Determining the Critical Micelle Concentration of Surfactants Using a Binary Mixing System

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A simple method is described for measuring the critical micelle concentration (CMC) of surfactants using a computer-driven mixing system and a variable wavelength spectrophotometer. Analyses using this method were typically done in 15 min. Detergents that were analyzed included sodium dodecyl sulfate (SDS), sodium cholate, Lubrol-PX and Triton X-100. Lubrol PX showed two phase transitions; other detergents appeared to have only one near their CMC. For sodium cholate, the CMC was more difficult to analyze than the others. This may have been due to its low aggregation number (≤ 4). Since this method successfully identified the CMC of these four detergents, it is likely that most, if not all, surfactants can be analyzed in the same manner.

Previously, methods for measuring the CMC of a surfactant have been tedious and time consuming. Some popular methods include mixing a series of solutions, each with a slightly different concentration of the surfactant, and measuring light scattering (1,2), surface tension (3), or flourescence of an added probe (4-7) on each solution. Additional techniques have also been described, all of which involve the analysis of multiple samples to determine each CMC (8-12). A method is described in which two solutions are combined using a carefully controlled mixing system. These solutions were mixed continuously such that gradient time correlated with the concentration of the surfactant. A variable wavelength detector was used to identify the CMC, or other phase transitions.

The detergents that were chosen for this study are typically used for the solubilization of membrane proteins. Solubilization, and subsequent chromatography of membrane proteins, often depends upon the ability of the detergent to form micelles (13-15). This paper represents an attempt at simplifying the determination of the CMC for studies involving membrane protein solubilization. The method that is reported here may also be applicable to other detergent systems.

MATERIALS AND METHODS

A gradient module equipped with a variable wavelength UV monitor (Model 1305A), a pulse damper and a Model 402 controller were obtained from Bio-Rad. A 10-μl low volume static mixer was used (Lee, Westbrook, CT) as well as a backpressure regulator (Rainin, Emeryville, CA). All analyses were done at room temperature. A 15-min linear gradient was used in all cases and the first solution always contained no surfactant. The second solution contained the same solvent as in A, but with detergent at a concentration higher than its CMC.

The variable wavelength UV monitor was adjusted between analyses to find the optimal wavelength for observing each phase transition. SDS and Triton X-100 were from Bio-Rad. Lubrol-PX and Sodium cholate were from Sigma (St. Louis, MO). All other reagents were of the highest purity available. Water was deionized and filtered using a Milli Q System (Millipore).

RESULTS

The four detergents analyzed in this study included SDS, Lubrol-PX, Triton X-100, and sodium cholate. The CMCs of these detergents ranged from 0.01 mM for Lubrol-PX to approximately 10 mM for sodium cholate (Table 1). Some of the values in the literature of various detergents differ slightly, although reasons for these discrepancies are not clear. Table 1 also summarizes

TABLE 1

Determination of the CMC Using Gradient Mixing

Detergent	Properties of detergents		
	CMC (mM)		Aggregation no.
	Previous work	Present study	
Lubrol PX	0.1[a]	0.04, 0.10	106[b]
Triton X-100	0.24[c], 0.3[a]	0.24	140[c]
SDS	8.2[c]	7.1	62[c]
Sodium cholate	8[a], 13-15 [c]	3.4	2-4[c]

For published data see references [a](19); [b](22); [c](18), and [d](4).

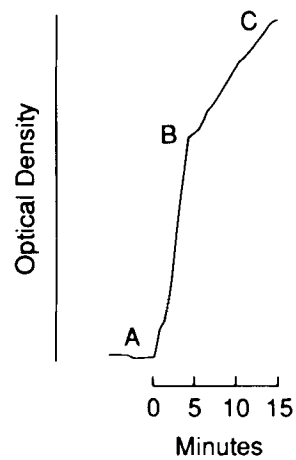

FIG. 1. A typical CMC analysis. In this case, SDS was used. The starting solution was 0.1 M NaCl, and the final solution contained 4 mM SDS in 0.1 M NaCl. Absorbance was measured at 280 nm with 0.02 absorbance units full scale (AUFS). A 15-min linear gradient was used between the two solutions. The region before A depicts the delay time associated with the volume of plumbing between the mixer inlets and the detector. The region between A and B represents the phase in which only detergent monomers are present in the aqueous system. The CMC is indicated at B as a sharp transition between monomer and micelle phases. Above the CMC (between B and C) monomers and micelles coexist. A 10-μl mixer was used to obtain this scan.

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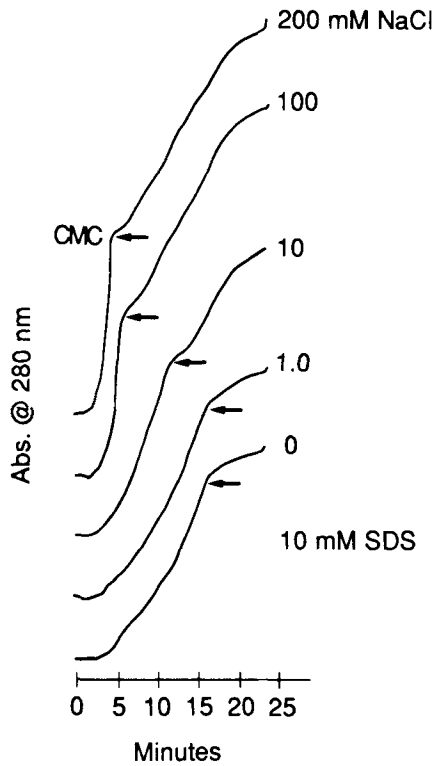

FIG. 2. The effect of NaCl on the CMC of SDS. Here, a 2.5 mL mixer was used. Conditions were the same as in Fig. 1 except the salt concentration remained constant for each of the scans. For each experiment, salt levels were adjusted according to the figure, but did not vary during the scan. Only the SDS concentration was varied from 0 to 10 mM SDS for each case. Detection was at 280 nm with 0.02 AUFS.

TABLE 2

Comparison of Mixing Volumes

[NaCl] (mM)	[SDS] at CMC (mM) 2.5-ml mixer	[SDS] at CMC (mM) 10-μl mixer	[SDS] at CMC (mM) Published
0	7.50	7.10	8.2[a], 8.13[b]
1	7.67	6.67	-
10	4.84	-	-
100	2.37	0.89	1.33[b]
200	2.00	1.00	0.92[b]
500	-	0.30	0.52[a]

Conditions were as described in Figure 2 except similar samples were evaluated using a 10-μl mixer. Published values were from either [a](18) or [b](17).

the results of the present study. The conditions that were used to obtain the values found in Table 1 are described in Figures 2-5.

Figure 1 shows a typical scan of the CMC for SDS using 280 nm for detection and a 15-min linear gradient from 0 to twice the CMC in 0.1 M NaCl. From these data, three regions can be identified. The first is the monomer region at which micelles are absent. The next is the phase transition at the CMC. The third region is that above the CMC, where micelles and monomers coexist. In this example, the observed transition is clear and appears as a change in slope between the two phase regions. The transition was followed in differing concentrations of sodium chloride. Similar effects by sodium chloride have been reported previously (16). Several scans were run to study how salt affects the CMC of SDS (Fig. 2). In each scan, both the starting and ending solutions contained the same salt concentration. The final solution also contained SDS at a concentration of 10 mM. In these examples, a 2.5-ml mixing volume was used and thus the transition was not as clear as when the 10-μl mixer was used (Table 2). In these cases, the CMC was not easily determined when it occurred either very early or very late in the scan. For example, when 200 mM NaCl was used, the concentrations at which micelles appeared to form was ca. 2.2 mM SDS. This is more than twice the published value of 0.92 mM (17). The same method was used with a smaller (10 μl) mixing volume and a value of 1.00 mM was obtained (Table 2).

Additional experiments were carried out to observe the CMC transition in Triton X-100. One difference between Triton X-100 and SDS is its strong absorbance at 280 nm. This makes the analysis of Triton X-100's CMC difficult at short wavelengths. However, when longer wavelengths were used, the CMC became clearly visible. The published value for the CMC of Triton X-100 in distilled water is 0.24 (18) or 0.3 mM (19). From Figure 3, the calculated CMC was 0.24 mM.

The CMC of Lubrol PX was studied as well. From Table 1 the CMC of Lubrol PX was close to 0.1 mM. The pattern shown in Figure 4 suggests that Lubrol undergoes more than one phase transition near the CMC. More than one phase transition near the CMC has been observed before. Two phase transitions that are related to forming and elongating micelles have been reported for at least one other detergent (20).

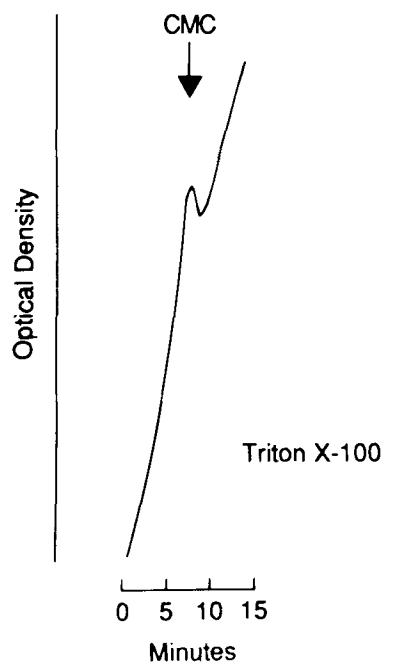

Fig. 3. CMC analysis of Triton X-100. Conditions were the same as described in Fig. 1, except both solutions contained distilled water and the ending solution also contained 0.48 mM Triton X-100 in place of SDS. Detection was by absorbance at 320 nm and 0.02 AUFS.

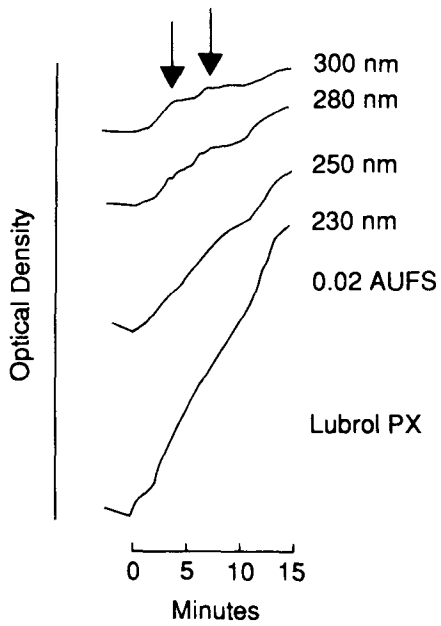

FIG. 4. CMC analysis of Lubrol PX. Conditions were the same as described in Fig. 3 except the final solution contained 0.21 mM Lubrol PX in place of Triton X-100. 0.02 AUFS and 230, 250, 280, and 300 nm were used for detection as indicated. The clearest transitions were observed at 280 and 300 nm.

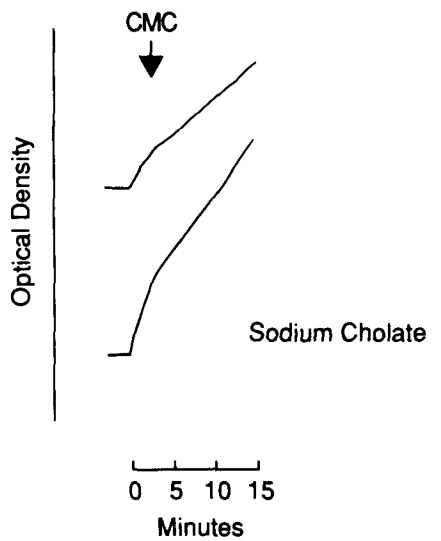

FIG. 5. CMC analysis of sodium cholate. Conditions were the same as described in Fig. 3, except 16.8 mM sodium cholate was used in the final solution in place of Triton X-100. The lower scan was measured at 0.08 AUFS, while the upper scan was at 0.16 AUFS. Both measurements were taken at 400 nm.

The CMC of sodium cholate was difficult to study because of its small aggregation number (Table 1). If only a few molecules form a micelle, the difference in absorbance between micelles and monomers may be so small that the transition cannot be observed. From Figure 5 it is clear that the CMC of sodium cholate is only slightly visible by this technique. The results shown in Table 1 further indicate that the observed transition may not be the CMC. The wavelength and sensitivity of the detector were adjusted for the highest sensitivity for observing the phase transition of sodium cholate (Fig. 5).

DISCUSSION

Methods for determining the CMC of surfactants have been actively studied. However, the purity of detergents has improved, and the number of surfactants has increased. Estimating the CMC by classical methods (e.g., surface tension, light scattering) is accurate, but these methods are tedious and time consuming. Studying several variables that may affect the CMC (e.g., buffer, salt and pH composition of the aqueous phase) represents a formidable task. In some instances, due to significant improvements or deviations in purity, the CMC for a surfactant should be re-evaluated. As a result, a number of new methods have been developed for determining the CMC.

Fluorescent probes have become popular tools for measuring CMCs (4-7). These methods rely upon probe sensitivity to environmental differences. For example, the fluorescence intensity may increase as the probe partitions from the aqueous to the micelle phase. Probes may also influence the micelle structure and/or CMC. Therefore, each analysis may require finding an optimal probe concentration for each surfactant. In addition, CMC analyses with fluorescent probes may require careful monitoring of both pH and buffer composition.

Dye probe methods are sensitive and less time consuming than surface tension or light scattering methods. Yet, regardless of the technique that is used, each CMC determination has in the past required numerous measurements. Gilpin (9) has described an alternative to dye probes using solute-solvent partitioning diferences on reversed phase HPLC supports. Gilpin used detergents in the solvent phase during reversed phase separations of selected pairs of solutes. The resolution of the solutes was maximum near the CMC of the detergents. Unfortunately, these columns deteriorated rapidly and the system was plagued by excessive foaming. These problems were costly and reduced the accuracy of their CMC determinations.

There is no chromophore in SDS which absorbs in the UV region, although Figures 1 and 2 indicate absorbance at 280 nm. However, the amount of apparent absorbance was low (0.02 AUFS, Fig. 1), and could have been caused by refractive index effects. Similar effects have been observed using this absorbance detector in comparable situations (21).

Absorption is roughly proportional to concentration for any compound in a single phase. The absorption of Triton X-100 is much greater than the apparent absorbance observed for SDS. The CMC of Triton X-100 is also lower than that of SDS (Table 1), and the wavelength that was used for Triton X-100 was 320 nm rather than 280 nm (SDS). Therefore, it is not surprising that the combined effect of these phenomena allows the absorbance near the CMC of Triton X-100 (Fig. 3) and that of SDS (Fig. 1) to be similar.

During these experiments it became clear that the scan pattern differed slightly as the number of analyses

progressed. These differences did not appear to influence the transition point, and may have been due to the process of coating the mixing system with a layer of surfactant.

For this reason, all the analyses were carried out after several repeat scans of each sample. Since this was a computer driven system, repeat scans could be done automatically and rapidly.

Some differences were seen when dynamic mixing volumes were altered (Table 2), which in some cases influenced the accuracy of the determination of the CMC. For example, Table 2 indicates that the CMC for SDS increased slightly with increased salt levels from 0 to 1 mM NaCl when the large volume (2.5 ml) mixer was used. However, with the smaller mixer, the CMC decreased under the same circumstances. This difference is due to the inaccuracies associated with the larger mixing volume. Other gradient mixing systems have been tested either with much larger mixing volumes or with other mixing configurations. In these cases, the CMC was sometimes difficult to detect. This was true even for SDS, which gave a clear transition under the conditions reported in this study.

Many CMC analyses have been avoided due to the complex, tedious and time-consuming methods that have been available. In this report, we describe a method that is fast and easy to use. Detergents with either high (≥ 140) or low (≥ 2) aggregation numbers, or CMC ranging from 0.1 to 10 mM, have been determined.

The success of this technique depends somewhat upon the kinetics of the micelle formation. The time allowed for mixing before detection between the detergent rich and detergent poor solutions depends on both the flow rate and the volume of solution between the mixer inlets and the detector. Very slow kinetics could be investigated simply by adjusting the flow rate of the mixing system. From the close correlations between CMC values shown in Table 1, it appears that the rate of micelle formation is rapid enough for these four detergents (Table 1) that under these conditions, decreasing the flow rate may have little effect.

We recommend the use of the mixing system described here, using a low dead volume mixer ($\leq 10\mu l$) for precise CMC measurements. This method could also utilize other types of detectors (infrared, conductivity, fluorescence, etc.) with low flow cell volumes. The UV monitor was selected in this study for convenience.

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[Received July 13, 1988; accepted December 26, 1988]