[Chem. Pharm. Bull. 27(9)1995—2002(1979)]

UDC 547.587.12-26.03.08 : 541.183.02

Interaction of Propyl *p*-Hydroxybenzoate with Polyoxyethylene Dodecyl Ethers

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(Received October 21, 1978)

The interaction of propyl *p*-hydroxybenzoate (PP) with polyoxyethylene dodecyl ethers (PDE) having various numbers of oxyethylene units in a homogeneous chain was studied by means of ultrafiltration and nuclear magnetic resonance spectrometry. The results indicate that PP molecules are bound to two distinct loci within the surfactant micelles. The primary class of sites had high affinity but a low capacity for PP molecules, whereas the secondary class of sites exhibited low affinity and a large binding capacity. In the primary class of sites, it seems probable that the PP molecules are situated at the interface of the hydrocarbon core and the polyoxyethylene mantle of the micelles. The interaction of the secondary class of sites is probably a simple partitioning of PP molecules into the polyoxyethylene region of the micelles. The interaction of PP with PDE was much greater than that of methyl *p*-hydroxybenzoate with PDE.

Keywords——nonionic surfactant; preservative; ultrafiltration; NMR spectrometry; binding parameters; micelle; solubilization

It is now generally accepted that the inactivation of preservatives in the presence of nonionic surfactants arises from an interaction between molecules of the preservative and micelles of the surfactant. The preservative molecules bound to or solubilized within surfactant micelles are devoid of antimicrobial activity. The most important factor affecting the activity of preservatives in a system containing nonionic surfactants is the concentration of the unbound or free preservative in the aqueous phase.[2] Therefore the physico-chemical parameters of the interaction can be used to calculate the total preservative concentration required to provide an effective concentration of free preservative adequate for the prevention of microbial spoilage. Previous studies[3] have dealt with a method utilizing an ultrafiltration technique in connection with the interaction of methyl *p*-hydroxybenzoate (MP) with polyoxyethylene dodecyl ethers (PDE).

In this paper, the binding of propyl *p*-hydroxybenzoate (PP), which is more lipophilic than MP, to PDE is studied using the ultrafiltration technique. The location of the solubilizate within the micelles is also discussed.

Experimental

Materials——Propyl *p*-hydroxybenzoate (PP) was of J.P. IX grade. Polyoxyethylene (10, 15, 20, 30 and 50)[4] dodecyl ethers (PDE-10, -15, -20, -30 and -50) were of commercial grade, supplied by Nihon Emulsion Co., Tokyo. The Diaflo membrane, UM-10, 43 mmϕ (cross-linked dextran gel membrane), was purchased from Amicon Corp., Mass., U.S.A. Deuterium oxide had a purity of 99.9%.

1) Location: *a) Juso-Honmachi, Yodogawa-ku, Osaka, 532, Japan*; *b) Yoshida-Shimoadachi-cho, Sakyo-ku, Kyoto, 606, Japan.*

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3) *a)* T. Shimamoto, Y. Ogawa, and N. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **21**, 316 (1973); *b)* T. Shimamoto and Y. Ogawa, *ibid.*, **23**, 3088 (1975).

4) The numbers in parentheses denote the nominal numbers of oxyethylene units per molecule.

Ultrafiltration and Quantitative Analysis——The technique employed was essentially the same as that described in the previous report.[3a] The ultrafiltration technique was used for separation of the free form of PP from the bound form within micelles. The procedures were carried out at a temperature of 25°. After ultrafiltration, the sample was diluted with water and the concentration of PP was determined spectrophotometrically at a wavelength of 256 nm, since PDE did not interfere with the assay of PP. The pH values of all the sample solutions were measured.

Nuclear Magnetic Resonance (NMR) Measurements——The NMR spectra were obtained in deuterium oxide using a Varian XL-100-12 high resolution spectrometer. Tetramethyl silane was used as an external reference. Chemical shifts were measured in Hertz and the precision was within about ±0.5 Hz.

Results and Discussion

pH Values of Sample Solutions

The pH values of sample solutions ranged from 4.5 to 6.3 and were sufficiently below 8.5, the pK_a of *p*-hydroxybenzoate,[5] so that all of the PP molecules could be regarded as in the undissociated form.

Membrane Permeability to PP and Ultrafiltration

Aqueous solutions of PP were filtered through the Diaflo UM-10 membrane. Figure 1 shows the PP concentration in each filtrate as a function of the fraction number. The concentration of PP in the first few fractions on ultrafiltration was rather low, but the PP concentration reached a constant level after 12 ml. Allowing for experimental error, the PP concentration of the filtrate was essentially equal to that of the initial PP solution. Next, solutions containing PP and PDE-15 were subjected to ultrafiltration. Figure 2 illustrates the PP concentration in the ultrafiltrate (4 ml fractions). The effluent concentration rose to a constant level after some time lag.

During filtration there was an initial delay in the appearance of preservative in the effluent for both PP and PP–PDE solutions. This time lag may be a consequence of the "void volume" of the system, which consists of a membrane, a membrane support of sintered glass and connecting tubing, as pointed out by Blatt.[6] The average PP concentration of the first six fractions in Fig. 1 (total volume, 12 ml) was about 70% of that of the non-filtered solution. This indicated that the apparent void volume of this system was *ca.* 3.6 ml. Blatt[6] reported that the apparent void volume was about 8 ml using a filtration cell of the same type as in this study. In the light of the construction of the system between the membrane and the collecting tube, the value obtained for the void volume seemed reasonable. Although the PP concentrations after 12 ml in Fig. 1 and 2 show minor variations and very slight increases on continued filtration, it can be assumed that the membrane is satisfactorily nonretentive for PP in approximately the same way as for MP.[3a] The errors inherent in this ultrafiltration are of the order of a few per cent, and should not interfere in determining the binding behavior of PP and PDE.

Some authors[7] described the limitations of ultrafiltration in studies of drug-protein binding, while many investigators[8] have reported that the binding parameters obtained by ultrafiltration were in good agreement with those obtained by other methods, and that the ultrafiltration method was applicable to the study of binding behavior. Advantages and disadvantages inherent in ultrafiltration have been discussed in detail by several authors.[6,9] Interfering factors are, (1) the retentivity of the membrane for macromolecules, (2) the binding

5) T.R. Aalto, M.C. Firman, and N.E. Rigler, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 449 (1953).
6) W.F. Blatt, S.M. Robinson, and H.J. Bixler, *Anal. Biochem.*, **26**, 151 (1968).
7) C.G. Bruck and J. Kuhne, *Arzneim. Forsch.*, **12**, 1116 (1962); W. Scholtan, *ibid.*, **15**, 1433 (1965).
8) E.R. Garrett, J. Tsau, and P.H. Hinderling, *J. Pharm. Sci.*, **61**, 1411 (1972); P.H. Hinderling, J. Bres, and E.R. Garrett, *ibid.*, **63**, 1684 (1974); D.S. Campion and R. Olsen, *ibid.*, **63**, 249 (1974); V.P. Shah, S.M. Wallace, and S. Riegelman, *ibid.*, **63**, 1364 (1974).
9) H. Kurz, H. Trunk, and B. Weitz, *Arzneim. Forsch.*, **27**, 1373 (1977).

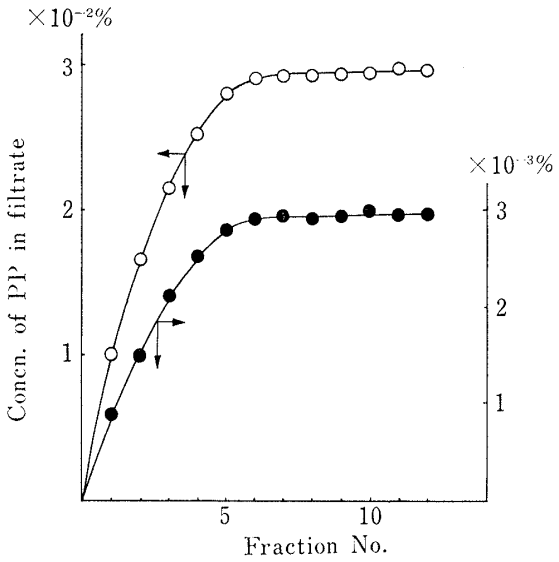

Fig. 1. Ultrafiltration of Aqueous PP Solution

The filtrates were fractionated in 2 ml portions. The PP concentrations of feed solutions were 3.0×10^{-2} % (—○—) and 3.0×10^{-3} % (—●—).

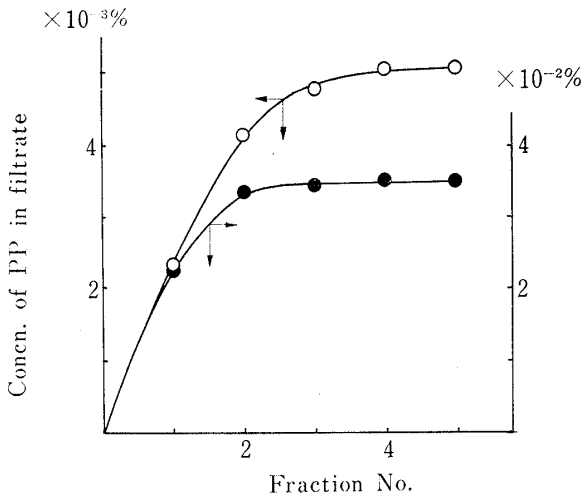

Fig. 2. Ultrafiltration of PP in PDE-15 Solution

The filtrates were fractionated in 4 ml portions. The PDE-15 concentration of feed solution was 1%. The total PP concentrations in solutions were 0.211% (—●—) and 0.0367 % (—○—).

and rejection of microsolutes by the membrane, (3) the Donnan effect and (4) the increase of macromolecule concentration during filtration. In this study, there was only a negligible leakage of PDE from the membrane[3a] and the critical micelle concentration of PDE was negligibly low,[10] so the amount of micelles in the filtration cell was assumed to be unchanged during ultrafiltration. There was no significant degree of preservative binding or preservative rejection by the membrane, *i.e.*, the membrane was regarded as nonretentive for PP. The filtration of undissociated preservative molecules would not be subject to Donnan effects. Increase of PDE concentration during filtration had only a slight effect on the PP concentration in the ultrafiltrate, as shown in Fig. 2. Therefore, ultrafiltration through a cell bounded by a Diaflo membrane is applicable for measurement of the binding characteristics of PP to PDE. However, it was necessary to carry out a careful inspection and preliminary test of the membrane to avoid experimental errors due to scratched membranes and the variation of membrane permeability from one batch to another.

Interaction of PP with PDE

The data and the apparent partition coefficients obtained from ultrafiltration experiments with PP in aqueous PDE-15 solutions are shown in Table I. Here, the concentration of total preservative in the solution, $[D_t]$, and the concentration of free PP in the aqueous phase, $[D_f]$, were determined directly by analyzing the initial solution and the filtrate. The concentration of bound PP based on the total volume of the non-filtered solution, $[D_b]$, and the apparent partition coefficient, K_m', were calculated using the following equations,[3b]

$$[D_b] = [D_t] - [D_f] \cdot \left(1 - \frac{[S]}{100}\right) \quad (1)$$

$$K_m' = \frac{[D_b]/[S]}{[D_f]/100} \quad (2)$$

where $[S]$ is the surfactant concentration in the non-filtered solution. The saturation ratio is given by $[D_f]/0.034\%$ (solubility of PP in water).

The calculated values of K_m' increased markedly as the saturation ratio of the sample solution was reduced, though K_m' was nearly constant for different concentrations of PDE-15

10) P.H. Elworthy and C.B. Macfarlane, *J. Pharm. Pharmacol.*, **17**, 65 (1965).

at a given saturation ratio. Thus, the results suggest that the distribution of PP between the aqueous and micellar phases is not governed by the simple partition law described by McBain.[11] Many authors[12] have studied the solubilization mechanism of preservative-surfactant systems and have observed variations of the apparent partition coefficients with free presevative concentration. If the preservative concentration bound within micelles is not proportional to the free preservative concentration in the aqueous phase, the above partition coefficient cannot fully characterize the interaction.

TABLE I. Apparent Partition Coefficients for PP between Micelles and the Aqueous Phase of PDE-15 Solutions

% of surfactant in total solution [S]	% of total PP in total solution [D_t]	% of free PP in aqueous phase [D_f]	Saturation ratio	% of bound PP in total solution [D_b]	Apparent partition coefficient K_m'
1.5	0.312	0.0358	1.05	0.277	516
1.0	0.211	0.0352	1.04	0.176	500
1.0	0.211	0.0345	1.01	0.177	513
1.0	0.169	0.0280	0.823	0.141	504
1.0	0.167	0.0269	0.791	0.140	520
1.5	0.154	0.0169	0.497	0.137	540
1.0	0.102	0.0161	0.474	0.0861	535
1.5	0.154	0.0160	0.471	0.138	575
1.0	0.102	0.0151	0.444	0.0871	577
1.0	0.0614	0.0088	0.259	0.0527	599
1.0	0.0504	0.0065	0.191	0.0440	677
1.0	0.0367	0.0050	0.147	0.0318	636
1.0	0.0368	0.0048	0.141	0.0320	667
1.5	0.0474	0.0044	0.129	0.0431	653
1.0	0.0223	0.0030	0.088	0.0193	643
1.0	0.0223	0.0028	0.082	0.0195	696

As an alternative and widely used method, binding phenomena in under-saturated system can be considered to obey the law of mass action, as suggested by Garrett.[13] The results may be usefully expressed in the form of a Scatchard plot,

$$\frac{r}{[D_f]} = n \cdot K - r \cdot K \qquad (3)$$

where r is $[D_b]/[S]$, n is a constant corresponding to the number of binding sites on the micelles, expressed in moles of PP per mole of PDE (or in grams of PP per gram of PDE), and K is the association constant for the binding. Figure 3 shows the binding results in the form of a Scatchard plot for the interaction of PP with PDE having various numbers of oxyethylene units in the homogeneous chain, including the data with PDE-15 presented in Table I. PDE-10 was not studied because a saturated solution of PP in 1% aqueous PDE-10 gave a white, cloudy precipitate at a temperature of 25°.

As noted previously,[3b,12c] the Scatchard plots are curved. This suggests the existence of more than one class of binding sites. In analyzing these curved plots, the binding data

11) M.E.L. McBain and E. Hutchinson, "Solubilization and Related Phenomena," Academic Press, New York, 1955, p. 75.

12) *a*) M. Donbrow, P. Molyneux, and C.T. Rhodes, *J. Chem. Soc.* (*A*), **1967**, 561; *b*) A.G. Mitchell and K.F. Brown, *J. Pharm. Pharmacol.*, **18**, 115 (1966); *c*) S.J.A. Kazmi and A.G. Mitchell, *ibid.*, **23**, 482 (1971); *d*) *Idem*, *J. Pharm. Sci.*, **62**, 1299 (1973).

13) E.R. Garrett, *J. Pharm. Pharmacol.*, **18**, 589 (1966).

were fitted to a four-parameter model by a modification of the method of Hart.[14] A multiple linear regression technique was incorporated into the fitting procedure using a JEC-5 spectrum computer. The data were adequately described as an interaction with two classes of binding sites, which could be written as

$$r = \frac{[D_b]}{[S]} = \frac{n_1 \cdot K_1 \cdot [D_f]}{1+K_1 \cdot [D_f]} + \frac{n_2 \cdot K_2 \cdot [D_f]}{1+K_2 \cdot [D_f]} \qquad (4)$$

The binding parameters for the interaction of PP with PDE are shown in Table II. The solid lines joining the experimental points in Fig. 3 were generated from the set of binding parameters using Eq. (4).

The data analysis indicated that PP molecules were bound to two distinct loci within the PDE micelles. The primary class of binding sites exhibited high affinity and a low capacity for the preservative, while the secondary class of sites had low affinity and a large binding capacity. The Scatchard plot in Fig. 3 appears to approach a horizontal asymptote (*i.e.* the value of $r/[D_f]$ becomes constant) when the value of r increases to the saturation point. As $[D_f] < 0.0019$ mole/liter and $K_2 \cdot [D_f] \ll 1$, Eq. (4) can be written as

$$r = \frac{[D_b]}{[S]} = \frac{n_1 \cdot K_1 \cdot [D_f]}{1+K_1 \cdot [D_f]} + n_2 \cdot K_2 \cdot [D_f] \qquad (5)$$

The dotted lines in Fig. 3 were calculated by substituting the binding parameters into Eq. (5). These curves showed substantial agreement with the curves generated by Eq. (4). It appears that the primary class of sites is governed by the Langmuir isotherm, and the solubilization involves binding to definite sites in the micelles; on the other hand, the secondary process is a weak nonspecific interaction of large binding capacity which is analogous to a simple partitioning mechanism. The value of $n_2 \cdot K_2$ may correspond to an apparent partition coefficient for the secondary process.

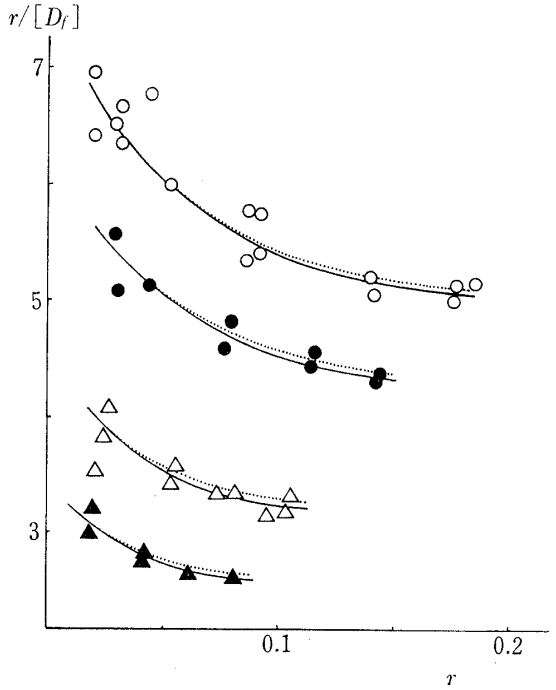

Fig. 3. Scatchard Plots for the Interaction of PP with PDE

Solid line and dotted line were calculated using Eq. (4) and (5), respectively.
○: PDE-15, ●: PDE-20, △: PDE-30, ▲: PDE-50.

TABLE II. Binding Parameters for the Interaction of PP with PDE at 25°

Surfactant	n_1		K_1		n_2		K_2		$n_2 \cdot K_2$	
	(g/g)	(mol/mol)	(100 ml/g)	(l/mol)	(g/g)	(mol/mol)	(100 ml/g)	(l/mol)	(100 ml/g)	(l/mol)
PDE-15	0.0230	0.108	137	2480	12.3	57.6	0.373	6.71	4.58	386
PDE-20	0.0197	0.117	125	2260	11.7	69.4	0.336	6.06	3.93	421
PDE-30	0.0135	0.113	144	2600	7.40	61.9	0.396	7.14	2.93	442
PDE-50	0.0088	0.116	135	2440	5.71	75.6	0.427	7.70	2.43	582

The Scatchard plot is useful for analysis of the binding process and to obtain the binding parameters, but it is difficult to get practical information about the bound preservative concentration at a given free preservative concentration. The saturation ratio *vs.* r curves,

14) H.E. Hart, *Bull. Math. Biophys.*, **27**, 87 (1965).

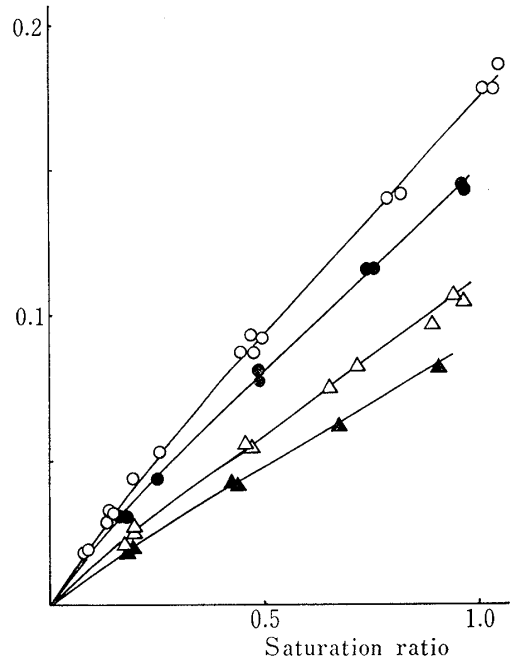

Fig. 4. Saturation Ratio *vs.* r Curves for PP in Aqueous PDE Solutions

Solid line was calculated using Eq. (5).
Saturation ratio: $[D_f]/0.034\%$
○: PDE-15, ●: PDE-20, △: PDE-30, ▲: PDE-50.

which correspond to adsorption isotherms, are given in Fig. 4. The lines were calculated by substituting the binding parameters for each surfactant into Eq. (5). Agreement between the calculated curves and the experimental values confirms that the binding process of PP with PDE can be adequately described in this way.

The binding parameters of PP to PDE are large in comparison with those of MP, so the ratio of free to toal preservative for PP is smaller than that for MP at a given concentration of surfactant. Although PP is effective in inhibiting the growth of microorganisms at a lower concentration than MP in water, the situation is complicated in the presence of surfactants. Therefore, the binding parameters may be helpful in selecting the most suitable preservative for a given preparation.

NMR Studies

Changes in environment resulting from interaction or solubilization can be followed by observing chemical shifts in the NMR spectra of the components. An upfield chemical shift corresponds to a change from a polar to a less polar environment.[15] Figure 5 shows the upfield chemical shift for the aromatic ring protons of PP in the presence of increasing concentrations of PDE-15. As the concentration increased, the ratio of bound PP to free PP increased, indicating that the environment of PP became less polar. The characteristic resonance lines of aqueous PDE-15 also showed an upfield shift in the presence of PP. The changes in chemical shifts for both the polyoxyethylene protons and the alkyl protons with

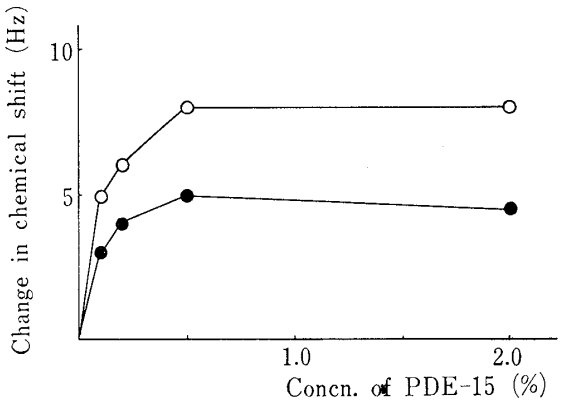

Fig. 5. Changes in Chemical Shift of Ring Protons of PP in the Presence of Varying Concentration of PDE-15

PP concn. : 0.04% in the total solution,
—○—: *o*-proton, —●—: *m*-proton.

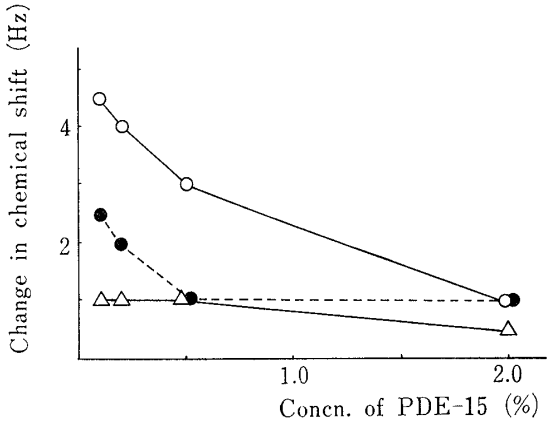

Fig. 6. Changes in Chemical Shift of PDE-15 Protons with Varying Concentrations of PDE-15 in the Presence of PP

PP concn. : 0.04% in the total solution,
——●——: polyoxyethylene protons,
——○——: methylene protons,
——△——: methyl protons.

15) J.C. Eriksson and G. Gillberg, *Acta Chem. Scand.*, **20**, 2019 (1966); J.J. Jacobs, R.A. Anderson, and T.R. Watson, *J. Pharm. Pharmacol.*, **23**, 148 (1971).

respect to the signals from the PDE-15 solution are presented in Fig. 6, for various concentrations of surfactant in the presence of 0.04% PP. There was a greater shift of the methylene signal than of the polyoxyethylene signal at high concentrations of surfactant and hence at low ratios of PP to PDE-15. At low concentrations of surfactant and therefore at high ratios of PP to PDE-15, the upfield shift increments of the polyoxyethylene protons increased.

To interpret the results in terms of the location of the bound material, it is necessary to consider the structure of the micelles. It has been stated[16] that solubilized molecules may reside in one or more of three different loci of a micelle; (1) within the hydrocarbon core, (2) within the polyoxyethylene mantle and (3) at the interface of these two loci.

A possible interpretation is therefore as follows. The preservative molecules may exist in or near the hydrocarbon core of the micelles at low ratios of PP to PDE-15. The very low solubility of PP in dodecane (*ca.* 0.015%) and the very small change in chemical shift for methyl protons of the surfactant suggest that PP molecules may be located at the oxyethylene-hydrocarbon interface, with the lipophilic propyl chain protruding into the hydrocarbon core. This may correspond to the primary class of sites represented by the Langmuir isotherm. At high ratios of PP to PDE-15, preservative molecules probably accumulate within the polyoxyethylene region, judging from the significant change in chemical shift for polyoxyethylene protons. It is considered that this corresponds to the second class of sites discussed previously. It is apparent that the interaction of PP with PDE is similar to that of MP with PDE.[3b]

Location of the Solubilizate within Micelles

Although many authors[12a,17] have studied the interaction of preservatives with surfactants, considerable differences with regard to the location of solubilizate molecules in nonionic surfactant micelles have been found. This type of discrepancy in the literature may be ascribed to a failure to consider a wide range of ratios of preservative to surfactant.

Based on the present series of experiments, it may be concluded that PP molecules are bound to two distinct loci within the PDE micelle; one has high affinity and a low capacity for PP molecules, while the other has low affinity and a large binding capacity. The probable locations of the solubilized PP within the micelle are the interface of the hydrocarbon core and the polyoxyethylene mantle for the high affinity-low capacity sites, and the polyoxyethylene mantle for the low affinity-large capacity sites.

If the solubilized PP molecules are located at the interface, the value of n_1 may be calculated based on the following assumption; (1) the hydrocarbon core is spherical and has the same density as dodecane, *i.e.* 0.751, (2) in view of molecular area data for some compounds at an air-water interface,[18] an adsorbed PP molecule occupies an area of 50 Å^2 and (3) a surfactant occupies an area of 25 Å^2 across the interface.[18] The interfacial area of a micelle can be calculated from the density, the molecular weight of the alkyl chain and the micellar aggregation number. The area available for adsorption is obtained by subtracting the area occupied by the surfactant portion from the interfacial area. The maximum number of PP molecules that can be situated at the interface of a micelle can then be calculated by dividing the available area by 50 Å^2, and division by the aggregation number gives the n_1 value. The aggregation number is expected to decrease with increasing ethylene oxide content and hence n_1 should increase with increasing chain length.

Application of this procedure to PDE-15 and PDE-30, taking 125 and 55 as the micellar

16) M. Donbrow and C.T. Rhodes, *J. Chem. Soc.*, **1964**, 6166; *idem*, *J. Pharm. Pharmacol.*, **18**, 424 (1966); P. Mukerjee, *J. Pharm. Sci.*, **60**, 1528 (1971).
17) J.J. Jacobs, R.A. Anderson, and T.R. Watson, *J. Pharm. Pharmacol.*, **23**, 786 (1971); T.C. Corby and P.H. Elworthy, *ibid.*, **23**, *Suppl.*, 49S (1971); M.J. Crooks and K.F. Brown, *ibid.*, **26**, 235 (1974).
18) The Chemical Society of Japan (ed.), "Jikken Kagaku Koza, Vol. 7, Kaimen Kagaku," Maruzen, Tokyo, 1956, pp. 325—332.

aggregation numbers, respectively,[19] gives n_1 values of 0.50 for PDE-15 and 0.82 for PDE-30. The experimental values for n_1 are much lower than these values, and do not show a tendency to increase as the number of oxyethylene units increases. This disagreement is not surprising in view of the oversimplification involved in the above assumptions. This treatment fails to consider (1) the configuration of ethylene oxide chains in the micelle, (2) the orientation of PP molecules relative to the oxyethylene chains and (3) the reorganization of the micelle with micellar solubilization. Although it is not strictly comparable, a surface tension study[20] has shown that the ethylene oxide chains of a surfactant form coils in the aqueous phase and their size (molecular area at the interface) increases markedly with increasing number of chain segments. Hence, a vertical orientation of the ethylene oxide chains can probably be ruled out in the micellar structure too, resulting in a decrease of the area available for adsorption with increasing chain length. This effect appears to cancel out completely the tendency of n_1 to increase with oxyethylene chain length. Moreover, it seems reasonable that the experimental values for n_1 are lower than the calculated ones, considering the steric hindrance of the oxyethylene chain to the adsorption of PP. It is well known that the solubilization process is accompanied by a reconstitution of the micelles when solubilizate molecules are dissolved in the micellar core,[21] but little is known about the micellar aggregation when solubilizate molecules are situated at the core-mantle interface or in the exterior of micelles. Further data are necessary, but the above calculation suggests that the values of n_1 are not necessarily unity.

The very low solubility of PP in dodecane and its remarkably high solubility in polyethylene glycol 400 (*ca.* 30%) support the view that the low affinity-large capacity sites are in the polyoxyethylene region. The values of $n_2 \cdot K_2$, shown in units of liters per mole in Table II, are not proportional to the number of oxyethylene units of the surfactants, though these values increase with increasing ethylene oxide chain length. This may be due to hydration of the polyoxyethylene mantle of the micelle. Some authors[22] have studied the hydration of nonionic surfactant micelles and found that the number of hydrating water molecules per ethylene oxide unit increased with increasing chain length. This effect may cause the above-mentioned change in $n_2 \cdot K_2$ values.

19) M.J. Schick (ed.), "Nonionic Surfactants," Marcel Dekker, Inc., New York, 1967, p. 495.
20) M.J. Schick, *J. Colloid Sci.*, **17**, 801 (1962).
21) M.J. Schick (ed.), "Nonionic Surfactants," Marcel Dekker, Inc., New York, 1967, p. 570.
22) M. Rosch, *Kolloid Z.*, **147**, 78 (1956); P.H. Elworthy, *J. Pharm. Pharmacol.*, **12**, 260T (1960); P.H. Elworthy and C.B. Macfarlane, *J. Chem. Soc.*, **1964**, 311.