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Gel Filtration of Solubilized Systems. I.[1] On the Gel Filtration of Aqueous Sodium Lauryl Sulfate Solution Solubilizing Alkyl Paraben on Sephadex G-50

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The gel filtration of a micellar solution on Sephadex G-50 was applied for the study of partition of methyl, ethyl and butyl parabens between the micellar and aqueous phases.

When, after the gel layer having been pre-equilibrated with 8 mM sodium lauryl sulfate (SLS), SLS solutions solubilizing alkyl parabens were eluted with 8 mM SLS nearly equal to critical micelle concentration (cmc) as an eluent, decomposition of micelles might be prevented. In this case, however, liberation of alkyl paraben from a micellar phase was observed, accordingly to a property of alkyl paraben or a sample volume (correlated to the gel volume). It might therefore be difficult to investigate quantitatively the distribution state of alkyl paraben in micelles.

It was tried that the gel pre-equilibrated with a large sample was eluted with water as an eluent (the so-called tail analysis). As a result, it was found from the elution curves of alkyl parabens obtained by the tail analysis that partition ratios of the alkyl parabens between the micellar and aqueous phases may be determined. These results correspond to the data from the solubility method. However, information from the solubility method is only obtained about the saturated solution from the nature of experiment. From gel filtration, on the other hand, it may be possible to get information not only about a partition ratio for a solute unsaturated in a detergent solution, but also about the state of detergent micelles.

Keywords——SLS; alkyl paraben; Sephadex G-50; partition ratio; micellar phase; aqueous phase; tail analysis; zone analysis; solubilization

Preservatives are often added to micellar solubilized preparations. In such systems, it is recognized that a partition of preservatives in a micellar phase results in an alteration of an antifungal activity.[3] When a poorly water soluble substance is solubilized in micellar solutions, the phenomenona have been investigated quantitatively by means of solubility method,[4] ultrafiltration,[5] equilibrium dialysis,[6] molecular sieve,[7] gel filtration, *etc.* The

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[4] S.M. Blaug and S.S. Ahsan, *J. Pharm. Sci.*, **50**, 441 (1961).

[5] S.J. Dougherty and J.C. Berg, *J. Colloid Interface Sci.*, **48**, 110 (1974).

[6] *a)* N.K. Patel and H.B. Kostenbauder, *J. Amer. Pharm. Assoc., Sci. Ed.*, **47**, 289 (1958); *b)* H. Matsumoto, H. Matsumura, and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **14**, 398 (1966).

[7] *a)* R.W. Ashworth and D.D. Heard, *J. Pharm. Pharmacol.*, **18**, 98S (1966); *b)* M. Donbrow, E. Azaz and R. Hamburger, *J. Pharm. Sci.*, **59**, 1427 (1970).

gel filtration was at first applied to the solubilized systems by Herries, *et al.*[8] and Borgström.[9] The elution behaviors of solubilizates were noticed in their experiments, but those of micelles were not considered by them at all.

On the other hand, Suzuki and Sasaki[10] analyzed the elution curves (Fig. 1) obtained by the gel filtration of aqueous sodium lauryl sulfate (SLS) solutions, and it is as follows. The elution curve of the SLS solution of a concentration above critical micelle concentration (cmc) shows two plateaus. The higher one is equal to the total concentration of SLS in a sample ($[SLS]_t$), and may be considered to be consisted of SLS forming micelles and monomer SLS equal to cmc. On the other hand, the lower one may be considered to be monomer SLS which is equal to cmc.

This elution behavior of the SLS miceller solution is worthy of remark. Therefore, the gel filtration of aqueous SLS solutions solubilizing various alkyl parabens under different experimental conditions was tried in order to investigate not only elution behaviors of a solubilizate, but also those micelles. As a result, partition ratios of the solubilizates between the micellar and aqueous phases could be determined as are reported in this paper. Sephadex G-50 was used as the gel according to Suzuki and Sasaki.[10] Measurement of solubilities of alkyl parabens in aqueous SLS solutions and water was made for the comparison of the results obtained by the gel filtration.

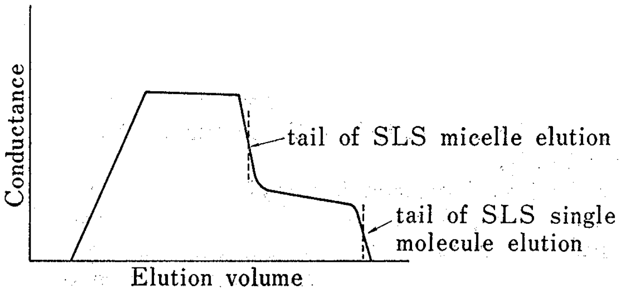

Fig. 1. Typical Elution Curve of SLS Solution of Concentration above cmc

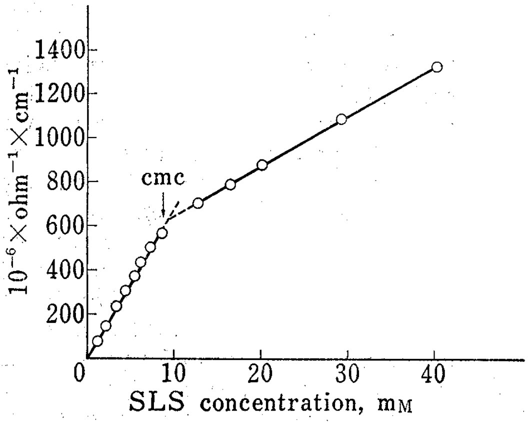

Fig. 2. Conductance (κ) of Aqueous SLS Solution plotted against the Concentration of SLS at 27°

Experimental

Materials——SLS was purified according to the method of Miyamoto.[11] Methyl, ethyl and butyl parabens used were of reagent grades. The water was used after the treatment of distilled water by ion exchanger and the distillation from an all Pyrex glass distillation apparatus.

Preparation of Sample Solutions——The SLS solutions solubilizing alkyl parabens were prepared by dissolving alkyl parabens in 40 mM SLS solutions.

Gel Filtration——The gel of Sephadex G-50 was put in a column with a diameter of 2.5 cm and a height of 45 cm made by Pharmacia. The column was jacketed and maintained at 27°. The gel bed was 41 cm in high. The gel filtration of sample solutions was made as follows. Method-I: On the gel column pre-equilibrated with 8 mM SLS solution, nearly equal to cmc (Fig. 2 shows cmc of SLS solution measured by an electrical conductivity method.), the various volumes of sample solutions were applied and then eluted with 8 mM SLS solution. Method-II: On the gel column pre-equilibrated with water, 100 ml of the sample solution was

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[8] D.G. Herries, W. Bishop, and F.M. Richards, *J. Phys. Chem.*, **68**, 1842 (1964).

[9] B. Borgström, *Biochim. Biophys. Acta*, **106**, 171 (1965).

[10] *a*) H. Suzuki and T. Sasaki, *Bull. Chem. Soc. Japan*, **44**, 2630 (1971); *b*) H. Suzuki, *Bull. Chem. Soc. Japan*, **49**, 375 (1976); *c*) H. Suzuki, *Bull. Chem. Soc. Japan*, **49**, 1470 (1976).

[11] S. Miyamoto, *Bull. Chem. Soc. Japan*, **33**, 371 (1959).

applied and then eluted with water. Method-III: On the gel column pre-equilibrated with 250 ml of the sample solution, the sample was eluted with water. The elution rate was about 24 ml/hr. Portions of 2 ml were exactly fractioned by an automatic fraction collector (Toyo Kagaku SF-160K type). The concentrations of alkyl paraben and SLS in each fraction were determined after the dilution with water. The total volume of the gel bed used (V_t) was 201 ml. The void volume of the gel bed (V_0) was 71 ml.[12] The elution volume of NaCl obtained by eluting 50 mM NaCl of 2 ml with water (so-called zone analysis) was 171 ml. Any change of V_0 and of the elution volume of NaCl was not observed in the presence of alkyl paraben. It was therefore suggested that gel conformation might not be changed in the presence of alkyl paraben. By elution of each 2 ml of the aqueous solutions of SLS (4 mM), methylparaben (15 mM), ethylparaben (6 mM), and butylparaben (1 mM), the elution volume (V_e) and $K_{av}=V_e-V_0/V_t-V_0$ of each solute were determined in the gel column.[13]

Determination of SLS and Alkyl Paraben——The concentration of alkyl paraben in SLS solution was determined by measuring the absorbance at 256 mμ (HITACHI 101 Spectrophotometer). A concentration of alkyl paraben was measured by diluting each fraction to be below 1 mM with respect to SLS, since no interference by the presence of SLS below 1 mM was observed for the determination of alkyl paraben.

A concentration of SLS was measured by electrical conductivity method (TOA DENPA type CM-2A). Plots of conductivities of SLS solutions against SLS concentrations (Fig. 2) consist of a straight line passing through the origin in the lower concentration region and another straight line in the higher concentration with a breakpoint (arrow in Fig. 2), which corresponds to cmc. The SLS concentration in each fraction was therefore diluted to be below cmc. The presence of alkyl paraben has little or no effect on the conductivity of SLS solution of a concentration below cmc.

Measurements of Solubilities of Alkyl Parabens in Water and SLS Solutions——The excess alkyl paraben was put in glass stoppered flasks containing 10 ml of water or SLS solutions. The flasks were then shaken at 27.0±0.1° in an incubator (Taiyo incubator M-I type) to attain an equilibrium. After a given volume of the supernatant was diluted adequately with water, the concentration of alkyl paraben in the solution was determined

Results

Solubilities of Alkyl Paraben in SLS Solutions

Table I shows that the respective alkyl parabens have higher solubilities in 40 mM SLS solutions (S) than in water (S_0). The $(S-S_0)/S_0$ ratio of each alkyl paraben increased markedly with the length of alkyl chain in alkyl parabens (Table III).

TABLE I. Solubility of Alkyl Paraben in Aqueous SLS Solution at 27°

	Solubility in water (S_0) mM	Solubility in 40 mM SLS (S) mM
Methylparaben	14.5	33.9
Ethylparaben	5.4	22.7
Butylparaben	1.1	24.3

TABLE II. K_{av} Values for Alkyl Parabens, SLS and NaCl

	V_e, ml	K_{av} [a]
Methylparaben	210	1.1
Ethylparaben	210	1.1
Butylparaben	230	1.2
SLS	190	0.92
NaCl	171	0.77

a) The K_{av} value was calculated from the equation, $K_{av}=V_e-V_0/V_t-V_0$: V_0 and V_t were 71 and 201 ml, respectively. (See Experimental)

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[12] The elution volume of Blue Dextran 2000.

[13] T.C. Laurent and J. Killander, *J. Chromatog.*, **14**, 317 (1961).

Elution Parameters of SLS and Alkyl Paraben

The K_{av} values were calculated from the elution volume (V_e) for each of alkyl parabens and SLS obtained by the gel filtration.[13] From the result that the K_{av} values of all alkyl parabens are larger than 1.0, it may be assumed that alkyl parabens are in the condition to be easily adsorbed on the gel by the presence of aromatic ring in the molecules.[14]

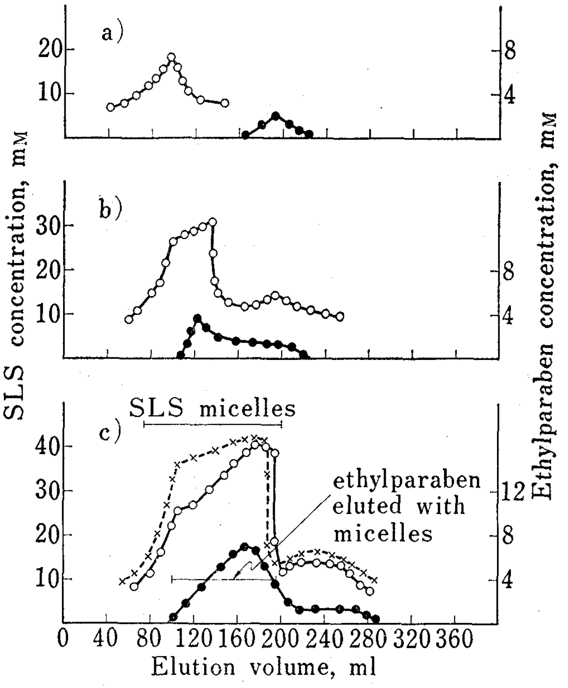

Fig. 3. Gel Filtration of 7 mM Ethylparaben in 40 mM SLS

After Sephadex gel in the column was pre-equilibrated with 8 mM SLS, the sample of each volume of a) 10 ml, b) 30 ml and c) 100 ml was applied on top of the column, and then eluted with 8 mM SLS.
—○—: SLS concentration in the eluate
—●—: ethylparaben concentration in the eluate
---×---: SLS concentration in the eluate using 40 mM SLS without ethylparaben as a sample

On the Elution Curves of 40 mM SLS Solutions Solubilizing Alkyl Paraben eluted with 8 mM SLS Solution

The gel filtration of SLS solutions solubilizing alkyl parabens was made according to the method–I (See Experimental). The results are shown in Fig. 3—5. The concentration of each alkyl paraben in the sample solution (Fig. 3—5) is lower than the saturated concentration in 40 mM SLS solution (Table I). The cumulative volume of elution fraction is shown on the abscissa, in which a zero point exhibits the point that influx of the sample in the gel column started.

The elution curves obtained by the gel filtration of various volume sizes of the 40 mM SLS solution solubilizing ethylparaben are shown in Fig. 3. When 10 ml of a sample was used (Fig. 3a), the appearance of ethylparaben was further delayed from the end of SLS micelle elution which was observed ranging in elution volume of about 70 to 110 ml. But in the case of 30 ml of a sample volume (Fig. 3b), the appearance of ethylparaben was observed in the range of SLS micelle elution which was observed in about 70 to 140 ml. Such a phenomenon that micelles and ethylparaben coexist in the eluate was observed to a marked degree with a sample size of 100 ml (Fig. 3c). In this case, two peaks were observed on the elution curves of SLS micelles. Appearance of the micelles in about 70 ml nearly agrees with that of SLS micelles in the absence of alkyl paraben (dotted line in Fig. 3c). After the first peak (about 100 ml) was observed, the concentration of SLS increased again with the appearance of ethylparaben in eluate (about 110 ml). The elution curve of SLS became finally to show the second peak (about 180 ml). The respective concentrations of SLS and ethylparaben in this peaks were approximately equal to those in a sample solution used. A steep lowering of the SLS concentration (the tail in the elution curve of SLS micelles) was observed. Simultaneously the concentration of ethylparaben also decreased quickly.

After SLS micelles have been eluted, another peak (about 230 ml) is observed in the elution curve of SLS, as shown in Fig. 3c. The phenomena seem to be characteristic of SLS elution, since no increase in the concentration of coexisting ethylparaben in parallel with SLS was observed and the peak was observed in the elution curve for the SLS solution without ethylparaben.

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[14] B. Gellote, *J. Chromatog.*, **3**, 330 (1960).

The elution curves obtained by the gel filtration of 40 mM SLS solution solubilizing methylparaben are shown in Fig. 4. In a sample size of 30 ml, the result was different from that of ethylparaben (Fig. 4a); methylparaben began to be eluted distinctly later than SLS micelles. The elution curves for 40 mM SLS solution solubilizing butylparaben are shown in Fig. 5. There is a marked tendency for butylparaben having a more hydrophobic property to be eluted with micelles. It is observed that butylparaben is eluted in parallel with micelles over the whole range where micelles are eluted, even in a sample size of 30 ml.

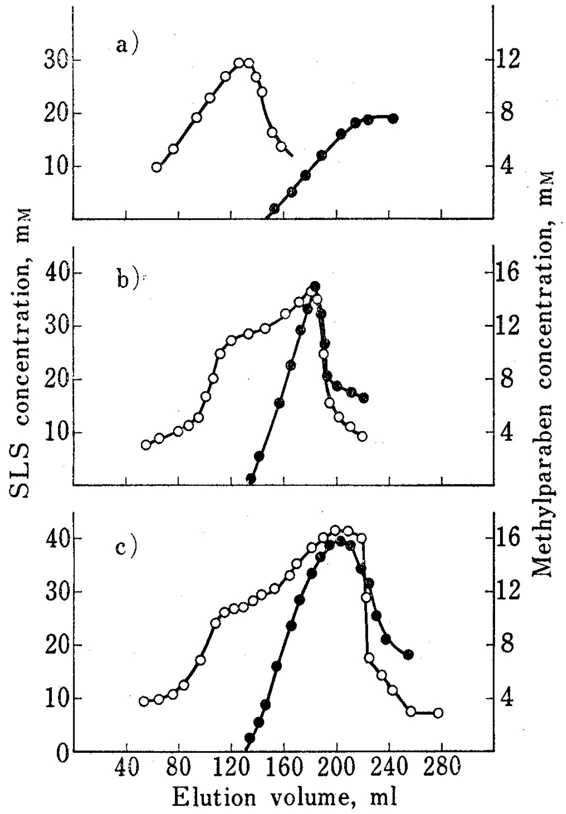

Fig. 4. Gel Filtration of 16 mM Methylparaben in 40 mM SLS

After Sephadex gel in the column was pre-equilibrated with 8 mM SLS, the sample of each volume of a) 30 ml, b) 70 ml, and c) 100 ml was applied on top of the column, and then eluted with 8 mM SLS.
—○—: SLS concentration in the eluate
—●—: methylparaben concentration in the eluate

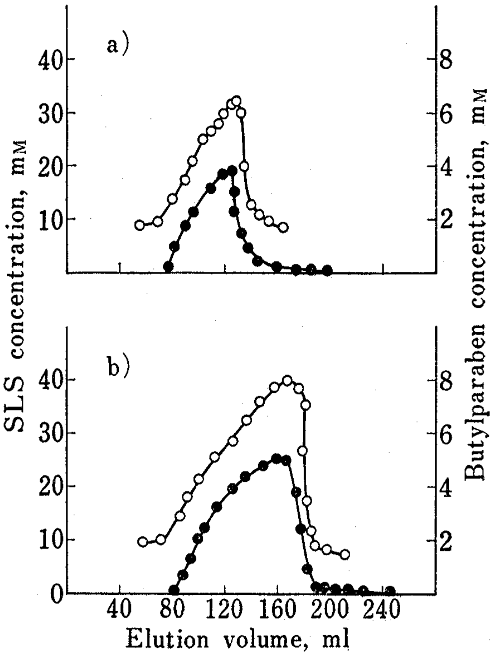

Fig. 5. Gel Filtration of 5 mM Butylparaben in 40 mM SLS

After Sephadex gel in the column was pre-equilibrated with 8 mM SLS, the sample of each volume of a) 30 ml and b) 70 ml was applied on top of the column, and then eluted with 8 mM SLS.
—○—: SLS concentration in the eluate
—●—: butylparaben concentration in the eluate

On the Elution Curves of SLS Solutions Solubilizing Alkyl Parabens Eluted with Water

Elution curves of 40 mM SLS solubilizing alkyl parabens obtained according to the method -II are shown in Fig. 6. These elution patterns are different from each other. Methylparaben begins to appear slightly later than SLS micelles, as shown in Fig. 6a. Two peaks are observed on the elution curve of SLS as well as on that in Fig. 4c. Ethylparaben is eluted with micelles over the whole range where the micelles are eluted, as in Fig. 6b. The elution behavior of butylparaben (Fig. 6c) is remarkably different from the two cases just described; that is, butylparaben appears in eluate prior to the elution of micelles. In this case, it was observed that a precipitate of butylparaben comes out and result in turbidity of the eluate. After the peak has appeared once, butylparaben exhibits a decrease, followed by the steady plateau. A plateau is also observed on the elution curve of SLS in parallel with that of butylparaben.

Attention was chiefly focused upon the front parts in the elution curves, and observations were made of differences between elution behaviors resulted from the solubilized systems. The so-called tail analysis[10] is then tried according to the method-III (See Experimental). The result of 40 mM SLS solution solubilizing ethylparaben is shown in Fig. 7. The concentration of ethylparaben in the sample was adjusted near the solubility limit in 40 mM SLS solution,

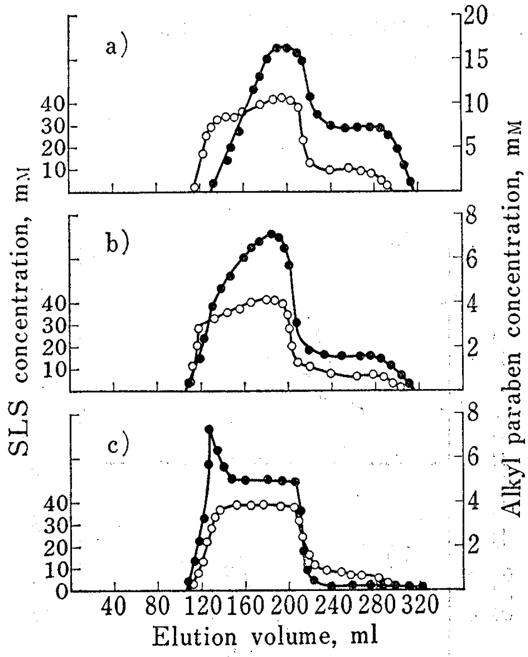

Fig. 6. Gel Filtration of Alkyl Parabens in 40 mM SLS

After Sephadex gel in the column was pre-equilibrated with water, the 100 ml samples solubilizing a) 16 mM methylparaben, b) 7 mM ethylparaben, and c) 5 mM butylparaben were applied on top of the column, and then eluted with water.

—○—: SLS concentrartion in the eluate
—●—: alkyl paraben concentration in the eluate

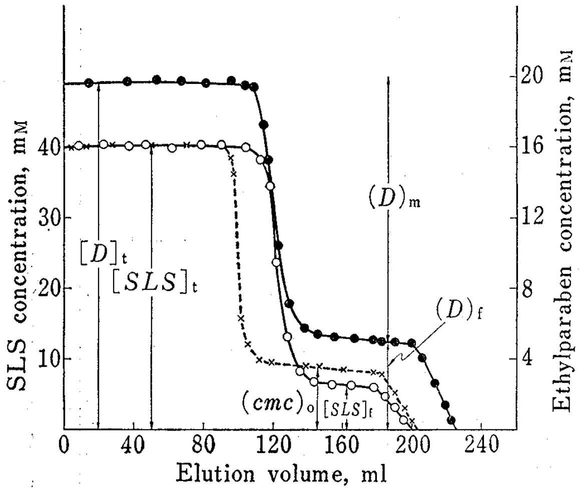

Fig. 7. Tail Analysis for Elution Curves of 19.5 mM Ethylparaben in 40 mM SLS

On the column of Sephadex G-50 pre-equilibrated with the sample solution of 250 ml, the sample was eluted with water.

—○—: SLS concentration in the eluate
—●—: ethylparaben concentration in the eluate
---×---: SLS concentration in the eluate using 40 mM SLS without ethylparaben as a sample

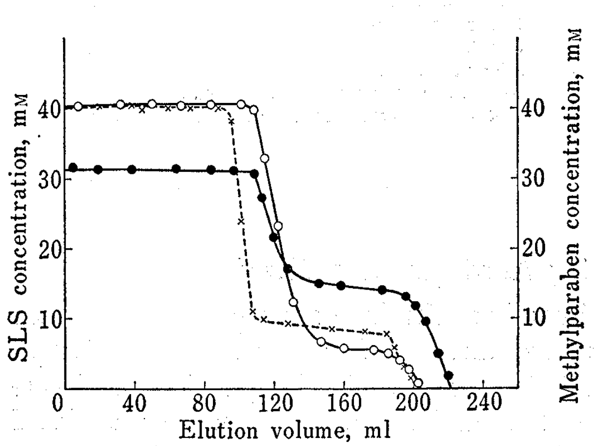

Fig. 8. Tail Analysis for Elution Curves of 30.9 mM Methylparaben in 40 mM SLS

On the column of Sephadex G-50 pre-equilibrated with the sample solution of 250 ml, the sample was eluted with water.

—○—: SLS concentration in the eluate
—●—: methylparaben concentration in the eluate
---×---: SLS concentration in the eluate using 40 mM SLS without methylparaben

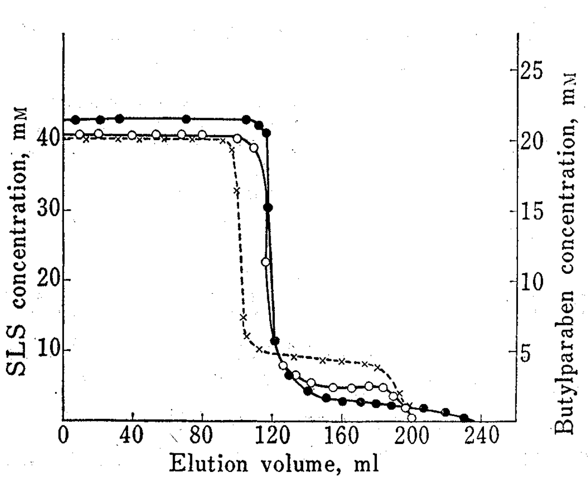

Fig. 9. Tail Analysis for Elurtion Curves of 21.0 mM Butylparaben in 40 mM SLS

On the column of Sephadex G-50 pre-equilibrated with the sample solution of 250 ml, the sample was eluted with water.

—○—: SLS concentration in the eluate
—●—: butylparaben concentration in the eluate
---×---: SLS concentration in the eluate using 40 mM SLS without butylparaben as a sample

because the result was to be compared with that from the solubility method. Zero point on the abscissa in Fig. 7 represents the point at which influx of water as an eluent started into the column having been saturated with the sample solution. It may be noticed that the elution curves are already in stable plateaus at the zero point. The heights of plateaus, $[SLS]_t$ and $[D]_t$, correspond to the concentrations of SLS and ethylparaben, respectively, in the original sample. In the range of tail (about 120 ml in Fig. 7) in SLS micelle elution, a steep lowering of the curve was observed. Simultaneously, the concentration of ethylparaben in eluate also decreased quickly. The second plateaus then appeared in both of the elution curves.

The elution curve of SLS solution without alkyl paraben under the same condition is shown in Fig. 7. The elution volume on the tail (about 100 ml in Fig. 7) in the SLS micelle elution is obviously smaller than that in the presence of ethylparaben.

Elution patterns of 40 mM SLS solutions solubilizing methylparaben and butylparaben are shown in Fig. 8 and 9. Each pattern tends to be similar to that of the sample solubilizing ethylparaben.

Discussion

Herries, *et al.*[8] and Borgström[9] applied the gel filtration to the solubilized systems. Herries, *et al.*[8] eluted SLS micellear solutions solubilizing water-insoluble substances (anithyl thioethane *etc.*) with the SLS micellar solution and investigated the distribution state of solutes between the micellar and aqueous phases. Borgström[9] subjected sodium taurodeoxycholate micellar solution solubilizing various ^{14}C-labeled fatty acids. He applied the so-called zone analysis to the gel filtration. In their experiments, consideration was given to only the elution behaviors of solubilizates, but was not to the elution behaviors of micelles. In the process of the gel filtration of solubilized systems, a solute solubilized in micelles might be assumed under a given condition to be liberated from the micellar phases because of a partition equilibrium of the solute between the micellar and aqueous phases, or bacause of decomposition of micelles. When characteristics of distribution of solubilizates between the micellar and aqueous phases in solubilized systems are investigated, it is thought to be significant that any additional information is obtained about elution behaviors of micelles. And it seems possible to determine accurately the concentration of a solubilizate in micelles by the information.

When a micellar solution of SLS is eluted with SLS solution of a concentration approximately equal to cmc, with which the gel was pre-equilibrated,[15] it might be assumed that micelles are eluted without decomposition in the process of the gel filtration. On the basis of this assumption, the authors carried out the gel filtration of solubilized systems according to the method-I. It was observed that ethylparaben was eluted by being liberated from micelles in the case of a small volume of the sample (Fig. 3). This phenomenon was more markedly observed in the case of methylparaben which is less hydrophobic than ethylparaben (Fig. 4). It is explained as follows; alkyl paraben solubilized in micelles must quantitatively be liberated in the surrounding of aqueous phase in order to maintain a partition equilibrium of the alkyl paraben between the micellar and aqueous phases, since the micelles migrate through the gel preceding the free alkyl paraben in aqueous phase. However, it was observed that a part of micelles are eluted accompanying alkyl paraben (Fig. 3b—c and Fig. 4), when a larger volume of a sample was used. By increasing sample volume, it will be resulted that a part of micelles pass through the gel layer where alkyl paraben liberated from preceding micelles presents. Accordingly, the succeeding micelles might be considered to be eluted without liberating alkyl paraben in them.

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15) T. Sasaki, *Yukagaku,* **16,** 49 (1967).

On the other hand, the elution patterns of butylparaben having a more hydrophobic property shows that most of the solubilizate is eluted with micelles, even in a sample size of 30 ml (Fig. 5a). It might be considered that these phenomena arised mainly from a difference in partition property of each solubilizate between the micellar and aqueous phases.

The decomposition of micelles in the solubilized system as described above might be prevented by pre-equilibration of gel with 8 mM SLS solution. However, a property of a solubilizate or a sample volume has a great influence on experimental results. It might be therefore difficult to consider accurately the solution state of a solubilizate in a sample solution and especially to investigate quantitatively the distribution state of a solubilizate in micelles. In addition, the determination of cmc from the elution curve of SLS solution obtained by such a gel filtration is impossible because of use of 8 mM SLS solution as an eluent. In the case of use of a moderately large volume of a sample (for example, Fig. 3c), alkyl paraben eluted with micelles might be assumed to be consisted of alkyl paraben in both of the micellar and aqueous phases. It seems possible to investigate quantitatively with respect to the partition of alkyl paraben between the micellar and aqueous phases by the tail analysis, described below.

Suzuki and Sasaki[10] studied the elution curve obtained by eluting a large volume of an aqueous SLS solution with water, as shown in Fig. 1. The authors applied this method to solubilized systems and investigated on elution behaviors of a solubilizate and a detergent (Fig. 6). Under the experimental condition, SLS micelles may be migrated prior to monomer SLS and free alkyl paraben. Consequently, micelles must pass the gel layer in which water alone is present. Since part of micelles decompose irreversibly as a result, alkyl paraben in the micelles might be liberated in an aqueous phase. The presence of free alkyl paraben and monomer SLS given in such a way strongly influences on the behavior of succeeding micelles in the gel layer. The K_{av} value of monomer SLS is smaller than that of alkyl paraben (Table II), and this fact indicates that the rate of migration of monomer SLS is higher than that of alkyl paraben. Thus, only monomer SLS given from decomposition of micelles might be considered to exist at the lower head of a sample in the process of elution. As a result, micelles flow down without decomposition in the gel layer in which monomer SLS equal to cmc are present, but alkyl paraben in the micelles must be released according to the partition equilibrium between the micellar and aqueous phases. Repeating the process as described above, the sample flows down in the gel layer, and micelles containing no alkyl paraben and monomer SLS come to coexist at the lower head of the sample in the process of elution. After the appearance of monomer SLS, alkyl paraben-free micelles were eluted with monomer SLS equal to cmc. Then, alkyl paraben is eluted by distributing to both of the micellar and aqueous phases. Eventually, the composition of eluate will have to come to the same as that of the original sample. This consideration seems to be reasonable in the elution curves of SLS solution solubilizing methylparaben (Fig. 6a).

On the other hand, in the case of ethylparaben having a less hydrophilic property, it may be inferred from Fig. 6b that even the micelles which are eluted at first from the lower end of the column appeared with incomplete release of ethylparaben. The elution behaviors of butylparaben solubilized in micelles are remarkably different from other alkyl parabens (Fig. 6c). Butylparaben liberated to the aqueous phase through decomposition of micelles partly separates as a heterogeneously phase, because this alkyl paraben is very slightly soluble in water (Table I). Since the solid phase of butylparaben given in such a way should be migrated the layer in prior to micelles, the succeeding micelles will result in passing through the layer saturated with butylparaben. The micelles will therefore be eluted without releasing butylparaben solubilized. A stable plateau is hence obtained in regard to the elution curve of each of micelles and butylparaben eluted at the same time, as shown in Fig. 6c.

The front part of elution curve obtained by the gel filtration of the solubilized solution was chiefly investigated, as described above. However, such elution behaviors may be influenced by the volume of a sample (or the gel volume in a column). Fig. 7 shows that stable plateaus on the elution curves of micelles and ethylparaben are made by increasing a sample volume. That is, $[SLS]_t$ consists of both of micellar and monomer SLS and $[D]_t$ consists of alkyl paraben in both of the micellar and aqueous phases. $[SLS]_f$ may be assumed to be equal to cmc in the presence of ethylparaben, if the consideration in Fig. 1 is applied here. This suggests that cmc in the presence of ethylparaben is lower than that in the absence of the alkyl paraben represented in $(cmc)_0$ in Fig. 7. On the other hand, on the second plateau for ethylparaben which appeared after all the micelles have been eluted, its height, $(D)_f$, may be assumed to be equal to the concentration of free alkyl paraben in the aqueous phase at equilibrium with alkyl paraben in the micelles in the first plateau. Therefore, the concentration of alkyl paraben solubilized in micelles, $(D)_m$, is obtained by substracting $(D)_f$ from $[D]_t$. In the discussion a partition ratio of alkyl paraben between the micellar and aqueous phases was defined as $(D)_m/(D)_f$. $(D)_f$, $(D)_m$ and $(D)_m/(D)_f$ of each alkyl paraben estimated by the tail analysis are shown in Table III. However, both of the concentration of $(D)_m$ and $(D)_f$ denote concentrations expressed in terms of the total volume of the system, rather than the individual phase volume.

Table III. Micellar Distribution of Alkyl Parabens in 40 mM SLS Solution from Fig. 7—9

	In 40 mM SLS			In 40 mM SLS
	$(D)_f$ mM	$(D)_m$ mM	$(D)_m/(D)_f$	$(S-S_0)/S_0$
Methylparaben	14.0	16.9	1.2	1.3
Ethylparaben	5.0	14.5	2.9	3.2
Butylparaben	1.0	20.0	20	21

The concentrations of alkyl paraben in 40 mM SLS were represented in footnote of Fig. 7—9.
$(S-S_0)/S_0$ was calulated from S_0 and S in Table I.

Difference in partition ratio between the respective alkyl paryabens may be considered to be highly influenced by differences in distribution of respective alkyl parabens into an aqueous phase ($(D)_f$). For comparison, $(S-S_0)/S_0$ of each alkyl paraben calculated from the results of the solubility method (Table I) is included in Table III. As a result, it was found that these values are nearly equal to the $(D)_m/(D)_f$ values. This suggests that $(S-S_0)/S_0$ from the solubility method corresponds to $(D)_m/(D)_f$ from the tail analysis. However, information from the solubility method is only obtained about the saturated solution from the nature of experiment. From the gel filtration, on the other hand, it may be possible to get information not only about a partition ratio for a solute unsaturated in a detergent solution but also about the state of detergent micelles.

The elution volume at the tail part in the curve of SLS micelles solubilizing alkyl paraben is larger than that in the curve of micelles solubilizing no alkyl paraben (Fig. 7—9). This suggests that a size of micelle solubilizing alkyl paraben is smaller than that of micelle solubilizing no solute. If that is the case, this solubilized system has a property different from the solubilized system of dodecanol by SLS: In the latter,16) it was observed that a size of SLS micelle solubilizing the alcohol is larger than that of micelle solubilizing no solute. In conventional methods4–9) by which a partition ratio of a solubilizate between the micellar and aqueous phases is determined, there are seen no examples investigated at the same time in regard to behaviors of detergent micelles. The authors found that the determination of a partition ratio of a solubilizate between the micellar and aqueous phases is possible by the so-called tail analysis. In addition, it was suggested that it is also possible to investigate the state of micelles in the presence of a solubilizate judging from the elution curve of a detergent.

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16) H. Suzuki, *Bull. Chem. Soc. Japan*, **49**, 381 (1976).