

Effect of hydrophobic tail on the mixed micelles of diheptanoylphosphatidycholine with cationic and zwitterionic surfactants: a fluorescence study

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Abstract

The pyrene fluorescence measurements have been performed for the mixtures of diheptanoylphosphatidycholine (DHPC) with dimeric cationic, phosphonium cationic, and zwitterionic surfactants of C₁₀ to C₁₆ hydrocarbon tail length over the whole mixing range. From the I_1/I_3 versus total surfactant concentration plots, the mixed critical micelle concentration (cmc) of various mixtures was computed. The nature of the mixed micelles between the unlike components was determined by applying the regular solution theory. It was observed that all the mixtures show attractive interactions between the binary components and the interactions becomes more intense with the increase in length of the hydrocarbon tail of cosurfactant. The increase in the length of cosurfactant allowed greater amount of DHPC to be accommodated in the mixed micelles, which in return brought stronger synergism. A variation in the Stern–Volmer constant, K_{SV} , further supports these results. © 2004 Elsevier B.V. All rights reserved.

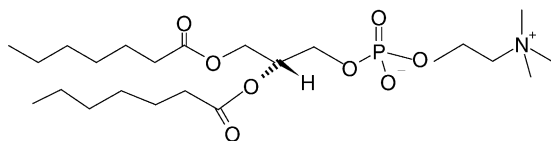
Keywords: Diheptanoylphosphatidycholine; Mixed micelles; Synergism; Fluorescence measurements

1. Introduction

The phospholipids form micelles similar to that of conventional surfactants only when the hydrocarbon tails are very short [1–3]. They are the derivatives of glycerol phosphorylcholine containing two long fatty acid chains. There is a wide variation in chain composition possible in both synthetic and natural phospholipids, and these can affect the solution properties of the phospholipids. The phospholipids with long hydrocarbon tails disperse in water, which may lead to the formation of many different structures including unilamellar and multilamellar vesicles [1]. Such kinds of aggregates [4] act as models for transportation across living cell membranes and hence find potential applications in drug delivery systems. A significant research work has re-

cently been carried out in the field of bilayer solubilization by ionic surfactants [5,6]. The complex mechanism of bilayer solubilization and the evaluation of its properties are still subjects of active debate. It is thought that bilayer solubilization by ionic surfactants involves the mixed micelle process. In such a complicated aggregation process, a precise quantitative analysis generally eludes physical insight when a transformation of bilayer assemblies into mixed micelles takes place [7]. It is, therefore, desirable to first understand the mixed micelle formation process of short chain phospholipids, which form micelles rather than bilayer assemblies at dilute concentration. This would help us to understand the bilayer solubilization process by surfactants more precisely. At this end, in the present study, we have selected binary combinations of a micelle forming phospholipid, i.e. diheptanoylphosphatidycholine (DHPC) [3,8,9] with zwitterionic, cationic monomeric, and dimeric surfactants. The different varieties of surfactants have been selected so as to have a

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Scheme 1.

wide understanding about the nature of mixed micelles with DHPC.

2. Experimental

The lipid, DHPC (see Scheme 1), was obtained from Avanti Polar Lipids as a lyophilized powder. Cationic geminis, dimethylenebis(alkyldimethylammonium bromide) (*m*-2-*m*, where *m* is 10, 12, 14, and 16), were synthesized according to the method reported elsewhere [10]. Decyl- (DeTPB), tetradecyl- (TTPB), and hexadecyltriphenylphosphonium bromides (HTPB), all more than 99% pure from Lancaster Synthesis, England, were used as received. 3-(*N,N*-dimethyldodecylammonio)propanesulphonate (DPS), 3-(*N,N*-dimethyltetradecylammonio)propanesulphonate (TPS), and 3-(*N,N*-dimethylhexadecylammonio)propanesulphonate (HPS), all more than 99% pure from Fluka, were used as received. Double distilled water was used in the preparation of all solutions. All solutions were prepared by mass within the accuracy of ± 0.01 mg. The mole fractions were accurate to ± 0.0001 units.

2.1. cmc measurements

The cmc values for each binary surfactant mixture were obtained by monitoring the pyrene I_1/I_3 ([pyrene] = $0.5 \mu\text{mol L}^{-1}$) [11–13]. The composition of the solutions was

expressed in molar fraction (X_i) of the respective surfactant, defined as

$$X_i = \frac{[S_i]}{[S_i] + [S_j]} \quad (1)$$

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 approximately 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 (see Fig. 1). The errors in cmc values were estimated to be less than 10%. All the steady-state fluorescence measurements were recorded on a Hitachi F-2500 fluorescence spectrophotometer at 25 °C.

3. Results

The cmc values thus obtained as a function of the bulk molar fraction of DHPC for DHPC + 10-2-10/12-2-12/14-2-14/16-2-16, DHPC + DeTPB/TTPB/HTPB, and DHPC + DPS/TPS/HPS mixtures have been shown in Figs. 2–4, respectively. The data for DHPC + DTAB/TTAB/HTAB mixtures plotted in Fig. 2c and d have been taken from our previous work [14]. All the figures also show the theoretical cmc values calculated using the Clint model [15], 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 cmc's of the pure components

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

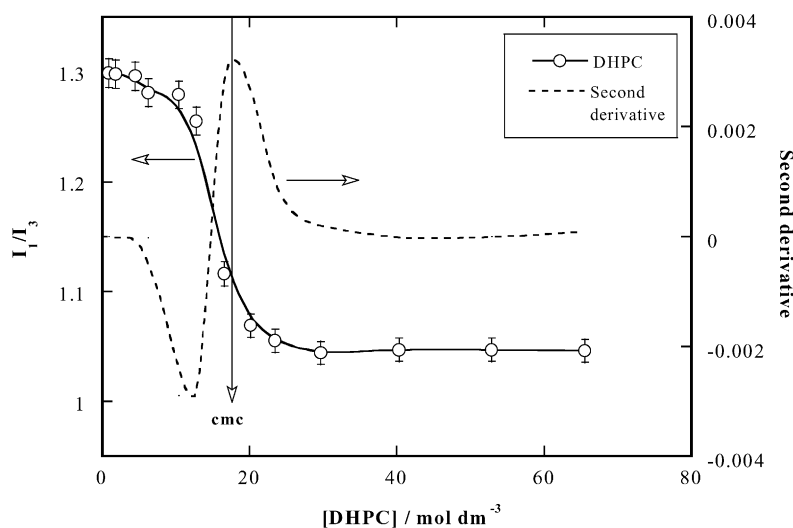


Fig. 1. Variation of the pyrene intensity I_1/I_3 ratios with the total concentration, [DHPC], of pure DHPC in water at 25 °C: (O) experimental points; the solid line represents the best sigmoidal fit to the experimental data; the dashed line corresponds to the second derivative of the sigmoidal curve; the vertical arrow denotes the cmc value.

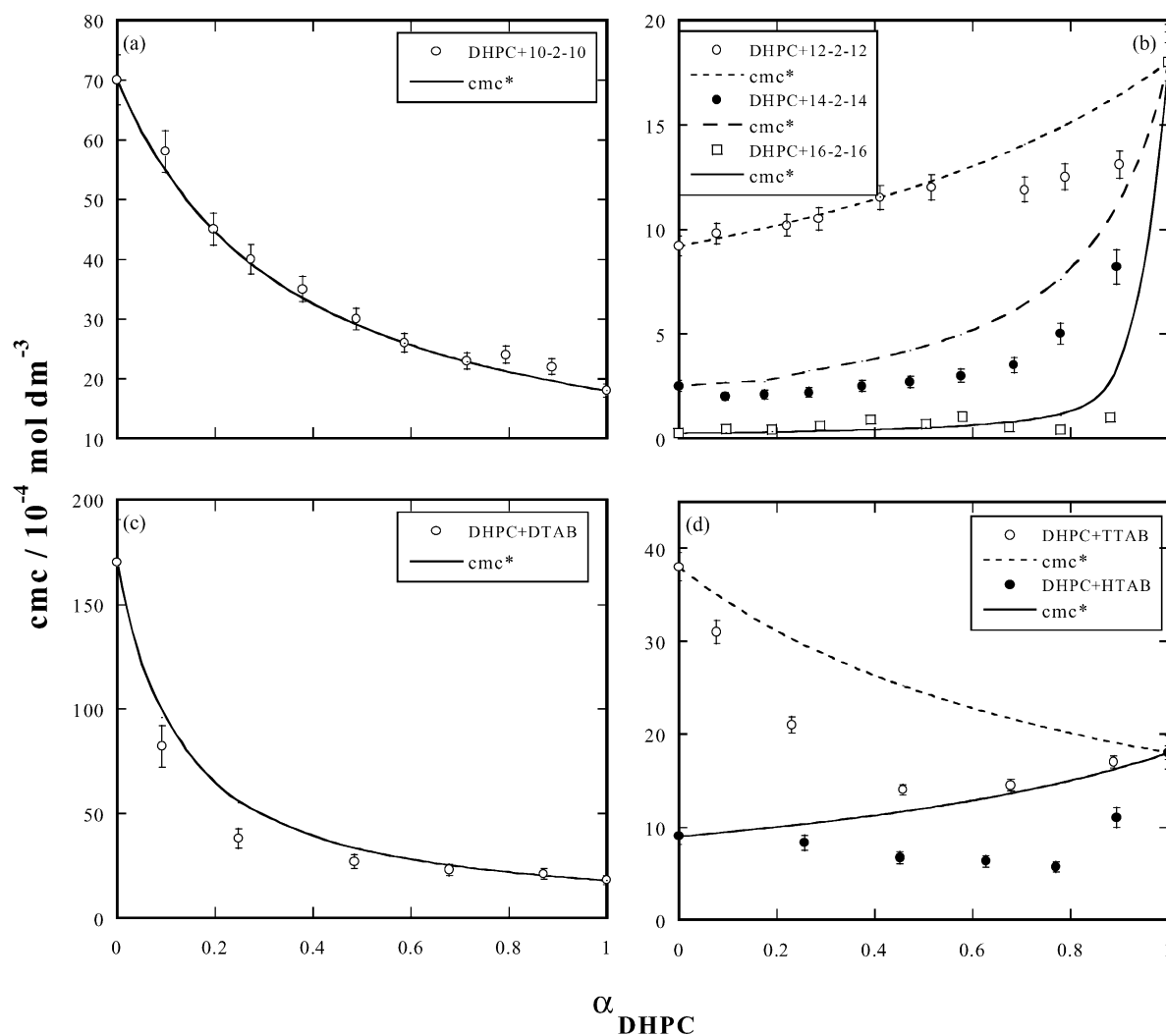


Fig. 2. Plot of cmc vs. α_{DHPC} of: (a) DHPC + 10-2-10, (b) DHPC + 12-2-12/14-2-14/16-2-16, (c) DHPC + DTAB, (d) DHPC + TTAB/HTAB mixture in pure water. Experimental cmc (points); predicted cmc* (line).

where X_i is the molar fraction of the surfactant i (DHPC) in the total mixed solute, and cmc_i and cmc_j are the cmc's of the pure components i and j . The data presented in Fig. 2b clearly show the non-ideal behavior of DHPC + 14-2-14 over the whole mixing range, where the experimental cmc values are always smaller than those predicted by Clint model. Whereas this behavior is mainly observed in the DHPC rich region of DHPC + 12-2-12 and DHPC + 16-2-16 mixtures. In the case of DHPC + 10-2-10, the experimental cmc values are somewhat higher than the cmc* values over the whole mole fraction range (Fig. 2a). The cmc values of all three DHPC + DTAB/TTAB/HTAB mixtures also lie lower to cmc* (Fig. 2c and d). On the other hand, the cmc values become systematically smaller from cmc* with the increase in the length of hydrophobic tail in the case of DHPC + DeTPB/TTPB/HTPB mixtures (Fig. 3). In the case of DHPC + zwitterionic surfactant mixtures (Fig. 4), the cmc values are always less than cmc*. A smaller cmc value than the corresponding cmc* value is accounted for the attractive interactions between the

unlike monomers in the mixed state and indicate a non-ideal mixing behavior.

Non-ideality of surfactant interactions (either antagonism or synergism) can be analyzed by using the regular solution theory (RST) [16], 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

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

$$\gamma_2 = \exp \beta_{12}x_1^2 \quad (4)$$

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

$$\frac{x_1^2 \ln(X_1 \text{cmc}^*/x_1 \text{cmc}_1)}{(1 - x_1)^2 \ln[(1 - X_1) \text{cmc}^*/(1 - x_1) \text{cmc}_2]} = 1 \quad (5)$$

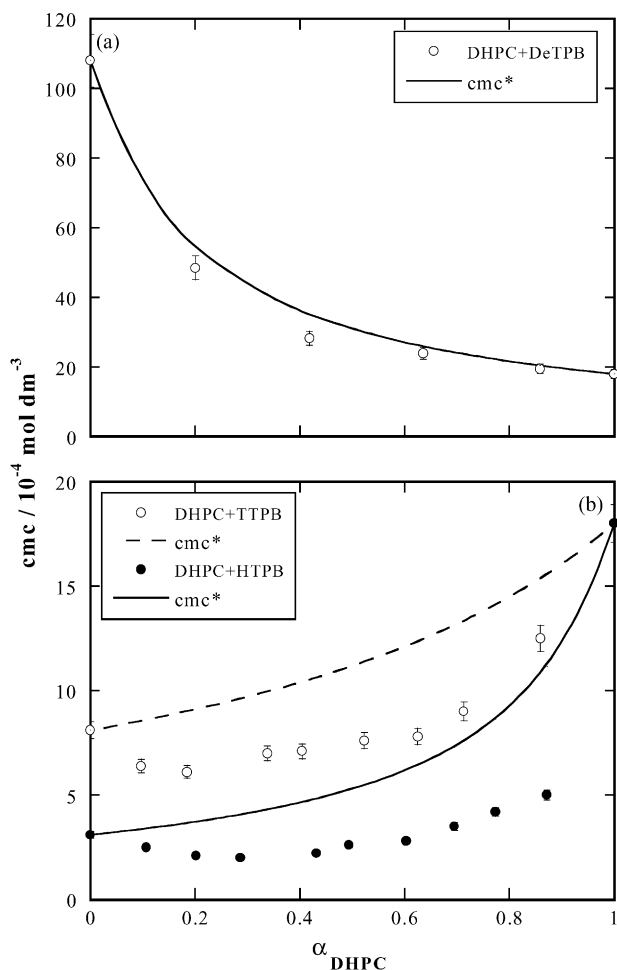


Fig. 3. Plot of cmc vs. α_{DHPC} of: (a) DHPC + DeTPB, (b) DHPC + TTPB/HTPB mixture in pure water. Experimental cmc (points); predicted cmc* (line).

The interaction parameter β_{12} can be evaluated from

$$\beta_{12} = \frac{\ln(X_1 \text{cmc}^* / x_1 \text{cmc}_1)}{(1 - x_1)^2} \quad (6)$$

The β_{12} is an index of the degree of interaction between the two surfactants and also accounts for the system deviation from ideality. A negative β value demonstrates the mixed micellization due to synergism whereas a positive β value represents antagonism. The β values thus computed for various mixtures have been plotted in Fig. 5. Fig. 5a demonstrates the β values of DHPC + dimeric surfactant mixtures. We could not get the β values for DHPC + 12-2-12/16-2-16 mixtures over the whole mixing range due to non convergence of Eq. (3). A relative comparison among the β values suggests that the values are slightly positive for the DHPC + 10-2-10, however, they decrease and become negative with the further increase in the length of hydrophobic twin tails. Similarly, the β values becomes more negative with the increase in the length of hydrophobic tail in the case of monomeric alkyl ammonium surfactants especially in the DHPC rich region of the mixtures (Fig. 5b). This behavior is also clearly demon-

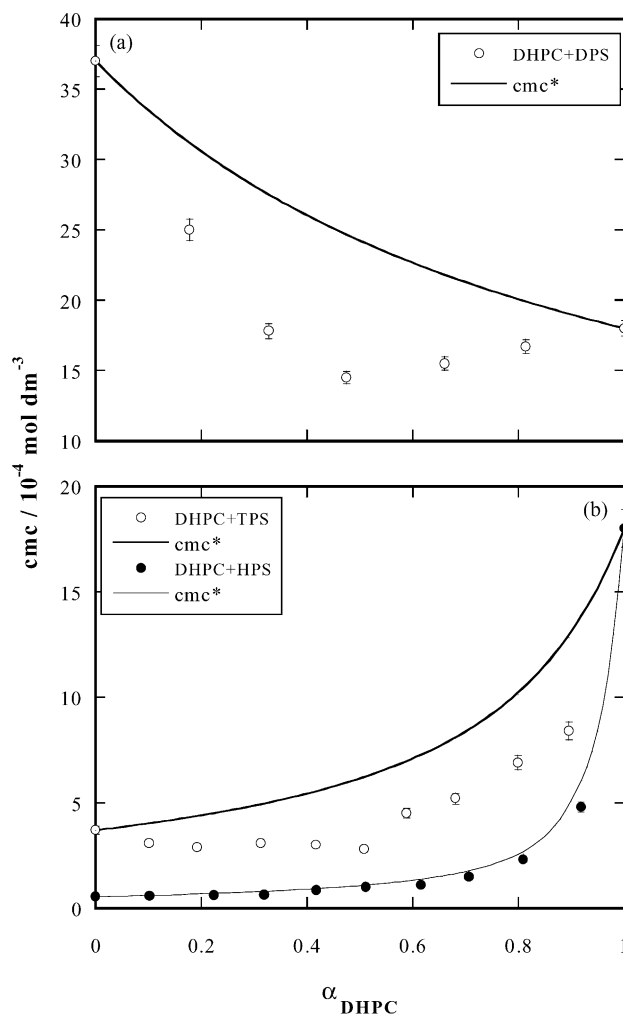


Fig. 4. Plot of cmc vs. α_{DHPC} of: (a) DHPC + DPS, (b) DHPC + TPS/HPS mixture in pure water. Experimental cmc (points); predicted cmc* (line).

strated by the phosphonium surfactants over the whole mole fraction range (Fig. 5c). In the case of DHPC + zwitterionic surfactants (Fig. 5d), β again depends upon the length of hydrophobic tail especially in the cosurfactant (CS) rich region of the mixtures.

The results are further evaluated by plotting micelle mole fraction of DHPC (x_1) in Fig. 6. In the same figure, the mole fraction of DHPC in the ideal state (X_{ideal}) has also been plotted and which has been computed by applying the Motomura's theory on the basis of excess thermodynamic quantities in the ideal state [17]. It is given by the following equation

$$X_{\text{ideal}} = 1 - \frac{X_2 \text{cmc}_1}{X_2 \text{cmc}_1 + (1 - X_2) \text{cmc}_2} \quad (7)$$

One would see that x_1 and X_{ideal} are almost the same in the case of DHPC + 10-2-10 (Fig. 6a). The x_1 is less than the X_{ideal} over the limited mixing range in the case of DHPC + 12-2-12 while mainly higher than X_{ideal} in the case of DHPC + 14-2-14/16-2-16 mixtures. A comparison with monomeric alkyl ammonium surfactants (Fig. 6b) suggests that x_1 is mainly

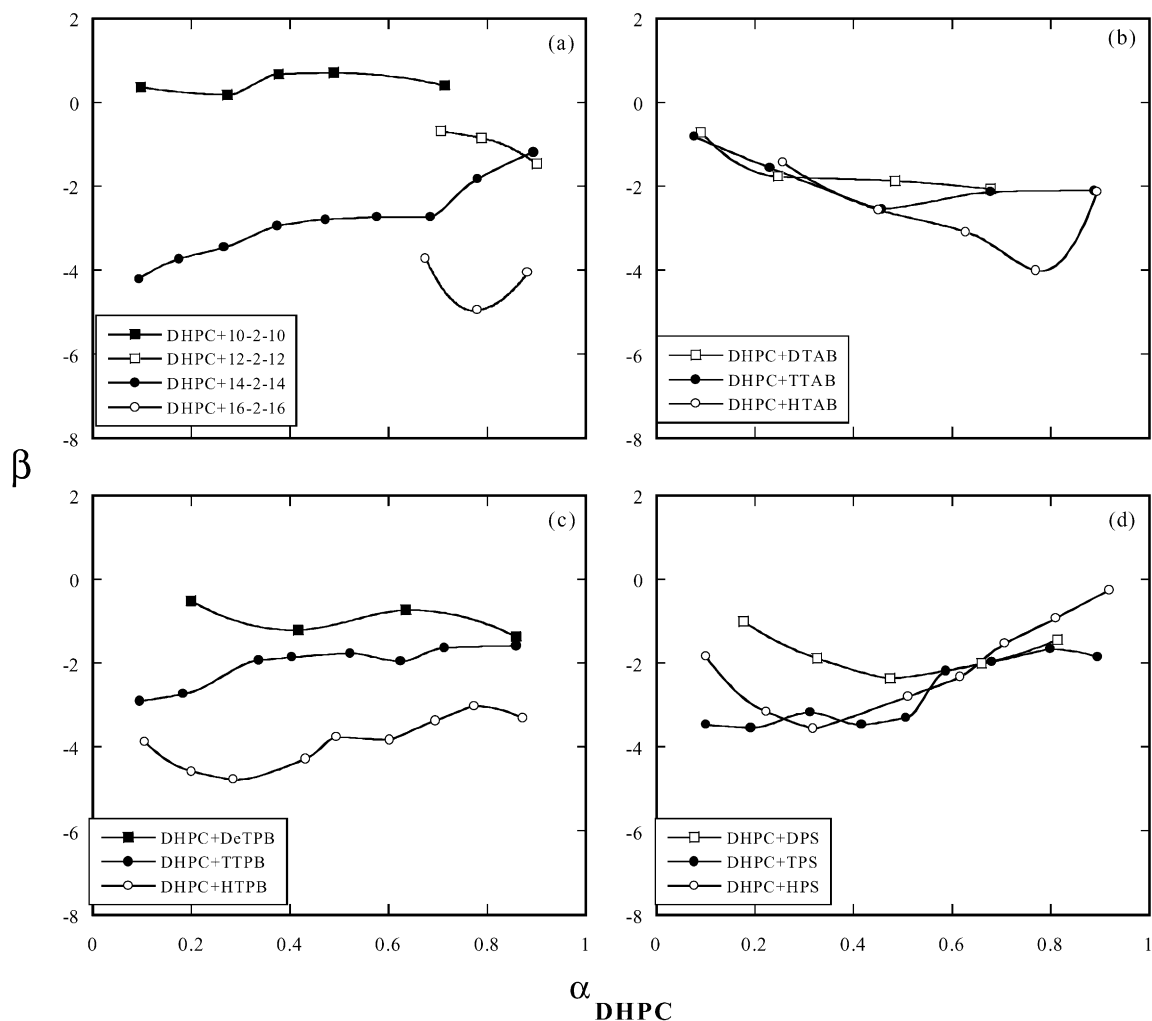


Fig. 5. Plot of β vs. α_{DHPC} of: (a) DHPC + 10-2-10/12-2-12/14-2-14/16-2-16; (b) DHPC + DTAB/TTAB/HTAB; (c) DHPC + DeTPB/TTPB/HTPB (d) DHPC + DPS/TPS/HPS mixtures.

less than X_{ideal} in the DHPC rich region while it becomes regularly more than X_{ideal} in the CS rich region with the increase in hydrophobic tail. The variation of the x_1 for DHPC + phosphonium/zwitterionic surfactant mixture is almost identical with each other (Fig. 6c and d). The x_1 is less than X_{ideal} in DHPC rich region for both DHPC + DeTPB/DPS mixtures, while it becomes much greater than X_{ideal} over most of the mixing range in all other mixtures. A lesser or greater x_1 value than X_{ideal} accounts for the fact that the corresponding mixed micelles are poor or rich in DHPC content, respectively.

4. Discussion

A collective comparison of Figs. 5 and 6 demonstrates that all the mixtures of DHPC with monomeric surfactants of 10 or 12 carbon chain length, i.e. DTAB, DeTPB, and DPS have x_1 values always less than X_{ideal} over most of the mixing range especially in the DHPC rich region. This gives negative β values in all these cases (Fig. 5). However, when instead

of monomeric, dimeric surfactants such as 10-2-10 and 12-2-12 have been taken, we get complete ideal mixing in the former case (Fig. 6a) with slightly positive β values (Fig. 5a). In the latter case, still slight synergisms observed (Fig. 5a) as in the case of monomeric C_{12} surfactants. As the hydrocarbon tail length both in the case of monomeric and dimeric cosurfactants increases from 12 to 16 carbon atoms, x_1 values start increasing from X_{ideal} and the magnitude becomes more prominent in the CS rich region of the mixtures. This produces large negative β values (Fig. 5), which increase as the length of hydrophobic tail increases.

These results indicate two categories of DHPC + CS mixtures. One in which the amount of DHPC in the mixed micelles is less than the ideal state as in the case of DHPC + 12-2-12/DTAB/DPS/DeTPB, the other in which the amount of DHPC is much more mainly in the CS rich region of the mixtures as in the case of all DHPC + surfactants mixtures of C_{14} and C_{16} hydrocarbon tails. This demonstrates that as the amount of DHPC in the mixed micelle increases, the synergism increases. Thus, the increase in the amount of DHPC

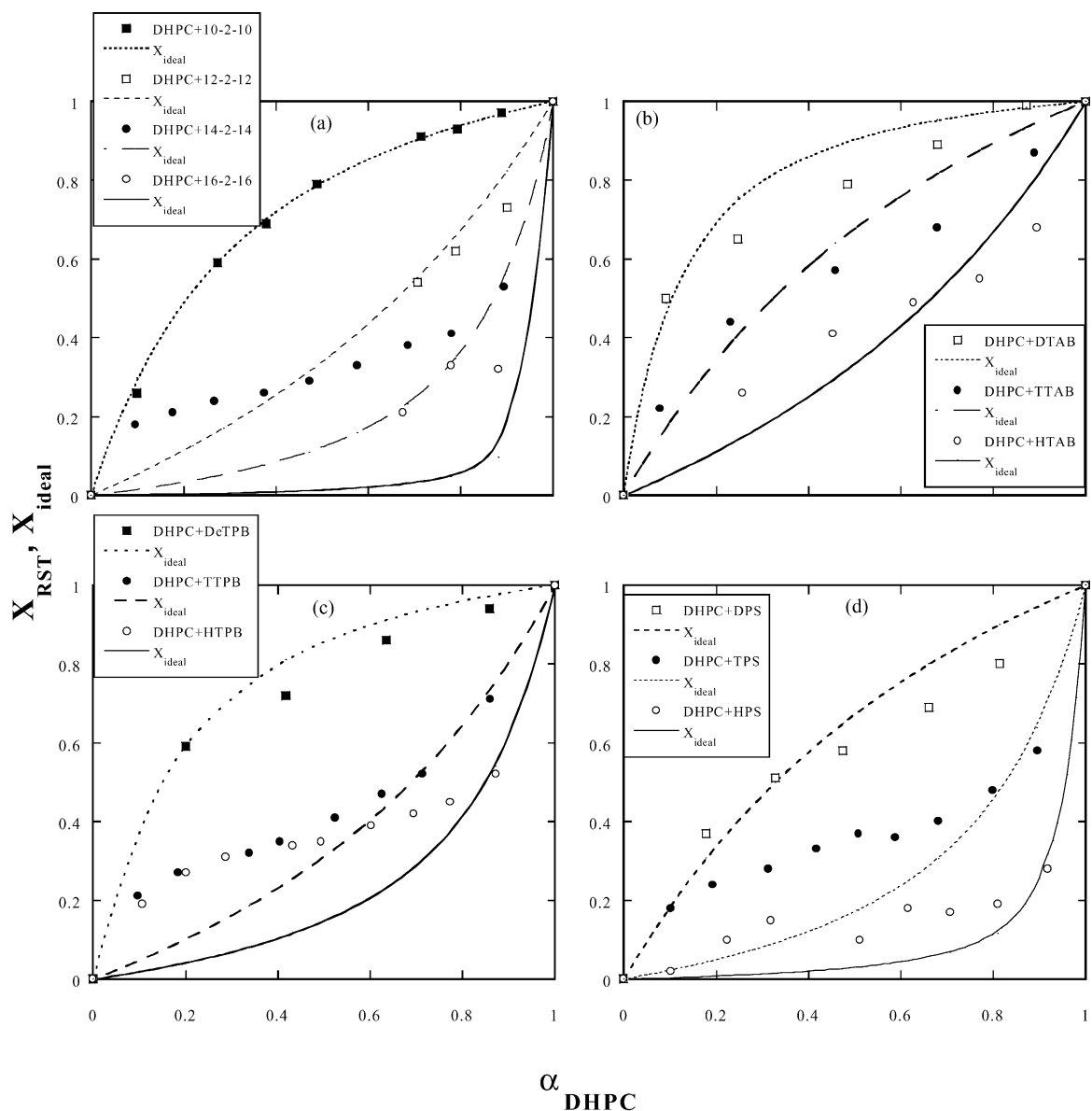


Fig. 6. Plot of X_{RST} and X_{ideal} vs. α_{DHPC} of: (a) DHPC + 10-2-10/12-2-12/14-2-14/16-2-16; (b) DHPC + DTAB/TTAB/HTAB; (c) DHPC + DeTPB/TTPB/HTPB (d) DHPC + DPS/TPS/HPS mixtures.

in the mixed micelle is related to the length of hydrocarbon tail of the CS. As the length of the tail of CS increases, the compatibility with twin tail of DHPC decreases and hence less amount of CS with longer hydrocarbon tail, i.e. C_{14} or C_{16} is allowed to accommodate in the mixed micelles. This could be due to the folding of C_{14} or C_{16} tail to such an extent that it may produce steric problem in accommodating with twin tail of DHPC in a compact micelle arrangement. A relative comparison among all the mixtures indicates that CS of particular tail length has more or less same degree of synergistic interactions with DHPC. This is primarily accounted for short-range electrostatic interactions existing between the phosphocholine head group of DHPC and cationic or zwitterionic head group of cosurfactants.

4.1. Quenching behavior

The above results can further be explained on the basis of quenching of pyrene by a suitable quencher such as cetylpyridinium chloride (HPyCl) under steady state conditions. It is being ensured that the time of the quencher in the micelle remains longer than the fluorescence lifetime of the probe. A suitable $[Pyrene]/[mixed\ micelle]$ and $[quencher]/[mixed\ micelle]$ ratio ensures the Poisson distribution. A fluorescence intensity of the first vibronic band of pyrene decreases with the increase in the $[quencher]$ without appearance of any new band (Fig. 7a). A Stern–Volmer relationship can be used to explain the collision quenching under the steady state condition [18–21]

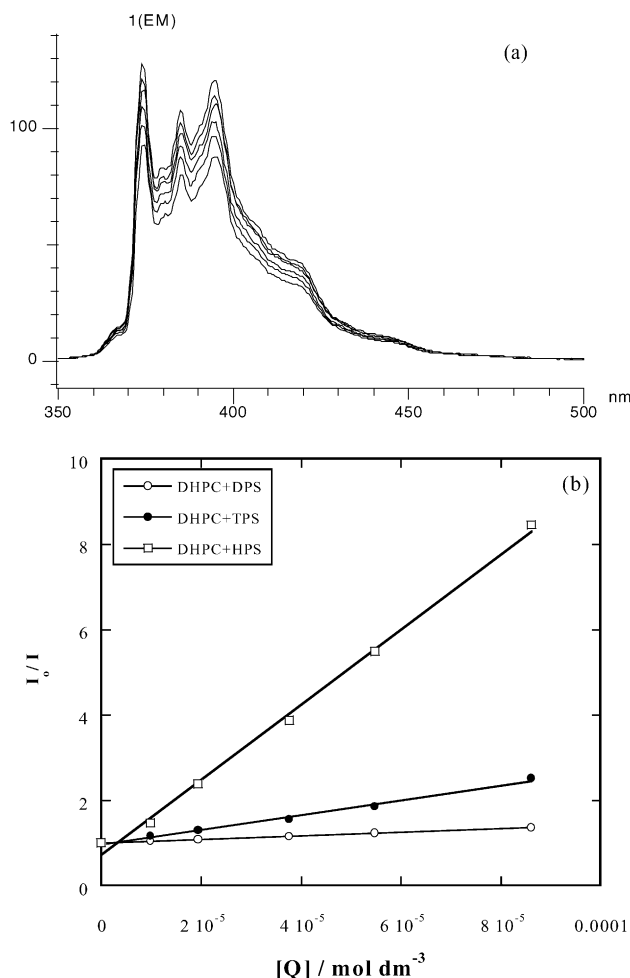


Fig. 7. (a) Fluorescence spectra of DHPC + 12-2-12 (at equimolar concentration) showing the fall in intensity with the increase in the concentration of quencher from 0 to 9×10^{-5} (from top to bottom). (b) Plot of I_0/I vs. the $[Q]$ for DHPC + DPS/TPS/HPS.

$$\frac{I_0}{I} = 1 + K_{SV}[Q] \quad (8)$$

where I_0 and I are the fluorescence intensities without and with quencher, respectively, and K_{SV} is the collisional quenching constant, called the Stern–Volmer constant. A linear variation of I_0/I versus $[Q]$ will give the value of K_{SV} (Fig. 7b). Such values calculated for all the present equimolar mixtures have been listed in Table 1. Similar considerations can be used to evaluate the mean micelle aggregation number (N_{agg}) by using the following equation

$$\ln \left(\frac{I_0}{I} \right) = \frac{[Q]N_{agg}}{([Surf] - cmc)} \quad (9)$$

where $[Surf]$ is the total surfactant concentration. The N_{agg} , thus, computed for equimolar mixtures have also been listed in Table 1. The N_{agg} values of some of the pure components such as DTAB, TTAB, and HTAB have been found to be, 50, 57, and 60, respectively, which are in excellent agreement

Table 1

The Stern–Volmer constant (K_{SV}) and the aggregation number (N_{agg}) of DHPC + CS ($\alpha_{DHPC} \approx 0.5$)

Systems	$K_{SV} \times 10^{-3}$ ($\text{dm}^{-3} \text{mol}^{-1}$)	N_{agg}
DHPC + DTAB	0.97	13
DHPC + TTAB	3.85	38
DHPC + HTAB	6.26	42
DHPC + DPS	3.52	47
DHPC + TPS	10.5	56
DHPC + HPS	31.0	48
DHPC + DeTPB	2.60	45
DHPC + TTPB	3.30	27
DHPC + HTPB	3.86	17
DHPC + 10-2-10	3.38	32
DHPC + 12-2-12	4.34	29
DHPC + 14-2-14	13.4	25
DHPC + 16-2-16	28.2	–

with those reported in literature 48 [22]/50 [23], 55 [22]/60 [24], and 61 [25].

The second column of Table 1 suggests that as the length of hydrophobic tail of cosurfactant increases, the value of K_{SV} also increases in all cases. The increase is quite significant for DHPC + zwitterionic/gemini surfactant mixtures, which demonstrates that the quenching is facilitated in these mixtures in comparison to that in case of DHPC + alkyl ammonium/triphenylphosphonium mixtures. This can be attributed to the presence of suitable hydrophobic environment provided by mixed micelles of the former mixtures for the favorable solubilization of pyrene where effective quenching can take place. It is to be mentioned here that the quencher, HPyCl is also a surfactant and is also expected to be solubilized in the mixed micelles in its monomeric form since the $[HPyCl]$ is always less than its cmc during the quenching process. The favorable hydrophobic environment is created by a significant neutralization of zwitterionic charged group of DHPC in mixed state with other conventional zwitterionic surfactants (i.e. DPS, TPS, and HPS) arranging alternatively in mixed state in the case of DHPC + zwitterionic mixtures. On the other hand, strong hydrophobic arrangement is again achieved when double hydrophobic tails of DHPC arrange themselves with the similar ones of gemini surfactants in the case of DHPC + gemini mixtures. Thus, increases in the K_{SV} value with the increase in the length of the hydrophobic tail of CS fully satisfy the increase in negative β value in the similar manner of Fig. 4. However, N_{agg} value (Table 1) increases with the increase in the length of hydrophobic tail of CS in the case of DHPC + DTAB/TTAB/HTAB, while it decreases more or less in the case of DHPC + zwitterionic/phosphonium/gemini surfactant mixtures. The steric factors can be attributed to a decrease in the N_{agg} value in the latter case.

5. Conclusions

The following conclusions can be made from this study.

1. All the present mixtures of DHPC + dimeric cationic/alkyl ammonium/phosphonium/zwitterionic show attractive interactions between the unlike monomers in the mixed state. The attractive interactions are mainly attributed to the electrostatic interactions between phosphocholine group of DHPC and cationic or zwitterionic head group of cosurfactants.
2. The degree of synergism increases with the increase in the length of the hydrophobic tail of cosurfactant in all the cases.
3. The increase in the length of hydrophobic tail of cosurfactant in fact produces mixed micelle with poor cosurfactant component and rich with DHPC. Thus, the synergism in all cases is related to rich amount of DHPC in the mixed micelles even in the cosurfactant rich region of the mixture.
4. A quenching process indicates the stronger hydrophobicity of the mixed micelles with longer hydrophobic tails.

Acknowledgments

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