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Micelle Formation of a Sulfobetaine Derivative of Cholic Acid

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A gel filtration chromatographic study of CHAPS, a nondenaturing zwitterionic surfactant, reveals a step-wise self-association of CHAPS, which provides important information about its use for membrane biochemistry and about micelle formation of bile salts.

3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) is a new zwitterionic surfactant that combines useful features of both the bile salts and the N-alkyl sulfobetaines. It can break protein-protein interactions much more effectively than either sodium cholate or Triton X-100, and it is capable of disaggregating P-450 to its monomeric form without denaturation.[1,2] The study of the micellar aggregation behavior of bile salts has a long history. The reported values of critical micellization concentrations (cmc) and micellar aggregation numbers (n) vary widely beyond experimental errors.[3] This lacking in general agreement would be ascribed to small aggregation numbers and the charge effects of the bile salts.[3] Since CHAPS has no net charge, one can expect a more detailed study. By light scattering, NMR, surface tension, and fluorescence probe methods, a range of cmc=6-10 mM (mmol dm^{-3}) and n= 4-14 has been reported.[1]

In previous reports, we established methodology for determining monomer concentration (C_1) using gel filtration chromatography (GFC).[4] In this work we report a GFC study on CHAPS with Sephadex G-10 at 25 °C and demonstrate the usefulness of GFC for the investigation of surfactants with small n values. The detail of GFC experiments has been reported for other surfactants with long alkyl chains.[4]

From a frontal GFC profile we can determine the centroid volume (V_c). As Fig. 1 shows, V_c decreases rapidly around a total CHAPS concentration of C=5 mM. Under the present conditions, V_c equals weight-averaged elution volumes for monomers (V_1) and micelles (V_m):[4]

$$V_c = [C_1V_1 + (C - C_1)V_m]/C \quad (1)$$

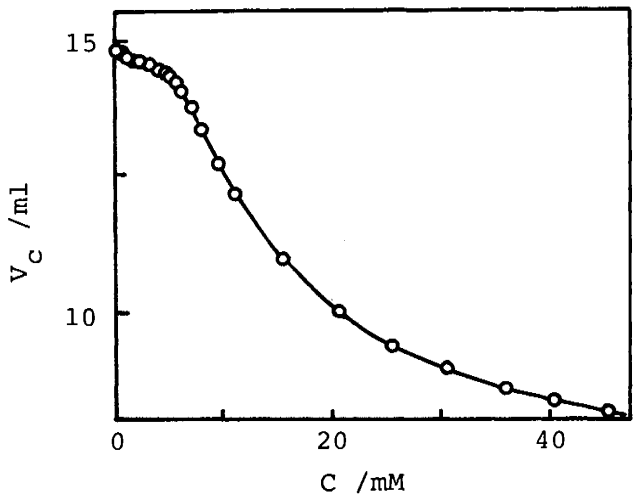

Fig. 1. Centroid volumes vs C plots for experiments and theory (solid line).

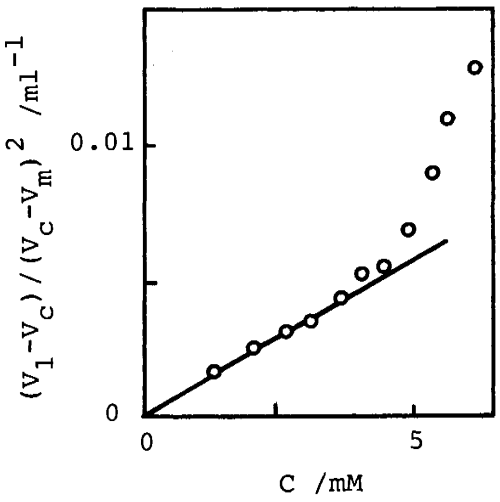

Fig. 2. Plots following Eq. 3 for dimerization.

Here as already reported,[4] V_1 and V_m were estimated by extrapolation of V_c to C=0 and ∞, respectively; V_1=14.81 ml and V_m=6.36 ml. From Eq. 1, therefore, we can calculate C_1 as a function of C. When i-mer is formed by aggregation of i-1-mer (A_{i-1}) and monomer, we can write the step-wise aggregation constant (k_i) as

$$k_i = [A_i]/[A_{i-1}]C_1 \quad (2)$$

If only dimerization occurs, we can expect the following equation from Eqs. 1 and 2:

$$(V_1-V_c)/(V_c-V_m)^2 = 2k_2C/(V_1-V_m) \quad (3)$$

As Fig. 2 shows, the plots of V_c (Fig. 1) following Eq. 3 hold true at low concentrations, and we determined a k_2 value of 0.0049 mM^{-1} from the linear portion.

Further evidence for dimerization at low concentrations comes from comparison between the derivative GFC patterns at the leading and trailing boundaries. As Fig. 3 shows, the trailing peaks are broader than the leading peaks and shift toward smaller volumes with an increase in C. These are characteristic of dimerization. As Fig. 4 shows, the derivative patterns at the trailing boundary and high concentrations are markedly different from those of ordinary surfactants possessing alkyl chains.[4]

When $k_2 \neq k_3 \neq k_4 \neq k_5 = k_6 = \cdots\cdots = k$, one can write C as

$$C = C_1 + 2k_2C_1^2 + 3k_2k_3C_1^3 + k_2k_3k_4C_1^4(4-3kC_1)/(1-kC_1)^2 \quad (4)$$

By nonlinear least squares fitting of the V_c values (Fig. 1) to Eqs. 1 and 4,[5] we determined values of k_2=0.0033 mM^{-1}, k_3=0.00048 mM^{-1}, k_4=23.2 mM^{-1}, and k=0.093 mM^{-1}; the solid line in Fig. 1 shows the fitted result.

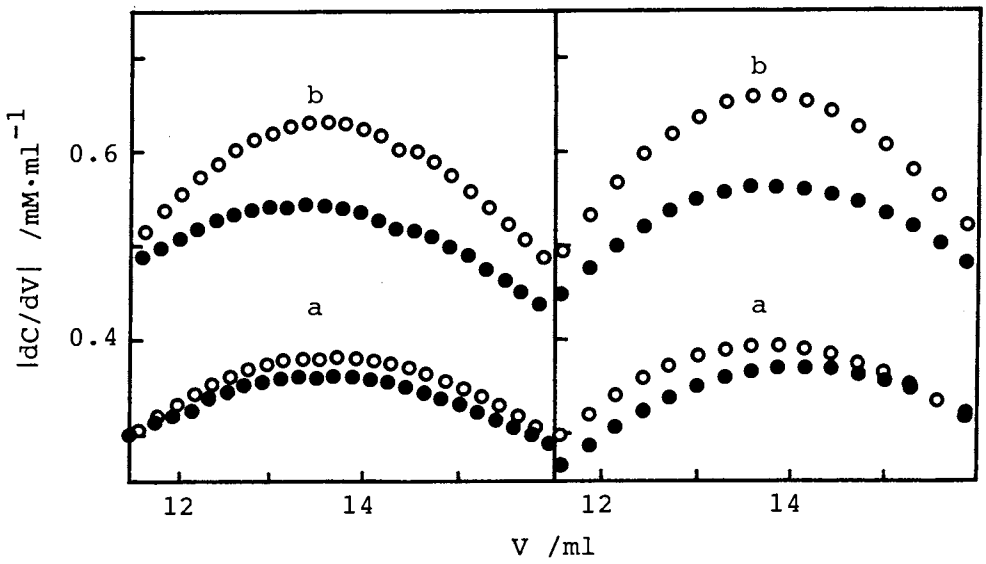

Fig. 3. Experimental (left) and theoretical (right) derivative GFC patterns at two low concentrations (Table 1) and the leading (o) and trailing (●) boundaries.

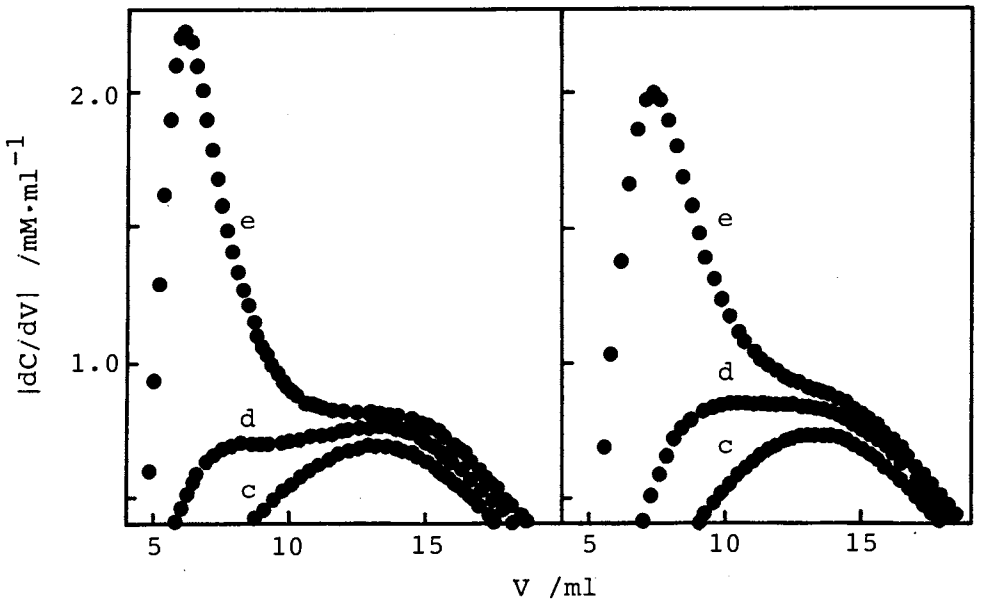

Fig. 4. Experimental (left) and theoretical (right) derivative patterns at three high concentrations (Table 1) and the trailing boundary.

Furthermore, by using these aggregation constants, V_1 and V_m, we simulated the derivative GFC patterns (Figs. 3 and 4) by plate theory,[4] where we used values of a plate number of 14 and of a void volume of 4.0 ml as adjustable parameters. Agreement between theory and experiment is very good, supporting the validity of our model. Judging from these aggregation

Table 1. Calculated concentrations of monomer, dimer, trimer, tetramer, and pentamer at the five concentrations shown in Figs. 3 and 4

	C/mM	C_1/mM	$2[A_2]$/mM	$3[A_3]$/mM	$4[A_4]$/mM	$5[A_5]$/mM
a	3.0849	3.0074	0.0597	0.0001	0.0120	0.0042
b	4.8792	4.6043	0.1399	0.0005	0.0661	0.0354
c	7.1161	6.1907	0.2529	0.0011	0.2159	0.1554
d	9.6878	7.3232	0.3540	0.0019	0.4228	0.3599
e	15.4520	8.4352	0.4696	0.0029	0.7442	0.7297

constants, tetramer is the most stable micelle of CHAPS. By using these aggregation constants, we can calculate concentrations of all species as a function of C. The C_1 value above the cmc is often assumed to be a constant(cmc) independent of C, but as Table 1 demonstrates, this assumption does not hold for CHAPS.

Since CHAPS possesses a small n value and a large cmc value, it can be easily permeable from protein solutions by dialysis across a membrane (probably permeable to monomer and very small micelles). Catalytic activity of some enzymes has a maximum at 2 mM.[2] The data illustrated in Table 1 will be useful for such applications and data analyses. The present data on CHAPS would serve for understanding the micelle formation of bile salts, and demonstrate that GFC is a powerful tool for the study of surfactant aggregation.

References

1) L. M. Hjelmeland, Proc. Natl. Acad. Sci. U. S. A., 77, 6368 (1980); J. Neugebauer, "A Guide to the Properties and Uses of Detergents in Biology and Biochemistry," CALBIOCHEM Biochemicals (1988).
2) S. L. Wagner, W. L. Dean, and R. D. Gray, J. Biol. Chem., 259, 2390 (1984).
3) J. P. Kratohvil, Adv. Colloid Interface Sci., 26, 131 (1986).
4) N. Funasaki, S. Hada, and S. Neya, J. Phys. Chem., 92, 3488, 7112 (1988); Bull. Chem. Soc. Jpn., 62, 380 (1989).
5) K. Yamaoka, "Analysis of Pharmacokinetics with Microcomputers," Nankodo, Kyoto (1984).

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