J Fluoresc (2009) 19:327–332
DOI 10.1007/s10895-008-0420-4

ORIGINAL PAPER

Spectrofluorimetric Determination of Second Critical Micellar Concentration of SDS and SDS/Brij 30 Systems

Ana Paula Romani · Antonio Eduardo da Hora Machado · Noboru Hioka · Divinomar Severino · Mauricio S. Baptista · Lúcia Codognoto · Maira R. Rodrigues · Hueder Paulo Moisés de Oliveira

Received: 30 April 2008 / Accepted: 12 September 2008 / Published online: 25 September 2008

Abstract Potentially useful stead-state fluorimetric technique was used to determine the critical micellar concentrations (CMC_1 and CMC_2) for two micellar media, one formed by SDS and the other by SDS/Brij 30. A comparative study based on conductimetric and surfacial tension measurements suggests that the CMC_1 estimated by the fluorimetric method is lower than the value estimated by these other techniques. Equivalent values were observed for SDS micelles without Brij 30 neutral co-surfactant. The use of acridine orange as fluorescent probe permitted to determine both CMC_1 and CMC_2. Based on it an explanation on aspects of micelle formation mechanism is presented, particularly based on a spherical and a rod like structures.

A. P. Romani
Departamento de Química–FFCLRP, Universidade de São Paulo, Ribeirão Preto, Brazil

A. E. da Hora Machado
LAFOT–Instituto de Química, Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil

N. Hioka
Departamento de Química, Univeridade Estadual de Maringá, Maringá, Paraná, Brazil

D. Severino · M. S. Baptista
Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brazil

L. Codognoto · M. R. Rodrigues · H. P. M. de Oliveira (✉)
Instituto de Pesquisa e Desenvolvimento—IP&D, Universidade do Vale do Paraíba—UNIVAP, Av. Shishima Hifumi 2911, Urbanova, CEP: 12244-000 São José dos Campos, SP, Brazil
e-mail: hueder@univap.br

Keywords SDS · CMC · Fluorescence · Dyes · Brij-30

Introduction

Surfactants tend to form micelles in aqueous solutions at a characteristic concentration named critical micellar concentration (CMC). When the concentration of a surfactant is increased, the structure in water may change from single molecules to spherical, rod- and lamellar-shaped micelles. Spherical micelles are formed at the called first critical micellar concentration (CMC_1) [1–4]. The second CMC indicates the structural transition from spherical micelles to rod-like ones (CMC_2). Many methods such as surface tension, conductivity, solubilization, and light scattering [1] have been used to determine the CMC_1. These methods are simple and easily utilized. However, few techniques have been applied to determine the second CMC [3, 4]. The occurrence of this second aggregation condition is due to a new rearrangement of the micellar structures. The maximum effective concentration for each surfactant falls within the concentration range defined by the CMC_1 and CMC_2 values. The concentration latitude of this plateau region depends upon the structure and hydrophobicity of the particular surfactant, and the presence of hydrolilic substances, and as such may extend over orders of magnitude [5]. In consequence, the profile of some physical properties of the system as a function of the surfactant concentration shows a break point (*ex.* conductivity, surface tension, osmotic pression, etc).

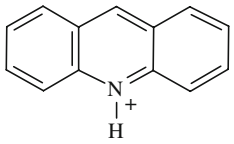
Organized colloidal systems such as micelles, vesicles and micro and nanoparticles have been focus of intense scientific research, mainly considering the capability of some of them as efficient drug delivery system [6–12].

The formation of mixed micelles, involving two surfactants is considered to be interesting in view of their more energetically favorable properties than individual surfactants in various commercial products [13]. Ionic/nonionic and anionic/cationic mixtures of surfactants show significant non-ideal behavior whereas mixtures of unlike surfactant monomers with identical polar head groups and different alkyl chains generally exhibit ideal mixing [13–16].

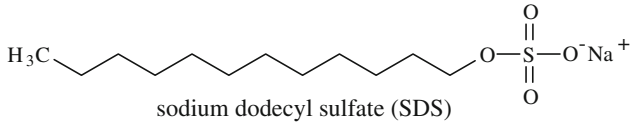
There are several methods to determine the CMC of mixed surfactants systems: conductimetry, surface tension, spectroscopy, etc [13]. Sodium dodecyl sulphate, SDS, in water presents a CMC_1 of 8 mM measured by surface tension [16]. In anionic micelles there are strong ionic interactions at the electrical double layer. Adding a neutral co-surfactant, are expected to bring additional favorable interactions to low down the energy in the micellar system formation. Neutral surfactants present no ionic counter-ion and attractive interactions with cationic counter-ion from SDS. So, a lower cmc is expected in a mixed micellar system.

Photophysical processes have high sensitivity to investigate the dynamics and the structure of the surfactant systems [17–19]. Dyes have used with success as fluorescent probes to estimate the CMC [16, 17, 19, 20] and in the sensing of other physical properties of organized media [10–12, 21–25].

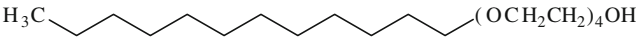
In the present work, acridine orange was used as fluorescent probe in the SDS-Brij 30 (polyoxyethylene 4 lauryl ether) system. An easy and useful method that uses the spectroscopic acridine orange properties to study the micelle characteristics of SDS and Brij 30 system is presented (chemical structures of SDS, BRIJ 30 and acridine orange are exhibited in Fig. 1).



Acridine



sodium dodecyl sulfate (SDS)



polyoxyethylene-4-lauryl ether (Brij 30)

Fig. 1 Chemical structure of acridine (probe), SDS and Brij-30

Experimental

The measurements were performed by the steady-state spectrofluorimetric technique using cuvette with 1.00 cm optical path length. The dye acridine orange (Merck) was recrystallized from methanol. Sodium dodecyl sulfate (Sigma, 99%) was used after recrystallization from acetone and treated by solvent extraction to remove traces of long-chain *N*-alkyl alcohol. Tetraoxyethylene dodecyl ether (Brij 30, Sigma) was used as purchased. The surfactants and dye concentrated stock solutions were prepared using Milli-Q bidistilled and deionised water.

Electronic absorption spectra were measured using a HITACHI U-2000 spectrophotometer, and the corrected steady-state emission spectra were recorded using an F-4500 Hitachi spectrofluorimeter. The analyses in water were performed with solutions of SDS (concentration fixed at 120 mM) and of Brij 30 (concentration range from 0 to 120 mM) in the presence of the dye (1.0×10^{-5} M).

The conductimetric measurements were performed using a conductimetric cell, with the solutions prepared by adding, via a microsyringe, the appropriate amounts of concentrated stock solution of the fluorophores and quencher to the SDS/Brij 30 solution, followed by stirring and a stabilization period of time before measurements.

The surfacial tension measurements were obtained in a ring Tensiometer using 35 mL of solution containing SDS at different concentrations. More details of this technique were presented earlier [37]. Briefly, small volumes of a SDS stock solution (500 mM) was injected with a syringe into the cuvette with water and acridine at 10 μM.

Results and discussion

Conductimetry and surfacial tension analysis were used as comparative methods. The results were compared with the fluorimetric ones in order to detect the micellar transitions more accurately due to its sensitivity and reproducibility. The obtained CMC values of SDS by fluorescence and conductimetric measurements are shown in Table 1. The same procedures were used for both techniques and as expected, these properties changed with the Brij 30 content in the SDS system [21]. These CMC values were obtained at the inflexion point of the curves (fluorimetric determination exemplified in Fig. 2). The fluorescence variation occurs due to the interaction between the probe and surfactant monomer [19] and dye–dye interactions at the negatively charged surface of the SDS micelles. The CMC_1 values obtained by fluorescence are lower than the values obtained by conductimetric measurements (without probe). This difference probably is due to the dye-monomer attractive charge interaction forming the ion pair species (dye^+-DS^-), which shows low solubility in water medium. This ion pair can induce the pre-micelle formation (before

Table 1 CMC values for the SDS as function of Brij-30 mole fraction obtained by fluorimetric (F) and conductimetric (C) techniques, T=298 K

Brij 30 mole fraction	CMC_1, mM (F)	CMC_2, mM (F)	CMC_1, mM (C)	CMC_2, mM (C)
0	7.70	50.00	8.40	48.00
0.05	4.70	9.20	6.40	12.00
0.10	2.80	7.40	5.60	8.90
0.15	1.70	3.72	4.70	7.30
0.20	1.40	3.88	4.30	7.00
0.30	0.80	4.02	3.80	6.80
0.40	0.50	3.67	3.10	6.50
0.50	0.40	3.50	2.90	6.20

the CMC_1) and it is plausible to consider that the probe located in the micelle surface contributes to the micelle formation (firstly CMC_1 and, after that, CMC_2) [20, 27–30]. The same fact was observed for SDS/methylene blue micelle system [26]. At low [SDS], during the pre-micellar phase, the sharp decrease in the fluorescence emission spectra is showed in Fig. 3, which should be due to the ion pair formation with consequent dye-dye self-aggregation process (leading to dimer, trimer and other acridine self-aggregates), which reduces the fluorescence yield by self-quenching processes. Indeed, the presence of Brij 30 in the SDS system did not change the dye fluorescence profile as [SDS] increases. As illustrated in Fig. 2, this fluorescence reduction is observed at [SDS] $<0.2\times10^{-2}$ mol.L^{-1}. At the micelles formation point (CMC_1), the molecules of acridine are re-distributed to the SDS micelles leading to a dye de-aggregation process (probe monomerization with sharp fluorescence enlargement) where the ratio [CMC_1]/[probe] is approximately 770 in 0.5×10^{-2} mol.L^{-1}<[SDS]<1.5×10^{-2} mol.L^{-1} (at Brij 30 absence) (Fig. 2). For CMC_2, despite the fluorescence changes much less remarkable than for CMC_1, this critical point could be detected (Fig. 2). The fluorescence results for CMC_2 can be ascribing on the basis of structural micellar changes corresponding to the second break (structural transition from spherical to rod-like micelles) with the probe rearrangement [31, 32].

The addition of Brij-30 in water SDS system promotes a decrease in the CMC values, both CMC_1 and CMC_2 (Fig. 4 and Table 1), due to the elimination of water molecules of the micelle core. The addition of a nonionic surfactant also promotes an increase in the aggregation number of the micelle, accompanied by size enlargement [13]. Such changes in the CMC_2 may be due to the sphere to rod transitions that could be accompanied by a change in the degree of counter ion binding. This can be achieved by the inclusion of additives, which at higher surfactant concentration could lead to the change in the geometry of the micelle. Additionally the decrease in the CMC values is explained by the fact that Brij-30 enhances the micelle formation tendency at lower SDS concentration. The micellization process is also thermodynamically favored when Brij-30 is added to the SDS solution [13]. An increase in the polidispersity is also expected [4]. This fact is in accordance with the thermodynamic theory of the aggregation of amphiphilic species [16]. The mixed system has a decrease in the micelle surface polarity due to the

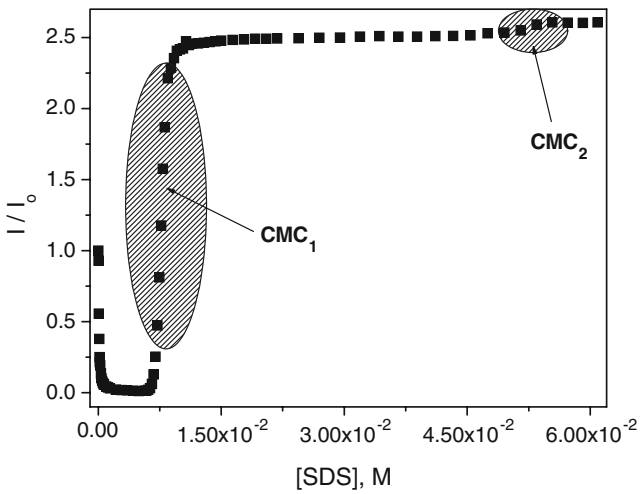


Fig. 2 Relative fluorescence intensity of acridine orange as a function of SDS concentration showing the regions of CMC_1 and CMC_2, T=298 K

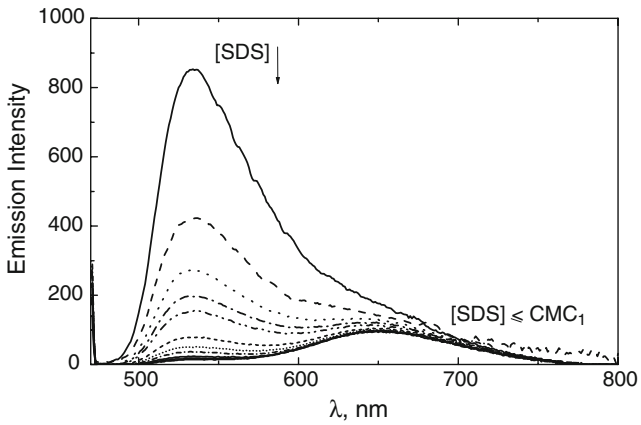


Fig. 3 Emission spectra of acridine orange at increasing concentrations of SDS, T=298 K. λ_{Exc}=420 nm

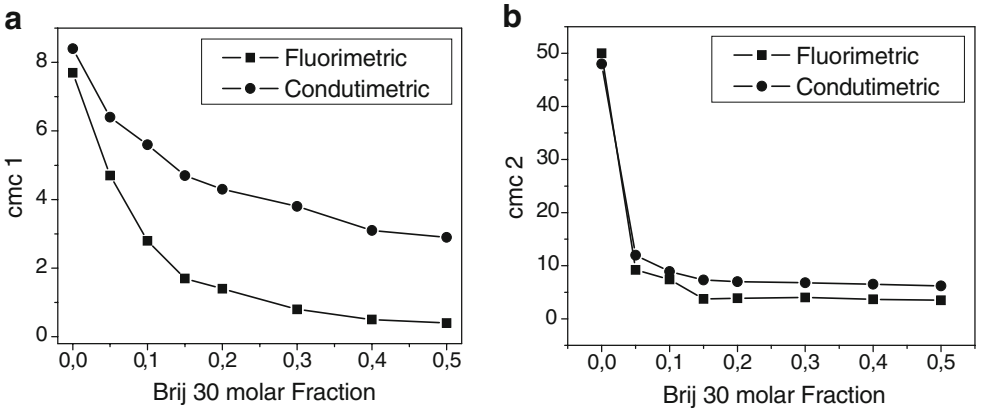


Fig. 4 Comparative effect of the BRIJ 30 molar fraction on the CMC_1 (**a**) and CMC_2 (**b**) by conductimetric and fluorimetric methods

penetration of Brij-30 molecules into the micelle that could be accompanied by a increase in the counter ion (Na^+) binding to DS^- contributing for CMC decreasing.

The Brij-30 at CMC_2 alters this micellar transition phase (sharp decrease from 0 to 0.05 molar fraction, after it the measured CMC_2 suffers a small decrease), however the probe presence or absence does not seem to interfere in this value (noticed as small changes of probe fluorescence emission at this CMC_2 (see Fig. 2)).

It seems clear that these two CMC (the first and second CMC) obtained for SDS and SDS/Brij-30 mixtures are related to the formation of mixed micelles with predominant DS^- presence. The apparent discrepance between the fluorimetric and conductimetric results of CMC_1 can be ascribed to the decrease of charge density by area in the micellar surface because the increase of the amount of Brij 30, which tends to diminish the interaction between the dye and the micelle surface. However, the present results are corroborating with the data from surface tension measurements (Fig. 5), estimated in a previous work on the CMC_1 for this system [13]. In these surface tension measurements (Fig. 5), it is showed that the positively charged acridine orange interacts with negatively charged of SDS to induce a small decrease in CMC_1 (from 7.5 to 6.0 mM). This kind of phenomenon has been reported by several researchers. A value of 4.6 mM was reported for SDS in PBS buffer by Machado et al. [21], very close to the estimated by Benito and co-workers [33] (5.0 mM), using methylene blue as fluorescent probe, in buffered medium, whereas the present CMC value of SDS using pure water as solvent was 7.5 mM [21], in good agreement with the value reported in the literature [34, 35]. The values found for buffered solutions are not properly the CMC of SDS, but a critical aggregation concentration (CAC). These lower values are an indication of a cooperative effect due to the presence of electrolytes in solution, since the electrolytes tend to actuate reducing the repulsion between the hydrophilic groups of the micelle [36]. A similar effect is also observed when certain molecules, as long chain alcohols, emulsifying agents, salts, etc., are added to the solution containing the surfactant, resulting in quasi-micelle aggregates with the surfactant [33, 36, 37].

Conclusions

The fluorimetric technique is a powerful tool to investigate the first and second CMC of the mixed system. Acridine orange was suitable as fluorescence probe to estimate CMC_1 and CMC_2 in a simple micelle system of SDS and in a mixed one with Brij 30—a neutral co-surfactant. The first CMC is more pronouncedly affected by BRIJ 30 molar

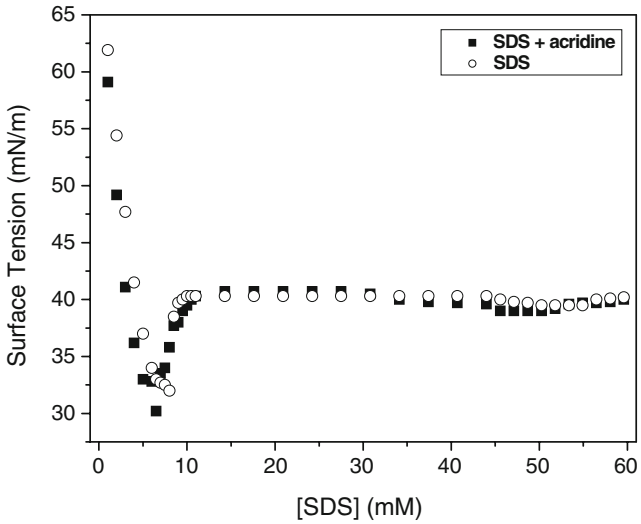


Fig. 5 Effect of the acridine orange on the CMC_1 and CMC_2 of SDS measured by the surface tension method

fraction than CMC_2. Fluorimetry was much more sensitive to detect the decrease in both CMC values due to external contaminants or by the presence of a co-surfactant.

Acknowledgments Financial support from FAPESP (06/56701-3; 02/00272-6) and CNPq are gratefully acknowledged. H.P.M.O. thanks to Vinicius C. C. Vieira for the help, discussion and suggestions in this work.

References

1. Aniansson EAG, Wall S (1974) Kinetics of step-wise micelle association. J Phys Chem 78(10):1024–1030 doi:10.1021/j100603a016
2. Kalyanasunadaram K (1987) Photochemistry in microheterogeneous systems. Academic, New York
3. Rosen MJ (1978) Surfactant and the interfacial phenomena. Wiley Interscience, New York
4. Kalyanasundaram K, Thomas JK (1977) Micellization, solubilization and microemulsion. Plenum, New York
5. Thomas L, Penner PB (1976) Spectral shifts and physical layering of sensitizing dye combinations in silver halide emulsion. Photogr Sci Eng 20(1):32–36
6. Sakthivel T, Florence AT (2003) Dendrimers & Dendrons: facets of pharmaceutical nanotechnology. Drug Deliv. Technol. 3: 73–78
7. Anwer K, Meaney C, Kao G, Hussain N, Shelvin R, Earls RM et al (2000) Cationic lipid-based delivery system for systemic cancer gene therapy. Cancer Gene Ther 7(8):1156–1164 doi:10.1038/sj.cgt.7700218
8. Ostro MJ, Cullis PR (1989) Use of liposomes as injectable-drug delivery systems. Am J Hosp Pharm 46(8):1576–1587
9. Oliveria CA, Machado AEH, Pessine FBT (2005) Preparation of 100 nm diameter unilamellar vesicles containing zinc phthalocyanine and cholesterol using ethanol injection method. Chem Phys Lipids 133(1):69–78 doi:10.1016/j.chemphyslip.2004.09.010
10. Gomes AJ, Assunção RMN, Rodrigues Filho G, Espreafico EM, Machado AEH (2007) Preparation and characterization of poly (D,L-lactic-co-glycolic acid) nanoparticles containing 3-(benzoxazol-2-yl)-7-(N,N-diethyl amino) cromen-2-one. J Appl Polym Sci 105 (2):964–972 doi:10.1002/app.26204
11. Gomes AJ, Faustino AS, Lunardi CN, Laurelúcia O, Machado AEH (2007) Evaluation of nanoparticles loaded with benzopsoralen in rat peritoneal exudate cells. Int J Pharm 332(1-2):153–160 doi:10.1016/j.ijpharm.2006.09.035
12. Gomes AJ, Faustino AS, Machado AEH, Zaniquelli ME, Rigoletto TP, Lunardi CN (2006) Characterization of PLGA microparticle as a drug carrier for the compound 3-ethoxycarbonyl-2H-benzofuro[3,2-d]-1-benzopyran-2-one. Ultrastructural study of cellular uptake and intracellular distribution. Drug Deliv 13(6):447–454 doi:10.1080/10717540600640369
13. de Oliveira HPM, Gehlen MH (2002) Characterization of mixed micelles of sodium dodecyl sulfate and tetraoxyethylene dodecyl ether in aqueous solution. Langmuir 18(10):3792–3796 doi:10.1021/la011501g
14. Chaudhuri R, Pradeep J, Sengupta PK (1996) Luminescence behaviour of phenosafranin in reverse micelles of AOT in n-heptane. J Photochem Photobiol A 101(2):261–265
15. Holland PM, Rubingh DN (1992) Mixed surfactant systems. ACS Symp Ser 501:2
16. Attwood D, Florence AT (1984) Surfactant systems: their chemistry, pharmacy and biology. Chapmann and Hall, London
17. Goon P, Manohar C, Kumar VV (1997) Determination of critical micelle concentration of anionic surfactants: comparison of internal and external fluorescent probes. J Colloid Interface Sci 189(1):177–180 doi:10.1006/jcis.1997.4804
18. Casero I, Sicilia D, Rubio S, Bendito DP (1997) Study of the formation of dye-induced premicellar aggregates and its application to the determination of quaternary ammonium surfactants. Talanta 45(1):167–180 doi:10.1016/S0039-9140(97)00117-3
19. Neumann MG, Gehlen MH (1990) The interaction of cationic dyes with anionic surfactants in the premicellar region. J Colloid Interface Sci 135(1):209–217 doi:10.1016/0021-9797(90)90301-4
20. Kalyanasundaram K, Grätzel M, Thomas JK (1975) Electrolyte-induced phase transitions in micellar systems. Proton and carbon-13 nuclear magnetic resonance relaxation and photochemical study. J Am Ceram Soc 97(14):3915–3922
21. de Paula R, Machado AEH, de Miranda JA (2004) 3-benzoxazol-2-yl-7-(N,N-diethylamino)-chromen-2-one as fluorescence probe for investigation of micellar microenvironments. J Photoch Photobiol A Chem 165(1-3):109–114
22. Gehlen MH, de Schryver FC (1993) Time-resolved fluorescence quenching in micellar assemblies. Chem Rev 93(2):99–221
23. Gao F, Li H-R, Yang Y-Y (2000) Influence of the molecular structure and medium on the absorption and emission character of ketocoumarin derivatives and probability of as fluorescence probes. Dyes Pigm 47(3):231–238 doi:10.1016/S0143-7208(00)00052-8
24. Kundu S, Maity S, Bera SC, Chattopadhyay N (1997) Twisted intramolecular charge transfer of dimethylaminobenzonitrile in micellar environments a way to look at the orientation of the probe within the apolar microenvironment. J Mol Struct 405(2-3):231–238 doi:10.1016/S0022-2860(96)09596-8
25. Krishnamoorthy G, Dogra SK (1999) Dual fluorescence of 2-(4′-*N,N*-dimethylaminophenyl)benzoxazole: effect of solvent and pH. Chem Phys 243(1-2):45–49 doi:10.1016/S0301-0104(99)00074-9
26. Severino D, Junqueira HC, Gugliotti M, Gabrielli DS, Baptista MS (2003) Influence of negatively charged interfaces on the ground and excited state properties of methylene blue. Photochem Photobiol 77(5):459–468 doi:10.1562/0031-8655(2003)077<0459:IONCIO>2.0.CO;2
27. Thomas JK (1987) Characterization of surfaces by excited states. J Phys Chem 91(2):267–276 doi:10.1021/j100286a008
28. Martens FM, Verhoeven JW (1981) Charge-transfer complexation in micellar solutions. Water penetrability of micelles. J Phys Chem 85(13):1773–1777 doi:10.1021/j150613a001
29. Croonen Y, Geladé E, Van der Zegel M, Van der Auweraer M, Vandendriessche H, De Schryver FC et al (1983) Influence of salt, detergent concentration, and temperature on the fluorescence quenching of 1-methylpyrene in sodium dodecyl sulfate with m-dicyanobenzene. J Phys Chem 87(8):1426–1431 doi:10.1021/j100231a029
30. Baumgardt K, Klar G, Strey R (1982) On the concentration-dependence of the mean aggregation number of ionic micelles as determined by stopped-flow experiments. Phys Chem (Kyoto) 86 (10):912–915
31. Treiner C, Makayssi A (1992) Structural micellar transition for dilute solutions of long chain binary cationic surfactant systems: a conductance investigation. Langmuir 8(3):794–800 doi:10.1021/la00039a012

32. Bakshi MS (2000) Cationic mixed micelles in the presence of β-Cyclodextrin: a host–guest study. J Incl Phenom Macrocycl Chem 227(1):78–83
33. Benito MA, García C, Monge JM, Saz ML (1997) Spectrophotometric and conductimetric determination of the critical micellar concentration of sodium dodecyl sulfate and cetyltrimethylammonium bromide micellar systems modified by alcohols and salts. Colloids Surf A Physicochem Eng Asp 125(2-3):221–224 doi:10.1016/S0927-7757(97)00014-9
34. Chaudhuri R, Guharay J, Sengupta PK (1996) Fluorescence polarization anisotropy as a novel tool for the determination of critical micellar concentrations. J Photochem Photobiol Chem 101 (2-3):241–244 doi:10.1016/S1010-6030(96)04412-7
35. Sharkar N, Datta A, Das S, Bhattacharyya K (1996) Solvation dynamics of coumarin 480 in micelles. J Phys Chem 100 (38):15483–15486 doi:10.1021/jp960630g
36. Capek I (2002) Fate of excited probes in micellar systems. Adv Coll Int Sci 97(1–3):91–149
37. Junqueira H, Severino D, Baptista MS, Dias LG, Gugliotti M (2002) Modulation of the methylene blue photochemical properties based on the adsorption at aqueous micelle interfaces. Phys Chem 4(11):2320–2328 doi:10.1039/b109753a