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Effect of Polyols on the Interaction of *p*-Hydroxybenzoic Acid Esters with Polyoxyethylene Dodecyl Ether

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The effect of polyols, *i.e.*, glycerol, propylene glycol and 1,3-butylene glycol, on the interaction between *p*-hydroxybenzoates and polyoxyethylene (15 units of oxyethylene) dodecyl ether was investigated by an ultrafiltration technique using a Diaflo membrane. Polyols had little effect on the binding of *p*-hydroxybenzoate to the primary class of sites in the nonionic surfactant micelles. In this binding process, bound preservative molecules are thought to be located at the oxyethylene-hydrocarbon interface, so it seems possible that polyols added to the system were too hydrophilic to penetrate deeply into the micelles and to compete effectively with this class of bound preservative. However, polyols modified the binding to the secondary class of sites to some extent; namely, these compounds could displace a part of the bound preservative from the polyoxyethylene region of the micelles. 1,3-Butylene glycol was the most effective polyol; glycerol was somewhat less effective in enhancing the preservative activity.

Keywords——nonionic surfactant; polyol; preservative; ultrafiltration; binding parameter; solubilization; micelle

Many reports have appeared on the inactivation of preservatives in the presence of nonionic surfactants. It is generally accepted that preservative molecules bound within surfactant micelles are inactive, and that the antimicrobial activity is directly related to the concentration of free preservative in the aqueous phase.[2] From a practical point of view, some authors[3] reported a potentiating effect of propylene glycol on the antimicrobial activity of preservatives in liquid pharmaceutical preparations. Hibbott[4] studied the prevention of spoilage of cosmetic emulsions by microorganisms and found that when propylene glycol was present in the aqueous phase, a more favorable distribution of methyl *p*-hydroxybenzoate between the water and oil phases was attained. Recently, Blanchard[5] reported the effect of sorbitol on the interaction of phenolic preservatives with polysorbate 80, showing that sorbitol had little effect on the binding of preservatives with surfactant micelles.

The present paper is concerned with the effect of polyols on the interaction between *p*-hydroxybenzoic acid esters and polyoxyethylene (15 units of oxyethylene) dodecyl ether (PDE-15). Glycerol, propylene glycol and 1,3-butylene glycol were studied to determine how these materials function and contribute to the activation of bound methyl *p*-hydroxybenzoate (MP) or propyl *p*-hydroxybenzoate (PP) within PDE-15 micelles.

Experimental

Materials——Methyl *p*-hydroxybenzoate (MP), propyl *p*-hydroxybenzoate (PP), glycerol and propylene glycol were of J.P. IX grade. Polyoxyethylene (15 units of oxyethylene) dodecyl ether (PDE-15) and 1,3-

1) Location: *Juso-Honmachi, Yodogawa-ku, Osaka, 532, Japan.*
2) M. Aoki, A. Kamata, I. Yoshioka, and T. Matsuzaki, *Yakugaku Zasshi*, **76**, 939 (1956); N.K. Patel and H.B. Kostenbauder, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 289 (1958); F.D. Pisano and H.B. Kostenbauder, *ibid.*, **48**, 310 (1959); M. Barr and L.F. Tice, *ibid.*, **46**, 445 (1957); S.M. Blaug and S.S. Ahsan, *J. Pharm. Sci.*, **50**, 138 (1961).
3) M. Barr and L.F. Tice, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 217 (1957); P.S. Prickett, H.L. Murray, and N.H. Mercer, *J. Pharm. Sci.*, **50**, 316 (1961).
4) H.W. Hibbott and J. Monks, *J. Soc. Cosmetic Chemists*, **12**, 2 (1961).
5) J. Blanchard, W.T. Fink, and J.P. Duffy, *J. Pharm. Sci.*, **66**, 1470 (1977).

butylene glycol were of commercial grade, supplied by Nihon Emulsion Co., Tokyo and Eastman Organic Chemicals, N.Y., U.S.A., respectively. A Diaflo membrane, UM-10, 43 mmϕ (cross-linked dextran gel membrane), was purchased from Amicon Corp., Mass., U.S.A.

Measurements of Surfactant Concentration and Critical Micelle Concentration (CMC)——The concentration of PDE-15 was measured by a surface tension method, and the CMC of PDE-15 was determined by a conventional method, using an ST-1 surface tensometer, Shimadzu Seisakusho, Kyoto.

Ultrafiltration and Quantitative Analysis——The binding of *p*-hydroxybenzoate with PDE-15 was characterized by an ultrafiltration technique and spectrophotometric assay as described previously.[6] The assay procedure of *p*-hydroxybenzoate presented no problems, since neither PDE-15 nor polyols interfered with the spectrophotometric assay of the preservatives. All procedures were carried out at a temperature of 25°.

Results and Discussion

CMC of PDE-15

Plots of the surface tension of PDE-15 solutions against PDE-15 concentration showed a sharp break at a concentration of 6.0×10^{-2}%, which corresponded to the CMC. This value is in reasonable agreement with the reported value.[6a,7]

Effect of Polyols on Ultrafiltration

Aqueous solutions of PDE-15 in the presence and absence of polyols were filtered through a Diaflo UM-10 membrane. Figure 1 shows the surfactant concentration in each fraction. Although a small amount of PDE-15 passed through the membrane, the concentrations in the filtrates were far below the CMC. Thus polyols had little effect on the ability of ultrafiltration to remove PDE-15 micelles from the surfactant solution.

The effect of polyols on the ultrafiltration of *p*-hydroxybenzoate solution was also studied. The plots (Fig. 2) show the *p*-hydroxybenzoate concentration in each fraction from the ultrafiltration in the presence and absence of polyol. As the curves in the presence and absence of polyol coincided well, it appears that polyols had no effect on the ultrafiltration of *p*-hydroxybenzoate solution under these experimental conditions. Thus, ultrafiltration using the Diaflo membrane should be suitable for measurement of the binding characteristics of *p*-hydroxybenzoate-surfactant systems containing polyols.

Effect of Polyols

There are many ways to express interaction data of preservatives with surfactant micelles. If the preservative concentration bound within the micelles is not proportional to the free preservative concentration in the aqueous phase, simple partition indices have little meaning.[6b,c,8] However, binding phenomena in under-saturated systems can be represented by the following expression,[9]

$$r=\frac{[D_b]}{[S]}=\frac{n\cdot K\cdot[D_f]}{1+K\cdot[D_f]} \qquad (1)$$

where n and K are constants corresponding to the number of binding sites and the association constant, respectively; $[S]$ is the concentration of the surfactant; $[D_b]$ and $[D_f]$ are the concentrations of bound preservative in the total solution and free preservative in the aqueous phase, respectively; and r is the ratio of bound preservative to surfactant. The value of $[D_b]$ can be calculated from the data on $[D_f]$, $[S]$ and the concentration of total preservative

6) *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); *c*) T. Shimamoto, H. Mima, and M. Nakagaki, *ibid.*, **27**, 1995 (1979).
7) P.H. Elworthy and C.B. Macfarlane, *J. Pharm. Pharmacol.*, **17**, 65 (1965).
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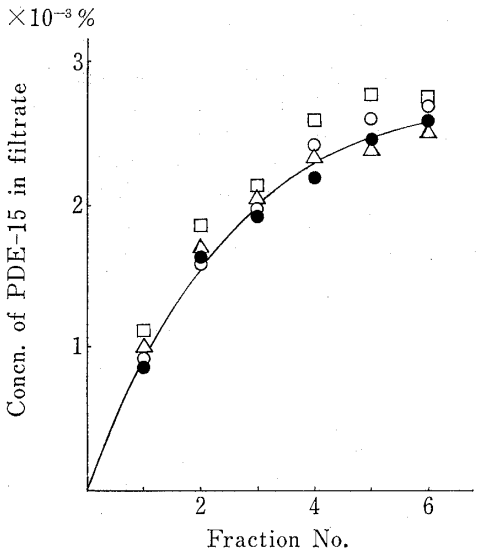

Fig. 1. Ultrafiltration of PDE-15 Solution in the Presence and Absence of Polyols

The filtrates were collected in 4 ml fractions. The PDE-15 concentration of feed solution was 0.5%.
Closed symbols: in the absence of polyol.
Open symbols: in the presence of 1% polyol.
○, glycerol; △, propylene glycol; □,1,3-butylene glycol.

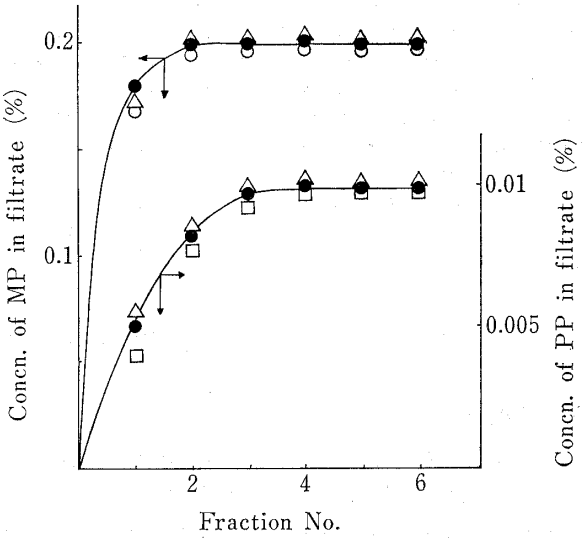

Fig. 2. Ultrafiltration of *p*-Hydroxybenzoate Solution in the Presence and Absence of Polyols

The filtrates were collected in 4 ml fractions. The MP and PP concentrations of feed solutions were 0.2% and 0.01%, respectively.
Closed symbols: in the absence of polyol.
Open symbols: in the presence of 1% polyol.
○, glycerol; △, propylene glycol; □, 1,3-butylene glycol.

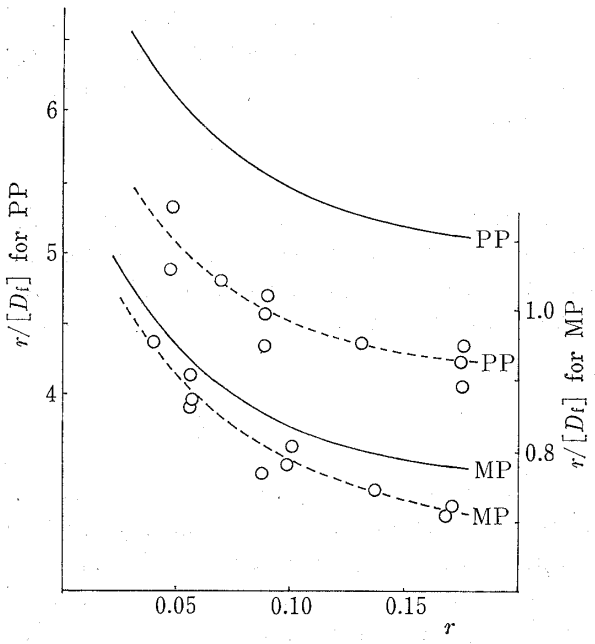

Fig. 3. Scatchard Plots for the Interaction of *p*-Hydroxybenzoate with PDE-15 in the Absence and Presence of Glycerol

The concentrations of PDE-15 and glycerol were maintained at 1%.
———, in the absence of glycerol;
—○—, in the presence of glycerol.

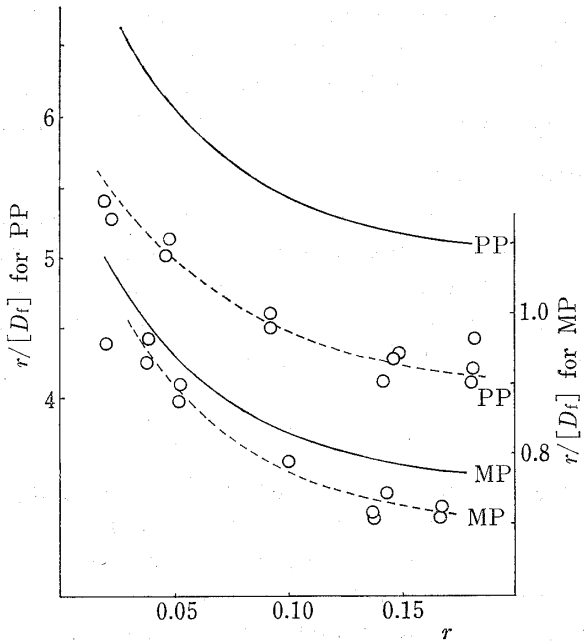

Fig. 4. Scatchard Plots for the Interaction of *p*-Hydroxybenzoate with PDE-15 in the Absence and Presence of Propylene Glycol

The concentrations of PDE-15 and propylene glycol were maintained at 1%.
———, in the absence of propylene glycol;
—○—, in the presence of propylene glycol.

in the solution, $[D_t]$, as described previously.[6b] For graphical treatment of experimental data, a Scatchard plot is generally useful; this can be written as follows,

$$\frac{r}{[D_f]} = n \cdot K - r \cdot K \qquad (2)$$

Scatchard plots of the interaction of MP and PP with PDE-15 in the presence and absence of polyols are shown in Fig. 3, 4 and 5. The concentrations of surfactant and polyol in the sample solutions were both maintained at 1% so that the ratio of polyol to surfactant was constant in a given experiment. The plots of the binding of *p*-hydroxybenzoate with PDE-15 in the absence of polyol are cited from previous studies.[6b,c] The presence of polyol in the system resulted in a downward displacement of the binding curve, indicating a possible inhibition of the interaction between *p*-hydroxybenzoate and PDE-15 in the presence of polyol.

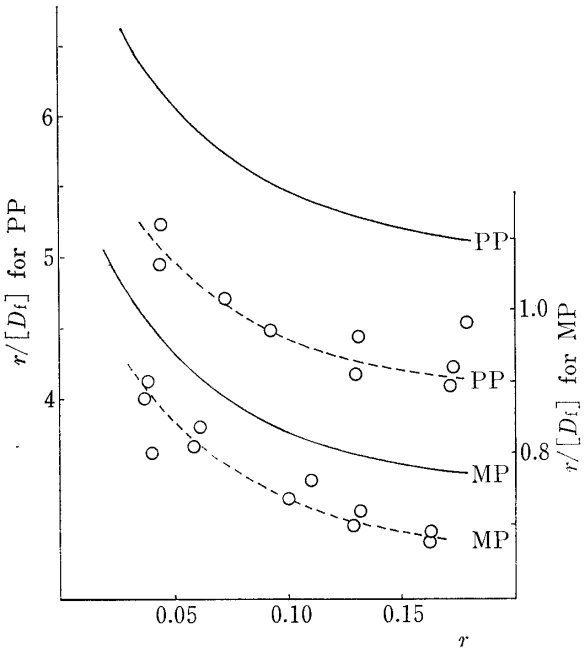

Fig. 5. Scatchard Plots for the Interaction of *p*-Hydroxybenzoate with PDE-15 in the Absence and Presence of 1,3-Butylene Glycol

The concentrations of PDE-15 and 1,3-butylene glycol were maintained at 1%.
————, in the absence of 1,3-butylene glycol;
—○—, in the presence of 1,3-butylene glycol.

All the plots were curved, suggesting the presence of more than one class of binding sites. In resolving these complex interactions, data were fitted to a four-parameter model using a modification of the method of Hart[10] and a programmable computer (JEC-5 spectrum computer). The binding data can be considered in terms of an interaction with two classes of sites, and the calculated parameters are shown in Table I. Thus the interaction in this study was expressed by the following relation.

$$r = \frac{[D_b]}{[S]} = \frac{n_1 \cdot K_1 \cdot [D_f]}{1 + K_1 \cdot [D_f]} + \frac{n_2 \cdot K_2 \cdot [D_f]}{1 + K_2 \cdot [D_f]} \qquad (3)$$

At high values of r, the curve approached a horizontal asymptote, indicating that this class of binding sites has a high capacity but a low affinity, resembling a simple partition process. Since $[D_f] < 0.0145$ mol/l (for MP) or $[D_f] < 0.0019$ mol/l (for PP) and $K_2 \cdot [D_f] \ll 1$, Eq. (3) could be rewritten as:

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

The polyols studied here had little effect on the binding of *p*-hydroxybenzoate to the primary class of sites, but in the second class of sites, the values of $n_2 \cdot K_2$ showed a decrease of about 10—15%.

The effects of polyols on the ratios of free to total preservative are shown in Table II. These values were calculated by substituting the binding parameters into Eq. (4). A slight increase in the ratio of free (or available) to total preservative was noted in the presence of polyols, indicating that a more favorable distribution of preservative between water and micelles was obtained. Although no pronounced differences in the effect of polyols could

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

Table I. Binding Parameters for the Interaction of *p*-Hydroxybenzoate with PDE-15[a] at 25° in the Presence of Polyol[a]

p-Hydroxy-benzoate	Polyol	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)
MP	None[b]	0.0223	0.124	27.0	410	3.70	20.6	0.192	2.92	0.710	60.1
	GL[c]	0.0238	0.133	27.5	418	3.30	18.4	0.200	3.04	0.660	55.9
	PG[c]	0.0219	0.122	27.4	417	3.75	20.9	0.177	2.69	0.663	56.2
	BG[c]	0.0222	0.124	26.6	404	3.32	18.5	0.189	2.88	0.628	53.3
PP	None[d]	0.0230	0.108	137	2480	12.3	57.6	0.373	6.71	4.58	386
	GL	0.0223	0.105	126	2270	10.8	50.9	0.353	6.36	3.82	324
	PG	0.0224	0.105	122	2190	12.0	56.6	0.315	5.67	3.79	321
	BG	0.0219	0.103	126	2280	11.7	55.0	0.319	5.75	3.73	316

a) The concentrations of surfactant and polyol were maintained at 1%.
b) T. Shimamoto and Y. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **23**, 3088 (1975).
c) GL, glycerol; PG, propylene glycol; BG, 1,3-butylene glycol.
d) T. Shimamoto, H. Mima and M. Nakagaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 1995 (1979).

Table II. Binding of Preservative with PDE-15[a] at 25° in the Absence and Presence of Polyol[a]

Preservative	Polyol	% of $[D_t]$[b]	% $[D_f]$	% of $[D_b]$	$[D_f]/[D_t]$
MP	None	0.350	0.195	0.157	0.557
		0.250	0.137	0.114	0.548
		0.160	0.0851	0.0756	0.532
		0.0800	0.0403	0.0401	0.504
	GL[c]	0.350	0.200	0.152	0.571
		0.250	0.140	0.111	0.560
		0.160	0.0868	0.0741	0.543
		0.0800	0.0409	0.0396	0.511
	PG[c]	0.350	0.201	0.151	0.574
		0.250	0.141	0.111	0.564
		0.160	0.0874	0.0734	0.546
		0.0800	0.0414	0.0392	0.518
	BG[c]	0.350	0.205	0.148	0.586
		0.250	0.144	0.108	0.576
		0.160	0.0892	0.0716	0.558
		0.0800	0.0422	0.0382	0.528
PP	None	0.180	0.0291	0.151	0.162
		0.130	0.0204	0.110	0.157
		0.0800	0.0118	0.0683	0.148
		0.0400	0.00543	0.0346	0.136
	GL	0.180	0.0337	0.147	0.187
		0.130	0.0235	0.106	0.181
		0.0800	0.0137	0.0665	0.171
		0.0400	0.00627	0.0338	0.157
	PG	0.180	0.0339	0.147	0.188
		0.130	0.0237	0.106	0.182
		0.0800	0.0138	0.0663	0.173
		0.0400	0.00633	0.0337	0.158
	BG	0.180	0.0343	0.146	0.191
		0.130	0.0240	0.106	0.185
		0.0800	0.0140	0.0662	0.175
		0.0400	0.00640	0.0337	0.160

a) The concentrations of PDE-15 and polyol were maintained at 1%.
b) Calculated using the equation, $[D_t]=[D_b]+[D_f]\ (1-[S]/100)$.
c) GL, Glycerol; PG, propylene glycol; BG, 1,3-butylene glycol.

be found, these results showed that 1,3-butylene glycol was the most effective, while glycerol was somewhat less effective in enhancing the preservative activity. The greatest increase in the ratio was observed at the highest preservative concentration, because polyols influenced only the second class of binding sites.

As has been reported in the previous papers,[6b,c] bound preservative molecules are probably located at the junction of the hydrocarbon core and the polyoxyethylene mantle of micelles in the primary class of sites. On the basis of these results, it can be speculated that polyols added to the system are too hydrophilic to penetrate deeply into the micelle and to compete effectively with bound preservative situated at the oxyethylene-hydrocarbon junction. However, polyols modify the binding in the secondary class of sites. It has been assumed that this binding involves a nonspecific partitioning between the micelles and the aqueous phase, and that the preservative molecules are associated with the polyoxyethylene region.[6b,c] The polar polyols are thought to be incorporated into the polyoxyethylene chain part of the micelle structure, displacing a part of the bound preservative from the polyoxyethylene mantle. These considerations are consistent with the relative polarity of the intramicellar regions, polyols and preservative.

Crooks *et al.*[11] studied the competitive interaction of preservative mixtures with cetomacrogol, and found that chloroxylenol and dichloroxylenol did not compete significantly with MP in the first class of sites, but that substantial competition occurred in the second class. Blanchard and co-workers[5] reported that sorbitol had little effect on the binding of phenolic preservatives with polysorbate 80 in either class of sites when the concentration of sorbitol added was fairly low compared with the concentration of surfactant. In the present study, the concentrations of polyols used are considered to be sufficient to affect the binding of *p*-hydroxybenzoates in the second class of sites. Based on these results and the foregoing discussion, the interaction with the primary class of sites seems to require considerable structural specificity, while the secondary class of sites appears to interact less specifically. The second class of sites makes the major contribution to the overall binding. Polyols are effective in reducing the inactivation of preservatives by nonionic surfactant by acting at the latter sites, but the effect is not very great.

11) M.J. Crooks and K.F. Brown, *J. Pharm. Pharmacol.*, **26**, 235 (1974).