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A b s t r a c t

Pyrene fluorescence intensity and conductivity measurements were used to determine the cmc of two monomeric surfactants namely, dodecyltrimethylammonium bromide and cetyltrimethylammonium bromide as well as two gemini surfactants, 16-3-16 2Br and 16-4-16 2Br of the alkanediyl-α,ω-bis(alkyldimethylammonium bromide) type. Cmc measurements were confirmed using fluorescence anisotropy measurements employing fluorescein as probe. Anisotropy–concentration plots showed sigmoidal behaviour and the obtained values for cmc were in good agreement with those determined using conductivity and pyrene fluorescence intensity. Also, the anisotropy of both fluorescein and another fluorescent probe, perylene, were used to provide information on the micellar structure.

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1. Introduction

Gemini surfactants first became of interest some fifteen years ago [1]. They consist of two hydrophilic headgroups connected by a spacer of variable length which bears one hydrophobic chain each. Gemini surfactants show very interesting properties with respect to their ‘‘monomers’’ [1,2] namely, lower critical micellar concentration (cmc), excellent adsorption at both air/water and solid/water interfaces as well as the ability to form micelles of different shapes and dimensions (e.g. spherical, rod-like, thread-like, etc.) even at low concentration. Cationic gemini surfactants have enjoyed widespread interest, as exemplified by the preparation of mesoporous materials [3,4] gene transfection [5], solubilization of oils [6] and the emulsification of monomers [7]. Cationic surfactants have received attention in terms of their possible use in TiO_2 preparation [8] and application in dye-sensitized solar cells (DSSC) as ionic liquids [9].

The use of dyes fluorescence for the determination of their interaction with surfactants is a well known topic. However, there is always a need for the exploitation of fluorescent dyes in a different way in order to detect changes in the colloidal medium. Sometimes it was found useful to resort to well-known dyes that can obtain new life from a new application, like the recently introduced SPQ (6-methoxy-N-(3-sulfopropyl)quinolinium) fluorescence quenching method for the cmc and the micellar degree of counterion binding determination [10]. Here we propose the use of a very cheap and well known dye, like fluorescein sodium salt, for the cmc determination, based on the measurement of the fluorescence anisotropy of the dye. The change in fluorescence anisotropy is a good way to follow surfactant aggregation, being very low in premicellar state and higher when micelles form. The fluorescence anisotropy of dyes was already used for the cmc determination [11–13], but in most cases the probes used were very hydrophobic probes that normally reside in the micellar core or were some amphiphilic dyes [12]. In general the use of dyes for both absorption and emission spectroscopies was sometimes questioned since the introduction of a probe could modify the micellar organization. In several cases however those methods work fine and are undoubtedly useful for colloidal characterization.

In the present work, we show that the use of the anisotropy of fluorescein sodium salt gives very good results for the cmc determination and also gives a qualitative indication of the micellar surface compactness. We applied this technique for the cmc determination of two monomeric surfactants DTAB (dodecyltrimethylammonium bromide) and CTAB (cetyltrimethylammonium bromide) and two gemini surfactants (16-3-16 2Br and 16-4-16 2Br) of the alkanediyl-a,u-bis(alkyldimethyl ammonium bromide) type, referred to as 16-n-16 2Br, where 16 and n are the carbon numbers of the surfactant alkyl chain and of the alkanediyl (polymethylene) spacer group, respectively and Br stands for bromide (see Fig. 1).

The 16-n-16 2Br surfactants are one of the most interesting family of surfactants belonging to the more general class of the m-s-m 2Br amphiphiles. They show very interesting properties in aqueous solutions [14–16]: their aggregate morphologies are strongly dependent on the chain and spacer lengths as Cryo-TEM studies suggested [16]. Those amphiphiles were involved in several applications even if their solution behaviour was not completely elucidated.

During the study of this particular class of surfactants, we developed an approach for the cmc determination based on fluorescence anisotropy using fluorescein sodium salt as the probe, also characterizing them with both the conductivity technique and pyrene fluorescence. This last well established method was never applied to those compounds. Moreover, the anisotropy of fluorescein sodium salt and of another fluorescent probe, i.e. perylene, has been used for clarifying the formation of micelles [17]. Since the two probes are located in a different position in the micelle (fluorescein in the palisade region while perylene in the core) [17,18], the use of this technique can also provide qualitative information that can be related to the micellar compactness and microenvironmental constraints of the micellar probe solubilization site.

2. Experimental section

2.1. Materials

CTAB, pyrene, fluorescein sodium salt and perylene were purchased from Fluka while DTAB was purchased from Sigma Aldrich. Pyrene, fluorescein sodium salt and perylene were fluorescent grade; all chemicals were used as received without further purification.

The two gemini surfactants were synthesized from the reaction of α,ω-dibromoalkanes with N,N,N-hexadecyldimethylamine as reported by Zana et al. [14,19]. The purity of the surfactants was checked using ^{1}H NMR, TLC (Thin Layer Chromatography) and LC-MS Mass Spectrometry.

16-3-16 2Br: ^{1}H NMR ($CDCl_3$): δ (ppm) 0.86 (t, 6H, CH_3-alkyl chain), 1.27 (bs, 52H, $26CH_2$), 1.76 (t, 4H, NCH_2CH_2-alkyl chain), 2.66 (m, 2H, $NCH_2CH_2CH_2N$, spacer), 3.38 (s, 12H, $4CH_3$), 3.52 (t, 4H, NCH_2-alkyl chain), 3.78 (t, 4H, $NCH_2CH_2CH_2N$).

16-4-16 2Br: ^{1}H NMR ($CDCl_3$): δ (ppm) 0.84 (t, 6H, CH_3-alkyl chain), 1.26 (bs, 52H, $26CH_2$), 1.72 (t, 4H, NCH_2CH_2-alkyl chain) 2.07 (m, 4H, NCH_2CH_2 CH_2CH_2N, spacer), 3.28 (s, 12H, 4 CH_3), 3.41 (t, 4H, NCH_2-alkyl chain), 3.85 (t, 4H, NCH_2CH_2

CH_2CH_2N).

2.2. Conductivity measurements

Conductivity measurements were performed on a conductivity meter equipped with a conductivity cell having cell constant of 0.943 cm^{-1} as already reported [20]. The addition of concentrated surfactant solution by a titrator and the collection of the conductivity data were performed by using a computer controlled auto-mated system, working with a program written in Quick Basic, available from the authors. Water of MilliQ quality (conductivity: 0.05 mS; surface tension: 72.8 mN/m at 20°C) was used for the measurements.

2.3. Fluorescence measurements

The steady-state intensity and anisotropy fluorescence measurements were performed on a Perkin Elmer LS 55 spectrofluorimeter. Pyrene stock solution was prepared by dissolving 5.3 mg of pyrene in 10 ml of methanol and 0.5 ml of this solution was diluted to 10 ml; perylene stock solution was prepared by dissolving 3.3 mg of perylene in 10 ml of ethanol and 0.25 ml of this solution was diluted to 10 ml; fluorescein stock solution was prepared by dissolving 3.9 mg of fluorescein in 10 ml of water and 1 ml of this solution was diluted to 5 ml. 10 ml of the probe stock solution was added to the sample solutions prepared by dilution of the different surfactants stock solutions. Pyrene spectra were collected in the 360–600 nm range, checking carefully that no excimer was formed: final concentration 5 x 10^{-7} M, excitation wavelength 320 nm; excitation slit 5 nm and emission slit 2.5 nm. The anisotropy settings were: (a) for fluorescein: final concentration 5 x 10^{-8} M, excitation wavelength 491 nm; emission wave-length 523 nm; excitation slit 2.5; emission slit 2.5 nm, integration 5 s; (b) for perylene final concentration 3.5 x 10^{-7} M, excitation wavelength 412 nm; emission wavelength 472 nm; excitation slit 2.5; emission slit 5.0 nm, integration 5 s. Measurements were taken until equilibrium was attained. As for anisotropy measurements for the micellar structure investigation, the surfactant solutions had a concentration ten times the estimated cmc value. To these solutions, 10 ml of an ethanolic solution of perylene or an aqueous solution of fluorescein sodium salt was added.

3. Results and discussion

3.1. Conductivity

The change in the specific conductivity of aqueous ionic surfactant solutions at the cmc is due to the different degree of surfactant ionization below and above the cmc. Actually, below the cmc, where no micelles are formed, the specific conductivity of a surfactant depends on the contributions of anions and cations in solution while above the cmc, the conductivity of ionic surfactants usually decreases because of the inclusion at the micellar surface of ions having opposite charge (counterions) to the long-chain ions.

The cmc values were obtained from specific conductivity vs. surfactant concentration by the intersection of the lines fitted in the diluted and concentrated regions before and after the cmc. The evaluation of the degree of counterion dissociation α and of counterion binding β ($\beta = 1 - \alpha$) is normally carried out as the ratio of the slope of the lines graphically fitted in the premicellar and postmicellar ranges, respectively. Since for gemini surfactants with spacer n = 3, 4 the shape of the plots is smooth (Fig. 2) and the lines are quite difficult to be precisely defined, we also used a different approach in the data analysis based on a non-linear fit introduced by Carpena et al. [21] which we already successfully applied to elaborate conductivity data obtained on gemini pyridinium surfactants [22]. The main power of this method resides in the fact that no personal taste or choice has to be applied on raw data, thus avoiding artefacts from both the researcher opinion and the introduction of noise, which is usual when the data are, by some way, manipulated or transformed.

In Table 1 the cmc values for both monomeric and gemini surfactants obtained by this method are reported and are in agreement with conductivity data found in literature [1,14,23]. β values are also in agreement with literature for DTAB, CTAB and 16-3-16 2Br while for 16-4-16 2Br we obtained a higher value than that first reported in the literature by Zana, but very similar to that reported by Oliviero et al. [24]. These results will be discussed again, and confirmed, in view of the results collected with fluorescence measurements we can say that, from our data, it seems that b would indicate a slightly more compact micellar surface for 16-4-16 2Br, with respect to 16-3-16 2Br. In general a high value of β means that the headgroups are kept closer to each other, due to a higher number of counterions, lying among the positive heads, that reduces coulombic repulsion. Since fluorescein is singly deprotonated at pH 7, the interaction of the dye with the two charges of a single gemini molecule is highly probable. β is lower for geminis than for DTAB and CTAB, due to the difficulty for geminis to pack two hydrophobic chains per molecule in the micellar core.

3.2. Fluorescence intensity of pyrene

Pyrene (benzophenanthrene) has been widely used in literature [25–27] to study surfactants aggregation. Its emission characteristics (I_1/I_3, ratio of intensities of the first and third vibronic peaks) are considered to estimate the polarity level of its solubilization environment and this peculiarity was applied to monitor the micelles formation in solution. The resultant plots of the pyrene I_1/I_3 ratio as a function of the total surfactant concentration show, around the cmc, a typical sigmoidal decrease. Below the cmc, the pyrene I_1/I_3 ratio value corresponds to a polar environment of water solution; as the surfactant concentration increases, the pyrene I_1/I_3 ratio decreases rapidly, indicating that the pyrene is sensing a more hydrophobic environment. Above the cmc, the pyrene I_1/I_3 ratio reaches a roughly constant value due to the incorporation of the probe into the hydrophobic region of the micelles. A problem arises from the fact that there is no objective and unified method to obtain the cmc value from the plots of pyrene ratio I_1/I_3 vs. surfactant concentration, and different authors seem to take different criteria to choose this point. As stated above, Zana and co-workers [28] suggested that the cmc can be alternatively obtained from two singular points in the pyrene I_1/I_3 ratio plots. On the contrary, a recent approach to the evaluation of the curve shape [29] is based on the assumption that the pyrene I_1/I_3 ratio plots can be adequately described by a decreasing sigmoid of the Boltzmann type, which is given by equation (1):

(1)

where the variable y corresponds to the pyrene I_1/I_3 ratio value, the independent variable x is the total concentration of surfactant, A_1 and A_2 are the upper and lower limits of the sigmoid, respectively, x_0 is the centre of the sigmoid, and Δx is directly related to the independent variable range where the abrupt change of the dependent variable occurs. This method shows a mathematical way to establish where the transition does occur and which part of the graph should indicate the cmc.

The cmc determination based on pyrene fluorescence (I_1/I_3 ratio) was never accomplished for the present gemini compounds (IIIa and IIIb in Fig. 1). The resultant plots of the pyrene I_1/I_3 ratio as a function of the surfactant concentration show a typical sigmoidal decrease [29] for both monomeric (I and II) and gemini surfactants (IIIa and IIIb), giving cmcs consistent with conductivity (see Table 2). However, the transition between the monomeric and the micellar state is abrupt for DTAB and CTAB (see CTAB in Fig. 3) and gradual for gemini surfactants (Fig. 4, 16-3-16 2Br as an example).

I_1/I_3 is measuring the wetting/dewetting of the pyrene solubilization site and follows the reverse order of the degree of counterion binding. The I_1/I_3 value, when the micellization process is concluded (see Table 2), suggested that the micelle shape

and/or micellar surface compactness influence significantly the pyrene solubilization site; consequently, the probe senses a different polarity in the different micelles.

3.3. Fluorescence anisotropy of fluorescein sodium salt

3.3.1. Fluorescence anisotropy principle

Anisotropy measurements [30] are based on the molecular motion of fluorescent molecules in solution in the time window occurring between absorption and emission of light. According to equation (2), the fluorescence anisotropy (r) values were determined as:

(2)

where I_{vv} and I_{vh} represent the vertically and horizontally polarized emission intensities, respectively, following instrumental excitation with vertically polarized light and G is a correction factor which detects the instrumental sensitivity of the polarization direction of emission. G is defined as $G = I_{hv}/I_{hh}$, where I_{hv} and I_{hh} represent the vertically and horizontally polarized emission intensities obtained by excitation with horizontally polarized light. G factor was estimated every day before starting measurements.

3.3.2. Cmc determination by fluorescence anisotropy of fluorescein sodium salt

Several phenomena can decrease the measured anisotropy: the most common one is the probe rotational diffusion, which occurs during the lifetime of the excited state and displaces the emission dipole of the fluorophore. In this work we applied this simple principle for the determination of the cmc taking into account that:

(1) if a fluorescent probe is added to a surfactant solution whose concentration is below the cmc, the measured anisotropy will be low due to the fact that the fluorophore will remain in the water phase and will have a high rotational rate; (2) when the surfactant solution reaches a concentration at which the micelles start to form (i.e. at the cmc), the anisotropy will obviously increase

because the rotational diffusion of the probe will be decreased owing to the constraints inside the micelles.

Since fluorescence anisotropy comes out from the restriction of the mobility of the probe during the light absorption and light emission events, it seems reasonable to relate the anisotropy of the micellar state, if a constant value can be obtained, to a restricted environment where the probe is located. Few literature attempts [11–13] have appeared on the surfactant cmc determination using anisotropy, giving sometimes poorly defined plots and results not in good agreement with data obtained by other techniques. An hydrophobic probe such as DPH [31] or perylene [17] was used to reveal the micellar core microviscosity. Apart from the use of an amphiphilic derivative of the fluorescein [12], probes that can monitor the micellar surface or the palisade layer were never used for cationic surfactants working with fluorescence anisotropy, as far as we know.

We used fluorescein sodium salt fluorescence anisotropy for the determination of the cmc of the above mentioned surfactants. The use of commercial fluorescein gave excellent results from both the plot shape and the cmc values, by sigmoidal fitting of the experimental points as for pyrene fluorescence intensity measurements. The cmc values are in excellent agreement with the data obtained by conductivity and pyrene fluorescence in the present work and with literature data as reported in Table 2. The anisotropy vs. C plot for gemini surfactants shows that the concentration range (Dx) where the transition between the pre-micellar and the micellar state occurs is larger than that observed for monomeric surfactants (see 16-3-16 vs. CTAB in Figs. 5 and 6).

An analysis of the plots by sigmoidal fit gives the CMC/Dx param-eter [29] as large as 21.12 for CTAB with respect to 2.97 for 16-3-16, where Dx is the transition amplitude (4.45×10^{-5} for CTAB and 8.73×10^{-6} for 16-3-16). This different behaviour between gemini and monomeric surfactants is also observed by comparing data obtained by other techniques and could be correlated to a greater difficulty in the micelles formation for gemini surfactants: a gradual micellization process occurs because of the difficulty of arrangement of two hydrophobic chains per molecule in the micellar core.

This technique could be in principle exploited to detect micellar shape transitions [32] since they are normally accompanied by a reduction of the available volume or space among surfactant headgroups making the volume probe more constraint.

3.3.3. Fluorescence anisotropy of fluorescein sodium salt and perylene for micellar structure investigation

Fluorescence anisotropy can qualitatively give information on the micellar compactness and can help in making hypothesis on micelles shape. Actually, if other literature data are available, or if a deeper investigation using particular techniques (SANS, Cryo-TEM studies or dynamic light scattering) is performed, fluorescence anisotropy can confirm micellar structures and

compactness. Two probes such as fluorescein sodium salt and perylene were taken into account in order to monitor the different probe mobilities in the micellar surface (or palisade) and core respectively. Actually, fluorescein sodium salt is located in the palisade region while perylene is placed in the micellar core as reported in Fig. 7 [17,18].

The anisotropy obtained for the micellar state is increasing, for both probes, when the chain is lengthened, i.e. from DTAB to CTAB, suggesting that a more compact and rigid micellar structure is obtained. By comparing CTAB and 16-3-16, i.e. the monomer and a gemini surfactant, a similar behaviour is shown, evidencing that in gemini surfactants micelles the probe mobility is more restricted. This is in agreement with SANS evidences [33] of large prolate ellipsoidal structures for 16-n-16 (n = 3, 4). This could account for the higher restriction of the probe mobility since those arrange-ments are more compact at the micellar surface than spherical micelles typical for CTAB like surfactants. We can recall from literature that, at concentrations ten times the cmc, CTAB and DTAB form spherical micelles [34], while 16-3-16 2Br [35] and 16-4-16 2Br [33,36] present rod-like micelles. In a qualitative way the micellar anisotropy value can indicate that gemini aggregates could not have a spherical shape since the r values for geminis are higher in respect with monomers for which the probe can rotate more easily in a spherical structure. Actually, the compactness of micellar surface is lower for geminis than for CTAB and, by only inspecting the compactness, we should expect a lower r value for geminis with respect to CTAB since from the degree of counterion binding the micellar surface compactness is higher for CTAB than for gemini surfactants. The fact that the opposite occurs is probably due to both ionic interaction of the negative charge of fluorescein with the two positive headgroups of a single gemini molecule and hydro-phobic effect due to its interaction with the spacer. Li et al. [37] showed that the same kind of surfactants keep one of the two bromide counterions in between the two positive heads, forming in practice an ion pair. Nearly the same can occur with fluorescein, for which also the hydrophobic nature of the dye can play a role in the interaction with the alkyl chains and the spacer of the surfactant molecule, like benzoic acid does for similar cationic gemini surfactants [38]. This interaction can highly restrict the probe rotational motion, thus raising the fluorescence anisotropy.

Fluorescence anisotropy, for geminis, follows the correct order along with the b value trend. This means that 16-4-16 2Br forms micelles having more compact surface than 16-3-16 2Br. The longer spacer can permit the hydrophobic chains of 16-4-16 2Br to accommodate more easily (finding more space) in the micellar core than that of 16-3-16 2Br. In this case the headgroups can be kept closer to each other, giving a higher b value (see Section 3.1) and raising the fluorescein anisotropy. More studies are needed, however, to elucidate if the obtained anisotropy can also be due, at least in part, to differences in the micellar shape (spherical for ‘‘monomers’’ and rod-like for ‘‘geminis’’).

Perylene anisotropy monitors the probe mobility restriction in the micellar core (Table 2). The micellar core appears more

compact when the chain is longer (see DTAB vs. CTAB), and for gemini surfactants (see CTAB vs. 16-3-16 2Br), probably due to a higher compactness of the core, which is usual for geminis. While being small, the difference for both fluorescein and perylene anisotropy for 16-3-16 2Br and 16-4-16 2Br surfactants seems to indicate a more compact micellar surface and, at the same time, a higher fluidity of the micellar core for 16-4-16 2Br vs. 16-3-16 2Br. In a few words, the probe mobility in the micellar core is raised when the spacer is longer, while the reverse is observed for the micellar surface, in qualitative agreement with the change of micellar shape.

4. Conclusions

Fluorescence anisotropy was demonstrated to be a successful method for the determination of cmc of two monomeric and two gemini cationic surfactants whose results are in agreement with other more applied methods such as conductivity and pyrene fluorescence intensity. Fluorescence anisotropy with fluorescein is a simple straightforward method, fast to apply, that requires quite standard instrumentation. Furthermore, the use of fluorescein sodium salt and perylene as probes in the anisotropy measurements helped us in a qualitative understanding of the surface compactness of the tested surfactant micelles. The micelles of monomeric surfactants become more compact by lengthening the hydrophobic chain and gemini surfactants (16-s-16 2Br) give more compact micelles than CTAB. Hence, this technique can be used to easily explore aggregation of other cationic surfactants.

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References

[1]Rosen MJ, Tracy DJ. Gemini surfactants. J Surfactant Deterg 1998;1:547–54.

[2]Fisicaro E, Compari C, Rozicka-Roszak B, Viscardi G, Quagliotto P. Curr Top Colloid Interface Sci 1997;2:54–68.

[3]Huo Q, Margolese DI, Stucky GD. Surfactant control of phases in the synthesis of mesoporous silica-based materials. Chem Mater 1996;8:1147–60.

[4]Gianotti E, Berlier G, Costabello K, Coluccia S, Meneau F. In-situ synchrotron small-angle X-ray scattering study of MCM-41 crystallisation using gemini surfactants. Catal Today 2007;126(1–2):203–10.

[5]Chen X, Wang J, Shen N, Luo Y, Li L, Liu M, et al. Gemini surfactant/DNA complex monolayers at the air–water interface: effect of surfactant structure on the assembly, stability, and topography of monolayers. Langmuir 2002;18:6222–8.

[6]Chen H, Han L, Luo P, Ye Z. The interfacial tension between oil and gemini surfactant solution. Surf Sci 2004;552:L53–7.

[7]Summers M, Eastoe J. Applications of polymerizable surfactants. Adv Colloid Interface Sci 2003;100–102:137–52.

[8]An HJ, Jang SR, Vittal R, Lee J, Kim KJ. Cationic surfactant promoted reductive electrodeposition of nanocrystalline anatase TiO_2 for application to dye-sensitized solar cells. Electrochim Acta 2005;50:2713–8.

[9]Suryanarayanan V, Lee KM, Ho WH, Chen HC, Ho KC. A comparative study of gel polymer electrolytes based on PVDF-HFP and liquid electrolytes, containing imidazolinium ionic liquids of different carbon chain lengths in DSSCs. Sol Energy Mater Sol Cells 2007;91:1467–71.

[10] Kuwamoto K, Asakawa T, Ohta A, Miyagishi S. Degree of micelle ionization and micellar growth for gemini surfactants detected by 6-methoxy-N-(3-sulfo-propyl)quinolinium fluorescence quenching. Langmuir 2005;21(17):7691–5.

[11] Zhang X, Jackson JK, Burt HM. Determination of surfactant critical micellar concentration by a novel fluorescence depolarization technique. J Biochem Biophys Methods 1996;31:145–50.

[12] Thorsteinsson MV, Richter J, Lee AL. 5-Dodecanoylaminofluorescein as a probe for the determination of critical micelle concentration of detergents using fluorescence anisotropy. Anal Biochem 2005;340(2):220–5.

[13] Chaudhuri R, Guharay J, Sengupta PK. Fluorescence polarization anisotropy as a novel tool for the determination of critical micellar concentrations. J Photochem Photobiol A Chem 1996;101:241–4.

[14] Zana R, Benrraou M, Rueff R. Alkanediyl-α,ω-(dimethylalkylammonium bromide) surfactants. 1. Effect of the spacer

length on the critical micelle concentration and micelle ionization degree. Langmuir 1991;7:1072–5.

[15] Alami E, Beinert G, Marie P, Zana R. Alkanediyl-α,ω-(dimethylalkylammonium bromide) surfactants. 3. Behaviour at the air–water interface. Langmuir 1993;9:1465–7.

[16] Danino D, Talmon Y, Zana R. Alkanediyl-α,ω-(dimethylalkylammonium bromide) surfactants. 5. Aggregation and microstructure in aqueous solutions. Langmuir 1995;11:1448–56.

[17] Garcia-Sanchez F, Ruiz CC. Intramicellar energy transfer in aqueous CTAB solutions. J Lumin 1996;69:179–86.

[18] Ruiz CC. Fluorescence anisotropy of probes solubilized in micelles of tetradecyltrymethylammonium bromide: effect of ethylene glycol added. J Colloid Interface Sci 2000;221:262–7.

[19] Zana R. Alkanediyl-α,ω-bis(dimethylalkylammonium bromide) surfactants. 10. Behavior in aqueous solution at concentrations below the critical micellization concentration: an electrical conductivity study. J Colloid Interface Sci 2002;246:182–90.

[20] Viscardi G, Quagliotto P, Barolo C, Savarino P, Barni E, Fisicaro E. Synthesis and surface and antimicrobial properties of novel cationic surfactants. J Org Chem 2000;65(24):8197–203.

[21] Carpena P, Aguiar J, Bernaola-Galvan P, Ruiz CC. Problems associated with the treatment of conductivity-concentration data in surfactant solutions: simu-lations and experiments. Langmuir 2002;18:6054–8.

[22] Quagliotto P, Viscardi G, Barolo C, Barni E, Bellivia S, Fisicaro E, et al. Gemini pyridinium surfactants: synthesis and conductometric study of a novel class of amphiphiles. J Org Chem 2003;68(20):7651–60.

[23] Buckingham SA, Garvey CJ, Warr GG. Effect of head-group size on micelliza-tion and phase behavior in quaternary ammonium surfactant system. J Phys Chem 1993;97(39):10236–44.

[24] Oliviero C, Coppola L, La Mesa C, Ranieri GA, Terenzi M. Gemini surfactant– water mixtures: some physical–chemical properties. Colloids Surf A: Physicochem Eng Aspects 2002;201:247–60.

[25] Dominguez A, Fernandez A, Gonzalez N, Iglesias E, Montenegro L. Determination of critical micelle concentration of some surfactants by three techniques. J Chem Educ 1997;74:1227–31.

[26] van Stam J, Depaemelaere S, De Schryver C. Micellar aggregation numbers – a fluorescence study. J Chem Educ 1998;75(1):93–8.

[27] Kalyanasundaram K, Thomas JK. Environmental effects on vibronic band intensities in pyrene monomer fluorescence and their application in studies of micellar systems. J Am Chem Soc 1977;99(7):2039–44.

[28] Zana R, Levy H, Kwetkat K. Mixed micellization of dimeric (gemini) surfactants and conventional surfactants. I. Mixtures of an anionic dimeric surfactant and of the non ionic surfactants $C_{12}E_5$ and $C_{12}E_8$. J Colloid Interface Sci 1998;197(2):370–6.

[29] Aguiar J, Carpena P, Molina-Bolivar JA, Ruiz CC. On the determination of the critical micelle concentration by the pyrene 1:3 ratio method. J Colloid Interface Sci 2003;258:116–22.

[30] Lakowicz JR. Principles of fluorescence spectroscopy. New York: Springer-Verlag; 2006.

[31] Zana R, In M, Levy H, Duportail G. Alkanediyl-a,u-bis(dimethylalkylammo-nium bromide). 7. Fluorescence probing studies of micelle micropolarity and microviscosity. Langmuir 1997;13:5552–7.

[32] Gao L, Song Q, Huang X, Huang J. A new surfactant-fluorescence probe for detecting shape transitions in self-assembled systems. J Colloid Interface Sci 2008;323:420–5.

[33] De S, Aswal VK, Goyal PS, Bhattacharya S. Role of spacer chain length in dimeric micellar organization. Small angle neutron scattering and fluorescence studies. J Phys Chem 1996;100:11664–71.

[34] Shikata T, Hirata H, Kotaka T. Micelle formation of detergent molecules in aqueous media: viscoelastic properties of aqueous cetyltrimethylammonium bromide solutions. Langmuir 1987;3:1081–6.

[35] Aswal VK, De S, Goyal PS, Bhattacharya S, Heenan RK. Transition from disc to rod-like shape of 16-3-16 dimeric

micelles in aqueous solutions. J Chem Soc Faraday Trans 1998;94:2965–7.

[36] Aswal VK, De S, Goyal PS, Bhattacharya S, Heenan RK. Small-angle neutron scattering study of micellar structures of dimeric surfactants. Phys Rev E 1998;57(1):776–83.

[37] Li ZX, Dong CC, Thomas RK. Neutron reflectivity studies of the surface excess of gemini surfactants at the air water interface. Langmuir 1999;15(13):4392–6.

[38] Kabir-ud-Din Fatma W, Khan ZA, Dar AA. ^{1}H NMR and viscometric studies on cationic gemini surfactants in presence of aromatic acids and salts. J Phys Chem B 2007;111(30):8860–7.

Fig. 1. Structures of the studied surfactants.

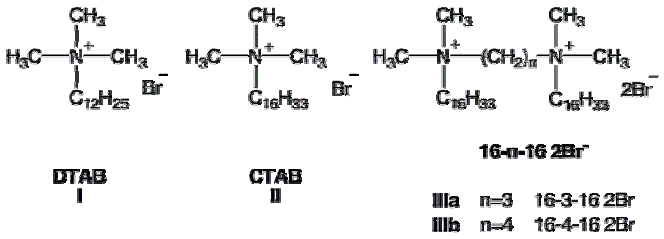

Fig. 2. Plot of conductivity vs. concentration for ●16-3-16 2Br and ▲16-4-16 2Br

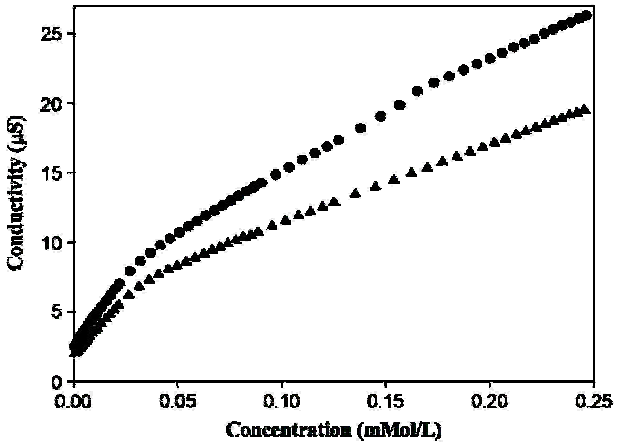

Fig. 3. Plot of fluorescence pyrene I1/I3 vs. concentration data for CTAB

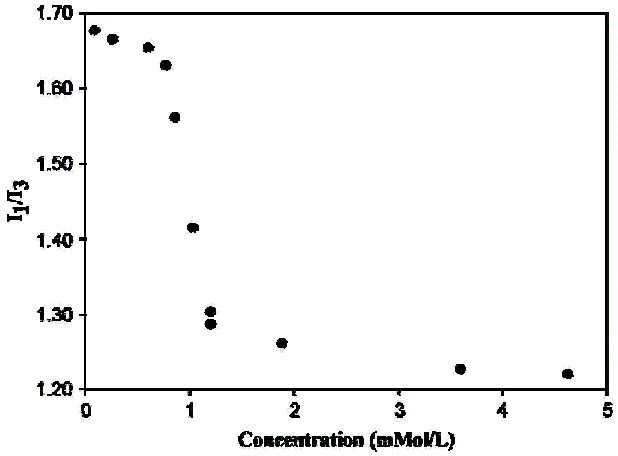

Fig. 4. Plot of the fluorescence pyrene I_1/I_3 intens

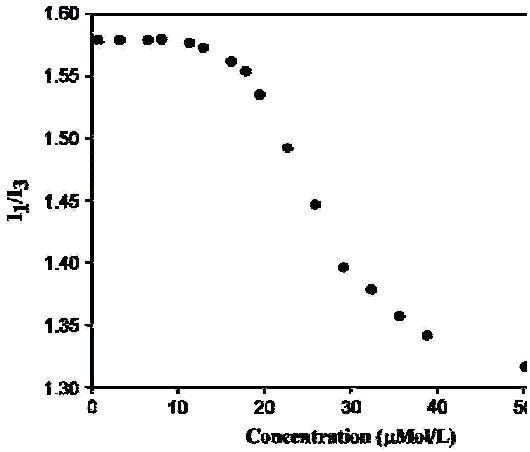

Fig. 5. Plot of the fluorescence anisotropy of fluo for 16-3-16 2Br.

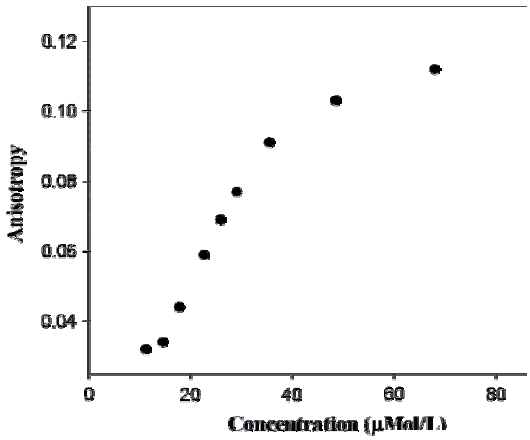

Fig. 6. Plot of the fluorescence anisotropy of fluo