Effects of interactions on the formation of mixed micelles of 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine with sodium dodecyl sulfate and dodecyltrimethylammonium bromide

Carolina Vautier-Giongo [a], Mandeep Singh Bakshi [b], Jasmeet Singh [b], Radha Ranganathan [a,*], Joseph Hajdu [c], Barney L. Bales [a]

[a] *Department of Physics and Astronomy and The Center for Supramolecular Studies, California State University, Northridge, CA 91330-8268, USA*
[b] *Department of Chemistry, Guru Nanak Dev University, Amritsar 143005, Punjab, India*
[c] *Department of Chemistry and The Center for Supramolecular Studies, California State University, Northridge, CA 91330-8268, USA*

Abstract

Mixed micelles of the phospholipid 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (DHPC) with sodium dodecyl sulfate (SDS) or dodecyltrimethylammonium bromide (DTAB) in aqueous solutions and the effects of interactions between the components were studied by fluorescence and NMR measurements. The regular solution theory (RST) was applied to analyze the experimental critical micelle concentration values determined from the fluorescence spectra of pyrene in the mixed micelles. Negative values for the interaction parameter (β_{12}) were obtained for both DHPC + SDS and DHPC + DTAB mixtures, with the value being more negative in the former case. The negative β_{12} values for the two systems imply that the interaction between the phospholipid and the two ionic surfactants is attractive in nature, being more intense in the case of DHPC + SDS. The interaction parameter, β_{12}, varies with composition of the mixtures indicating changes in packing. The proton NMR shifts are quite different for the two systems and also vary with composition. An interpretation of these experimentally determined chemical shifts in terms of the degree of compactness attributed to electrostatic and steric interactions in the mixed micelle supports the conclusions derived from the fluorescence cmc experiments.
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1. Introduction

Aggregates of synthetic lipids and their mixtures with ionic surfactants are model systems for investigating aggregated assemblies of phospholipids that occur naturally in biological systems [1,2]. In the human digestive tract, phospholipids form mixed micelles with bile salts and are hydrolyzed by the phospholipase enzymes [3–5]. Although several studies exist on the mixed micelle formation between conventional surfactants and long chain phospholipids upon a breakdown of the vesicular structures [6–9], little is known about direct mixed micelle formation between phospholipids and detergents. Hence, it is important to elucidate the nature of mixed micellization on the basis of different categories of the surfactants. Furthermore, information on the properties of these mixed aggregates remains scarce. Characterization of assemblies containing lipids, undertaken here, is motivated by their biological significance [10] and is believed to be a relevant first step toward better understanding of phospholipase enzymology where phospholipid containing mixed micelles serve as substrates.

In the present work, we report studies of binary combinations of a micelle forming phospholipid diheptanoylphosphatidycholine (DHPC, Fig. 1) [11,12] with two ionic surfactants: sodium dodecyl sulfate (SDS) and dodecyltrimeth-

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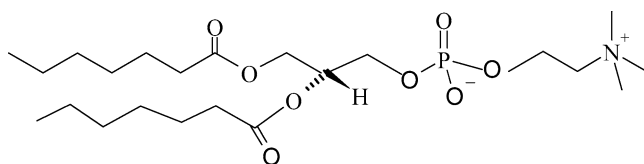
DHPC

Fig. 1. Chemical structure of the phospholipid 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (DHPC).

ylammonium bromide (DTAB). Micelles are formed directly upon mixing DHPC and SDS and DHPC and DTAB. The choice of surfactants was based on the fact that the ionic surfactants, SDS and DTAB, are expected to interact more strongly with zwitterionic DHPC in view of the electrostatic interactions than the surfactants of any other category. We have specifically selected two ionic surfactants with identical hydrophobic tail in order to distinguish between the effect of anionic and cationic head groups on the mixed micellization with DHPC. Critical micelle concentration (cmc) measurements and proton NMR shift studies were carried out with the goal of understanding the effects of headgroup interactions on the structural organization of the constituent components in the mixed micelles. The results from these experiments are examined for a mutually consistent interpretation. Interactions in mixed micelles are conventionally studied by cmc measurements [13]. Electrostatic and steric interactions between headgroups play a major role in the formation of mixed micelles [13]. At cmc, where the aggregation numbers are small, electrostatic interactions have been shown to play the dominant role [13]. In this work, ^{1}H NMR is used to probe the compactness of the mixed micelles at concentrations well above the cmc in order to determine the effects of steric as well as electrostatic interactions.

2. Materials and methods

2.1. Materials

DHPC (lyophilized powder from Avanti Polar Lipids Inc., >99%), sodium dodecyl sulfate, SDS (Sigma, 99%), and dodecyltrimethylammonium bromide, DTAB (Aldrich, 99%), were used as received. Pyrene (Aldrich) was purified by recrystallizations from ethanol. Nanopure water from Sybron/Barnstead Nanopure II was used as solvent.

2.2. Critical micelle concentration (cmc) from pyrene fluorescence measurements

The cmc values for each binary surfactant mixture were obtained by monitoring the pyrene I_1/I_3 ratio ([pyrene] = 0.5 µmol L^{-1}) [14–16]. Different binary stock solutions with a total surfactant concentration (S_t) of 10, 20 and 30 mM

were prepared for the two systems investigated. The composition of the solutions was expressed in molar fraction (X_i) of the respective surfactant, defined as

$$X_i = \frac{[S_i]}{[S_i] + [S_j]}, \tag{1}$$

where $[S_i]$ and $[S_j]$ are the molar concentrations of the surfactants i and j in the mixed solution. Fluorescence emission spectra of these solutions were recorded employing an excitation wavelength of 334 nm, and the intensities I_1 and I_3 were measured at the wavelengths corresponding to the first and third vibronic bands located at ca. 373 and 384 nm. The ratios I_1/I_3 were plotted as a function of the total surfactant concentration. The cmc was taken from the maximum of the second derivatives of the least-square sigmoidal best fits of the experimental data as represented in Fig. 2a with the example of SDS. The estimated errors in cmc values were less than 15%. All the steady-state fluorescence measurements were recorded on an FS 900 CT steady-state T-geometry fluorometer of Edinburgh Analytical Instruments (EAI). The apparatus uses a 450 W steady-state xenon lamp as the excitation source and is equipped with thermostat cells housing. For some of the samples, the cmc was checked by surface tension measurements, using a Krüss K12 tensiometer and the Wilhelmy plate technique [17].

2.3. NMR measurements

^{1}H NMR spectra were recorded on a Bruker AC200E instrument. Measurements were conducted on mixtures of SDS and DHPC (SDS + DHPC) and DTAB and DHPC (DTAB + DHPC) at various compositions. The total surfactant concentration was kept at 50 mmol L^{-1}. All chemical shifts were measured relative to the sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS), which acted as an internal standard [18]. Deuterium oxide (D$_2$O; 99.9%) from Aldrich was used as solvent instead of water to weaken the water signal for all solutions. Only the chemical shift differences were considered in this study. The downfield shift (to lower magnetic fields) of the resonance relative to the first component is represented by a negative sign ($-\Delta\delta$) and an upfield shift (to higher magnetic fields) is shown by a positive sign ($+\Delta\delta$) [18]. In terms of the micelle structure, an increase in the compactness of the micelle gives rise to an increased shielding of the proton from the magnetic field. Therefore a positive or upfield shift in the proton resonance signifies tighter packing of the monomers in a micelle [18]. Conversely, a negative shift means a deshielding or a loosening of the micelle.

3. Results and discussion

3.1. Critical micelle concentration and synergism

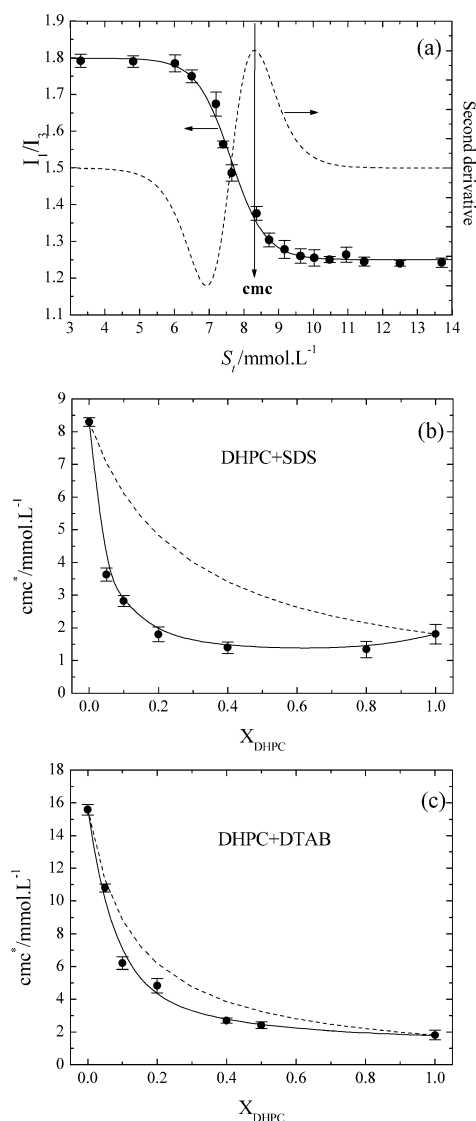The cmc values obtained for pure SDS and DTAB are 8.3 and 15.6 mmol L^{-1}, which are in good agreement with

Fig. 2. (a) Variation of the pyrene intensity ratios, I_1/I_3, with the total concentration, S_t, of pure SDS in water at 30 °C: (●) experimental points; the solid line represents the best sigmoidal fit to the experimental data; the dashed line corresponds to the second derivative of the sigmoidal curve; the vertical arrow denotes the cmc value. Experimental mixed critical micelle concentrations, cmc*, for (b) DHPC + SDS and (c) DHPC + DTAB mixed mixtures as a function of the total DHPC molar fraction in solution. The dashed line represents the theoretical cmc values calculated using the Clint model [29], which supposes an ideal behavior for the mixtures.

literature values, i.e., 8.3 mmol L^{-1} [19–21] and 14.6–16.0 mmol L^{-1} [22–25], respectively. For DHPC, the cmc of 1.8 mmol L^{-1} determined in this work from pyrene fluorescence compares well with the previously reported values of 1.4 and 2 mmol L^{-1} at 25 °C [11,26–28] and with the value of 1.6 mmol L^{-1} obtained in this work by surface tension measurements.

The changes in cmc values as a function of the bulk molar fraction of DHPC, X_{DHPC}, for the two mixed systems are shown in Figs. 2b and 2c (see also Tables 1 and 2) along with the error bars, which were determined from four independent measurements. The figures also show the theoretical cmc

values calculated using the Clint model [29], which supposes an ideal behavior for the mixtures. In this model, the cmc* of mixtures of two surfactants is expressed as the weight average of the cmcs of the pure components,

$$\frac{1}{\text{cmc}^*} = \frac{X_i}{\text{cmc}_i} + \frac{(1 - X_i)}{\text{cmc}_j}, \tag{2}$$

where X_i is the molar fraction of the surfactant i in solution and cmc$_i$ and cmc$_j$ are the cmcs of the pure components i and j. The data presented in Figs. 2b and 2c clearly show the nonideal behavior of both systems, where the experimental cmc values are always smaller than those predicted using Clint model. In addition, the deviation from the ideality is more pronounced in the case of DHPC + SDS system. The lowering of the cmc on mixing two types of surfactants arises from attractive interactions between the components. It is generally observed that anionic/nonionic interactions are stronger than those of cationic/nonionic assemblies [30]. The electrostatic shielding for the positive charge in the DTAB headgroup is likely to be greater than that for the negative charge on the sulfate headgroup in SDS. This is so because in the cationic trimethylammonium head group in DTAB, three methyl groups surround the positively charged nitrogen while the negatively charged oxygen in the sulfate headgroup in SDS is exposed (Fig. 4). The presence of a stronger attractive interaction in SDS + DHPC than in DTAB + DHPC is consistent with this explanation.

Nonideality of surfactant interactions (either antagonism or synergism) may be analyzed by using the regular solution theory (RST) [28,29,31–33], which includes an interaction parameter (β_{12}) to characterize the interactions between the two surfactant species in the mixed micelles. This parameter is related to the activity coefficients (γ) of the surfactants within the micelle by

$$\gamma_1 = \exp \beta_{12}(1 - x_1)^2, \tag{3a}$$

$$\gamma_2 = \exp \beta_{12} x_1^2, \tag{3b}$$

where x_1, the mole fraction of the surfactant 1 in the mixed micelle, can be extracted from an iterative solution of

$$\frac{x_1^2 \ln(X_1 \text{cmc}^*/x_1 \text{cmc}_1)}{(1 - x_1)^2 \ln[(1 - X_1)\text{cmc}^*/(1 - x_1)\text{cmc}_2]} = 1. \tag{4}$$

The interaction parameter β_{12} can be evaluated from

$$\beta_{12} = \frac{\ln(X_1 \text{cmc}^*/x_1 \text{cmc}_1)}{(1 - x_1)^2}. \tag{5}$$

The calculated values of β_{12} for the systems DHPC + SDS and DHPC + DTAB, as a function of the bulk DHPC molar fraction, X_{DHPC}, are shown in Fig. 3a as summarized in Tables 1 and 2. As expected from the analysis of Figs. 2b and 2c, the β_{12} values are negative over the whole range of composition for both systems, which indicates that formation of mixed micelles is favored. The values being more negative for DHPC + SDS system means that the attractive interactions between SDS and DHPC are stronger
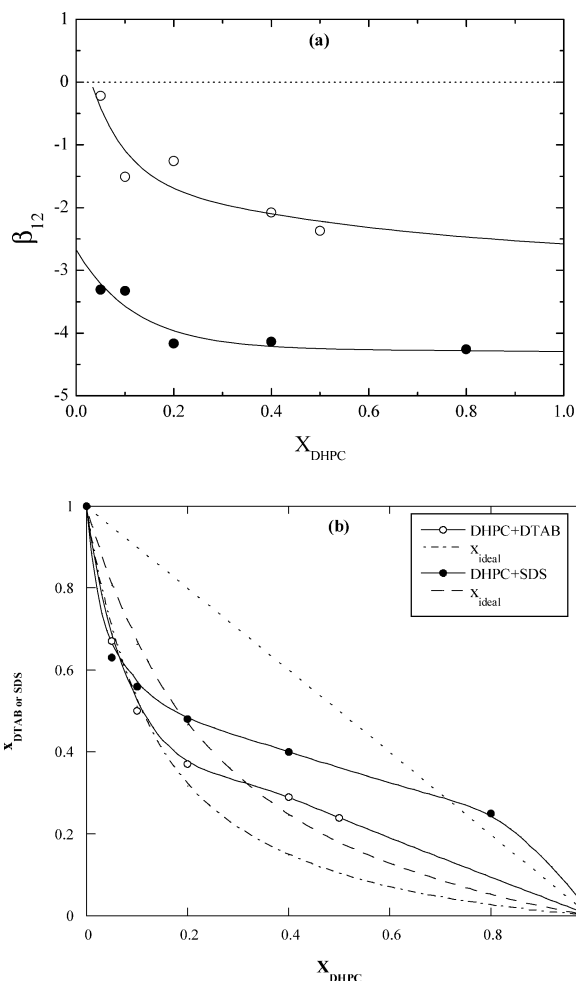
Fig. 3. (a) β_{12} parameter as a function of the bulk molar fraction of DHPC in the mixed systems: (●) DHPC + SDS and (○) DHPC + DTAB. The dashed line describes an ideal behavior. (b) Micelle molar fraction of SDS or DTAB, x_{SDS} or x_{DTAB}, as a function of the bulk molar fraction of DHPC, X_{DHPC}, in the mixed systems: (●) DHPC + SDS and (○) DHPC + DTAB. The dashed lines describe an ideal mixing behavior.

Table 1
cmc* (cmc of mixtures determined experimentally), interaction parameter β_{12}, and the SDS micelle molar fraction, x_{SDS}, for the mixed system DHPC + SDS at different DHPC bulk molar fraction, X_{DHPC}

X_{DHPC}	cmc* (mmol L^{-1})	β_{12}[a]	x_{SDS}[b]
0	8.3 ± 0.1		1
0.05	3.6 ± 0.2	-3.31	0.63
0.10	2.8 ± 0.2	-3.33	0.56
0.20	$1.8 \pm 0.2\ (1.7)$[c]	-4.17	0.48
0.40	1.4 ± 0.2	-4.14	0.29
0.80	$1.3 \pm 0.3\ (1.4)$[c]	-4.26	0.24
1.00	$1.8 \pm 0.3\ (1.6)$[c]		0

[a] Evaluated from Eq. (5).
[b] Iterative solution of Eq. (4).
[c] cmc obtained by surface tension measurement.

Table 2
cmc* (cmc of mixtures determined experimentally), interaction parameter β_{12}, and the DTAB micelle molar fraction, x_{DTAB}, for the mixed system DHPC + DTAB at different DHPC bulk molar fraction, X_{DHPC}

X_{DHPC}	cmc* (mmol L^{-1})	β_{12}[a]	x_{DTAB}[b]
0	15.6 ± 0.3		1
0.05	10.8 ± 0.3	-0.22	0.67
0.10	6.2 ± 0.4	-1.51	0.50
0.20	4.8 ± 0.4	-1.26	0.37
0.40	$2.7 \pm 0.2\ (2.5)$[c]	-2.08	0.29
0.50	2.4 ± 0.2	-2.37	0.24
1.00	$1.8 \pm 0.3\ (1.6)$[c]		0

[a] Evaluated from Eq. (5).
[b] Iterative solution of Eq. (4).
[c] cmc obtained by surface tension measurement.

than those between the phospholipids and DTAB. Since there is no difference between the alkyl chains of DTAB and SDS, the distinct behavior of the two mixed systems is clearly attributed to the differences in the interactions in the headgroup region [34–37]. In RST a single interaction parameter characterizes the nonideality of mixing. The component molecules are assumed to be of comparable volume, completely interchangeable and the interaction energy is expressed as a sum of pairwise nearest neighbor interactions [34,35]. A recent phenomenological approach argues that in ionic/nonionic mixed micelles, the origin of synergism and the variation of β_{12} with composition lies in the electrostatic contributions to the excess electrostatic free energy [38]. The procedure proposed is applicable to micelle solutions of high and medium ionic strengths and includes the contributions of the surface charge density as it changes with composition. The method may not be directly apply to the present set of data that are on additional electrolyte free solutions.

Nevertheless the idea does apply and is useful in understanding that the charged micelle surface interaction with the medium, counterion binding, and intermicelle interactions play a role in synergism [38]. A varying β_{12} could also indicate an interaction that depends on the relative arrangement of the molecules or the packing structure of the monomers in the mixed micelle in our case, where the two components are structurally different because of differences in the geometry of the headgroups and tails. This interaction makes a nonelectric contribution to the excess free energy. The variation is most pronounced in the region $0 \leqslant X_{DHPC} \leqslant 0.2$, that is when the phospholipid is present in smaller amounts. Such a behavior observed in other ionic/nonionic mixed micellar systems is attributed to changes with composition in the packing of the components and micelle hydration in the head group region [39]. The proton NMR chemical shift data given later below seem certainly consistent with the existence of such changes.

We next consider the micelle composition in Fig. 3b. In micelles of either system the DHPC molar fraction is generally higher than that in the total solution. The micelle composition curves lie below the solution composition line. This is to be expected because DHPC being less hydrophilic has the lower cmc and will preferentially partition into the micelles. Nonideality also results in micelle composition being different not only from the solution composition but also that

calculated for ideal mixtures, particularly at the cmc. A plot of the micelle molar fractions, estimated using Eq. (4), of SDS (x_{SDS}, values in last column of Table 1) or DTAB (x_{DTAB}, values in last column of Table 2), versus the molar fraction of DHPC in solution (in column 1 of Tables 1 and 2) are shown in Fig. 3b. The micelle mole fraction in the ideal mixing case (x_{ideal}) for the respective mixtures computed by using Motomura's theory [40] based on excess thermodynamic quantities,

$$x_{ideal} = \left[(X_1 cmc_2) / (X_1 cmc_2 + (1 - X_1)cmc_1) \right], \qquad (6)$$

is also plotted in Fig. 3b. It becomes very clear that both systems are far from an ideal behavior. The DHPC mole fraction in DHPC + SDS mixed micelles is greater than that predicted for ideal mixtures while DHPC + DTAB mixed micelles remain close to ideal for $X_{DHPC} < 0.2$. At about $X_{DHPC} = 0.2$ the micelle composition curve crosses the ideal mixing composition curve. The mixed micelles become richer in their respective ionic components over their values in ideal mixtures. This mixed micelle behavior of DHPC + SDS is somewhat analogous to that of SDS + hexaethylene glycol monododecylether ($C_{12}E_6$) [13]. Molecular thermodynamic calculations for SDS and $C_{12}E_6$ show that at cmc (i) mixed micelles are always richer in $C_{12}E_6$ than its composition in solution; (ii) the electrostatic free energy of mixed micelles decreases steeply for solution concentrations of $C_{12}E_6$ from 0 to 0.2 mol% and more gradually thereafter whereas the steric free energy increases at a relatively much smaller degree [13]. Therefore the dominant contribution to lowering the free energy is due to the reduction in the electrostatic free energy. A similar conclusion may be drawn for the present system of DHPC + SDS micelles. Steric interactions are expected to influence packing and this is indicated in the NMR results.

3.2. ^1H NMR

The shifts in the proton resonances of some of the selected protons of pure components (Fig. 4) of both binary mixtures are shown in Fig. 5 (data values are given in Tables 3 and 4). These measurements are at total surfactant concentrations of 50 mM, well above the cmc. An instant comparison of the Figs. 5a and 5b shows that the addition of DHPC to SDS micelles (Fig. 5a) brings shielding (upfield or positive shift) to all of the hydrophobic tail protons of SDS from the magnetic field. The addition of DHPC to DTAB micelles has the opposite effect (deshielding) on the DTAB protons in the DHPC + DTAB micelles (Fig. 5b). This seems to indicate that the SDS + DHPC micelles are more compact than pure SDS micelles and the DTAB + DHPC micelles are less compact than the pure DTAB micelles.

The increase or decrease and the finer features of maxima and minima in compactness as registered by the changing chemical shift values for any given system or differences between micellar systems, is an indication of the presence of and interplay between more than one type of interaction. The effects on three of the protons of SDS, including the terminal methyl group proton (H_{C12}), the proton nearest to the headgroup (H_{C1}), and one representing the other tail methylene protons (H_{C11}) are shown in Fig. 5a and on the protons of DTAB in Fig. 5b. Hydrophobic interactions and electrostatic attractions promote spherical and compact micelles whereas steric repulsion causes separation between components leading to exposure and deshielding of the protons. In SDS + DHPC, just as in SDS + $C_{12}E_6$ the small size of the SDS head causes insignificant steric hindrance [13]. The reduced electrostatic repulsion due to the presence of the zwitterionic head produces more compactness, which continues to increase with X_{DHPC} up to $X_{DHPC} \approx 0.5$. A decrease in shielding for higher DHPC concentrations signifies perhaps an increasing steric repulsion and the beginning of formation of large micelles that might be cylindrical. DHPC is indeed known to form very large cylindrical micelles [41,42] and the same may be expected at DHPC rich compositions. The deshielding in DTAB + DHPC micelles may be understood along the same line of reasoning. The steric hindrances between the large headgroups of DHPC and DTAB produce
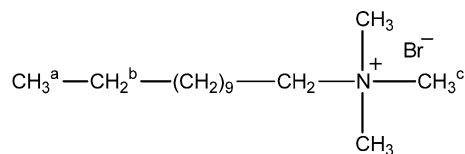
Proton chemical shifts used for ^{1}H-NMR studies in DTAB: ^{a}H$_{C12,}$ ^{b}H$_{C11,}$ ^{c}H$_{N-CH3}$
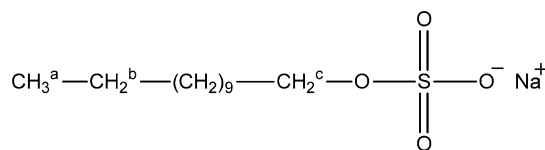
Proton chemical shifts used for ^{1}H-NMR studies in SDS: ^{a}H$_{C12,}$ ^{b}H$_{C11,}$ ^{c}H$_{C1}$
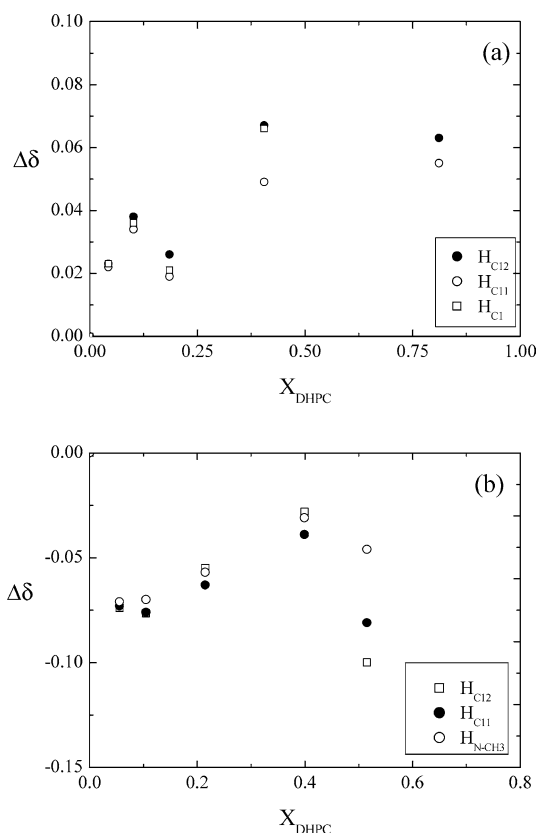
Fig. 4. Formulae and proton labeling in DTAB and SDS molecules.

Fig. 5. (a) ^{1}H chemical shifts (ppm) of SDS protons versus mole fraction of DHPC (X_{DHPC}): (●) H_{C12}^{SDS}; (○) H_{C11}^{SDS}; (□) H_{C1}^{SDS}. (b) ^{1}H chemical shifts (ppm) of DTAB protons versus mole fraction of DHPC (X_{DHPC}): (□) H_{N-CH3}; (●) H_{C12}^{DTAB}; (○) H_{C11}^{DTAB} (see Fig. 4). The total surfactant concentration is 50 mM.

Table 3
Values of proton NMR chemical shift differences ($\Delta\delta$) of the protons labeled in Fig. 4 in DHPC + SDS micelles at various mole fractions

X_{DHPC}	$\Delta\delta$		
	H_{C12}	H_{C11}	H_{C1}
0.0000	0.000	0.000	0.000
0.0435	0.023	0.022	0.023
0.1020	0.038	0.034	0.036
0.1851	0.026	0.019	0.021
0.4053	0.067	0.049	0.066
0.8118	0.063	0.055	

Table 4
Values of proton NMR chemical shift differences ($\Delta\delta$) of the protons labeled in Fig. 4 in DHPC + DTAB micelles at various mole fractions

X_{DHPC}	$\Delta\delta$		
	H_{C12}	H_{C11}	H_{N-CH3}
0.0000	0.000	0.000	0.000
0.0554	−0.074	−0.073	−0.071
0.1049	−0.0765	−0.076	−0.070
0.2147	−0.055	−0.063	−0.057
0.3991	−0.028	−0.039	−0.031
0.5150	−0.100	−0.081	−0.046

more openness in the micelle and the DTAB + DHPC micelles are always less compact than DTAB micelles. The drop in compactness at $X_{DHPC} \approx 0.4$ is rather sharp and could be brought about by a transformation to large cylindrical micelles. Scattering methods and relative viscosity measurements are necessary to confirm the changes in micelle shapes. In mixed micelles, a mismatch in the length of the component molecules can cause the surface of the micelle to be rough in the sense of a curvature that varies along the surface as opposed to a smooth spherical surface. This causes additional exposure of the protons. Steric repulsion is generally greater when aggregation numbers are large. In both SDS + DHPC and DTAB + DHPC, existence of a weak minimum in the chemical shift curves around $X_{DHPC} \approx 0.15$ (for $X_{DHPC} < 0.5$) signifies that this is the composition where the least compact micelles are formed, predicting a maximum in the aggregation number at that composition.

4. Summary and conclusions

Mixed micelles are formed between diheptanoyl phosphatidylcholine and the ionic detergents, SDS and DTAB at

all compositions. Critical micelle concentration and NMR measurements were conducted and the results of these experiments complement each other. Electrostatic and steric interactions play the dominant roles in the formation of the mixed micelles.

An analysis of the cmc data based on RST shows that both DHPC + SDS and DHPC + DTAB mixtures undergo synergistic interactions, which are stronger in the former case than later. However deviations from the RST model are clearly observed. Proton NMR shifts in the mixed micelles reveal that SDS + DHPC micelles are more compact than SDS micelles and DTAB + DHPC micelles are less compact than DTAB micelles. The shielding of the positive charge by the bulky trimethylammonium head group and its steric hindrances in the course of mixed micelle formation likely oppose the compacting effects of the attractive interactions between the components to a greater extent in DHPC + DTAB than the properties of the SDS headgroup in DHPC + SDS micelles. Thus, the collective interpretation of NMR results for both mixtures support our conclusions from cmc data that stronger attractive interactions exist in the case of DHPC + SDS than DHPC + DTAB. This is significant from the biological point of view because naturally occurring mixed micelles include zwitterionic phospholipids in conjunction with negatively charged surfactants.

Micelle compactness varies with composition as determined from the chemical shifts of the detergent hydrophobic tail proton resonance measured by NMR. This is now attributed to interplay between electrostatic and steric interactions.

Acknowledgments

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