[Chem. Pharm. Bull. 27(11)2557—2563(1979)]

UDC 547.587.12.03 : 615.451.011.4.014.2

Interaction of *p*-Hydroxybenzoic Acid Esters with Polyethylene Glycol

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(Received January 27, 1979)

The interactions of methyl and propyl *p*-hydroxybenzoate with polyethylene glycol (PEG) 4000 were investigated by means of an ultrafiltration technique. The results show that the interaction is a weak and nonspecific process of large binding capacity, and is a type of partitioning phenomenon between the macromolecule and the aqueous phase. The characteristics of this interaction are quite similar to those of the binding of *p*-hydroxybenzoates with the polyoxyethylene region of polyoxyethylene dodecyl ether (PDE) micelles. A solubility study of *p*-hydroxybenzoates in aqueous solutions of PEG-400 was also performed. Assuming that the increase in solubility of the preservatives in the presence of PEG was due to the interaction with PEG, the ratio of bound preservative concentration to PEG concentration was calculated. The ratio increased as the concentration of PEG in the solvent increased, and this was thought to be due in part to a dehydrating effect. A comparison of solubility data with the binding parameters of *p*-hydroxybenzoates for the secondary binding sites of PDE micelles provided information concerning the micellar hydration. The amount of water trapped by nonionic surfactant micelles was estimated and the values thus obtained were consistent with those reported elsewhere.

Keywords——nonionic surfactant; polyethylene glycol; preservative; ultrafiltration; binding parameters; solubilization; micellar hydration

Our previous studies[2] were concerned with the interaction of *p*-hydroxybenzoates with polyoxyethylene dodecyl ethers (PDE) and the location of the bound preservative molecules within the surfactant micelles. Experimental evidence was offered to support the existence of two kinds of interaction mechanisms between the preservative and the micelles; one class of sites showed a low affinity but a large binding capacity, and this binding appeared to involve a nonspecific partitioning of the preservative molecules into the polyoxyethylene region of the micelles. These findings suggest that there may be some interaction between *p*-hydroxybenzoates and polyethylene glycol (PEG). As PEG is widely used for pharmaceutical formulations, additional information on the interaction of *p*-hydroxybenzoate with PEG is desirable to determine the total preservative concentration required to provide an effective free preservative concentration sufficient for protection from microbial spoilage.

Several workers[3] have reported the existence of an interaction between phenolic compounds and PEG, though the experimental method employed was a solubility technique, which would not permit full characterization of the binding phenomena since the free preservative concentration was invariant throughout the experiments. A convenient method for investigating an under-saturated system of this type is an ultrafiltration technique using a Diaflo membrane which is permeable to the preservative and impermeable to the macromolecule.

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3) *a*) T. Higuchi and J.L. Lach, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 465 (1954); *b*) D. Guttman and T. Higuchi, *ibid.*, **45**, 659 (1956); *c*) J.L. Lach, K. Ravel, and S.M. Blaug, *ibid.*, **46**, 615 (1957); *d*) G.M. Miyawaki, N.K. Patel, and H.B. Kostenbauder, *ibid.*, **48**, 315 (1959).

In the present paper, we report a study on polyethylene glycol 4000 (PEG-4000) and *p*-hydroxybenzoate binding utilizing an ultrafiltration technique over a wide range of preservative–macromolecule ratios. Studies were also performed on the solubility of *p*-hydroxybenzoate in polyethylene glycol 400 (PEG-400) in order to substantiate our previous interpretation of the binding phenomena of *p*-hydroxybenzoate with PDE.

Experimental

Materials——Methyl *p*-hydroxybenzoate (MP), propyl *p*-hydroxybenzoate (PP), polyethylene glycol 400 (PEG-400) and polyethylene glycol 4000 (PEG-4000) were J.P. IX grade. The Diaflo membrane, UM-10, 43 mmϕ, a cross-linked dextran gel membrane, was purchased from Amicon Corp., Mass., U.S.A.

Ultrafiltration Procedure and Quantitative Analysis——PEG-4000 was suited to ultrafiltration studies by virtue of its high molecular weight. The technique used in characterizing the interaction between *p*-hydroxybenzoate and PEG-4000 was essentially the same as that described previously.[2] All procedures were carried out at a temperature of 25°.

Solubility Determination——Aqueous PEG-400 solution was used as a solvent. An excess of *p*-hydroxybenzoate was placed in a 50 ml glass-stoppered bottle together with 20 ml of solvent. The bottle was kept at a temperature of 25° for at least a week with occasional stirring to allow the solution to reach equilibrium. An aliquot of the suspension was filtered through a No. 5 sintered glass filter. The first few milliliters of the filtrate were discarded. The sample was greatly diluted with water and the concentration of *p*-hydroxybenzoate in the solution was determined spectrophotometrically at a wavelength of 256 nm.

Results and Discussion

Interaction of *p*-Hydroxybenzoate with PEG-4000

The ultrafiltration data for MP and PP in the presence of PEG-4000 are shown in Table I. The concentrations of total preservative in the solution, $[D_t]$, and of free preservative in the aqueous phase, $[D_f]$, were determined directly by analyzing the initial solution and the filtrate obtained by means of ultrafiltration. The concentration of bound preservative in the total solution, $[D_b]$, and the apparent partition coefficient, K'_m were calculated using the following equations as described previously.[2b]

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

$$K_m' = \frac{[D_b]/[P]}{[D_f]/100} \tag{2}$$

where $[P]$ is the concentration of PEG in the total solution. The saturation ratio is given by $[D_f]/0.22\%$ (solubility of MP in water) for MP or $[D_f]/0.034\%$ (solubility of PP in water) for PP.

The results support the existence of an interaction between the preservatives and PEG-4000. As already reported,[2b,c] K'_m values for *p*-hydroxybenzoates between micelles and the aqueous phase of polyoxyethylene (15 mol of ethylene oxide) dodecyl ether (PDE-15) solutions were *ca.* 100 for MP and *ca.* 600 for PP. A comparison of these values with the results in Table I indicates that *p*-hydroxybenzoates are bound more extensively to PDE micelles than to PEG.

In Fig. 1 the data are plotted in the form of a Scatchard plot, which can be written as follows,

$$\frac{r}{[D_f]} = n \cdot K - r \cdot K \tag{3}$$

where n and K are the number of binding sites and the association constant, respectively; and r is the ratio of bound preservative concentration to PEG-4000 concentration. The plot appears to be horizontal, within the limits of experimental error. Inspection of Eq.

Table I. Binding of Preservative to PEG-4000

PR[a]	% of PEG-4000 in total solution [P]	% of total PR in total solution [D_t]	% of free PR in aqueous phase [D_f]	Saturation ratio	% of bound PR in total solution [D_b]	Apparent partition coefficient K_m'
MP	1	0.242	0.225	1.02	0.0193	8.58
	1	0.193	0.176	0.800	0.0188	10.7
	1	0.125	0.115	0.523	0.0112	9.74
	1	0.121	0.114	0.518	0.0081	7.11
	2	0.122	0.107	0.486	0.0171	7.99
	2	0.121	0.106	0.482	0.0171	8.07
	1	0.0486	0.0453	0.206	0.0038	8.39
	2	0.0477	0.0410	0.186	0.0075	9.15
PP	2	0.0439	0.0329	0.968	0.0117	17.8
	2	0.0348	0.0264	0.776	0.0089	16.9
	1	0.0216	0.0182	0.535	0.0036	19.8
	2	0.0216	0.0168	0.494	0.0051	15.2
	1	0.0092	0.0078	0.229	0.0015	19.2
	1	0.0086	0.0073	0.215	0.0014	19.2
	2	0.0092	0.0070	0.206	0.0023	16.4

a) Preservative.

(3) indicates that, since the slope is nearly zero, the value of K is almost zero and the value of n becomes infinity if $n \cdot K$ is constant. Thus, Eq. (3) can be written as

$$\frac{r}{[D_f]} = n \cdot K \tag{4}$$

As $r=[D_b]/[P]$, it follows from Eqs. (2) and (4) that

$$K_m' = \frac{r}{[D_f]/100} = 100 \cdot n \cdot K \tag{5}$$

Thus, the value of $100 \cdot n \cdot K$ corresponds to an apparent partition coefficient. These values were 8.7 for MP and 18 for PP.

In this case, the interaction can be interpreted as a weak and nonspecific process of large binding capacity which is analogous to a simple partitioning of the preservative between the macromolecules and the aqueous phase. The characteristics of this interaction are quite similar to those of the interaction of *p*-hydroxybenzoate with the polyoxyethylene region of nonionic surfactant micelles, as described in previous papers.[2b,c] The values of $100 \cdot n_2 \cdot K_2$ for PDE-15, which correspond to the apparent partition coefficients for *p*-hydroxybenzoates between the polyoxyethylene mantle of the micelles and the aqueous phase, were 71 for MP and 458 for PP in terms of grams per 100 milliliters. These values and the K'_m values shown in Table I indicate that PP is bound to a greater extent than MP, but both the difference and the ratio of binding for these two preservatives with PEG are less than those with PDE.

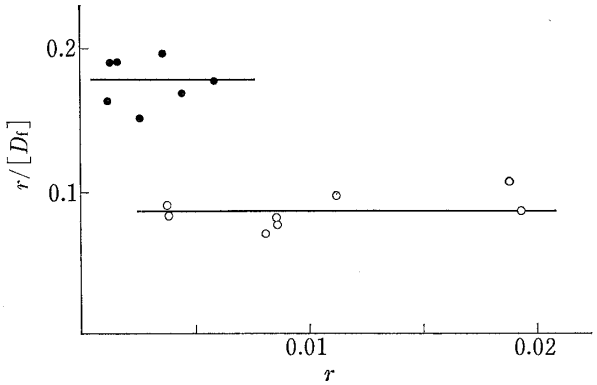

Fig. 1. Scatchard Plots for the Interaction of *p*-Hydroxybenzoate with PEG-4000

—○—, MP; —●—, PP.

The solubility data for MP and PP in aqueous solutions of PEG-4000 reported by Kostenbauder[3d] were also available for the calculation of the apparent partition coefficients. The values thus obtained were about 9 for MP and about 14 for PP at 2% PEG-4000. The values obtained here by means of the ultrafiltration technique are in satisfactory agreement with those reported using a solubility method. This suggests that the binding process is essentially a simple interaction similar to a partitioning mechanism over a wide range of free preservative concentration, and that the increase in the solubility of preservative in the presence of PEG-4000 is attributable to the interaction between them.

Solubility of *p*-Hydroxybenzoate in Aqueous PEG-400 Solution

The tentative conclusion drawn from the ultrafiltration study might hold only at a definite macromolecule concentration. Moreover, further experiments to elucidate the difference in binding between PEG and the polyoxyethylene region of PDE micelles were necessary. Since PEG does not form a distinct structure in aqueous solution, whereas nonionic surfactants form micelles above the critical micelle concentration, solubility studies were conducted at a much higher concentration of PEG than that used in the ultrafiltration experiments.

Kostenbauder[3d] determined the solubilities of MP and PP in aqueous solutions containing up to 10% PEG-4000. In the present study, solubility determinations of MP and PP in aqueous solutions containing various concentrations of PEG-400 from 0 to 75% were performed. Semi-logarithmic plots of solubility as a function of PEG-400 concentration are shown in Fig. 2. An exponential increase in solubility can be seen over the range from 0 to 75% PEG-400.

The effect of electrolytes on the solubilities of various nonelectrolytes in water has been widely studied. The salting-in phenomenon is described by the equation[4]

$$\log \frac{\gamma_0}{\gamma} = \log \frac{L}{L_0} = k_s \cdot C_s \qquad (6)$$

where γ_0 and γ are the activity coefficients of the nonelectrolyte in salt-free solution and in salt solution, respectively; L_0 and L are the solubilities of the nonelectrolyte in pure water and in salt solution at a salt concentration of C_s, respectively; and k_s is the salting-in parameter. In this study, the exponential increase in the solubility of *p*-hydroxybenzoate is nearly proportional to the PEG-400 concentration, and so the effect of PEG-400 is quite similar to that of electrolytes on the solubilities of various nonelectrolytes in water; PEG-400 behaves as a "structure-breaker" since $k_s > 0$. This result suggests that the interaction between *p*-hydroxybenzoate and PEG is relatively simple and nonspecific, as the solubility behavior of the preservative in PEG solution conforms to the general rule expressed by Eq. (6).

Assuming that the increase in solubility of the preservative in the presence of PEG-400 is due to the binding between them, the concentration of bound species can be calculated using Eq. (1), where $[D_f]$ is the solubility of *p*-hydroxybenzoate in water. Thus, the ratio of the bound preservative concentration to the PEG-400 concentration can be obtained. The ratio thus estimated is plotted against the PEG-400 concentration in Fig. 3.

It has been suggested that the interaction between *p*-hydroxybenzoate and PEG-4000 is similar to a simple partitioning phenomenon in a certain range of PEG concentration, but the results depicted in Fig. 3 show that the interaction is undoubtedly more complex than this. The most significant feature of this interaction is the remarkable dependency on the concentration of PEG-400; namely, the ratio of bound preservative concentration to polymer concentration increases with increasing concentration of PEG in the solvent. This may be due in part to a dehydrating effect. It can be considered that the hydrophilic polymer is

4) F.A. Long and W.F. McDevit, *Chem. Rev.*, **51**, 119 (1952); The salt parameter in this report corresponds to $(-k_s)$ in the present paper.

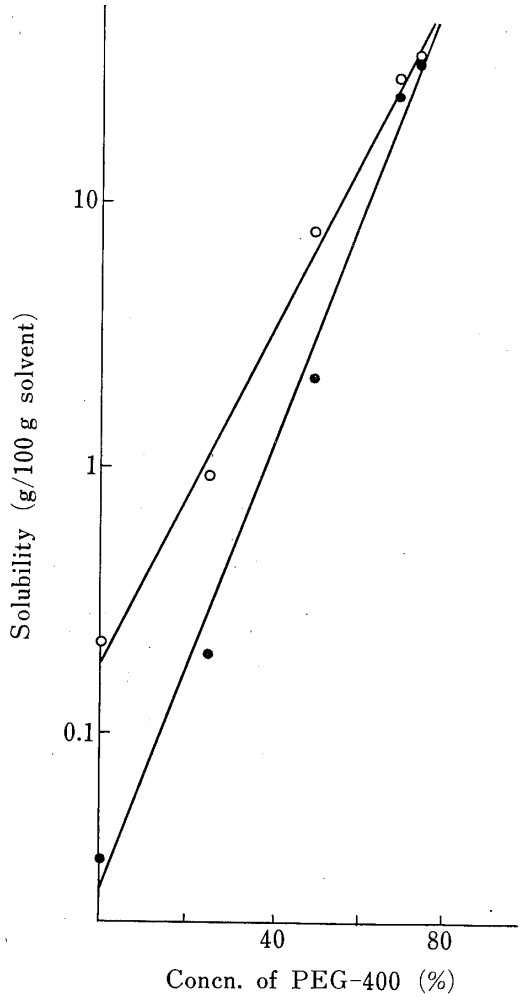

Fig. 2. Solubility of MP and PP in Aqueous Solutions of PEG-400

—○—, MP; —●—, PP.

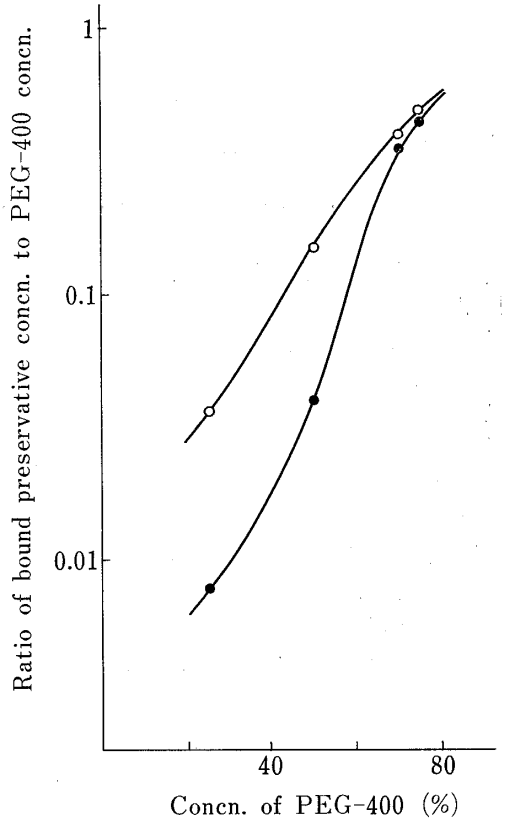

Fig. 3. Ratio of Bound Preservative Concentration to PEG-400 Concentration as a Function of PEG-400 Concentration

—○—, MP; —●—, PP.

associated with water molecules in the aqueous solution. When the concentration of this polymer in the solvent increases, the solvation is suppressed and this effect may favor the competing preservative-macromolecule interaction.

Binding Mechanism between *p*-Hydroxybenzoate and the Polyoxyethylene Mantle of Micelles

Since the polyoxyethylene mantle of the micelles can be taken to have properties similar to those of an aqueous solution of polyethylene glycol, a comparison of the solubility behavior of preservative in PEG-400 solution with the binding parameters for the interaction of the preservative with a nonionic surfactant of the polyoxyethylene type may provide valuable information concerning the hydration of the micelles. The binding of *p*-hydroxybenzoate with PDE can be adequately described as an interaction with two classes of sites,[2b,c]

$$r = \frac{[D_b]}{[S]} = \frac{[D_b]_1+[D_b]_2}{[S]} = \frac{n_1 \cdot K_1 \cdot [D_f]}{1+K_1 \cdot [D_f]} + n_2 \cdot K_2 \cdot [D_f] \qquad (7)$$

where $[S]$ is the concentration of surfactant and a numerical subscript indicates the class of binding sites. The binding parameters for the interaction between the preservative and the polyoxyethylene mantle, *i. e.*, for the second class of sites, are cited as the product of n_2 and K_2 in Table II, which are expressed in terms of grams per 100 ml instead of moles per liter. For convenience in the following discussion, these parameters describing the distribu-

tion of preservative between the micellar and aqueous phases were used to calculate the preservative concentration in the polyoxyethylene portion of the micelle. From Eq. (7), we have

$$\frac{[D_b]_2}{[S]} = n_2 \cdot K_2[D_f] \tag{8}$$

If the molecular weights of the surfactant and polyoxyethylene portion are M_s and M_p respectively, it follows that

$$\frac{[D_b]_2}{[S]} \cdot \frac{M_s}{M_p} = \frac{[D_b]_2}{[P]} = n_2 \cdot K_2 \cdot [D_f] \cdot \frac{M_s}{M_p} \tag{9}$$

where $[P]$ is the concentration of polyoxyethylene in the total solution. The ratio of $[D_b]_2$ to $[P]$ at any given value of $[D_f]$ in the PDE-preservative system can be evaluated using Eq. (9). It has been stated that the interaction of *p*-hydroxybenzoate with the polyoxyethylene mantle of micelles is similar to a simple partitioning phenomenon. Accordingly, when the PDE solution is saturated with the preservative, *i. e.* $[D_f]$ is 0.22% for MP or 0.034% for PP, the value of $[D_b]_2/[P]$ may correspond to the solubility of *p*-hydroxybenzoate in the polyoxyethylene portion of micelles. These values, shown in Table II, can be directly compared with the results in Fig. 3. It is now possible to estimate the amount of water held within the polyoxyethylene region of a micelle. In Table II, the results are expressed as the concentration of PEG-400 in the aqueous solution and also as the number of water molecules per ethylene oxide unit of PEG-400 (assuming that the average molecular weight and the number of oxyethylene units are 414.5 and 9, respectively).[5]

Table II. Micellar Hydration estimated from $n_2 \cdot K_2$ Values and Solubility Data

Preservative	Surfactant[a]	$n_2 \cdot K_2$[b]	$[D_b]_2/[P]$	% of PEG[c]	Water molecules/ethylene oxide unit
MP	PDE-8	1.00	0.335	65	1.4
	PDE-10	0.787	0.246	59	1.8
	PDE-15	0.710	0.200	55	2.1
	PDE-20	0.648	0.173	52	2.4
	PDE-30	0.559	0.140	49	2.7
	PDE-50	0.452	0.108	44	3.3
PP	PDE-15	4.58	0.199	64	1.4
	PDE-20	3.93	0.162	61	1.6
	PDE-30	2.93	0.114	59	1.8
	PDE-50	2.43	0.090	57	1.9

a) Each number denotes the nominal number of oxyethylene units per molecule.
b) T. Shimamoto and Y. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **23**, 3088 (1975); T. Shimamoto, H. Mima and M. Nakagaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 1995 (1979).
c) Estimated from Fig. 3.

It is presumed that here the hydration includes all the arrangements of water molecules relative to polyoxyethylene chains, such as hydrogen bonding, orientation by dipole–dipole forces and physically trapped water both within and between the hydrophilic chains. The amount of hydrating water around the ether oxygen or within the glycol structure of a PDE micelle is likely to range from 1.4 to 3.3 molecules for each ethylene oxide and to increase as the number of oxyethylene units in the chain increases. From viscosity and micellar studies, Kushner *et al.*[6] estimated that 43 water molecules were associated with a polyoxy-

5) M. Windholz (ed.), "The Merck Index," 9th ed., Merck and Co., Inc., Rahway, N.J., 1976, p. 984.
6) L.M. Kushner and W.D. Hubbard, *J. Phys. Chem.*, **58**, 1163 (1954).

ethylene (10 units of oxyethylene) chain in a micelle of Triton X100,[7] and suggested that 20 of these molecules were held by hydrogen bonding to the ether oxygen atoms, and the rest were trapped by the chain. Other authors[8] gave a value of 2 molecules of water per ether oxygen for polyoxyethylene (7 units of oxyethylene) cetyl ether and 6 molecules for polyoxyethylene (24 units of oxyethylene) cetyl ether using viscosity and vapor pressure data. They also showed that the number of water molecules per oxygen atom increased with increasing ethylene oxide chain length. Another value,[9] of 1 to 4 water molecules per ether oxygen, depending upon the surfactant used, was based on measurements of viscosity and heat of hydration. Various arrangements of water molecules relative to the ethylene oxide chains can be considered. For example, there is less space for water molecules close to the hydrocarbon core of the micelle than nearer the outside of the micelle, so the number of hydrating water molecules per ethylene oxide unit may increase with increasing chain length. Furthermore, this may be related to the fact that the micellar aggregation number for a given hydrophobic group decreases as the polyoxyethylene chain length increases.

Although the hydration of nonionic surfactant micelles has been estimated by an indirect method in the present study, the values obtained are in fair agreement with those reported elsewhere. This finding substantiates the view that the secondary binding process described in the previous papers[2b,c] is a type of partitioning phenomenon and that the polyoxyethylene mantle of the micelle has properties similar to those of an aqueous solution of PEG. The amount of hydration estimated using the binding data for PP is rather small compared with that in the case of MP. This implies that PP molecules cause some reorganization of the micelles, leading to a greater binding capacity.

7) Poloxyethylene (10 mol) octylphenyl ether.
8) 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.
9) G. Boehmke and R. Heush, *Fette, Seifen, Anstrichm.*, **62**, 87 (1960).