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Gel Filtration of Aqueous Sodium Dodecyl Sulfate

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Gel filtration of an aqueous solution of sodium dodecylsulfate (SDS) was carried out using Sephadex G-50 as a gel column, and the overall elution rate (R_f) of SDS was measured. The value of R_f was found to be independent of the concentration C of SDS below a certain point, above which it increased with increasing C. A sharp break point observed coincided with the critical micelle concentration (CMC) of SDS. The intermicellar concentration of SDS was estimated from the shape of the elution curve for the solutions beyond CMC, and shown independent of the total concentration C. The value of R_f for the micelle was further determined by direct measurement. The value was found to increase slightly with C above CMC, becoming practically constant above about 1.4×10^{-2} mol/l. It was concluded from the results that the size of the micelle also increase with the concentration above CMC. Theoretical equations were derived for the relation between R_f and C below and above CMC. Observed R_f *vs.* C relation fitted the equations satisfactorily, from which CMC was calculated. A rapid equilibrium between micelles and monomer ions and the independence of intermicellar concentration on C were also confirmed.

A study on the dissolved state of sodium dodecyl sulfate (SDS) by the gel filtration method using Sephadex G-50 was reported in a previous paper.[1] Equations were proposed which predict a relation between a relative elution rate and the concentration of SDS solution. The observed data fitted the equation from which a rapid equilibrium between micelles and monomer ions, uniformity of micellar size and the independency of intermicellar concentration on the total concentration were concluded. Further studies made on the direct measurement of the relative elution rate of micelles suggested a slight increase of the micellar size above CMC. This made it necessary to re-examine the former plots. We have derived a revised equation for gel filtration which involves a well defined parameter for the rate of micelle decomposition. Verification was carried out without the method of trial and error which was adopted in the previous case.[1] Constancy of the intermiceller concentration of SDS was also checked experimentally.

Experimental

Materials. Sodium dodecyl sulfate (SDS) was synthesized from dodecyl alcohol and chlorosulfonic acid by the usual method[2] and was purified by solvent extraction using ethyl ether and recrystallization from ethanol. Purity was checked by the absence of a minimum in the surface tension-concentration curve.

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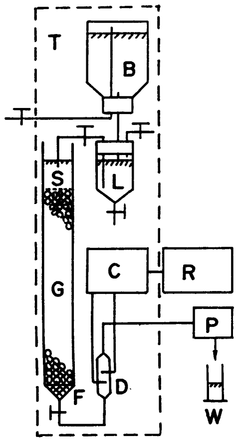
[1] T. Sasaki and Y. Ogihara, Proceedings of 5th International Congress on Surface Activity, Barcelona (1968).

[2] S. J. Rehfeld, *J. Phys. Chem.*, **71**, 738 (1967).

Sodium chloride was purified by recrystallization of the reagent grade product.

The gel used for packing the column was Sephadex G-50[3] fine, manufactured by Pharmacia, Uppsala, Sweden.

Apparatus. The apparatus used for gel filtration is schematically shown in Fig. 1. The main parts of the apparatus consisted of a gel column G prepared in the usual manner[3,4] (diameter 1.2 cm and gel height 32.5 cm), a conductance cell D, a conductometer C and a recorder R. The conductance cell D for detecting SDS recorded the conductance against the elution volume. All experiments were carried out at 30±1°C.



G	gel column
S	sample solution
F	glass wool
D	conductance cell
C	conductometer
P	steady flow pump
R	recorder
W	weighing vessel
T	thermostated chamber
B	reservoir
L	leveler

Fig. 1. Arrangement for gel filtration.

Methods. To measure the retention volume of SDS, the gel column was previously equilibrated with distilled water and 25.0 ml of SDS solutions of varying concentration (C) were put on the gel column. Elution was then started using distilled water at a flow rate of 20 ml/hr, and the retention volume (V) was determined from the conductance curve recorded. The results were conventionally expressed as the relative elution rate

$$R_f = V_0/V \tag{1}$$

where V_0 denotes the retention volume of solvent water.

To measure R_f value of the micelles in the aqueous solution of SDS above CMC, the gel was previously equilibrated with SDS solution of concentration C larger than CMC (C_m), and SDS solution with concentration C' larger than C was added to the gel column. The solution was then eluted with water. From the elution volume $V_m(C')$ thus obtained for the SDS solution of concentration C', the relative elution rate of the micelle $R_f{}^m(C')=V_0/V_m(C')$ was calculated. $R_f{}^m(C')$ was then plotted against C' and the value $R_f{}^m(C)$ was estimated by extrapolating C' to C. The value $R_f{}^m(C)$ can be considered as the relative elution rate of the micelle flowing through its own intermiceller solution of concentration C without decomposition.

Results and Discussion

Intermiceller concentration of SDS. Figure 2 shows two typical elution curves of SDS solution of concentrations below and above CMC, through the gel column filled with water. We see that the curve of SDS solution of the concentration below CMC shows one plateau and that above CMC two. The higher one corresponds to concentration C of the solution eluted. The lower one might be considered to be the concentration of the intermicellar SDS left after the fast flowing micelles have been eluted, provided that the plateau is sufficiently horizontal and the change between the two plateaus is fairly sharp. Figure 3 shows the plots of the conductance and concentration corresponding to one (solid circles) or two plateaus (open circles and triangles) of the elution curves against the analytical concentration of SDS. We see a straight line passing through the origin and a horizontal straight line starting from CMC. The latter might indicate constant intermicellar concentration which is independent of the SDS concentration and is equal to C_m.

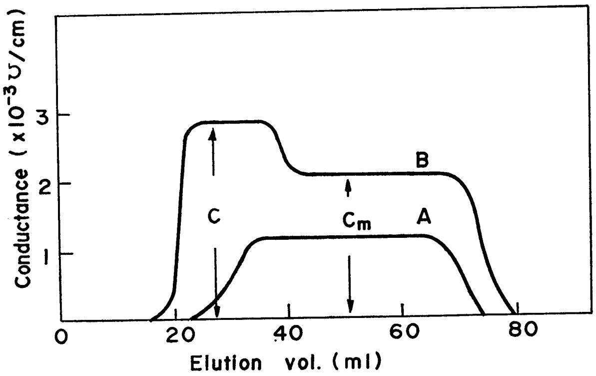


Fig. 2. Elution curve of SDS solution.

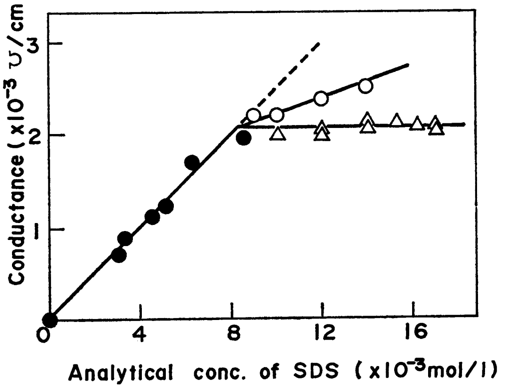


Fig. 3. Conductance of SDS solution.

Conductance of the solution below CMC(●) and above CMC(○); conductance of intermicellar solution(△)

R_f vs. C relation. The observed values of R_f for the aqueous solution of SDS below and above CMC are plotted against the concentration as shown in Fig. 4. Since V_s, the retention volume of the lower concentration region of SDS, was found practically independent of SDS concentration,[1,5,6] its mean value $\bar{V}_s$ was calculated, and $R_f=\bar{V}_s/V$ instead of $R_f=V_0/V$ was used as a relative elution rate. The relative

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[3] P. Flodin, "Dextran Gels and Their Application in Gel Filtration," Pharmacia, Uppsala, Sweden (1962).

[4] H. Determann, "Gel Chromatography," Springer-Verlag, Berlin, Heidelberg, New York (1969), p. 42.

[5] T. Sasaki, *Yukagaku*, **16**, (2) 49 (1967).

[6] F. Tokiwa, K. Ohki, and I. Kokubo, This Bulletin, **41**, 2285 (1968).

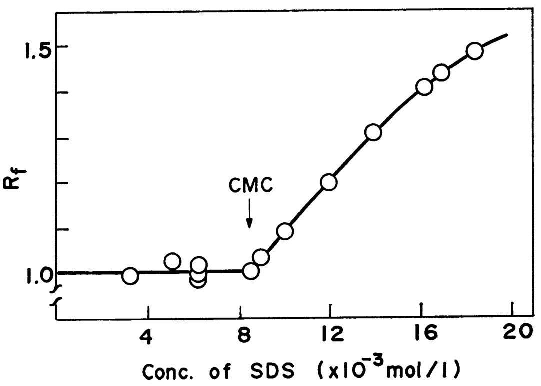

Fig. 4. R_f *vs.* concentration of SDS.

elution rate of monomer ion $R_f{}^s$ is therefore expressed as

$$R_f{}^s = \bar{V}_s/V_s \doteqdot 1$$

It is evident from Fig. 4 that R_f *vs.* C curve consists of a horizontal straight line $R_f = R_f{}^s \doteqdot 1$ in the lower concentration region and a curve in the higher concentration region, with a break point between them corresponding to the CMC of SDS (8.5×10^{-3} mol/l). The constancy of $R_f{}^s$ value in the lower concentration region might indicate the presence mainly of monomer molecule of SDS in this region.

The results of the direct measurement of $R_f{}^m(C')$ together with the process of extrapolation are shown in Fig. 5. The extrapolated $R_f{}^m(C)$ value (solid circles) increases from about 2.2 near CMC with increasing SDS concentration until it becomes constant, 2.54 above 1.4×10^{-2} mol/l SDS. It is worthy to note that the change in $R_f{}^m$ is exclusively ascribed to the change in size of solute particle, since the effect of adsorption and other conceivable factors can be excluded from the present experimental conditions, and there is the possibility for a slight increase of micellar size with concentration above CMC.

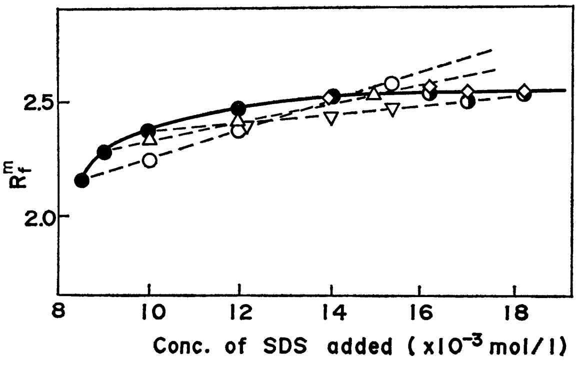

Fig. 5. $R_f{}^m$ *vs.* concentration of SDS.

Conc. of SDS in gel ($\times10^{-3}$mol/l): ○, 8.5; △, 9.0; ▽, 10.0; ◇, 12.0; ◑, 14.0; ●, extrapolated value of $R_f{}^m$ for each case.

Let us discuss the relation between R_f and SDS concentration for the solution especially above CMC. For this purpose, it is convenient to use such a diagram as shown in Fig. 6a,[5] for the elucidation of the mechanism of gel filtration and the calculation of R_f as a function of the concentration above CMC. In this diagram, the effective cross sectional area A_m of a packed gel column through which micelles can flow (total cross-section of interspace of gel particles), and a similar area A_s for the passage of sufficiently small molecules (monomer surfactant molecules and solvent water molecules), are taken on OY axis. The distance travelled by the monomer molecules and the micelles during the flow of a solution is taken on OX axis. Thus, the vertical line RS indicates the front of the micelles in sample solution (large circles) just reaching the lower end of the gel column while the solution is continuously supplied and flows down, assuming no decomposition into monomer takes place. The vertical line PQ indicates the front of monomer molecules (small circles) when the front of the micelles reaches RS. Since the volumes occupied by these solute components are equal, we have

$$\square\, A_sOPQ = \square\, A_mORS \tag{3}$$

where □ denotes the area of the square specified which actually expresses the volume. A simple geometry shows that the intersection M of the two straight lines A_mS and PQ lies on the diagonal OU. The micelles in □ MPRS are unstable since they are isolated from monomer molecules which should exist in equilibrium with them. Therefore, such micelles as in □ RR'S'S actually decompose into monomer molecules to fill the space □ QPR'U' with the concentration $C_m{}'$ ($C_m{}' \leqq C_m$) as shown by the small circles in this area (Fig. 6b). For further calculation, we put OR$=t$

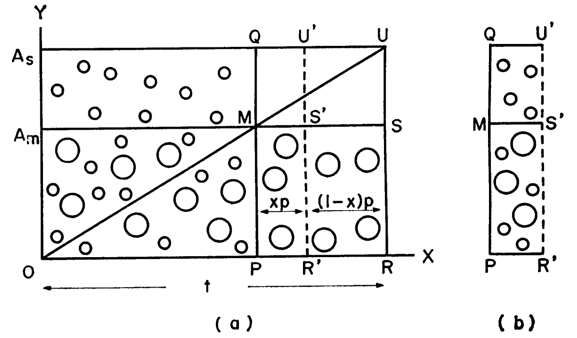

Fig. 6. Mechanism of gel filtration.

○; monomer molecule, ◯; micelle

which represents the total length of the gel column, PR'$=xp$, RR'$=(1-x)p$ as shown in Fig. 6. Then we obtain the relation

$$A_mp(1-x)(C-C_m) = A_spxC_m{}' = A_spxC_m\alpha$$

or

$$A_m(1-x)(C-C_m) = A_sxC_m\alpha \tag{4}$$

Here α, being equal to $C_m{}'/C_m$, denotes a parameter of the rate of micelle decomposition or the rate of equilibration between micelles and monomer. Namely, the decomposition is instantaneous when $\alpha=1$ and no decomposition occurs when $\alpha=0$. Further, the actual retention volume V of the solute is given by

$$tA_m : V = [t-(1-x)p] : t$$

or

$$V[t-(1-x)p] = t^2A_m \qquad (5)$$

Eq. (3) is rewritten as

$$(t-p)A_s = tA_m \qquad (6)$$

From Eqs. (4), (5), and (6), we obtain

$$\frac{V}{V-tA_m} = \frac{A_mC}{(A_s-A_m)C_m\alpha} + \frac{A_s\alpha-A_m}{(A_s-A_m)\alpha} \qquad (7)$$

It can be understood from Fig. 6 that $A_st=V_s$ and $A_mt=V_m$ represent the retention volumes of monomer molecule and micelle (assumed to flow without decomposition), respectively, and the relations

$$R_f = \bar{V}_s/V,\ R_f{}^m = \bar{V}_s/A_mt,\ R_f{}^s = \bar{V}_s/A_st$$

express the relative elution rates of solution and micelle, and mean relative elution rate of monomer molecule, respectively.

Substituting V, A_s, and A_m in Eq. (7) by R_f's, we obtain

$$\frac{R_f{}^m-R_f{}^s}{R_f{}^s}\cdot\frac{R_f}{R_f{}^m-R_f} = \frac{C}{C_m\alpha} - \frac{1-\alpha}{\alpha} \qquad (8)$$

Eq. (8) predicts a linear relationship between $\frac{R_f{}^m-R_f{}^s}{R_f{}^s}\cdot\frac{R_f}{R_f{}^m-R_f}$ and C above CMC, from which α and C_m can be calculated. Below CMC we can also express the R_f term in a form similar to Eq. (8) for comparison:

$$\frac{R_f{}^m-R_f{}^s}{R_f{}^s}\cdot\frac{R_f}{R_f{}^m-R_f} = \frac{R_f{}^m-R_f{}^s}{R_f{}^s}\cdot\frac{R_f{}^s}{R_f{}^m-R_f{}^s} \doteqdot 1 \qquad (9)$$

To verify Eqs. (8) and (9), $\frac{R_f{}^m-R_f{}^s}{R_f{}^s}\cdot\frac{R_f}{R_f{}^m-R_f}$ was plotted against C, using the observed data of $R_f{}^m(C)$, $R_f(C)$, and $R_f{}^s$ (Fig. 7).

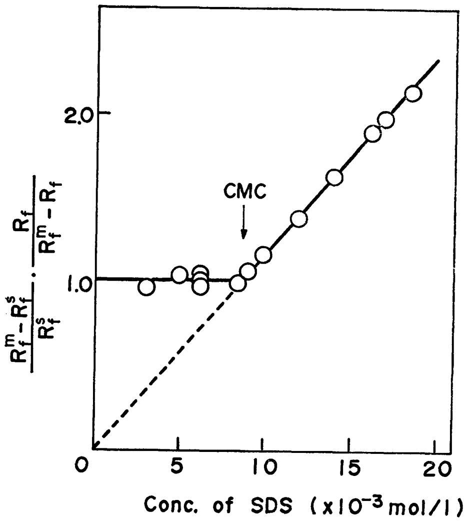

Fig. 7. Concentration dependence of R_f.

We see two straight lines corresponding to Eqs. (8) and (9) with a sharp break point between them which again gives the CMC of SDS. The fact that the plots corresponding to Eq. (8) show a straight line which passes through the origin ($\alpha=1$) indicates the constancy of the intermicellar concentration and a rapid equilibrium between micelles and monomer molecules, at least under the present conditions of the experiment. The value of CMC obtained from the break point was 8.6×10^{-3} mol/l, and the same value was obtained from the inclination of Eq. (8). These values are compared with similar values in literature.[7]

In the above study, the elution volume of SDS monomer molecule was used in place of V_0, the elution volume of solvent water or such a simple substance as sodium chloride. This may be justified since the molecular weight of SDS (about 500) is well below the lower limit of the fractionation range of Sephadex

TABLE 1. GEL CHROMATOGRAPHY OF SDS AND NaCl ON SEPHADEX GEL

concn. of aq. SDS or NaCl filling gel medium	concn. of SDS solution added afterwards	elution volume
0 mol/l	SDS 3.2 ~6.4×10⁻³ mol/l	mean value 31.0 ml
0 mol/l	NaCl 5.0~ 10.0×10⁻² mol/l	mean value 28.8 ml
SDS 2.5×10⁻³ mol/l	SDS[a)] 2.5×10⁻³ mol/l NaCl 5.0×10⁻³ mol/l	28.45 ml (NaCl)[b)]
SDS 2.5×10⁻³ mol/l	SDS[a)] 2.5×10⁻³ mol/l NaCl 2.5×10⁻³ mol/l	28.45 ml (NaCl)
NaCl 1.9×10⁻³ mol/l	NaCl[a)] 1.9×10⁻³ mol/l SDS 5.0×10⁻³ mol/l	31.58 ml (SDS)
NaCl 2.5×10⁻³ mol/l	NaCl[a)] 2.5×10⁻³ mol/l SDS 7.0×10⁻³ mol/l	31.59 ml (SDS)

a) mixed solution.
b) compound in parenthesis indicates the substance for which elution volume was measured.

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7) M. Miura and T. Matsumoto, *J. Science of the Hiroshima University*, **21**, 51 (1957); K. Sinoda, "Colloidal Surfactants," Academic Press, New York (1963).

G-50. In a separate experiment, however, the elution volumes of urea, NaCl, and dodecylpyridinium bromide have been measured under the same conditions as for SDS. The retention volumes for urea and NaCl found were 29.1 m*l* and 28.8 m*l*, respectively, while the retention volumes of SDS and dodecylpyridinium bromide obtained were 31.0 m*l* and 30.9 m*l*, respectively, the latter two being slightly but distinctly larger. This presents another problem since an elution volume not larger than 28.8 m*l* is expected for SDS. Various factors can be pointed out to explain the anomalies, such as adsorption of SDS by the gel matrix,[8] swelling or syneresis of the gel by SDS or NaCl respectively, so-called ion exclusion effect,[9] and the effect due to the amphipathic nature of the internal surface of gel matrix.[10] In the present study, however, the effect of adsorption need not be considered since in the measurements above CMC the elution of the solution was made into the gel previously equilibrated with the solution of the same concentration. Elution of the solutions below CMC was also carried out under similar conditions and the possibility of adsorption was also confirmed to be excluded. In order to confirm the effects, if any, of syneresis and swelling, elution volumes of aqueous NaCl and SDS solutions into the gels previously equilibrated with SDS and NaCl, respectively, were measured for comparison with the untreated gels as shown in Table 1. However, the elution volumes were not affected by this gel-treatment. Thus these factors are considered to be insignificant. The possibility of ion exclusion effect which had been expected was also excluded, since the elution volume was independent of the concentration of the electrolyte in the present study. Further study is required concerning the effect of amphipathic nature of the gel matrix as a cause of the anomalies.[10] It may be inferred further that the micelles are uniform in size, since the conductance rise in the elution curve indicating the micellar front is as sharp as that for single ions below CMC.

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

[9] D. Saunders and R. L. Pecsok, *Anal. Chem.*, **40**, 44 (1968); P. A. Neddermeyer and L. B. Rogers, *ibid.*, **40**, 755 (1968).

[10] H. Small and D. N. Bremer, *I & EC Fundamentals*, **3**, 361 (1964).