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Characterization of sodium dodecylsulphate and dodecylphosphocholine mixed micelles through NMR and dynamic light scattering

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The complexity of biological membranes leads to the use of extremely simplified models in biophysical investigations of membrane-bound proteins and peptides. Liposomes are probably the most widely used membrane models due, especially, to their versatility in terms of electric charge and size. However, liquid-state NMR suffers the lack of such a model, because even the smallest liposomes slowly tumble in solution, resulting in a dramatic signals broadening. Micelles are typically used as good substitutes, with sodium dodecylsulphate (SDS) and dodecylphosphocholine (DPC) being the most widely employed surfactants. However, they are always used separately to mimic prokaryotic and eukaryotic membranes, respectively, and accurate investigations as a function of surface charge cannot be performed. In this work, the critical micelle concentration (CMC) of binary mixtures with different SDS/DPC ratios has been determined by following the chemical shift variation of selected ^{1}H and ^{31}P NMR signals as a function of total surfactant concentration. The regular solution theory and the Motomura's formalism have been applied to characterize the micellization both in water and in phosphate buffer saline, and results were compared with those obtained directly from the experimental NMR chemical shift. The ζ-potential and size distribution of the mixed micelles have been estimated with dynamic light scattering measurements. Results showed that SDS and DPC are synergic and can be used together to prepare mixed micelles with different negative/zwitterionic surfactants molar ratio. Copyright © 2013 John Wiley & Sons, Ltd.

Supporting Information may be found in the online version of this article.

Keywords: NMR; ^{1}H; ^{31}P; dynamic light scattering; regular solution theory; Motomura's model; membrane models; critical micelle concentration

Introduction

One of the main difficulties encountered in the biophysical investigations of proteins and peptides–membrane interactions is the complexity of the biological membranes themselves. A typical biological membrane is a complex dynamical structure, whose major components are lipids belonging to various classes, such as glycerophospholipids, sphingolipids, sterols and glycolipids. These amphiphiles constitute the main structural element of the membrane, the lipid bilayer, where, in addition, a multitude of different membrane proteins are embedded. Moreover, lipids can have different headgroups, ranging from negatively charged to neutral zwitterionic. The exact protein and lipid composition depends on the specific membrane being considered and, rarely, the outer and inner leaflets have the same composition.[1] This complexity limits the application of the majority of the standard *in vitro* biophysical techniques to investigate the structure and functions of membrane proteins and peptides, somehow forcing to employ enormously simplified membrane models. One of the most used membrane models is the liposome, also referred to as vesicle. A comprehensive description about liposomes preparation and applications as membrane models in biophysics can be found in refs[2,3] and in the references quoted therein.

Among the various biophysical techniques, despite NMR is certainly one of the most powerful ones to study both the structure and dynamics of proteins and peptides in solution,[4] when proteins or peptides are assembled with a lipid bilayer, such as a vesicle, the molecular mass of the assembly readily exceeds 10^5 Da and molecular tumbling is correspondingly slow. This leads to an excessive broadening of the NMR resonances, which, in turn, makes the achievement of a high resolution 3D structure difficult, often impossible.[3,5] Surfactants micelles represent a good compromise between the need for relatively fast tumbling assemblies and a suitable membrane model to study the protein/peptide structure and obtain, still, physiologic relevant results.[3,5] On the other hand, solid-state NMR progressed remarkably in the last decade, being up to date a more than valid alternative for structure solving. Numerous examples and references can be found at the following URL: http://www.drorlist.com/nmr/SPNMR.html.

Surfactants are, generally speaking, amphipathic molecules consisting of a polar headgroup and a hydrophobic tail. In this respect, they closely resemble lipids but instead of forming bilayered structures in solution, they spontaneously form com-

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pact structures above a characteristic surfactant concentration, namely, the critical micelle concentration (CMC). In a micelle, the headgroups are in contact with the external aqueous solvent, whereas the tails are packed in the micelle core. Thus, compared with liposomes, micelles (i) do not have a water core, (ii) are not delimited by a bilayer, and (iii) are smaller, having a typical diameter of less than 10 nm.[6] Surfactants are classified on the basis of their headgroup as ionic, with a net positive or negative charge, non-ionic, with a polar but uncharged headgroup, and zwitterionic, which combine the properties of the ionic and non-ionic surfactants, characterized by a headgroup containing both a net positive and a net negative portion that neutralize each other.[6] Among the various well-known and characterized surfactants, the negatively charged sodium dodecylsulphate (SDS) and the zwitterionic dodecylphosphocholine (DPC) are certainly the most widely used in the field of NMR structural biology.[2,3,5–12] Importantly, both these surfactants are commercially available, even in the perdeuterated form, at a relatively low price compared with phospholipds, which is particularly suitable for ^{1}H NMR investigations focused on the proteins/peptides. Their use is especially widespread in the field of antimicrobial peptides. Because the latter are usually positively charged, whereas bacterial membranes usually display a higher content of negatively charged lipids than the eukaryotic ones, the electrostatics clearly plays a fundamental role in the peptide–membrane interaction. In particular, pure DPC micelles are used to mimic eukaryotic membranes, because DPC has exactly the same headgroup as phosphatidylcholines that are the predominant class of lipids in eukaryotes. On the other hand, pure SDS micelles are used to mimic bacterial membranes, even if rarely, they are constituted only by negatively charged phospholipids.[2,3]

Thus, many different biophysical techniques are usually employed beyond liquid-state NMR, such as for instance circular dichroism,[13–15] fluorescence spectroscopy,[13,16–18] molecular dynamics simulations,[19,20] and solid-state NMR,[3,15,21,22] where more sophisticated membrane models can be employed, allowing for investigations as a function of the charge, that is, by mixing different types of lipids in the model.

Actually, in the literature, there are examples of the use of SDS/DPC mixtures,[23–25] but as far as we are aware, a characterization of this binary system is still lacking and it is merely assumed that these two surfactants are synergic and form mixed micelles in the experimental conditions employed. Indeed, this is not a trivial issue at all, because a mixture of two or more surfactants can, depending on the experimental conditions such as composition, temperature, pH, ionic strength and the type of counterions, exhibit either an ideal or a non-ideal micellization behavior.[26]

This work aimed at verifying whether SDS and DPC are synergic in the formation of mixed micelles, to be used as differently charged models in liquid-state NMR. In order to investigate and characterize their micellization behavior, we have applied both the regular solution theory (RST)[27–30] and the Motomura's model.[31] The CMC of the two surfactants and that of different mixtures have been estimated by following the chemical shift variation of selected ^{1}H and ^{31}P NMR resonances as a function of total surfactant concentration. The measures have been performed at room temperature both in deionized water and in one of the most widely used buffers in biophysics, namely, phosphate buffer saline (PBS). Then, using dynamic light scattering (DLS), we have characterized the mixed micelles in terms of their ζ-potential and average size, at a concentration up to two orders of magnitude above the CMC. Finally, the present work has shown that SDS and DPC are synergic in both the environments and can actually be used to prepare mixed micelles with different negative/zwitterionic surfactant ratio. Some of the advantages offered by NMR in the CMC determination of surfactants mixtures, when compared with the commonly used conductivity or surface tension measurements are also discussed.

Experimental

Samples preparation

Sodium dodecylsulphate (electrophoresis purity reagent) was purchased from Bio-Rad Laboratories, CA, USA, DPC (purity ≥99%) from Avanti Polar Lipids, USA. PBS (10 mM phosphate buffer + 150 mM NaCl) was prepared according to the specifics reported in the Calbiochem buffers guide,[32] adjusting the pH to 7.4 before use. Either deionized water and PBS were always passed through a 0.2 mm filter in order to remove the (eventually present) dust, which is particularly important for accurate DLS measurements. Millipore water was always employed. In addition, PBS was also prepared at a NaCl concentration of 100 and 50 mM, in order to investigate the effect of the ionic strength on a selected sample (see the Results and Discussion).

Proper amounts of SDS and DPC have been weighted to prepare separate stock solutions (~200 mM). After surfactants solubilization, either in water or PBS, the stock solutions have been vortexed and then sonicated in an ultrasonic bath for 5 min. Proper amounts of these solutions have been then mixed to obtain the final desired SDS/DPC molar ratio and total surfactant concentration. Final samples have been vortexed, sonicated for 10 min and then left to equilibrate overnight at room temperature. The pH of the water samples was found to vary in the 6–8 range.

In particular, samples with the following DPC molar fraction have been prepared: 0, 0.25, 0.50, 0.75, 0.90 and 1.00. For each one of these, a concentrated stock (total surfactant ~40 mM) has been prepared and used as a basis to prepare all the other NMR samples by dilution, until ~0.2 mM. As far as the DLS measurements are concerned, samples were prepared in PBS with a total surfactant concentration of 20 and 200 mM.

^{1}H NMR

Spectra have been acquired with a Unity Inova 500NB high-resolution spectrometer (Agilent Technologies, CA, USA) operating at a ^{1}H frequency of 500 MHz, equipped with a high-field indirect detection probe. A total of 700 µl samples were loaded into a 5-mm test tube, then 50 µl of D_2O were added for the frequency lock. Experiments were carried out at 298 K. Chemical shifts were referenced to the methyl resonance of 3-(trimethylsilyl)-2,2′,3,3′-tetradeuteropropionic acid (TSP) loaded in a capillary tube as external reference (removed before spectrum acquisition). The acquisition parameters for the one-dimensional spectra were as follows: 10 ppm spectral width; 10 k data points; 128 ÷ 1024 transients; 1 s acquisition time; 2 s delay and 6.7 µs (90°) pulse length. Suppression of the water signal was achieved with 1 s pre-saturation, applied just before the 90° pulse, during the recycle delay. The FT was always performed zero-filling up to 64 kB (0.15 Hz digital resolution).

^{31}P NMR

Spectra have been acquired with a Unity Inova 500NB high-resolution spectrometer operating at a ^{31}P frequency of 203 MHz,

equipped with a gHX-nano (direct detection) probe. A total of 700 µl samples were loaded into a 5-mm test tube, then 50 µl of D_2O were added for the frequency lock. Experiments were carried out at 298 K. Chemical shifts were referenced to the DPC signal recorded for the most concentrated sample of each of the investigated compositions. The acquisition parameters for the proton-decoupled one-dimensional spectra were as follows: 20 ppm spectral width; 16 k data points; 128 ÷ 4096 transients; 2 s acquisition time; 2 s delay, and 8.6 µs (90°) pulse length. The FT was always performed with zero-filling up to 64 kB (0.12 Hz digital resolution).

Critical micelle concentration and mixed micelles composition from NMR data

Similarl to what is observed for other parameters, such as conductivity or surface pressure, also, the ^{1}H chemical shift shows a remarkable slope variation as function of surfactant concentration. The abscissa of the intersection point between the two straight lines, obtained with a least square data fitting, corresponds to the CMC.

Because both SDS and DPS were found in fast exchange between the bulk and the mixed micelles (see the Results and Discussion), a two-state model was applied. This is the simplest assumption; one can take in the absence of any evidence about the presence of multiple phases. This assumption was justified by the synergism observed in the mixed micelle formation.

The chemical shift observed for the most diluted solution was taken as a reference for the monomeric state. The chemical shift corresponding to $C^{-1} = 0$, where C is the total surfactants' concentration could be extrapolated and taken as a reference for the micelle state. The chemical shift observed at the different concentrations was assumed to be equal to a weighted average of these two reference chemical shifts. The weighting factors were the corresponding molar fraction for the specific detergent investigated.

Thus, on the basis of the NMR chemical shift data, the concentration of the detergents present in the micelles could be calculated for SDS and DPC independently and their molar fraction accordingly evaluated with increasing the total surfactants' concentration. The results could be fitted with an exponential function ($y = a \cdot e^{bx} + c$), obtaining a root mean square deviation ≤ 0.01. Finally, the molar fraction for the two surfactants in the mixed micelles was extrapolated at a total surfactants' concentration equal to the experimental cmc, and at 20 mM, which is well beyond the cmc.

Dynamic light scattering

The micelles size distribution was determined by photon correlation spectroscopy[33,34] using a Zetasizer nano-ZS (Malvern Instruments, UK), with a 4 mW He-Ne laser operating at a wavelength of 633 nm and an angle of 173°. ζ-Potential was estimated with the same instrument using the M3-PALS (Phase Analysis Light Scattering) technique, which measures the particles electrophoretic mobility. All the measurements were performed at 298 K. At least three measurements were carried out for each of the samples in appropriate disposable cuvettes (sample volume 0.75–1.00 ml). The refractive index and viscosity of the solvent were properly taken into account within the instrument software manager.

Results and discussion

An ideal micellization behavior usually arises from the mixing of homologous surfactants having the same (or very similar) headgroup, differing, for instance, in the chain length.[26,29] In this case, mixed micelle composition simply reflects the molar fraction of the different species present in solution, and the CMC of the mixed system can be straightforwardly calculated on the basis of the CMC of each of the involved surfactants, using Clint's model[35]

$$\frac{1}{cmc_{mix}} = \sum_i \frac{\alpha_i}{cmc_i} \quad (1)$$

where, cmc_{mix} is the CMC of the mixture, α_i is the molar fraction of the *i*th species in solution, and cmc_i is the CMC of the *i*th species in the same experimental conditions. However, this model, which works especially well for binary mixtures of homologous surfactants, was developed in the case of non-ionic molecules, where the driving force for micellization is mainly given by the hydrophobic interactions between the surfactants molecules, while the electrostatic contribution is negligible. Mixed nonionic/ionic and ionic/ionic surfactant systems have been studied extensively and it was found that, when the electrostatics plays a major role in surfactants interactions, a non-ideal micellization behavior is often observed.[28,29,36] Either a synergistic or antagonistic behavior can be expected in these cases, depending on whether attractive or repulsive interactions between the surfactants are favorite, respectively. This, in addition, strictly depends on the experimental conditions. For instance, a pH variation might change the protonation state and thus the net charge of the headgroup, or the solution ionic strength and the type of the counterions might significantly alter the headgroups charge shielding. Indeed, experimental conditions are known to have a large influence on the CMC (as well as on the micelle size and shape), even of a single surfactant,[37] and it is thus very difficult to predict the behavior of a particular mixture at given conditions. Moreover, in the particular case of SDS and DPC, we are interested in, the latter compound is zwitterionic. To date, zwitterionic surfactants have been generally much less studied than other classes of surfactants. Their behavior is often described in relation to that of the ionic ones, but their electrically neutral nature clearly distinguishes them from the latters.[29] On the other hand, they cannot be considered as non-ionic, because they actually have both a localized net positive and a localized net negative charge in the headgroup.

For non-ideal mixtures, the strength and nature of the interaction between two surfactants can be measured in terms of the so-called β parameter within the RST.[27–30] Positive values of β indicate repulsive interactions, that is, the two surfactants are antagonist in the micelle formation process; negative values of β indicate attractive interactions, thus, the formation of mixed micelles is favored with respect to the formation of micelles formed by the two surfactants separately. It is possible to determine β from CMC measures, using the following equation:

$$\beta = \frac{\ln\left(\alpha_1 \, cmc_{mix} / \chi_1 \, cmc_1\right)}{(1-\chi_1)^2} \quad (2)$$

where, α_1 is the molar fraction of the surfactant 1, cmc_{mix} and cmc_1 are the CMC of the mixture and the surfactant 1,

respectively, and χ_1 is the molar fraction of the surfactant 1 in the mixed micelle. χ_1 can be calculated solving the following equation:

$$\frac{(\chi_1)^2 \ \ln\left(^{\alpha_1} \ cmc_{mix}/_{\chi_1} \ cmc_1\right)}{(1-\chi_1)^2 \ \ln\left[^{(1-\alpha_1)} \ cmc_{mix}/_{(1-\chi_1)} \ cmc_2\right]} = 1 \qquad (3)$$

where, cmc_2 is the CMC of the surfactant 2. From a theoretical point of view, the β parameter should be composition independent and its magnitude might be evaluated by fitting Equation 4 to the experimental CMC versus surfactant[36]

$$cmc_{mix} = \left(\frac{\alpha_1}{f_{1,mix}\, cmc_1} + \frac{\alpha_2}{f_{2,mix}\, cmc_2}\right)^{-1} \qquad (4)$$

where the activity coefficients $f_{i,mix}$ for the surfactants in the micelles are given by

$$f_{i,mix} = \exp\left[\beta(1-\chi_i)^2\right] \qquad (5)$$

However, in various cases, this is not possible because a variable β parameter is found, which is ultimately determined iteratively for each composition of interest.[28–30,38–41]

Finally, the following two conditions must be met in order for synergism to exist in mixed micelle formation as follows: (i) β must be negative and (ii) $|\beta| > |\ln(cmc_1/cmc_2)|$.[29] Thus, with the RST approach, synergism between two surfactants in mixed micelles formation can be ascertained and the actual mixed micelle composition at the cmc estimated, which might be different from that of the monomers in solution at total surfactant concentration below the CMC.[27–30,36]

In this study, CMC were determined by measuring the NMR chemical shift variation of selected ^{1}H and ^{31}P resonances with decreasing total surfactant concentration. Figure 1 shows, for instance, the ^{1}H NMR spectra of ~40 mM SDS, DPC, and the SDS/DPC 1/1 mol/mol mixture in water. Resonances are progressively labeled using capital letters with increasing chemical shift. Resonances have been assigned on the basis of data from the SDBS database,[42] signals multiplicity, and their relative integrated area, and are schematically reported in Figure 2. The corresponding data are also displayed in Table S1 for the sake of completeness. The number and multiplicity of the resonances did not change with SDS/DPC mixture composition, total surfactant concentration, or moving from water to PBS. Chemical shift variation with decreasing total surfactant concentration was the method of choice to determine the CMC. Compared with the most commonly used conductivity and surface tension measurements, which can only give a measure reflecting the entire mixture (i.e., the average response of all the system components at once), NMR offers the opportunity to separately follow the evolution of a signal for each of the different surfactants. In this way, it is possible to monitor CMC variation with varying the experimental conditions for both the surfactants and, in addition, to verify if they are actually characterized by the same CMC when mixed together under the given conditions. Therefore, at least one resonance has to be selected for each of the surfactants under investigation, and, thus, resonances that overlap in the mixtures' spectra are not suitable. In the case of SDS, the unique resonance satisfying this criterion was the C(1)H_2 (resonance *G* in Figure 1). In the case of DPC, there were four possibilities, that is, the C(1)H_2 and all of the three resonances due to the choline headgroup. However, the resonance *H* was immediately discarded because it is too close to the water signal tail, thus suffering from baseline distortions. Chemical shift variation was then monitored with decreasing surfactant concentration for the three remaining resonances in pure DPC samples and the C(1)H_2 (resonance *F* in Figure 1) finally resulted to be the most sensitive one. The second advantage offered by NMR in this kind of applications is represented by the possibility to study nuclei other than the ^{1}H. This might be very useful when some of the surfactants do not have any well-resolved resonance in the ^{1}H spectrum of the mixture or, anyway, to cross-check the results obtained from the latter improving the statistics. In the present study, for instance, the ^{31}P choline resonance was also used to determine the CMC of DPC. In the spectra recorded from PBS samples, the phosphate buffer resonance was found to be ~2.5 ppm high-frequency shifted with respect to the phosphocholine signal and they have never found to overlap. It is important to stress here that, both in water and PBS, the observation of only one resonance for each of the proton groups, as well as only one phosphorus DPC resonance, in the spectra of all of the

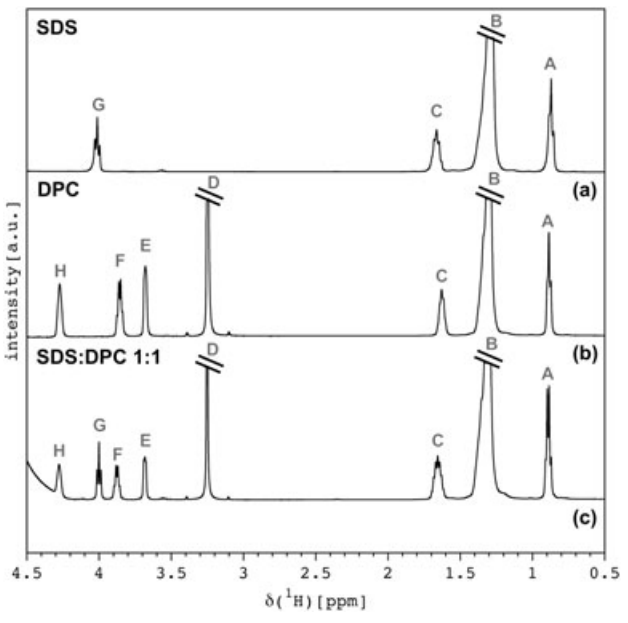

Figure 1. Proton spectra. ^{1}H NMR spectra of **(a)** sodium dodecylsulphate (SDS), **(b)** dodecylphosphocholine (DPC), and **(c)** SDS/DPC 1/1 mol/mol ~40 mM in water, recorded with a 500 MHz spectrometer at 298 K. Water resonance was suppressed using pre-saturation during the recycle delay. Chemical shift scale was calibrated using TSP as external reference. Resonances are progressively labeled using capital letters with increasing chemical shift.

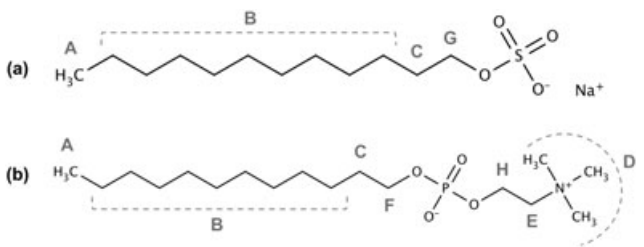

Figure 2. Sodium dodecylsulphate (SDS) and dodecylphosphocholine (DPC) molecular structure with proton resonances assignments. Schematic molecular structure of **(a)** SDS and **(b)** DPC. The majority of hydrogen is not explicitly represented for clarity. The capital letters indicate the proton groups responsible for the observed ^{1}H NMR resonances that are accordingly labeled in Figure 1.

investigated mixtures, both below and above the corresponding CMC, leads to conclude that surfactant monomers are in fast exchange with the surfactant molecules assembled in the micelles.

Figure S1 shows the chemical shift of $C(1)H_2$ resonance as a function of the surfactant concentration reciprocal, obtained for pure SDS in water and PBS. Figure S2 shows the results obtained for pure DPC solutions, monitoring either ^1H $C(1)H_2$ (Figure S2a) or the ^{31}P resonance (Figure S2b). Figure 3 shows, for instance, the results obtained for the SDS:DPC 1 : 1 mixture in PBS.

The resonance shifts towards lower frequency values with increasing the surfactant concentration, indicating an increased shielding of the corresponding protons from the main magnetic field. In terms of micelle formation, this is due to an increased local concentration of surfactants (i.e., the compactness of surfactants in the micelle) with respect to the monomers dispersion below the CMC.[30] The CMC of pure SDS at 298 K resulted to be 8.22 and 1.73 mM in water and in PBS, respectively, in a very good agreement with data reported in the literature.[43–46] With increasing the solution ionic strength, that is, moving from water to the PBS, headgroups repulsion is attenuated by the presence of the electrolytes, which tend to neutralize headgroups charge, causing CMC to decrease.[6,37,44,45]

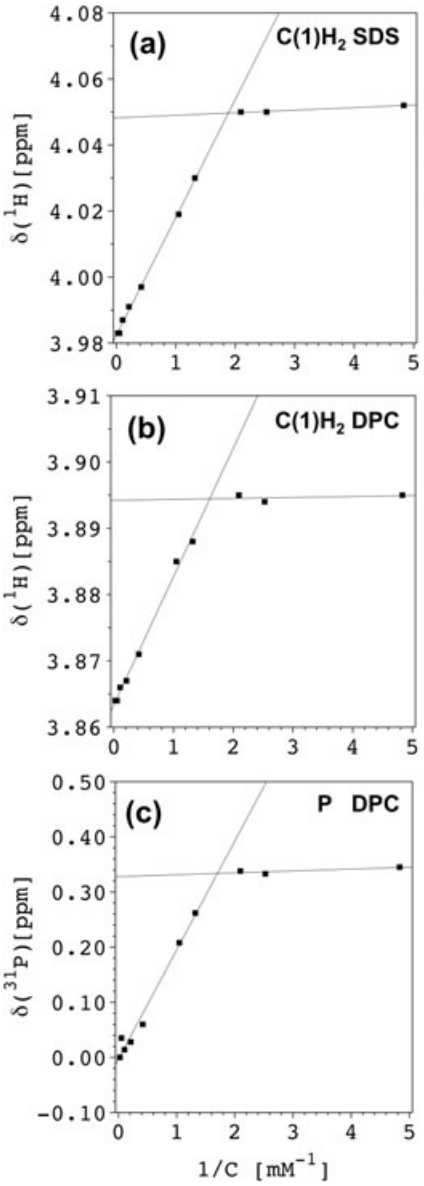

Figure 3. Critical micelle concentration determination. Chemical shift of **(a)** sodium dodecylsulphate (SDS) ^1H $C(1)H_2$, **(b)** dodecylphosphocholine (DPC) ^1H $C(1)H_2$ and **(c)** DPC ^{31}P resonance as a function of total surfactants' concentration reciprocal from a SDS/DPC 1 : 1 mixture in phosphate buffer saline. Least square fittings below and above the CMC are shown as solid lines.

The CMC of pure DPC at 298 K resulted to be 1.52 ± 0.05 mM and 1.36 ± 0.03 mM in water and in PBS, respectively. In the literature, there is still no general consensus about DPC CMC in water at 298 K, but values are reported in the relatively narrow range between ~1.5 and ~0.9 mM.[47] Again, CMC decreases with increasing the solution ionic strength, even if for a zwitterionic surfactant such as DPC, this effect is less pronounced than for the anionic SDS, because of the overall neutral charge of DPC headgroup.[48] For comparison, CMC values from the literature for both pure SDS and DPC are provided in Table S2.

Following the same procedure, CMC was determined for the different SDS/DPC mixtures using all of the three selected NMR resonances (SDS: ^1H-$C(1)H_2$; DPC: ^1H-$C(1)H_2$; and ^{31}P-phosphate). The iterative solution of Equation 3 provided the effective molar fraction of the two surfactants in the micelles χ_i, whose values were substituted in Equation 2 to finally calculate the β parameter for each composition. Results obtained in water and PBS are reported in Tables 1 and 2, respectively. The negative values of β, obtained for all the mixtures in both solvents, clearly indicate that SDS and DPC are synergic in the formation of mixed micelles both in water and PBS at all the investigated compositions. This is further supported by the fact that $|\beta| > |\ln(cmc_1/cmc_2)|$ for all the cases.[29] Indeed, Figure 4 shows that the CMC

Table 1. Critical micelle concentration and micelles' composition for different sodium dodecylsulphate/dodecylphosphocholine mixtures in water at 298 K

α_{DPC} [a]	CMC[b] [mM]	χ_{DPC} [c]	β [d]	ψ_{DPC} [e]	ψ^*_{DPC} [f]
0.00	8.22	0.00	−2.25	0.00	0.00
0.25	1.81 ± 0.29	0.56		0.61	0.26
0.51	1.41 ± 0.30	0.67		0.74	0.53
0.74	1.44 ± 0.14	0.79		0.79	0.75
0.90	1.32 ± 0.19	0.84		0.85	0.90
1.00	1.52 ± 0.05	1.00		1.00	1.00

CMC, critical micelle concentration; DPC, dodecylphosphocholine.

[a] α_{DPC} is the molar fraction of DPC in solution.

[b] Error is given as three times the standard deviation of the results obtained following the selected ^1H and ^{31}P resonances, independently.

[c] χ_{DPC} is the molar fraction of DPC in the micelles, calculated on the basis of the RST.

[d] $\beta = 0$ indicate ideal behavior; $\beta > 0$ indicate antagonism, whereas $\beta < 0$ indicate synergism, between the two surfactants, on the basis of RST.

[e] ψ_{DPC} is the molar fraction of DPC in the micelles, on the basis of a two-state model applied to the NMR chemical-shift data, at a total surfactant concentration equal to the CMC.

[f] ψ^*_{DPC} is the molar fraction of DPC in the micelles, on the basis of a two-state model applied to the NMR chemical-shift data, at a total surfactant concentration of 20 mM.

Table 2. Critical micelle concentration and micelles' composition for different sodium dodecylsulphate/dodecylphosphocholine mixtures in PBS at 298 K

α_{DPC} [a]	CMC[b][mM]	χ_{DPC} [c]	β [d]	χ'_{DPC} [e]	ψ_{DPC} [f]	ψ^*_{DPC} [g]
0.00	1.73	0.00	—	0.00	0.00	0.00
0.25	0.64 ± 0.02	0.43	−3.99	0.28	0.27	0.25
0.51	0.58 ± 0.05	0.52	−3.78	0.56	0.45	0.51
0.74	0.43 ± 0.04	0.59	−5.50	0.59	0.59	0.74
0.90	1.35 ± 0.06	0.90	−0.35	0.89	0.83	0.90
1.00	1.36 ± 0.03	1.00	—	1.00	1.00	1.00

CMC, critical micelle concentration, DPC, dodecylphosphocholine.

[a] α_{DPC} is the molar fraction of DPC in solution.

[b] Error is given as three times the standard deviation of the results obtained following the selected ^{1}H and ^{31}P resonances, independently.

[c] χ_{DPC} is the molar fraction of DPC in the micelles, calculated on the basis of the RST.

[d] $\beta = 0$ indicate ideal behavior; > 0 indicate antagonism, whereas $\beta < 0$ indicate synergism, between the two surfactants, on the basis of RST.

[e] χ'_{DPC} is the molar fraction of DPC in the micelles, calculated on the basis of the Motomura's approach.

[f] ψ_{DPC} is the molar fraction of DPC in the micelles, on the basis of a two-state model applied to the NMR chemical-shift data, at a total surfactant concentration equal to the CMC.

[g] ψ^*_{DPC} is the molar fraction of DPC in the micelles, on the basis of a two-state model applied to the NMR chemical-shift data, at a total surfactant concentration of 20 mM.

as a function of DPC molar fraction in solution remarkably deviates from the ideal behavior described by Clint's model (Equation 1).[35] The synergism between SDS and DPC appears to be more pronounced in the PBS than in water, as shown by the larger deviation from Clint's model and the correspondingly lower β values. This observation can be attributed to the headgroups charge shielding provided by the electrolytes of the PBS. However, although a constant β parameter was found in water (Table 1), it varied with system composition in PBS (Table 2). This does not mean, *per se*, that the RST model is not valid in the latter case but cross-checking the results, applying another theoretical approach, is advisable. Indeed, the RST is based upon the assumption that a surfactants mixture could be treated as a homogeneous solvents mixture, and the concept of activity coefficients is applied to correct Clint's model for ideal behavior in order to describe a non-ideal mixing.[36] The main issue, however, is the molar fraction definition for each surfactant when charged species are employed as in our case. Possible dissociation of the components, that is, between the charged surfactant and its counter-ions, should be considered in the bulk as well as in the micelle. An alternative theoretical approach, which takes the number of sufactants' counterions into account, was derived by Motomura and coworkers.[31] Basically, the new α'_i and cmc'_{mix} are calculated from α_i and cmc_{mix}, respectively, with the following equations:

$$\alpha'_i = \frac{v_i \alpha_i}{v_i \alpha_i + v_j \alpha_j} \tag{6}$$

$$cmc'_{mix} = \left(v_i \alpha_i + v_j \alpha_j\right) cmc_{mix} \tag{7}$$

where $v_i = v_{i,a} + v_{i,c}$ and $v_j = v_{j,b} + v_{j,d}$, that is, surfactant i is composed by $v_{i,a}$ ions and $v_{i,c}$ counterions, whereas surfactant j comprises $v_{j,b}$ ions and $v_{j,d}$ counterions. Finally, the molar fraction of the i-species in the mixed micelle can be calculated as

$$\chi'_i = \alpha'_i - \frac{\left(\frac{\alpha'_i \alpha'_j}{cmc'_{mix}}\right)\left(\frac{\partial cmc'_{mix}}{\partial \alpha'_i}\right)_{T,\ p}}{\left[1 - \delta^c_d v_{i,c} v_{j,d} \Big/ \left(v_{i,c} v_j \alpha'_i + v_{j,d} v_i \alpha'_j\right)\right]} \tag{8}$$

where δ^c_d is the Kronecker delta, which is 0 for $c \neq d$, 1 for $c = d$. In our case, $v_{SDS,a} = v_{DPC,b} = v_{SDS,c} = 1$ and $v_{DPC,d} = 0$. Results for χ'_{DPC} in the mixed micelles formed in PBS are reported in Table 2. The values are very close to those predicted by the RST, suggesting viability of both models for the SDS/DPC system under the experimental conditions employed in the present study. The only significant difference is observed at the lowest DPC molar fraction (0.25). Although the RST estimated a relatively high difference between α_{DPC} and χ_{DPC}, Motomura's model resulted in comparable values.

A two-state model was applied to the NMR chemical shift data, allowing for independent determination of both SDS and DPC molar fractions in the mixed micelles as a function of the total

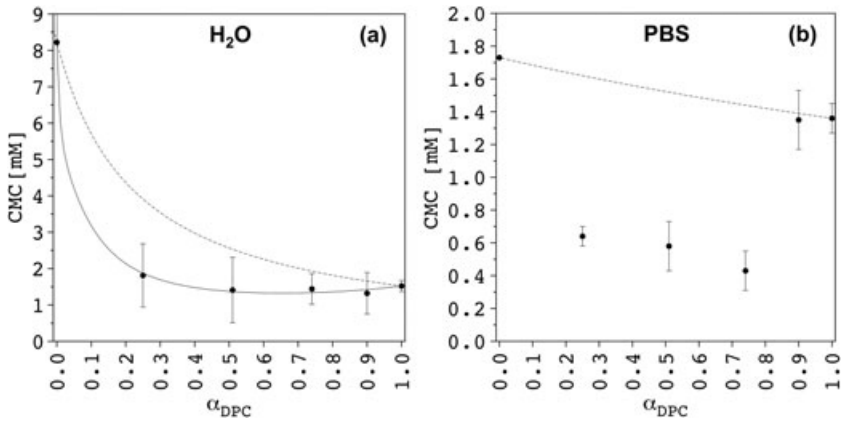

Figure 4. Critical micelle concentration (CMC) versus composition. CMC determined for different sodium dodecylsulphate/dodecylphosphocholine (DPC) mixture in **(a)** water and **(b)** PBS are reported with filled circles. The error bars were calculated as 3σ, three times the standard deviation of the results obtained following the selected ^{1}H and ^{31}P resonances, independently. The solid line in **(a)** corresponds to the least squares fitting of Equation 4. Data calculated on the basis of Clint's model (Equation 1) are represented with the dashed line.

surfactants' concentration. In the absence of any evidence about the presence of multiple phases, this is the simplest model applicable to the NMR data, in the light of the synergism shown by the CMC values and the RST application.

In Tables 1 and 2, the results of the application of the two-state model are shown. In particular, DPC molar fraction in the mixed micelles is reported for two different values of surfactants concentration, that is, at the corresponding CMC (ψ_{DPC}) and at 20 mM (ψ^{*}_{DPC}). In the case of water (Table 1), ψ_{DPC} turned out to be similar to the molar fractions obtained with the RTS at all the mixture compositions and a constant value of beta was obtained for all detergent ratios, supporting the validity of this model. In the case of PBS, ψ_{DPC} values were similar to those obtained with both models, but at the lowest DPC molar fraction (0.25), that is, the only case where a significant difference was observed, ψ_{DPC} was comparable with the molar fraction obtained with Motomura's model.

Because this difference was observed only in PBS, in order to investigate whether it was due to electrolytes' concentration, we measured the CMC of the same mixture ($\alpha_{DPC} = 0.25$) at different NaCl concentrations, namely, 50 and 100 mM, resulting in 1.49 and 1.04 mM, respectively. Recalling the values obtained in water (1.81 mM) and in the PBS with 150 mM NaCl (0.64 mM), we observed that, as expected, the CMC decreases with increasing the solution ionic strength. From the NMR chemical shift data, DPC molar fraction in the mixed micelles resulted to be 0.58 and 0.36 at 50 mM and 100 mM NaCl, respectively. Thus, comparing these results to those obtained in water (0.61) and in PBS with 150 mM NaCl (0.27), it can be seen that the difference between DPC molar fraction in the mixed micelles and that in the bulk increases with decreasing the solution's ionic strength.

At such a relatively high SDS molar fraction, our results show that, when SDS and DPC are mixed together, their CMC decreases reflecting a facilitated (synergistic) micellization. In terms of mixed micelle composition, when the two surfactants interact in water, the molar fraction of DPC in the mixed micelle at the CMC was found to be significantly higher than that of the bulk solution, suggesting that a relatively higher local concentration of the zwitterionic detergent is needed to effectively compensate for the electrostatic repulsion between the negatively charged SDS headgroups. On the other hand, with the increasing solution ionic strength, micellization of both pure surfactants and of their mixtures becomes easier because of the presence of the electrolytes. They tend to neutralize the electrostatic repulsion between the headgroups and, in turn, such a higher local concentration of DPC in the mixed micelle is no more needed during synergistic micellization.

However, as expected, the results obtained for DPC molar fraction in the mixed micelles at a total surfactants concentration of 20 mM (ψ^{*}_{DPC} in Tables 1 and 2), show that the micelles' composition derived from either the RST and/or Motomura's formalism refer only to a relatively narrow range above the CMC. Indeed, both in water and PBS, as soon as total surfactant concentration exceeds the CMC by ca. one order of magnitude, micelles' composition closely matches the mixture's one, in all of the investigated cases.

Biophysical studies are usually performed in a controlled environment at physiologically relevant pH and ionic strength, such as PBS, while pure water is rarely employed as solvent. Thus, we focused our attention on the former environment, estimating the average size and ζ-potential for the different SDS/DPC mixtures with DLS measurements. Usually, the systems used for biophysical studies of membrane proteins and peptides are characterized by lipids/surfactants concentration about 100 times higher than that of the protein/peptide under investigation. This means that for a peptide/protein concentration of about 2 mM, which is common in liquid-state NMR, lipids/detergents concentration easily reaches 200 mM. Thus, we performed our measurements at a total surfactant concentration of 20 and 200 mM, that is, about one and two orders of magnitude above the CMC. Micelles with different composition resulted to have comparable size at both these concentrations. This is in agreement with data reported in the literature, because, within the range investigated in the present study, micelles average size is usually found to be only weakly dependant on surfactant concentration.[36,49,50] Assuming a spherical shape, micelles diameter was estimated to be 4.3 ± 0.9 nm, which is compatible to the values reported in the literature for pure SDS and DPC micelles.[9,49,51,52] For instance, SDS micelles in water at 298 K are found to be characterized by an almost constant aggregation number ($N_{ag} = 64$) above the CMC in the range 10–500 mM, corresponding to a constant average diameter of 3.7 nm.[49] Finally, Figure 5 shows the ζ-potential values determined at both 20 and 200 mM as a function of mixture composition. As expected, it increases with decreasing SDS molar fraction in the mixed micelles but does not show any significant difference at the two concentrations employed. This result, together with the observed comparable size and recalling that micelle's composition matches that of the mixture, as soon as total surfactant concentration is about one order of magnitude larger than the CMC, indicates that no further variation in the mixed micelles composition occurs up to two orders of magnitude above the CMC.

However, it has to be taken into account that the presence of a peptide might significantly alter the binary system investigated in this work. It might be possible that it preferentially interacts with one of the two detergents and that, eventually, its presence might lead to a partial demixing and/or a compositional change. Thus, results have to be carefully analyzed, and a preliminary investigation on the stability/modifications of the mixed-micellar system should be taken into consideration.

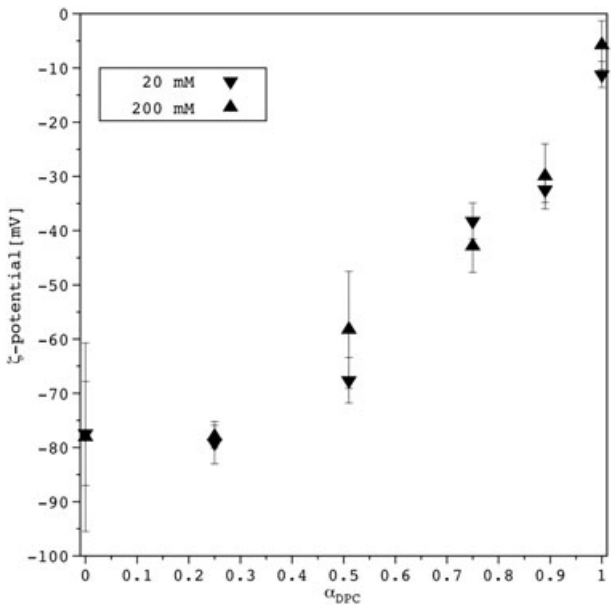

Figure 5. ζ-Potential as a function of dodecylphosphocholine (DPC) molar fraction. The dynamic light scattering measurements were performed at 298 K in PBS, with total surfactant concentration of 20 and 200 mM.

Conclusions

Sodium dodecylsulphate and DPC are the most widely employed surfactants in biophysical investigations that uses micelles as biological membrane models. In particular, the former is used to mimic the bacterial plasma membrane, whereas the latter, the eukaryotic one. However, SDS and DPC are always used separately and when the electrostatics plays a fundamental role in the phenomena under investigation, liposomes are preferentially employed, because differently charged vesicles can be easily prepared depending on the type of the phospholipids included. Because of their slow motional regime, liposomes cannot be employed in liquid-state NMR, resulting in the use of a very highly negatively charged model such as the SDS micelle, or, on the other side, a purely zwitterionic model, such as the DPC one.

The determination of the CMC for different SDS/DPC mixtures and the application of both the RST and Motomura's formalism showed that these two surfactants are synergic and form mixed micelles in both pure water and phosphate buffer saline (pH 7.4) at 298 K.

Overall, the results presented in this work have shown that it is possible to prepare mixed SDS/DPC micelles with different composition to be used as a sufficiently versatile membrane model, allowing for investigations as a function of surface charge. It is clear that these binary micelles represent an extremely simple membrane model compared with bicelles, liposomes, or reconstituted membranes; but nevertheless, we believe they are interesting because they are really stable, easy, and cheap to prepare.

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References

[1] D. L. Nelson, M. M. Cox, *Lehninger Principles of Biochemistry*, W.H. Freeman And Company, New York, **2008**.
[2] L. Maler, A. Graslund. *Methods Mol. Biol.* **2009**, *480*, 129–139.
[3] E. Strandberg, A. S. Ulrich. *Concepts Magn. Reson. A* **2004**, *23*, 89–120.
[4] J. Cavanagh, W. J. Fairbrother, A. G. Palmer III, M. Rance, N. J. Skelton, *Protein NMR Spectroscopy - Principles and Practice*, Elsevier Academic Press, Oxford, **2007**.
[5] P. Damberg, J. Jarvet, A. Graslund. *Methods Enzymol.* **2001**, *339*, 271–285.
[6] A. M. Seddon, P. Curnow, P. J. Booth. *Biochim. Biophys. Acta* **2004**, *1666*, 105–117.
[7] D. V. Tulumello, C. M. Deber. *Biochemistry* **2009**, *48*, 12096–12103.
[8] T. S. Ulmer, A. Bax, N. B. Cole, R. L. Nussbaum. *J. Biol. Chem.* **2005**, *280*, 9595–9603.
[9] D. P. Tieleman, D. Van der Spoel, H. J. C. Berendsen. *J. Phys. Chem. B* **2000**, *104*, 6380–6388.
[10] A. Rozek, C. L. Friedrich, R. E. W. Hancock. *Biochemistry* **2000**, *39*, 15765–15774.
[11] V. Beswick, R. Guerois, F. Cordier-Ochsenbein, Y. M. Coïc, H. D. Tam, J. Tostain, J. P. Noël, A. Sanson, J. M. Neumann. *Eur. Biophys. J.* **1998**, *28*, 48–58.
[12] D. A. Kallick, M. R. Tessmer, C. R. Watts, C. Y. Li. *J. Magn. Reson.* **1995**, *109*, 60–65.
[13] M. Jelokhani-Niaraki, R. S. Hodges, J. E. Meissner, U. E. Hassenstein, L. Wheaton. *Biophys. J.* **2008**, *95*, 3306–3321.
[14] F. Abrunhosa, S. Faria, P. Gomes, I. Tomaz, J. C. Pessoa, D. Andreu, M. Bastos. *J. Phys. Chem. B* **2005**, *109*, 17311–17319.
[15] S. R. Farver, F. D. Mills, V. C. Antharam, J. N. Chebukati, G. E. Fanucci, J. R. Long. *Biophys. J.* **2010**, *99*, 1773–1782.
[16] S.-T. Yang, E. Zaitseva, L. V. Chernomordik, K. Melikov. *Biophys. J.* **2010**, *99*, 2525–2533.
[17] K. B. Smith-Dupont, L. Guo, F. Gai. *Biochemistry* **2010**, *49*, 4672–4678.
[18] V. V. Andrushchenko, M. H. Aarabi, L. T. Nguyen, E. J. Prenner, H. J. Vogel. *Biochim. Biophys. Acta* **2008**, *1778*, 1004–1014.
[19] Z. Zhuang, A. I. Jewett, S. Kuttimalai, G. Bellesia, S. Gnanakaran, J.-E. Shea. *Biophys. J.* **2011**, *100*, 1306–1315.
[20] V. Beswick, A. Isvoran, P. Nédellec, A. Sanson, N. Jamin. *Biophys. J.* **2011**, *100*, 1660–1667.
[21] E. S. Salnikov, B. Bechinger. *Biophys. J.* **2011**, *100*, 1473–1480.
[22] A. A. De Angelis, C. V. Grant, M. K. Baxter, J. A. McGavin, S. J. Opella, M. L. Cotten. *Biophys. J.* **2011**, *101*, 1086–1094.
[23] C. Esposito, A. Tedeschi, M. Scrima, G. D'Errico, F. M. Ottaviani, P. Rovero, A. M. D'Ursi. *J. Peptide Sci.* **2006**, *12*, 766–774.
[24] K. F. Morris, X. Gao, T. C. Wong. *Biochim. Biophys. Acta* **2004**, *1667*, 67–81.
[25] R. M. Watson, R. W. Woody, R. V. Lewis, S. D. Bohle, A. H. Andreotti, B. Ray, K. W. Miller. *Biochemistry* **2001**, *40*, 14037–14046.
[26] S. Mele, S. Murgia, M. Monduzzi. *J. Fluorine Chem.* **2004**, *125*, 261–269.
[27] M. Chen, C. Dong, J. Penfold, R. K. Thomas, T. J. P. Smyth, A. Perfumo, R. Marchant, I. M. Banat, P. Stevenson, A. Parry, I. Tucker, R. A. Campbell. *Langmuir* **2011**, *27*, 8854–8866.
[28] S. Ghosh, D. Khatua, J. Dey. *Langmuir* **2011**, *27*, 5184–5192.
[29] A. A. McLachlan, G. D. Marangoni. *J. Colloid Interf. Sci.* **2006**, *295*, 243–248.
[30] C. Vautier-Giongo, M. S. Bakshi, J. Singh, R. Ranganathan, J. Hajdu, B. L. Bales. *J. Colloid Interf. Sci.* **2005**, *282*, 149–155.
[31] K. Motomura, M. Yamanaka, M. Aratono. *Colloid Polym. Sci.* **1984**, *262*, 948–955.
[32] C. Mohan, *Buffers. A Guide for the Preparation and Use of Buffers in Biological Systems*, Calbiochem - Merck, Darmstadt, **2007**.
[33] W. Schärtl, *Light Scattering from Polymer Solutions and Nanoparticle Dispersions*, Springer, Berlin, **2007**.
[34] W. Brown, *Dynamic Light Scattering: The Method and Some Application*, Clarendon Press, Oxford, **1993**.
[35] J. H. Clint. *J. Chem. Soc., Faraday Trans. 1* **1975**, *71*, 1327–1334.
[36] R. Nagarajan. *Langmuir* **1985**, *1*, 331–341.
[37] K. S. Birdi, S. U. Dalsager, S. Backlund. *J. Chem. Soc., Faraday Trans. 1* **1980**, *76*, 2035–2042.
[38] C. Das, T. Chakraborty, S. Ghosh, B. Das. *Colloid Polym. Sci.* **2008**, *286*, 1143–1155.
[39] T. Chakraborty, S. Ghosh. *Colloid Polym. Sci.* **2007**, *285*, 1665–1673.
[40] K. Din, M. A. Rub, A. Z. Naqvi. *Colloids Surf. B* **2012**, *92*, 16–24.
[41] A. Rodríguez-Pulido, A. Casado, M. Muñoz-Ubeda, E. Junquera, E. Aicart. *Langmuir* **2010**, *26*, 9378–9385.
[42] AIST-SDBS: spectral database for organic compounds. http://riodb01.ibase.aist.go.jp/sdbs/ [January 2012].
[43] E. Fuguet, C. Ràfols, M. Rosés, E. Bosch. *Anal. Chim. Acta* **2005**, *548*, 95–100.
[44] R. De Paula, A. E. da Hora Machado, J. A. de Miranda. *J. Photochem. Photobiol. A* **2004**, *165*, 109–114.
[45] C. Honda, H. Kamizono, K. Matsumoto, K. Endo. *J. Colloid Interf. Sci.* **2004**, *278*, 310–317.
[46] P. Mukerjee, K. J. Mysels, National standards reference data series, national bureau of standards (U.S.A.) **1971**, *36*, 1–227.
[47] M. Yaseen, Y. Wang, T. J. Su, J. R. Lu. *J. Colloid Interf. Sci.* **2005**, *288*, 361–370.
[48] P. Palladino, F. Rossi, R. Ragone. *J. Fluoresc.* **2010**, *20*, 191–196.
[49] G. Duplâtre, M. F. Ferreira Marques, M. da Graça Miguel. *J. Phys. Chem.* **1996**, *100*, 16608–16612.
[50] F. H. Quina, P. M. Nassar, J. B. S. Bonilha, B. L. Bales. *J. Phys. Chem.* **1995**, *99*, 17028–17031.
[51] C. D. Bruce, M. L. Berkowitz, L. Perera, M. D. E. Forbes. *J. Phys. Chem. B* **2002**, *106*, 3788–3793.
[52] T. Lazaridis, B. Mallik, Y. Chen. *J. Phys. Chem. B* **2005**, *109*, 15098–15106.