Effect of the Aggregation Number on Gel Chromatographic Patterns of Micellar Systems

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Synopsis. Gel chromatographic patterns and their derivatives of micellar systems are predicted as a function of the total surfactant concentration and the aggregation number m by plate theory and asymptotic theory. These predicted patterns provide the basis for estimation of monomer concentration and m from experimental chromatographic data.

Gel chromatography has provided important information about aqueous surfactant solution; e.g., micellar size,[1,2] monomer concentration,[2-4] and mixed micelle formation.[5] This technique has been also used for investigations of self-association of proteins,[6,7] but a wealth of knowledge obtained from such studies has not well been utilized for surfactants.

For quantitative analysis of self-associating systems, a large amount of sample is usually charged into a gel column so that a plateau region may appear on the elution curve.[2-7] For a nonassociable solute, asymptotic theory presumes the elution curve of a rectangle possessing the same concentration and volume with those of the charged sample.[3,4,6] For a self-associable solute with the aggregation number m, this theory predicts the equation[4,6]

$$V_c = C_1V_1/C_0 + (C_0 - C_1)V_m/C_0 \quad (1)$$

Here V_c is the centroid of elution profile defined as

$$V_c = \int_0^{C_0} V\mathrm{d}C/C_0 \quad (2)$$

C_0 and C_1 denote the total concentration and the monomer concentration, and V_1 and V_m denote the elution volumes of monomer and m-mer, respectively. For proteins, first derivative of the elution curve often provides more detailed information than the original curve,[6,7] but has not yet been reported for surfactants.

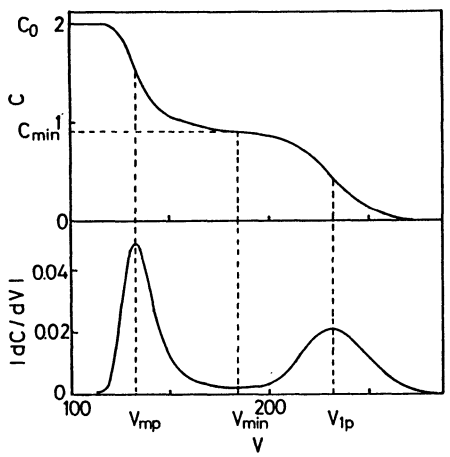

Fig. 1. Elution curve and its derivative simulated by using values of m=40 and C_0=2 and definitions of characteristic values.

Plate theory, based on phase separation model for micelle formation, was applied for simulation of gel chromatograms of surfactants.[5] This theory may predict a more realistic elution pattern than the above asymptotic theory, though no comparison has been reported for surfactants.

In this work, we develop plate theory based on mass action model for micellization of surfactants. This model is more rigorous than phase separation model and may also apply for proteins. Based on this theory, we investigate the effects of the total concentration and the aggregation number upon gel chromatographic patterns of self-associating systems. Derivative elution patterns are also simulated. These simulations make possible a better analysis of experimental results of gel chromatograms and reveal the scope and limitations of the asymptotic theory.

Methods

The procedure of computer simulation for plate theory developed by Nakagawa[5] was employed. It was assumed that the equilibrium of partition of solute between the mobile phase and the stationary phase is established instantaneously. At time zero, the column bed is saturated with surfactant solution of concentration C_0 and water as eluent begins to enter onto the gel bed. That is, the trailing boundary of frontal elution chromatography[6] is investigated.

For the monomer-m-mer equilibrium, the association constant K can be written as

$$K = (C_0 - C_1)/C_1^m \quad (3)$$

This reaction was assumed to be rapid. The value of C_1 was calculated by the Newton-Raphson method from Eq. 3.

To simulate a chromatogram, we must input the number N of plate, the void volume V_0, K, V_1, V_m, m, and C_0; N=100, K=1, V_1=230, V_m=130, and m=2, 3, 6, 10, 40, 80, 200, or ∞ were used together with a wide range of concentration C_0 Derivation of the elution curve was replaced by differentiation $\Delta C/\Delta V$.

Results and Discussion

Figure 1 shows a sample of simulation and definitions of C_{min}, V_{min}, V_{1p}, and V_{mp}. In general, derivative pattern has two peaks of V_{1p} and V_{mp} and a minimum of V_{min}. The C_{min} value is defined as the concentration at V_{min} on the elution curve. The V_c value can be calculated from the elution curve by using Eq. 2. For only cases of m=10, 40, and ∞, V_{1p}, V_c, and V_{mp} are shown as a function of C_0. Other cases are omitted, since we can infer such patterns from Fig. 2 roughly. For the case of m=2, derivative patterns had a single peak, regardless of C_0, whereas for cases of $m \geqq 3$, those patterns had two peaks. These results are consistent

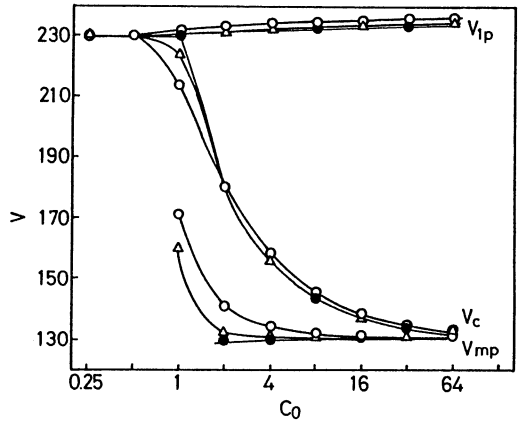

Fig. 2. Plots of V_{1p}, V_c, and V_{mp} against the logarithm of C_0 for cases of m=10 (○), m=40 (△), and m=∞ (●).

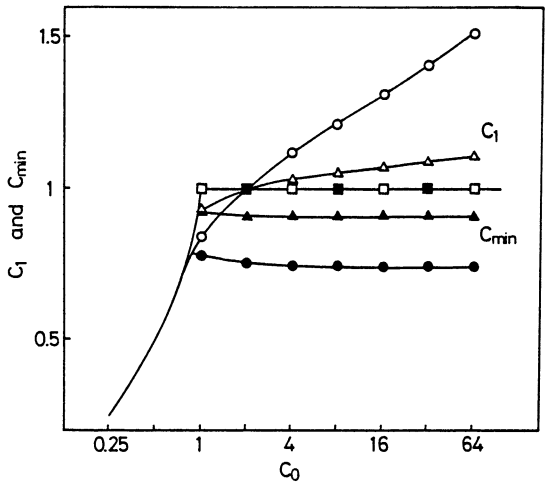

Fig. 3. Plots of C_1 (hollow symbols) and C_{min} (filled symbols) against the logarithm of C_0 for cases of m=10 (○,●), m=40 (△,▲), and m=∞ (□,■).

with predictions from asymptotic theory.[6] The V_c value simulated was in excellent agreement with that from Eq. 1: this equation, though obtained from asymptotic theory, holds rigorously for self-associating systems. As C_0 decreases, V_{1p} decreases and approaches V_1. This result indicates that V_1 should be evaluated at zero concentration. When m is small, V_{mp} decreases with increasing C_0 and approaches V_m. These results indicate that V_m should be evaluated at infinite concentration and that variation of V_{mp} with C_0 does not always reflect true size changes of the micelle. Concentration dependence of V_{mp} for hexamerization of α-chymotrypsin[7] is in qualitative agreement with the above prediction. Accurate estimation of micellar size of hexaethylene glycol dodecyl ether from V_{mp}[2] cannot be justified, though we do not deny micellar growth of this surfactant with C_0. According to asymptotic theory, V_{min} is expected to be $[(2m-1)V_1+(m-2)V_m]/(3m-3)$, regardless of C_0,[6] whereas the V_{min} value simulated increased with increasing C_0 (data are not shown). The latter prediction is in agreement with the experimental result.[8]

The C_1 value calculated from Eq. 3 is shown as a function of C_0 in Fig. 3. As is well known, the increase in C_1 decreased with increasing m above a concentration, called the critical micelle concentration. At $m \to \infty$, (phase separation model) C_1 was a constant of unity. As Fig. 3 shows, the C_{min} value simulated slightly decreased with increasing C_0 and increased with increasing m. According to asymptotic theory,

$$C_{min}=2(m^2-1)[(m-2)/K]^{1/(m-1)}[m(2m-1)]^{m/(1-m)} \quad (4)$$

The C_{min} values calculated from Eq. 4 at m=10, 40, and ∞ were 0.733, 0.940, and 1, respectively. These values are close to the simulated values at large values of C_0 (see Fig. 3). Values of monomer concentration of hexaethylene glycol dodecyl ether were estimated from Eq. 1[4] and from C_{min}.[2] These values are different from each other, as expected.

The present results provide the basis for analysis of experimental chromatographic data. If V_1 and V_m can be estimated from the data, we can determine C_1 as a function of C_0. As we have shown herein, V_1 can be evaluated from V_{1p} or V_c at C_0=0 and V_m from V_{mp} or V_c at infinite concentration. Concentration dependence of C_1 changes with m, as shown in Fig. 3. This suggests the possibility for determination of m from the C_1 vs. C_0 relationship. In gel chromatography the elution volume of solute decreases with increasing size of the solute. For self-associating systems, however, the value of V_{mp} depends on C_0 as well as on size.[7] This must be taken into consideration, when we estimate micellar size from V_{mp}. Values of V_c and V_{mp} at infinite concentration correspond to correct micellar size. As m increases, the difference between C_{min} and C_1 decreases (Fig. 3). When $m>200$, C_{min} is practically equal to C_1 and then we can estimate C_1 from C_{min}, without use of Eq. 1. Asymptotic theory can predict a correct value of V_c and a rough value of C_{min}. Derivative elution patterns can provide useful information about micellar size and monomer concentration of surfactants. The above predictions based on plate theory are in agreement with experimental results, which will be published elsewhere.[8]

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