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Gel Filtration of Surfactants on Sephadex

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An attempt has been made to apply gel filtration on Sephadex to micellar solutions of surfactants. The data on the gel filtration of surfactants were interpreted by taking into account the fact that there exist both micellized and unmicellized species in the solutions. A simple method for obtaining the retention volume of the micelle (V_M) of a surfactant directly from the elution curve was developed. Further, the relation between the values of V_M and the micellar molecular weights (MMW) was examined for various types of surfactants. A linear relation was found between V_M and log MMW, with the exception of a surfactant with a heterocyclic structure. It appears that gel filtration offers a convenient way for roughly estimating the MMW's of surfactants.

Gel filtration is a type of partition chromatography in which substances are separated largely on the basis of the molecular size. If a mixture of low-molecular-weight substances and high-molecular-weight substances moves through a gel column, the former are slowed down as a result of their penetration into the gel, enabling the latter to flow faster down through the column. The sizes of surfactant micelles are, in general, within the range to which this gel filtration is applicable. However, few studies have been made of the application of gel filtration to micellar solutions of surfactants.[1]

In the present work, an attempt has been made to apply gel filtration on Sephadex, a cross-linked dextran gel, to micellar solutions of surfactants, especially those of sodium dodecyl sulfate (SDS) and sodium tetradecyl sulfate (STS). The results were interpreted by taking into account the fact that micelles always exist in equilibrium with monomolecularly-dispersed species above the critical micelle concentration (CMC). Further, the relation between the retention volumes of micelles and their molecular weights was examined for various types of surfactants.

Experimental

Materials. Sephadex G-50 and G-75, with particle sizes of 20—80 and 40—120 micron respectively, and Blue Dextran 2000, with a molecular weight of 2×10^6, were obtained from Pharmacia, Sweden. The sodium decyl-, dodecyl-, and tetradecyl-sulfates were prepared from the respective alcohols by the method of Dreger *et al.*[2] and were purified by repeated recrystallizations from a mixture of isopropanol-water and by extraction with petroleum ether. The sodium decylbenzene sulfonate and sodium dodecyltrioxyethylene sulfate were the same samples as those used in previous works.[3,4] The sodium dodecane sulfonate was a product of Farbenfabrik Bayer, Germany. The dodecyl decaoxyethylene ether and octylphenyl decaoxyethylene ether were samples prepared and purified by a method described elsewhere.[4] The dodecylamine hydrochloride and hexadecyl pyridinium chloride were obtained from Tokyo Kasei and were purified by repeated recrystallizations from acetone. The oil-soluble dyes Yellow OB and Aniline Blue, which were used as solubilizates, were specially-prepared commercial samples.

Procedure. Sephadex G-50 or G-75 was allowed to swell in distilled water for at least 24 hr, and then decanted before being poured into a jacketed column, 1.3 cm in diameter $\times$38 cm in height, with a sintered glass plate at the bottom. After the column had then been filled with the Sephadex gel, a filter paper disk was placed on the top of the gel to prevent its disturbance when the sample solution was added. The column was maintained at 25 ± 0.1°C or 32 ± 0.1°C. The gel column was then washed with distilled water overnight. The void volume of the column, V_0, was checked on the first run of each freshly-packed column, and then on alternate runs, using Blue Dextran 2000.

The sample solution of a surfactant was applied continuously to the top of the column through a siphon connected to the sample reservoir. After about 30 m*l* of the sample solution had sunk into the gel, the eluant, which was the same solution as that which had previously filled the column, was allowed to flow down. An automatic fraction-collector was employed, and fractions of approximately 1 m*l* were collected. The electrical conductivity of each fraction was measured in

1) T. Sasaki and T. Siki, Paper presented at Symposium on Oil Chemistry in Japan, Nagoya, Oct., 1966; T. Sasaki, *Yukagaku (J. Japan Oil Chemists' Soc.)*, **16**, 49 (1967).

2) E. E. Dreger, G. I. Keim, G. D. Miles, L. Shedlovsky and J. Ross, *Ind. Eng. Chem.*, **36**, 610 (1944).

3) F. Tokiwa and K. Ohki, *Kolloid-Z. Z. Polymere*, **223**, 38 (1968).

4) F. Tokiwa and K. Ohki, *J. Phys. Chem.*, **71**, 1343 (1967).

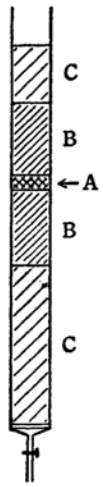

Fig. 1. The gel filtration in measurement of the retention volume of the micelle tagged with Yellow OB.
A: 1 m*l* of the sample solution in 2.0 g/d*l* tagged with Yellow OB.
B: 10 m*l* of the sample solution in 2.0 g/d*l* untagged.
C: the solution in 0.5 g/d*l* of the same surfactant as that in the sample solution.

order to determine the retention volume of the surfactant.

An alternative procedure was employed to examine the relation between the retention volumes of the micelles and their molecular weights. A 1 m*l* portion of the sample solution in a concentration of 2.0 g/d*l*, which had been tagged by solubilized Yellow OB, was allowed to flow down, in the form of a sandwich (shown in Fig. 1) with two untagged 10 m*l* portions of the sample solution, through a column filled with the solution in a concentration of 0.5 g/d*l* of the same surfactant as that in the sample solution. This minimizes the widening of the sample band by diffusing the tagged micelles. The volume of the solution flowing down before and after the tagged sample solution was set at 10 m*l* after several examinations. The retention volume of the sample was determined with the naked eye.

Results and Discussion

Figure 2 shows the elution curves of SDS in water at various concentrations, using a Sephadex G-50 column. Below the CMC the retention volume obtained from the curve is independent of the concentration of the surfactant. Above the CMC, on the other hand, the retention volume decreases, at first rapidly and then more and more gradually, with an increase in the concentration. The surfactant solution, the concentration of which is higher than the CMC, contains both micellized and unmicellized (monomeric) species. The retention volume observed, V_{obs}, may, therefore, be separated into the retention volume of the monomer, V_m, and that of the micelle, V_M. The values of

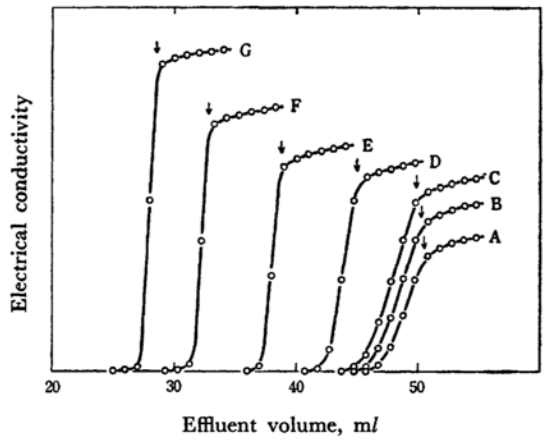

Fig. 2. Elution curves of sodium dodecyl sulfate in water at various concentrations with a Sephadex G-50 column at 32°C:
A 0.0030 M; B 0.0060 M; C 0.0080 M;
D 0.010 M; E 0.013 M; F 0.020 M;
G 0.040 M. The arrow indicates the retention volume of the surfactant at each concentration.

V_{obs}, V_m, and V_M are related by the expression:

$$V_{obs} = xV_m + (1-x)V_M = V_M + x(V_m - V_M),\ x = C_m/C \qquad (1)$$

where C is the total concentration of the surfactant and C_m is the concentration of the monomer species. Below the CMC, $C_m = C$; therefore $V_{obs} = V_m$, since x is equal to unity. Above the CMC, C_m is nearly a constant ($C_m \simeq$ CMC).[5] Thus, plots of V_{obs} *vs.* $1/C$ consist of two straight lines; one intersects the ordinate at V_M, and one is parallel to the abscissa at $V_{obs} = V_m$. These two lines intersect at $1/C = 1/\text{CMC}$. Figure 3 shows the V_{obs}-*vs.*-$1/C$ plots for SDS and STS in water. The

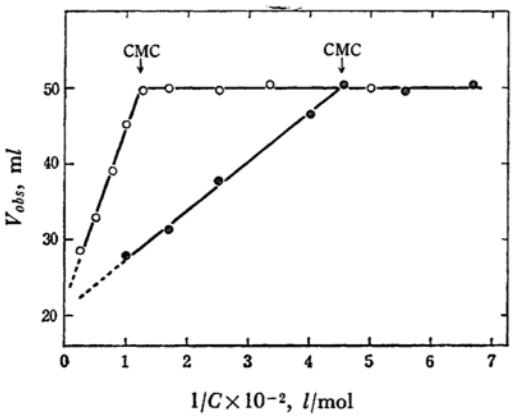

Fig. 3. Plots of V_{obs} *vs.* $1/C$ for sodium dodecyl sulfate (○) and sodium tetradecyl sulfate (◍) in water with a Sephadex G-50 column at 32°C.

5) E. Hutchinson, *Z. Physik. Chem. (Frankfurt)*, **21**, 38 (1959); E. Hutchinson, V. E. Sheaffer and F. Tokiwa, *J. Phys. Chem.*, **68**, 2818 (1964).

values of V_m for SDS and STS are almost the same, while the value of V_M for SDS is slightly larger than that for STS. The CMC values of SDS and STS obstained from the breakpoint of the V_{obs}-*vs.*-$1/C$ plots are in good agreement with their published values.[6,7]

If the gel column has previously been filled with the solution of the surfactant, which is the same as that in the sample solution, of a concentration of C_s, the x in Eq. (1) may be written in the following forms. When C_s is smaller than CMC:

$$x = (C_m - C_s)/(C - C_s)$$

When C_s is larger than CMC:

$$x = 0$$

Therefore, we have:

$$V_{obs} = V_M + \frac{C_m - C_s}{C - C_s}(V_m - V_M) \qquad \text{for } C_s < \text{CMC} \quad (2)$$

and:

$$V_{obs} = V_M \qquad \text{for } C_s \geqq \text{CMC} \quad (3)$$

Figure 4 shows the V_{obs}-*vs.*-$1/(C-C_s)$ plots for SDS at different values of C_s. When $\text{CMC} > C > C_s$, $C_m = C$; therefore, the value of V_{obs} is again equal to V_m, and the plots of V_{obs} *vs.* $1/(C-C_s)$ in this region become straight lines parallel to the abscissa at $V_{obs} = V_m$. When $C \geqq \text{CMC} > C_s$, C_m is nearly equal to the CMC and is independent of C, so the slope of the plots of V_{obs} *vs.* $1/(C-C_s)$ (*i. e.*, the value of $(C_m - C_s)\cdot(V_m - V_M)$) decreases with an increase in C_s and becomes zero at $C_s = C_m (\simeq \text{CMC})$, as may be expected from Eq. (2). When $C > C_s \geqq$ CMC, the plots of V_{obs} *vs.* $1/(C-C_s)$ are straight lines parallel to the abscissa at $V_{obs} = V_M$, as described in Eq. (3). Thus, the value of V_M can easily be obtained from the retention volume observed, V_{obs}, at $C_s \geqq$ CMC.

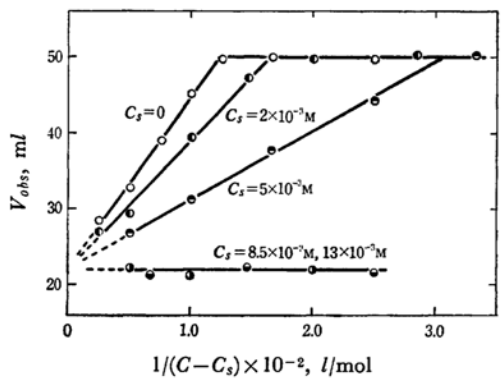

Fig. 4. Plots of V_{obs} *vs.* $1/(C-C_s)$ for sodium dodecyl sulfate at different values of C_s with a Sephadex G-50 column at 32°C.

6) K. J. Mysels and L. H. Princen, *J. Phys. Chem.*, **63**, 1696 (1959).
7) H. Lange, *Kolloid-Z.*, **131**, 96 (1953).

The retention volumes of substances have some relation with their molecular weights. In the field of biochemistry numerous attempts have been made to determine the molecular weights of proteins from their retention volumes.[8-10] It is

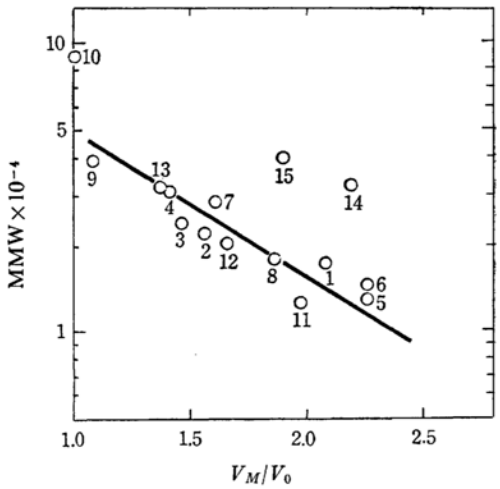

Fig. 5. Relation between retention volumes and micellar molecular weights for various types of surfactants at 25°C, using a Sephadex G-75 column.

(1) $C_{12}H_{25}OSO_3Na$ in water[a]
(2) $C_{12}H_{25}OSO_3Na$ in 0.05 M NaCl[a]
(3) $C_{12}H_{25}OSO_3Na$ in 0.10 M NaCl[a]
(4) $C_{12}H_{25}OSO_3Na$ in 0.25 M NaCl[a]
(5) $C_{10}H_{21}OSO_3Na$ in water[b]
(6) $C_{12}H_{25}SO_3Na$ in water[b]
(7) $C_{12}H_{25}O(CH_2CH_2O)_3SO_3Na$ in 0.10 M NaCl[c]
(8) $C_{10}H_{21}$-C$_6$H$_4$-SO_3Na in 0.10 M NaCl[d]
(9) $C_{12}H_{25}O(CH_2CH_2O)_{10}H$ in water[e]
(10) C_8H_{17}-C$_6$H$_4$-$O(CH_2CH_2O)_{10}H$ in water[f]
(11) $C_{12}H_{25}NH_2HCl$ in water[b]
(12) $C_{12}H_{25}NH_2HCl$ in 0.016 M NaCl[b]
(13) $C_{12}H_{25}NH_2HCl$ in 0.046 M NaCl[b]
(14) $C_{16}H_{33}$-N-Cl in 0.018 M NaCl[g]
(15) $C_{16}H_{33}$-N-Cl in 0.058 M NaCl[g]

MMW was taken a) from Ref. 6; b) from Ref. 11; c) from Ref. 4; d) from Ref. 3; e) from Ref. 12; f) from Ref. 13; g) from Ref. 14.

8) J. R. Whitaker, *Anal. Chem.*, **35**, 1950 (1963).
9) T. I. Pristoupil, *J. Chromatog.*, **19**, 64 (1965).
10) A. A. Leach and P. C. O'shea, *ibid.*, **17**, 245 (1965).
11) H. V. Tarter and A. L. M. Lelong, *J. Phys. Chem.*, **59**, 1185 (1955).
12) F. Tokiwa and T. Isemura, This Bulletin, **35**, 1737 (1962).
13) L. M. Kushner, W. D. Hubbard and A. S. Doan, *J. Phys. Chem.*, **61**, 371 (1957).
14) E. W. Anacker, *ibid.*, **62**, 41 (1958).
15) B. Gelott, *J. Chromatog.*, **3**, 330 (1960).

of interest and also of importance to learn whether there is any possibility of estimating the micellar molecular weights (MMW) of surfactants directly from their gel filtration data. For this purpose, the V_M values for various types of surfactants of known MMW were determined. In the present experiment the micellar solution of the surfactant examined, which was tagged with solubilized Yellow OB, was allowed to flow down in the form of a sandwhich, as has been described in the Experimental section, through a Sephadex G-75 column. The Sephadex G-75 was chosen after taking into account its fractionation range of molecular weight (3×10^3—6×10^4). The effect of oil-soluble dyes on the values of V_M was checked by using two different types of dyes, Yellow OB and Aniline Blue. It was confirmed that the retention volumes are not influenced by the addition of the dyes.

In Fig. 5, the MMW's of various types of surfactants are plotted against the V_M/V_0 ratios, where V_0 is the void volume of the column. There is a linear relation between log MMW and V_M/V_0 except in the cases of hexadecyl pyridinium chloride and octylphenyl decaoxyethylene ether, as may be seen in Fig. 5. The values of V_M for these surfactants deviate from the linear relation. Probably the derivation in the case of hexadecyl pyridinium chloride may be attributed to the interaction of the dextran gel with the pyridine ring in the surfactant molecule. Such interactions have been found in experiments with heterocyclic substances.[15] The reason for the deviation found in octylphenyl decaoxyethylene ether is not clear, because its MMW is outside the fractionation range of the gel used.

From the data presented above, it appears that gel filtration on Sephadex offers a convenient way of roughly estimating the MMW of a surfactant unless the surfactant has a heterocyclic structure.

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