9	27.4
With HCO-60	73.6	11.2	15.2
With HP-α-CyD	90.4	5.1	4.6
With HP-β-CyD	92.1	3.4	4.5
With HP-γ-CyD	68.9	13.0	18.1

a) The average of two or three determinations, which coincided with each other within S.D. 2.6%.

order of permeated amounts of eugenol being fragrance alone > HP-γ-CyD ≫ HP-α-CyD ≧ HP-β-CyD. The suppressive effect of HCO-60 was much lower than that of HP-α-CyD or HP-β-CyD. Table 3 summarizes the amount of eugenol recovered in the donor phase, skin and receiver phase at the end of the 28 h diffusion experiments. The total amount of eugenol recovered was almost 100% in all experiments. The amount of eugenol in the donor phase increased in the order of eugenol alone < HP-γ-CyD < HCO-60 ≪ HP-α-CyD < HP-β-CyD, reflecting that of eugenol which permeated through skin. Furthermore, the amount of eugenol remaining in the skin was markedly low in the presence of HP-α-CyD and HP-β-CyD.

It has been reported that CyDs release some membrane components such as cholesterol, phospholipids and proteins from erythrocytes, and the hemolytic activity of CyDs is in the order of γ-CyD < α-CyD < β-CyD.[18] Furthermore, the intrinsic irritancy of CyDs on skin has been reported, the irritating mechanism being similar to that of the hemolysis.[19] Therefore, HP-CyDs may affect the intrinsic cutaneous permeation of eugenol, although HP-CyDs have less hemolytic activity than unmodified CyDs.[20] In order to gain insight into the suppressing mechanism of HP-CyDs, therefore, the diffusion and partition parameters and mean transit time (MTT) for the skin permeation of eugenol were calculated with Eqs. 1 and 2,[21] using the nonlinear regression program MULTI(FILT).[22]

Table 4. Diffusion and Partition Parameters and MTT for Skin Permeation of Eugenol[a]

System	D ($\times 10^4$ cm^2/h)	K	MTT (h)
Without additives	7.59±0.69 (100)	14.13±1.59 (100)	77.0
With HCO-60	2.13±0.09 (28)	24.94±1.95 (177)	160.4
With HP-α-CyD	4.06±0.28 (53)	3.42±0.34 (24)	575.2
With HP-β-CyD	2.97±0.36 (39)	4.22±0.81 (30)	639.1
With HP-γ-CyD	6.89±0.28 (91)	11.83±0.61 (84)	100.6

a) Mean±standard deviations. Each value in parentheses represents the percentage of D or K value without additives.

$$\bar{Q}_B = \frac{KV_2X_0}{S[V_1 \cdot L \cdot (s/D)^{1/2} \cdot \sinh(L \cdot (s/D)^{1/2}) + KV_2 \cdot \cosh(L \cdot (s/D)^{1/2})]} \quad (1)$$

$$\mathrm{MTT} = \frac{L^2}{2D} + \frac{V_1 \cdot L}{DKA} \quad (2)$$

where A is the effective diffusion area, X_0 is eugenol concentration applied on the donor phase, D is the diffusion constant in skin, K is the partition constant between skin and donor solution, L is the effective length of diffusion through skin, s is the Laplace variable with respect to time t, and V_1 and V_2 are the volume of donor solution and skin, respectively. Table 4 summarizes the parameters and MTT values calculated from the permeation profiles of eugenol shown in Fig. 6. The diffusion constant (D) and the partition coefficient (K) values of eugenol decreased in the order of HP-γ-CyD ≫ HP-α-CyD ≧ HP-β-CyD systems, indicating that the suppression of the cutaneous permeation of eugenol by HP-α-CyD and HP-β-CyD can be explained by a lowering of D and K values, where the latter parameter had a greater effect. It has been reported that ^{14}C labeled HP-β-CyD is not absorbed in an intact form from hairless mouse skin, suggesting that HP-β-CyD or the complex is transfered to the skin only in minor amounts, if at all.[23] Therefore, the present reduced permeation of eugenol by HP-CyDs is attributable mainly to the complex formation of eugenol with HP-CyDs leading to a decrease in the free fraction of the guest molecule.[24,25] On the other hand, HCO-60 decreased the D value, while increasing the K value, which was different from the reported results of polysorbate 80, a non-ionic surfactant.[26,27] Thus, HP-CyDs and HCO-60 may have different effects on the percutaneous permeation of eugenol. The MTT values of eugenol with HP-α-CyD and HP-β-CyD were 7—8 times as high as that of eugenol alone. Thus, it is apparent that HP-CyDs, especially HP-α-CyD and HP-β-CyD, greatly suppress the cutaneous permeability of eugenol.

The above results suggest that HP-β-CyD suppresses the release of fragrance materials from water and the cutaneous permeation of eugenol. Therefore, HP-β-CyD has a significant advantage over surface active agents, with respect to providing high aqueous solubility while maintaining a lack of toxicity,[28] in topical liquid preparations of fragrance materials that can be used in medicines and cosmetics.

Acknowledgment The authors would like to thank Miss Yuki Kaneko for her technical assistance.

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