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A new surfactant-fluorescence probe for detecting shape transitions in self-assembled systems

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ABSTRACT

A new kind of fluorescence probe, a fluorophore-labeled anionic surfactant, sodium 12-(*N*-dansyl)aminododecanate (12-DAN-ADA), was designed and synthesized. The applications of 12-DAN-ADA as a fluorescence probe in molecular assemblies, especially in the transitions between micelles and vesicles, were investigated systematically. It was found that 12-DAN-ADA can efficiently differentiate the two different aggregate types (shapes) in mixed cationic and anionic surfactant systems and double-chain cationic surfactant systems. Experimental results showed that the fluorescence anisotropy of 12-DAN-ADA increased sharply, the emission maxima became blue-shifted, and the fluorescence lifetime rose notably when the aggregates transformed from micelles to vesicles in mixed cationic and anionic surfactant systems. The fluorescence anisotropy can also distinguish different aggregate types in single-component double-chain cationic surfactant systems. Further studies demonstrated that 12-DAN-ADA is a more useful probe of transitions between micelles and vesicles than commonly used fluorescence probes, such as pyrene and 1,6-diphenyl-1,3,5-hexatriene (DPH).

1. Introduction

Formation and transformation of differently organized molecular assemblies, such as spherical and rodlike/wormlike micelles, vesicles, and lamellar phases, have attracted increasing attentions in the past decades [1–11]. Therefore, characterization of the transitions of different aggregates has become a hot issue. In these studies, electron microscopy and light scattering have become two popular characterizing methods, since they can provide straightforward images and detailed size information about the aggregates. However, these techniques can only provide limited information on the aggregates at molecular level. Fluorescence spectroscopy, in contrast, is well known to provide more detailed information at molecular level. For example, fluorescence probes are highly sensitive to their local environment, such as micropolarity, and they have multiple interesting photophysical properties such as excimer/exciplex, fluorescence intensity, and lifetime. Therefore, the fluorescence probe technique has been extensively employed to investigate the formation and transition of aggregates [12–16].

To ideally probe organized molecular assemblies, the desired fluorophores should have attractive features such as being sensitive to their molecular packing. Among many fluorescence probes, pyrene [17] and 1,6-diphenyl-1,3,5-hexatriene (DPH) [18,19] are two commonly used ones that are thought to be effective in the measurement of micropolarity and microviscosity, respectively. However, the location of the two probes in various aggregates is not fixed or affirmable. Thus, the variation of their related fluorescence parameters is usually a combined contribution from both the transition of aggregates and the change of the location of probes. In some cases, the variation in fluorescence signals cannot reflect the real situation of transitions between different aggregates. This problem may be expected to be solved by the use of fluorophore-labeled amphiphiles, since this kind of fluorescence probe as part of the surfactant can take part in the formation of aggregates and provide more reliable information. Based on the considerations mentioned above, some fluorophore-labeled amphiphiles have been used to detect the correlative physical properties of various aggregates [20–30]. However, most of these studies focus on micelle [20–24] or membrane [25–29] systems. The application of this kind of fluorescence probe in the transitions of different aggregates, especially micelles and vesicles [30], is still rare. Therefore, systematic study in this field is necessary.

The intramolecular charge transfer (ICT) compounds containing electron donors and acceptors groups in their moiety have attracted growing interest in recent investigations [31–33]. When excited, the fluorophore could give a dual fluorescence emission that is very sensitive to the solvent polarity. Thus, this emissive property of ICT compounds is important for applications, for example, as a fluorescence marker [34] and a fluorescence probe [35–37] or in materials science [38]. 1-(Dimethylamino)-naphthalene-5-

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sulfonyl (dansyl) derivatives may undergo an ICT phenomenon in which the dimethylamino moiety functions as a donor part and naphthalenesulfonyl as an acceptor part. As a fluorescence probe, it can exhibit a large Stoke's shift that varies with the changes of environment. Therefore, the ICT emission of dansyl has been widely used to study microscopic viscosity and micropolarity when dansyl was incorporated into biological membranes, or bonded to polymers and solid substrates [39–41]. But similarly to other fluorescence probe systems, the problem of locating the probe molecules in aggregates still needs to be solved.

In the present work, we synthesized a new surfactant-fluorescence probe, sodium 12-(*N*-dansyl) aminododecanate (12-DAN-ADA), by choosing dansyl and 12-aminolauric acid as the fluorophore and the starting anionic surfactant, respectively. In this study, the transition between two different aggregates, namely micelles and vesicles, was investigated systematically in three mixed cationic and anionic surfactant systems and two double-chain cationic surfactant systems by using 12-DAN-ADA as a fluorescence probe. As expected, the experimental results showed that the emission maxima and fluorescence anisotropy of 12-DAN-ADA changed greatly along with aggregates transforming between micelles and vesicles. Moreover, 12-DAN-ADA showed obvious advantages in probing transitions of micelles and vesicles over the popularly used fluorescence probes, pyrene and DPH.

2. Materials and methods

2.1. Chemicals

Dansyl chloride (99%), pyrene (98%), DPH (98%), sodium dodecyl sulfate (SDS), and didodecyldimethylammonium bromide (DDAB) were purchased from Acros. 12-Aminolauric acid and dimethyldimyristylammonium bromide (DMAB) were purchased from Tokyo Kasei Kogyo Chemicals. Triton X-100 was from E. Merck Co., Darmstadt. Branched-chain sodium dodecylbenzenesulfonate (SDBS) and sodium octyl sulfate (SOS) were obtained from Beijing Chemical Co. All the above regents were used directly without further purification. Dodecyltrimethylammonium bromide (DTAB), dodecyltriethylammonium bromide (DEAB), and cetyltrimethylammonium bromide (CTAB) were synthesized from *n*-alkyl bromide and corresponding trialkylamine. The three products were recrystallized five times from acetone, acetone, and acetone–ethanol, respectively. The purity of all the synthesized cationic surfactants was examined and no surface tension minimum was found in the surface tension curve. All other reagents were products of Beijing Chemical Co. and were of analytical grade at least. Deionized water was treated with $KMnO_4$ for over 24 h and distilled before use.

2.2. General instruments

^{1}H NMR spectra were recorded on a Mercury Plus 300 M (USA) instrument in DMSO-*d* solvent. Analyses of C, H, and N were conducted on an Elementar Vario EL elemental analyzer (Germany).

2.3. Fluorescence measurements

All of the fluorescence measurements were performed at room temperature (23–25 °C) on a time-correlated single-photon-counting Edinburgh FLS 920 fluorescence spectrometer. The samples containing 12-DAN-ADA, pyrene, and DPH were excited at 337, 337, and 355 nm, respectively, and their stock solutions (1.0×10^{-4} M) were prepared in ethanol, ethanol, and tetrahydrofuran, respectively. A certain amount of stock solution was added to a tube and heated slightly to remove the solvent. Then the final concentration of the probes was adjusted by adding an appropriate amount of the analyte solutions. A FLS 920 fluorescence spectrometer equipped with filter polarizers that use the L-format configuration using a 1-cm quartz cuvette was used for fluorescence depolarization measurements. An average of three fluorescence anisotropy values was recorded. Fluorescence lifetimes were calculated from time-resolved fluorescence intensity decays using an FLS 920 fluorescence spectrometer in the time-correlated single-photon-counting mode. FLS 920 is equipped with a thyratron-gated nanosecond flash lamp filled with hydrogen as the plasma gas (0.38–0.40 bar) and is operated at 40 kHz. For each measurement, at least 3000 photon counts were collected in the peak channel to ensure the decay quality. The goodness of fit of decay curves, χ^2, was no more than 1.3.

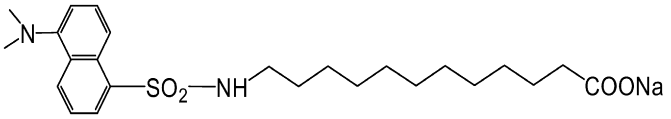

Chart 1. Chemical structure of sodium 12-(*N*-dansyl)amino-dodecanate (12-DAN-ADA).

2.4. Synthesis of 12-DAN-ADA

The synthesis procedure of 12-DAN-ADA is described below. Dansyl chloride (0.235 g, 0.87 mmol, 1 equiv) and 12-aminolauric acid (0.563 g, 2.61 mmol, 3 equiv) were combined in 2 M NaOH aqueous solution (40 ml) and stirred overnight at room temperature until the suspension changed from dark yellow to light yellow. After the reaction was completed, plenty of deionized water was added until the mixture became a clear solution. Then the solution was neutralized with concentrated HCl and 2 M HCl aqueous solution, successively, to adjust the pH value to 5–6. The resulting light yellow cloudy mixture was extracted with ethyl acetate three times. The organic layer was filtered to remove the excessive 12-aminolauric acid. After being dried over anhydrous Na_2SO_4 for at least 12 h, the filtrate was evaporated under reduced pressure to give a yellow-green oil. 2 M NaOH aqueous solution was added drop by drop to the oil until the oil vanished. (*Caution*: The addition of NaOH solution must be handled very carefully, and no excessive NaOH was added.) The residue was precipitated with plenty of acetone and the coarse product was filtered. Then the product was dissolved in ethanol and filtered to remove indissoluble residues. The filtrate was evaporated and a light yellow precipitate was given. ^{1}H NMR (DMSO-*d*): $\delta = 8.75$ (1 H, d, Ar–*H*), 8.12 (1 H, d, Ar–*H*), 7.88 (1 H, d, Ar–*H*), 7.34 (2 H, m, Ar–*H*), 7.07 (1 H, d, Ar–*H*), 3.37 (2 H, m, –NH–C*H*$_2$–), 2.73 (6 H, s, –N(C*H*$_3$)$_2$), 2.57 (2 H, t, COONa–C*H*$_2$–), 1.85 (2 H, t, COONa–CH$_2$–C*H*$_2$–), 1.39 (2 H, m, –NH–CH$_2$–C*H*$_2$–), 1.17–1.02 (14 H, b, –NH–CH$_2$–CH$_2$–(C*H*$_2$)$_7$–CH$_2$–CH$_2$–COONa). Anal. Calcd. for 12-DAN-ADA $C_{24}H_{35}N_2SO_4Na$: C, 61.25; H, 7.50; N, 5.95. Found: C, 60.59; H, 7.73; N, 5.51. As an anionic surfactant, its general surface properties were investigated. The critical micelle concentration (CMC) of 12-DAN-ADA is about 2.2×10^{-3} M, which was determined by surface tension measurements (drop volume method) and fluorescence techniques (cf. Fig. 1 in the supplementary materials).

3. Results and discussion

3.1. Determination of the polarity and viscosity dependence of 12-DAN-ADA

A new fluorescently labeled anionic surfactant, 12-DAN-ADA, was synthesized and its chemical structure is shown in Chart 1. As an ICT compound, dansyl is very sensitive to the polarity and viscosity of its surroundings. Hence it can be anticipated that

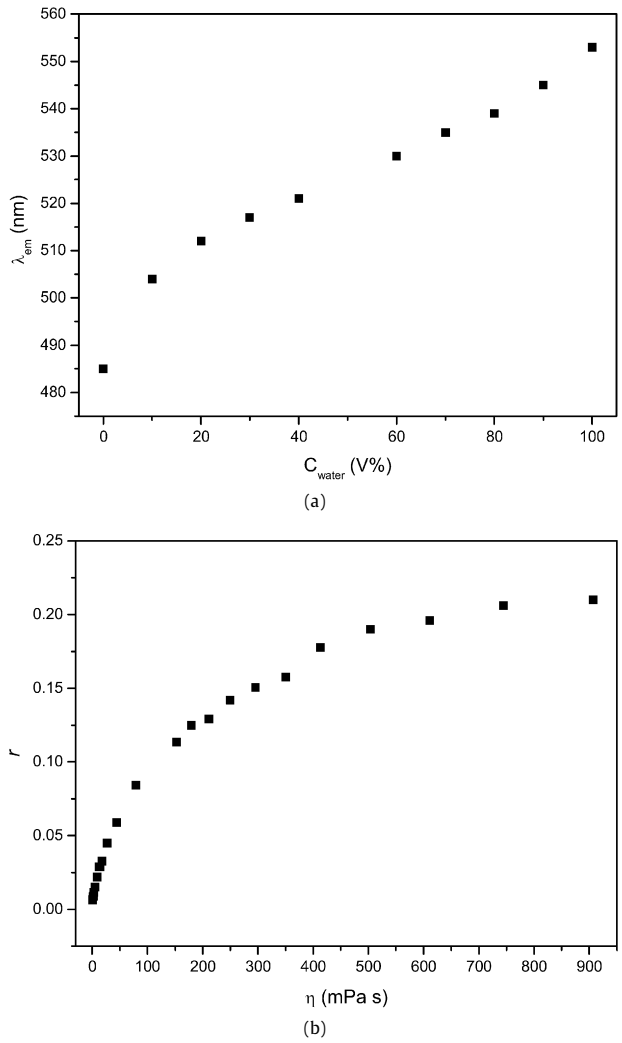

Fig. 1. Emission maxima of 12-DAN-ADA in 1,4-dioxane/water binary systems (a) and fluorescence anisotropy in glycerine/water mixture solvents (b). The viscosity values are obtained from Refs. [43] and [44]. $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-5}$ M.

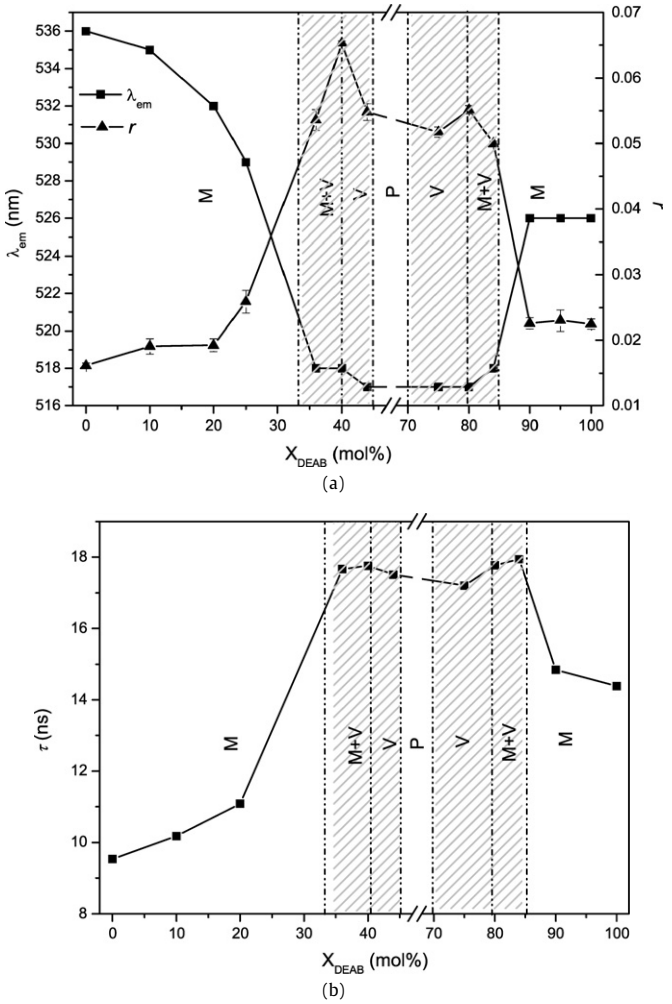

Fig. 2. Emission maxima, fluorescence anisotropy (a), and fluorescence lifetime (b) of 12-DAN-ADA in the SDS/DEAB system. $C_T = 1.0 \times 10^{-2}$ M, $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M. P: precipitate; M: micelles; V: vesicles.

this polarity and viscosity dependence of dansyl can be restored when it is labeled to 12-aminolauric acid to form a novel anionic surfactant, 12-DAN-ADA. Based on this consideration, the fluorescence emission spectra of 12-DAN-ADA in 1,4-dioxane/water binary solvents [42] and various solvents with different polarities were measured. As expected, the emission maxima of 12-DAN-ADA red-shifted obviously, along with the increasing of the solvent polarity. Fig. 1a shows the emission maxima of 12-DAN-ADA in 1,4-dioxane/water binary systems with different water content. From Fig. 1, it can be seen that the emission maxima red-shifted about 70 nm, from 480 to 550 nm, as the solvent changed from 1,4-dioxane to water. The normalized fluorescence emission spectra of 12-DAN-ADA in six different solvents are shown in Fig. 2 in the supplementary materials. Meanwhile, the fluorescence anisotropy of 12-DAN-ADA in the binary solvents of glycerine/water (with different viscosity [43,44]) was also examined (cf. Fig. 1b). The curve in Fig. 1b suggests that the fluorescence anisotropy of 12-DAN-ADA increased along with increasing viscosity. These observations demonstrated that the properties of polarity and viscosity dependence of dansyl were well reserved in 12-DAN-ADA. Based on the consideration of its solubility (see Section 2.4 in Experimental and Fig. 1 in the supplementary materials) and polarity and viscosity dependence, it may be suitable to use 12-DAN-ADA as a fluorescence probe to detect the formation and transition of aggregates since it can take part in the formation of the aggregates. Thus, the variation of related fluorescence parameters in the formation of aggregates and the corresponding transition between different aggregates were examined.

3.2. Probing the transition between micelles and vesicles in mixed cationic and anionic surfactant systems

Considering the rich aggregation behaviors and the challenging characterization in mixed cationic and anionic surfactant systems, the transition between micelles and vesicles in these systems was studied systematically. In the present work, three mixed cationic and anionic surfactant systems, SDS/DEAB, SOS/CTAB, and SDBS/DTAB, were selected to study the transition. It is known that the molecular arrangement will become more closely and more orderly [45,46], and the polarity of the hydrophobic region will decrease obviously when the aggregate transforms from micelles to vesicles. Therefore, the variation of the emission maxima, fluorescence anisotropy, or fluorescence lifetime will respond to the changes of the aggregates.

12-DAN-ADA was applied to the micelle region, the vesicle region, and their mixed region of SDS/DEAB system [11] as a fluorescence probe. The total concentration of SDS and DEAB was 1.0×10^{-2} M in the following experiments. The emission maxima, fluorescence anisotropy, and fluorescence lifetime of 12-DAN-ADA in the SDS/DEAB system are shown in Figs. 2a and 2b. When the

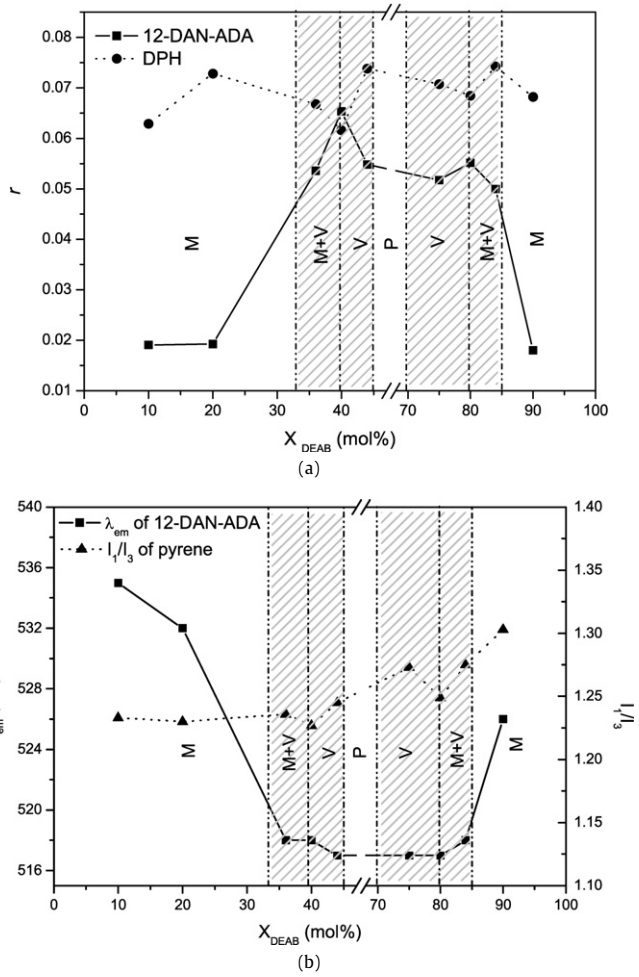

Fig. 3. Fluorescence anisotropy of DPH and 12-DAN-ADA (a) and emission maxima of 12-DAN-ADA and I_1/I_3 of pyrene (b) in the SDS/DEAB system. $C_T = 1.0 \times 10^{-2}$ M, $C_{DPH} = 1.0 \times 10^{-6}$ M, $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M, and $C_{pyrene} = 1.0 \times 10^{-7}$ M.

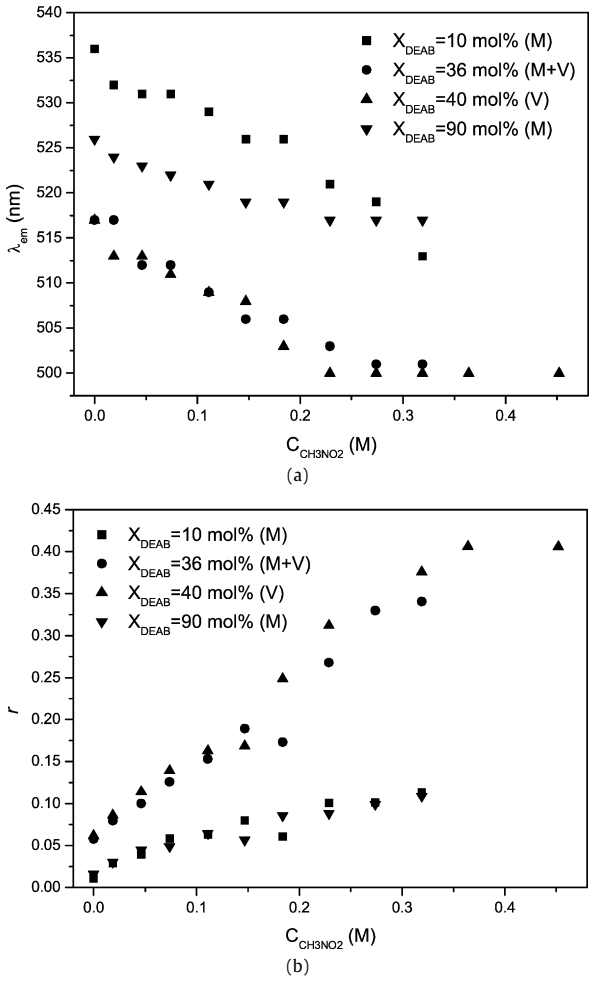

Fig. 4. Emission maxima (a) and fluorescence anisotropy (b) of 12-DAN-ADA in different concentrations of nitromethane in the SDS/DEAB system. $C_T = 1.0 \times 10^{-2}$ M and $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M.

organized assemblies changed from micelles to vesicles, it was found that the emission maxima of 12-DAN-ADA blue-shifted from 536 to 517 nm while the fluorescence anisotropy increased sharply along with this transition. In contrast, the emission maxima of 12-DAN-ADA shifted back to nearly 530 nm and the fluorescence anisotropy decreased while the vesicles transformed back to micelles by increasing the molar ratio of DEAB in the SDS/DEAB system. Similarly, the fluorescence lifetime increased from 10 to 17 ns along with the micelles transforming to vesicles, and vice versa. It should be noted that the emission maxima of 12-DAN-ADA in the DEAB-rich micelle region is smaller than that in the SDS-rich micelle region. Correspondingly, the fluorescence anisotropy and lifetime are larger than those in the SDS-rich region. These observations may be attributed to the electrostatic interaction between DEAB and 12-DAN-ADA. Considering the changes of polarity and viscosity during the transition between micelle and vesicle, it may be concluded that 12-DAN-ADA is an ideal fluorescence probe in probing the transition between different aggregates, especially for micelle and vesicle, in mixed cationic and anionic surfactant systems.

Further investigation was also performed in two other mixed cationic and anionic surfactant systems, SOS/CTAB [47] and SDBS/DTAB [48]. Similar results were also obtained in these systems. The variation of the corresponding fluorescence parameters was also consistent with that of the corresponding aggregate forms (Figs. 3 and 4 in the supplementary materials).

As a controlled experiment, pyrene and DPH were introduced into the SDS/DEAB system to examine their fluorescence responses (including I_1/I_3 of pyrene and fluorescence anisotropy of DPH) in different aggregate forms. Differently from the observations of 12-DAN-ADA, as discovered from the experimental results (cf. Fig. 3), there were no obvious variation in all the determined fluorescence signals over the whole tested concentration range. Consequently, it may be concluded that the synthesized fluorescence probe, 12-DAN-ADA, has advantages over traditional fluorescence probes, such as pyrene and DPH, in probing transitions of micelles and vesicles in mixed cationic and anionic surfactant systems.

Despite 12-DAN-ADA exhibiting ideal characteristics in determining transitions between micelles and vesicles, however, as an anionic surfactant, 12-DAN-ADA can be dissolved in water, with its CMC of 2.2×10^{-3} M (cf. Fig. 1 in the supplementary materials). Therefore, the resulting fluorescence signals must be the combined information about 12-DAN-ADA in free aqueous solution and in aggregated micelles or vesicles. To unveil this puzzle, a water-soluble quencher, nitromethane [49], was introduced to examine the fluorescence signals of 12-DAN-ADA in aggregated form. In fact, the emission maxima of 12-DAN-ADA blue-shifted and the fluorescence anisotropy increased as the concentration of nitromethane increased (cf. Fig. 4). That is, the changing of corresponding fluorescence signals became more obvious, along with the aggregates transition, when nitromethane was introduced. This phenomenon can be explained by the consideration that the more nitromethane in the solution, the smaller the contribution to the fluorescence

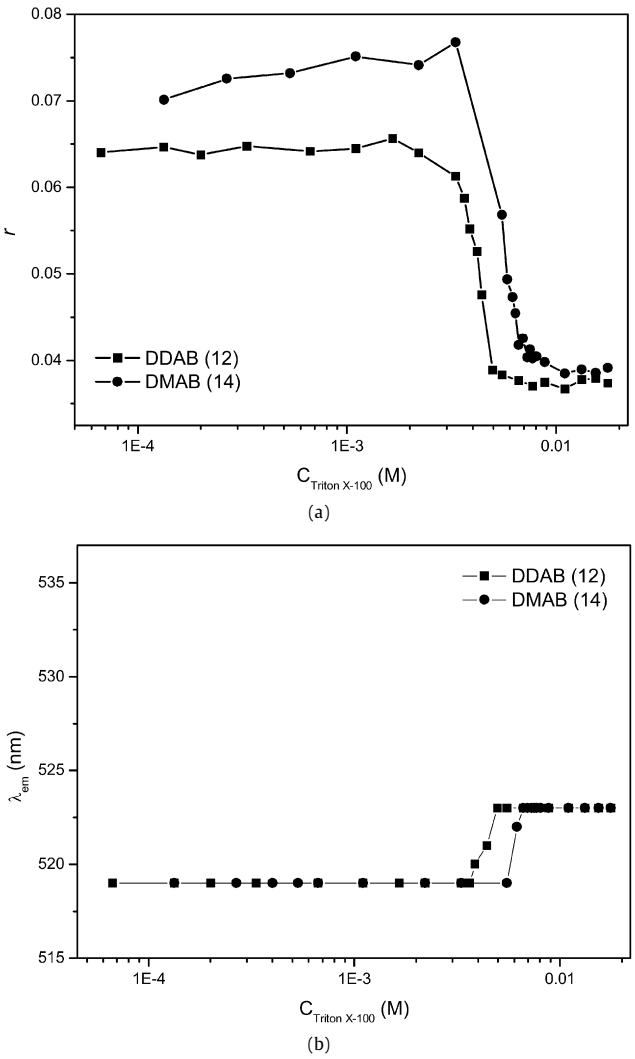

Fig. 5. Fluorescence anisotropy (a) and emission maxima (b) of 12-DAN-ADA in 5.0×10^{-3} M double-chain cationic surfactant systems with different chain lengths varying with the concentration of Triton X-100. $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M.

signals from free 12-DAN-ADA in solution, and the more from the aggregated forms. This is a powerful proof to confirm that the variation of fluorescence parameters of 12-DAN-ADA can distinguish the two types of aggregates, even with the absence of quencher in systems. Thus, when 12-DAN-ADA was incorporated into the double-chain cationic surfactant systems in the following experiments, the quenching of nitromethane did not carry out.

Careful examination of Fig. 4 reveals that the changing extent of the fluorescence signals in vesicle and vesicle/micelle systems was larger than that in micelle systems, and the fluorescence anisotropy almost reached its top value, 0.4 [50], indicating that the microenvironment of 12-DAN-ADA was closely packed, and the fluorescence probe was hard to rotate when 12-DAN-ADA was located in vesicles compared with in micelle.

3.3. Expended applications in double-chain cationic surfactant systems

To further extend the applications of 12-DAN-ADA in probing the transition between micelles and vesicles in other systems, 12-DAN-ADA was introduced into double-chain cationic surfactant systems to detect the transition from vesicles to micelles.

In double-chain cationic surfactant systems, Triton X-100 was used as a breaker of vesicles [51]. Fig. 5 shows the fluorescence anisotropy and emission maxima of 12-DAN-ADA in double-chain

Table 1
Fluorescence lifetime of 12-DAN-ADA in SDS/DEAB and DDAB systems

SDS/DEAB ($C_T = 1.0 \times 10^{-2}$ M)				DDAB ($C = 1.0 \times 10^{-2}$ M)			
X_{DEAB} (mol%)	Aggregates	τ (ns)	χ^2	$C_{Triton\ X\text{-}100}$ (10^{-3} M)	Aggregates	τ (ns)	χ^2
0	M	9.54	1.172	0	V	15.26	0.987
20	M	11.08	1.146	0.53	V	15.38	1.105
40	M + V	17.76	1.184	3.31	V	15.65	1.182
44	V	17.51	1.206	7.72	V	15.97	1.194
80	V	17.77	1.057	10.26	M	15.76	1.012
84	M + V	17.94	1.159	11.03	M	15.84	1.053
90	M	14.84	1.087	15.44	M	15.76	1.109
100	M	14.39	1.262				

Note. $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M. M: micelles; V: vesicles.

cationic surfactant systems (5.0×10^{-3} M) of different chain length varying with the concentration of Triton X-100. Similarly to the results observed in mixed cationic and anionic surfactant systems, it can be seen from Fig. 5 that the fluorescence anisotropy decreased and the emission maxima red-shifted when the vesicles were broken to micelles by the addition of an amount of breaker, Triton X-100. It is noted that the longer the chain length, the larger a breakdown concentration of Triton 100 is needed, which is consistent with the literature results [52].

Comparing the results in Fig. 2a with those in Fig. 5b, it can be found that the changing of emission maxima of 12-DAN-ADA in double-chain cationic surfactant systems is notably smaller than that in mixed cationic and anionic surfactant systems, along with the transition from vesicles to micelles. These results may be attributed to the nature of double-chain cationic surfactants or the influence of the breaker, and thereby, the micropolarity of 12-DAN-ADA should be barely changed in these systems. This consideration is also supported by the results of fluorescence lifetime measurements (cf. Table 1). As a polarity-relative parameter, the fluorescence lifetime of 12-DAN-ADA changed slightly when it was incorporated into the double-chain surfactant systems.

The changing of the fluorescence parameters of 12-DAN-ADA in the different surfactant systems during the transition from micelles to vesicles can be attributed to the closer molecular arrangement and decreasing polarity of the microenvironment. Thus, it should reflect these changes by employing 12-DAN-ADA as a fluorescence probe once the polarity and viscosity of aggregates changed. That is to say, it may be useful in probing the formations and transitions of different aggregates, not only for micelles and vesicles, but also among spherical micelles, rodlike micelles, wormlike micelles, lamellar structures, and vesicles. The relating works and synthesis of other novel similar fluorescently labeled surfactants are in progress in our laboratory. In fact, as to the formation of micelles, 12-DAN-ADA was also suitable for probing the CMC of anionic and nonionic surfactants (cf. Fig. 6) and the CMC values are consistent with literature values [53], but not for cationic surfactants due to the electrostatic interaction between 12-DAN-ADA and cationic surfactant molecules.

4. Summary

The experimental results in the present work showed that the emission maxima of 12-DAN-ADA blue-shifted obviously, the fluorescence anisotropy increased sharply, and the fluorescence lifetime rose clearly when the aggregates transformed from micelles to vesicles in mixed cationic and anionic surfactant systems, and these changes were attributed to the closer molecular arrangement and decreasing polarity of the microenvironment. Fluorescence anisotropy of 12-DAN-ADA is an effective parameter to observe the transition of vesicles to micelles in double-chain cationic surfactant systems. Moreover, controlled experiments showed that

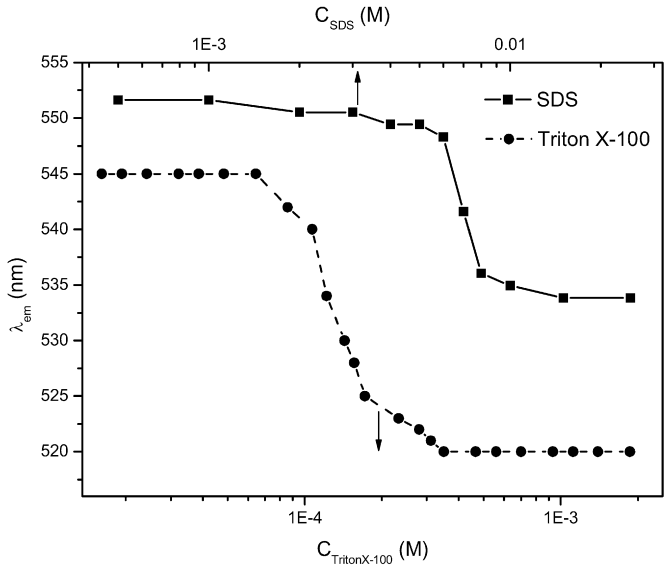

Fig. 6. Emission maxima change of 12-DAN-ADA with the concentrations of SDS and Triton X-100. $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M. CMC of SDS: 8.2×10^{-3} M (literature value [53]: 8.9×10^{-3} M); CMC of Triton X-100: 3.0×10^{-4} M (literature value [53]: 3.3×10^{-4} M).

12-DAN-ADA is a better fluorescence probe in studying the transition between micelles and vesicles than the two popular fluorescent probes pyrene and DPH. We hope our work can provide a new method for studying organized assemblies in solution and be useful in obtaining further understanding in this field.

The surface tension curve and fluorescence characterization of 12-DAN-ADA in water, the normalized fluorescence emission spectra of 12-DAN-ADA in different solvents, and the emission maxima, fluorescence anisotropy, and fluorescence lifetime of 12-DAN-ADA in SOS/CTAB and SDBS/DTAB systems are available as supplementary materials.

Acknowledgments

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Supplementary materials

Supplementary materials associated with this article can be found in the online version, at DOI: 10.1016/j.jcis.2008.04.015.

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