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# Estimation of Distribution of *p*-Hydroxybenzoic Acid Esters between Non-ionic Surfactant Micellar and Aqueous Phases[1)]

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**The partition of *p*-hydroxybenzoic acid esters (parabens) between polyoxyethylene alkyl ether (PAE) micelles and an aqueous phase was studied by a solubility method and ultrafiltration. Paraben solubility in aqueous PAE solution and the extent of interaction between parabens at a low concentration and the micelles varied with the structure of PAEs. The binding capability for methyl, ethyl, propyl and butyl paraben per unit hydrocarbon of PAE molecules increased with oxyethylene chain length but decreased with an increase in hydrocarbon chain length. The interactions between parabens and PAEs thus appear to be influenced by the rigid or bulky character of the micelles, which is dependent on the structures of the surfactant micelles.**

**The extent of the interaction is related to the hydrophile–lipophile balance of the surfactants reported by Davies, and to the carbon number in the alkyl group of parabens. We proposed an empirical formula to estimate the magnitude of the interaction, which was successfully applied not only to PAEs but also to ester-type non-ionic surfactants generally used in eye drops and syrups. In the case of aqueous based pharmaceuticals with various non-ionic surfactants, it is highly probable that the paraben concentration required for antibacterial effectiveness could be determined by the empirical formula.**

**Key words** hydroxybenzoic acid ester; non-ionic surfactant; apparent partition coefficient; interaction; Davies' hydrophile–lipophile balance value; micelle formation

*p*-Hydroxybenzoic acid esters (parabens) are widely used for pharmaceuticals and cosmetics as preservatives. The antibacterial activity of parabens in oil-in-water emulsions is reduced by incorporation into oil droplets and surfactant micelles.[2–4)] The extent of interaction between the parabens of methyl and propyl esters and non-ionic surfactants in water was reported to be affected by the oxyethylene chain length of the surfactants.[5)] The solubilization of some hydrophobic drugs increased in proportion to the hydrocarbon content of non-ionic surfactants whose oxyethylene chains differed in length.[6)] The solubilization of barbituric acid derivatives increased with the hydrocarbon chain length of polyoxyethylene alkyl ethers (PAEs) as a solubilizer.[7)] Maximal solubilization of azobenzene, menaphthone, cortisone acetate and griseofulvin was attained at a certain molecular size of homologous PAEs.[8)] The properties of non-ionic surfactants are influenced by the structures of the surfactants. Changes in critical micelle concentration (CMC) of non-ionic surfactants is attributable to changes in oxyethylene and hydrocarbon chain length.[9–11)] The interactions between parabens and non-ionic surfactant micelles may be influenced by changes in micellar properties. Little has been reported on the influence of non-ionic surfactant structural differences on interactions with parabens.

To determine the effective concentration of parabens for sterilization in various non-ionic surfactant solutions, the interactions between parabens from methyl to butyl ester and polyoxyethylene alkyl ethers were studied by a solubility method and ultrafiltration. Ester-type non-ionic surfactants widely used for pharmaceutical formulations were also examined.

## Experimental

**Materials** Methyl *p*-hydroxybenzoate (MP), ethyl *p*-hydroxybenzoate (EP), propyl *p*-hydroxybenzoate (PP) and butyl *p*-hydroxybenzoate (BP) were of JPXII grade. PAE, (Nikko Chemicals Co., Tokyo), shown in Table 1, and polyethylene glycol 20000 (PEG, Nippon Oil and Fats Co., Tokyo) were of commercial grade (JSCI-II). Polysorbate 80 or polyoxyl 40 stearate and polyoxyethylene hydrogenated castor oil 60 were of JPXII and JPE 1993 grade (Nikko Chemicals Co.). These parabens, surfactants and PEG were used without purification. All other reagents and solvents were of analytical reagent grade.

**Solubility Measurement** Excess paraben was added to aqueous solutions with PAE or PEG of 0—8 mg/ml. After being stirred on a boiling water bath, paraben solutions were cooled and maintained at 25 °C. The solutions were filtered through a membrane filter (pore size 0.45 $\mu$m) followed by the determination of paraben concentration in the filtrate at a wavelength of 256 nm using a spectrophotometer (model

Table 1. Abbreviations and the Hydrophile–Lipophile Balance (HLB) of PAE

| PAE[a)] | Abbreviation[b)] | Hydrocarbon content[c)] | Davies' HLB[d)] | Griffin' HLB[e)] |
|---|---|---|---|---|
| Polyoxyethylene 21 lauryl ether | $C_{12}E_{21}$ | 0.167 | 10.1 | 16.6 |
| Polyoxyethylene 20 cetyl ether | $C_{16}E_{20}$ | 0.215 | 7.90 | 15.7 |
| Polyoxyethylene 30 cetyl ether | $C_{16}E_{30}$ | 0.154 | 11.2 | 16.9 |
| Polyoxyethylene 40 cetyl ether | $C_{16}E_{40}$ | 0.120 | 14.5 | 17.6 |
| Polyoxyethylene 20 stearyl ether | $C_{18}E_{20}$ | 0.234 | 6.95 | 15.3 |
| Polyoxyethylene 20 behenyl ether | $C_{22}E_{20}$ | 0.269 | 5.05 | 14.6 |

*a)* Numbers show moles of added oxyethylene units per molecule. *b)* $C_nE_m$ represents the hydrocarbon (*n*) and oxyethylene numbers (*m*). *c)* Weight ratio of hydrocarbon chain to whole PAE molecule. *d)* Evaluated based on the extent of polarity of each functional group.[12)] *e)* Evaluated based on the molecular weights of hydrophilic and hydrophobic groups.[13)]

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UV-2100, Shimadzu Co.). The measurements were repeated until the concentration remained unchanged.

**Ultrafiltration of Aqueous Paraben Solutions** A paraben was dissolved in aqueous solution with 1, 2, 3, 4, 6 or 8 mg/ml of PAE or PEG. Its concentration was adjusted to *ca.* 0.01% (w/v) as *p*-hydroxybenzoic acid ($7.24\times10^{-4}$ M). An ultrafiltration cell (50 ml, model 8050, Amicon Co.) and ultrafiltration membrane (43 mm i.d., Diaflo YM5, Amicon Co.) were used for filtration. After being poured into the ultrafiltration cell (50 ml), the aqueous paraben solutions with surfactants were filtered under a constant pressure (3 kg/cm$^2$) at 25 °C. The filtrates were collected to determine the concentration, rejecting the first *ca.* 20 ml of the filtrate to avoid possible adsorption of the paraben to the membrane and cell. Paraben concentrations in the feed solution and filtrate, except for those using polysorbate 80, were determined spectrophotometrically. HPLC (model LC100, Yokogawa Electric Co., Tokyo) was used for the determination of parabens in the paraben–polysorbate 80 system to avoid ultraviolet absorption interference from polysorbate 80 under the following conditions: reversed-phase column (Inertsil ODS-2 4.6 × 150 mm, GL Sciences, Inc., Tokyo); detection (UV at 256 nm); mobile phase (methanol : water = 3 : 2, v/v); flow rate of 1.0 ml/min; temperature at 40 °C. Paraben molecules in the filtrate were regarded as free paraben (unbound form) in the aqueous phase. No surfactants or PEG in the filtrate were detectable by the phosphomolybdic acid method.[14)]

**Data Treatment** The partition coefficient of a paraben between the surfactant micellar and aqueous phases, $K$, is given by Eq. 1:

$$K=\frac{D_s/V_s}{D_f/V_f} \tag{1}$$

where $D_s$ and $D_f$ are the amounts of paraben in the micellar and aqueous phases, respectively, and $V_s$ and $V_f$ are the volumes of the micellar and aqueous phases, respectively. $V_s$ is generally unknown; however, it is considered to be proportional to the amount of the surfactant. By using the amount of surfactant instead of $V_s$, since the CMC of the surfactant is negligibly low compared with the total concentration, Eq. 1 can be expressed as follows:

$$K=\frac{D_s/k\cdot s}{D_f/V_f} \tag{2}$$

where $k$ is a proportional constant, and $s$ is the amount of the surfactant in the solution. The apparent partition coefficient of the paraben, $K_{app}$, is defined by Eq. 3:

$$K_{app}=K\cdot k=\frac{D_s/s}{D_f/V_f} \tag{3}$$

$D_f/V_f$ is the paraben concentration in the aqueous phase of the solution, $C_f$, and is given directly as the paraben solubility in water without a surfactant (solubility method) or the paraben concentration in the ultrafiltrate obtained from paraben–surfactant systems (ultrafiltration technique). Because $V_s$ is negligibly small, $D_s$ is calculated according to Eq. 4:

$$\begin{aligned} D_s &= D_t - D_f \\ &= D_t - C_f\cdot V_t \end{aligned} \tag{4}$$

where $D_t$ is the total amount of the paraben and $V_t$ is the total volume of the solution ($V_t=V_f+V_s$). The apparent partition coefficient of paraben obtained from the solubility method was expressed as $K_{s,app}$, whereas that obtained from ultrafiltration was expressed as $K_{u,app}$ to distinguish it from $K_{s,app}$.

## Results and Discussion

**Solubility of Parabens in Aqueous PAE or PEG Solution** Figure 1 shows typical changes in paraben solubilities in the presence of increasing $C_{16}E_{20}$. Paraben solubilities increased in proportion to the surfactant concentration, and the intercept on the ordinate agreed with the solubilities in water at 25 °C (MP, $15.4\times10^{-3}$; EP, $5.53\times10^{-3}$; PP, $1.97\times10^{-3}$ M). The EP solubility in water without surfactant agreed with that in the ultrafiltrate from aqueous $C_{16}E_{20}$ solutions saturated with EP, confirming the validity of the ultrafiltration technique. The good linearity in Fig.1 thus gives the paraben concentration solubilized in $C_{16}E_{20}$ micelles, since its CMC is extremely low. The slope corresponds to $D_s/s$ in Eq. 3. Nearly the same slope of three parabens indicates that about equimolecular amounts of parabens are incorporated into the micelles. Similar results were obtained for paraben–other PAEs or PEG systems, except for BP, whose solubility in the presence of PAE was unable to be determined due to its emulsification in aqueous PAE solution. Slopes obtained from paraben solubility *vs.* PAE or PEG concentration plots are summarized in Table 2, corresponding to paraben solubility in PAE micelles or the binding capacity of PEG.

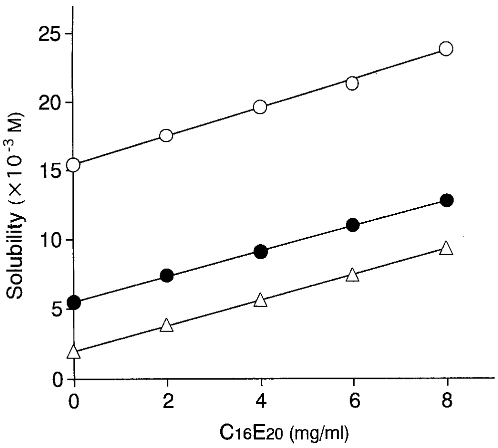

Fig. 1. Changes in Solubility of Parabens as a Function of $C_{16}E_{20}$ Concentration in Aqueous Solution at 25 °C

Parabens: ○, MP; ●, EP; △, PP. Each point is the mean of three experiments.

Table 2. Slopes Obtained from Plot of Paraben Solubility *vs.* PAE or PEG Concentration[a)]

| Compound | Slope (mol/g × 10$^3$) | | |
|---|---|---|---|
| | MP | EP | PP |
| $C_{12}E_{21}$ | 1.05 ±0.181 | 0.856 ±0.023 | 0.854 ±0.006 |
| $C_{16}E_{20}$ | 1.02 ±0.071 | 0.908 ±0.028 | 0.916 ±0.008 |
| $C_{16}E_{30}$ | 0.969 ±0.143 | 0.742 ±0.024 | 0.743 ±0.009 |
| $C_{16}E_{40}$ | 0.761 ±0.042 | 0.653 ±0.012 | 0.631 ±0.008 |
| $C_{18}E_{20}$ | 1.12 ±0.137 | 0.962 ±0.019 | 0.975 ±0.005 |
| $C_{22}E_{20}$ | 0.886 ±0.049 | 0.924 ±0.014 | 0.982 ±0.012 |
| PEG | 0.0790±0.035 | 0.0597±0.008 | 0.0232±0.002 |

*a)* Data are the mean ± S.D. ($n=3$).

**Effects of PAE Structure on Apparent Partition Coefficient of EP Obtained from the Solubility Method** Figure 2 shows the apparent partition coefficients, $K_{s,app}$, of EP between PAE micellar and aqueous phases obtained from the solubility method. In four surfactants with varying hydrocarbons and almost the same oxyethylene chain length ($C_{12}E_{21}$, $C_{16}E_{20}$, $C_{18}E_{20}$ and $C_{22}E_{20}$), maximal $K_{s,app}$ was given by $C_{18}E_{20}$ (Fig. 2A). $K_{s,app}$ for $C_{16}E_{20-40}$ decreased with an increase in the length of the oxyethylene chain (Fig. 2B). The hydrophilic oxyethylene chain in PAE molecules has the same structure as PEG, and the EP which bonded to PEG was small compared with that incorporated into PAE micelles (Table 2). EP molecules thus seem to be mainly solu-

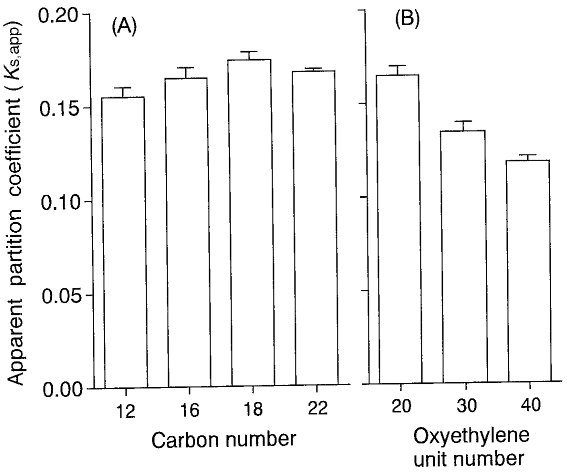

Fig. 2. Effects of Hydrocarbon (A) and Oxyethylene Chain Length (B) of Various PAEs on $K_{s,app}$ of EP between Micellar and Aqueous Phases Obtained from the Solubility Method

PAEs with 20 or 21 oxyethylene units (A) and 16 carbon atoms in the hydrocarbon chain (B). Each column is the mean and S.D. ($n=3$), and only (B) was significantly different ($p<0.01$) from the others.

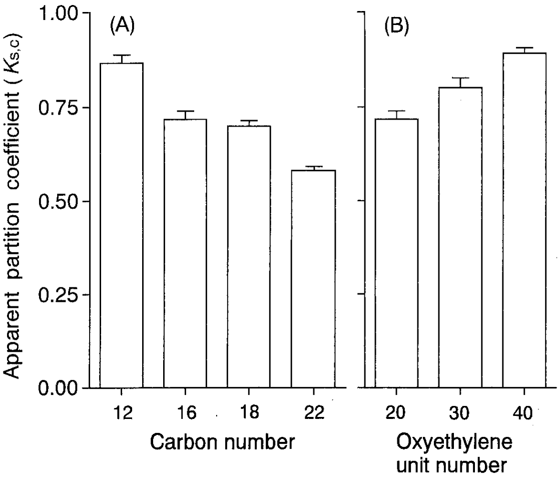

Fig. 3. Effects of Hydrocarbon (A) and Oxyethylene Chain Length (B) of Various PAEs on $K_{s,c}$ of EP Calculated Based on Hydrocarbon Content in PAE between Micellar Core and Aqueous Phase Obtained from the Solubility Method

PAEs with 20 or 21 oxyethylene units (A) and 16 carbon atoms in hydrocarbon chain (B). Each column is the mean and S.D. ($n=3$) and significantly different ($p<0.05$) from the others in (A), except for $C_{16}E_{20}$ *vs.* $C_{18}E_{20}$, and in (B). $K_{s,c}$ was evaluated by deducting EP in the oxyethylene layer from that in PAE micelles based on the assumption in the text.

bilized in the hydrophobic region of PAE micelles.

The site of incorporation of drugs in non-ionic surfactant micelles is generally accepted to be the oxyethylene layer, the hydrocarbon core and the interface between them. In this paper, solubilized regions of parabens were discussed, including the oxyethylene layer and hydrocarbon core. Assuming that the solubility in the hydrophilic region (oxyethylene layer) of the PAE micelles is the same as that in equivalent PEG, the EP incorporated into the hydrophobic region may be calculated by subtracting the EP content in the oxyethylene layer from that in the micelles. The apparent partition coefficient of EP between the micellar hydrophobic core and aqueous phase ($K_{s,c}$) is given using the amount of hydrocarbon provided by the hydrocarbon content in PAE and the EP amount in the hydrophobic core calculated based on the above assumption in a similar manner, as $K_{s,app}$. $K_{s,c}$ increased with decreasing hydrocarbon content in the PAE molecules, as shown in Fig. 3. Thus, $C_{12}E_{21} > C_{16}E_{20} > C_{18}E_{20} > C_{22}E_{20}$ and $C_{16}E_{40} > C_{16}E_{30} > C_{16}E_{20}$. Similar results were obtained for other classes of parabens and PAE systems, except for emulsified BP.

Paraben molecules may be dissolved in hydrophilic and hydrophobic solvents because they consist of hydrophilic (phenolic hydroxyl and carbonyl groups) and hydrophobic groups (alkyl and phenyl groups). This suggests that parabens require a hydrophobic environment to be solubilized, whereas they are not likely to dissolve in a hydrocarbon-like core. Although an increase in hydrocarbon chain in the non-ionic surfactant molecules is responsible for a decrease in the CMC,[9,10] an increasing oxyethylene chain brings about an increase in the CMC.[11] Some oxyethylene chains were predicted to intrude into the hydrocarbon core of non-ionic surfactant micelles.[15] An increase in micellar inner polarity occurs with an increase in oxyethylene chain in non-ionic surfactants,[16] whereas decreasing polarity occurs with an increase in hydrocarbon chain.[17] The distribution of polar molecules, parabens, in the hydrocarbon core of non-ionic surfactants may thus decrease on turning into rigid micelles containing non-polar cores of long hydrocarbon chains. It is therefore presumed that bulky micelles with some hydrate-like cores comprised of long oxyethylene chains facilitate the distribution of paraben molecules in the micellar interior.

**Effects of PAE Structure on Apparent Partition Coefficient of Parabens Obtained from Ultrafiltration** Plots of $D_s/s$, which corresponded to the apparent concentration of parabens in PAE micelles, against free paraben concentrations in the ultrafiltrate, $C_f$, gave straight lines, and their correlation coefficients were above 0.99. The slope of the line describes the apparent partition coefficients of parabens between PAE micellar and aqueous phases obtained from ultrafiltration,[1] expressed as $K_{u,app}$ to distinguish them from $K_{s,app}$ obtained by the solubility method. Figure 4 shows the $K_{u,app}$ of parabens for PAE; this parameter increased with carbon number in the alkyl group of parabens. Maximal $K_{u,app}$ was attained by $C_{18}E_{20}$ in four surfactants ($C_{12}E_{21}$, $C_{16}E_{20}$, $C_{18}E_{20}$ and $C_{22}E_{20}$, Fig. 4A). $K_{u,app}$ for $C_{16}E_{20-40}$ decreased with an increase in oxyethylene chain length (Fig. 4B). $K_{u,app}$ obtained from aqueous PAE solution with $7.24 \times 10^{-4}$ M of EP (0.189—0.264 of $K_{u,app}$ in Fig. 4) was larger than $K_{s,app}$ at the solubility of EP (0.118—0.174 of $K_{s,app}$ in Fig. 2). $K_{u,app}$ decreased with an increase in total concentration of EP in aqueous $C_{16}E_{20}$ solution, as shown in Table 3. Thus, the extent of interaction with PAE micelles at low concentrations of EP was larger than that at high concentrations. This may be due to a concentration dependence of the partition of parabens between surfactant micellar and aqueous phases.[18]

Although EP was somewhat bound to PEG in aqueous solution due to some increases in EP solubility with increasing PEG, PEG in aqueous paraben solution resulted in only a very small decrease in free paraben at a low concentration of parabens in the solution. This indicates

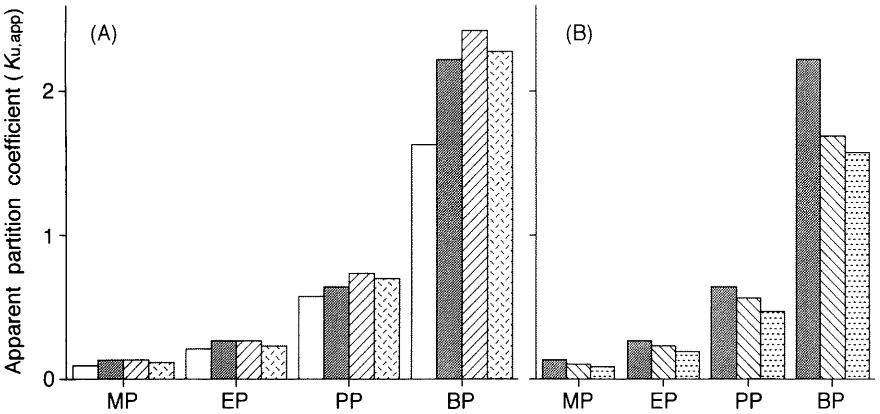

Fig. 4. Effects of Hydrocarbon (A) and Oxyethylene Chain Length (B) of Various PAEs on $K_{u,app}$ of Parabens between Micellar and Aqueous Phases Obtained from Ultrafiltration

(A) □, $C_{12}E_{21}$; ▦, $C_{16}E_{20}$; ▨, $C_{18}E_{20}$; ▧, $C_{22}E_{20}$; (B) ▦, $C_{16}E_{20}$; ▧, $C_{16}E_{30}$; ▩, $C_{16}E_{40}$.

Table 3. Change in $K_{u,app}$ of EP between $C_{16}E_{20}$ Micellar and Aqueous Phases as a Function of Total EP Concentration Obtained from Ultrafiltration[a)]

| EP concentration ($M \times 10^4$) | $K_{u,app}$ |
|---|---|
| 7.24 | 0.264 |
| 14.48 | 0.227 |
| 28.96 | 0.225 |
| 43.44 | 0.206 |

a) $C_{16}E_{20}$ concentration was 4 mg/ml except for 1—8 mg/ml at $7.24 \times 10^{-4}$ M of total EP.

that parabens at low concentrations only slightly form complexes with PEG in aqueous solution. Assuming that paraben molecules at low concentrations are incorporated into the hydrophobic region in PAE micelles, the apparent partition coefficients of parabens between the hydrocarbon core in PAE micelles and aqueous phase ($K_{u,c}$) can be similarly calculated from the amount of hydrocarbons in PAE. Figure 5 shows the $K_{u,c}$ of parabens between the hydrocarbon core in PAE micelles and aqueous phase. It is evident that $K_{u,c}$ decreases with an increase in hydrocarbon chain length of the surfactants by considering the results of $K_{s,c}$ obtained from the solubility, except for the BP–$C_{12}E_{21}$ system (Fig. 5A). $K_{u,c}$ increased with oxyethylene chain length (Fig. 5B).

$K_{u,app}$ and $K_{u,c}$ obtained from ultrafiltration show the same tendency as those of the paraben solubilities in aqueous PAE solution. It is highly probable that an increase in oxyethylene content in PAE molecules is responsible for the large distribution of parabens in the micelles. Paraben molecules at low concentrations may thus become distributed in the interior of PAE micelles with an increase in oxyethylene content of PAE owing to increased polarity in the hydrocarbon core, and binding capability per unit hydrocarbon of PAE may increase with a decrease in hydrocarbon content in PAE molecules.

MP, EP and PP showed greater $K_{u,c}$ against $C_{12}E_{21}$, whereas the $K_{u,c}$ of the BP–$C_{12}E_{21}$ system was less than that of the BP–$C_{16}E_{20}$ and BP–$C_{18}E_{20}$ systems. The reason for the small $K_{u,c}$ of the BP–$C_{12}E_{21}$ system is considered to be as follows. BP molecules seem to require the large free energy of hydrocarbon chains in surfactants for interactions because the hydrophobicity of BP is larger than that of other parabens. The characteristics of PAE become similar to those of PEG having little binding affinity for parabens by decreasing the hydrocarbon content. Thus, the small $K_{u,c}$ of BP for $C_{12}E_{21}$ is probably caused by the large hydrophobicity of BP and the small binding affinity of $C_{12}E_{21}$.

**The Relationship between the Apparent Partition Coefficient and the Hydrophile–Lipophile Balance (HLB)** The HLB is used to assess characteristics of non-ionic surfactants. The apparent partition coefficients of parabens between various PAE micellar and aqueous phases may likely be related to the HLB of PAE due to the dependence of the interaction on hydrocarbon and oxyethylene contents in the surfactants. Figure 6 shows the relationship between the $K_{u,app}$ of parabens and the reciprocal HLB of PAE. The HLB proposed by Davies (Table 1) was evaluated based on the extent of polarity of each functional group in the PAE molecule.[12)] Plots of $K_{u,app}$ *vs.* reciprocal HLB were roughly linear except for those obtained from $C_{22}E_{20}$ (*ca.* 0.20 of 1/HLB). The deviation of $K_{u,app}$ for $C_{22}E_{20}$ may result from its deficient binding capacity for parabens, as described previously. Although the interactions of some parabens were reported to be related to the oxyethylene content of non-ionic surfactants having the same hydrocarbon and various oxyethylene chains,[5)] it was found here that Davies' HLB of various PAEs was proportional to the $K_{u,app}$ of parabens, except in PAEs with small HLB, as $C_{22}E_{20}$, and presumably in those with great HLB.

We reported that the extent of the interaction between parabens and $C_{16}E_{20}$ micelles logarithmically varied in proportion to the carbon number in the alkyl group of parabens.[1)] Therefore, not only the HLB of PAE but also the carbon number in the alkyl group of parabens must be considered for this parameter. Figure 7 shows typical relationships between $K_{u,app}$ and carbon number in the alkyl group of parabens, in which the curves are linear with respect to $C_{16}E_{20-40}$. Similar results were obtained for other PAE systems, and slopes for the lines of semilogarithmic plots of $K_{u,app}$ *vs.* carbon number in the alkyl group of parabens were roughly in accord

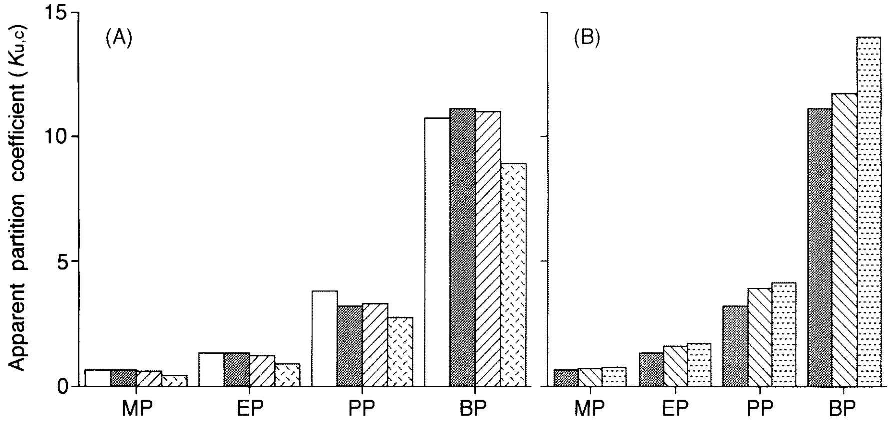

Fig. 5. Effects of Hydrocarbon (A) and Oxyethylene Chain Length (B) of Various PAEs on $K_{u,c}$ of Parabens between Micellar Hydrocarbon Core and Aqueous Phase Obtained from Ultrafiltration

(A) □, $C_{12}E_{21}$; ▩, $C_{16}E_{20}$; ▨, $C_{18}E_{20}$; ⬚, $C_{22}E_{20}$; (B) ▩, $C_{16}E_{20}$; ▧, $C_{16}E_{30}$; ▦, $C_{16}E_{40}$. $K_{u,c}$ was evaluated on the basis of the hydrocarbon content of PAE.

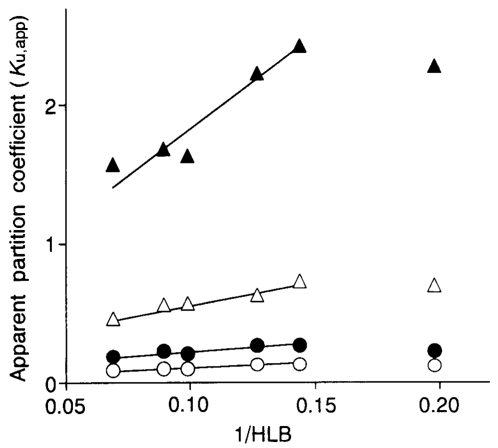

Fig. 6. Relationship between $K_{u,app}$ and Reciprocal HLB of Various PAEs

Parabens: ○, MP; ●, EP; △, PP; ▲, BP.

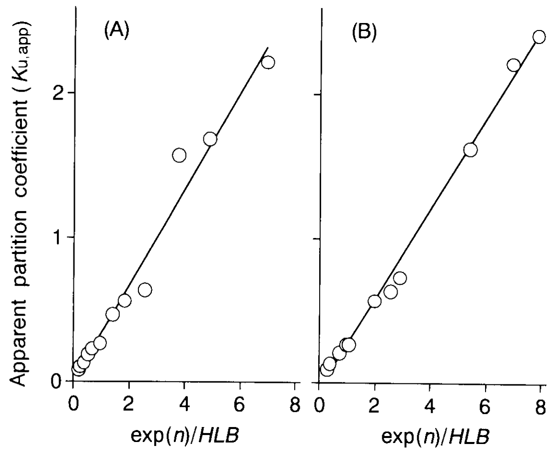

Fig. 8. Relationship between $K_{u,app}$ of Four Parabens for Various PAEs and exp($n$)/$HLB$

$C_{16}E_{20-40}$ (A) or $C_{12}E_{21}$, $C_{16}E_{20}$ and $C_{18}E_{20}$ (B) were used. Regression line: (A) $K_{u,app}=0.337\exp(n)/HLB$, $r=0.987$; (B) $K_{u,app}=0.322\exp(n)/HLB$, $r=0.997$.

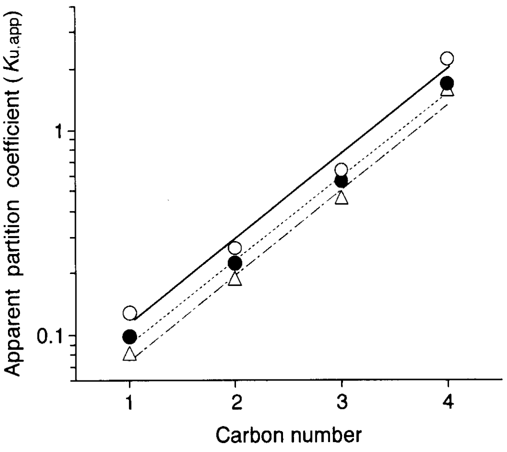

Fig. 7. Relationship between $K_{u,app}$ for $C_{16}E_{20-40}$ and Carbon Number in Alkyl Group of Parabens

PAEs: ○, $C_{16}E_{20}$; ●, $C_{16}E_{30}$; △, $C_{16}E_{40}$.

(0.946—1.02), regardless of structural differences in PAEs.

The $K_{u,app}$ of parabens for PAE may thus be expressed using the exponential carbon number in the alkyl group of parabens and the reciprocal HLB of PAE. Figure 8 shows plots of the $K_{u,app}$ obtained from four parabens and $C_{16}E_{20-40}$ (A) or three PAEs ($C_{12}E_{21}$, $C_{16}E_{20}$ and $C_{18}E_{20}$) (B) systems *vs.* exp($n$)/$HLB$, where $n$ is the carbon number in the alkyl group of paraben and $HLB$ is the HLB of Davies. The regression equation of Fig. 8 (slopes of 0.337 (A) and 0.322 (B)) can be expressed by Eq. 5:

$$K_{u,app}=[\text{slope}]\cdot\exp(n)/HLB \qquad (5)$$

However, the plots of the $K_{u,c}$ *vs.* exp($n$)/$HLB$ and those of the $K_{u,app}$ obtained by the widely utilized HLB of Griffin,[13] based on the molecular weights of hydrophilic and hydrophobic groups (Table 1), indicated a low correlation, different from Fig. 8. From these results, the $K_{u,app}$ of paraben between micellar and aqueous phases can be estimated by Eq. 5 with regard to the majority of PAEs which have a wide range of HLB.

**Application of the Empirical Formula to Ester-Type Non-ionic Surfactants** Table 4 shows the results of applications of Eq. 5 to four parabens and ester-type non-ionic surfactant systems in the same manner as PAE. Three surfactants, polysorbate 80, polyoxyl 40 stearate and polyoxyethylene hydrogenated castor oil 60, are generally available for use in eye drops and syrups as additions.[19] The slopes from polyoxyl 40 stearate (0.322) and polyoxyethylene hydrogenated castor oil 60 (0.348)

Table 4. Results of Eq. 5 Applied to Four Parabens and Ester-Type Non-ionic Surfactant Systems

| Non-ionic surfactant | Davies' HLB | Slope | Correlation coefficient |
|---|---|---|---|
| Polysorbate 80 | 16.5 | 0.504 | 0.999 |
| Polyoxyl 40 stearate | 16.4 | 0.322 | 1.00 |
| Polyoxyethylene hydrogenated castor oil 60 | 14.1 | 0.348 | 0.999 |

were nearly the same as those of PAEs. Polysorbate 80 indicated a larger slope (0.504) than that of PAEs and other ester-type non-ionic surfactants. Generally, additional polar groups in surfactant molecules, carbon double bonds, the branching of hydrocarbon chains and increasing oxyethylene chains increase CMC by inhibiting the association of the molecules due to repulsion between the polar groups.[20] Therefore, the discrepancy of the slope from polysorbate 80 may be attributable to the distribution of the polar paraben molecules in the bulky micellar interior of polysorbate 80, which contains carbon double bonds in the hydrocarbon chain.

The interactions between parabens and non-ionic surfactants may be influenced by the following factors: polarity changes in the micellar core with oxyethylene and hydrocarbon chain length, functional groups of the solubilizate, and steric structure of the surfactant molecule. Even if the structures of various non-ionic surfactants were fairly different from that of PAE, Eq. 5 could be applied not only to PAEs but also to ester-type non-ionic surfactants, except for polysorbate 80. Consequently, the empirical formula is applicable, provided that surfactants containing double bonds (polysorbate 80) and having small HLB ($C_{22}E_{20}$) are eliminated.

Substitution of Eqs. 4 and 5 in Eq. 3 yielded Eq. 6 using the experimental data of $K_{u,app}$ obtained from PAEs in Fig. 8 gives:

$$\frac{C_t}{C_f}=1+\frac{0.33\cdot\exp(n)\cdot S}{HLB} \qquad (6)$$

$$S=s/V_t$$

where $C_t$ is the total concentration of parabens and $S$ the concentration of the surfactants. In the case of methyl, ethyl, propyl and butyl parabens for pharmaceutical formulations, it is highly probable that based on paraben concentration in various non-ionic surfactant solutions, the $C_t$ required for antibacterial effectiveness may be determined using Eq. 6.

## References and Notes

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