Gel Filtration of Aqueous Sodium Dodecyl Sulfate

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The tail analysis of gel filtration was applied to a sodium dodecyl sulfate (SDS) solution using Sephadex G-50. This method is more simple and useful than the frontal analysis method formerly reported. The relative elution rate of SDS micelle, R_m, increased with an increase in the SDS concentration above CMC, reached a maximum near 16 mmol/l and then decreased up to 100 mmol/l. The application of the Laurent-Killander equation gave a micellar radius of 17.6 Å at CMC and 11.7 Å at the maximum R_m, corresponding to aggregation numbers of 35 and 80, respectively. The apparent decrease of R_m observed above 20 mmol/l was due to an structural change of the Sephadex gel caused by the addition of SDS. The micellar weight was estimated referring to a substance of known molecular weight; it was found to be constant in the region from 15 to 100 mmol/l SDS. The results of gel filtration of the SDS solution using CPG-10 as a gel column were similar to the case of Sephadex G-50; a micellar weight and an aggregation number of 25000 and 88, respectively, in the constant R_m range were obtained.

As for the study of the dissolved state of the solute by gel filtration, zone and band analyses are possible. The former method is used to measure the position of a sharp peak of the elution curve obtained by charging a small amount of the sample, while the latter method is used to determine the positions of the front F and the tails M and S of the elution curve as shown in Fig. 1, obtained by charging a relatively large amount of material in band form. Band analysis enables the detection of species of solutes in rapid equilibrium with each other as has been mentioned previously.[1)]

Using the latter method, the present authors have measured the elution rate (R_m) of micelles, a measure of the micellar size, and the CMC from the elution rate of the front (R_f) for sodium dodecyl sulfate assuming the size of the micelles and the intermicellar concentration to be independent of the total concentration.[1)] Also the R_m have been measured directly using frontal analysis applying the extrapolation method[2)] and the same results as those employing the former method[1,2)] were obtained.

However, the frontal method with extrapolation, requires fairly large amounts of the substance and a troublesome procedure. Compared with this, tail analysis, namely, the analysis of the tail part of elution curve obtained by charging a large amount of the sample, proved to be quite simple and useful. Reliable information concerning the dissolved state of the surfactants, especially the state of the micelles, are directly obtained under some assumptions. The present paper reports the results obtained by applying the tail analysis of gel filtration to aqueous solutions of SDS using Sephadex G-50 and CPG-10 glass gel column.

Experimental

Materials. Sodium dodecyl sulfate was prepared by the same method as reported in a preceding paper.[2)] Sodium chloride of the first reagent grade was used after recrystallization. Distilled water was boiled and degassed before use. Blue Dextran 2000, a product of Pharmacia, Uppsala, was used for the measurement of the void volume of the gel bed used. Poly(vinyl alcohol)s (PVA) and the molecular weight marker kits of non-enzymatic proteins of known molecular weight were used as molecular weight standards. The PVA were kindly supplied by Nippon Gosei Kogyo Co., Ltd.

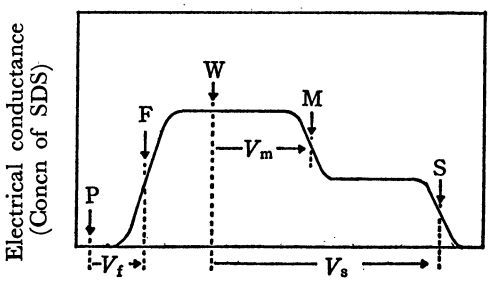

Fig. 1. The elution curve for an SDS micelle (frontal and tail analysis).
P and W: the points of sample and water charge, respectively, F: the front of the solution, M and S: the tails of micelles and single ion, V_f, V_m, and V_s: the elution volumes of front, micelles, and single ions, respectively.

and the proteins were obtained from Mann Research Laboratories Inc., New York.

The elution curve obtained is shown in Fig. 1. Elution volumes V_f, V_m, and V_s of the front F, the tails of the micelles M, and single ions S were measured as the volumes between the charge point of the sample P and the point F, between the charge point of water W, and between the points M and S on the elution curve, respectively. Relative elution rates of the front and micelles were respectively calculated from

$$R_f = V_s/V_f,\ R_m = V_s/V_m,\ \text{together with}\ R_s = 1, \quad (1)$$

R_s being the elution rate of the single ions.

Results and Discussion

Gel Filtration Using Sephadex Gel. (A) *SDS Solution*: The elution rate of micelles, R_m, calculated from V_m obtained by tail analysis is plotted against the total concentration of SDS as shown by open circles in Fig. 2. As is seen, R_m increases slightly at first, reaches a maximum near 16 mmol/l, and then decreases with increasing concentration of SDS, in agreement with previous results obtained by frontal analysis.[1,2)] A similar change with a change in the concentration was also observed with the Sephadex G-75 gel column. A linear relation between $(R_m - R_s)R_f/(R_m - R_f)$ and C predicted in a preceding paper[2)] was confirmed to be valid, and the CMC of 8.6 mmol/l

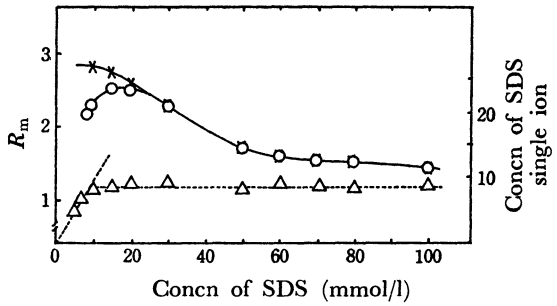

Fig. 2. R_m and the concn of SDS single ion *vs.* SDS concn using tail analysis.
×: Commercial SDS, ○: pure SDS, △: the concn of SDS single ion (CMC).

was obtained from the crossing point of this line with the horizontal straight line passing through unity on the ordinate; this CMC value is in agreement with the previous results.[2] However, the above plot deviates from linearity above 20 mmol/l, probably due to a change in gel matrix as discussed below. The lower plateau of the elution curve of Fig. 1 was also plotted against the concentration shown by the triangles in Fig. 2. As is seen, the value remains constant up to 100 mmol/l. This may indicate that the micelle-monomer equilibrium is relatively simple for SDS up to about 100 mmol/l, excluding the existence of the so-called second CMC.[3] But this does not necessarily mean that the intermicellar SDS concentration is constant. A detailed study will be reported in the near future on this subject.

(B) *Concentration Dependence of* R_m: The increase of R_m with C obtained from the tail analysis for pure SDS (open circles in Fig. 2) reflects the increase of micellar size up to about 16 mmol/l. The following experiment confirms this increase in micellar size.

An SDS solution of concentration C was charged on top of the gel which had been previously equilibrated with the SDS solution of the concentration C' between C and CMC, and was eluted with water. If the elution rate of micelles in the solution of higher concentration C is larger than that in the solution of lower concentration C', the front of the former solution goes ahead of the tail of the latter and a peak of high concentration $C+C'$ is expected to appear at the front. However, the peak was not actually observed and the front of the elution curve exhibited two simple plateaus corresponding to the concentrations C and C'. In addition, the R_f of the apparent front of the solution of concentration C is slightly but distinctly smaller than the R_m of the same solution. Since the elution volumes of NaCl and Blue Dextran confirm the absence of the effect of SDS below 20 mmol/l on the gel structure of Sephadex G-50, the above phenomena may be explained if we assume that the intermicellar concentration of single SDS ions is not equal in the solutions of differing concentrations C and C'. Figure 3 illustrates the phenomena which occurs according to such reasoning, where (a) is the initial and (b) the intermediate states of gel filtration. In this figure, the abscissa denotes the position and direction of the eluting solute, point 0 being the upper end of the gel column, and

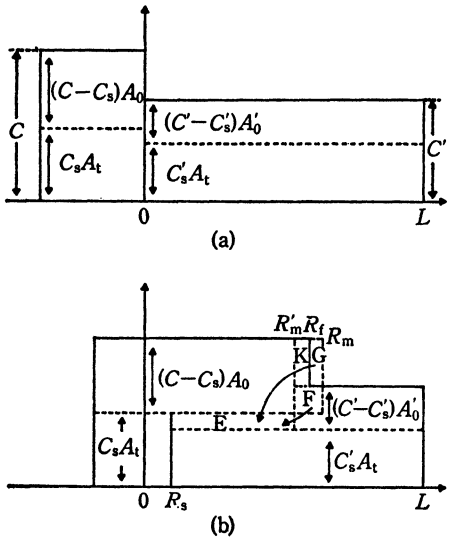

Fig. 3. The schematic representation of gel filtration of an SDS micellar solution near to the CMC.
(a) Initial and (b) intermediate state of gel filtration.

the ordinate shows the quantities of SDS single ions, (C_sA_t), and micelles, $(C-C_s)A_0$, per unit column length for both the solution of concentration C and that of concentration C'. Furthermore, $A_0(A_0')$ and A_t denoted the cross-sectional areas of the gel column through which the micelles and single ions, respectively, can pass. R_s, R_m, R_m', and R_f represent the elution rates of SDS single ions, of micelles in solution of concentrations C and C' and of the front of the solution of concentration C, respectively. In the course of gel filtration, the front of fast flowing micelles of concentration C advances ahead of the tail of the slowly flowing micelles in the solution of concentration C' by an amount $K+G+F$, as is shown in Fig. 3. Material balance gives the amount of SDS of

$$F = [(C'-C_s')A_0'+C_s'A_t-C_sA_t]\ (R_m-R_m') \quad (2)$$

and the amount of SDS of

$$G = [(C-C_s)A_0+C_sA_t-(C'-C_s)A_0'-C_s'A_t](R_m-R_f) \quad (3)$$

that should fill the concentration gap of the portion

$$E = (C_sA_t-C_s'A_t)(R_m'-1) \quad (4)$$

as indicated by the curved arrows in Fig. 3. Here, C_s and C_s' denote the intermicellar concentration of the solutions of concentrations C and C', respectively. Then, from Eqs. (1)—(4), the following equation results for CMC$<C'<C$

$$(C-C_s)(1-R_f/R_m) - (C'-C_s')(1-R_f'/R_m') = (C_s-C_s')(R_f-1) \quad (5)$$

From a similar consideration, the following equation is obtained instead of Eq. (5), in the case of

$C' <$ CMC $< C$

$$(1-R_f/R_m)(C-C_s) = (C_s-C')(R_f-1) \quad (6)$$

Gel filtration conducted for the system of $C=10$ mmol/l and $C'=8.5$ mmol/l solutions gave $R_f=2.24$. This value together with $R_m=2.37$ for $C=10$ mmol/l (Fig. 2) when substituted into Eq. (6) gives $C_s=8.55$ mmol/l

for $C=10$ mmol/l. Successive experiments for the system of $C'=10$ mmol/l and $C=14$ mmol/l solutions gave $R_f=2.44$. From this value together with $R_m=2.52$, $R_m'=2.37$, and $C_s'=8.55$ mmol/l, $C_s=8.73$ mmol/l for $C=14$ mmol/l was obtained using Eq. (5).

The data suggest a small but not negligible increase of the intermicellar concentration above CMC in accord with the results obtained by the electromotive force measurement.[4] A detailed gel filtration study of this problem will appear in the near future. A short extrapolation of the C_s *vs.* C relation at a point at which $C_s=C$ gives CMC=8.50 mmol/l, a value close to the value of 8.6 mmol/l obtained in a preceding paper[1,2] under the assumption of a constant intermicellar concentration.

As regards the decrease of R_m for SDS solutions of concentrations greater than 30 mmol/l, the change in gel structure as mentioned in below can be taken into account. However, a similar experiment as that shown in Fig. 3 also offers some information concerning the behavior of micelles flowing through a gel of high SDS concentration, where aqueous SDS solutions of $C=60$, 70, and 80 mmol/l were eluted through a gel column filled with a $C'=50$ mmol/l SDS solution. Figure 4(a) represents the initial and Fig. 4(b) the intermediate states during gel filtration. The notations used are the same as in Fig. 3. In this case, contrary to the dilute system described above, the apparent R_m for the solution of concentration C is considered to be smaller than that for the solution of concentration C'. Therefore, as the filtration proceeds, a gap or a space containing no micelles may form between the tail of foregoing micelles and the front of the micelles which follow, and an elution curve resembling the dotted line in Fig. 4 is expected. However, in this case as in the former case, two simple plateaus corresponding to the concentrations C and C' appeared without any gap. As in Fig. 3, the elution rate R_f

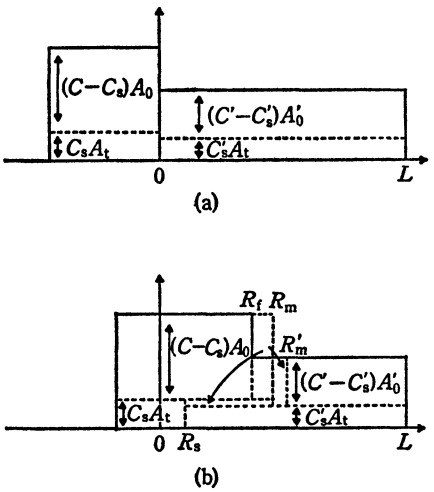

Fig. 4. The schematic representation of the front and the tail during gel filtration in higher concn of SDS. R_m': the tail of micelle in 50 mmol/l solution, R_m and R_f: the imaginary and actual fronts of micelles in C mmol/l solution, R_s: the tail of single ion in 50 mmol/l solutoin. The arrows indicates the eventual shift of solute.

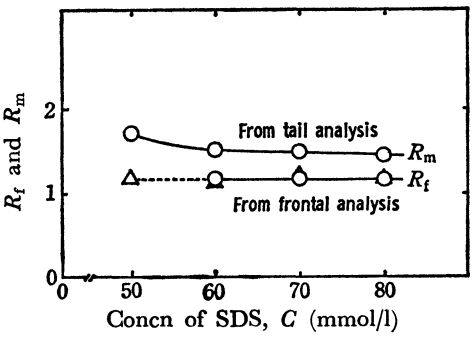

Fig. 5. The relationship between the elution rate for frontal analysis and that from tail analysis.
○: Observed, △: calculated.

observed in Fig. 4 is smaller than R_m, but the difference is fairly large compared with the case of Fig. 3, shown by circles in Fig. 5, and the value of R_f extrapolated to $C=50$ mmol/l does not agree with R_m at the same concentration, as was the case in the lower concentration region.[2]

These phenomena are explained by the higher concentration of micelles filling the above mentioned gap as is shown by the curved arrows in Fig. 4. Eq. (5) applies also in this case although the situation is somewhat different from the case shown in Fig. 3. The rate of the elution front, R_f, is calculated by putting the R_m obtained from the tail analysis into Eq. (5). The results are shown in Fig. 5 as triangles, which are seen to be in good agreement with observed values. By the trial-and-error-method best-fit values are obtained by setting $S_s=C_s'=8.7$ mmol/l. The results indicate the probable mechanism of filling at the elution front mentioned above and may suggest that the intermicellar concentration C_s is approximately constant over the region of SDS concentration from CMC to 80 mmol/l, further study being under way on this point.

(*C*) *Micellar Size*: The size of micelles can be estimated either from the theoretical formula taking account of the gel structure or from the elution volume using the appropriate substances as molecular weight standards. The former method utilizes the Laurent-Killander equation[5]

$$(V_e-V_0)/(V_t-V_0) = \exp[-\pi L(r_r-r_s)^2] \qquad (7)$$

where V_t is the total volume of the gel bed, V_0 the void volume, V_e the elution volume of the substance, L and r_r are the concentration and radius of rods considered as an approximation of the structure of gel matrix (the values being 8.2×10^{12} cm/cm^3 and 7×10^{-8} cm, respectively, for Sephadex G-50) and r_s is the

Table 1. Micellar size of SDS

Concn of SDS	V_m	Radius(Å)	n
8.5 mmol/l	14.35 ml	17.6	35
10.0 mmol/l	13.20 ml	19.9	54
15.0 mmol/l	12.20 ml	22.7	80
20.0 mmol/l	12.47 ml	21.0	63

$n=89$ (K. J. Mysels)[9,10]
$n=40-70$ (H. V. Tartar)[8]

radius of the eluted particles. The hydrodynamic radii of the micelles calculated by setting $V_e = V_m$ in Eq. (7) are shown for SDS in Table 1.

The aggregation number of the micelles is calculated by dividing the micelle volume (calculated as a sphere of radius r_s) by 368.6 ml/mol, the molar volume of hydrated SDS molecules considered to be the constituent of hydrated micelles. Here, the amount of hydration was estimated from the conductivity measurement proposed by Nakagaki,[6] the value being in good agreement with that obtained from the intrinsic viscosity measurement.[7]

Calculation were not made for solutions with concentrations higher than 20 mmol/l because of the uncertainty of the V_m value due to the change in Sephadex gel structure. The aggregation numbers given by Tartar[8] and Mysels[9,10] are also listed in Table 1 for comparison. Table 1 suggests the possibility that the wide range of aggregation numbers reported is not due exclusively to experimental error but in part due to an actual increase in size with concentration. It may also be inferred that such a change in micellar size with concentration taking place in the solution near CMC may not be detectable by methods other than gel filtration.

It is noted that the micellar weight shown in Table 1 is in agreement with the value obtained by CPG gel filtration at 15 mmol/l SDS concentration, which is described below.

To confirm the probable change in the gel matrix of Sephadex beyond 20 mmol/l, and to determine the micellar weight of SDS more accurately, molecular weight standards were used. For each concentration of SDS with which the gel column is equilibrated, gel filtration was carried out with such standards of known molecular weight as sodium chloride, Blue Dextran, myoglobin, cytochrome C, and chymotrypsinogen. An calibration curve of the elution volume *vs.* molecular weight was obtained as shown in Fig. 6.

The actual molecular weight of the protein–SDS complex used for this plot was estimated by tail analysis of the elution curve, obtained by gel filtration of the protein–SDS solution in a manner similar to the measurement of the poly(vinyl acetate)–SDS system.[11] Further details of the measurement will be reported elsewhere. The results show that the protein–SDS complex contains 0.45 g of SDS per g of protein in the concentration range of SDS studied which is compared with the saturated amount of about 1 g per g of protein obtained after prolonged contact of SDS with the protein[12,13] as compared with about 1 hr contact in the present experiment.

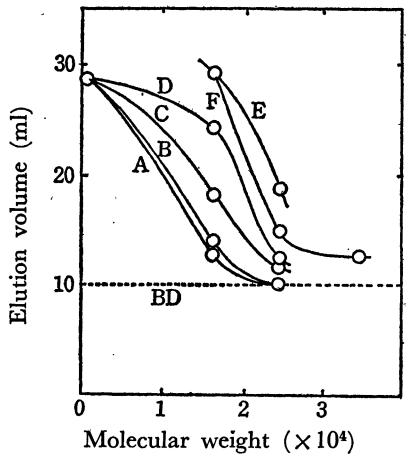

Fig. 6. The calibration curve for SDS solution.
(A): 8.5 mmol/l, (B): 15 mmol/l, (C): 30 mmol/l, (D): 50 mmol/l, (E): 70 mmol/l, (F): 100 mmol/l, BD: Blue Dextran.

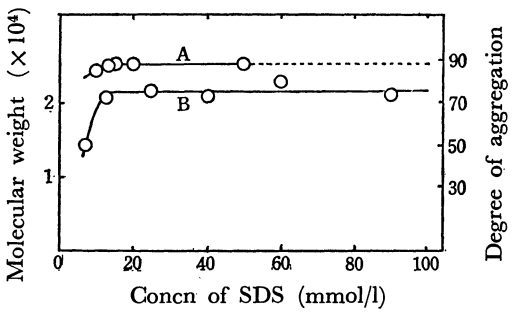

Fig. 7. The dependence of the micellar weight on the concentration of SDS measured using CPG-10 gel.
(A): CPG-10 gel, (B): Sephadex gel.

As is seen in Fig. 6, a marked change in the gel structure was found to occur beyond 30 mmol/l. The micellar weights were calculated using these calibration curves and were plotted against the concentration, as is shown in Fig. 7. It is evident that the micellar size increases from CMC to about 15 mmol/l of SDS beyond which it remains constant over a wide range of concentrations contrary to the apparent R_m and the micellar weight decreases with increasing concentration in the case of Sephadex gel. The aggregation number of SDS micelles corresponding to this plateau is about 75. In the above calculation, hydration of the protein is assumed to be nearly equal to that of the SDS which may be permissible when both amounts of hydration[14] are compared.

Gel Filtration of an Aqueous SDS Solution by CPG-10. (*A*) *Elution Curve for Aqueous SDS Solution*: As mentioned above, the pore distribution of Sephadex gel is affected by a concentrated SDS solution. To avoid such a disturbance, CPG-10, a porous rigid glass gel was used instead of the Sephadex gel and the elution curve was measured for varying concentrations of SDS. A curve similar to that in Fig. 1 was obtained and the conductivity corresponding to the height of each plateau of the curve was plotted against the total concentration of SDS, as is shown in Fig. 8. Three straight lines met at one point giving CMC=8.6 mmol/l as in the case of Sephadex G-50[2]. From the observed values of V_f and V_m, R_f and R_m were calculated using Eq. (1). R_m, plotted against the SDS concentration, shows a slight increase in micellar size with concentration from CMC up to about 15 mmol/l beyond which it remains constant at least up to 50 mmol/l, as is shown in Fig. 9. This confirms the previous results obtained using Sephadex G-50 shown in Fig. 7.

Furthermore, the $(R_m - R_s)R_f/(R_m - T_f)R_s$ *vs.* C plot was made below and above CMC, and two straight lines, one passing through the origin and the other parallel to the c-axis, were obtained as shown in Fig.

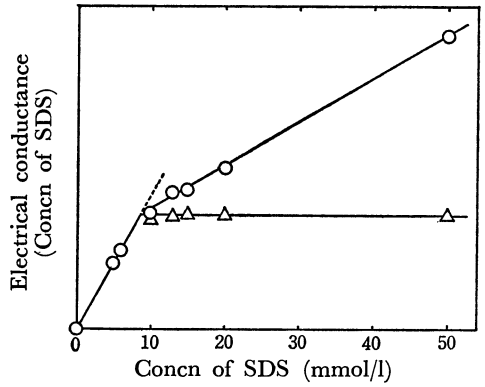

Fig. 8. The conductance of an SDS solution obtained by gel filtration using CPG-10.
○: conductance of the solution, △: conductance of the tail part of the solution (CMC).

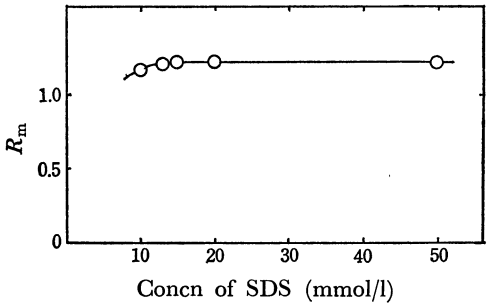

Fig. 9. The dependence of R_m on the concentration of SDS measured using CPG-10 gel.

10. The crossing point of these straight lines gives CMC=8.6 mmol/l in agreement with the value obtained previously. The fact that a straight line passes through the origin indicates the decomposition of micelle being instantaneous as was pointed out in a previous paper[2] using Sephadex G-50. It is emphasized in addition that the linearity shown in Fig. 10 extending up to and the probably beyond 50 mmol/l of SDS, as compared with the deviation from linearity over 20 mmol/l in the case of the Sephadex gel, clearly confirms the inert nature of CPG towards SDS.

(*B*) *Measurement of Micellar Weight*: To obtain the micellar weight of SDS, a calibration curve of the elution volume *vs.* molecular weight of the unhydrated SDS micelles, shown as SDS in Fig. 11, was constructed from the relation of the elution volume *vs.* molecular weight of the hydrated poly(vinyl alcohol) obtained by the conductance method,[6] shown as PVA in Fig. 11. To obtain the calibration curve, the amount of hydration of SDS (0.6 g of H_2O/g of SDS) was taken into account. To avoid disturbances due to the adsorption of poly(vinyl alcohol), the gel was treated with a dilute solution of albumin, then with a sufficient amount of concentrated SDS solution and washed with water. From this curve and the observed V_m, the micellar weight was calculated and plotted against the concentration as shown in Fig. 7. Again, a slight increase in the micellar weight is seen up to about 20 mmol/l, above which a constant micellar weight of approximately 25000 was obtained up to about 50 mmol/l. An aggregation number of about 88 was obtained for the maximum

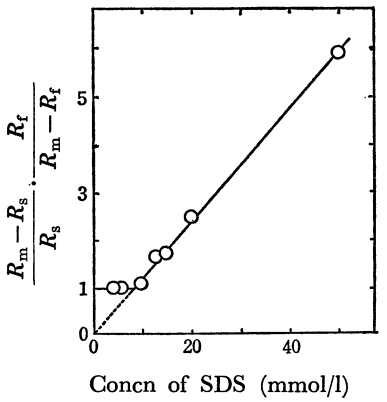

Fig. 10. The relationship between R_f and the concentration of SDS using CPG-10 gel.

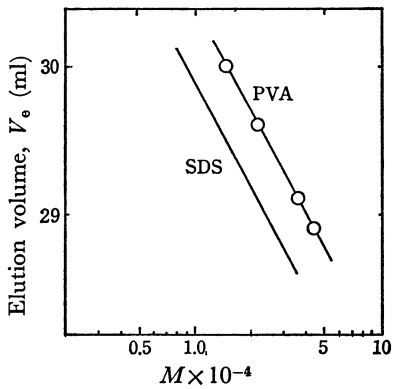

Fig. 11. The CPG-10 system calibration curve, showing the molecular weights of poly(v nyl alcohol)s relative to V_e plotted as a linear log function.

micellar weight. The value is larger than the value obtained using Sephadex G-50 with standards of known molecular weight but is near the maximum aggregation number shown in Table 1 calculated using the Laurent-Killander equation. The results obtained by the CPG gel method are considered to be more reliable considering the stability of the gel matrix.

In conclusion, it is emphasized that the frontal and tail analysis of gel filtration is useful for the study of the state and behavior of micelles in surfactant solutions. It is particularly suited for the study of micellar solutions near CMC, where other methods such as X-ray analysis, osmometry, and the light scattering method are not so effective.

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References

1) T. Sasaki and Y. Ogihara, Proceedings of 5th International Congress on Surface Activity, Barcelona (1968).
2) H. Suzuki and T. Sasaki, This Bulletin, **44**, 2630 (1971).
3) M. Kodama and M. Miura, *ibid.*, **45**, 2259 (1972).
4) T. Sasaki, M. Hattori, J. Sasaki, and K. Nukina, *ibid.*, **48**, 1397 (1950).
5) T. C. Laurent and J. Killander, *J. Chromatog.*, **14**,

317 (1964).
6) M. Nakagaki and S. Shimabayashi, *Nippon Kagaku Kaishi*, **1973**, 207.
7) P. Mukerjee, *J. Colloid Sci.*, **19**, 722 (1964).
8) H. V. Tartar, *ibid.*, **14**, 115 (1959).
9) D. Stigter, R. J. Williams, and K. J. Mysels, *J. Phys. Chem.*, **59**, 330 (1955).
10) K. Shinoda, T. Nakagawa, B. Tamamushi, and T. Isemura, "Colloidal Surfactants," Academic Press, New York (1963).
11) T. Sasaki, K. Tanaka, and H. Suzuki, Proceedings of 6th International Congress on Surface Activity, Zürich (1972).
12) R. Pitt-Rivers and F. S. A. Impiombato, *Biochem. J.*, **109**, 829 (1968).
13) W. W. Fish, J. A. Reynolds, and C. Tanford, *J. Biol. Chem.*, **245**, 5166 (1970).
14) J. A. Reynolds and C. Tanford, *ibid.*, **245**, 5161 (1970).