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# A surfactant type fluorescence probe for detecting micellar growth

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ABSTRACT

We report on the detection of micellar growth in anionic, cationic, and catanionic surfactant systems using a novel surfactant type fluorescence probe, sodium 12-(N-dansyl)amino-dodecanate (12-DAN-ADA). The fluorescent group was incorporated in the tail of the surfactant which tethers the fluorescent group deep inside the apolar micellar cores. The fluorescence anisotropy of 12-DAN-ADA was found to be very sensitive for directly detecting the micellar growth in micelles containing oppositely charged surfactants, including cationic CTAB systems and mixed systems of oppositely charged surfactants (DEAB/SDS); in regard to the like charged SDS micellar systems, the sensitivity can be greatly enhanced by addition of a water soluble quencher which quenches the background fluorescence from the equilibrium population of free 12-DAN-ADA.

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## 1. Introduction

Surfactant self-assemblies have attracted great attention in the last two decades owing to their important role in the field of biomimetic and material science [1,2]. Among which, the transition between different self-assemblies is of special interest because this helps to understand the formation process of these self-assembled structures [3,4]. In order to have a better insight on the self-assembly transition, many efforts were made to detect the shape changes occurred in surfactant systems. However, most of the efforts involve using of expensive apparatus, such as small angle neutron or X-ray scattering (SANS or SAXS) and Cryo-TEM [5–7], which are not accessible for many researchers. Under this background, developing cheap and convenient method to detect the self-assembly transition becomes an important issue. Fluorescence method was proved to be one of the competitive methods in this regard. It is well-known that the transition of the self-assembly of amphiphiles is always accompanied by the changes of the microenvironments in the molecular membrane. This can be sensitively detected by the tethered fluorescence probe molecules, since the fluorescence emission and relating parameters are very sensitive to the local environments where the probe molecules locate [8,9]. This, therefore, can be utilized to detect the self-assembly transition in surfactant and other amphiphilic systems [10–12].

However, in practical applications, using fluorescent probes to detect the self-assembly transition has two weak points. One is the uncertainty of the location of the probe molecules in the membrane, and the other is the possible influence of the probe molecules on the structure of the self-assemblies [13–15]. This means that the variation of fluorescence parameters is usually the combined contribution from both the transition of the self-assemblies and the change of the location of the probes in the assemblies [16]. To solve this problem, fluorescence-labeled amphiphiles (FLAs) were synthesized [17–21]. It is expected that the FLAs may attend the self-assembly meanwhile detect the local environments where the fluorophores are located [22–26]. However, so far most the reported FLAs are with a fluorescent group incorporated close to the head group or in the middle of their hydrophobic chain, few of them are designed with the fluorescent group attached to the tail of an amphiphilic molecule. Therefore, those probes are not able to detect changes in the most interior of the surfactant self-assemblies, thus no convincing data were provided regard to the changes occur in the deep interior of surfactant self-assembly.

To solve this problem, our group developed a FLA molecule with the fluorescent groups attached to the end of the hydrophobic chain. In our design, the fluorescent group, dansyl, was linked to the tail of an anionic surfactant (12-aminolauric acid), which is abbreviated as 12-DAN-ADA in the following text [27]. We were surprised to find that such a FLA is much more sensitive in detecting the micelle–vesicle transition than other commonly used fluorescence probes, such as pyrene and DPH [27]. The experimental results revealed that the emission maximum and the fluorescence anisotropy vary significantly as a transition between micelles and

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vesicles in catanionic surfactant systems occurs, whereas the characteristic $I_1/I_3$ value of pyrene, or the anisotropy of DPH hardly give significant responsiveness [27]. This extremely high sensitivity may be related to the deep location of the fluorescent group of our FLA molecules in the self-assemblies. We are therefore especially curious about the sensitivity of this probe: can it detect the growth of micelles, namely, transition from spherical to rod-like, and to worm-like micelles?

It is known that the physicochemical properties of micelles change rather subtly with increasing the micellar size, making them difficult to study by many more direct techniques. To our surprise, every stage of the micellar growth was successfully tracked by using our surfactant like probe. Especially, this surfactant type fluorescent molecule can effectively detect micellar growth in catanionic surfactant systems, where other common probes are disabled. This finding for the first time provides a detailed view for the environmental change in the micellar core along with the micellar growth, which is of significant importance to the fundamental of colloid and interface science, and may help scientists in relating field to consider all the possible changes in the process of the micellar growth more roundly.

## 2. Materials and methods

### 2.1. Materials

12-DAN-ADA was synthesized from 12-aminolauric acid and dansyl chloride according to the reported method [27]. Sodium dodecyl sulfate (SDS) was purchased from Acros and was used directly without further purification. Dodecyltriethylammonium bromide (DEAB) and cetyltrimethylammonium bromide (CTAB) were synthesized from *n*-alkyl bromide and corresponding trialkylamine. These two products were recrystallized five times from 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 above analytical grade. Deionized water was treated with $KMnO_4$ for over 24 h and distilled before use.

### 2.2. Fluorescence measurements

All of the fluorescence measurements were performed on a time-correlated single photon counting Edinburgh FLS 920 fluorescence spectrometer. The samples containing 12-DAN-ADA were excited at 337 nm, and the stock solution ($1.0 \times 10^{-4}$ M) was prepared in ethanol. A certain amount of stock solution was added to a tube and heated slightly to remove the solvent. Then the final concentration of the probe was adjusted by adding appropriate amount of the analyte solutions. All these analyte solutions were kept in relevant temperature for 24 h before measurement. 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. Every measurement was repeated two times to confirm the results. Errors for the wavelength are about ±0.5 nm; for the fluorescence anisotropy are about 5%.

## 3. Results and discussion

In the present work, three typical micellar systems, namely SDS/NaX, CTAB/KBr, and SDS/DEAB, which are known in literature to have a full spectrum of micellar shape were selected to study the morphological transition of different micelles. At given surfactant concentrations, the morphology of the micelles will transform from spheres, ellipsoids, rods, to worms in proper conditions. In addition, these three micellar systems represent classical anionic, cationic, and catanionic surfactant systems. Therefore, by measuring the fluorescence response in these systems with the growth of micelles, one may have a full view of the ability of 12-DAN-ADA in detecting the growth of various micelles.

### 3.1. Probing the micelle transition in SDS/NaX system

First, we report on the micellar transition in anionic surfactant SDS system probed by 12-DAN-ADA. It is known that the micelles change from spheres to rods, and then to worms with increasing the concentration of NaBr from 0 to 1 M in 35 mM SDS system at 40 °C (the result from SANS studies [28]). In this process, the SDS molecules are supposed to pack more compact, and the local environment in the micelles changes accordingly. These changes are checked by using 12-DAN-ADA as the probe. It can be found in Fig. 1 that the fluorescence anisotropy increases slowly from 0.010 to 0.018 while the emission maximum blue-shifts from 537 nm to 534 nm. Upon increasing the SDS concentration to 175 mM, where the micelles are much longer, the fluorescence anisotropy of 12-DAN-ADA varies more obviously than that in the 35 mM SDS system. At the beginning where no salt was added, the micelles are ellipsoidal [29] which allows the anisotropy of 0.012 for 12-DAN-ADA; whereas the anisotropy increases about 1.5 times as the micelles become long worms of 4240 Å. One should notice that the fluorescence anisotropy values for the worm-like micelles are actually much lower if they are compared with those for vesicles [27], which indicates that the molecular packing in micelles is much looser than that in vesicles. This suggests two possibilities: (1) the 12-DAN-ADA molecule is not sensitive enough in detecting the growth of SDS micelles; (2) the quantity of the free 12-DAN-ADA molecules in the bulk solution which in equilibrium with the SDS micelles is rather large, so that signals from these free 12-DAN-ADA molecules mask the real response of those tethered in the SDS micelles.

To exclude the latter effect, we measured the fluorescence parameters in the presence of a water soluble quencher, nitromethane [30]. Since the fluorescent group of 12-DAN-ADA is designed at the end of the hydrophobic chain, it will locate in the interior of the micellar core. Therefore, water soluble quenchers that can only access to the water–micelle interface will fail to quench the fluorescence of 12-DAN-ADA inside the micelles.

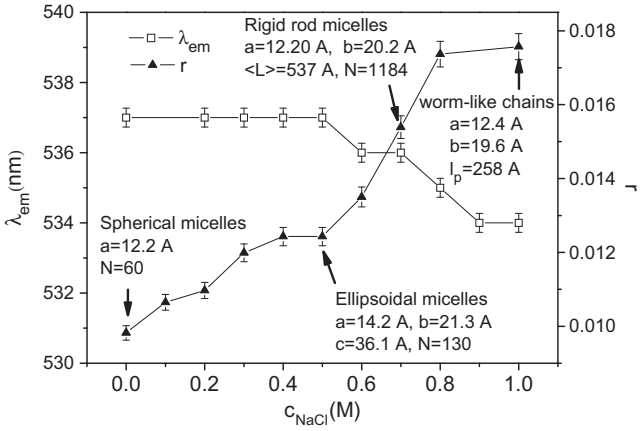


**Fig. 1.** Fluorescence anisotropy and emission maxima of 12-DAN-ADA in SDS/NaBr system ($C_{SDS}$ = 35 mM, $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M, $T$ = 40 °C). (The related data of three points in the figure are from the results of SANS and indicating the different micelle type [28]).

However, the fluorescence from the free 12-DAN-ADA molecules which do not participate in the micellar formation will be effectively quenched, so that the remained fluorescence signal must be from those probe molecules tethered in the SDS micelles. With this idea in mind, we first examined the variation of the fluorescence parameter with the concentration of nitromethane in 175 mM SDS/1 M NaCl system. It was so pleasant to find that the fluorescence anisotropy increases considerably with increasing the concentration of nitromethane (cf. Fig. 3). This means that after quenching the fluorescence contributed by the free 12-DAN-ADA molecules, the change of the fluorescence anisotropy is indeed very significant in the process of micellar growth. As can be read from Fig. 2, the fluorescence anisotropy is enhanced about 3.4 times as the concentration of nitromethane amounts to 0.55 M. In addition, the emission maximum of 12-DAN-ADA blue-shifts significantly up to 20 nm as the quencher concentration amounts to 0.55 M. In contrast, without the presence of quencher, the blue shift is only around 3 nm. To make a parallel comparison with results in Fig. 1, we also measured the variation of above parameters in 35 mM SDS system at a fixed nitromethane concentration while changing the NaCl concentration. As demonstrated in Fig. 4 that in the presence of only 0.02 M nitromethane, the blue shift of the fluorescence maxima is about 5 nm, about 60% higher than that in the solution without nitromethane. Meanwhile, the improvement in the anisotropy is much more significant: the fluorescence anisotropy was increased 16 times, whereas it is only around two times (read from Fig. 1) when no nitromethane was added. These data clearly demonstrate that 12-DAN-ADA can sensitively detect the growth of micelles as well as it does with the transition between micelles and vesicles if the fluorescent noise from the free 12-DAN-ADA molecules is excluded.

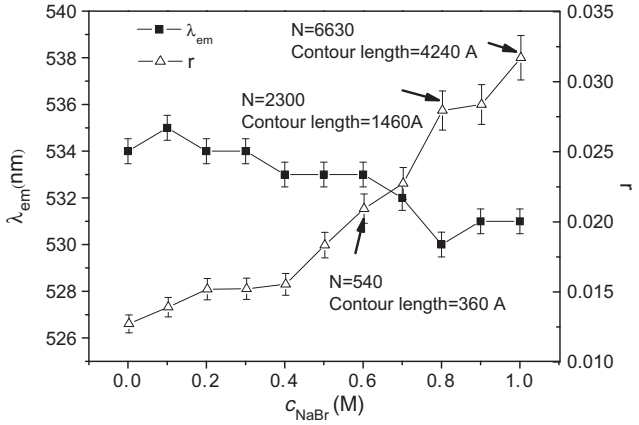


**Fig. 2.** Fluorescence anisotropy and emission maxima of 12-DAN-ADA in SDS/NaBr system ($C_{SDS}$ = 175 mM, $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M, $T$ = 40 °C). The related data of three points in the figure are from the results of SANS and indicating the different micelle type [29].

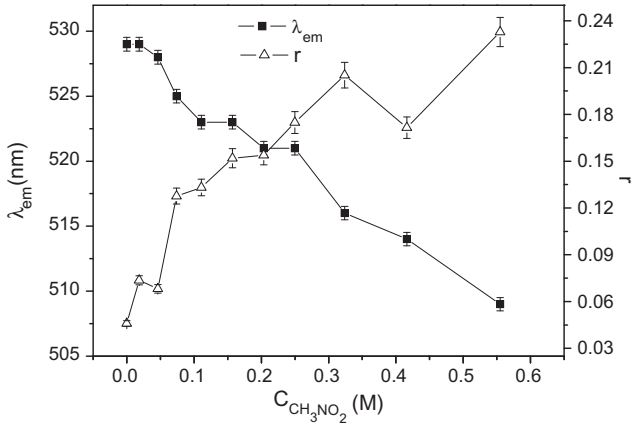


**Fig. 3.** Fluorescence anisotropy and emission maxima of 12-DAN-ADA in different concentrations of nitromethane in 175 M SDS/1 M NaCl system ($C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M, $T$ = 40 °C).

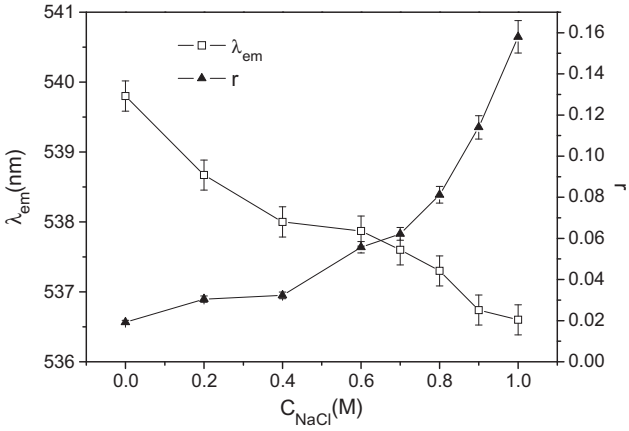


**Fig. 4.** Fluorescence anisotropy and emission maxima of 12-DAN-ADA in SDS/NaCl system in the presence of 0.02 M nitromethane ($C_{SDS}$ = 35 mM, $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M, $T$ = 40 °C).

### 3.2. Probing the micelle transition in CTAB/NaBr system

In Section 3.1 we have shown that the sensitivity of 12-DAN-ADA in detecting the micellar growth in SDS micelles can be improved upon quenching the background fluorescence. Actually, the background fluorescence is unavoidable due to the surfactant nature of 12-DAN-ADA: there will always be monomers coexisting with micelles. Since SDS and 12-DAN-ADA carries charges of the same sign, the CMC of 12-DAN-ADA (2.2 mM, [27]) is not significantly reduced in the SDS/12-DAN-ADA mixed system, so that it allows considerably large amount of 12-DAN-ADA molecules floating in the bulk solution which in equilibrium with the mixed micelles. However, in the oppositely charged surfactant systems, this situation may change significantly. It is well-known that strong synergistic interaction exists in the latter case [31–33]. As a result, the CMC of both components will be greatly reduced, so that the amount of free molecules will be much less and most of them will take part in the micelles. This suggests that the sensitivity of the anionic 12-DAN-ADA in probing the micellar growth in cationic surfactant systems might be very high even without smearing the background fluorescence. In the following we try to verify this deduction in the cationic surfactant CTAB system.

The micellar growth was realized according literature by addition of 0–0.6 M KBr to 10 mM CTAB aqueous solution at 30 °C [34,35]. The fluorescence anisotropy and the emission maxima of 12-DAN-ADA in CTAB/KBr system are shown in Fig. 5. One can find that the fluorescence anisotropy increases from 0.017 to 0.039 as the concentration of KBr increases from 0 to 0.6 M. This anisotropy change is more significant than that in SDS/NaX system without the presence of quencher, which indicates that the electrostatic interaction between 12-DAN-ADA and CTAB indeed helps to promote the sensitivity of 12-DAN-ADA. That is, the electrostatic interaction allows enriching of 12-DAN-ADA in the micelles, so that few free molecules are remained in the bulk solution [27]. As a result, the fluorescence contributed by the free 12-DAN-ADA molecules that floating in the bulk was reduced in the CTAB systems. Therefore, the apparent fluorescence anisotropy for this system became more pronounced owing to the smearing of the

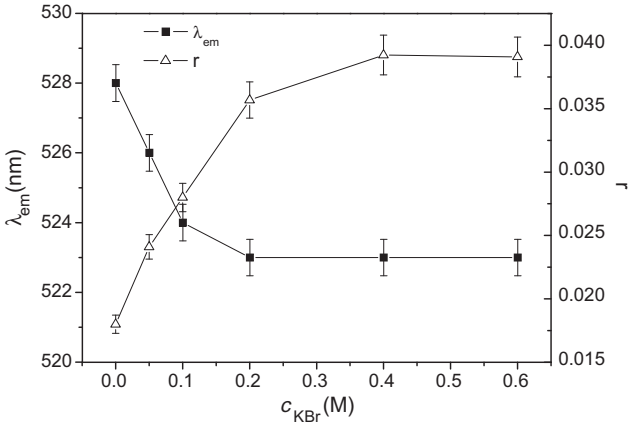


**Fig. 5.** Fluorescence anisotropy and emission maxima of 12-DAN-ADA in CTAB/KBr system ($C_{CTAB}$ = 10 mM, $C_{12\text{-DAN-ADA}}$ = 1.0 × $10^{-6}$ M, $T$ = 30 °C).

fluorescence from the free 12-DAN-ADA. Here, the variation of the fluorescence maxima (about 6 nm) does not change significantly, which is in contrast with the 20 nm blue shift in the process of micelle–vesicle transition [27]. This suggests that the polarity in the micellar core is not considerably changed in the process of micellar growth.

Nevertheless, the presence of quencher will also increase the sensitivity of the micellar growth in the CTAB/NaBr system, as revealed in Fig. 6. Compared with results in Fig. 5, where no quencher is present, the fluorescence anisotropy shifts from 0.1 to about 0.4 in the presence of 0.1 M quencher; which is considerably larger than the result in Fig. 5. Meanwhile, the blue shift of the fluorescence maxima is increased as well in the latter case. These clearly demonstrate that 12-DAN-ADA indeed sensitive enough to probe micellar growth in both the cationic and anionic surfactant systems; the sensibility can be considerably enhanced if the background noise from the free probe molecules is smeared out by using a water soluble quencher.

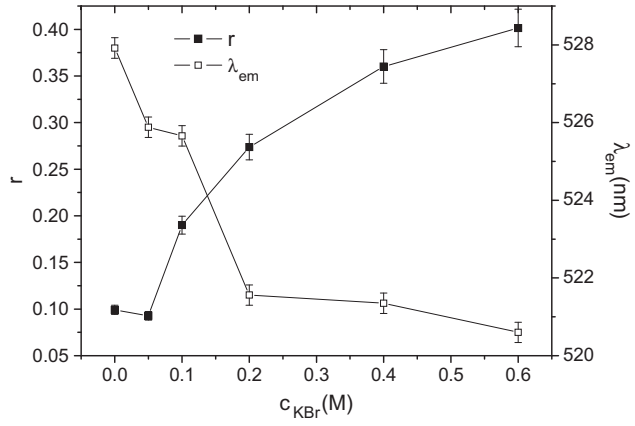


**Fig. 6.** Fluorescence anisotropy and emission maxima of 12-DAN-ADA in CTAB/KBr system in the presence of 0.1 M nitromethane. ($C_{CTAB}$ = 10 mM, $C_{12\text{-DAN-ADA}}$ = 1.0 × $10^{-6}$ M, $T$ = 30 °C).

### 3.3. Probing the micelle transition in catanionic surfactant system

The above experimental results demonstrate that 12-DAN-ADA is a suitable probe for detecting the growth of micelles in both anionic and cationic surfactant systems. The sensitivity of this probe alone is much better for detecting the micellar growth in the oppositely charged cationic CTAB system; for the application of this probe into the micellar growth in the like charged SDS system, the presence of a water soluble quencher is necessary to obtain high enough sensitivity. In the following, we would like to examine the efficiency of this probe in detecting another important category of micellar growth, namely micelles formed by mixed systems of oppositely charged surfactants. We expect that the presence of positively charges surfactants, which is opposite to the anionic surfactant type 12-DAN-ADA probe, will help in providing good enough sensitivity. The catanionic surfactants chosen in our study are SDS/DEAB mixed system, which is the classical system investigated by our group [36–38]. On the other hand, using of this system helps to understand whether the presence of cationic DEAB makes some difference on the sensitivity of 12-DAN-ADA from that in SDS single systems. It is well-known that with variation of the mixing ratio between SDS and DEAB, the micelles grow gradually, and transform into vesicles as SDS:DEAB approaches 1:1, no matter start from which side [37]. However, according to our previous work, the wormlike micellar region is much broader in the SDS rich side [36], so that micelles formed in this side, as listed in Table 1, were taken out for this study. We have found in our previous study that the two very often used fluorescent probes pyrene and DPH are not able to detect this micellar growth, although they may effective in probing the growth of micelles of single surfactants [27]. For example, Chattopadhyay et al. have reported that along with the growth of SDS micelles, the $I_1/I_3$ values of pyrene may change accordingly [39]. However, the $I_1/I_3$ values of pyrene were found to be almost constant in the process of micellar growth in SDS/DEAB catanionic surfactant systems [27]. In contrast, by using 12-DAN-ADA, we observed that the fluorescence anisotropy increases pronouncedly with the growth of the catanionic micelles (Fig. 7), meanwhile the fluorescence maximum decreases considerably, suggesting that 12-DAN-ADA has obvious advantage over pyrene and DPH in detecting the micellar growth in catanioinc surfactant systems. It is worth noting that both the variation of the fluorescence anisotropy and the fluorescence maxima in this SDS rich system shift in a much larger extent than those in the SDS single system (Fig. 1) even without the presence of quenchers. This suggests that the presence of cationic surfactant DEAB indeed helps to decrease the background noise of 12-DAN-ADA, which is probably owing to the electrostatic interaction between cationic DEAB and anionic 12-DAN-ADA. This greatly lowers the concentration of free 12-DAN-ADA in the bulk solution, so that improves the sensibility of 12-DAN-ADA as a probe in detecting the subtle environmental change accompanied with micellar growth. One may question why such smearing of background fluorescence can still occur in the SDS rich catanionic surfactant systems. This can be attributed to the aggregation equilibrium in this micellar system: the presence of CMC in this system suggests that there will be DEAB monomers that do not participate the mixed micelle

**Table 1**
The apparent phenomena and aggregates' type of SDS/DEAB system in different SDS/DEAB ratio ($C_T$ = 200 mM and $T$ = 25 °C). Data were taken from Ref. [36].

| Ratio of SDS/DEAB | 89/11 | 80/20 | 73/27 | 65/35 | 60/40 |
|---|---|---|---|---|---|
| Apparent phenomena | Clear<br>Transparent<br>Low viscous | Clear<br>Transparent<br>Low viscous | Clear<br>Transparent<br>High viscous | A little turbid | Turbid low viscous |
| Aggregates' type | Rod-like micelles | Rod-like micelles | Worm-like micelles | Vesicle and micelles | Vesicle and micelles |

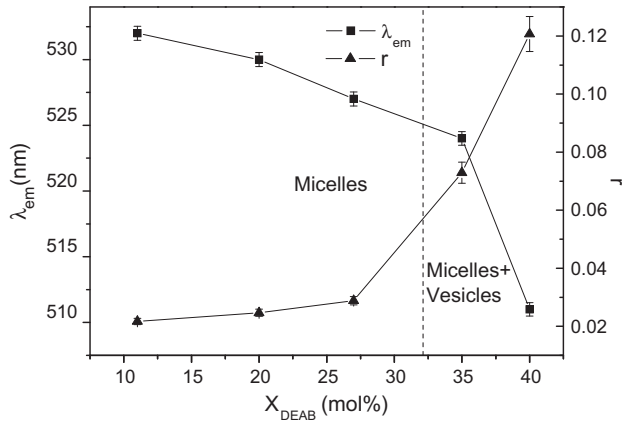


**Fig. 7.** Fluorescence anisotropy of 12-DAN-ADA in SDS/DEAB system ($C_T$ = 200 mM, $C_{12\text{-DAN-ADA}} = 1.0 \times 10^{-6}$ M, $T$ = 25 °C).

formation in the solution. This enables the interaction of cationic DEAB with anionic 12-DAN-ADA so that smears the background emission.

### *3.4. The influence of solution viscosity on the fluorescence anisotropy of 12-DAN-ADA*

Another important point should be addressed for the results in Section 3.3 is the effect of viscosity on the fluorescence anisotropy. It is well-known that the apparent viscosity increases as the micelles grow. One may argue that this bulk viscosity might affect the fluorescence anisotropy since the latter reflects the rotation difficulties of the fluorescent molecules. Therefore, it is very necessary to consider the influence of bulk viscosity on the fluorescence anisotropy before any powerful conclusion is made. As shown in Table 1, firstly, the apparent viscosity increases as the micelles grow, and then decreases as the worm-like micelles transform into vesicles. In contrast, the fluorescence anisotropy of 12-DAN-ADA in the whole process increases monotonously (cf. Fig. 7). This result indicates that the increase of the fluorescence anisotropy is indeed caused by the dense packing of molecules in the micelles, not by the increase in bulk viscosity.

The influence of bulk viscosity on the fluorescence anisotropy can also be excluded by controlled experiments made in glycerine solution. The fluorescence anisotropy of 12-DAN-ADA in 1 Pa s glycerine solution is about 0.2 [27]. In contrast, it is only 0.027 in a 100 Pa s SDS/DEAB (73:27) system ($C_T$ = 180 mM) [36]. This result further confirms that the fluorescence anisotropy is only influenced by the compact surfactant molecular arrangement, not by the bulk viscosity.

## 4. Summary

We have reported in the present work that the fluorescence labeled surfactant12-DAN-ADA can be used to detect the growth of micelles formed in cationic, anionic and especially catanionic surfactant systems. With the fluorescent group positioned in the tail of the hydrophobic chain, the fluorescence anisotropy of 12-DAN-ADA can sensitively detect subtle changes in the molecular packing accompanied with the micellar growth. The sensitivity of the 12-DAN-ADA probe in detecting micellar growth in the cationic surfactant system and the mixed system of oppositely charged surfactant system is much higher than that in the anionic system without the presence of water soluble quenchers. This is attributed to reduce of the amount of free 12-DAN-ADA molecules in the bulk solution so that smearing the noise by the electrostatic interaction between the cationic surfactant and the anionic 12-DAN-ADA. However, in the presence of water soluble quenchers, the sensitivity of 12-DAN-ADA to detect the anionic micelles can be very high as well owing to the smearing of the background fluorescence. These findings experimentally verified the environmental changes in the process of micellar growth in molecular level, which on the one hand provides an effective method to detect micellar growth regardless the type of the micelles, on the other hand, enriches the fundamental knowledge about subtle changes inside the micelles along with micellar growth. These are very helpful for researchers in the field of colloid and interface science to understand better all the possible changes in the process of surfactant self-assembly transition.

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