

## A Model for the Evaluation and Prediction of Preservative Activity in Oil-in-Water Emulsions

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A simple mathematical model was derived to evaluate the antimicrobial activity of *p*-hydroxybenzoates in emulsified systems containing nonionic surfactants of the polyoxyethylene type. The model consists of preservative bound within the surfactant micelles, species partitioned into the oil phase, the ionized form in the water phase and the free effective form in the aqueous phase. The validity of the derived equations was confirmed by means of ultrafiltration experiments. The total concentration of a preservative necessary to give the desired concentration of free preservative in the aqueous phase of the emulsion can be calculated using this model. It was also shown that the amount of bound preservative within a mixture of several surfactants could be predicted from the binding parameters which characterize the interaction with each surfactant constituting the mixture, and that the binding parameters of a preservative for a surfactants mixture could be estimated from the hydrophile-lipophile balance value of the mixture and the binding parameters of some appropriate surfactants. The limitations of this simple mathematical model are discussed.

**Keywords**—nonionic surfactant; preservative; binding parameters; distribution; oil-in-water emulsion; antimicrobial activity; mathematical model; ultrafiltration; hydrophile-lipophile balance

The antimicrobial activity of a preservative in pharmaceutical and cosmetic emulsions is dependent on many factors. A preservative added to an oil-water mixture partitions between the two phases, and its antimicrobial activity is directly related to the concentration in the continuous aqueous phase in contact with microorganisms.<sup>2)</sup> In oil-in-water emulsion systems, the third components are the emulsifying agents, which usually form micelles in the aqueous phase. Preservatives commonly used in emulsions are solubilized or bound within surfactant micelles. Consequently, their activity is reduced,<sup>3)</sup> though the micelles function as a reservoir of the preservative.

Moreover, it has been reported by some authors<sup>4)</sup> that the preservative activity of organic acids in the aqueous phase is primarily attributable to the undissociated acid and not to the ionic form. Therefore, the most important factor affecting the antimicrobial activity of a preservative in emulsion systems is the concentration of the unbound and undissociated preservative in the aqueous phase.<sup>5)</sup>

1) Location: *Juso-Honmachi, Yodogawa-ku, Osaka, 532, Japan.*

2) M.G. deNavarre and H.E. Bailey, *J. Soc. Cosmetic Chem.*, **7**, 427 (1956); M. Aoki, M. Matsumoto, I. Yoshioka, and Y. Isa, *Yakuzaigaku*, **17**, 231 (1957); H.S. Bean and S.M. Heman-Ackah, *J. Pharm. Pharmacol.*, **16**, Suppl., 58T (1964); S.M. Heman-Ackah and G.H. Konning, *ibid.*, **19**, Suppl., 189S (1967).

3) 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); F.W. Goodhart and A.N. Martin, *J. Pharm. Sci.*, **51**, 50 (1962); W.P. Evans, *J. Pharm. Pharmacol.*, **16**, 323 (1964).

4) O. Rahn and J.E. Conn, *Ind. Eng. Chem.*, **36**, 185 (1944); E.R. Garrett and O.R. Woods, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 736 (1953).

5) M.G. deNavarre, *J. Soc. Cosmetic Chem.*, **8**, 68 (1957); S.M. Blaug and S.S. Ahsan, *J. Pharm. Sci.*, **50**, 138 (1961); A.G. Mitchell, *J. Pharm. Pharmacol.*, **16**, 533 (1964); N.K. Patel and J.M. Romanowski, *J. Pharm. Sci.*, **59**, 372 (1970).

Several models for calculating the concentration of available preservative in the water phase of emulsions have been reported. Bean *et al.*<sup>6)</sup> devised a mathematical model based on the assumption of a simple partitioning of the preservative between the micellar and aqueous phases. On the other hand, Garrett<sup>7)</sup> derived a model for the quantification of preservative action by considering the Langmuir adsorption of preservative molecules with surfactant micelles. Recently, Kazmi and Mitchell<sup>8)</sup> calculated the free preservative concentration in emulsified systems using two kinds of independent Langmuir-type mechanisms to describe the interaction between the preservative and the nonionic surfactant micelles. However, there is considerable uncertainty as to the actual interaction mechanism between them.

In our preceding papers<sup>9)</sup> of this series, the existence of two kinds of interaction mechanisms between preservative molecules and polyoxyethylene dodecyl ether micelles was suggested. It is thought that the primary class of binding sites can be represented by a Langmuir isotherm and the second class of sites by a simple partition law. On the basis of these findings, the present paper presents a new model describing the relation between the available preservative concentration in the aqueous phase and the total preservative concentration of the oil-in-water emulsion. The validity of the derived equation was tested by means of the ultrafiltration technique.

### Theoretical

#### Ionization of Preservative in the Aqueous Phase

Many preservatives are weak acids and ionize in the aqueous phase as a function of pH. The dissociation constant,  $K_a$ , for an acid preservative,  $HA$ , is defined by

$$HA \rightleftharpoons H^+ + A^-, \text{ and } K_a = \frac{[H^+] \cdot [A^-]}{[HA]} \quad (1)$$

where the parentheses indicate the concentration of each species in the aqueous phase. Designating the volume of the aqueous phase as  $V_w$ , the amount of ionized preservative in the aqueous phase,  $A^-$ , is

$$A^- = [A^-] \cdot V_w = \frac{K_a \cdot [HA] \cdot V_w}{[H^+]} \quad (2)$$

#### Partition of Preservative between Oil and Aqueous Phase

In a simple dispersion of oil and water, the undissociated preservative molecules are distributed between the two phases according to the partition law,

$$P = \frac{[HA_o]}{[HA]} \quad (3)$$

where  $P$  is the partition coefficient,  $[HA_o]$  is the concentration of undissociated molecules in the oil phase and  $[HA]$  is that in the aqueous phase. When the volume ratio of oil to water is  $a$ , the volume of the oil phase is given by  $a \cdot V_w$ . Hence the amount of the preservative in oil,  $HA_o$ , is

$$HA_o = [HA_o] \cdot a \cdot V_w = P \cdot [HA] \cdot a \cdot V_w \quad (4)$$

#### Binding of Preservative to Surfactant Micelles

The interaction of a preservative with nonionic surfactant micelles can be adequately expressed by the following equation,<sup>9)</sup>

6) H.S. Bean, G.H. Konning, and S.A. Malcolm, *J. Pharm. Pharmacol.*, **21**, Suppl., 173S (1969).

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

8) S.J.A. Kazmi and A.G. Mitchell, *J. Pharm. Sci.*, **67**, 1260 (1978).

9) a) T. Shimamoto and Y. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **23**, 3088 (1975); b) T. Shimamoto, H. Mima, and M. Nakagaki, *ibid.*, **27**, 1995 (1979).

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

where  $[D_f]$  is the concentration of free preservative in the aqueous phase,  $[D_b]$  is that of bound preservative based on the total volume of the solution,  $[S]$  is the concentration of surfactant,  $n$  and  $K$  are constants, and the subscripts indicate the class of sites.

The volume of the micellar phase,  $V_m$ , is generally not known. Since the critical micelle concentrations of nonionic surfactants are negligibly low,<sup>10)</sup> the value of  $V_m$  is considered to be proportional to the amount of surfactant,  $S$ , over a limited concentration range. As the density of polyoxyethylene-type surfactant is about 1 g/ml,<sup>11)</sup> the volume of the micellar phase may be approximated by the amount of surfactant. Thus,  $[S]$  can be calculated as follows:

$$[S] = \frac{S}{V_w + V_m} \approx \frac{S}{V_w + S} \quad (6)$$

On the basis of the same approximation, the amount of bound preservative,  $D_b$ , is

$$D_b = [D_b] \cdot (V_w + S) = \left( \frac{n_1 \cdot K_1 \cdot [D_f]}{1 + K_1 \cdot [D_f]} + n_2 \cdot K_2 \cdot [D_f] \right) \cdot [S] \cdot (V_w + S) \quad (7)$$

Putting  $b$  equal to  $(V_w + S)/V_w$ , Eq. (7) can be rearranged to

$$D_b = b \cdot V_w \cdot [D_f] \cdot [S] \cdot \left( \frac{n_1 \cdot K_1}{1 + K_1 \cdot [D_f]} + n_2 \cdot K_2 \right) \quad (8)$$

#### Total Preservative Concentration in the Emulsified System

The total amount of preservative in the emulsified system,  $D_t$ , is

$$D_t = D_f + A^- + HA_o + D_b \quad (9)$$

where  $D_f$  is the amount of free undissociated preservative in the aqueous phase. The total volume of this system,  $V_t$ , consists of the sum of the volumes of the oil, aqueous and micellar phases. In this study, the volume of the micellar phase is expressed in terms of  $S$  from a practical viewpoint;

$$V_t = a \cdot V_w + V_w + V_m \approx a \cdot V_w + V_w + S = (a+b) \cdot V_w \quad (10)$$

The term  $[HA]$  in Eqs. (2) and (4) must be equal to  $[D_f]$ , and the amount of free undissociated preservative in the aqueous phase is  $[D_f] \cdot V_w$ . Thus, the total preservative concentration based on the total volume of the emulsion,  $[D_t]$ , is

$$[D_t] = \frac{D_t}{V_t} = \frac{[D_f]}{a+b} \cdot \left[ 1 + \frac{K_a}{[H^+]} + a \cdot P + b \cdot [S] \cdot \left( \frac{n_1 \cdot K_1}{1 + K_1 \cdot [D_f]} + n_2 \cdot K_2 \right) \right] \quad (11)$$

When the required concentration of free undissociated preservative in the aqueous phase and necessary parameters are substituted into Eq. (11), the total preservative concentration required in the emulsified system can be obtained.

For a mixture of several surfactants of concentrations  $[S_1]$ ,  $[S_2]$ , ...,  $[S_m]$  with binding parameters  $n_{11}$ ,  $n_{12}$ , ...,  $n_{1m}$ ;  $n_{21}$ ,  $n_{22}$ , ...,  $n_{2m}$ ;  $K_{11}$ ,  $K_{12}$ , ...,  $K_{1m}$  and  $K_{21}$ ,  $K_{22}$ , ...,  $K_{2m}$ , respectively, Eq. (11) becomes

$$[D_t] = \frac{[D_f]}{a+b} \cdot \left[ 1 + \frac{K_a}{[H^+]} + a \cdot P + \sum_{i=1}^m b_i \cdot [S_i] \cdot \left( \frac{n_{1i} \cdot K_{1i}}{1 + K_{1i} \cdot [D_f]} + n_{2i} \cdot K_{2i} \right) \right] \quad (12)$$

where

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

11) M.J. Schick (ed.), "Nonionic Surfactants," Marcel Dekker Inc., New York, 1967, Chapter 3, 4 and 5.

$$b = \frac{V_w + S_1 + S_2 + \dots + S_m}{V_w}$$

$$b_i = \frac{V_w + S_i}{V_w}$$

$$[S_i] = \frac{S_i}{V_w + S_i}$$

and  $S_1, S_2 \dots S_m$  are the amounts of the surfactants in the emulsion.

### Experimental

**Materials**—Methyl *p*-hydroxybenzoate (MP), propyl *p*-hydroxybenzoate (PP) and light mineral oil were of J.P. IX grade. Polyoxyethylene (20, 30 and 50)<sup>12)</sup> dodecyl ethers (PDE-20, -30 and -50) were of commercial grade, supplied by Nihon Emulsion Co., Tokyo. The Diaflo membranem UM-10, 43 mm  $\phi$  (a crosslinked dextran gel membrane) was purchased from Amicon Corp., Mass., U.S.A.

**Preparation of Emulsion and Separation of the Aqueous Phase of the Emulsion**—The emulsions were prepared according to the formula shown in Table I. All the procedures for the preparation of emulsions and separation of the aqueous phase of emulsions were the same as in the previous work.<sup>13)</sup> The sample emulsions were equilibrated at a temperature of 25° for two weeks prior to the separation of the aqueous phase.

**Ultrafiltration and Quantitative Analysis**—The technique employed was essentially the same as that described in the previous paper.<sup>13)</sup> Ultrafiltration at a temperature of 25° was used for the separation of the free form of *p*-hydroxybenzoates from the bound form within micelles. The concentration of free preservative in the filtrate was determined spectrophotometrically at a wavelength of 256 nm. The pH values of all the sample solutions were measured on completion of ultrafiltration.

TABLE I. Formulation of Oil-in-Water Emulsions

Ingredients	Composition (g) Sample No.				Remarks
	1	2	3	4	
Light mineral oil	30.0	30.0	30.0	30.0	35.3 ml
PDE-20	0.9	0.9	0.9	0.9	
PDE-30	1.4	1.4	1.4	1.4	
PDE-50	1.0	1.0	1.0	1.0	
MP	0.4	0.2	—	—	
PP	—	—	0.2	0.1	
Distilled water	66.3	66.5	66.5	66.6	
Total	100	100	100	100	ca. 105 ml

### Results and Discussion

#### Free Preservative Concentration in the Aqueous Phase of the Emulsion

Table II shows the pH values of the aqueous phase, the concentration of total preservative incorporated in the emulsions and the free preservative concentrations in the aqueous phase determined by ultrafiltration of the samples. Compared with the  $pK_a$  of *p*-hydroxybenzoates, *i.e.*, 8.5,<sup>14)</sup> the pH values were low, so all of the preservative molecules were regarded as being in the undissociated form in this emulsified system. The results indicate that PP is bound or distributed within the micellar or oil phases to a greater extent than MP, as the ratio of free preservative concentration to the total preservative concentration for PP is smaller than that for MP.

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

13) T. Shimamoto, Y. Ogawa, and N. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **21**, 316 (1973).

14) T.R. Aalto, M.C. Firman, and N.E. Rigler, *J. Am. Pharm. Assoc., Sci. Ed.*, **42**, 449 (1953).

TABLE II. Comparison between Calculated and Observed Free *p*-Hydroxybenzoate Concentrations in the Aqueous Phase of the Emulsions shown in Table I

	Sample No.			
	1	2	3	4
pH	4.10	4.20	5.15	5.28
$[D_t]$ , % (w/v)	0.381	0.190	0.190	0.0952
$[D_f]$ (obsd.), % (w/v)	0.150	0.0641	0.0142	0.0067
$[D_f]$ (obsd.)/ $[D_t]$	0.394	0.337	0.075	0.070
$[D_f]$ (calcd.-I), <sup>a)</sup> % (w/v)	0.145	0.0678	0.0153	0.0071
$[D_f]$ (calcd.-II), <sup>b)</sup> % (w/v)	0.144	0.0670	0.0158	0.0073

a) Calculated using the binding parameters for each surfactant and Eq. (12).

b) Calculated using the binding parameters for PDE-30 and Eq. (11).

### Calculation of Free Preservative Concentration in the Aqueous Phase of the Emulsion

The simple mathematical model derived in this paper was used to calculate the free preservative concentrations in the aqueous phase of oil-in-water emulsions in order to compare the results with those obtained experimentally. Physico-chemical constants and various parameters necessary for calculation are shown in Table III. Assuming that the distribution of *p*-hydroxybenzoate between mineral oil and water obeys the simple partition law, the partition coefficient was determined by taking the ratio of preservative solubility in mineral oil to that in water. In this computation, each of the values of  $[D_t]$  for sample emulsions was substituted into Eq. (12) and the corresponding value of  $[D_f]$  was obtained by trial and error. The results are shown in Table II.

TABLE III. Various Constants Necessary for the Calculation of  $[D_f]$

Parameters	Values
Solubility of MP at 25°	0.01% (in mineral oil), <sup>14)</sup> 0.22% (in water) <sup>9a)</sup>
Solubility of PP at 25°	0.03% (in mineral oil), <sup>14)</sup> 0.034% (in water) <sup>9b)</sup>
<i>P</i>	0.0455 (for MP), 0.882 (for PP)
<i>a</i>	0.532 (for sample No. 1), 0.531 (for sample No. 2 and 3), 0.530 (for sample No. 4)
<i>b</i>	1.05 (for all samples)
<i>pK<sub>a</sub></i>	8.5 <sup>14)</sup>
<i>n<sub>1</sub></i> , <i>K<sub>1</sub></i> and <i>n<sub>2</sub></i> · <i>K<sub>2</sub></i> <sup>a)</sup>	0.0204, 32.5 and 0.648 (for MP and PDE-20) <sup>9a)</sup> 0.0127, 40.1 and 0.559 (for MP and PDE-30) <sup>9a)</sup> 0.0078, 23.5 and 0.452 (for MP and PDE-50) <sup>9a)</sup> 0.0197, 125 and 3.93 (for PP and PDE-20) <sup>9b)</sup> 0.0135, 144 and 2.93 (for PP and PDE-30) <sup>9b)</sup> 0.0088, 135 and 2.43 (for PP and PDE-50) <sup>9b)</sup>

a) Expressed in terms of grams per 100 milliliters.

The  $[D_f]$  values calculated by substituting the constants, including the binding parameters determined for each surfactant, into Eq. (12) were in good agreement with the experimental values. This indicates that, though mixtures of several surfactants in various ratios may form mixed micelles of different compositions and different binding characteristics, the extent of preservative binding within a surfactant mixture is a simple summation of the bindings observed with the individual surfactants. Moreover, the results shows that the total concentration of preservative required to provide a desired concentration of free preservative

in the aqueous phase of the emulsion can be calculated using Eq. (12).

Another attempt was made to estimate the binding parameters of a preservative with a surfactant mixture. It was anticipated that the relation between the hydrophile-lipophile balance (HLB) values of surfactants and the binding parameters of the preservative with surfactants would be relatively simple. Thus, the parameters determined experimentally in the previous paper<sup>9)</sup> were plotted as a function of the HLB value of the surfactant, as shown in Figs. 1 and 2. In these figures, the binding parameters are expressed in terms of grams per 100 milliliters. The HLB values of the individual surfactants were determined simply by dividing the weight percent of oxyethylene chain in the surfactant molecule by five.<sup>15)</sup> Allowing for experimental error, it appeared that the  $K_1$  value was independent of the HLB value of the surfactant, whereas the  $n_1$  and  $n_2 \cdot K_2$  values both decreased as the HLB value increased. The relationship between the polyoxyethylene chain length of surfactants and the binding parameters was discussed in preceding papers.<sup>9,16)</sup>

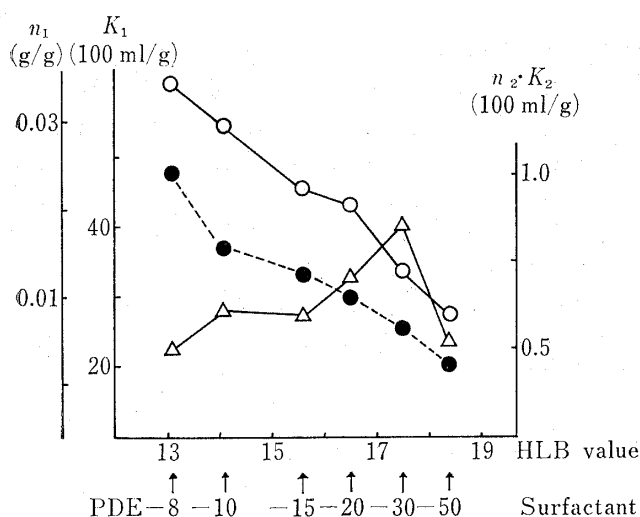


Fig. 1. Relation between HLB Value of Surfactant and Binding Parameters of MP to Surfactant

Parameters were expressed in terms of gram per 100 milliliters, and cited from T. Shimamoto and Y. Ogawa, *Chem. Pharm. Bull.* (Tokyo), **23**, 3088 (1975).

—○—:  $n_1$ , —△—:  $K_1$ , ---●---:  $n_2 \cdot K_2$ .

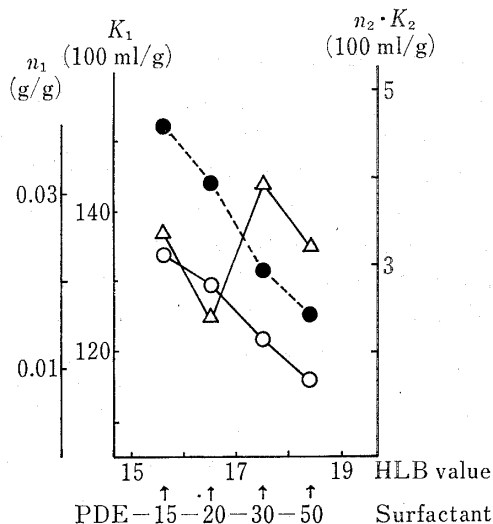


Fig. 2. Relation between HLB Value of Surfactant and Binding Parameters of PP to Surfactant

Parameters were expressed in terms of gram per 100 milliliters, and cited from T. Shimamoto, H. Mima, and M. Nakagaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 1995 (1979).

—○—:  $n_1$ , —△—:  $K_1$ , ---●---:  $n_2 \cdot K_2$ .

This simple relationship between the HLB value and the binding parameters enables us to estimate the binding parameters for any homologous surfactant for which experimental data are not available. In addition, this can be extended to include formulations containing more than one surfactant, because commercial surfactants are not chemically pure and have a wide range of ethylene oxide chain lengths. The HLB value of the surfactant mixture used in the present experiment was determined by taking the weight-average of the individual surfactant HLB values. This value was 17.5, equal to the HLB of PDE-30. Assuming that the interaction of *p*-hydroxybenzoates with this surfactants mixture was equivalent to that with PDE-30, the value of  $[D_f]$  was obtained using the binding parameters for PDE-30 and Eq. (11). The values of  $[D_f]$  calculated in this manner are also shown in Table II. These values agree well not only with the experimental values but also with the values calculated

15) W.C. Griffin, *J. Soc. Cosmetic Chem.*, **1**, 311 (1950); *idem, ibid.*, **5**, 249 (1954).

16) T. Shimamoto, H. Mima, and M. Nakagaki, *Chem. Pharm. Bull.* (Tokyo), **27**, 2557 (1979).

from the binding constants of the individual surfactants and Eq. (12). This result shows that the simple mathematical model devised in this report can be extended to include formulations containing several surfactants. There is no need to determine the binding parameters of a preservative for a surfactants mixture, nor those of every one of the surfactants constituting a mixture, since the interaction of a preservative with a surfactants mixture can be evaluated from the binding parameters that characterize the interaction with each surfactant constituting the mixture, or can be predicted from information about the HLB values and binding parameters of the appropriate surfactants. Thus the concentration of preservative required for antimicrobial effectiveness in emulsified systems can be calculated by substituting the necessary constants into a suitable equation.

### Limitations of the Simple Mathematical Model

Limitations of the simple model studied here may be encountered with actual complex emulsions in practice. Many emulsions contain surfactants which do not have enough hydrophilic character to be soluble in water and are for the most part soluble in oils. As the borderline between these substances and the water-soluble surfactants is not sharp, it is difficult to differentiate between a surfactant which distributes itself into the oil phase and one which does so into the aqueous phase. Even if a single water-soluble surfactant is used in the emulsion system, commercial compounds of the polyoxyethylene type are heterogeneous and have a distribution of ethylene oxide chain lengths, so a part of the surfactant may partition into the oil phase. Furthermore, some of the surfactant molecules will be adsorbed at the oil-water interface, and some will be present in the monomer form (nonmicellar) in the aqueous phase. From a practical viewpoint, the amount of nonmicellar surfactant is small enough to be neglected, as discussed previously. The surfactant adsorbed at the oil-water interface can be assumed to have binding characteristics similar to those of micellar surfactant, while the distribution of surfactant molecules between oil and aqueous phases reduces the amount of surfactant available for binding with the preservative and changes the partition coefficient of the preservative between oil and water.

Emulsions studied in a previous report<sup>13)</sup> are an example of such a complex dispersion system, in which not all of the surfactant molecules will be involved in binding to the preservative. As shown in Table IV, a mixture of four surfactants was used in one emulsion. Both polyoxyethylene (2 mol) palmityl ether and polyoxyethylene (4 mol) stearyl ether were quite insoluble in water, polyoxyethylene (6 mol) stearyl ether was also insoluble but dispersed easily on gentle shaking, and polyoxyethylene (10 mol) butyl ether was completely soluble in water. Consequently, it was difficult to determine the value of  $[S]$  and other parameters. On the assumption that the whole of the polyoxyethylene (10 mol) butyl ether and one-half of the polyoxyethylene (6 mol) stearyl ether species contributed to the binding with preservative present in the aqueous phase or at the oil-water interface, and that the rest of the

TABLE IV. Formulation of Oil-in-Water Emulsions<sup>a)</sup>

Ingredients	Amount (g)	Remarks <sup>b)</sup>
Light mineral oil	30.0	35.3 ml
Polyoxyethylene (2 mol) palmityl ether	0.8	HLB= 5.4
Polyoxyethylene (4 mol) stearyl ether	0.9	HLB= 7.9
Polyoxyethylene (6 mol) stearyl ether	2.0	HLB= 9.8
Polyoxyethylene (10 mol) butyl ether	1.9	HLB=17.2
MP	0—0.5	
Distilled water	Add to 100	ca. 105 ml

a) T. Shimamoto, Y. Ogawa, and N. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **21**, 316 (1973).

b) Values of HLB were calculated by dividing the weight percent of oxyethylene chain in the molecule by five.

TABLE V. Comparison of Calculated with Observed Free MP Concentrations in the Aqueous Phase of the Emulsions Shown in Table IV

% (w/v) of MP in emulsion [ $D_i$ ]	% (w/v) of free MP in aqueous phase [ $D_f$ ]	
	Obsd. <sup>a)</sup>	Calcd.
0.0049	0.0010	0.0010
0.0195	0.0043	0.0040
0.0488	0.0116	0.0108
0.0971	0.0242	0.0234
0.193	0.0566	0.0522
0.292	0.0958	0.0846
0.484	0.196	0.150

a) T. Shimamoto, Y. Ogawa and N. Ohkura, *Chem. Pharm. Bull.* (Tokyo), **21**, 316 (1973).

surfactants affected the partition coefficient of preservative between the oil and water phases, being present in the oil, various constants necessary for calculation using Eq. (11) were estimated. As the HLB value of the mixture of two surfactants present in water was 14.6, the binding parameters of MP to the surfactants mixture were presumed, based on Fig. 1, to be as follows;  $n_1=0.027$ ,  $K_1=30$  and  $n_2 \cdot K_2=0.75$ . The solubility of MP in the oil phase containing surfactants was approximately 0.05% (w/v), so the value of  $P$  was roughly estimated to be 0.23. Other parameters,  $a$ ,  $b$  and  $[S]$ , were determined as described before. The ionization term was negligible, since the pH values of the samples were 5.0—5.6. The  $[D_f]$  values calculated on the present assumptions are shown in Table V together with the experimental values quoted from the previous paper.<sup>13)</sup>

There is generally good agreement between the calculated and observed values over a wide range. Some discrepancy between the two values is seen at high concentrations of the preservative, but this is considered to be allowable for practical purposes. This difference may result from the complexity of the system. Further studies are required on pharmaceutical and cosmetic emulsions, which usually contain a humectant such as glycerol, propylene glycol or polyethylene glycol, because the presence of these additives may cause changes in the binding parameters of preservatives to the micelles.<sup>17)</sup> As an alternative to mathematical prediction for complex emulsion systems in which calculation using a model is difficult or impossible, a direct method to estimate the total concentration of preservative necessary for the preservation of emulsions has been suggested.<sup>18)</sup>

17) T. Shimamoto and H. Mima, *Chem. Pharm. Bull.* (Tokyo), **27**, 2602 (1979).