Interaction between Hydroxybenzoic Acid Esters and Polyoxyethylene Cetyl Ether[1]

Masahiro Fukahori,[*,a] Yasuo Takatsuji,[a] Masako Seki,[a] Hiroaki Takahashi,[a] Hiroshi Sato,[a] and Toshihisa Yotsuyanagi[b]

Central Research Laboratories, Zeria Pharmaceutical Co., Ltd.,[a] 2512–1, Oshikiri, Konan-machi, Osato-gun, Saitama 360–01, Japan and Faculty of Pharmaceutical Sciences, Nagoya City University,[b] Mizuho-ku, Nagoya 467, Japan.

The mechanism of interaction between *p*-hydroxybenzoic acid esters (parabens) and polyoxyethylene cetyl ether (PCE) was studied by ultrafiltration, a fluorescence probing method and NMR spectroscopy. Apparent partition coefficients of parabens from methyl to butyl ester between the PCE micellar and aqueous phase increased logarithmically in proportion to the carbon number in the alkyl group of parabens. Changes in the chemical shift of paraben protons in the presence of PCE were also enlarged with the carbon number. Hydrocarbon proton signals of PCE were shifted in the presence of ethyl paraben, whereas the signal of oxyethylene protons was changed only at high concentrations of the paraben. In addition, the incorporation of parabens into PCE micelles brought about a decrease in the polarity of the palisade layer in the micelles. The interaction magnitude of ethyl benzoate derivatives with PCE tended to be correlated with the hydrophobicity of the derivatives. The affinity of ethyl benzoate derivatives for PCE micelles, however, was affected by the possession or position of the hydroxyl group in the derivatives. The paraben molecules may thus be concluded to locate in the PCE micellar interior by a hydrophobic interaction between the alkyl chain of the paraben and the hydrocarbon core of the micelles, and to be distributed from the hydrocarbon core close to the oxyethylene layer to the core interior, as the carbon number in the alkyl group of parabens increased.

Key words hydroxybenzoic acid ester; polyoxyethylene cetyl ether micelle; complex formation; hydrophobic interaction; apparent partition coefficient

p-Hydroxybenzoic acid esters (parabens) added to oil-in-water emulsions become inactivated because they are incorporated into oil droplets and surfactant micelles in the dispersed system.[2,3] The interactions between parabens and non-ionic surfactants in aqueous solution were investigated by utilizing dialysis,[2,4] gel-filtration[5] and ultrafiltration.[3] Those techniques enabled direct measurements of paraben concentration of the unbound form in aqueous surfactant solution. Polyethylene glycol (PEG) forms a complex with phenolic compounds resulting from hydrogen bond formation between a hydroxyl group in the compounds and an ether oxygen atom of PEG.[6] Patel and Foss[4] reported that the binding affinity of polysorbate 80 or PEG for parabens decreased or increased with increasing temperature, respectively. They thus concluded that the mechanism of interactions between parabens and polysorbate 80 was a hydrophobic interaction different from that between parabens and PEG. Benzoic acid molecules were estimated to be located at the oxyethylene–hydrocarbon interface of non-ionic surfactant micelles by means of NMR spectroscopy.[7] In addition, differences in the hydrophobicity or polarity of benzoic acid derivatives affect the solubilized site of the derivatives in surfactant micelles.[8]

Generally, the concentration of parabens in eye drops or syrups is less than that in oil-in-water emulsions. The present study was conducted to clarify the interaction mechanism between parabens at low concentrations and non-ionic surfactants. In this paper, ultrafiltration, a fluorescence probing method and NMR spectroscopy were used to study the interactions between parabens or ethyl benzoate derivatives and polyoxyethylene cetyl ether (PCE).

Experimental

Materials Methyl *p*-hydroxybenzoate (MP), ethyl *p*-hydroxybenzoate (EP), propyl *p*-hydroxybenzoate (PP) and butyl *p*-hydroxybenzoate (BP) were of JP XII grade. Ethyl benzoate, ethyl *m*-hydroxybenzoate, ethyl *o*-hydroxybenzoate and pyrene (Wako Pure Chemical Industries, Ltd.) were of analytical reagent grade. PCE possessing 20 units of oxyethylene (Nikko Chemicals Co., Tokyo) and PEG 20000 (Nippon Oil and Fats Co., Tokyo) were of commercial grade (JSCI-II). These parabens, ethyl benzoate derivatives, pyrene, PCE and PEG were used without purification. All other reagents and solvents were of analytical reagent grade.

Sample Preparation of Ultrafiltration The parabens or ethyl benzoate derivatives were dissolved in aqueous PCE solution (0—8 mg/ml) prepared by distilled water. Concentrations of parabens were adjusted to *ca.* 0.005, 0.01 and 0.015% (w/v) as *p*-hydroxybenzoic acid (3.62×10^{-4}, 7.24×10^{-4} and 10.86×10^{-4} M, respectively), reflecting the paraben concentrations added to eye drops or syrups.[9] The concentration of ethyl benzoate derivatives was adjusted to only 7.24×10^{-4} M. Paraben and isomer molecules in aqueous solution were regarded as an undissociated form of the phenolic hydroxyl group since the pK_a of *p*-hydroxybenzoic acid esters $(8.4)^{[10]}$ was larger than the pH of the solutions (*ca.* 5—6).

Ultrafiltration and Determination of Parabens or Ethyl Benzoate Derivatives An ultrafiltration cell (50 ml, model 8050, Amicon Co.) and ultrafiltration membrane (43 mm i.d., Diaflo YM5, Amicon Co.) were used. The YM5 membrane rated at a cutoff molecular weight of 5000 as spherical proteins. After being poured into the ultrafiltration cell, the aqueous paraben or ethyl benzoate derivative solutions containing PCE were filtered at 25 °C.[11]

The concentrations of parabens and ethyl benzoate derivatives in the feed solution and ultrafiltrate were determined spectrophotometrically at a wavelength of maximum adsorption using a spectrophotometer (model UV-2100, Shimadzu Co.). The paraben and ethyl benzoate derivative molecules in the ultrafiltrate were regarded as free form, or antibonding molecules with PCE, in the aqueous phase. The PCE content in the filtrate could not be detected by the phosphomolybdic acid method.[12]

Measurement of Surface Tensions The surface tension of aqueous PCE solutions prepared by purified water passed through a Milli-Q system (Nihon Millipore Co.) was measured at 25 °C using a modified

Wilhelmy-type surface tensiometer (model ST-1, Shimadzu Co.).

Solubility Measurement Excess paraben was added to aqueous PCE solutions (0 or 4 mg/ml) and to n-hexane. After being stirred in a boiling water bath, the solutions were cooled and maintained at 25 °C. These solutions were filtered through a membrane filter (pore size 0.45 μm) followed by spectrophotometric determination of paraben in the filtrate. The measurements were repeated until the concentration remained unchanged.

Retention Time (t_R) Measurement by HPLC The t_R of parabens and ethyl benzoate derivatives was measured as an indication of their hydrophobicity using HPLC (model LC100, Yokogawa Electric Co., Tokyo) under the following conditions: reversed-phase column (Inertsil ODS-2 4.6 × 150 mm, GL Sciences, Inc., Tokyo); detection (UV at adsorption maxima); mobile phase (50 mM phosphate buffer solution (pH 3):methanol = 3:2, v/v); flow rate of 1.0 ml/min; temperature at 40 °C.

Evaluation of Pyrene Fluorescence Intensity The fluorescence spectra of pyrene were taken with a fluorescence spectrophotometer (model RF-540, Shimadzu Co.) at 25 °C and an excitation wavelength of 338 nm. After being stirred at 25 °C for 24 h, the aqueous paraben solutions with pyrene (1.0×10^{-6} M) or with PCE (1 mg/ml) and pyrene (1.0×10^{-7} M) were used for fluorescence measurement. The ratio of pyrene fluorescence intensity at the first peak (ca. 373 nm) to the third peak (ca. 383 nm) was defined as I_1/I_3 in analogy with previous literature.[13]

Evaluation of ^{1}H-NMR Spectra ^{1}H-NMR spectra were evaluated at room temperature with a NMR spectrometer (model JNM GSX-270, JEOL Co., Tokyo). Chemical shifts were measured in deuterium oxide solution containing parabens and PCE or PEG. As an external reference, 3-(trimethylsilyl) propionic acid sodium salt-d_4 was used.

Data Treatment The partition coefficient of a drug between the surfactant micellar and aqueous phases, K, is defined by Eq. 1:

$$K = \frac{D_b/V}{D_f/(1-V)} \tag{1}$$

where D_b and D_f are the amounts of the drug in the micellar and aqueous phase, respectively, and V is the volume of the micellar phase. V is generally unknown; however, it is negligibly small and can be given as a function of the surfactant concentration. By using a surfactant concentration instead of V, Eq. 1 is converted to Eq. 2:

$$K' = \frac{(C_t - C_f)/S}{C_f} \tag{2}$$

where K' is the apparent partition coefficient of the drug, C_t and C_f are the concentrations of the drug in total surfactant solution and in its aqueous phase, regarded as free form, respectively, and S is the surfactant concentration (w/v) in the initial solution.

Results and Discussion

Critical Micelle Concentration (cmc) of PCE The cmc of PCE was given as 5.0×10^{-3} mg/ml (ca. 4.5×10^{-6} M) as determined from the abrupt change in the slope of surface tension against PCE concentration plots. This concentration was in reasonable agreement with reported values for homologous surfactants.[14] A change in free paraben in the presence of PCE (will be shown later) was determined at a significantly larger concentration of PCE (1—8 mg/ml) than the cmc.

Complex Formation between Parabens and PCE Figure 1 shows plots of apparent concentrations of parabens in PCE micelles, $(C_t - C_f)/S$, against free paraben concentrations in the ultrafiltrate, C_f. Plots gave a straight line, and the correlation coefficient of each line was above 0.98. The slope of the line thus describes the apparent partition coefficient of parabens between the micellar and aqueous phase, K'. The linear relationship of such plots represents that the complex formation between parabens and PCE micelles is accounted for by the partition theory. Similar results were reported on some paraben and other non-
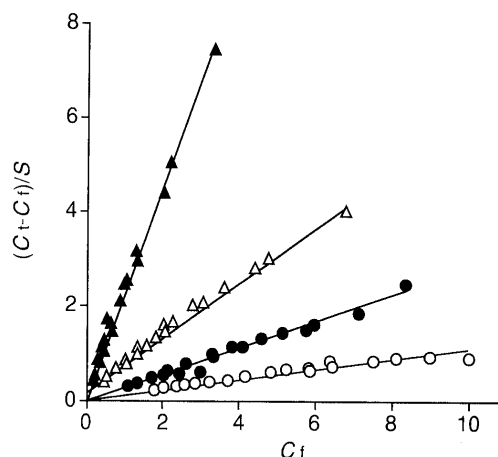

Fig. 1. Relationship between C_f and $(C_t - C_f)/S$ Values

Parabens: ○, MP; ●, EP; △, PP; ▲, BP. Initial concentrations: parabens, 3.62×10^{-4}, 7.24×10^{-4} and 10.86×10^{-4} M; PCE, 1—8 mg/ml.
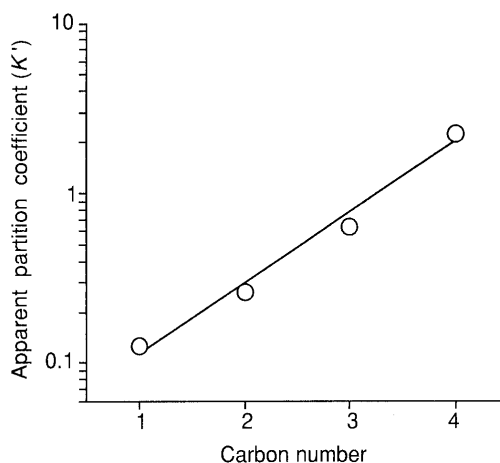

Fig. 2. Relationship between K' and Carbon Number in Alkyl Group of Parabens

ionic surfactant systems.[4,15]

Figure 2 shows the relationship between K' and carbon number in the alkyl group of parabens. An increase in carbon number in the alkyl group caused a linear change in the logarithms of K'. A good correlation of the line demonstrates that the alkyl group contributes to the partition of parabens into PCE micelles. An additional methylene group of the drug molecule results in a logarithmic change in the partition coefficient of the drug between a non-polar solvent and water, this change being attributable to a free energy change based on transfer of the methylene group from water to a non-polar solvent phase.[16] Therefore, the logarithmic change in K' is considered to result from distribution of the alkyl group in the parabens into a non-polar environment, i.e., the hydrophobic region of the PCE micelles.

Relationship between K' and Hydrophobicity of Parabens or Ethyl Benzoate Derivatives Figure 3 shows the relationship between the K' of parabens or ethyl benzoate derivatives and their t_R. The t_R is an indication of hydrophobicity, since the endcapped octadecylsilylated silica phase in the HPLC column possesses negligible neutral silanols and hydrogen bonding ability. The t_R of parabens were correlated to K' (solid line), while plots of ethyl
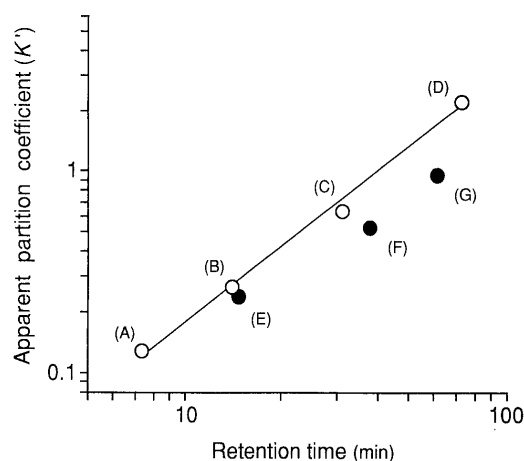
Fig. 3. Relationship between K' and t_R of Parabens or Ethyl Benzoate Derivatives

(A), MP; (B), EP; (C), PP; (D), BP; (E), ethyl m-hydroxybenzoate; (F), ethyl benzoate; (G), ethyl o-hydroxybenzoate. Initial concentration: (E), (F) and (G), 7.24×10^{-4} M; PCE, 1—8 mg/ml.

Table 1. Solubilities of Parabens in n-Hexane and PCE Micelles

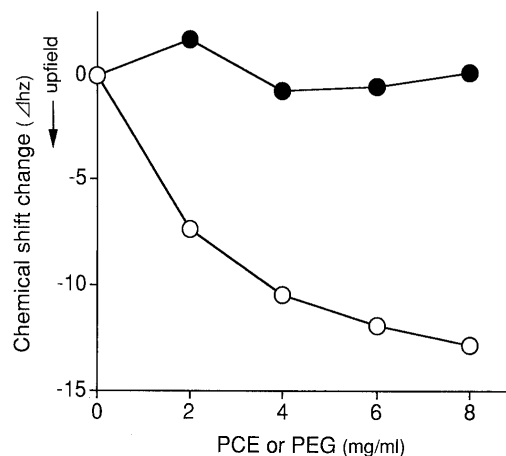| Paraben	Solubility	
	n-Hexane ($M \times 10^3$)	PCE micelles (mmol/g)
MP	0.460	1.01
EP	0.723	0.870
PP	1.05	0.914
BP	3.15	—[a]

a) Emulsification.

Fig. 4. Changes in Chemical Shift of Methylene Proton of EP in the Presence of PCE or PEG

○, with PCE; ●, with PEG. EP concentration in deuterium oxide solution: 7.24×10^{-4} M.

benzoate derivatives were different from those anticipated from their t_R and the paraben line.

The ethyl group of ethyl benzoate derivatives may be oriented into the hydrocarbon core of PCE micelles due to a hydrophobic interaction. Contrarily, the phenolic hydroxyl group of the derivatives is liable to be directed toward the oxyethylene layer because of hydrogen bond formation. Deletion of the hydrogen bond between the hydroxyl group and the oxyethylene layer probably accounts for the small K' of ethyl benzoate compared with its relatively large hydrophobicity (t_R). Tomida $et\ al.$[17] reported that the substituent position of benzoic acid derivatives affected the magnitude of the partition coefficient of the derivatives between polyoxyethylene lauryl ether micelles and the aqueous phase, because hydrogen bonding between the compounds and the oxyethylene moiety of the micelles was changed by the proton-donating ability of the substituted derivatives. Little difference in K' between the $para$ substituted EP and ethyl m-hydroxybenzoate was probably attributed to a slight difference in the liability to hydrogen bond between the hydroxyl group and the oxyethylene chain of PCE. The K' of ethyl o-hydroxybenzoate for PCE was notably larger than that of the $para$ and $meta$ forms. The reason for this difference would be the large hydrophobicity of ethyl o-hydroxybenzoate due to intramolecular hydrogen bonding between the phenolic hydroxyl group and the carbonyl oxygen atom. The K' of ethyl o-hydroxybenzoate was, however, smaller than that anticipated from the t_R and the line of parabens. These results thus suggest that the complex formation between parabens and PCE micelles occurs not only through hydrophobic interaction but also by hydrogen bonding of the hydroxyl group in the parabens.

Position of Paraben Molecules in PCE Micelle Table 1 shows paraben solubilities in n-hexane or PCE micelles in aqueous solution. The solubilities in n-hexane increased with carbon number in the alkyl group of parabens, but roughly the same amounts of parabens were incorporated into the PCE micelles in spite of differences in the alkyl

chain length. Binding between parabens and PEG decreases with increasing carbon number in the alkyl group of the parabens.[18] Therefore, the hydrocarbon core of the micelle having the same environment as n-hexane would contribute to the logarithmic change in K' of parabens at low concentrations, depending on the alkyl chain length.

Figure 4 shows the effects of PCE or PEG on chemical shifts for methylene protons of EP. Upfield chemical shifts for the methylene protons, as well as methyl, $ortho$ and $meta$ protons of EP, were observed in the presence of PCE, but remained unchanged in the presence of PEG. Figure 5 shows the effects of EP on chemical shifts for PCE or PEG protons. The signals of methyl and methylene protons from PCE shifted upfield more than those of oxyethylene protons, which changed above 7.24×10^{-4} M of EP. However, the oxyethylene signal change from PEG was negligibly small, regardless of the EP concentration examined.

The solubilization of some hydrophobic drugs by polyoxyethylene alkyl ethers has been assumed to occur in the oxyethylene rich layer close to the micellar core because PEG is a much better solvent for those drugs than liquid hydrocarbon.[19] PP in non-ionic surfactant micelles was presumed to be located in the palisade and oxyethylene layers at 2—30 times higher the total PP concentration compared to that in the present study.[15] Interactions between EP and the hydrocarbon core in the PCE micellar interior was demonstrated by upfield shifts of the EP protons in the presence of PCE and those of
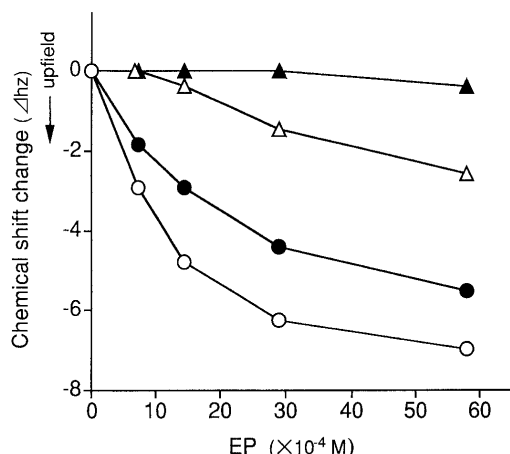
Fig. 5. Changes in Chemical Shift of PCE or PEG Proton in the Presence of EP

Protons: ○, methylene of PCE; ●, methyl of PCE; △, oxyethylene of PCE; ▲, oxyethylene of PEG. Concentration of PCE or PEG in deuterium oxide solution: 4 mg/ml.

0.0	16.7	33.5	50.2	66.9	MP
0.0	8.4	16.8	25.2	33.6	EP
0.0	4.8	9.6	14.4	19.2	PP
0.0	2.7	5.4	8.1	10.8	BP

Total paraben in aqueous PCE solution ($\times 10^{-4}$ M)
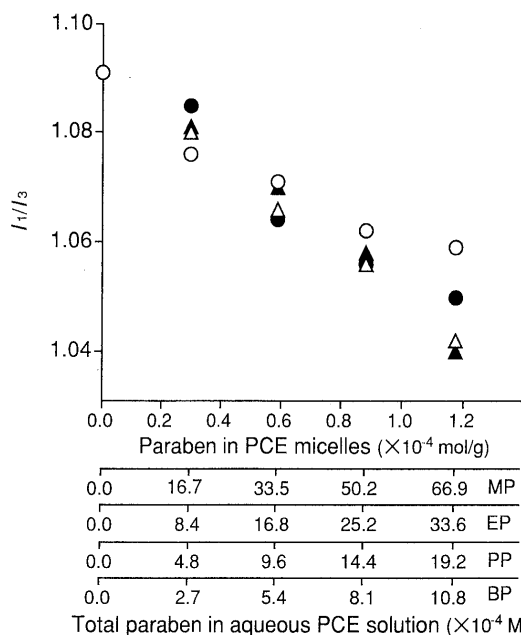
Fig. 6. Effect of Paraben Concentration in PCE Micelles on I_1/I_3 Obtained from Pyrene Fluorescence Spectra

Parabens: ○, MP; ●, EP; △, PP; ▲, BP. PCE concentration in paraben solutions, 1 mg/ml; paraben concentrations in PCE micelles were calculated using Eq. 2 and apparent partition coefficients (MP, 0.0940; EP, 0.273; PP, 0.571; BP, 2.19) obtained from Fig. 1. Each point is the mean of three experiments.
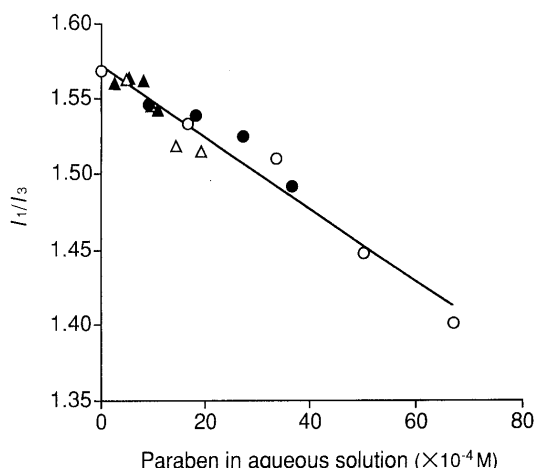
PCE hydrocarbon protons in the presence of EP. In the meantime, little interaction between EP and PEG occurred because there were no signal changes of the EP protons in the presence of PEG nor of those of the PEG protons in the presence of EP. The change in the oxyethylene signal of PCE resulting from increasing EP is attributable to the distribution of the EP molecules, probably after EP saturation in the core, in the oxyethylene rich layer, as noted in the literature.[15]

Figures 6 and 7 show the effects of paraben on I_1/I_3 obtained from pyrene fluorescence spectra in the presence and absence of PCE, respectively. The I_1/I_3 in the presence of PCE was smaller than that in the absence of PCE, but both values decreased with increasing parabens.

A pyrene fluorescence technique has been used to investigate surfactant micellar properties based on the fluorescence intensities of the vibronic bands being dependent on solvent polarity.[20] Pyrene molecules are solubilized at the interface between the oxyethylene layer and hydrophobic core in polyoxyethylene nonylphenyl ether micelles,[21] and I_1/I_3 decreases with a decrease in the polarity of the pyrene environment in surfactant micelles.[13] The smaller I_1/I_3 in the presence of PCE (Fig. 6) may be accounted for by incorporation of the pyrene molecules to low polar environment, i.e., the PCE micellar interior. The decrease in I_1/I_3 in the absence of PCE (Fig. 7) would be due to a decrease in the polarity of aqueous pyrene solution by increasing parabens. The I_1/I_3 change observed at low concentrations of parabens in the PCE micelles, involving a greater decrease of I_1/I_3 by a short alkyl chain of parabens, may thus be remarkably influenced by a higher concentration of paraben in the aqueous phase. A long alkyl chain of parabens may likely increase the exclusion of water molecules near the fluorescent in the micelles; thus I_1/I_3 would become progressively smaller in the high concentration ranges of parabens in the micelles, as the alkyl chain length increased. Malliaris[22] described that n-alkane was solubilized in the inner hydrocarbon core of surfactant micelles, as no I_1/I_3 change of pyrene was detected while n-alkanol was

Fig. 7. Effect of Paraben Concentration on I_1/I_3 Obtained from Pyrene Fluorescence Spectra in the Absence of PCE

Parabens: ○, MP; ●, EP; △, PP; ▲, BP. Paraben concentrations are the same as those in aqueous PCE solution in Fig. 6. Each point is the mean of three experiments.

solubilized near the hydrophilic region, resulting in decrease of I_1/I_3. Similarly, the decrease in I_1/I_3 observed in the paraben–PCE system suggests that the paraben molecules are located near the site and at the outside of pyrene molecules, i.e., in the palisade and oxyethylene layers in the micelle. Consequently, parabens may be incorporated into the PCE micellar core at low concentrations, being gradually distributed from the palisade layer to the oxyethylene layer as the concentration increases.

Figure 8 shows the change in chemical shifts of the ortho and methyl protons of parabens in the presence of PCE. The upfield shifts of the ortho protons became greater with an increase in the alkyl chain length, and similar
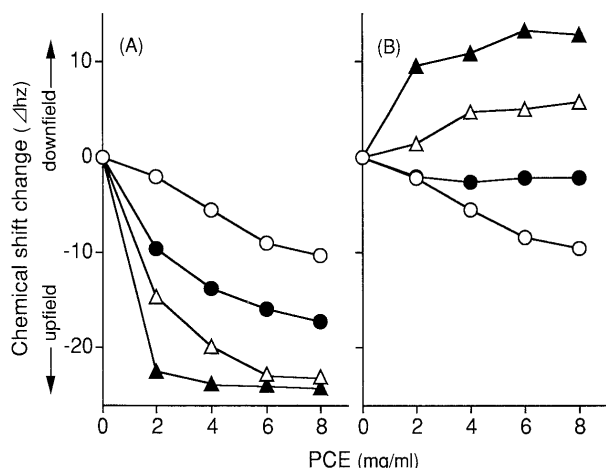
Fig. 8. Changes in Chemical Shift of *Ortho* (A) and Methyl (B) Protons of Paraben in the Presence of PCE

Parabens: ○, MP; ●, EP; △, PP; ▲, BP. Paraben concentration in deuterium oxide solution: 7.24×10^{-4} M.

changes were observed for the *meta* and methylene protons (not shown). The methyl protons of MP and EP also showed upfield shifts. However, those of PP and BP shifted downfield, in the opposite direction.

An increase in the distribution of paraben molecules into the micelles is explained in terms of enhanced upfield shifts of the molecular protons, except for methyl protons, depending on the carbon number of the alkyl group. Protons held over the plane of the aromatic ring or aliphatic double bond resonate at a higher magnetic field, whereas those near the coplane of the ring or double bond resonate at a lower field by interaction with π electrons of the functional groups, *i.e.*, the magnetic anisotropy effect.[23] Shifts of the methyl proton signals to a lower field may thus be due to the location of the methyl group on the coplane of the benzene ring or carbonyl double bond. Mukerjee[8] suggested that solubilization sites for benzoic acid derivatives in polyoxyethylene stearate micelles turn into micellar interior, with increasing hydrophobicity and decreasing polarity of the derivatives. It seems that paraben molecules which possess a long alkyl chain exist stably in the micellar hydrocarbon core by directing a terminal methyl group toward the benzene ring coplane to presumably stack the phenyl group vertically.

In an aqueous surfactant solution at relatively low concentrations of parabens, such as eye drops or syrups, it is highly probable that the alkyl and phenyl groups of parabens exist in the hydrocarbon core of the micelle, whereas the hydroxyl group may point to the oxyethylene layer. Based on the present results, paraben molecules are considered to be distributed from the hydrocarbon core close to the oxyethylene layer to the core interior, as the carbon number of the alkyl group in parabens increases.

References and Notes

1) A part of this study was presented at the 112th Annual Meeting of the Pharmaceutical Society of Japan, Fukuoka, March 1992.
2) Patel N. K., Romanowski J. M., *J. Pharm. Sci.*, **59**, 372—376 (1970).
3) Shimamoto T., Ogawa Y., Ohkura N., *Chem. Pharm. Bull.*, **21**, 316—322 (1973).
4) Patel N. K., Foss N. E., *J. Pharm. Sci.*, **53**, 94—97 (1964).
5) Goto A., Nihei M., Endo F., *J. Phys. Chem.*, **84**, 2268—2272 (1980).
6) Higuchi T., Lach J. L., *J. Am. Pharm. Assoc. Sci. Ed.*, **43**, 465—470 (1954); Guttman D., Higuchi T., *ibid.*, **45**, 659—664 (1956).
7) Donbrow M., Rhodes C. T., *J. Pharm. Pharmacol.*, **18**, 424—428 (1966).
8) Mukerjee P., *J. Pharm. Sci.*, **60**, 1528—1531 (1971).
9) Notification No. 651 of the Ministry of Health and Welfare of Japan, dated May 12, 1973; Shimazaki J., *Ganka*, **33**, 533—538 (1991).
10) Büchi V. J., Hansen J. B., Tammilehto S. A., *Pharm. Acta Helv.*, **46**, 620—626 (1971).
11) Fukahori M., Takatsuji Y., Yamakita T., Takahashi H., Sato H., Yotsuyanagi T., *Chem. Pharm. Bull.*, **44**, 245—248 (1996).
12) Hall M. J., *J. Assoc. Off. Anal. Chem.*, **47**, 685—688 (1964).
13) Turro N. J., Kuo P. L., *J. Phys. Chem.*, **90**, 4205—4210 (1986).
14) Abe M., Uchiyama H., Yamaguchi T., Suzuki T., Ogino K., Scamehorn J. F., Christian S. D., *Langmuir*, **8**, 2147—2151 (1992).
15) Shimamoto T., Mima H., Nakagaki M., *Chem. Pharm. Bull.*, **27**, 1995—2002 (1979).
16) Davis S. S., Higuchi T., Rytting J. H., *J. Pharm. Pharmacol.*, **24**, 30P—46P (1972).
17) Tomida H., Yotsuyanagi T., Ikeda K., *Chem. Pharm. Bull.*, **26**, 2824—2831 (1978).
18) Shimamoto T., Mima H., *Chem. Pharm. Bull.*, **27**, 2557—2563 (1979).
19) Elworthy P. H., Patel M. S., *J. Pharm. Pharmacol.*, **34**, 543—546 (1982).
20) Nakajima A., *Bull. Chem. Soc. Jpn.*, **44**, 3272—3277 (1971).
21) Turro N. J., Kuo P. L., *Langmuir*, **2**, 438—442 (1986).
22) Malliaris A., *Adv. Colloid Interface Sci.*, **27**, 153—168 (1987).
23) Chamberlain N. F., "The Practice of NMR Spectroscopy," Plenum Press, New York, 1974, pp. 8—9.