Gel Filtration of Surfactants [1,2]

TOSHIO NAKAGAWA and **HIROAKI JIZOMOTO**,
Shionogi Research Laboratory, Fukushima-ku, Osaka, Japan

ABSTRACT

A simulation technique was developed for analyzing the gel filtration chromatography of surfactants. Theoretical elution curves obtained by this technique were compared with the experimental curves for the following five systems: an ionic or a nonionic surfactant of a single component, an ionic surfactant in the presence of an electrolyte, a mixture of two nonionic surfactants, a mixture of two ionic surfactants, and a mixture of an ionic and a nonionic surfactant. In the first four systems, good agreement was found between the theoretical and experimental elution curves. A possible explanation for the disagreement in the last system is presented.

INTRODUCTION

Gel filtration chromatography is a powerful and convenient method for separating individual components of different molecular size from a solution of several constituents, and it frequently permits the estimation of the molecular weights of the solutes. This is due to the fact that large molecules enter gel cavities with difficulty whereas small molecules enter almost freely; thus the ratio of populations between gel interior and exterior is smaller for larger molecules. When a solution containing two or more solutes of different molecular size is injected into the top of a gel column and subjected to the gel filtration procedure, only the portion outside gel cavities can flow down, and therefore, the larger molecules tend to pass through the column more rapidly than the smaller molecules.

The same phenomenon is expected for a solution of a surfactant or surfactants whose concentration is high enough to produce micelles. The micelles correspond to the larger molecules, and coexisting monomolecularly-dispersed surfactant molecules (monomers) correspond to the smaller molecules. In this case, however, the filtration process is complicated by the disruption of micelles into monomers and the conglomeration of monomers into micelles, and thus the theoretical elution curve cannot be obtained by a conventional mathematical technique. Using an electronic computer, the authors have developed a simulation technique which can derive the theoretical elution curve. This paper first describes the technique in outline, and then discusses and compares the theoretical and experimental elution curves obtained for the following five systems: a surfactant of a single component, an ionic surfactant in the presence of an electrolyte, a mixture of two nonionic surfactants, a mixture of two ionic surfactants, and a mixture of an ionic and a nonionic surfactants.

Although this paper is not directly concerned with detergency, there are several reasons why it should be presented at the symposium on "Basic Aspects of Detergency":

1. Gel filtration chromatography and its analysis by the simulation technique afford us with various physicochemical data of detergents. The data obtained may help elucidate fundamental aspects of detergent action.
2. In order to clarify the detergent action, it is usually desirable to purify test materials. Gel filtration is a powerful and convenient method to isolate the component which has the lowest critical micelle concentration from a detergent mixture. Simulation calculation is useful to predict the best condition for the isolation.
3. In applied colloid science such as detergency, the system to be studied is usually very complicated. As has been shown in this work, simulation is a powerful tool to investigate a complex system. It is hoped that some day the simulation technique will be successfully used to elucidate detergent action.

EXPERIMENTAL PROCEDURES

Only the experimental procedure in case 5 (a combination of a nonionic (A) and an ionic surfactant (B)) is described below because those in the other cases have already been reported in previous papers (2,4,5,7). Octyl β-D-glucoside was synthesized by a conventional method (9) and purified by repeated recrystallizations from a mixed solvent of acetone and petroleum ether. High purity was ascertained by elemental analysis, melting point (67-69) C, IR spectra, thin layer chromatography, and surface tension measurements. An aqueous solution of a 1:1 mixture of sodium decyl sulfate (SDeS) and octyl glucoside was poured slowly onto the top of a Sephadex column (Sephadex G-25 fine made by Pharmacia, diameter 2.5 cm, length 45 cm, 30 C) which had been pretreated with pure water. After the charge of the mixture, it was eluted with water. The effluent was poured into a series of tubes by means of a fraction collector. Each fraction was divided into two aliquots. With one of them, the concentration of SDeS was determined by the titration with hexadecyltrimethyl-ammonium chloride using Eosin Red as an indicator (10). The other one was dried in vacuo to determine the weight of the residue.

Simulation calculation was carried out on an IBM-1620 computer by assuming K=0.5.

PRINCIPLE OF SIMULATION

To establish a quantitative theory for a natural phenomenon, one often proceeds by the following steps: setting up a hypothetical model that may explain the phenomenon; stating the results expected from the model in a mathematical form, frequently in a differential equation; solving the equation; comparing the solution with the experimental result. If these agree with each other within the limit of experimental accuracy, the proposed model is considered plausible, and additional evidence supporting this model is sought. If they do not agree, the model is rejected.

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[1] One of five papers presented at the Symposium, "Basic Aspects of Detergency," AOCS-ISF World Congress, Chicago, September 1970.

[2] Special Abbreviations: T_A: the total quantity of A component in a plate. Q_A: the quantity of A component in a mobile phase. x_A: the concentration of monomeric A in a solution. C_A: the total concentration of A component in a solution. C_{OA}: the critical micelle concentration of A in the absence of B. K: a constant, the value of which is near 0.5. V_f: the volume of a mobile phase. V_{As}: the effective volume of a stationary phase with respect to monomeric A. V_m: the effective volume of a stationary phase with respect to a micelle. $q_A=Q_A/V_f$: the concentration of A component in a mobile phase. $t_A=T_A/V_f$: Note that t_A is not an actual concentration though its dimension is mole per volumne. $P_{As}=V_{As}/V_f$: the distribution ratio of monomeric A between the stationary and mobile phases. $P_m=V_m/V_f$: the distribution ratio of micelles between the stationary and mobile phases. $P_m=V_m/V_f$: the distribution ratio of micelles between the stationary and mobile phases. $Y=\{t_B-x_B(1+P_{Bs})\}/\{t_A-x_A(1+P_{As})+t_B-x_B(1+P_{Bs})\}$: the mole fraction of B component in a mixed micelle of A and B. $p=C_{OB}(1+P_{Bs})-C_{OA}(1+P_{As})$. $P=C_{OB}^{1+K}(1+P_{Bs})-C_{OA}^{1+K}(1+P_{As})$. Similar symbols with a subscript B refer to B component.

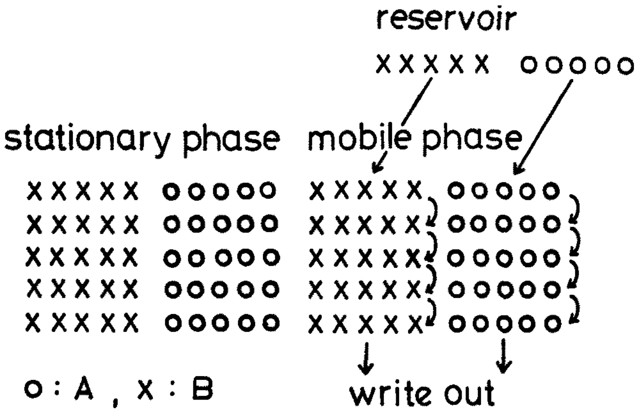

FIG. 1. Model of gel column.

In the field of colloid chemistry, the system to be studied is usually very complicated. Mathematical formulation for the proposed model is not always possible, and even when possible the equation obtained is frequently insolvable. Provided the hypothetical model itself can be simulated in a digital computer, the solution will be calculated automatically even when the formulation is impossible, and also when the formulated equation cannot be solved analytically. Thus, the adequacy of the hypothesis can be tested.

Our simulation technique may be best understood by using an example in which a solution containing two solutes, A and B, is subjected to the chromatography. The gel column is considered to be composed of many plates piled on top of one another, each of which consists of a mobile phase and a stationary phase. The stationary phase stands for the gel interior (cavities) and the mobile phase for the interparticle void space through which the solution flows down. For every plate, two pairs of memory fields and descriptions of the quantities of A and B components in the mobile phase and in the stationary phase are prepared in a computer.

When the concentration equilibrium has been attained in all plates, the data in the lowest mobile phase are written out. The data written out are the quantities of both components in an aliquot taken by a fraction collector. The data in the other mobile phases are shifted to the neighboring lower phases, and the quantities supplied from a reservoir are written in the highest mobile phase as illustrated schematically in Figure 1. This process corresponds to the flowing down of the mobile phase by one plate. After the flowing down, every plate is no more in equilibrium, and a reequilibration process is applied as follows.

For each plate, the total quantity of A component, T_A is computed by the addition of the data contained in the two memory fields that are assigned to A component in the mobile and in the stationary phases. In a similar way, T_B is obtained. Knowing T_A and T_B, one can calculate, at least in some cases, the quantities of both components, Q_A and Q_B, in the mobile phase at a newly established equilibrium; the quantities in the stationary phase are T_A-Q_A and T_B-Q_B. For all plates, the four quantities thus obtained are put into the appropriate memory fields. The above mentioned procedure is repeated until the whole elution curves of both components are obtained from the data written out successively.

This technique is applicable to any type of chromatography in which the column can be regarded to consist of the mobile and stationary phases, and at least in principle, to any solution containing a finite number of solutes. If the solution contains only one solute, the terms T_B and Q_B are eliminated. For a solution containing three or more solutes, additional terms must be used to express the quantities of C, D, etc.

EQUILIBRIUM CONCENTRATIONS

In the preceding section, it has been shown that the gel filtration procedure can be simulated in a computer if it is possible to calculate Q_A and Q_B from T_A and T_B. To achieve the calculation, it is necessary to know how the monomer concentrations in a solution vary with the total concentrations of both components. The theory of Shinoda (1) predicts the following relations under the definitions of $x_A(x_B)$ = the concentration of monomeric A(B), $C_A(C_B)$ = the total concentration of component A(B), $C_{OA}(C_{OB})$ = the cmc of A(B) in the absence of B(A), and K = a constant, the value of which is near 0.5.

Case 1: A is an ionic or a nonionic surfactant and B is absent (2). When $C_A \geqq C_{OA}$ then $x_A = C_A$, and when $C_A > C_{OA}$ then $x_A = C_{OA}$.

Case 2: A is an ionic surfactant and B is a 1:1 type electrolyte (3,4).

$$\ln x_A = -K\ln(x_A+x_B) + (1+K)\ln C_{OA}$$

$$x_B = C_B$$

When the solution of the above simultaneous equations satisfies $x_A < C_A$, then x_A obtained is a correct answer. Alternatively, when the solution satisfies $x_A \geqq C_A$, then the correct answer is $x_A = C_A$.

Case 3: Both A and B are nonionic surfactants (5).

$$\ln x_A = \ln C_{OA} + \ln\{(C_A-x_A)/(C_A-x_A+C_B-x_B)\}$$

$$\ln x_B = \ln C_{OB} + \ln\{(C_B-x_B)/(C_A-x_A+C_B-x_B)\}$$

Case 4: Both A and B are ionic surfactants (6,7).

$$\ln x_A = -K\ln(x_A+x_B) + (1+K)\ln C_{OA} + \ln\{(C_A-x_A)/(C_A-x_A+C_B-x_B)\}$$

$$\ln x_B = -K\ln(x_A+x_B) + (1+K)\ln C_{OB} + \ln\{(C_B-x_B)/(C_A-x_A+C_B-x_B)\}$$

Case 5: A is a nonionic and B is an ionic surfactant. (Discussion will be presented in a later section.)

$$\ln x_A = \ln C_{OA} + \ln\{(C_A-x_A)/(C_A-x_A+C_B-x_B)\}$$

$$\ln x_B = \ln C_{OB} + \{(2K+1)/(K+1)\}\ln\{(C_B-x_B)/(C_A-x_A+C_B-x_B)\}$$

For cases 3, 4 and 5, it can be shown that if $x_A < C_A$ then $x_B < C_B$ and also that if $x_A \geqq C_A$ then $x_B \geqq C_B$. Correct answers can be found out by the following claims. When the solution satisfies $x_A < C_A$, then x_A and x_B obtained are the correct answers. When the solution satisfies $x_A \geqq C_A$, then the correct answers are $x_A = C_A$ and $x_B = C_B$.

The concentration relations for cases 4 and 5 reduce to those for case 3 if K is equated to zero. In this sense, K may be regarded as a measure of ionic effect.

Our problem was to calculate Q_A and Q_B from T_A and T_B However, from a practical standpoint, it is more convenient to use "concentrations" rather than quantities. For this reason, the mathematical procedure to derive the concentrations of A and B components in a mobile phase, $q_A = Q_A/V_f$ and $q_B = Q_B/V_f$, from $t_A = T_A/V_f$ and $t_B = T_B/V_f$ is explained below by using the following notations: V_f, the volume of a mobile phase; $V_{As}(V_{Bs})$, the effective volume of a stationary phase with respect to monomeric A(B); V_m, the effective volume of a stationary phase with respect to a micelle; P_{As}, V_{As}/V_f; P_{Bs},V_{Bs}/V_f; P_m, V_m/V_f. The latter three stand respectively for the distribution ratios of monomeric A, of monomeric B, and of micelles between the stationary and mobile phases.

The quantity of monomeric A in a plate is given by the product of "the concentration of monomeric A" and "the effective volume of the plate for monomeric A", namely $x_A(V_f+V_{As}) = x_AV_f(1+P_{As})$. Therefore, the quantity of micellar A in the plate is given by $T_A - x_AV_f(1+P_{As})$. Since the micellar A is distributed between the stationary phase

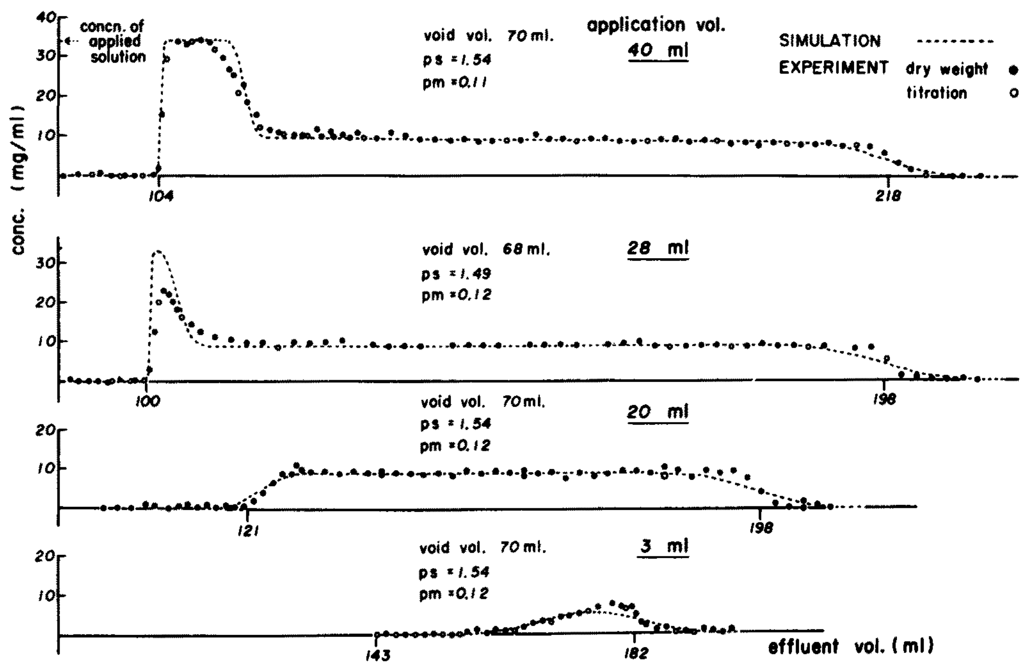

FIG. 2. Elution curves of sodium decyl sulfate.

and the mobile phase in a ratio of $P_m : 1$, the quantity of micellar A in the mobile phase must be equal to $\{T_A - x_A V_f(1+P_{As})\}/(1+P_m)$. On the other hand, the quantity of monomeric A in the mobile phase is $x_A V_f$. The sum of the latter two quantities is, of course, equal to Q_A.

$$Q_A = x_A V_f + \{T_A - x_A V_f(1+P_{As})\}/(1+P_m)$$

Dividing both sides by V_f, one obtains Equation 1, and in a similar way, 2.

$$q_A = x_A + \frac{t_A - x_A(1+P_{As})}{1+P_m} = \frac{t_A}{1+P_m} + \frac{P_m - P_{As}}{1+P_m} x_A \qquad [1]$$

$$q_B = x_B + \frac{t_B - x_B(1+P_{Bs})}{1+P_m} = \frac{t_B}{1+P_m} + \frac{P_m - P_{Bs}}{1+P_m} x_B \qquad [2]$$

The next section will show that the equilibrium concentrations in a mobile phase can be obtained by combining these equations with the previous concentration relations, which correlate monomer concentrations and total concentrations, applied to the mobile phase. When applied to the mobile phase, C_A and C_B in these relations should be replaced by q_A and q_B respectively.

CALCULATION OF EQUILIBRIUM CONCENTRATIONS FOR PARTICULAR CASES

For case 1, the concentration relation says that when $q_A \leqq C_{OA}$ then $x_A = q_A$, and that when $q_A > C_{OA}$ then $x_A = C_{OA}$. This implies that q_A can be expressed as a function of t_A by either of the two methods, one of which (a) is to replace x_A by q_A in Equation 1 and the other (b) is to replace by C_{OA}. Which method should be applied depends on conditions. The condition $t_A \leqq C_{OA}(1+P_{As})$ will be examined first. If on applies method b, as a trial, Equation 1 reduces to $q_A = C_{OA} + \{t_A - C_{OA}(1+P_{As})\}/(1+P_m)$. From this relation, it follows that $q_A \leqq C_{OA}$, because the numerator of the second term is negative or zero. This conflicts with the requisite that method a should be applied when $q_A \leqq C_{OA}$. In other words, he has chosen the wrong method. Alternatively, if one applies method a, Equation 1 reduces to $q_A = q_A + \{t_A - q_A(1+P_{As})\}/(1+P_m)$, and thus to $q_A = t_A/(1+P_{As})$. This relation combined with the condition $t_A \leqq C_{OA}(1+P_{As})$ leads to $q_A \leqq C_{OA}$, showing that method a is consistent with the requisite that when $q_A \leqq C_{OA}$ then $x_A = q_A$.

Next, the condition $t_A > C_{OA}(1+P_{As})$ will be examined. If one applies method a, $q_A = t_A/(1+P_{As})$ is derived from Equation 1. This relation combined with the condition $t_A > C_{OA}(1+P_{As})$ results in $q_A > C_{OA}$ which requires method b. On the other hand, if method b is applied, the resulting equation $q_A = C_{OA} + \{t_A - C_{OA}(1+P_{As})\}/(1+P_m)$ indicates that $q_A > C_{OA}$, because the numerator of the second term is positive. Thus, one knows that method b is the correct method for treating this case. In summary, it is concluded that when $t_A \leqq C_{OA}(1+P_{As})$ then $q_A = t_A/(1+P_{As})$, and that when $t_A > C_{OA}(1+P_{As})$ then $q_A = t_A/(1+P_m) + (P_m - P_{As})C_{OA}/(1+P_m)$.

In case 2, $q_B = t_B/(1+P_{Bs})$ is readily obtained by combining Equation 2 and the second concentration relation $x_B = q_B$. On the other hand, the first relation can be transformed into $x_A(x_A+q_B)^K = C_{OA}^{1+K}$. The solution of this equation is obtained by defining $F(x_A) = x_A(x_A+q_B)^K - C_{OA}^{1+K}$ and applying the successive approximation method using the well known formula $x_{n+1} = x_n - F(x_n)/F'(x_n)$, where x_{n+1} and x_n stand for the n+1th and nth order approximation. (One may use C_{OA} as the zero order approximation. Similar considerations as presented for case 1 lead to the following conclusions. When the ultimate x_A thus obtained satisfies the relation $t_A > x_A(1+P_{As})$, q_A can be calculated by inserting this value of x_A into Equation 1. Alternatively, when the ultimate x_A satisfies the relation $t_A \leqq x_A(1+P_{As})$, q_A must be equated to $t_A/(1+P_{As})$.

For cases 3, 4 and 5, the concentration relations can be transformed into

$$\ln x_A = \ln C_{OA} + \ln \frac{t_A - x_A(1+P_{As})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [3a]$$

$$\ln x_B = \ln C_{OB} + \ln \frac{t_B - x_B(1+P_{Bs})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [3b]$$

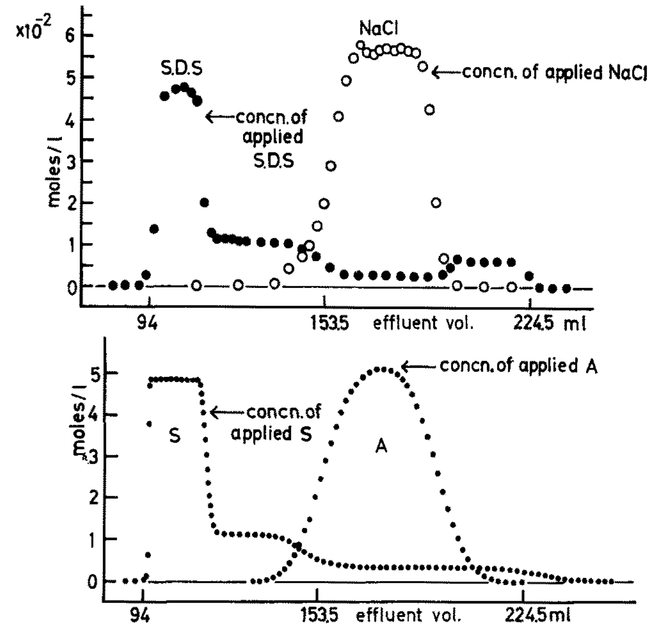

FIG. 3. Elution curves of sodium dodecyl sulfate (S, surfactant) and sodium chloride (A, added salt). Application volume 40 ml, void volume 70 ml, $P_s \equiv P_{As} = 1.71$, $P_a \equiv P_{Bs} = 1.21$, $P_m = 0.08$, $C_{OA} = 8.60 \times 10^{-3}$ moles/liter.

$$\ln x_A = -K\ln(x_A+x_B) + (1+K)\ln C_{OA} + \ln\frac{t_A - x_A(1+P_{As})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [4a]$$

$$\ln x_B = -K\ln(x_A+x_B) + (1+K)\ln C_{OB} + \ln\frac{t_B - x_B(1+P_{Bs})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [4b]$$

$$\ln x_A = \ln C_{OA} + \ln\frac{t_A - x_A(1+P_{As})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [5a]$$

$$\ln x_B = \ln C_{OB} + \frac{2K+1}{K+1}\ln\frac{t_B - x_B(1+P_{Bs})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [5b]$$

by substituting $\{t_A - x_A(1+P_{As})\}/(1+P_m)$ for $q_A - x_A$ and $\{t_B - x_B(1+P_{Bs})\}/(1+P_m)$ for $q_B - x_B$. (cf. Equations 1 and 2). Similar considerations as in case 1 lead to the conclusion that when the values of x_A and x_B obtained by solving simultaneous Equations a and b satisfy the relation $t_A > x_A(1+P_{As})$ or $t_B > x_B(1+P_{Bs})$, then q_A and q_B can be calculated by inserting these values into Equations 1 and 2. When $t_A \leqq x_A(1+P_{As})$ is satisfied, $q_A = t_A/(1+P_{As})$ and $q_B = t_B/(1+P_{Bs})$ are deduced.

The remaining problem is the mathematical method for solving Equations a and b.

SOLUTION OF SIMULTANEOUS EQUATIONS a AND b

Equations 3a and 3b

Defining $Y = \{t_B - x_B(1+P_{Bs})\}/\{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})\}$, one obtains $x_A = C_{OA}(1-Y)$ from equation 3a and $x_B = C_{OB}Y$ from Equation 3b. Insertion of these expressions into $(1-Y)/Y = \{t_A - x_A(1+P_{As})\}/\{t_B - x_B(1+P_{Bs})\}$ yields $(1-Y)/Y = \{t_A - C_{OA}(1+P_{As})(1-Y)\}/\{t_B - C_{OB}(1+P_{Bs})Y\}$. From this relation, one gets

$$pY^2 - (p+t_A+t_B)Y + t_B = 0$$

under the definition of a constant $p = C_{OB}(1+P_{Bs}) - C_{OA}(1+P_{As})$. The proper solution of Y is given by

$$Y = t_B/(t_A+t_B) \text{ for } p = 0$$

$$Y = \{p + t_A + t_B - \sqrt{(p+t_A+t_B)^2 - 4pt_B}\}/2p \text{ for } p \neq 0.$$

One can obtain q_A and q_B by the following steps; calculation of p, calculation of Y by the former formula if $p = 0$ and by the latter formula if $p \neq 0$, calculation of $x_A = C_{OA}(1-Y)$ and $x_B = C_{OB}Y$, calculation of q_A and q_B by inserting x_A and x_B into Equations 1 and 2 if $t_A > x_A(1+P_{As})$, calculation of $q_A = t_A/(1+P_{As})$ and $q_B = t_B/(1+P_{Bs})$ if $t_A \leqq x_A(1+P_{As})$.

Equations 4a and 4b

From [4a] and [4b] one obtains

$$x_A = (x_A+x_B)^{-K}C_{OA}^{1+K}\frac{t_A - x_A(1+P_{As})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [5]$$

$$x_B = (x_A+x_B)^{-K}C_{OB}^{1+K}\frac{t_B - x_B(1+P_{Bs})}{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})} \qquad [6]$$

Division of [5] by [6] gives

$$x_A/x_B = C_{OA}^{1+K}\{t_A - x_A(1+P_{As})\}/C_{OB}^{1+K}\{t_B - x_B(1+P_{Bs})\}$$

from which one can derive

$$x_B = C_{OB}^{1+K}t_Bx_A/(C_{OA}^{1+K}t_A+Px_A) \qquad [7]$$

where $P = C_{OB}^{1+K}(1+P_{Bs}) - C_{OA}^{1+K}(1+P_{As})$.

Up to the present, no restriction has been imposed on the notations of surfactants; either of them may be denoted by A. We shall now specify the notations as to make P positive. Then, it is easy to show that x_B is a positive increasing function of positive x_A. On the other hand, the addition of $[5]/C_{OA}^{1+K}$ and $[6]/C_{OB}^{1+K}$ gives

$$(x_A+x_B)^K(x_A/C_{OA}^{1+K} + x_B/C_{OB}^{1+K}) = 1$$

which can be transformed into

$$F(x_A,x_B) = (x_A+x_B)^K(C_{OB}^{1+K}x_A + C_{OA}^{1+K}x_B) - C_{OA}^{1+K}C_{OB}^{1+K} = 0$$

It is apparent that $F(x_A,x_B)$ is an increasing function with respect to positive x_A and x_B.

Suppose a value of x_A in a range from zero to C_{OA}, calculate the corresponding value of x_B by Equation 7, and insert these values into $F(x_A,x_B)$. If $F(x_A,x_B)$ thus obtained is equal to zero, the correct answer has been obtained accidentally. Generally, however, $F(x_A,x_B)$ is not equal to zero. The value of $F(x_A,x_B)$ is negative at $x_A = 0$, increases monotonically with an increase in x_A, and is positive at $x_A = C_{OA}$. Therefore, if $F(x_A,x_B)$ obtained is positive, the correct x_A must be somewhere between zero and the supposed x_A; if $F(x_A,x_B)$ is negative, the correct x_A is between the supposed x_A and C_{OA}. In a similar way, one can successively limit the range of x_A to be examined and approach the correct solution. This procedure may be called the limiting-range method. When a sufficiently accurate solution has been obtained, q_A and q_B are calculated as described in the preceding section.

Equations 5a and 5b

From [5a] and [5b] one gets

$$\{t_A - x_A(1+P_{As})\}/\{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})\} = x_A/C_{OA}$$

$$\{t_B - x_B(1+P_{Bs})\}/\{t_A - x_A(1+P_{As}) + t_B - x_B(1+P_{Bs})\} = (x_B/C_{OB})^{(K+1)/(2K+1)}$$

The first equation can be transformed into

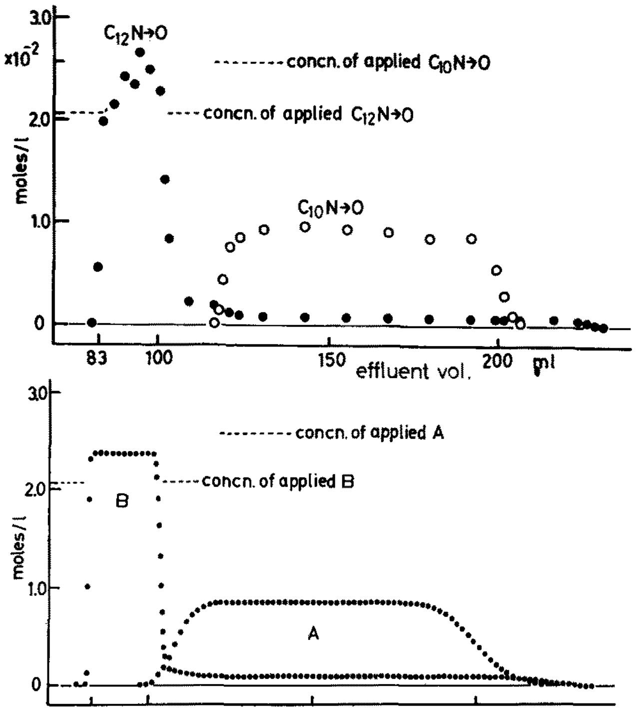

FIG. 4. Elution curves of decyl- and dodecyl-dimethylamine-N-oxides. Application volume 30 ml, void volume 70 ml, $P_{As} = 1.42$, $P_{Bs} = 1.73$, $P_m = 0.04$, $C_{OA} = 1.82 \times 10^{-2}$ moles/liter, $C_{OB} = 1.70 \times 10^{-3}$ moles/liter.

$$x_B = \frac{1}{1+P_{Bs}}\left\{t_A + t_B - (1+P_{As})x_A - \frac{C_{OA}t_A}{x_A} + C_{OA}(1+P_{As})\right\} \qquad [8]$$

Addition of both equations results in

$$(x_A/C_{OA}) + (x_B/C_{OB})^{(K+1)/(2K+1)} = 1$$

which can be transformed into

$$F\ (x_A,x_B) = C_{OA}x_B^{(K+1)/(2K+1)} + C_{OB}^{(K+1)/(2K+1)}\,x_A - C_{OA}C_{OB}^{(K+1)/(2K+1)} = 0$$

It is obvious that the solution of [8] and $F\ (x_A,x_B) = 0$ satisfies [5a] and [5b] simultaneously. Examination of the mathematical features of [8] and $F\ (x_A,x_B)$ reveals that the limiting-range method is applicable also to this case. The value of x_A that satisfies [5a] and [5b] must be larger than

$$\left\{t_A+t_B+C_{OA}(1+P_{As}) - \sqrt{\{t_A+t_B+C_{OA}(1+P_{As})\}^2 - 4(1+P_{As})C_{OA}t_A}\right\}/2(1+P_{As}),$$

and smaller than both of C_{OA} and $t_A/(1+P_{As})$. This means that the higher end of the range of x_A is C_{OA} if $C_{OA} < t_A/(1+P_{As})$, and is $t_A/(1+P_{As})$ if $C_{OA} > t_A/(1+P_{As})$. The value of $F\ (x_A,x_B)$ is negative at the lower end, and increases with an increase in x_A. When the higher end is C_{OA} the value of x_A which satisfies $F(x_A,x_B) = 0$ can always be found, because $F(x_A,x_B)$ at $x_A = C_{OA}$ is positive. When the higher end is $t_A/(1+P_{As})$, the value of $F(x_A x_B)$ at this point is not necessarily positive. If it is negative, q_A must be equated to $t_A/(1+P_{As})$ and q_B to $t_B/(1+P_{Bs})$. In any event, one can calculate q_A and q_B for any combination of t_A and t_B.

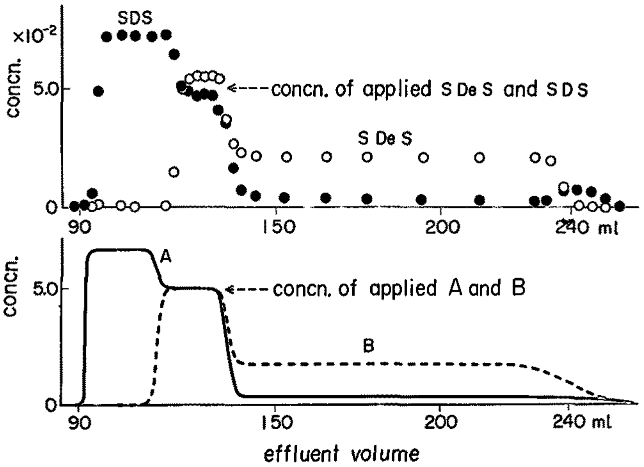

FIG. 5. Elution curves of sodium decyl and dodecyl sulfates. Application volume 58 ml, void volume 70 ml, $P_{As} = 1.71$, $P_{Bs} = 1.61$, $P_m = 0.10$, $C_{OA} = 8.60 \times 10^{-3}$ moles/liter, $C_{OB} = 3.35 \times 10^{-2}$ moles/liter.

This method has, however, a disadvantage. It becomes apparent in the case of extremely small t_A. For small t_A, the value of x_A must be small too. The evaluation of the fourth term in the right hand side of Equation 8 is inaccurate when both the numerator and the demoninator are near zero.

INPUT FOR COMPUTER

Gel filtration chromatography is usually carried out by the following steps: pretreatment of the column with pure water, charging an aqueous solution of certain A and B concentrations slowly, and elution with pure water. In some cases, however, a solution containing A or B, or both, is used for the pretreatment or the elution, or both. Therefore, these six concentrations are required as input data. The amount of charge can be expressed by the number of plates to be charged if one specifies beforehand the number of plates which are supposed to constitute the whole column. For the latter, too large or too small a number is not recommended, because the larger the number, the more accurate the result but the longer the calculation time. It is the authors' experience that 200 plates can produce reasonably good results in most cases.

Aside from the above-mentioned input data, five parameters, C_{OA}, C_{OB}, P_{As}, P_{Bs} and P_m, must be determined. This can be done by the filtration experiments of two solutions containing A or B alone. The experimental elution curve of the solution of A is compared with the theortical one which is obtained by assuming a set of values of C_{OA}, P_{As} and P_m. By the trial and error method, one seeks for a set that brings excellent agreement between both curves. This is not as difficult as might be supposed. After several trials, one can find the required set of values. The C_{OA} thus obtained agrees well with the cmc determined by other methods, and P_{As} is far larger than P_m when a suitable gel, such as Sephadex G-25, is employed. These facts support the adequacy of the concentration relation assumed in case 1. Using the other solution, one determines C_{OB}, P_{Bs} and P_m. The arithmetical mean of the two P_m's thus obtained is regarded as the P_m of the mixture of A and B. The adequacy of the concentration relations assumed in cases 2-5 can be examined by comparing the experimental elution curves with the theoretical ones which are obtained by using these five parameters.

IDEAL CONDITIONS ASSUMED IN THE THEORY

The ideal conditions assumed in the theory are uniform

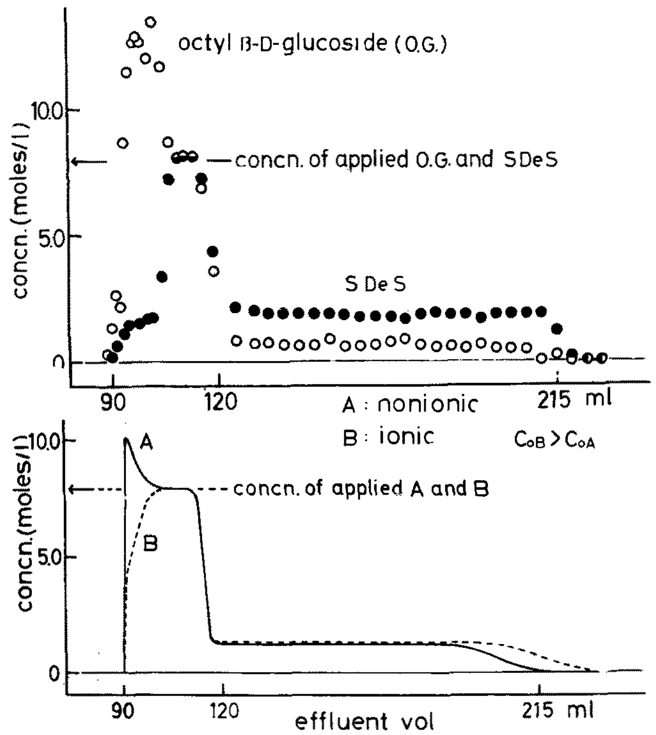

FIG. 6. Elution curves of octyl β-D-glucoside and sodium decyl sulfate. Application volume 39 ml, void volume 70 ml, $P_{As} = 1.34$, $P_{Bs} = 1.54$, $P_m = 0.08$, $C_{OA} = 2.50 \times 10^{-2}$ moles/liter, $C_{OB} = 3.35 \times 10^{-2}$ moles/liter.

packing of the column, no flow of solution in gel cavities, no adsorption on gel surface, rapid establishment of concentration equilibrium between mobile and stationary phases (this requires rapid establishment of exchange equilibrium between micelles and monomers), no vertical diffusion along the column, no convection in the column, and constance of P_m throughout the whole course of chromatography, in spite of possible change of micelle structure. In practice, these conditions can not be fully realized.

COMPARISON OF THEORETICAL AND EXPERIMENTAL ELUTION CURVES

Figure 2 represents the elution curves of SDeS. Different amounts of an aqueous surfactant solution were applied to a column and eluted with pure water. Generally speaking, the agreement between the theoretical and experimental curves seems satisfactory. The concentration in the plateau region coincides with the cmc (8.7 mg/ml). In all examples shown in Figure 2 (and in other figures), the slope of the tailing part of the experimental curve is steeper than that of the theoretical curve, which converges asymptotically to the abscissa. This may be due to the incomplete realization of the second condition assumed in the preceding section.

In the presence of sodium chloride, the theoretical elution curve of an ionic surfactant, sodium dodecyl sulfate (SDS), becomes more complicated, as shown in the lower half of Figure 3. It rises sharply from zero to a definite value exceeding the concentration of the applied solution. The curve remains at this level for a short period, then it drops sharply to a value close to the cmc. In line with the elution of NaCl, the concentration decreases further and finally converges to zero. The above mentioned features are recognized also in the experimental curve, which displays, however, a slight recovery after the elution of NaCl has been completed. This difference of behavior is probably due to the adsorption of SDS promoted by the presence of NaCl; the surfactant adsorbed on gel surface is desorbed after NaCl has been eluted out. It may be said that the theoretical and experimental elution curves show fairly good agreement on the whole, in spite of the over idealized conditions, especially the third condition assumed in the theory.

A mixture of two nonionic surfactants, decyl- and dodecyl-dimethylamine-N-oxides, gave the elution curves reproduced in Figure 4. The effluent at an early stage consists of pure dodecyl homolog, the cmc of which is lower than that of the partner surfactant. At a later stage, the effluent is rich in the decyl homolog, but includes a small but measurable amount of the other component. The heights of elution curves in the plateau region agree with the monomer concentrations of both species in the applied solution.

Figure 5 illustrates the elution curves of a mixture of two ionic surfactants, SDeS and SDS. Three regions appear in this figure because a fairly large amount was applied (When a small amount was applied, the shape of the elution curves resembled Figure 4 (7)): the flowing out of a solution of pure SDS whose concentration is higher than that in the applied solution, a solution whose composition is the same as that of the applied solution, a solution in which the concentrations of both components are equal to the concentrations of monomeric surfactants in the applied solution. A small hump in the experimental curve of SDS may be ascribed to the desorption promoted by the diminution of the partner surfactant.

Excellent agreement between the theoretical and experimental curves in the above examples and in some additional examples reported in previous papers (2,4,7) supports the adequacy of the concentration relations for cases 1-4. The same is not true for case 5. Figure 6 shows the elution curves of a mixture of an ionic surfactant SDeS and a nonionic surfactant octyl β-D-glucoside. The agreement between the theoretical and experimental curves is unsatisfactory. The heights of curves of both components at the plateau region show a great discrepancy between theory and experiment. The discrepancy is too large to be ascribed solely to the experimental inaccuracy or the mathematical inaccuracy pertaining to the solution of Equations 5a and 5b, or both; the concentration relations derived for case 5 must be inapplicable to a mixture of SDeS and octyl glucoside. The derivation of these relations shall, therefore, be outlined and reexamined below.

Shinoda has derived an equation which correlates the monomer concentration and total concentration of an ionic surfactant in the presence of a solubilizate such as a long chain alcohol (8). In other worlds, he derived a concentration relation for a system in which B is an ionic surfactant but A is a nonionic solubilizate. Supposing the case where A is a nonionic and B is an ionic surfactant, the authors have traced his logic and arrived at the same relation for component B. (Assuming K = 0.56, Shinoda replaced the coefficient (2K+1)/(K+1) with 1.36.) On the other hand, an appropriate relation for component A has been derived on assuming ideal mixing of the components in mixed micelles (5). As shown in a previous section (Equilibrium Concentrations), the latter relation has the same form as that of the relations for case 3.

The derivation has been performed by considering a mixed micelle composed of an ionic and a nonionic surfactants. The electrical potential of the micelle surface is proportional to the square of charge density. Therefore, the electrical work required to bring a surfactant ion from the surrounding water phase into the micelle changes with the micellar composition. The required work was estimated as a function of the mixing ratio by taking into account only the change of charge density and disregarding the change of dielectric constant. If the size of the hydrophilic group of the nonionic surfactant molecule had been small, this treatment would have been allowable.

In the present case, the nonionic surfactant is octyl glucoside. A considerable portion of the micelle surface must be covered with bulky glucose residues. In the proximity of the micelle surface, the effective dielectric constant may be lowered from that in the water phase. The degree of the lowering depends on the mixing ratio. The bulkiness of the hydrophilic group may bring about another effect. The mechanical work to transfer a nonionic surfactant molecule from the water phase into the micelle may vary with the mixing ratio in a complicated manner. These effects are difficult to evaluate and were neglected in the calculation. The neglect of these effects may have led to the inadequate concentration relations.

REFERENCES

1. Shinoda, K., T. Nakagawa, B. Tamamushi and T. Isemura "Colloidal Surfactants, Some Physicochemical Properties," Academic Press, New York, 1963, p. 37.
2. Nakagawa, T., and H. Jizomoto, "Proceedings of the Fifth International Congress on Surface Active Substances," Vol. 1, 1968, p. 299.
3. Shinoda, K., T. Nakagawa, B. Tamamushi and T. Isemura, "Colloidal Surfactants, Some Physicochemical Properties," Academic Press, New York, 1963, Equation 1.55, p. 58.
4. Nakagawa, T., and H. Jizomoto, Kolloid Z. Z. Polym. 236:79 (1970).
5. Nakagawa, T., and H. Jizomoto, Ibid. 234:1124 (1969).
6. Shinoda, K., T. Nakagawa, B. Tamamushi and T. Isemura, "Colloidal Surfactants, Some Physicochemical Properties," Academic Press, New York, 1963, Equations 1.69, 1.70, p. 65.
7. Nakagawa, T., and H. Jizomoto, Kolloid Z. Z. Polym. 239:606 (1970).
8. Shinoda, K., T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants, Some Physicochemical Properties," Academic Press, New York, Equation 1.82, p. 73.
9. Noller, C.R., and W.C. Rockwell, J. Amer. Chem. Soc. 60:2076 (1938); Fishher, E., Ber. 49:584 (1916).
10. Aoki, M., and Y. Iwayama, J. Pharm. Soc. Jap. 79:522 (1959).

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