Micellar Properties of Drugs: Micellar and Nonmicellar Patterns of Self-Association of Hydrophobic Solutes of Different Molecular Structures — Monomer Fraction, Availability, and Misuses of Micellar Hypothesis

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Abstract □ The nature of multiple, sequential, self-association equilibria and the significance of the average degrees of association are discussed. Characteristic patterns of association are examined on the basis of available experimental evidence and suitable theoretical models of self-association and their relationship to molecular structure is indicated. The meaning and significance of the simple two-phase approximation of the micellar hypothesis to the CMC are examined and its application is discussed.

Keyphrases □ Micellar properties of drugs—symposium □ Micelle and nonmicelle patterns of self-association, hydrophobic solutes of different molecular structures—consideration of monomer fraction, availability, and correct application of micellar hypothesis □ Self-association of hydrophobic solutes—micellar and nonmicellar patterns, discussion and equations □ Solutes, hydrophobic—micellar and nonmicellar patterns of self-association, discussion and equations

Self-association of hydrophobic molecules in aqueous solution is not confined to micelle formation of soaps and detergents. Many hydrophobic drugs, dyes, physiological surfactants such as bile salts, and hydrophobic proteins show self-association. However, the *patterns* of self-association may be very different for solutes with different molecular structures and geometry. Hydrophobic solutes are classified on the basis of their structures. The nature of multiple, sequential, self-association equilibria and the significance of the average degrees of association are discussed. Some characteristic patterns of association are examined on the basis of available experimental evidence and suitable theoretical models of self-association. These include formation of unique oligomers, *e.g.*, a dimer, open-ended continuous self-association, and cooperative association to form micelles of limited and wide size distributions. The relationship of these patterns to molecular structures is indicated.

Since self-association in various situations is often described using the simple two-phase approximation of the micellar hypothesis after the isolation of a critical micelle concentration (CMC), the meaning and significance of this hypothesis with respect to the CMC in practical situations are examined in detail. It is shown by some suitably constructed examples that different experimental approaches may give rise to apparent CMC values for many systems exhibiting nonmicellar patterns of association. However, the application of the micellar hypothesis to such systems may be seriously misleading. The importance of the monomer fraction as a measure of the activity or "availability" of the self-associating system is underscored. The micellar hypothesis is especially inappropriate for estimating the monomer fraction in systems showing nonmicellar patterns of association and may seriously underestimate the extent of self-association in dilute solutions, *i.e.*, below the apparent CMC. These conclusions should be particularly relevant for many physiological surfactants and aggregating drug systems.

HYDROPHOBIC INTERACTIONS AND HYDROPHOBIC ASSOCIATION

In aqueous media, essentially all molecules containing exposed organic groups, *i.e.*, ones that are not protected by polar groups on more than one side, show some degree of hydrophobicity. Methanol (CH_3OH) and phenol (C_6H_5OH), for example, containing the smallest alkyl and aromatic groups substituting for a proton in the water molecule itself, reduce the surface tension of water and are, therefore, surface active. In a qualitative sense, this surface activity is a result of the tendency of the organic groups of the molecules constituting the hydrophobic moiety to leave the contact of water and seek out relatively nonpolar substrates. The hydrophobicity also expresses itself in many other ways. For example, lipophilicity, as reflected in the ability of the molecules to partition into nonpolar phases, is closely related to hydrophobicity. Similarly, adsorption and binding to nonpolar substrates, which may be as diverse as charcoal, proteins, enzymes, membranes, or receptor sites, and solubilization in micelles or other lipoidal systems are often caused by the hydrophobicity of the molecules.

The intermolecular forces and water structure effects responsible for this hydrophobicity have been the focus of much attention but are still not well understood (1–4). The magnitude of the problems involved has been discussed (3). For correlation with molecular structure, one fruitful qualitative idea that has had considerable application in many guises and forms is the additivity of the hydrophobic interactions: as the hydrophobic moiety gets larger, its interfacial activity, lipophilicity, adsorbability, *etc.*, tend to increase and often do so in a parallel fashion (3, 5). This generalization seems to hold particularly well for a homologous series of compounds (3).

Another expression of the hydrophobicity of organic molecules in aqueous solution is their tendency to exhibit aggregation in solution, *i.e.*, self-association and mutual association. Here again, qualitatively, the larger the hydrophobic moiety the stronger is the tendency toward association. Thus, the association of small molecules may be appreciable only at high concentrations, whereas molecules containing large hydrophobic moieties can show self-association at extremely low concentrations. For example, many

long-chain nonionic surfactants have CMC's of the order of 10^{-5}–10^{-6} mole/liter (6). Detailed applications of the theories of micelle formation (7) to such systems suggest that the monomer concentration may not exceed this value by very much even if the total concentration is 10^{-1} mole/liter, *i.e.*, 10^{4}–10^{5} times as much as the CMC. Thus, at these high concentrations, the monomer fraction can be as low as 10^{-4}–10^{-5}. This can be an important factor if the concentration of free monomers is the important variable, as it often is.

The self-association and mutual association of hydrophobic solutes are usually ascribed to "hydrophobic bonding." This catch-all expression summarizes various interactions, most of which are difficult to quantify or even to examine separately (1–4). Van der Waals' forces, water-structure effects, and/or interfacial free energy changes are some of the attractive interactions involved. These are opposed by short-range electron cloud repulsions and repulsions between any charges or higher multipoles that may be present. As in all reactions between solute molecules in a liquid medium, the "net" interactions are the result of the balance between solute–solute and solvent–solvent interactions on the one hand and solute–solvent interactions on the other. The extent of association is determined by the interplay of these interactions with the ever present thermal energy of the molecules and the loss in the entropy of mixing on association (1, 4).

SELF-ASSOCIATION

Equilibria—Stepwise self-association of monomers can lead to an immense variety of distributions of aggregates of different sizes. Various techniques are available and are being developed for the analysis of such multiple equilibria (7–11). The equilibria for ideal, nonionic systems can be written as a set of stepwise equilibria involving addition of a monomer to a q-mer containing q monomers or as a set of overall equilibria in which the q-mer is depicted as a result of the association of q monomers. The equilibria (Scheme I) will often be applicable to charged systems if the ionic strength and the counterion concentration and composition are kept constant (7, 9) and the aggregates can be assumed to behave ideally, *i.e.*, the interaction between aggregates is negligible.

The simplest self-association equilibrium is dimerization, as represented by Scheme I and Eq. 1:

$$2b_1 \overset{K_2}{\rightleftharpoons} b_2$$

Scheme I

$$K_2 = \frac{[b_2]}{[b_1]^2} \qquad \text{(Eq. 1)}$$

where b_1 is the monomer, b_2 is the dimer, and K_2 is the association constant for the dimer.

All concentration units used here will be moles or equivalents per liter, which are practical units for dilute solutions. For thermodynamic analysis, the mole fraction concentration scales are more appropriate (1, 4). The equilibria described here can easily be transformed into the mole fraction or molality scale.

Dimerization is the first step in any self-association scheme. As stepwise association continues, larger multimers form (Scheme II).

$$b_2 + b_1 \overset{K_3}{\rightleftharpoons} b_3$$

$$b_3 + b_1 \overset{K_4}{\rightleftharpoons} b_4$$

$$b_{q-1} + b_1 \overset{K_q}{\rightleftharpoons} b_q$$

Scheme II

The overall association constant for the formation of b_q from b_1 in Scheme III:

$$qb_1 \rightleftharpoons b_q$$

Scheme III

is represented by:

$$\beta_q = \frac{[b_q]}{[b_1]^q} \qquad \text{(Eq. 2)}$$

where:

$$\beta_q = \prod_{2}^{q} K_q \qquad \text{(Eq. 3)}$$

β_q is the product of all stepwise association constants K_2, K_3, . . . , up to K_q.

The total molar concentration of all species, M, is given by:

$$M = \sum [b_q] \qquad \text{(Eq. 4)}$$

The total equivalent concentration of all species, C, the concentration that is usually known experimentally from the composition of a solution, is given by:

$$C = \sum q[b_q] \qquad \text{(Eq. 5)}$$

If the quantity G is defined as:

$$G = \sum q^2[b_q] \qquad \text{(Eq. 6)}$$

then the average degrees of association of all species, including the monomer, can be defined as:

$$N_n' = \frac{C}{M} \qquad \text{(Eq. 7}a\text{)}$$

$$N_w' = \frac{G}{C} \qquad \text{(Eq. 7}b\text{)}$$

where N_n' is the number average degree of association, and N_w' is the corresponding weight average value. N_n' is usually determined from colligative measurements such as freezing-point depression or vapor pressure lowering, and N_w' is obtained from light scattering and equilibrium ultracentrifugation. Various other methods also are available.

For many systems, particularly micelle-forming systems, the average degree of association of interest is that pertaining to the aggregates only, *i.e.*, *excluding the monomer.* Such degrees of association are defined as:

$$N_n = \frac{C - [b_1]}{M - [b_1]} \qquad \text{(Eq. 8}a\text{)}$$

$$N_w = \frac{G - [b_1]}{C - [b_1]} \qquad \text{(Eq. 8}b\text{)}$$

In experimental situations, the assumption is usually made that the monomer concentration above the CMC in micellar systems is represented by the value of the CMC itself. This assumption leads to the approximation:

$$N_w \approx \frac{G - \text{CMC}}{C - \text{CMC}} \qquad \text{(Eq. 9)}$$

These equilibria lead to some general conclusions about the degree of association from the fact that $[b_q]$ is proportional to $[b_1]^q$. Therefore, as $[b_1]$ increases, the concentrations of all q-mers increase. Thus, irrespective of which or how many q-mers form, N_n' and N_w', from their definitions (Eqs. 7*a* and 7*b*), must increase as the total concentration, C, increases. On the other hand, as infinite dilution is approached, *i.e.*, as $C \to 0$, then $N_n' \to 1$, $N_w' \to 1$, and $[b_1] \to C$.

If one considers the N_n and N_w values, *i.e.*, the degrees of association of the aggregates only, then if a *unique*, single oligomer (containing few monomers) or multimer (containing many monomers) forms, *i.e.*, the aggregate is *monodisperse*, clearly $N_w = N_n$ and both are independent of concentration. However, if there is a distribution in aggregate sizes, *i.e.*, the aggregates are polydisperse, since $[b_q]$ is proportional to $[b_1]^q$ and therefore the fractional increase in $[b_q]$ with increasing $[b_1]$ is larger as q increases, the definitions of N_n and N_w (Eqs. 8*a* and 8*b*) and Eqs. 4–6 lead to the important result that N_n and N_w must increase with concentration (7). For polydisperse aggregates, $N_w > N_n$ and the ratio N_w/N_n is a measure of the polydispersity. For a monodisperse system, this ratio is unity.

Structural and Geometrical Factors—In the self-association

equilibria discussed previously, the absolute values of K_q and β_q are the quantitative measure of the tendency toward self-association. Thus, the higher these values are, the more extensive is the association and the lower is the concentration at which the association becomes important. The pattern of the association, however, *i.e.*, whether the products of the self-association are unique oligomers (containing a few monomers) or multimers (containing many monomers), a narrow distribution of oligomers or multimers, or a broad distribution of oligomers or multimers, depends upon the *relative* variation of K_q or β_q with q. The geometrical and structural characteristics of the hydrophobic solutes are reflected both in the absolute and the relative values of K_q and β_q.

Hydrophobic solutes can be separated into four broad classes depending upon their structures.

Class I—The first class consists of flexible chain compounds containing polar head groups. Soaps, many synthetic detergents, cationic and nonionic surfactants, and phospholipids fall into this class. The association of these molecules is mainly due to the flexible chains, although these sometimes include unsaturated groups or aromatic moieties. These compounds are characterized by having nonpolar and polar ends joined together.

Class II—The second class consists mainly of aromatic or heteroaromatic ring or fused ring structures which are rigid. Many of these molecules may be quite flat or planar. Many dyes, purines, pyrimidines, *etc.*, fall into this class, which also contains a large number of drugs. Some of these molecules may possess one or more short flexible chains, with or without a polar group attached to the ring structure. If these chains are not large in comparison to the rings, the self-association is expected to be controlled by the planar portion of the molecule. Because of the presence of π-electron systems and slightly polar groups such as ring nitrogens, which may be capable of hydrogen bonding, these molecules tend to be less hydrophobic than the hydrocarbon chains of the same molecular weight.

An important characteristic that distinguishes this class of compounds from the Class I compounds is that the molecules do not possess a particularly polar or nonpolar end; they are roughly symmetrical with respect to their hydrophobicity on both sides of their flat or nearly flat structures. These molecules are thus capable of a "stacking"-type association in which each associating monomer can lie flat on top of a stack containing one or more monomers and there are no geometrical restrictions to open-ended continuous self-association. When polar or nonpolar groups are attached to the planar ring systems, these are radially disposed and, unless they are very bulky, they do not prevent face-to-face association.

Phenothiazines and similar molecules containing ring structures that are somewhat bent or kinked in the middle form a subclass of this class of solutes. The self-association of a monomer with another monomer probably involves the association of the convex side of one to the concave side of the other. The dimer in this configuration still has a concave and convex side open for further association, so stepwise self-association can continue indefinitely somewhat in the manner of a stacking of tiles.

Class III—This class consists mainly of alicyclic fused ring systems which may be inflexible but are not planar, *e.g.*, bile acids, cholesterol, and similar compounds. When polar functions are present, because of the tetrahedral geometry of most of the carbon atoms, the polar groups are directed away from the backbone of the molecule more to one side than the other. This tends to make one side more hydrophobic than the other (12).

Class IV—This class consists of macromolecular solutes, particularly proteins and enzymes. The self-association of proteins is of considerable importance in biochemistry and physiology. Hydrophobic interactions between exposed side chains probably play an important role in these association reactions, but complex conformational changes may be involved. The association equilibria can formally be treated in terms of stepwise self-association (10, 11). These solutes are not discussed further in this article.

DATA TREATMENT, METHODS, AND OTHER CONSIDERATIONS

The complete description of a self-associating system requires a knowledge of the values of K_q or β_q for all relevant values of q. Even when only a few oligomers or multimers are involved, this task is extremely difficult because experimental uncertainties of one K_q value affect those of all the others (8, 9).

Of the various possible experimental approaches available for studying self-associating systems, the nonequilibrium methods (*e.g.*, those based on spectroscopy of any kind, polarography, conductance, *etc.*) are usually unsuitable (13, 14). Such methods require the determination of a characteristic parameter for every oligomer or multimer other than the formation constant. For example, methods based on spectral changes require the estimate of the absorptivity for every oligomer or multimer (15), NMR methods require the estimate of a chemical shift, and conductance methods require the estimate of an equivalent conductance (16). This additional set of unknown parameters makes the determination of K_q or β_q values extremely difficult. The equilibrium methods generally depend upon the estimate of the free monomer concentration $[b_1]$ as a function of the total equivalent concentration C (8, 13, 14), the osmolarity M from colligative measurement, the number average degree of association $N_n' = M/C$, or the weight average degree of association $N_w' = G/C$. Different kinds of data usually require different kinds of analysis (7-11). Even when equilibrium data are available, if they are of ordinary precision, it is in general extremely difficult to extract more than two or three association constants precisely, although mathematical methods of data analysis are available in principle (8, 9).

It is, therefore, frequently necessary to devise suitable models of association in which the stepwise association constants are allowed to vary in some predetermined sequence and no more than two or three independent parameters are used to describe the models. For systems exhibiting unique oligomers or multimers (*e.g.*, dimers) or monodisperse micelles, two suitable parameters are β_q and q, for example. For systems showing continuous self-association, there may not be any restriction on allowed q values, and the association model may involve the use of one or two parameters which describe the variation of K_q with q, with q ranging from 2 to ∞, according to some predetermined sequence. Various models of this kind have been devised (8, 9).

Different Patterns of Self-Association: Structural and Geometrical Factors—Considering the complexities of interactions and geometrical factors involved, it is not surprising that self-association reactions are not simple. However, some limiting simple patterns of self-association are *approached* by some systems. Quantitative treatments based on these simple patterns can then be used to *approximate* the behavior of real systems.

The forces of interactions between monomers and multimers in hydrophobic association, as discussed earlier, are usually of short range unless coulombic repulsions are involved between similarly charged particles. The coulombic repulsions themselves are strongly dependent on the ionic strength of the medium. Higher ionic strengths, therefore, stabilize aggregates of ionic solutes. At high ionic strengths, salting-out and salting-in of the nonionic moiety may be appreciable also (3).

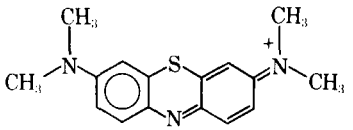
The different patterns of self-association are generated by the different variations in K_q or β_q with q. The patterns of association are seldom known completely. Even when they are known reasonably well from an experimental point of view, it is not often clear what factors control the patterns of association. In general, large multimers, *i.e.*, q values above 10 or 20, form as a result of stepwise association of monomers and not because of the concerted aggregation of 10 or more monomers. Collisional processes involving 10 or more monomers are extremely improbable in dilute solutions. Furthermore, it is unlikely that when a 20-mer, for example, is a major species, 19-mers and 21-mers are completely absent. Polydispersity should thus be the rule rather than the exception. On the other hand, the distribution in sizes can be quite narrow in many cases. A detailed understanding of the system requires not only the understanding of how stepwise self-association proceeds but how it is stopped.

Formation of Unique Oligomers—The formation of monodisperse aggregates, *i.e.*, q-mers where $(q - 1)$-mers or $(q + 1)$-mers are negligible, is more of a possibility for small oligomers than for large multimers. In hydrophobic association, only a few cases are known where the evidence suggests that a unique oligomer may exist over a range of concentrations. Long-chain fatty acid anions have been shown to be dimerized in dilute solutions (13). From equilibrium partition data, the average number of monomers in the average aggregate could be derived without assuming any

model of association. This value was found to be 2 over a wide range of concentrations varying by a factor of about 1000 (13). This means that K_3 and higher K_q values are negligible with respect to K_2. It has been argued that the association is limited to dimers for the ionic surfactants in dilute solution because stepwise trimerization entails greater charge repulsion (16) and less hydrophobic interaction when compared to the dimerization reaction (3).

For sodium cholate in aqueous solutions, light-scattering studies suggested a degree of association of 2, *i.e.*, dimer formation (17). Higher degrees of association are found in the presence of added salt (17–19). This fact, coupled with the uncertainty of the interpretation of light-scattering data in the absence of added salt and other lines of evidence, makes it difficult to assert that only dimers form. The structure of the cholate ion, however, suggests that it is not physically unreasonable to assume that dimerization may be somewhat favored in this system, *i.e.*, K_2 may be significantly higher than K_3 or K_4. Cholic acid, with three hydroxyl groups directed to one side of the molecule, has a hydrophobic back side which should allow dimerization in a back-to-back configuration. This configuration does not allow further association in the same manner because that would entail front-to-front or front-to-back association, involving the side containing the polar groups. Thus, the formation of trimers and higher multimers is likely to be reduced because of the special geometry of the molecule. Multimer formation may require different arrangements of the molecules (12).

Continuous Stepwise Self-Association—In contrast to sodium cholate, rigid planar molecules, aromatic or heteroaromatic, should allow stacking interactions, as described earlier. A model system, the cationic dye methylene blue, was investigated in detail recently (4, 9, 14, 20, 21); Structure I is one of four resonance forms of this dye ion.



I

It was possible, by using two new experimental techniques, to estimate the monomer concentration as a function of the total concentration of methylene blue directly. This information is valuable for deriving association constants and patterns of self-association (9). Many different models of association were examined. Some evidence was found for cooperative interactions in the early stages of association. Thus, in spite of the greater charge repulsion involved in stepwise trimerization, K_3 was found to be greater than K_2. The best fitting model indicated that higher K_q values decrease in a mild sequence, in agreement with the expected build-up of the charge repulsion. However, these variations were not very large.

This study (9) suggested that with stacking-type interactions for many charged systems undergoing self-association, if the charge repulsions in self-association are of minor importance compared to the hydrophobic interactions, and for uncharged systems generally, a particularly simple model of continuous self-association often provides a good description of the associating system. This model uses only one parameter, namely a stepwise association constant which is assumed to be the same for all oligomers and multimers (8). The model thus states that:

$$K_2 = K_3 = K_4 = K_q = K_\infty = K \qquad \text{(Eq. 10)}$$

This one-parameter model, because of its simplicity and widespread applicability, is examined in some detail.

Relationships between various observable quantities can be easily derived. The product of the equilibrium constant K and the equilibrium monomer constant $[b_1]$ is defined as a pure number X:

$$X = K[b_1] \qquad \text{(Eq. 11)}$$

The total molar concentration M is obtained as follows:

$$M = [b_1] + [b_2] + [b_3] + \ldots [b_q] \ldots \qquad \text{(Eq. 12a)}$$

$$M = [b_1]\{1 + K[b_1] + K^2[b_1]^2 + K^3[b_1]^3 \ldots + K^{q-1}[b_1]^{q-1} \ldots\} \qquad \text{(Eq. 12b)}$$

$$M = [b_1](1 + X + X^2 \ldots X^q \ldots) \qquad \text{(Eq. 12c)}$$

$$M = \frac{[b_1]}{1 - X} \text{ for } X < 1 \qquad \text{(Eq. 12d)}$$

In any real system obeying Eqs. 12a–12d, the value of X is less than 1. In a similar manner, the equivalent concentration C is given by:

$$C = \frac{[b_1]}{(1 - X)^2} \qquad \text{(Eq. 13)}$$

so that:

$$\left(\frac{[b_1]}{C}\right)^{1/2} = 1 - K[b_1] \qquad \text{(Eq. 14)}$$

Equation 14 permits the determination of K if $[b_1]$ is known as a function of the total concentration C (8, 9).

The molar osmotic coefficient ϕ can be defined as the ratio of the osmolarity M to the equivalent concentration C; ϕ is related to $[b_1]$ through the equation:

$$\frac{M}{C} = \phi = 1 - K[b_1] \qquad \text{(Eq. 15)}$$

K can be determined from osmotic coefficient data obtained from colligative measurements (20) by using:

$$K = \frac{1 - \phi}{C\phi^2} \qquad \text{(Eq. 16)}$$

The number average degree of association N of all species, including the monomer, namely N_n', is given by:

$$N_n' = \frac{C}{M} = \frac{1}{1 - X} \qquad \text{(Eq. 17)}$$

Similarly:

$$N_w' = \frac{G}{C} = \frac{1 + X}{1 - X} \qquad \text{(Eq. 18)}$$

The number average degree of association of all aggregate species, *i.e.*, excluding the monomer, can be derived from Eqs. 19–21 derived from the model:

$$\frac{M - [b_1]}{[b_1]} = \frac{X}{1 - X} \qquad \text{(Eq. 19)}$$

$$\frac{C - [b_1]}{[b_1]} = \frac{X(2 - X)}{(1 - X)^2} \qquad \text{(Eq. 20)}$$

$$\frac{G - [b_1]}{[b_1]} = \frac{2X}{(1 - X)^3} + \frac{X(2 - X)}{(1 - X)^2} \qquad \text{(Eq. 21)}$$

It follows from these equations that:

$$N_n = \frac{C - [b_1]}{M - [b_1]} = \frac{2 - X}{1 - X} \qquad \text{(Eq. 22)}$$

Similarly:

$$N_w = \frac{G - [b_1]}{C - [b_1]} = \frac{2}{(1 - X)(2 - X)} + 1 \qquad \text{(Eq. 23)}$$

This model of association fits or approximates the self-association behavior of many purine and pyrimidine bases of nucleosides that show stacking interactions (22, 23).

The characteristic features of the association pattern described by the one-parameter model, which leads to the well-known most probable distribution (7), are that N_n and N_w are slowly varying functions of the total concentration (compare, for example, Eqs. 13 and 22), that the aggregates are polydisperse so that N_w/N_n is greater than unity (compare Eqs. 22 and 23), and that the polydispersity index N_w/N_n approaches the value of 2.00 as X and, therefore, C increase, so the molecular weight distribution is

wide. The average degree of association of the aggregate species can be quite low, even when a major fraction of the molecules is associated. Thus, when the value of X is 0.30, $C/[b_1]$ from Eq. 13 is 2.04, so about half of the monomers are aggregated. At this concentration, however, $N_n = 2.43$ (Eq. 22) and $N_w = 2.68$ (Eq. 23). The value of $N_n = 11$ when $X = 0.9$. The corresponding $C/[b_1]$ ratio, however, is 100, indicating only 1% free monomers.

Several other models of continuous stepwise self-association involving changes in K_q values with q were devised and their predictions were examined (8, 9). In these models, a predetermined explicit relationship between the successive equilibrium constants K_q as a function of q above and including K_3 is invoked whereas K_2 is considered to be an independent parameter. The variation in K_q in the model might indicate that successive association constants become more favorable or less favorable, *i.e.*, the model might include an element of positive or negative cooperativity. These models are likely to be more realistic than the simple one-parameter model in many cases (9).

Formation of Large Multimers (Micelles) of Narrow Size Distributions—In contrast to the rigid monomers discussed, flexible chain surfactants with polar head groups produce aggregates of different geometry. The chains can coil around each other and fill up space by themselves. Thus, spheroidal aggregates can form, with the cores consisting of hydrocarbon chains, whereas the polar groups remain exposed to water at the surface. This arrangement partially satisfies both the hydrophobic and hydrophilic tendencies of the amphipathic monomers. The aggregates described are called micelles. Various physical properties of long-chain surfactants can be understood in terms of the formation of micelles. Micelle formation is one of the best known and most spectacular examples of self-association.

A characteristic feature of a typical micelle-forming system is that above the CMC, the first detectable aggregate (micelle) may be already quite large. Thus, only a small fraction of the solute may be aggregated and yet the value of N_w may be in the 30–120 range (24). The pattern of the association is thus quite different from those described earlier. The micelle sizes are *approximately* consistent with those expected for spherical micelles whose radii are equal to the lengths of the fully stretched monomers. The degree of aggregation, N_w, depends upon various factors including the chain length, the ionic strength, and the nature of the counterions for ionic surfactants, the nature of the head groups, and the temperature (24). The degree of aggregation, N_w, is often nearly constant and independent of the concentration above the CMC in many systems. When this is the case, the molecular weight (size) distribution is narrow, *i.e.*, $N_w \approx N_n$ (7), as shown recently by an analysis of the multiple equilibria involved.

The formation of large multimers of narrow molecular weight distributions requires some special types of variations in K_q with q. As discussed previously, when K_q is independent of q, polydisperse aggregates result and high N_n or N_w values can be obtained only when monomer fractions are extremely low. If K_q increases monotonically with q, a narrow molecular weight distribution independent of concentration cannot be obtained. If K_q decreases monotonically with q, the formation of large aggregates in preference to smaller ones is not possible. Therefore, typical micellar systems showing high N_w values close to the CMC require that K_q *increases* with q at the early stages of the growth of micelles, so that larger micelles are more stable than smaller ones. Thus, there must be a positive cooperativity of association in the early stages of growth. On the other hand, the narrow range of q values indicates that the growth must be limited beyond certain values of q, *i.e.*, K_q must eventually decrease with q at higher values of q. Thus, K_q values must show a maximum at some value of q.

When considering the factors responsible for micelle formation, the rationale for this pattern of the variation of K_q with q derives from the opposing effects of hydrophobic interactions and head-group interactions. Hydrophobic interactions are mainly responsible for the formation of micelles. The head groups usually oppose micelle formation. When the head groups are charged, the addition of a monomer to an aggregate involves a charge repulsion (25, 26). When the head groups are nonionic, the repulsive interactions arise from the crowding of the head groups at the micelle surface (3, 7). As small spherical micelles grow, the hydrophobic interactions involved in the addition of successive monomers increase because the total amount of hydrocarbon interface lost on the addition of a monomer is greater as the size of the sphere increases, as simple geometrical considerations will show. This factor, therefore, leads to an increase in K_q with q. As the sphere increases in size, however, the volume of the micelle and, therefore, q increase proportionately to r^3, where r is the radius of the sphere, whereas the surface increases as r^2 increases. Therefore, the density of the head groups also increases with q, as do the charge repulsion factor and the self-interaction of the head groups on crowding. These factors should, therefore, reduce the value of K_q as q increases.

It is generally accepted that when the micelles are very large, *i.e.*, for q values above several hundred, the micelles are rod-like (27, 28), presumably with spherical ends. When small micelles grow to be too large for spheres, there must be a transition in shape from a sphere to a sphero-cylinder-type structure (28). During this transition also, both the hydrophobic interactions and head-group and charge interactions increase. It is possible that the sphere–rod transition is ultimately responsible for limiting the growth of the micelles. Qualitatively, however, it seems that the interplay of geometrical factors, the hydrophobic interactions, and the charge and head-group effects is responsible for an increase in K_q with q at low values of q and a subsequent decrease at some higher values.

When q values are large, only small variations in the average free energy of micelle formation per monomer can produce narrow distributions in molecular weights. To demonstrate this, as is customary (3), the formation constants of the micelles from the monomers, the β_q values, are used. These provide a direct measure of the standard free energy changes associated with micelle formation for nonionic systems corresponding to the process of Scheme III:

$$q\,\Delta G_q = \Delta G_q' = -RT\,\ln \beta_q \qquad \text{(Eq. 24)}$$

Here ΔG_q is the average free energy change per monomer when the q-mer forms, $\Delta G_q'$ is the total change, R is the molar gas constant, and T is the absolute temperature. Since β_q is a product of the K_q values (Eq. 3), $\ln \beta_q/q$, which equals $-\Delta G_q/RT$, will not show a maximum at the same value of q as K_q itself.

Figure 1 illustrates a model calculation in which the following *arbitrary* relationship between β_q and q is assumed:

$$\ln \beta_q = 2(q-1)\ln(q-1) - 0.02(q-1)^2 + 2.7896(q-1) \qquad \text{(Eq. 25)}$$

An equation of this form (25) is chosen because it shows a slight minimum in ΔG_q (Fig. 1). The equivalent concentrations of the q-mers, $q[b_q]$, are then calculated using the appropriate β_q values and Eq. 25 for an assumed monomer concentration corresponding to $\ln[b_1] = -10.000$, ($[b_1] = 4.11 \times 10^{-5}$ mole/liter). A narrow size distribution with a maximum at about $q = 97$ and a half-width of less than 10 is obtained, even though ΔG_q changes by less than 2% over the whole range of 70–120 for q. Thus, only mild minima in the free energy profile (ΔG_q *versus* q curves) are needed to produce narrow size distributions of micelles. Sharper minima produce narrower distributions. This point also illustrates the difficulty of predicting such size distributions exactly, because extremely precise calculations of the ΔG_q values are required which involve the incompletely understood hydrophobic interactions and head-group effects.

Formation of Polydisperse Giant Micelles—In many micellar systems, particularly when the monomer chains are very long, very large rod-like micelles form in dilute solutions (7, 27–30). For these giant micelles, a relatively simple model of self-association can be derived which seems to account for many of their properties (7).

The basic assumption of the model is that for a cylindrical micelle, if the end effects can be neglected and if the growth of the micelle does not change the minor axes, the introduction of a monomer involves the same amount of hydrophobic and head-group interactions irrespective of the length of the cylinder (7). When q is very large, above 200–300 for most surfactants, K_q becomes independent of q. The real system, of course, must also have aggregates for which q values are low. For the giant micelles ($q > \sim 200$) to appear at low solute concentrations, K_q must increase with q over certain ranges of low q values as with ordinary small

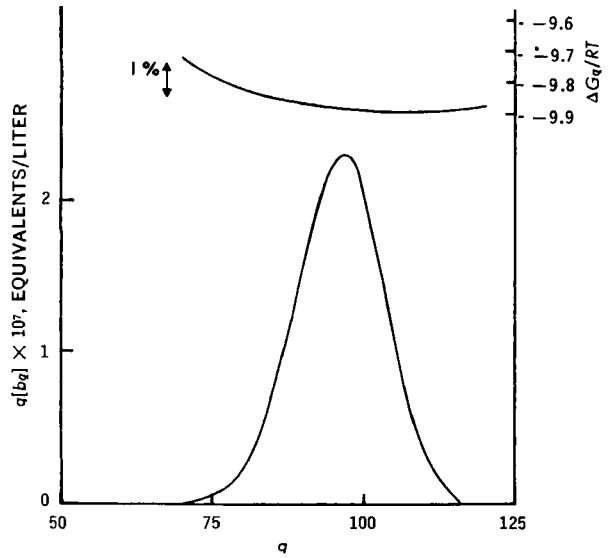


Figure 1—*Variation in the concentration of monomers existing as micelles,* $q[b_q]$, *as a function of the number of monomers in the micelle,* q, *for an assumed free energy profile,* ΔG_q versus q, *upper curve (see text).*

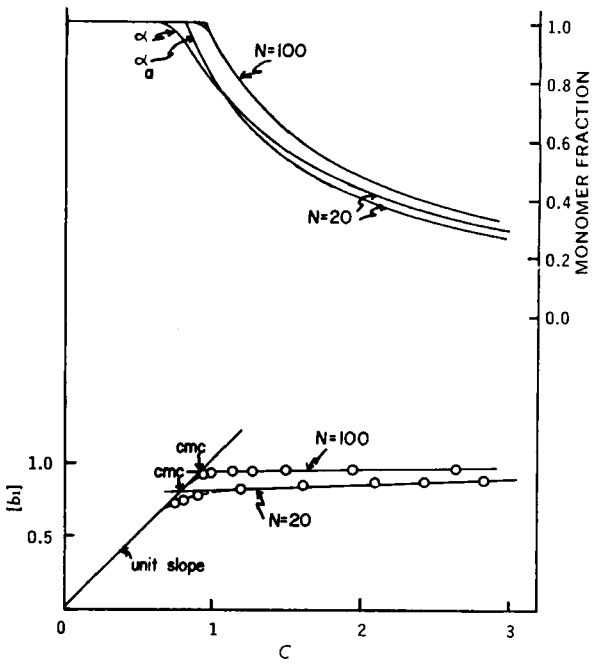


Figure 2—*Variation in the monomer concentration* $[b_1]$ *as a function of the total solute concentration* C *for monodisperse micelles containing 20 and 100 monomers/micelle.* α *is the true monomer fraction, and* α_n *is the fraction calculated using the micellar hypothesis (see text).*

micelles. This gradual increase is replaced by a step function in an approximate manner by invoking the following model of association:

$$K_2' < K \qquad K_3 = K_4 = K_q = K_\infty = K \qquad \text{(Eq. 26)}$$

Here, instead of a gradually increasing function of K_q with q at low q values, followed by an uncertain transition region around $q \approx 50$–200 corresponding to the sphere to rod transition which, in turn, is followed by a constant value of K_q at very high q values ($q > \sim 200$), a *hypothetical* K_2' is chosen which is probably a good deal lower than the real K_2, followed by all subsequent K_q values equal to each other. The replacement of the gradually increasing function by a step function has some important consequences. One is that the model is appropriate only when moderate q values do not make a major contribution to the micelle size distribution, *i.e.*, average N_w values that are large ($N_w > \sim 300$).

Given the limitations of the model, the predictions are easy to examine. The model differs from the previous one-parameter model for continuous self-association by having an extra parameter K_2'. On summing the series for M, C, and G in terms of $X = K[b_1]$, the following relationships are obtained (7):

$$\frac{M - [b_1]}{[b_1]} = \frac{K_2'}{K}\left(\frac{X}{1 - X}\right) \qquad \text{(Eq. 27)}$$

$$\frac{C - [b_1]}{[b_1]} = \frac{K_2'}{K}\left(\frac{X(2 - X)}{(1 - X)^2}\right) \qquad \text{(Eq. 28)}$$

$$\frac{G - [b_1]}{[b_1]} = \frac{K_2'}{K}\left(\frac{2X}{(1 - X)^3} + \frac{X(2 - X)}{(1 - X)^2}\right) \qquad \text{(Eq. 29)}$$

These relationships are identical with Eqs. 19–21 when $K_2' = K$.

The predictions of the model are in some ways similar to the previous one-parameter model. In particular, N_w and N_n values are given by Eqs. 22 and 23. When N_w is large, $N_w \approx 2N_n$ and the size distribution is wide. When X is close to unity, $C >> [b_1]$ and N_w is proportional to $\sqrt{C}$, a prediction that has been confirmed (7).

The difference between the two-parameter model leading to the formation of a series of giant aggregates and the one-parameter model leading to the formation of a continuous series of aggregates is a matter of degree and derives from the ratio K_2'/K. To make this comparison, let us take a numerical example in which $K = 10^5$ (liters/mole) and $X = 0.999$, so that $[b_1] = 9.99 \times 10^{-6}$ mole/liter and N_w is then about 2000 (Eq. 23), a value large enough so that the two-parameter model applies. From Eq. 20, the value of C needed to produce this degree of aggregation for the one-parameter model is 10 equivalents/liter, a very high concentration indeed. From Eq. 28, however, if $K_2'/K = 10^{-4}$, a value of 2000 for N_w is obtained at a concentration of only about 10^{-3} equivalent/liter. The values of K, K_2', and $[b_1]$ used for illustrative purposes are similar to some values pertaining to a real nonionic micelle-forming surfactant (7). Some other consequences of this model have been examined, including the prediction of a sharp CMC when K_2'/K is 10^{-3} or lower. The initial cooperativity of micellar association can thus be represented in these cases by the choice of a low ratio of K_2'/K (7).

Micellar Hypothesis and Significance of CMC—In contrast to the systems showing small oligomers only, either unique ones or those having a distribution over a narrow range, and systems showing continuous self-association in which all stepwise association constants are roughly of the same magnitude, typical micelle-forming systems exhibiting high degrees of association ($N_w > 20$) show no evidence of micelle formation until the CMC characterizing the system is reached. This is the result of the cooperativity of association in the initial growth of micelles. The significance of the CMC is of central importance in the discussion of micelle formation and the application of the micellar hypothesis to self-associating systems.

It has been shown that typical micellar systems involving small micelles (*i.e.*, the ones that are roughly consistent with spherical micelles of radius r where r is the length of the stretched-out monomer) in many cases have narrow distributions in molecular weight (7). Such a micellar system, showing an N_w value of 50, has negligible amounts of 10-mers, 20-mers, 30-mers, 70-mers, and 80-mers. Narrow distributions of this sort can be *approximated* by monodispersity, so that the monomer–micelle equilibrium for nonionic systems can be represented to a good approximation by Scheme IV:

$$Nb_1 \rightleftharpoons M$$

Scheme IV

where the micelle M contains N monomers. It is well known that when N is large ($N > 20$), Scheme IV predicts the existence of a critical concentration range somewhat below which micelles are absent and somewhat above which essentially all additional solute forms micelles (31).

Figure 2 illustrates this fact and the effect of N on the sharpness of the transition at the CMC. The equilibrium constant, K_m,

governing Scheme IV is given by:

$$K_m = \frac{[M]}{[b_1]^N} \qquad \text{(Eq. 30)}$$

for ideal uncharged systems. By assuming that $K_m = 1$, the monomer and micellar concentrations have been calculated for $N = 20$ and $N = 100$. Any other choice of the K_m value merely shifts the concentration scales and not the shapes of the curves. For $N = 100$, as C increases from zero, the value of $[b_1]$ increases with unit slope, *i.e.*, there is no detectable self-association, until C approaches 0.9. When $C = 0.929$, $[b_1]$ equals 0.915, *i.e.*, only about 1.5% of the solute is micellized. On increasing C further, however, the value of $[b_1]$ increases very slowly and the equivalent concentration of micelles, $C - [b_1]$, increases roughly as C itself. Thus, over a narrow range of concentrations of the order of a few percent only, as C changes from about 0.92 to 0.97, the distribution of the solute species changes abruptly. Any measured physical property of the system that reflects the concentration (activity) of the monomer, the equivalent concentration of the micelle, or any unequally weighted combination of the two will register or display an abrupt change of slope over this concentration range, *i.e.*, show a "kink" in the curve, when the property is plotted as a function of the concentration. Even when the value of N is as low as 20, corresponding to very small micelles, the change in composition of the solution around the critical region is still fairly sharp. Thus, when $C = 0.75$, the fraction of the solute micellized is only 4%, and the value of $[b_1]$ is 0.72. Beyond the transition region, as C increases, $[b_1]$ increases faster than in the case of $N = 100$ but is still only 0.88 when $C = 2.43$. If now N is allowed to increase continuously from a minimum value of 20, the rate of increase of $[b_1]$ with C will be reduced further. Thus, polydispersity of the giant micelles does not invalidate the argument about the sharpness of the changes but only strengthens it. For the giant micelles, N_w is large and increases with C.

The key requirement for the existence of a narrow concentration range across which the fraction of added solute that forms micelles changes from nearly zero to nearly unity is the initial cooperativity of association that makes large or intermediate sized micelles so much more stable with respect to smaller ones that the latter do not appear to any significant extent. However, no careful study has demonstrated a "break" in any curve, *i.e.*, a discontinuity, at the CMC. All properties of a solution in the CMC region vary in a continuous manner.

Although the change in the nature of the solute species distribution occurs over a narrow range of concentrations rather than at one concentration, only very precise techniques allow the transition range itself to be studied (3, 16). The usual practice is to extend observed segments of the curves of a chosen property plotted against a suitable concentration scale below the transition region and above the transition region linearly to meet at a point which is then defined as the CMC. Figure 2 gives examples of how CMC values may be determined from $[b_1]$ *versus* C curves. The CMC thus represents some value of the concentration within the transition range. Many methods are available for determining the CMC (6). Different experimental methods may give somewhat different estimates of the CMC, depending upon how monomers and micelles are reflected in the measured property (6). For typical micellar systems, however, they agree within a few percent. The CMC is an approximate measure of the concentration at which micelles become detectable.

The micellar hypothesis, therefore, represented by Scheme IV and its proper modification for charged systems (3), leads to the following approximations which are usually valid to within small degrees of error. These approximations are frequently used when micellar systems are encountered in many practical situations and are closely related to the micellar hypothesis itself.

1. In the dilute concentration range, up to very near the CMC (*i.e.*, below roughly 0.9 × CMC), micelle formation can be completely neglected. Unless there is some premicellar formation of small aggregates, the evidence for which is uncertain (3), one can assume that the monomer concentration equals the total concentration and the monomer fraction is unity.

2. Above the CMC, the monomer concentration for the ideal uncharged system and the monomer activity in general increase very slowly with total concentration so that the CMC is a reasonable measure of the activity of the monomers above the CMC up to fairly high concentrations. The monomer fraction above the CMC is given by CMC/C.

3. The micellar concentration is negligible until shortly before the CMC. Above the CMC, the equivalent concentration of micelles can be approximately equated to C − CMC.

4. The CMC can be used to calculate thermodynamic quantities related to micelle formation, such as the free energy of micelle formation, and the degree of dissociation for charged systems using various approaches (3).

5. Light-scattering and various data dealing with properties of micelles can be interpreted assuming monodisperse micelles. This approximation has been used for very large (giant) micelles also. In view of the polydispersity inferred for such systems (7), this particular approximation appears to be unsuitable for such systems.

These approximations are clearly of importance in all situations where monomer concentrations (activity) or micellar concentrations are involved. These include equilibrium or transport processes of the monomer–micelle system itself; adsorption, binding, and interfacial activity of the monomer; and processes involving micelles such as solubilization. In quantitative treatments of physiological activities of associating systems such as bile salts or hydrophobic drugs for which CMC values are reported, the ancillary approximations are often made. This need arises because the detailed information that would make the approximations unnecessary is not usually available. Indeed, properties of the micelles themselves above the CMC, such as molecular weights (24), charge (32), electrophoretic mobility and electrokinetic potentials (33), hydration (34), and solubilizing powers (35), are usually investigated by making the approximation that C − CMC represents the equivalent concentration of micelles.

These approximations are really not approximations but are exact consequences of the two-phase model of micelle formation (3) according to which the CMC corresponds to a phase separation point. Evidence from various sources (3) indicates that the two-phase model is only approximately valid for micellar systems. A theoretical framework has recently become available for examining the approximations for ideal systems, *i.e.*, systems in which intermicellar interactions can be neglected (7). For example, Eq. 31, representing the change in the concentration of the monomers above the CMC, is applicable to all such systems of any degree of polydispersity:

$$d \ln [b_1] = \frac{C - [b_1]}{G - [b_1]} d \ln (C - [b_1]) = \frac{d \ln [b_1]\left(\frac{C}{[b_1]} - 1\right)}{N_w(C)} \qquad \text{(Eq. 31)}$$

where $N_w(C)$ represents the fact that N_w can be a function of (C) if the system is polydisperse. Equation 31 indicates that the change in $[b_1]$ with C is reduced as N_w becomes higher, and as $C/[b_1]$ increases.

The approximations discussed are closely connected to the estimated value of the CMC. The meaning and significance of the CMC in many other connections were examined in detail as a part of a critical discussion of the methods of determining CMC values and of a critical compilation of some 4600 CMC values of 720 different compounds (6). The important point to be made here is that when a concentration value for a system is reported as a CMC, these approximately valid conclusions about the system usually become part of the description of the system. It is, therefore, of some general interest to examine how the micellar hypothesis can be misused for many systems and what some consequences of this misuse are for self-associating systems to which the micellar hypothesis does not apply to a suitable degree of approximation.

Apparent CMC Values that May Be Misleading—The genesis of a narrow critical concentration range in micellar systems from the initial cooperativity of association of monomers to micelles has been discussed. Whereas the CMC is usually determined from a change in the concentration dependence of some solution property, not all real or apparent changes in such dependence correspond to CMC's. Indeed, many solution properties of systems showing self-association, micellar or nonmicellar, show curvatures in graphical plots against concentration; many of these curved plots may appear to show kinks resembling critical con-

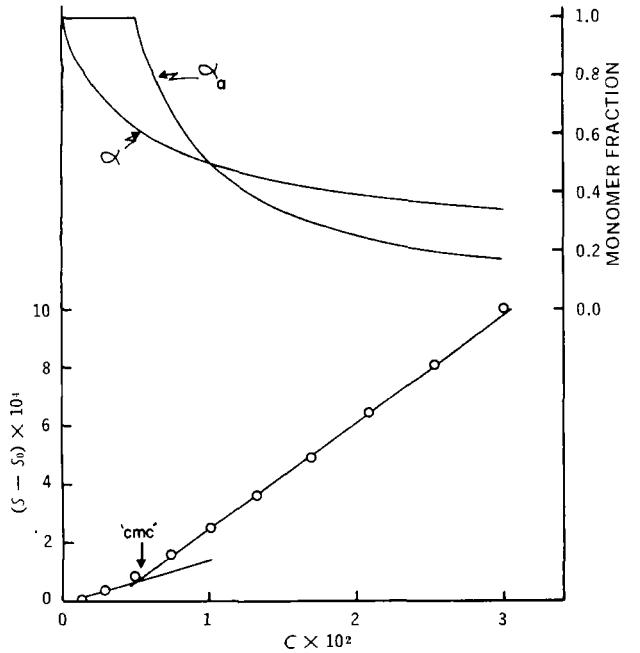


Figure 3—*Simulated solubilization curve for a system showing only dimerization. α is the true monomer fraction, and α_a is the fraction calculated using the micellar hypothesis (see text).*

centration ranges, particularly if a limited amount of experimental data is available. If all of these kinks are interpreted as CMC values and the micellar hypothesis is applied along with the approximations associated with the pre-CMC region, the post-CMC region, and the CMC itself for micellar systems, rather substantially erroneous conclusions about the self-associating system may be drawn. Several examples of how such apparent CMC values may arise in nonmicellar self-associating systems in practical situations are given here. These do not include such relatively trivial examples as the reaching of a solubility limit or the leveling of the degree of hydrolysis of a hydrolyzing system.

Figure 3 shows an example of a model system in which it is assumed that a nonionic hydrophobic solute undergoes only dimerization ($2b_1 \rightleftharpoons b_2$) with $K_2 = 10^2$ liters/mole. It is now assumed that a substance P, with a low solubility in water, is solubilized according to the association reaction shown in Scheme V:

$$b_1 + P \rightleftharpoons b_1P$$

Scheme V

At saturation, if the concentration of P in solution is S and the solubility in water is S_0, then $S - S_0$ is the amount solubilized, which equals $K_sS_0[b_2]$ where K_s is a constant. The ordinate of Fig. 3 plots this excess solubility, $S - S_0$, assuming $K_sS_0 = 10^{-2}$. The diagram thus simulates a solubilization experiment. The concentration of b_2P was neglected in calculating C. The data can now be represented by two lines, showing an apparent CMC value at about 5×10^{-3} equivalent/liter. Any other choice of K_sS_0 produces the same curve with a change in the ordinate scale. A different K_2 value does not change the shape of the curve and, therefore, the existence of the apparent CMC. Clearly, however, this apparent CMC cannot have the same significance as the CMC of a micelle-forming substance and the generalizations that hold *at least approximately* for micelle-forming systems are completely inapplicable here.

A second example is shown in Fig. 4. Here the model system is assumed to show continuous self-association, with all stepwise association constants having the identical value (Eq. 10). By assuming this value of K to be 10^2 liters/mole (any other value merely changes the scales of the ordinate and the abscissa), the calculated molar concentration M (Eq. 12) is plotted against the equivalent concentration C (Eq. 13). This is equivalent to plotting osmotic pressure data or osmolarity data from vapor pressure lowering (36). The system again shows an apparent CMC, which depends somewhat upon which segments of the curve are used to derive it. This apparent CMC again cannot have the same kind of significance as the CMC of a typical micellar system because of the assumption of the model chosen.

A third example is shown in Fig. 5. Here the function G (from Eq. 21) is plotted against C for the continuous self-association model in which all the stepwise association constants are the same, these values being 10^2 liters/mole. Any other value is represented by the same curve by a change in scale of the ordinate and the abscissa. The definition of G shows that this plot is equivalent to plotting turbidity data from light scattering, assuming that the optical and refractive characteristics of all aggregates and the monomer are the same. The curve again can be represented approximately by two straight lines to show an apparent CMC value. From the slope of the plot above the apparent CMC, a value of 7 is derived for N_w.

Figures 4 and 5 illustrate that systems showing typical stacking-type interactions lend themselves fairly easily to interpretation in terms of micelle formation. This interpretation and the use of the attendant generalizations about micellar systems can be substantially erroneous. Many drug systems with rigid structures are likely to show patterns of self-association of the kind used for Figs. 4 and 5 or some mild variants of it such as the ones examined for methylene blue self-association (9, 14). Considerable care should be exercised in the application of the micellar hypothesis to such systems. Close examinations of all such systems in terms of various models of multiple self-association equilibria are desirable before the micellar hypothesis is forced on the systems. In general, if the degree of association close to the CMC is low, less than 20, and if there is evidence of association well below the apparent CMC, care should be taken.

Monomer Fraction and Problems of Availability—An obvious result of self-association is to decrease the value of the monomer fraction, α, from unity. This is also one of the most important effects of self-association. Hydrophobic self-association equilibria are usually rapidly attained, so that for most processes any change in the total concentration C results in a rapid adjustment of the value of α to the equilibrium value. For many processes, the effective concentration is the monomer concentration, αC. Indeed, for ideal self-associating systems that are uncharged, α is the activity coefficient of the system (23) and $\alpha C = [b_1]$ measures the activity of the system. In any process where this activity is important, such as binding to a substrate or adsorption to an interface, α and its dependence on C are thus of crucial importance in all quantitative calculations. For self-associating drug systems in particular, one can expect that α will be an important parameter in the control and understanding of binding to proteins or re-

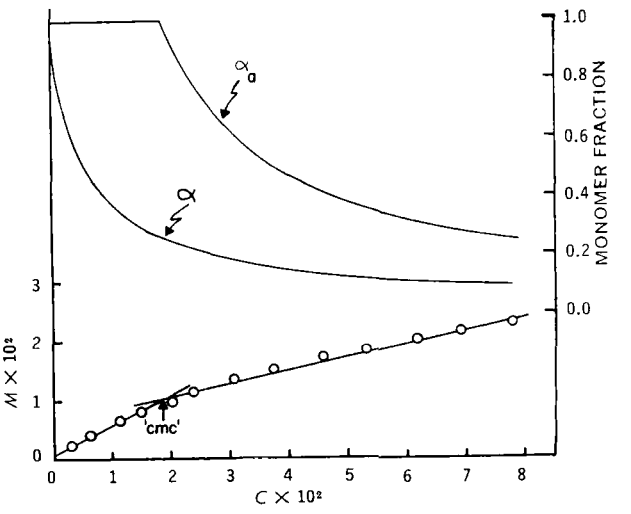


Figure 4—*Calculated osmolarity, M, for a system showing continuous self-association (Eq. 10) with all stepwise association constants having the same value, 10^2 liters/mole, plotted against the total solute concentration C. α is the true monomer fraction, and α_a is the fraction calculated using the micellar hypothesis (see text).*

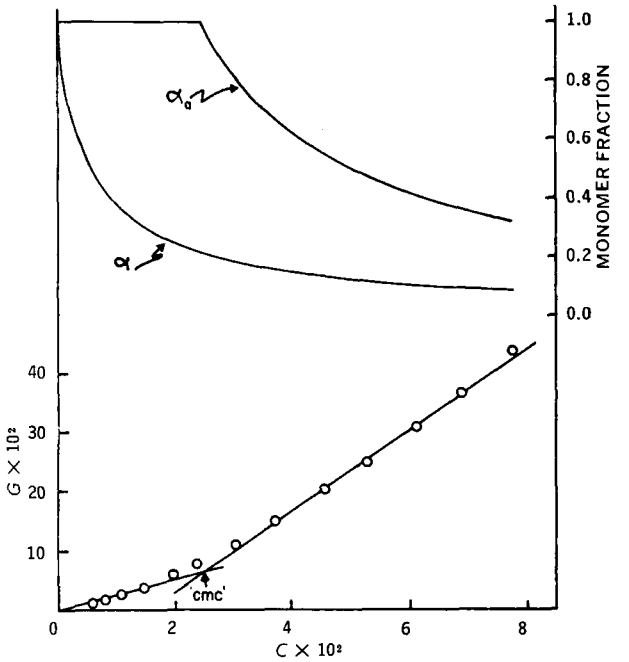


Figure 5—*Simulated turbidity–concentration plot for a system showing continuous self-association (Eq. 10) with all stepwise association constants having the same value, 10^2 liters/mole. Ordinate* G *(Eq. 6) is proportional to the solute turbidity obtained in light-scattering experiments under idealized conditions (see text), α is the true monomer fraction, and α_a is the fraction calculated using the micellar hypothesis (see text).*

ceptor sites, binding to membranes prior to transport, adsorption to interfaces, and rates of solution and diffusional transport; α is a measure of the "availability" of the drug or any other hydrophobic solute in many situations.

In view of the importance of this parameter and its close interrelationship with the micellar model or any other model of self-association, it is of interest to examine to what extent the approximations of the micellar hypothesis apply to other systems. These approximations are that α is unity up to the CMC and has the value of CMC/C above the CMC. The true values calculated for various model systems are compared here to this approximate value, designated as α_a.

Figure 2 shows how α compares with α_a for monodisperse micellar systems. Above the CMC, α decreases rapidly. As mentioned previously, for micellar systems α or CMC/C in some experimental situations may be as low as 10^{-4}–10^{-5}. In the limited range covered in Fig. 2, the agreement between α_a and α is better when N = 100 than when N = 20, as is to be expected. The important point to note is that even when N = 20, *i.e.*, when the micelle is rather small, α and α_a do not differ by more than 0.07 in the region covered. In dilute solutions in particular, somewhat below the CMC, α_a, with a value of unity, provides a very good approximation.

In contrast to these micellar systems, systems for which other patterns of self-association hold show considerable differences between the true α and the α_a calculated using the micellar hypothesis. For the dimerization case (Fig. 3), for example, α has a value of 0.62 at the apparent CMC and the monomer fraction is appreciably less than below the CMC unity. The deviations below the apparent CMC are even more marked in Figs. 4 and 5. Considerably below the CMC, in dilute solutions, when the micellar hypothesis would indicate negligible association, the value of α is well below unity. In the dilute solutions, therefore, which are often the most important in physiological systems, the true α may be considerably lower than that predicted by the micellar hypothesis, even though the latter hypothesis assumes large aggregates. Thus, in any situation where the availability or activity of the monomer is important, assuming α to be unity leads to errors. The misuse of the micellar hypothesis for such systems also

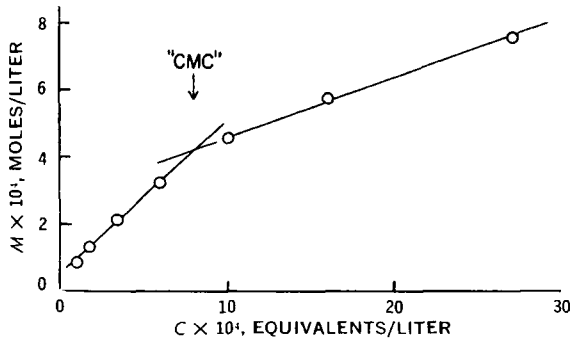


Figure 6—*Apparent CMC for methylene blue in aqueous solutions at 25°. (The data for* M *and* C *are from Ref. 14.)*

leads to serious errors in any quantitative calculation involving the CMC, such as the free energy of micelle formation, because the monomer fraction at the apparent CMC may be as low as 0.25 instead of being close to unity.

Lack of Significance of a CMC Value for Methylene Blue—Figure 6 shows an example of a real system for which an apparent CMC value can be obtained but for which the application of the micellar hypothesis leads to erroneous results. The self-association of methylene blue has been studied in detail and analyzed in terms of multiple stepwise association equilibria (4, 9, 14). From the experimentally determined variation in the equilibrium monomer concentration [b_1] with the total concentration C, the values of M, the molarity sums of all the methylene blue species (Eq. 4), were calculated as a function of C by graphical integration to examine the variation of N_n with C in the original work (14). The data were obtained at an approximately constant ionic strength so that Schemes I–III and Eqs. 1–8 apply because the charge effects can be assumed to be constant (9, 14). Changes in M for this system, therefore, represent changes in the osmolarity contribution of the aggregating species of methylene blue.

Figure 6 shows a plot of M *versus* C. An apparent CMC value can be obtained at a concentration of 8×10^{-4} mole/liter of methylene blue. This value, if interpreted as a true CMC, gives a very misleading description of the system. The aggregation of methylene blue is extensive at much lower concentrations, and the experimental value of the monomer fraction at this apparent CMC is only about 0.24 (14).

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Micellar Properties of Drugs: Determination of Molecular Weight, Size, and Molecular Interactions of Drug Micelles Using the Analytical Ultracentrifuge

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Abstract □ This article illustrates the usefulness of the analytical ultracentrifuge in investigating micellar properties of drugs. Also discussed are some examples of micelle forms of drugs and the working hypothesis that the same binding forces that hold together the aggregate found at high concentrations may be responsible for the binding of monomer (at physiological concentrations) to biological structures.

Keyphrases □ Micellar properties of drugs—symposium □ Ultracentrifugation—determination of molecular weight, size, and molecular interactions of drug micelles □ Sedimentation velocity and coefficient—determination of micellar properties of drugs □ Molecular weight of drug micelles—determination from sedimentation data □ Density gradient centrifugation—determination of micellar properties of drugs

The analytical ultracentrifuge is frequently used by physical, biological, and polymer chemists to investigate molecular weights, subunit structure, and purity of molecules and small particles from diverse sources. The investigation of drug micelles is facilitated by the described techniques since ultracentrifugation affords a direct view of some properties of molecules and small particles. For example, a recent controversy (1, 2) concerning whether or not theophylline aggregated in aqueous solutions was resolved when molecular weight studies performed with an analytical ultracentrifuge (3) showed that the self-association of theophylline is concentration dependent; that is, monomers are found at low concentration and multimers (dimers, trimers, and tetramers) are found at higher concentrations. This paper discusses some ultracentrifugal techniques, some physical properties of several drugs that form micelles, and the question of whether aggregation of these agents is a manifestation of a physical property required for activity *in vivo*.

All analytical ultracentrifuges operate on the same principle, *i.e.*, they cause molecules to move toward the bottom (outer edge) of the ultracentrifuge cell under the influence of the artificial gravitational field created by a rotor spinning from 800 to 60,000 rpm.

Results obtained with different instruments are similar (4), as expected from calculations based on thermodynamics and mass transfer phenomena.

Separations of the sample within the ultracentrifuge cell may be achieved by: *(a)* differences in size and shape, as in the sedimentation velocity methods; *(b)* differences in mass, as in approach-to-sedimentation equilibrium and equilibrium procedures; or *(c)* differences in density, as in the density gradient (isopycnic or isodensity method).

SEDIMENTATION VELOCITY

Figure 1*a* shows a cross section of the filled ultracentrifuge cell. Before the ultracentrifuge run begins, solute is distributed equally throughout the solution. As the cell is accelerated, solute molecules move toward the bottom of the cell. Complete redistribution by diffusion is prevented by maintaining a sufficiently high speed to cause net sedimentation of solute. This speed depends on the size, shape, and partial specific volume (which is related to recip-