Fluorimetric Determination of Critical Micelle Concentration
Avoiding Interference from Detergent Charge

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Upon mixing detergent solutions with the neutral fluorescent molecule 1,6-diphenyl-1,3,5-
hexatriene a large increase in fluorescence is observed if detergent exceeds the critical micelle
concentration. This property has been used to determine the critical micelle concentration of
anionic, uncharged, zwitterionic, and cationic detergents. Regardless of detergent charge, the
critical micelle concentrations obtained agree with the values obtained by other methods. This
fluorescence assay is both sensitive and rapid, and should provide a simple and general method
for determination of critical micelle concentration of any detergent.

KEY WORDS: detergents; critical micelle concentration; diphenylhexatriene; fluorescence; mi-
celles.

Detergents are extremely important in studies of biological membranes due to their ability to solubilize membrane proteins (1,2). It is known that above a particular concentration, called the critical micelle concentration (CMC),2 detergent molecules self-associa-
tie to form thermodynamically stable aggregates called micelles (1–3). The CMC of detergents has been studied by many physicochemical techniques. These include measurement of light scattering (4–6), surface ten-
sion (7–11), hydrodynamic properties (12) and changes in absorbance or fluorescence upon
dye solubilization (13–20). Methods based upon changes in fluorescence intensity of a dye upon incorporation into micelles are among the most sensitive and convenient assays for CMC (15–20). However, charged fluo-
rescent probes have been used in previous methods, and it has been demonstrated that

such assays usually do not work if probe and detergent have opposite charges (18–20). In
this report we show that this problem can be avoided by use of the neutral fluorescent probe
DPH. We have previously shown that DPH can be used to assay phospholipid vesicles and
noted a response of DPH fluorescence to detergent CMC (21). In this paper, we report the
determination of CMC of detergents with various electric charge using DPH. We show that
determination of CMC with DPH is simple and reliable.

MATERIALS AND METHODS

DPH was purchased from Aldrich. SDS (electrophoresis grade) was purchased from Bio-Rad. CHAPS and octyl glucoside were obtained from Calbiochem. CTAB was purchased from Baker. In some experiments it was twice recrystallized from ethanol before use. Cholic acid and Triton X-100 were pur-

CHASED from Sigma. Cholic acid was recrystallized from aqueous acetone. Solutions of sodium cholate were prepared by slow titration of cholic acid suspended in water with sodium hydride. The purity of the bile salt and bile salt derivatives was checked by TLC on silica

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2 Abbreviations used: CHAPS, 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; CMC, critical micelle concentration; CTAB, cetyltrimethylammonium bromide; DPH, 1,6-diphenyl-1,3,5-hexatriene; SDS, sodium dodecyl sulfate; THF, tetrahydrofuran; TLC, thin-
layer chromatography.
gel plates in chloroform:acetone:acetic acid 70:10:10 (v/v); ethyl acetate:acetic acid:water 85:10:5 (v/v); and methanol:conc. ammonia, 95:5 (v/v). A few percents of deoxycholic acid was found in the cholic acid. Surface tension was measured with a Du Nouy tensiometer. Fluorescence measurements were made with a Perkin-Elmer MPF-44A fluorescence spectrophotometer operating in ratio mode. The excitation wavelength was 358 nm and the emission wavelength was 430 nm. The excitation and emission slits were set at bandwidths of 1 and 20 nm, respectively. In all experiments, 1-cm path length quartz cuvettes were used. The usual protocol for CMC determination was as follows: 1 µl of 10 mM DPH dissolved in THF was added to various amounts of detergent dissolved in a total volume of 2 ml of aqueous solution. Tubes were incubated for 30 min in the dark at room temperature before measurement of fluorescence. Most of the experiments were done with duplicate sets of samples and average fluorescence is shown in the figures. To reverse any photoisomerization of DPH, samples were kept dark in the fluorimeter for 30 s before the excitation shutter was opened and fluorescence measured.

RESULTS

The principle of the DPH assay of CMC is that DPH fluorescence will be greatly enhanced above the CMC due to its incorporation into the hydrophobic interior of the micelle. In order to determine the best conditions for this assay we examined the dependence of fluorescence intensity upon detergent concentration, DPH concentration and time of incubation of the fluorescent probe with detergent. Figure 1 shows that as detergent concentration is increased, fluorescence is weak at the lowest detergent concentrations, then rises rapidly and finally levels off. This is most easily interpreted as follows: the rapid rise in fluorescence occurs at and above the CMC of the detergent. As the amount of detergent is increased, the number of micelles increase and the amount of bound DPH, and therefore fluorescence, increases. At very high detergent concentrations, all DPH is bound and so fluorescence levels off. The CMC is given by the intersection of the straight line through the fluorescence at low detergent concentrations with a straight line through the fluorescence values in the region of rapid intensity increase.

When this experiment is repeated at different DPH concentrations, no effect of DPH concentration upon CMC is observed (Fig. 1). However, the region of fluorescence linearity above the CMC is extended when using higher DPH concentrations, presumably due to the increased range of detergent concentration for which DPH is in excess. The maximum fluorescence obtained does not increase proportionally with DPH concentration. This is due to the inner filtering effect of DPH absorbance arising from the high extinction coefficient of this fluorescent probe.

The incubation time necessary for DPH to equilibrate between the aqueous solution and micelles is shown in Fig. 2. In these experiments DPH was incubated with several detergents of various charge, at detergent concentrations above their CMC (see below). DPH fluorescence levels off between 5 and 10
min and we used a 30-min incubation for our later studies only for convenience.

Figure 3 shows the dependence of DPH fluorescence upon detergent concentration for a series of detergents, and Table 1 lists the CMC determined from those plots, along with literature values determined by a variety of methods. The DPH assay gives a CMC value within 10% of that determined by other methods. This variation is expected and previous studies have noted that the exact value of CMC obtained varies from method to method (1-3,11).

The literature value we have used for the CMC of CTAB (0.92 mM) disagrees with the old literature value of 0.4-0.5 mM referred to in Venditti et al. (20,22,23), and our assay does not give a CMC value in agreement with their value determined by rhodamine 6G fluorescence. We examined this question further, first by twice recrystallizing our CTAB before use. This had no effect on CMC. Then we measured the CMC of CTAB by measuring the dependence of surface tension on detergent concentration. We found a value of 0.8 mM in agreement with our fluorescent results. Finally, we tried to use the rhodamine 6G method of Venditti et al. (20) with our CTAB. We found it difficult to obtain reproducible results, with some drift in apparent CMC during the 5-day incubation as used by these investigators (20). Therefore, we believe strongly that the value we obtained with DPH is correct in this case.

Salt does not interfere with the DPH assay. As expected the CMC values of the anionic detergents SDS and cholate decreased in the presence of salt (Table 1), but the CMC of uncharged Triton X-100 and zwitterionic CHAPS (data not shown) did not. The agreement of our CMC values with literature values shows that the small amount of THF (0.05%, v/v) added with DPH does not affect CMC determination. Nevertheless, we examined the effect of THF concentration on CMC. Table 2 shows that up to 1-2% (v/v) THF has little or no effect on CMC for several detergents.

**DISCUSSION**

In this report we have demonstrated the determination of detergent CMC using DPH. As might be expected, this uncharged fluorescent probe can be used with detergents of any charge. Using DPH one need not worry that differently charged impurities in a detergent will affect the measurement of CMC. For
## Table 1

<table>
<thead>
<tr>
<th>Detergent</th>
<th>Conditions</th>
<th>CMC by present method (%)</th>
<th>CMC by present method (mM)</th>
<th>Literature CMC (mM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Neutral</td>
<td>Triton X-100</td>
<td>Water</td>
<td>0.019</td>
<td>0.3</td>
<td>0.24-0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mM NaCl</td>
<td>0.018</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Octylglucoside</td>
<td>Water</td>
<td>0.72</td>
<td>24.5</td>
<td>23.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mM NaCl</td>
<td>0.69</td>
<td>23.4</td>
<td></td>
</tr>
<tr>
<td>(B) Zwitterionic</td>
<td>CHAPS</td>
<td>Water</td>
<td>0.48</td>
<td>7.4</td>
<td>8-10</td>
</tr>
<tr>
<td>(C) Anionic</td>
<td>SDS</td>
<td>Water</td>
<td>0.23</td>
<td>8.0</td>
<td>8.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mM NaCl</td>
<td>0.1</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mM NaCl</td>
<td>0.04</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sodium cholate</td>
<td>Water&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63</td>
<td>14.6</td>
<td>13-15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 mM NaCl&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.53</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 mM NaCl&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42</td>
<td>9.75</td>
<td></td>
</tr>
<tr>
<td>(D) Cationic</td>
<td>CTAB</td>
<td>Water</td>
<td>0.032</td>
<td>0.88</td>
<td>0.92</td>
</tr>
</tbody>
</table>

<sup>a</sup> At 0.1 M NaCl, 0.05 M Tris, pH 7.4.
<sup>b</sup> At pH 8.

Example, we have found in commercial preparations of the zwitterionic bile salt derivative CHAPS very small amounts of impurities with the mobility on TLC of charged bile salts. Additionally, this method should be useful when examining the pH dependence of the behavior of a detergent with an ionizable group.

One should note that some simple precautions can improve the reliability of this assay. First, it is important to take a sufficient number of measurements above the CMC so as not to accidentally mistake points of the excess detergent plateau for those in the straight line region used for CMC determination. Also, points within 5 and 10% of CMC should be ignored because the fluorescence intensity can have a curved dependence upon detergent concentration in this region. Finally, the excitation slit should be kept as narrow as practical to minimize DPH photoisomerization. We found in the worst cases DPH fluorescence could drop 5-10% after seconds of exposure to the excitation beam in our instrument even when the excitation slit was narrow. Nev-
Nevertheless, due to the tremendous increase in DPH fluorescence above the CMC this is not a significant source of error, and short periods of sample exposure to room light are not a problem. Furthermore, photoisomerization reverses in the dark in a minute or two, so any "overexposed" sample can be remeasured.

Upon preparing this manuscript we found old reports describing the use of the uncharged hydrocarbon methylcholanthrene to measure CMC (26-28). It also should be useful for any detergent regardless of charge. However, methylcholanthrene is extremely carcinogenic (29), so DPH is a preferable choice for a fluorescent probe.

Overall we have found the DPH assay of CMC to be reliable and convenient. We believe it provides a simple and general fluorescence assay for detergent CMC.

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REFERENCES