Determination of the Dimerization Constant in Self-Associating Systems

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The self-association of Methylene Blue (MB), a cholic acid derivative, chlorpromazine hydrochloride (CPZ), and octaethylene glycol decyl ether has been investigated by frontal gel filtration chromatography (GFC) and spectrophotometry at 25 °C. The stepwise aggregation constant k_i is estimated as a function of aggregation number i from the centroid volume V_c of the GFC profile by using appropriate association models. The dimerization constants k_2 for MB and CPZ determined by spectrophotometry are close to those from V_c values. The dimerization constants are also estimated from the concentration dependence of the peak volume V_p of the derivative GFC profile. The k_2 values determined from the V_p values are slightly larger than those from the V_c values. This discrepancy is ascribed to the approximation of the V_p approach. The minimum multimerization concentration (mmc), above which multimers higher than trimer are formed, is determined from each of V_c, V_p, and absorption spectral measurements. The relation between mmc and the critical micelle concentration (cmc) depends on the degree of criticalness of cmc. This degree of criticalness increases as the aggregation number at the maximum k_i value increases.

Hydrophobic organic solutes often self-associate in water to avoid contact with water. Depending on the chemical structure of the associating molecule, its concentration, and other factors, self-association can lead to small oligomers only, to an extended series of multimers or to micelles. For hydrophobic self-association we can distinguish, very roughly, between four classes of solutes on the basis of chemical structure.[1]

The first class consists of aliphatic chain compounds, which have polar heads attached at one end, such as surfactants (e.g., octaethylene glycol decyl ether ($C_{10}E_8$)). Although these compounds are established to form micelles above the critical micelle concentration (cmc), it is still a matter of controversy whether they form small oligomers (premicelles) below the cmc.[2–6] The second class of solutes has aromatic fused ring structures. Many of these molecules may be quite planar. Many dyes, nucleoside bases (e.g., purines), etc., fall into this class, which includes also a large number of drugs. These molecules self-associate by stacking of their planar rings.[1,3,7] Methylene Blue (MB) and chlorpromazine hydrochloride (CPZ) shown in Fig. 1 fall into this class. The third class of solutes consists of alicyclic compounds which are nonplanar. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and bile salts fall into this class. The fourth class of solutes consists of macromolecular solutes, particularly proteins. Hydrophobic interactions between exposed side chains probably play a role in association of subunits, such as hemoglobin.[1]

According to Mukerjee, the simplest type of association, i.e., dimerization, must take place in all the self-associating systems being considered.[1] The formation of higher multimers may often overshadow it and may lead to difficulties involved in even detecting it. In general, CPZ is believed not to dimerize, although it definitely forms micelles.[7] The mode of self-association, cmc, and aggregation numbers for bile salts and

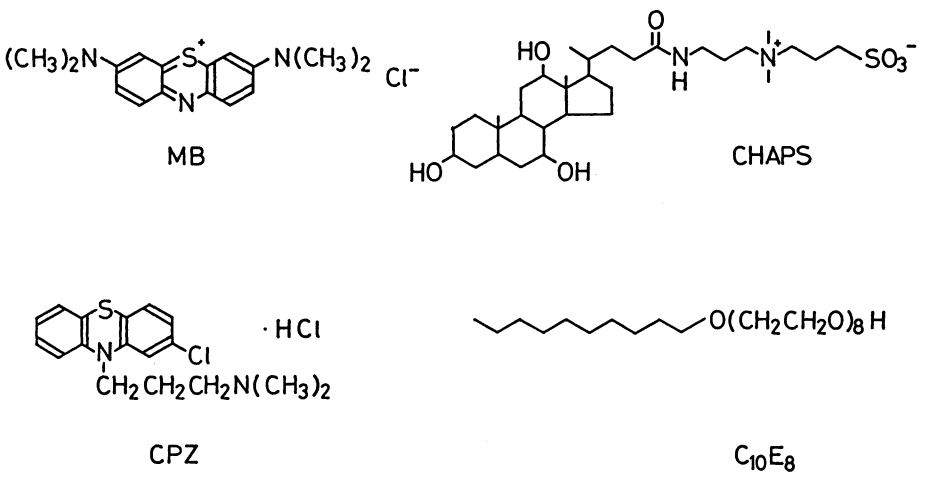

Fig. 1. Chemical structures of MB, CHAPS, CPZ, and $C_{10}E_8$.

their derivatives merit further investigations.[8,9] For instance, the aggregation number of CHAPS was estimated to be four from the concentration dependence of ^{13}C NMR chemical shifts and it was suggested from the concentration dependence of the refractive index that CHAPS dimerizes below the cmc.[10] The basis of these conclusions on CHAPS, however, is not very firm.

The dimerization constants of most dyes can be easily determined by spectrophotometry.[3] When higher multimers form in addition to dimer, however, this method is not very accurate, relative to other methods. For instance the trimer formation of MB has been suggested by vapor pressure osmometry,[11] polarography,[12] and isoextraction method.[13] Chromatography may be used for all classes of self-associating solutes including proteins.[14–17] The self-association of $C_{10}E_8$,[15] CPZ,[16] and CHAPS[17] has been investigated through analysis of the boundary position of frontal gel filtration chromatographic (GFC) pattern and the dimerization constant has been estimated for these compounds. The shape of the GFC boundary for the dimerizing system is theoretically predicted to be different from that for higher multimerizations and may allow us to evaluate the dimerization constant.[15–19] To our knowledge, however, this method has never been applied, probably since it was difficult to obtain accurate derivative chromatograms.

In this work we investigate the dimerization of $C_{10}E_8$, CPZ, CHAPS, and MB by means of the GFC profile, its derivative, and the absorption spectrum. For these compounds exhibiting different self-association patterns, the GFC derivative profile is useful for the detection of dimerization as well as for the estimation of the dimerization constant.

Experimental

Materials. A sample of MB (Merck) was recrystallized three times from ethanol. CHAPS (Dojindo),[17] CPZ (Sigma),[16] and $C_{10}E_8$ (Nikko Chemicals)[15] were the same with those already reported. Sephadex G-10 (Pharmacia) was treated according to the suggestions of the manufacturer. The ion-exchanged water was twice distilled and degassed before use.

Methods. A column of a total gel volume V_t of 6.91 cm^3 for MB was jacketed in order to maintain it at a constant temperature of 25±0.2 °C. The 1 mmol dm^{-3} hydrochloric acid was used as eluent for MB. The elution process was monitored continuously with a Shimadzu spectrophotometric detector and recorded with a data processor. Wavelengths of 415 and 290 nm were used for MB at high and low concentrations, respectively. The observed absorbance data were smoothed by the Savitzky-Golay method[20] before conversion into concentrations. The position and height of the peak in the derivative GFC profile were determined by a polynomial approximation. The GFC conditions for CHAPS,[17] CPZ,[16] and $C_{10}E_8$[15] have been already reported. The absorption spectra of MB were recorded with a Shimadzu MPS-2000 spectrophotometer. All measurements were carried out at 25 °C.

Results

Centroid Volumes of GFC Patterns. Figure 2a shows the schematic GFC profile for a dimerization system. When a large amount of sample of concentration C_0 is applied onto the gel column, a plateau region having the same concentration appears on the chromatogram. This method, called frontal chromatography, is often used for the investigation of self-associating systems. We may assume that the equivalent sharp boundary for the leading or trailing edge of the solute zone on the frontal chromatogram is approximately the beginning of the plateau region (centroid) of the elution profile and satisfies the relationships:

$$V_c' = \int_0^{C_0} V\mathrm{d}C / C_0 \qquad \text{(leading boundary)} \qquad (1)$$

and

$$V_c = \int_0^{C_0} V\mathrm{d}C / C_0 + S \qquad \text{(trailing boundary).} \qquad (2)$$

Here S is the applied volume of the sample. For these determinations the volume coordinate V is assigned a zero value when the leading boundary of the applied sample enters the column bed. According to this approximation (called asymptotic theory), the elution curve for a nonassociable solute is expected to fall within a rectangle of height C_0 and width S.

Hereafter, we assume that the equilibria for solute partition between the stationary and mobile phases and for self-association in the mobile phase are instantaneously established. When the gel pores are smaller than the dimer, one can take the elution volumes of all

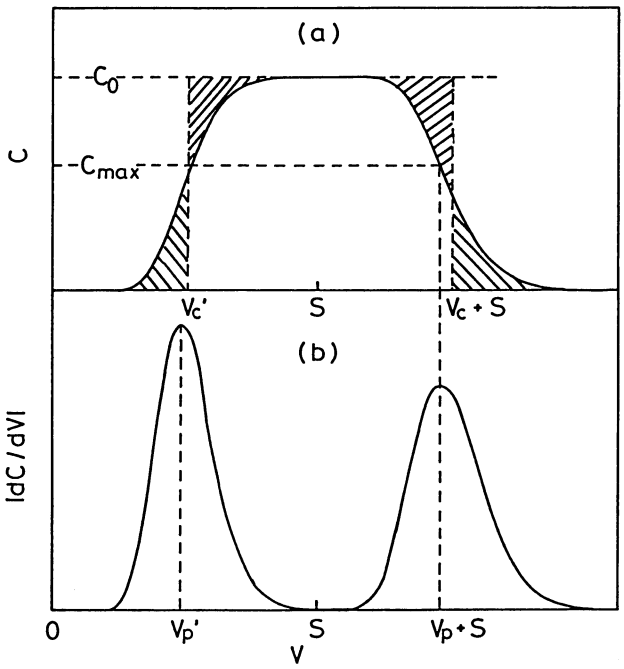

Fig. 2. Schematic frontal GFC profile (a), its derivative (b), and the definition of GFC parameters.

aggregated species as the void volume of the column. Under these conditions, from asymptotic theory, we may assume the following equation:[15–17]

$$V_c=[C_1V_1+(C-C_1)V_m]/C. \quad (3)$$

Here V_1 and V_m denote the elution volumes of monomer and micelles (including dimer), respectively and C_1 denotes the monomer concentration. Figure 3a shows the plot of V_c against C for MB. The V_c value shown herein is the average of V_c and V_c', since they are close to each other within experimental errors. The values of V_1 and V_m were estimated by the extrapolation of the V_c value to $C=0$ and $C=\infty$, as already reported for other systems.[15,16] Thus, values of $V_1=50.20$ cm^3 and $V_m=2.58$ cm^3 were estimated. Using these values, we can estimate C_1 from Eq. 3. In Fig. 3b, the C_1 values thus obtained for MB are shown as a function of C. The concentration dependence of C_1 reflects the aggregation pattern. The weight-average micellar aggregation number n_w can be estimated from[15–17]

$$n_w=\mathrm{d}\log(C-C_1)/\mathrm{d}\log C_1. \quad (4)$$

This equation holds for self-associating systems, irrespective of the kind and concentrations of micelles. From the slope of the plot of log $(C-C_1)$ vs. log C_1, shown in Fig. 4, we can estimate n_w as a function of C. The dashed line in Fig. 4 shows a slope of $n_w=2$. As Fig. 4 shows, n_w increases with increasing concentration and reaches about 3.8 at 0.8 mmol dm^{-3}.

When the i-mer is formed from the $(i-1)$-mer and monomer, the stepwise association constant k_i can be given by

$$A_{i-1}+A_1 \overset{k_i}{\rightleftarrows} A_i \quad (5)$$

and

$$k_i=[A_i]/[A_{i-1}]C_1. \quad (6)$$

Here $[A_i]$ denotes molarity of the i-mer A_i. When we regard the formation of the i-mer as a one-step reaction, we can write the one-step association constant K_i as

$$iA_1 \overset{K_i}{\rightleftarrows} A_i \quad (7)$$

and

$$K_i=[A_i]/C_1^i. \quad (8)$$

These equilibrium constants are connected by

$$K_i=\prod_2^i k_i. \quad (9)$$

Here it should be noted that $K_2=k_2$. By assuming a mode of self-association, we can write C as a function of C_1.

For the self-association of MB, we assumed a stepwise aggregation model; $k_2\neq k_3\neq k_4=\cdots=k$. For this case we can write C as

$$C=C_1+2k_2C_1^2+k_2k_3C_1^3(3-2kC_1)/(1-kC_1)^2. \quad (10)$$

Since the errors in observed V_c values are almost independent of C, we determined best fit values of k_2, k_3, and k so that the sum SS of the squares of residuals in V_c between theory and experiment over the number n of data is minimized by a nonlinear least-squares method.[21]

$$SS=\sum_{i=1}^{n}(V_{obsd}-V_{calcd})^2. \quad (11)$$

The theoretical V_c value is obtained from Eqs. 3 (with $V_1=50.20$ cm^3 and $V_m=2.58$ cm^3) and 10. Thus obtained were best fit values of $k_2=5.3$ dm^3 mmol^{-1}, $k_3=18.0$ dm^3 mmol^{-1}, and $k=3.2$ dm^3 mmol^{-1} and the final value of $SS=1.86$ cm^6. This k_2 value is shown in Table 1, together with the k_2 values similarly determined

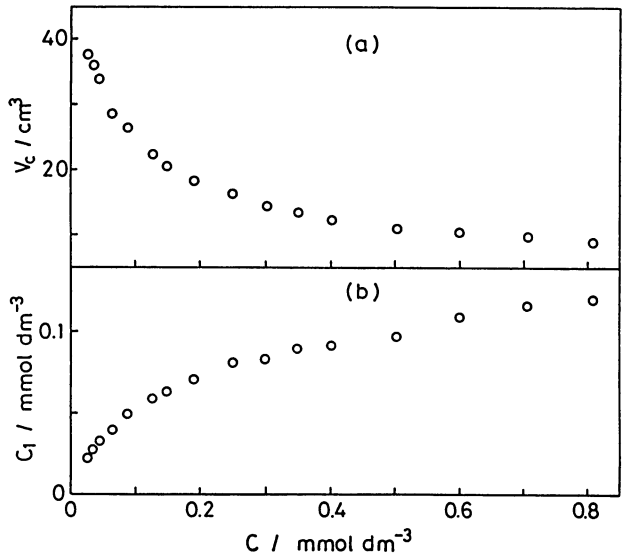

Fig. 3. Plots of centroid volumes (a) and monomer concentrations (b) against the total concentration for MB.

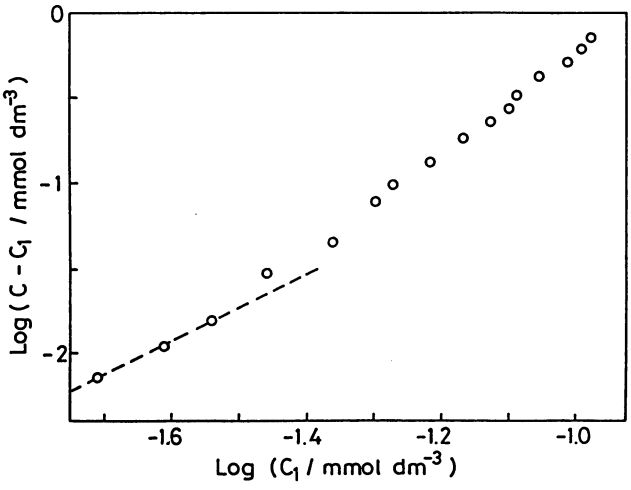

Fig. 4. log–log Plots of aggregate concentrations and monomer concentrations according to Eq. 4 for MB.

Table 1. Dimerization Constants Estimated by Three Methods

Compound	k_2/dm³ mmol⁻¹		
	V_c	V_p	Absorbance
MB	5.3	10.7	5.7
CHAPS	0.0046[a)]	0.0056	—
CPZ	0.124[b)]	0.142	0.127[b)]
$C_{10}E_8$	0.0077[c)]	0.0087	—

a) Calculated from model M9 of Ref. 17. b) Taken from Ref. 16. c) Calculated from Eq. 13.[15)]

for CHAPS, CPZ, and $C_{10}E_8$. Multimers larger than trimer form at the concentration where the log–log plot in Fig. 4 begins to deviate upward from the dashed line. Therefore we defined this concentration as a minimum multimerization concentration (mmc). As Table 2 shows, mmc is 0.044 mmol dm^{-3} for MB. In general, MB is regarded to have no cmc. The cmc values for CHAPS and CPZ shown in Table 2 were determined by several experimental methods.[16,17)]

The stepwise aggregation constants k_i for CHAPS[17)] have been already determined from the same analysis of the GFC data by using an appropriate self-association model and are shown as a function of aggregation number in Fig. 5. Those constants for $C_{10}E_8$[15)] and CPZ[16)] are calculated from a modification of Tanford's micellization theory:[22)]

$$i\Delta G^\circ_i/RT = -a(i-1)+b(i^{2/3}-1)+c(i^{4/3}-1) \quad (12)$$

and

$$\ln k_i = [(i-1)\Delta G^\circ_{i-1} - i\Delta G^\circ_i]/RT. \quad (13)$$

The k_2 value for CPZ is determined from another approach. The ΔG°_i value denotes the standard free energy of formation of i-mer.

Derivative GFC Patterns. The derivative GFC pattern provides some information about self-association. Figure 2b shows a schematic derivative pattern for a dimerizing system. Whenever some self-association occurs, the peak at the leading boundary becomes higher than that at the trailing boundary. For the dimerizing system the trailing derivative pattern consists of only one peak, but for the other multimerizing systems it splits into two peaks.[18,19)] This tendency may also hold for systems containing dimer and other multimers.

Figure 6 shows the concentration dependence of peak position V_p in the trailing derivative pattern for CPZ. Below C=5 mmol dm^{-3}, only one peak is present and the V_p value decreases with increasing concentration. These results suggest that CPZ dimerizes in this region. Above C=6 mmol dm^{-3} two peaks appear, suggesting

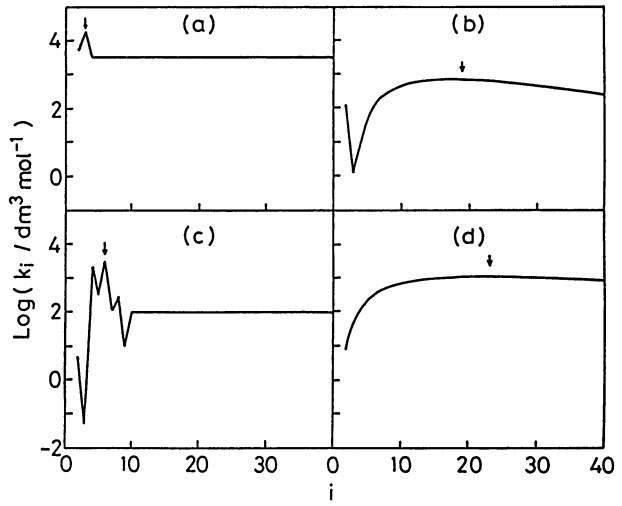

Fig. 5. Logarithm of the stepwise aggregation constants plotted against the aggregation numbers for MB (a), CPZ (b), CHAPS (c), and $C_{10}E_8$ (d).

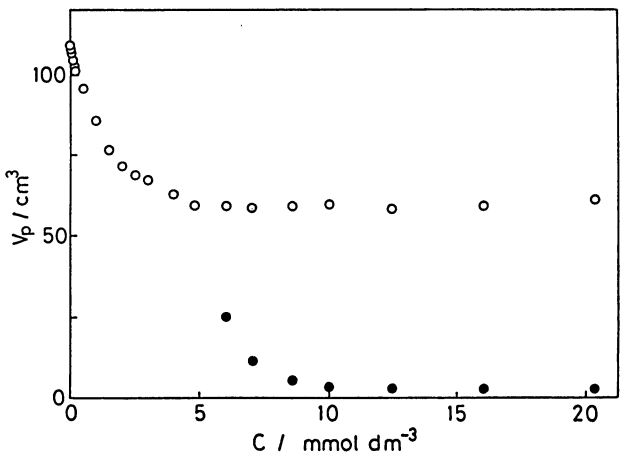

Fig. 6. Concentration dependence of the peak positions at the trailing derivative profile for CPZ: o, V_p; ●, V_{mp}.

Table 2. Values of the cmc and Minimum Multimerization Concentration (mmc) Determined by Four Methods

Compound	cmc/mmol dm^{-3}	mmc/mmol dm^{-3}			
		V_c	V_{mp}	V_p	Absorbance
MB	—	0.044	—	0.044	0.046
CHAPS	5—10[a)]	3	7	3.7	—
CPZ	4.4—6.4[b)]	4	6	4	—
$C_{10}E_8$	1.03[c)]	0.9—1.0	1.1	1.1	—

a) Taken from Ref. 30. b) Taken from Ref. 16. c) Taken from Ref. 15.

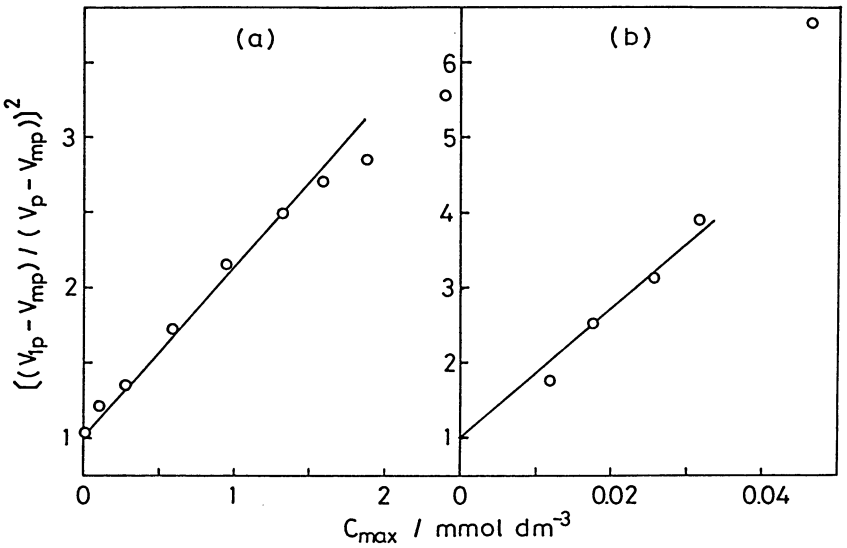

Fig. 7. Plots according to Eq. 14 for CPZ (a) with V_{1p}=111.5 cm^3 and V_{mp}=2.57 cm^3 and for MB (b) with V_{1p}=46.4 cm^3 and V_{mp}=5.1 cm^3.

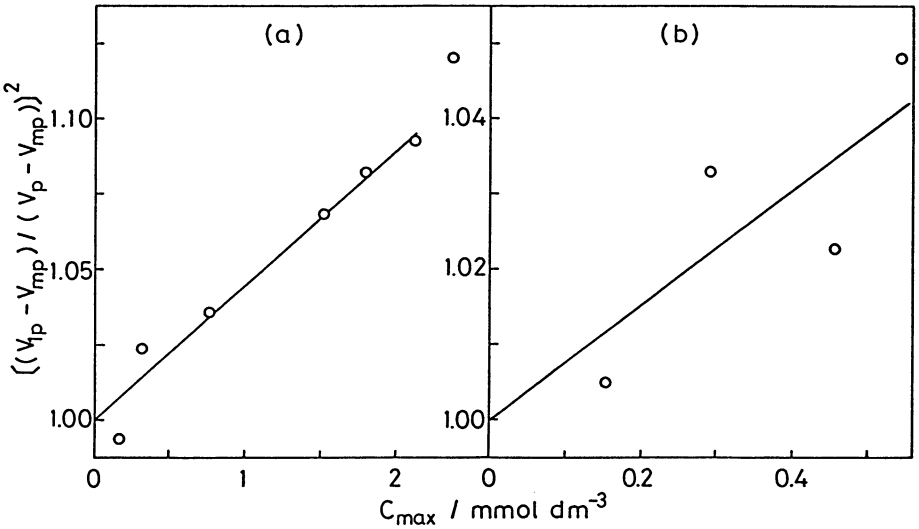

Fig. 8. Plots according to Eq. 14 for CHAPS (a) with V_{1p}=13.87 cm^3 and V_{mp}=5.62 cm^3 and for $C_{10}E_8$ (b) with V_{1p}=15.71 cm^3 and V_{mp}=7.63 cm^3.

that higher multimers form. Therefore, this concentration shown in Table 2 can be regarded as an mmc. In this table the mmc values similarly determined are shown for MB, CHAPS, and $C_{10}E_8$.

According to asymptotic theory, the concentration dependence of V_p at the trailing boundary for the dimerizing system may obey the following equation:[18,19]

$$[(V_{1p}-V_{mp})/(V_p-V_{mp})]^2 = 1 + 8k_2C_{max}. \quad (14)$$

This equation may also hold for systems including dimer and higher multimers. In Eq. 14, V_{1p} may be estimated by extrapolation of V_p (the hollow circles in Fig. 6) to C=0, V_{mp} may be estimated by extrapolation of V_p (the solid circles in Fig. 6) to $1/C$=0, and C_{max} denotes the concentration at V_p (see Fig. 2). Thus we obtained values of V_{1p}=111.5 cm^3 and V_{mp}=2.57 cm^3 from Fig. 6. By using these values, we can plot the V_p data (the hollow circles in Fig. 6) according to Eq. 14. As Fig. 7a shows, the V_p data at low C_{max} values show linearity, but those at high C_{max} values deviate from this linearity. By using the slope of this linear relationship, we can calculate a k_2 value of 0.142 dm^3 mmol^{-1} from Eq. 14. This k_2 value for CPZ is included in Table 1. The concentration above which a deviation from the linearity occurs may be regarded as an mmc. As Table 2 shows, this mmc value is 4 mmol dm^{-3} for CPZ. From the same plot we determined the values of k_2 and mmc for MB (Fig. 7b), CHAPS (Fig. 8a), and $C_{10}E_8$ (Fig. 8b). These values are shown in Tables 1 and 2. For $C_{10}E_8$, since k_2 is small, the data highly scattered around the

linearity, as shown Fig. 8b.

Absorbance Measurements. Figure 9 shows absorption spectra of MB at several concentrations in 1 mmol dm^{-3} HCl solution. The maximum wavelengths at 665 and 610 nm have been assigned to monomer and dimer.[23-26] The maximum wavelength of higher multimers is around 575 nm.[11] As is evident in this figure, there is an isosbestic point at 625 nm at concentrations lower than 0.042 mmol dm^{-3} (Table 1). The presence of the isosbestic point indicates that only two species, monomer and dimer, are present in this concentration range. Therefore an mmc value for MB is estimated to be 0.046 mmol dm^{-3} from curve d. In Fig. 10 the molar absorption coefficient at 665 nm are plotted against C below 0.042 mmol dm^{-3}. Under these conditions we can write the apparent molar absorption coefficient ε of MB solution as

$$\varepsilon=(\varepsilon_1-\varepsilon_2)[(1+8k_2C)^{1/2}-1]/4k_2C+\varepsilon_2, \quad (15)$$

where ε_1 and ε_2 denote the molar absorption coefficients for monomer and dimer. The ε_1 value for MB was estimated by extrapolation of ε to C=0. The values of k_2 and ε_2 were determined by best fitting k_2 and ε_2 to the observed ε values below C=0.042 mmol dm^{-3} by a nonlinear least-squares method: Values of k_2=5.7 dm^3 mmol^{-1} and ε_2=3.4 dm^3 mmol^{-1} cm^{-1} were obtained. The best fit curve is shown in Fig. 10.

For CPZ the absorption spectrum changes only slightly with C. This change allowed us to determine the k_2 value, but is not large enough to detect any isosbestic point.[16]

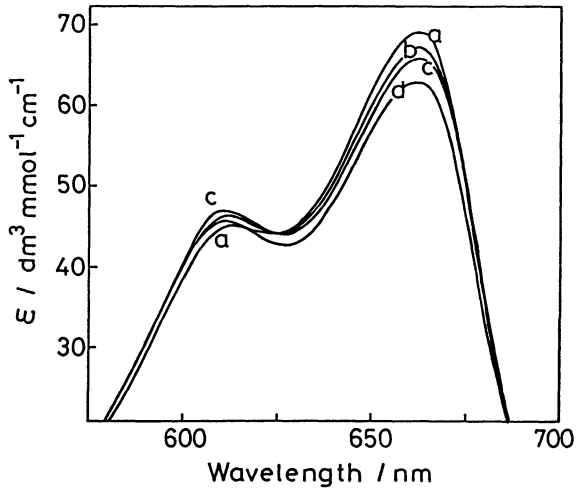

Fig. 9. Absorption spectra for MB at C (mmol dm^{-3}): a, 0.0288; b, 0.037; c, 0.0412; d, 0.0464.

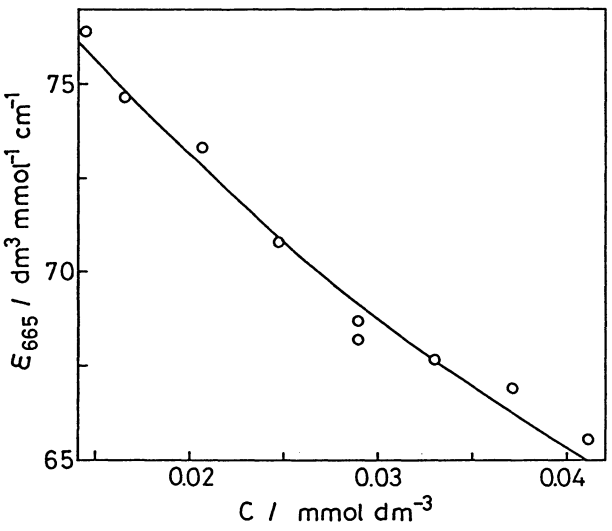

Fig. 10. Concentration dependence of the millimolar absorption coefficient at 665 nm for MB. The solid line is calculated from Eq. 15 with ε_1=86.4 dm^3 mmol^{-1} cm^{-1}, ε_2=3.4 dm^3 mmol^{-1} cm^{-1}, and k_2= 5.7 dm^3 mmol^{-1}.

Discussion

Although the self-association of MB was investigated by a number of experimental techniques such as spectrophotometry,[11,23-26] vapor pressure osmometry,[23] sedimentation equilibrium method,[27] isoextraction method,[13] polarography,[12] and conductance method,[28] spectrophotometry will be the best method for the determination of k_2 among these methods. The k_2 values for MB, determined by spectrophotometry, are 2.0,[11] 3.97,[23] 4.67,[23] 5.3,[26] 5.7 (this work), 5.9,[25] and 6.5 dm^3 mmol^{-1}.[24] This variation in k_2 would be ascribed to the data treatments and to the difference in the minor ionic compositions contained in the solutions. The k_2 values for MB are determined to be 6.7 dm^3 mmol^{-1} by sedimentation equilibrium,[27] and 2.1 dm^3 mmol^{-1} by isoextraction method.[13] Our k_2 values estimated from V_c and absorbance measurements fall within the above data, but the value obtained from V_p is slightly large. From measurements of spectrophotometry,[11] isoextraction,[13] and GFC (this work), it is shown that MB forms multimers larger than trimer. Although spectrophotometry has difficulties involved in the estimation of the absorption spectra for pure multimers, values of k_2=2.0 dm^3 mmol^{-1} and k_3=3 dm^3 mmol^{-1} are estimated.[11] Values of k_2=2.09 dm^3 mmol^{-1}, k_3=3.99 dm^3 mmol^{-1}, k_4=3.0 dm^3 mmol^{-1}, and k=2.00 dm^3 mmol^{-1} are obtained from isoextraction data.[13] Our stepwise constants estimated from the V_c data are k_2=5.3 dm^3 mmol^{-1}, k_3=6.2 dm^3 mmol^{-1}, and k=3.2 dm^3 mmol^{-1}. In comparison with CHAPS, CPZ, and $C_{10}E_8$, the k_2 value for MB is large, and these stepwise aggregation constants are almost independent of the aggregation number (Fig. 5). The latter result leads to low cooperativity in self-association and consequently there is no cmc for MB. These results are explicable on the basis of the aggregation mode that MB self-associates through stacking interactions between the planar aromatic rings.

The aggregation pattern of CHAPS appears to be similar to those of bile salts. Irrespective of many

studies, there are still controversies concerning cmc values, aggregation numbers, aggregation modes of bile salts.[8,9,17] Mysels adequately expresses the aggregation behavior of bile salts.[29] "In most bile salt systems, the breadth of the transitions precludes a precise determination of cmc, and measurements of other properties of the solution, which weigh the monomers and multimers differently, will give somewhat different results. Hence, the term critical is not appropriate and uncritical or, better, noncritical, seems indicated. Furthermore, micelles are large aggregates of 50 to 100 or more monomers. In bile salts, the aggregates are certainly often much smaller. Hence, the term micelle is also not appropriate. Thus, noncritical multimerization concentration would be a better term to describe this measure of onset of association." For CHAPS, cmc values range from 5 to 10 mmol dm^{-3}, and n_w values range from 4 to 14.[17,31] On the basis of V_c measurements, we showed that n_w gradually increases with increasing concentration, from 2 to 18 at 40 mmol dm^{-3}.[17] The fact that the mmc values determined from V_c and V_p are smaller than those cmc values (Table 2) reflects this gradual aggregation of CHAPS. The mmc value obtained from V_{mp} is larger than these mmc values. This result is mainly ascribed to the gradual aggregation of CHAPS in comparison with $C_{10}E_8$ and partially ascribed to the small difference between V_{1p} and V_{mp} in comparison with MB.

In contrast with MB, CPZ forms micelles of 37 to 64 above the cmc.[16] These compounds have similar ring structures, but a minor difference in the nonplanar middle ring and the presence of the side chain (Fig. 1) may lead to a marked difference in the cooperativity. A number of drugs are known to self-associate in water in micellar or nonmicellar modes. CPZ is classified into drugs self-associating micellarly, where micellar aggregation means that no premicelle forms below the cmc.[7] From the concentration dependence of V_c and V_p, however, we have found that CPZ dimerizes below the cmc (Tables 1 and 2).

For surfactants the presence of premicelles is still debated.[2,6] To our knowledge, there is no report on the premicelle formation of nonionic surfactants. The dimerization constant of $C_{10}E_8$ has been explained by a modification of the Tanford theory.[15]

Since the k_2 values obtained from V_c and absorbance measurements are close to each other (Table 1), the V_c method for k_2 determination will be generally reliable. As Table 1 shows, the k_2 value obtained from V_p is larger than that from V_c. Since the four compounds shown in this table self-associate in different modes, this will be a general tendency. This tendency is not unexpected, since asymptotic theory is based on an approximation.[18,19] For CPZ, CHAPS, and $C_{10}E_8$ the k_2 values obtained from V_p values are about 1.2 times as large as those from V_c values. This is ascribed to the approximation of Eq. 14. However, this ratio is about two for MB (Table 1). Reasons for this discrepancy are experimental errors in V_p and data treatments. Another reason may be the difference in aggregation pattern. For many compounds it is controversial whether dimer is present or not. For such compounds, the derivative GFC pattern can provide qualitative and semiquantitative information on the presence of dimer.

The criticalness of cmc may be related to the aggregation number i_{max} at which the stepwise aggregation constant shows a maximum. As Fig. 5 shows, i_{max}'s are 3, 6, 19, and 23 for MB, CHAPS, CPZ, and $C_{10}E_8$, respectively. In this order of the compounds the degree of criticalness of cmc increases. This criticalness influences the difference between cmc and mmc, as shown in Table 2. For $C_{10}E_8$ cmc is very close to mmc, suggesting that the fraction of oligomers excluding dimer is very small.

For the micelle-forming drug, the cmc is much larger than concentrations at which the drug shows physiological activities in vivo, though it is most likely that its surface-active characteristic is more important biologically.[7] For the possible implications of surface activity, there is extensive literature on the phenothiazines.[7] Since dimerization generally occurs at concentrations much lower than the cmc, it can affect some physiological activities of drug. For instance, CPZ lyses erythrocytes below 0.03 mmol dm^{-3}, whereas it stabilizes above this concentration.[31] MB at 0.01 mmol dm^{-3} can influence activity of drug metabolizing enzymes[32] and can amplify phleomycin-induced antibiotic activity.[33] At this concentration the one-tenth of MB dimerizes.

Dyes are often used as optical and fluorescent probes. In such applications the self-association of the dyes must be taken into consideration.

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References

1) P. Mukerjee, "Physical Chemistry: Enriching Topics from Colloid and Surface Science," ed by H. van Olphen and K. J. Mysels, Theorex, La Jolla, California (1975), Chap. 9.
2) H. Wennerstrom and B. Lindman, *Phys. Rep.*, **52**, 1 (1979).
3) S. Ikeda, "Koroido Kagaku (Colloid Chemistry)," Shokabo, Tokyo (1986), Chap. 6.
4) P. Mukerjee, K. J. Mysels, and C. I. Dubin, *J. Phys. Chem.*, **62**, 1390 (1958).
5) G. D. Parfitt and A. L. Smith, *J. Phys. Chem.*, **66**, 942 (1962).
6) P. Mukerjee, *Adv. Colloid Interface Sci.*, **1**, 241 (1967).
7) D. Attwood and A. T. Florence, "Surfactant Systems," Chapman and Hall, London (1983), Chap. 4.
8) M. C. Carey, "Sterols and Bile Acids," ed by H. Danielsson and J. Sjovall, Elsevier, Amsterdam (1985), p. 345.
9) J. P. Kratohvil, *Adv. Colloid Interface Sci.*, **26**, 131 (1986).
10) R. E. Stark, P. D. Leff, S. G. Milheim, and A. Kropf, *J. Phys. Chem.*, **88**, 6063 (1984).

11) E. Braswell, *J. Phys. Chem.*, **72**, 2477 (1968).
12) P. J. Hillson and R. B. McKay, *Trans. Faraday Soc.*, **61**, 374 (1965).
13) P. Mukerjee and A. K. Ghosh, *J. Am. Chem. Soc.*, **92**, 6043 (1970).
14) G. K. Ackers, *Adv. Protein Chem.*, **24**, 343 (1970).
15) N. Funasaki, H.-S. Shim, and S. Hada, *J. Chem. Soc., Faraday Trans.*, **87**, 957 (1991).
16) N. Funasaki, S. Hada, and J. Paiement, *J. Phys. Chem.*, **95**, 4131 (1991).
17) N. Funasaki, S. Hada, and S. Neya, *Chem. Lett.*, **1990**, 1075. N. Funasaki, S. Hada, and S. Neya, *J. Phys. Chem.*, **95**, 1846 (1991).
18) G. K. Ackers and T. E. Thomson, *Proc. Natl. Acad. Sci. U.S.A.*, **53**, 341 (1965).
19) N. Funasaki, S. Hada, and S. Neya, *Bull. Chem. Soc. Jpn.*, **62**, 380 (1989).
20) A. Savitzky and M. J. E. Golay, *Anal. Chem.*, **36**, 1627 (1964).
21) K. Yamaoka, "Yakubutsu Tainai Dotai Kaiseki Ho," Nankodo, Tokyo (1984).
22) C. Tanford, *J. Phys. Chem.*, **78**, 2469 (1974).
23) W. Spencer and J. R. Sutter, *J. Phys. Chem.*, **83**, 1573 (1979).
24) R. E. Ballard and H. Park, *J. Chem. Soc. A*, **1970**, 1340.
25) K. Bergmann and C. T. O'Konski, *J. Phys. Chem.*, **67**, 2169 (1963).
26) L. Fornili and G. Sgroi, *J. Chem. Soc., Faraday Trans. 1*, **77**, 3049 (1981).
27) E. Branswell and J. Lary, *J. Phys. Chem.*, **85**, 1573 (1981).
28) R. H. Boyd, *J. Phys. Chem.*, **65**, 1834 (1961).
29) K. J. Mysels, *Hepatology*, **4**, 80S (1984).
30) J. Neugebauer, "A Guide to the Properties and Uses of Detergents in Biology and Biochemistry," CALBIOCHEM Biochemicals, San Diego, California (1988).
31) P. Seeman and J. Weinstein, *Biochem. Pharmacol.*, **15**, 1737 (1966).
32) F. E. Beyhl, *Experientia*, **37**, 943 (1981).
33) G. W. Grigg, A. M. Gero, J. M. Hughes, W. H. F. Sasse, M. Bliese, N. K. Hart, O. Johansen, and P. Kissane, *J. Antibiot.*, **30**, 870 (1977).