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Near-Infrared Hydrophobic Probes as Molecular Light Switch for CMC Determination of Triton X-100 Solution

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The fluorescence behavior of two near-infrared (NIR) chromophores with linear alkyl chains of different lengths, 2-[4'chloro-7'(3"ethyl-2"benzothiazolinylidene)-3',5'-(1'",3'"-propanediyl)-1',3',5'-heptantriene-1'-yl]-3-ethylbenzothiazolium iodide (Probe I) and 2-[4'chloro-7'(3"hexadecyl-2"benzothiazolinylidene)-3',5'-(1'",3'"-propanediyl)-1',3',5'-heptantriene-1'-yl]-3-ethylbenzothiazolium iodide (Probe II), in aqueous solution containing different concentrations of surfactants was studied. The fluorescence of the probe with a short chain (probe I) was completely quenched in water and aqueous solution containing a low concentration (below the critical micelle concentration, CMC) of surfactant Triton X-100. However, the fluorescence reappeared and reached maximum rapidly once the concentration of the surfactant approached the CMC. The probe with a long chain (probe II) displayed a similar fluorescence behavior but more dramatically fluorescent recovery in Triton X-100 system, which gave a direct indication for the micelle forming process and provided a simple method for the determination of the critical micelle concentration of the surfactant. The CMC values determined by this method were in good agreement with those obtained by other techniques. The fluorescence behavior of the two probes in other surfactant systems was also investigated.

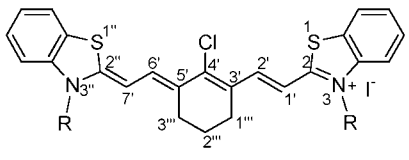
Keywords Triton X-100, critical micelle concentration (CMC), near-infrared (NIR) dye, fluorimetry

Introduction

Surfactant solutions with a concentration above their critical micelle concentration (CMC) are frequently utilized in fluorescence spectrometry to solubilize the dye and enhance its photophysical properties.[1-3] Micelle systems can also be used to study kinetics and mechanism of reactions in some cases. Therefore, the determination of CMC is important for scientific and practical understanding of the behavior of surfactant.[4]

A great deal of research has been devoted to the determination of CMC.[5-7] Fluorimetric approach is one of the most convenient and fast methods. It is desirable to find a suitable probe which is sensitive to microenvironmental changes and tends to change its fluorescent properties dramatically when it is transferred into the hydrophobic core of the micelle at CMC. The platinum complex $[Pt(terpy)(OH)]^+$ recently reported is a good example. The "light switch" function of the molecule makes it applicable to the CMC determination of SDS.[8]

The near infrared (NIR) spectrum has been widely proven to be very useful in the study of biological and environmental samples because the absorption of samples themselves in this spectral region is largely reduced and, thus, does not interfere with the absorption or fluorescence of an NIR chromophore.[9,10] Indeed, the analytical applications of NIR chromophores have been dramatically increased over the last few years since they have following advantages. First, they have a strong absorption band (with a molar absorptivity of 200,000) in the NIR spectral region (600—1000 nm) and reasonably good fluorescence quantum yield, which facilitates low detection limit. Second, good selectivity can be achieved due to the interference reduced in this region. When semiconductor lasers are used as the excitation source, the advantage becomes more obvious.[11]



R = Et (Probe I)
R = hexadecyl (Probe II)

Figure 1 Chemical structure of the NIR probes used in this study.

A near-infrared (NIR) dye, 2-[4'chloro-7'(3"ethyl-2"

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benzothiazolinylidene)-3',5'-(1''',3'''-propanediyl)-1',3', 5'-heptantriene-1'-yl]-3-ethylbenzothiazolium iodide (probe I in Figure 1), has already been presented by Patonay' group.[12] As a hydrophobic probe, it has been used for the determination of the hydrophobicity of albumins and other proteins,[12] for moderately hydrophobic aqueous solutions,[13] and also for the CMC measurement of surfactants.[14] The reported method for the determination of CMC is a spectrophotometry by means of measuring the intensity ratio of two absorption peaks in NIR region.

We have recently synthesized a new near-infrared dye with *n*-hexadecyl chain, 2-[4'chloro-7'(3"hexadecyl-2"benzothiazolinylidene)-3',5'-(1''',3'''-propanediyl)-1',3', 5'-heptantriene-1'-yl]-3-ethylbenzothiazolium iodide (probe II in Figure 2), and studied its fluorescence property. It was found that probe II could be used as an indicator for the CMC determination of nonionic surfactant solutions. This probe functions as a true molecular "light switch" (yes or no response), with an enhancement factor of emission intensity greater than 10^3. For comparison, probe I was also synthesized and investigated. As a result, similar fluorescence behavior was observed.

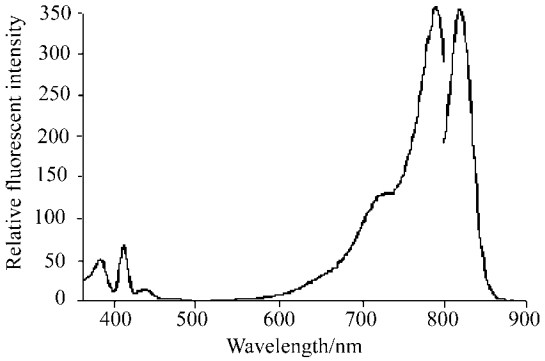

Figure 2 The fluorescence excitation (left) and emission (right) spectra of probe II in ethanol solution.

We herein report the microenvironmental dependence of the fluorescence characteristics of the above two NIR hydrophobic probes in surfactant solutions, and develop a new fluorimetric method by using the two probes for monitoring the micelle forming process and estimating the CMC values of some surfactants. Due to the utility of a "light switch" molecule, the measurement of CMC by fluorimetric method appears to be more convenient, direct, and reliable compared with the spectrophotometry and other techniques.

Experimental

Materials and instruments

A Perkin-Elmer LS-55 luminescence spectrometer equipped with a standard quartz cell with 10 mm path length was adopted to record the fluorescence spectra and relative fluorescence intensity. A Genesys-5 spectrophotometer was used to measure the absorption spectra. An Orion Model 818 standard pH-meter was employed to make the pH measurements.

Triton X-100, Brij-35, and cetyltrimethylammonium bromide (CTAB) were obtained from the Aldrich Chemical Co. Tween-20 and sodium dodecyl sulfate (SDS) were purchased from Shanghai Chemical Reagent Co. (Shanghai, China). Probe I was synthesized according to the Ref. 14 and the synthesis of probe II referred to the same reference. The purity of probe II was confirmed by NMR and MS analysis:

^{1}H NMR ($CDCl_3$) δ: 0.88 (t, J=7.0 Hz, 6H), 1.2—1.4 (m, 56H), 1.90—2.02 (m, 2H), 2.61—2.77 (m, 4H), 4.39 (t, J=6.5 Hz, 4H), 6.32 (d, J=13.5 Hz, 2H), 7.32 (t, J=7.5 Hz, 2H), 7.39 (d, J=8.0 Hz, 2H), 7.47 (t, J=8.0 Hz, 2H), 7.75 (d, J=8.0 Hz, 2H), 7.88 (d, J=13.0 Hz, 2H). MS *m/z*: 884.8 (M^++1−I^-), 883.8 (M^+−I^-), 659.8 (M^++1−$C_{16}H_{33}I$).

Probe I or II was dissolved in 1 mL of chloroform and diluted with ethanol to 10 mL to obtain a 1×10^{-3} mol/L stock solution. The stock solutions were stored below −15 ℃ to avoid possible decomposition of the dye. After allowing it to stand for two weeks, the concentration of the stock solution remained unchanged. However, the concentration of a diluted solution (1×10^{-5} mol/L) decreased after standing for one day. Therefore, a small volume (50 μL) of the stock solution was directly used as working solutions.

Method

Transfer appropriate volumes of standard surfactant solution and 0.1 mL of pH 5.0 H_3CCOOH-CH_3COONa buffer solution into a 10-mL volumetric tube and dilute the mixture with water to the mark. Then, 40 μL of the dye stock solution was added and mixed thoroughly. Fluorescence measurements were made at 823 nm with excitation at 784 nm after the mixture stood at room temperature for 5 min.

Results and discussion

Spectral characteristics

As shown in Figure 2, the excitation and emission wavelengths of probe II in ethanol solution were at 789 nm and 819 nm, respectively. The excitation and emission spectra of probe I were similar to those of probe II, with the excitation and emission wavelengths at 784 nm and 820 nm, respectively. The fluorescence intensity of both probe solutions decreased as the water was added. The fluorescence was completely quenched when the content of water was above 60%, suggesting that both probes be sensitive to the environmental change. The change in absorption spectra with the content of water was also evident. As the content of water increased, the peak absorbance of monomer at about 800 nm for both probes decreased and finally disappeared. Meanwhile, two new weak peaks at short wavelengths (about 578 nm and 695 nm) emerged (spectra not shown).

Representative absorption spectra of probe II in water and in the presence of the surfactant Triton X-100 are shown in Figure 3. It can be seen that as the amount of Triton X-100 is increased, the absorbance of dye dimer formed at about 680 nm decreases and the absorbance of dye monomer formed at 804 nm increases. Once the concentration of Triton X-100 is above the CMC, the absorption peak of the monomer (A_{monom}) becomes higher than that of the dimer (A_{dim}). With a small amount of Triton X-100 added, a sharp change of the $A_{dim}/(A_{dim}+A_{monom})$ ratio can be observed at the CMC. This change in the absorption spectrum of the probe can be used for discerning the transfer of the probe from a hydrophilic to a hydrophobic environment. Also, it allows the determination of micelle formation referring to the literature.[14] The experimental results show that a reliable CMC value can be gotten with probe II through measuring the change of the $A_{dim}/(A_{dim}+A_{monom})$ ratio with surfactant concentrations.

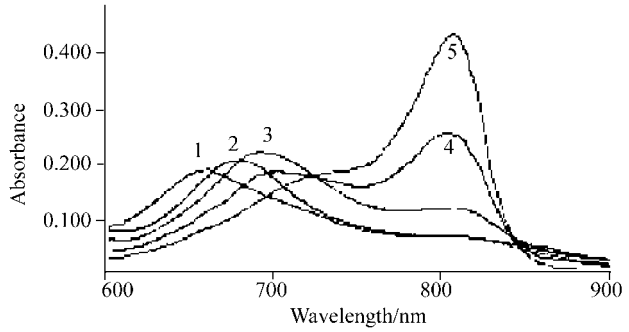

Figure 3 Representative absorption spectra of probe II in water (1) and Triton X-100 solution. The concentration of Triton X-100 (2—5): 5.0×10^{-5}; 1.5×10^{-4}; 2.0×10^{-4}; 8.0×10^{-4} mol/L.

The fluorescence of probe II is completely quenched in water and aqueous solution containing a low concentration (below the critical micelle concentration, CMC) of surfactant Triton X-100. However, the fluorescence reappears and reaches maximum rapidly once the concentration of the surfactant approaches the CMC (Figure 4). The surfactant concentration at the starting point of fluorescence recovery, which corresponds to the concentration value of 1.7×10^{-4} mol/L (Figure 5), is in good agreement with the CMC value of Triton X-100 reported in literature.[15] The results show that probe II is another ideal molecular "light switch" for the determination of CMC in Triton X-100 system. Obviously, estimating CMC value by means of fluorimetric method is simpler than the above spectrophotometry since the latter involves the measurements of A_{dim} and A_{monom} and the conversion of the $A_{dim}/(A_{dim}+A_{monom})$ ratio. In general, judging whether the micelle is formed (above the CMC) or not (below the CMC) by fluorescence method can be easily carried out by observing whether the surfactant solution is luminescent or not. The fluorescence method is, therefore, very rapid and convenient for the application studies of a micelle system, such as fluorescence titration of CMC.

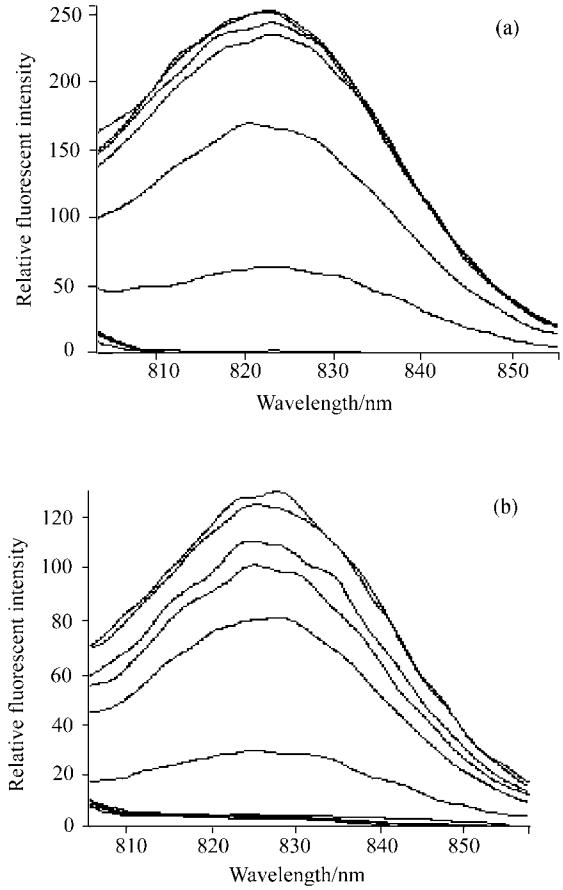

Figure 4 Representative fluorescence spectra of probe II (a) and probe I (b) in water and Triton X-100 solutions with an excitation wavelength at 784 nm. The concentration of Triton X-100 (from bottom to top): 0.00; 0.050; 0.080; 0.10; 0.15; 0.20; 0.30; 0.40; 0.60; 0.80; 1.0; 5.0 mmol/L.

For comparison, the fluorescence behaviors of probe I in water and Triton X-100 solutions were also investigated (Figure 4). The fluorescence titration curve of probe I titrated with Triton X-100 is similar to that of probe II (Figure 5a). As can be seen from Figure 5 that there is still a very weak fluorescence appearing before the CMC for probe I system, in addition, the enhancement in fluorescence intensity at the turning point of fluorescence titration is about a half of that for probe II system. Nevertheless, a sharp change of fluorescence intensity at CMC can still be observed for probe I system. The CMC value obtained by fluorescence method with probe I system is 2.6×10^{-4} mol/L, a little higher than the CMC value given by probe II system, which suggests that the transfer of probe I from a hydrophilic to a hydrophobic environment be more difficult than that of probe II. This result can be ascribed to the fact that probe II is more hydrophobic than the probe I.

Influence of experimental conditions on the CMC determination of Triton X-100

For the determination of CMC by fluorescence method, it is desirable to have a greater enhancement in fluorescence intensity at the turning point of fluores-

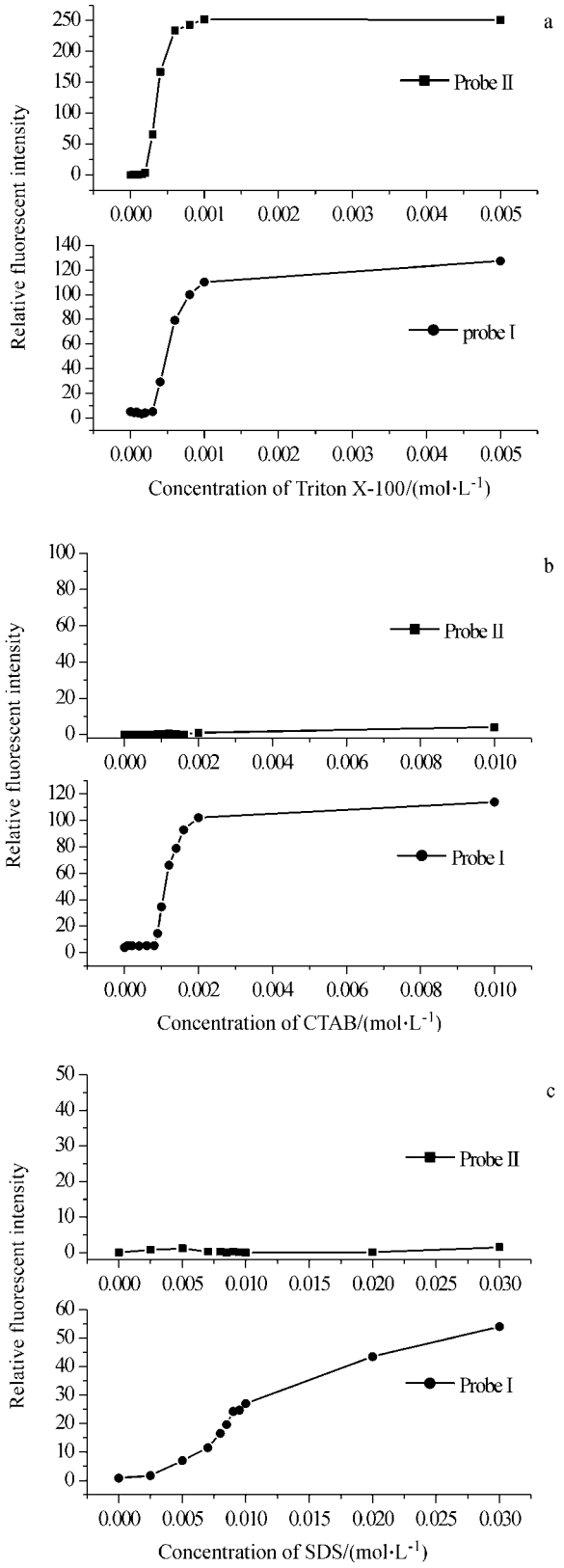

Figure 5 Fluorescence intensities of probe II and probe I as a function of surfactant concentration. Emission was measured at 823 nm (excited at 784 nm) with an excitation and emission bandpass of 10 nm.

cence titration. Our experiments show that a proper increase of the probe concentration is beneficial to the enhancement in fluorescence intensity at the turning point of fluorescence titration. But, when the concentration of the probe is too high, the probe can be hardly dissolved in the solution containing a small amount of surfactant (below the CMC) due to the hydrophobicity of the probe. In terms of the spectrophotometric method, the poor solubility of the probe would result in the poor stability and the reproducibility of the $A_{dim}/A_{dim}+A_{monom}$ ratio measurement, which would make it difficult to estimate the value of CMC. However, the fluorimetric method of CMC is not affected by the poor solubility of the probe since the fluorescence of probe is almost completely quenched below the CMC. Therefore, just a small amount of organic solvent was adopted to help the probe to be dispersed in aqueous solution without using sonic treatment in the whole process. In spite of these, the concentration of probe should be not kept too high. In fact, the fluorescence intensity did not increase remarkably with the increase of the probe concentration when the concentration of probe exceeded 10^{-5} mol•L^{-1}. If the probe concentration is too high, it will enlarge the concentration range of detergent needed from no luminescence at CMC to the fluorescence maximum after CMC, which would not lead to a clear fluorescence abrupt change. Taking the sensitivity of the fluorescence change into account, a final probe concentration of 4.0×10^{-6} mol•L^{-1} was chosen.

The solubility of these probes in ethanol or methanol is not well, especially for probe II. Therefore, a small amount of chloroform was firstly used to dissolve the probes, and then ethanol was added to dilute the solutions. The addition of ethanol can help the probe to be dispersed in the solution and, therefore, avoids a sonic treatment, which significantly simplifies the analytical procedure. Excessive sonic treatment would lead to the decomposition of the probes according to our observation. From the result examined in this work, small amounts of organic solvent ($<0.5\%$) do not introduce significant error to the determination of the CMC. Therefore, adding 40 μL of the dye stock solution to 10 mL of surfactant solution was recommended and adopted throughout.

The effect of pH and ionic strength on the determination of CMC was also investigated. The results show that the fluorescence intensity above the CMC basically keeps maximal and constant in the pH range of 1—8 at ionic strength below 0.2 mmol/L. In basic medium of pH 9, the fluorescence intensity above the CMC decreased a little, meanwhile, the absorbance of the monomer was also reduced, but the estimation of CMC value using the proposed fluorimetry was not seriously affected.

Evaluation of some CMC data

Similar “light switch” character for probe II was also observed in other nonionic surfactants such as Tween-20, Brij-35 systems, and satisfactory results for CMC determination in nonionic surfactant systems were exhibited (shown in Table 1). Its probe function for CMC, however, disappeared in ionic surfactant systems such

as cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulfate (SDS). In these ionic surfactant systems, no luminescence of probe II was observed even if the concentration of surfactants was well above the CMC.

Table 1 CMC values of some surfactants measured by NIR probes I and II

Surfactant	CMC [a]/ (mmol•L^{-1}) by probe I	CMC [a]/ (mmol•L^{-1}) by probe II	CMC [b]/ (mmol•L^{-1})
Triton X-100	0.26	0.17	0.17—0.30 (15,4)
Tween-20	0.05	0.01	0.05 (22)
Brij-35	0.10	0.05	0.048—0.17 (23)
CTAB	0.90		0.92 (24)
SDS			8.2 (24,25)

[a] The concentration corresponding to the starting point of sharp change in fluorescence intensity was denoted as the CMC in this method. [b] CMC values provided by the literature.

Probe I displayed a suitable sensitivity to microenvironmental change in nonionic surfactant systems (such as Tween-20 and Brij-35) and cationic surfactant system of CTAB in our experiments. It is desirable to find a suitable probe that can be used for all types of surfactants. Unfortunately, probe I also could not serve as an indicator for CMC measurement of SDS system since the fluorescence intensity increased gradually with the increase of SDS concentration without a sharp change in fluorescence intensity at the CMC.

The important feature of cyanine dyes is their ability of aggregation.[16] Cyanines are known as unique compounds to form aggregates of various composition and structures in solutions, films and layers. In general, addition of surfactants at concentrations above CMC leads to deaggregation of dyes.[17,18] Apart from preventing dye aggregation, the micellar aggregates also strongly enhance the fluorescence emission of cyanine dyes due to the inhibition of internal conversion or photo-isomerization.[3,19-21] Hydrophobic probe I has hydrophobic and polar groups and hence is expected to be solubilized in the palisade layer of the surfactant aggregate. A common behavior found with such a type of molecules is that the polar groups tend to be directed toward the surface region, while the rest trends to protrude into the micellar interior. Internal motion of cyanine molecule will be controlled by the local microviscosity, and the microviscosity is relatively higher in surfactant assembly than in solution. Thus, possible radiationless deactivation is retarded and the fluorescence quantum yield is increased. Therefore, as shown in Figure 5, probe I displays strong fluorescence emission in Triton X-100, CTAB and SDS micelles.

For probe II, a strong fluorescence emission was also observed in Triton X-100 solutions with concentrations above CMC, which suggests that probe II be restricted to the similar local environment as probe I even though probe II has two longer hydrophobic chains. In the case of CTAB or SDS, it is predicted from the structure of the dye that strong interaction of probe II with these surfactants exists. Besides, the micellar sizes of CTAB and SDS are smaller than those of Triton X-100. Thus, even if the two long hydrophobic chains of probe II protrude into the micellar interior, the main part of probe II molecule including its emission group might still stay in the interface of micelle-solution phase or even in solution phase. In this case, the encountered local environment of probe II in CTAB or SDS micelle may more resemble to that in solution without surfactants. Therefore, the fluorescence of probe II is still completely quenched in CTAB and SDS micelles (Figures 5b and 5c).

Table 1 gives the determination results of CMC of different surfactants with probe I and probe II. From Table 1, it can be seen that the CMC values given by probe II are a little lower than those provided by probe I. Under the optimized experimental conditions, the CMC values of some surfactants provided by the proposed method are in reasonable agreement with the values obtained from the literature, which indicates the feasibility of the proposed method for the CMC determination of some micelle systems.

Conclusion

A simple rapid near-infrared fluorimetry for the determination of CMC using hydrophobic probes was presented. As a molecular “light switch” for the determination of CMC, probe II can be used to nonionic surfactant systems under the experimental conditions, while probe I is suitable not only for nonionic surfactant systems, but also for the cationic surfactant system. It may be expected that other similar hydrophobic molecules, besides the two reported probes, may have the function of “light switch” as a probe for the CMC determination of surfactants. Also, it is possible that the emission properties and the function of “light switch” for the two reported probes can be tuned by careful design of the alkyl chains with a proper length in order to be suitable for the different micelle environments. Furthermore, the quenching effect of ionic surfactants on probe II is interesting. Further studies on the quenching effect would obtain more information about micelle structure and the interaction between probe and micelle, and this work is under way.

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