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Fluorescence study of solubilization of L-α-dilauroylphosphatidylethanolamine in the mixed micelles with monomeric and dimeric cationic surfactants

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Abstract

The mixed micelle formation between L-α-dilauroylphosphatidylethanolamine (DLPE) and a series of monomeric and dimeric cationic surfactants have been studied with the help of steady state fluorescence technique. The micelle formation process has been detected by plotting the emission intensities of solubilized fluorescence probes such as 5(6)-carboxyfluorescein (CF) and pyrene (Py) in the hydrophobic environment of mixed micelles. The mixed micelle process has been found to be facilitated in the presence of monomeric surfactants with higher hydrophobicities. In the case of dimeric surfactants, the steric hindrances generated by both the dimeric head group as well as twin hydrophobic tails have delayed the mixed micellization. The results have been further supported by evaluating the mean aggregation number (N_{agg}) of the mixed micelles and the collisional quenching rate constant.

Keywords: Phospholipid; Cationic surfactants; Fluorescence measurements; Mixed micelles; Aggregation number

1. Introduction

Phospholipids form molecular assemblies called liposomes or vesicles when dispersed in aqueous media. According to the classification of lipids [1], phospholipids belong to a class II amphiphiles. These lipids are virtually insoluble in water but swell to form usually lamellar liquid crystalline phases. In excess of water, the lipid molecules assemble to form closed spherical structures with lipid bilayer separating the inner and outer solutions. They are called liposomes or vesicles. The vesicle membranes well mimic the biomembranes, and hence, vesicles have occupied an important position as a useful model system in the investigation of biomembranes. On the other hand, surfactants belong to a class III amphiphiles [1]. They are capable of forming aggregates when dissolved in water above a specific concentration known as critical micelle concentration (cmc). The micellized surfactants have the ability to solublize even insoluble or sparingly soluble chemical species like lipids in the interior of the micelles [2–6]. Such a process leads to the breakdown of lamellar structures and the formation of lipid-surfactant mixed micelles [7–12]. The wide applications of surfactants as molecular tools in membranology, DNA extraction drug delivery vehicles, phase transfer catalysis in biomolecules, building blocks of supramolecular assemblies with biopolymers [13–15], etc. are due to the lipid solubilization in the micellar phase. The disintegration of biomembranes for the purpose of isolation and purification of membrane proteins [11,16–17] has a fundamental relevance to the mixed micelle formation.

These wide applications have prompted us to evaluate the fundamental nature of the mixed micelles of a phospholipid like L-α-dilauroylphosphatidylethanolamine (DLPE) with a variety of monomeric as well as dimeric cationic surfactants on the basis of their head group as well as hydrophobic tail

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modifications. The results have been analyzed by computing various micellar parameters for these surfactants in the presence of DLPE. Although, there is no reason to believe that DLPE should available in the form of vesicular structures in pure aqueous phase in the absence of surfactant, the presence of surfactant is expected to solubilize the bilayer structure into stable mixed micelles. Thus, the presence of stable mixed micelles with stronger hydrophobic environment with a particular cationic surfactant would be the best example of a surfactant and its applications in the field of lipid solubilization.

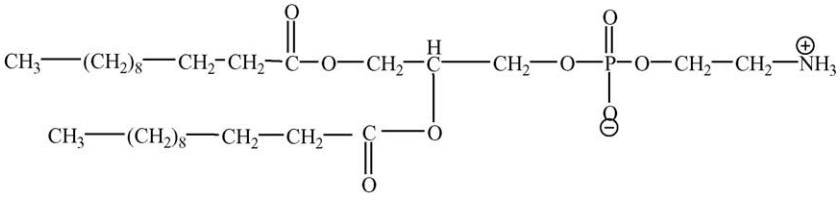
2. Experimental

2.1. Materials

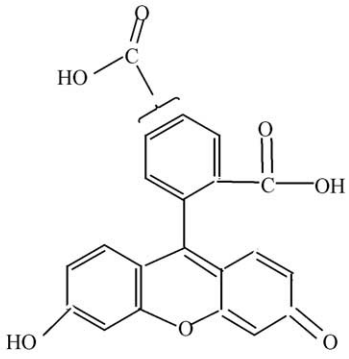
The lipid, L-α-dilauroylphosphatidylethanolamine (DLPE), 98% pure, was obtained from Sigma (Scheme 1). Cationic geminis, dimethylene bis (alkyldimethylammonium bromide) (*m*–2–*m*, where *m* is 12, 14, and 16), were synthesized according to the method reported elsewhere [18]. Dodecyl- (DTAB), tetradecyl- (TTAB), hexadecyltrimethylammonium bromides (HTAB), and hexadecylpyridinium chloride (HPyCl) all more than 99% pure from Lancaster Synthesis, UK, were used as received. The fluorescence probes, i.e. 5(6)-carboxyfluorescein (CF) 99% pure and pyrene (Py) were obtained from Acros Organics, Germany and Sigma, respectively, and were used as such (Scheme 1). 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.

2.2. Fluorescence measurements

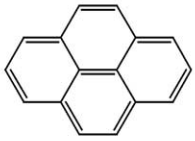
The micelle formation of the present monomeric/dimeric cationic surfactants has been carried out in the absence as well as in the presence of DLPE by employing fluorescence probes such as CF and Py in micromole range. The former is generally used to study its release from the vesicular systems during the structure transitions in the phospholipids vesicles [19], while the latter has been mainly used to evaluate the hydrophobicity of the micellar microenvironment [20]. Several



L-α-Dilauroylphosphatidylethanolamine



5(6)-Carboxy fluorescein



Pyrene

Scheme 1.

studies [21] demonstrate that the Py is mostly found in the palisade layer of the micelles. Since the structure of the vesicles is much different from that of the micelles, therefore, it is not necessary that the solubilization sites of CF and Py should be identical. Hence, we prefer to study the dissolution of the DLPE vesicles in the presence of surfactants by using both CF and Py as fluorescence probes.

The excitation wavelengths of CF and Py are 490 and 334 nm, respectively, while the emission wavelength for CF is 517 nm. In the case of Py, the ratio of first and third vibronic emission bands is generally used to determine the cmc. The errors in the cmc values have estimated to be less than 10%. All the fluorescence measurements have been recorded on Hitachi spectrofluorometer F-2500 at 25 °C by circulating the thermostated water by using the Julabo F-25 water thermostat bath. The concentrations of CF and Py were in the range of 10^{-6} mol dm^{-3} while that of quencher (HPyCl) was in the range of 10^{-5} mol dm^{-3}.

The stock solutions of DLPE + surfactant were prepared first by weighing the appropriate amounts of surfactants in clean glass vials and then adding the desired amount of DLPE. This solution was stirred for 10–15 min to ensure the complete solubilization of DLPE in surfactant solution and was kept for overnight in order to attain equilibrium before performing the fluorescence titration in pure water containing fixed amount of fluorescence probe. The composition of the solution was expressed in molar fraction (α_{DLPE}) of the respective surfactant, and defined as

$$\alpha_{DLPE} = \frac{[DLPE]}{[surfactant] + [DLPE]} \quad (1)$$

where [surfactant] and [DLPE] are the molar concentrations of the surfactant and DLPE in the mixed solution, respectively.

3. Results

Fig. 1 demonstrates the typical examples of micellization of 12–2–12 in the presence of DLPE by using both CF

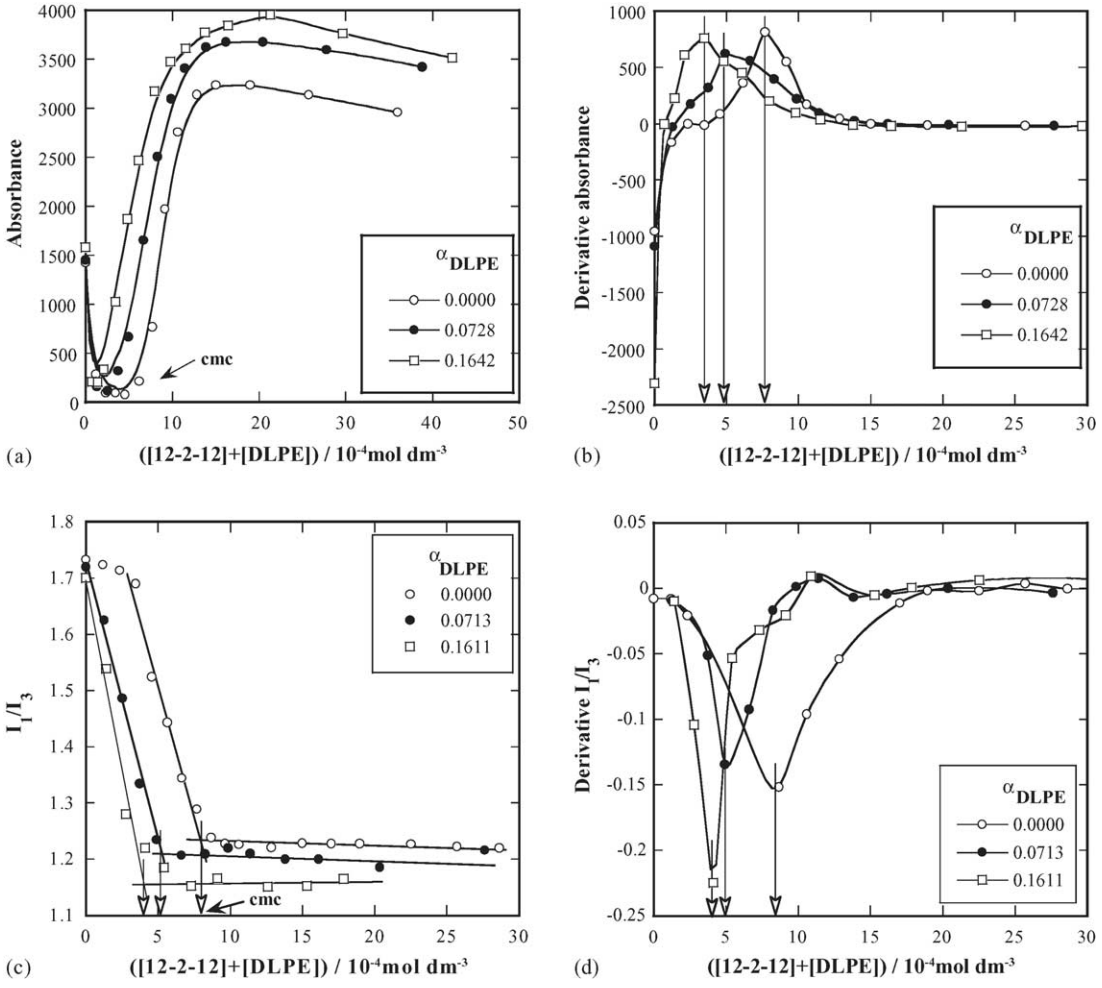


Fig. 1. Variation of (a) absorbance of CF; (b) first derivative of absorbance of CF; (c) pyrene intensity I_1/I_3 ratios; and (d) first derivative of pyrene intensity I_1/I_3 ratios with the total concentration, i.e. [12–2–12] + [DLPE] of 12–2–12 + DLPE binary mixture on some selected mole fractions at 25 °C: (◯) experimental points; the vertical arrow denotes the cmc value.

Table 1
Critical micelle concentration (cmc/10^{-4} mol dm^{-3}) of various DLPE + monomeric/dimeric binary mixtures

DLPE + DTAB		DLPE + TTAB		DLPE + HTAB	
α_{DLPE}	cmc	α_{DLPE}	cmc	α_{DLPE}	cmc
0.0000	160 ± 8	0.0000	37.5 ± 1.9	0.0000	9.09 ± 0.46
0.0024	131 ± 7	0.0095	30.5 ± 1.5	0.0333	6.37 ± 0.32
0.0048	121 ± 6	0.0189	24.5 ± 1.2	0.0689	3.22 ± 0.16
0.0072	108 ± 5	0.0281	23.5 ± 1.2	0.1034	2.78 ± 0.14
0.0096	89.5 ± 5	0.0371	21.5 ± 1.1	0.1212	1.85 ± 0.09
0.0119	82.5 ± 4	0.0459	19.0 ± 0.9	0.1470	1.79 ± 0.09
DLPE + 12–2–12		DLPE + 14–2–14		DLPE + 16–2–16	
α_{DLPE}	cmc	α_{DLPE}	cmc	α_{DLPE}	cmc
0.0000	7.60 ± 0.45	0.0000	2.85 ± 0.14	0.0000	0.25 ± 0.01
0.0378	7.35 ± 0.44	0.1442	2.70 ± 0.14	0.6398	0.26 ± 0.01
0.0728	5.00 ± 0.35	0.2520	2.55 ± 0.13	0.7804	0.27 ± 0.01
0.1054	4.90 ± 0.25	0.3364	2.45 ± 0.12	0.8420	0.30 ± 0.02
0.1358	4.65 ± 0.23	0.4002	2.45 ± 0.12	0.8766	0.30 ± 0.02
0.1642	3.60 ± 0.20	0.8423	2.45 ± 0.12	0.8988	0.30 ± 0.02

(Fig. 1a) and Py (Fig. 1c) as fluorescence probes. The cmc has been evaluated by taking the derivatives of these plots (Fig. 1b and d). Similar plots were obtained for other surfactants (not shown). The cmc values thus calculated for all the present surfactants in the presence of DLPE have been listed in Table 1 along with their uncertainties. The values for pure surfactants have been listed in Table 2 and compared with those already available in the literature. They have been found to be in good agreement with each other. Plots of normalized cmc (cmc/cmc_o, where cmc_o is the critical micelle concentration of the cationic surfactant in the absence of DLPE) have been shown in Fig. 2a and b for DLPE + monomeric and DLPE + dimeric cationic surfactants, respectively. A close inspection of Fig. 2a demonstrates that the increase in [DLPE] leads to a decrease in cmc/cmc_o value in the order of HTAB > TTAB > DTAB. In the case of binary mixtures of dimeric surfactants, a decrease in the cmc/cmc_o value is in the order of 12–2–12 > 14–2–14 > 16–2–16.

3.1. Quenching process

The above results can further be explained on the basis of quenching of Py by a suitable quencher such as HPyCl under steady state conditions. It is being ensured that the fluorescence lifetime of pyrene is longer than the residence time of the quencher in the micelle. A suitable [pyrene]/[mixed micelle] and [quencher]/[mixed micelle] ratios ensure the Poisson distribution. The fluorescence intensity of the first vibronic band of pyrene decreases with the increase in [quencher] without appearance of any new band

Table 2
Values of cmc/10^{-4} mol dm^{-3} of various surfactants from fluorescence measurements

Surfactant	cmc	Literature values
DTAB	160	160 [32], 145 [33]
TTAB	37.5	37.0 [34]
HTAB	9.09	9.20 [35], 10.0 [36]
12–2–12	7.60	8.40 [18,37]
14–2–14	2.85	–
16–2–16	0.25	0.20 [38]

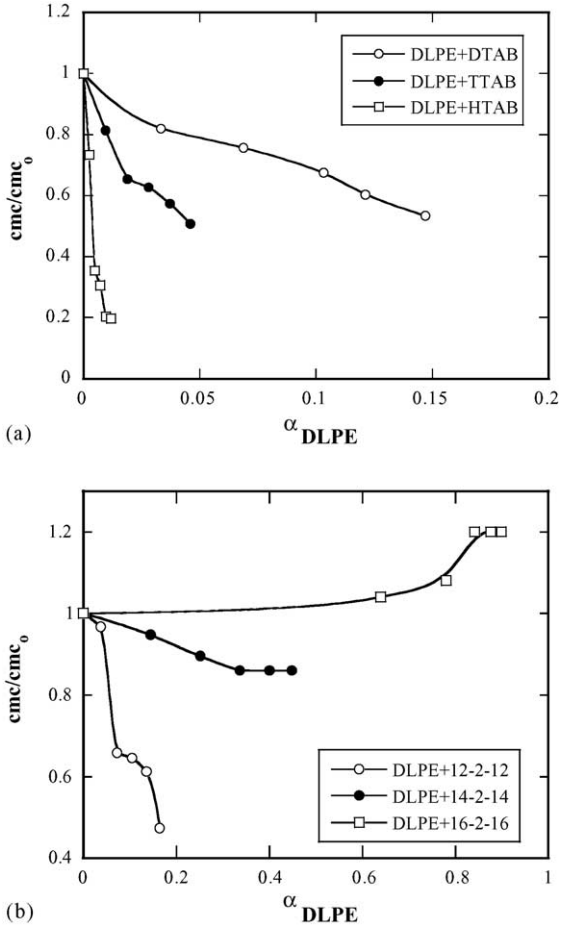


Fig. 2. Plot of cmc/cmc_o vs. α_{DLPE} of (a) DLPE + monomeric and (b) DLPE + dimeric surfactant mixtures in pure water.

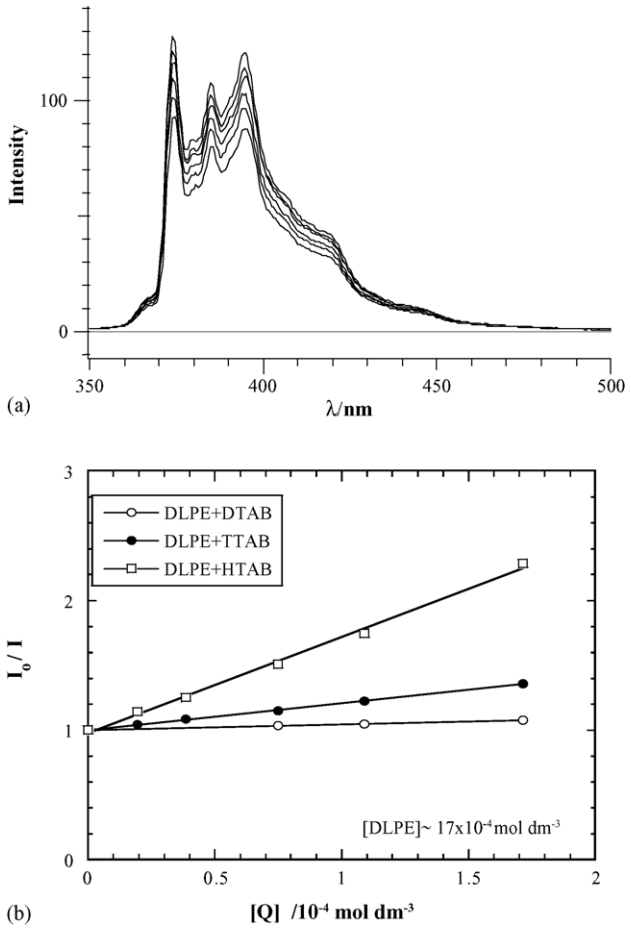


Fig. 3. (a) Fluorescence spectra of pyrene in aqueous DLPE + DTAB mixture showing the fall in intensity with the increase in the concentration of quencher from 0 to 9×10^{-5} top to bottom. (b) Plot of I_o/I vs. the [Q] for DLPE + DTAB/TTAB/HTAB.

(Fig. 3a). A Stern–Volmer relationship can be used to explain the collision quenching under the steady state conditions [22–25].

$$\frac{I_o}{I} = 1 + K_{SV}[Q] \quad (2)$$

where I_o 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_o/I versus [quencher] (Fig. 3b) will give the value of K_{SV}. Similar considerations can be used to evaluate the mean micelle aggregation number (N_{agg}) of DLPE + surfactant mixed micelles by using the following equation.

$$\ln\left(\frac{I_o}{I}\right) = \frac{[Q]N_{agg}}{[\text{Surf}] - \text{cmc}} \quad (3)$$

where [Surf] is the total concentration. The equation is valid only when probe and quencher reside in the same environment in order to undergo first order quenching. In the present systems, this is perfectly achieved by maintaining the suitable [pyrene]/[mixed micelle] and [quencher]/[mixed micelle] ratios. The values of N_{agg} and K_{SV} values thus calculated have been given in Table 3. The N_{agg} values have been plotted in Figs. 4a and b, for DLPE + monomeric/dimeric surfactant mixtures, respectively. Figs. 4c and d show a graphical representation of normalized aggregation number (N_{agg}/N_o, where N_o is the aggregation number of the cationic surfactant in the absence of DLPE) versus [DLPE]. One can see that the N_{agg}/N_o value increases in the order of HTAB < TTAB < DTAB for monomeric surfactants (Fig. 4a) and in the order of 12–2–12 < 14–2–14 < 16–2–16 for dimeric surfactant mixtures (Fig. 4d). A close inspection of all the curves of Fig. 4 demonstrate that both the mean aggregation numbers as well as its normalized values show a clear change at [DLPE] $\approx 7 \times 10^{-4}$ mol dm^{-3} (shown by dotted lines) for various combinations of DLPE + surfactants.

The results are further supported by evaluating K_{SV} from Eq. (2). The normalized K_{SV} values have been plotted in Fig. 5. Since the magnitude of K_{SV} can be related to the rate of quenching process, therefore, higher the rate of quenching between the probe and the quencher, higher will be the value of K_{SV}. Thus, the rate can be related to the probability of finding both probe and quencher in the same environment. Since both probe and quencher are expected to be favorably solubilized in the hydrophobic region, hence, the quenching of probe by quencher is expected to be much higher in the same region. As the mixed micelle composition varies by changing the mixing ratios between surfactant and phospholipid, the K_{SV} also varies over the whole mixing range demonstrating a change in the micellar environments. It is to be mentioned here that it was not possible to evaluate K_{SV} value by using CF as fluorescence probe along with HPyCl as quencher, since no quenching was observed for this combination. Fig. 5 shows that the normalized value mainly increases with the increase in [DLPE] in the case of DTAB + DLPE, while it remains mostly constant in case of TTAB/HTAB + DLPE mixtures. On the other hand, such value decreases instantaneously with the increase in [DLPE] in all the cases of dimeric + DLPE mixtures. The magnitude of the decrease in normalized value is in the order of 14–2–14 > 16–2–16 > 12–2–12. Here too, the change can be mainly seen around [DLPE] $\approx 7 \times 10^{-4}$ mol dm^{-3}, and beyond the latter concentration, the K_{SV}/K_{SVo} value predominantly tends to remain constant except in the case of DLPE + DTAB mixture.

4. Discussion

The above results can be explained on the basis of the mixed micelle formation between DLPE and monomeric/dimeric cationic surfactants. A clear micelle formation process of both monomeric as well as dimeric cationic surfactants in the presence of DLPE has been detected by both

Table 3
Values of N_{agg} and $K_{SV} \times 10^3$ of various monomeric/dimeric surfactants[a] in the presence of various (DLPE)

DLPE + DTAB			DLPE + TTAB			DLPE + HTAB		
[DLPE]	N_{agg}	K_{SV}	[DLPE]	N_{agg}	K_{SV}	[DLPE]	N_{agg}	K_{SV}
0.00	50 ± 3	0.33 ± 0.02	0.00	55 ± 3	2.1 ± 0.1	0.00	58 ± 3	8.2 ± 0.4
3.62	52 ± 3	0.40 ± 0.02	3.41	62 ± 3	2.1 ± 0.1	3.45	56 ± 3	8.5 ± 0.4
7.24	54 ± 3	0.40 ± 0.02	6.82	60 ± 3	1.9 ± 0.1	6.90	53 ± 3	7.5 ± 0.4
10.9	71 ± 4	0.53 ± 0.03	10.2	64 ± 3	2.1 ± 0.1	10.4	53 ± 3	7.7 ± 0.4
14.5	75 ± 4	0.56 ± 0.03	13.6	66 ± 3	2.1 ± 0.1	13.8	54 ± 3	7.5 ± 0.4
18.1	61 ± 3	0.44 ± 0.02	17.1	65 ± 3	2.1 ± 0.1	17.2	55 ± 3	7.4 ± 0.3
DLPE + 12–2–12			DLPE + 14–2–14			DLPE + 16–2–16		
[DLPE]	N_{agg}	K_{SV}	[DLPE]	N_{agg}	K_{SV}	[DLPE]	N_{agg}	K_{SV}
0.00	32 ± 2	5.3 ± 0.3	0.00	15 ± 1	17 ± 1	0.00	4 ± 1	6.6 ± 0.3
3.35	27 ± 1	4.5 ± 0.2	3.35	12 ± 1	9.7 ± 0.5	3.35	4 ± 1	–
6.90	28 ± 1	3.7 ± 0.2	6.90	9 ± 1	5.8 ± 0.3	6.90	4 ± 1	3.3 ± 0.2
10.3	26 ± 1	3.5 ± 0.2	10.3	11 ± 1	5.4 ± 0.3	10.3	7 ± 1	3.0 ± 0.2
13.8	25 ± 1	2.9 ± 0.1	13.8	12 ± 1	6.0 ± 0.3	13.8	9 ± 1	3.8 ± 0.2
17.3	25 ± 1	3.0 ± 0.2	17.3	12 ± 1	4.9 ± 0.2	17.3	9 ± 1	2.2 ± 0.1

[a] Concentration of each cationic surfactant ≈10 × cmc.

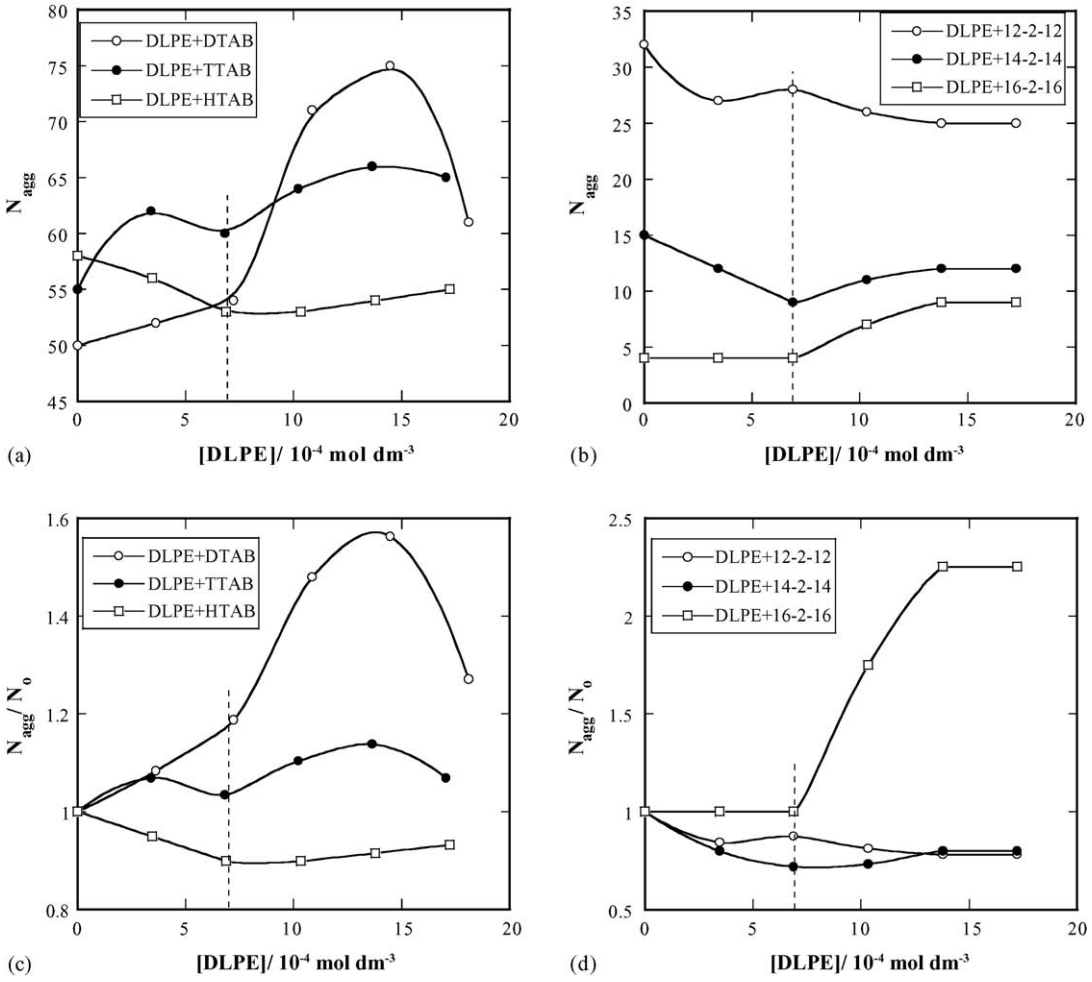


Fig. 4. Plot of N_{agg} vs. α_{DLPE} of (a) DLPE + DTAB/TTAB/HTAB; (b) DLPE + 12–2–12/14–2–14/16–2–16 mixture and plot of N_{agg}/N_o vs. α_{DLPE} of: (c) DLPE + DTAB/TTAB/HTAB; and (d) DLPE + 12–2–12/14–2–14/16–2–16 mixtures in pure water.

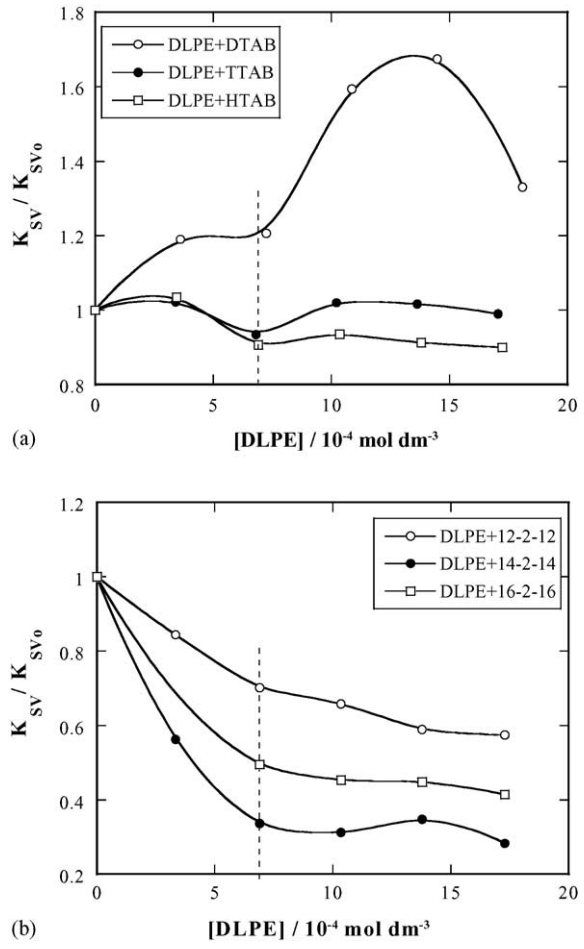


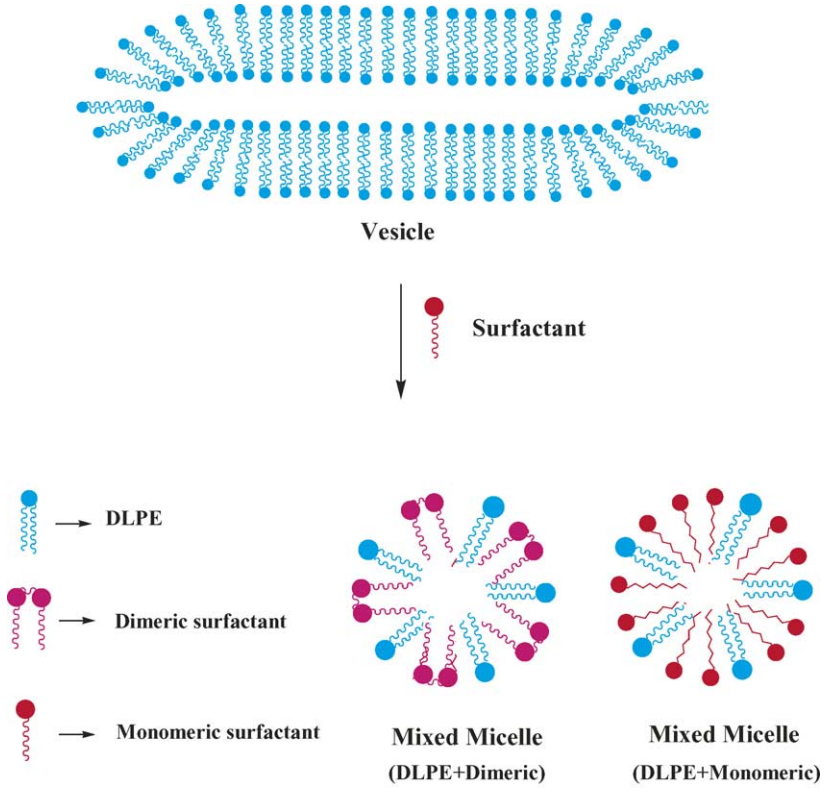
Fig. 5. Plot of K_{SV}/K_{SVo} vs. α_{DLPE} of (a) DLPE + DTAB/TTAB/HTAB and (b) DLPE + 12–2–12/14–2–14/16–2–16 surfactant mixtures in pure water.

CF as well as Py fluorescence probes (Fig. 1). The DLPE is a vesicle forming phospholipid due to its poor solubility in aqueous phase. The micellization of the cationic surfactants is expected to occur upon the dissolution of vesicular DLPE into mixed micellar environment. All parameters evaluated from the present results indicate the presence of DLPE + cationic surfactant mixed micelles rather than the presence of any vesicular structures.

The increase in the amount of DLPE in the mixed micelles of monomeric cationic surfactant (Fig. 2a) leads to a reduction in the mixed cmc value. This has been taken as an indication of the facilitation of the mixed micellization [26]. This trend is also significantly influenced by the hydrophobicity of the cationic surfactant. It suggests that the cationic surfactant with greater hydrophobicity facilitates its mixed micellization with DLPE in a much prominent way. The reduction in cationic polar head group repulsions upon incorporation of zwitterionic head group would reduce the polarity of the stern layer, thus strengthening the non-polar environment [27,28]. Similar explanation can be extended for all the mixtures of DLPE + DTAB/TTAB/HTAB. A typical schematic representation (Scheme 2) demonstrates the dissolution of DLPE vesicular structures into the mixed micelles. Fig. 2b demonstrates that the mixed micelle formation process of 12–2–12 still facilitates in the presence of DLPE, however, increase in the hydrophobicity of the twin tail leads to a significant reduction in the facilitation of mixed micelle formation. Thus, the delay in the latter micellization process can be related to the dimeric nature of both head group as well as twin tails. They may induce steric hindrances and packing geometry constraints at the interface and in the core, respectively [29].

This can be further explained on the basis of the variation of N_{agg}/N_o (Fig. 4c and d). The facilitation of the mixed micellization as observed in the case of DLPE + monomeric surfactants suggest the decrease in the N_{agg}/N_o value in the case of DLPE + HTAB. It seems that the incorporation of DLPE in HTAB micelles generates additional hydrophobicity with the result of which N_{agg}/N_o value decreases. The increase in N_{agg}/N_o in the case of DLPE + DTAB indicates the facilitation of the micellization only on the basis of reduction in the cationic head group repulsions while hydrophobicity is not playing any significant role. On the other hand, though N_{agg}/N_o values slightly less than unity, they prefer to remain predominantly constant over the range of [DLPE] in the case of DLPE + 12–2–12/14–2–14 mixtures. The N_{agg}/N_o shows a significant increase in the case of DLPE + 16–2–16 mixtures suggesting no contribution of the hydrophobicity in reducing this value contrary to what observed in the case of corresponding DLPE + HTAB mixture. At the same time, the K_{SV}/K_{SVo} value remains almost constant for DLPE + TTAB/HTAB mixtures (Fig. 5a) while it increases significantly in the case of DLPE + DTAB. As it has already been mentioned that increase in K_{SV}/K_{SVo} value can be directly related to facilitation of the quenching process, therefore, it seems that the rate of the quenching process is much significant in the mixed micelles of DLPE + DTAB mixtures. A greater quenching can further be related to the lower microviscosity experienced both by the probe and quencher. We expect that higher N_{agg}/N_o value for DLPE + DTAB (Fig. 4c) should in fact, reduce the quenching as normally observed. But increase in K_{SV}/K_{SVo} for DLPE + DTAB can be related to the reduction in microviscosity due to the smallest hydrocarbon tail length of DTAB among all monomeric cationic surfactants.

The present mixed micellar properties (Figs. 4 and 5) show a clear change in their variation especially at $[DLPE] \approx 7 \times 10^{-4}\,mol\,dm^{-3}$. It seems that the mixed micelles beyond this concentration become rich in DLPE component with the result of which the structure transitions from conventional spherical to rod shaped mixed micelles occur [30,31]. Hence, an increase in [DLPE] would lead to the vesicle formation through the rod shape mixed micelles. This transition would obviously bring a little variation in N_{agg}/N_o and K_{SV}/K_{SVo} value as observed in most of the cases.



Schematic Representation of Mixed Micellization

Scheme 2.

5. Conclusions

The induction of DLPE in the micellar phase of monomeric cationic surfactants such as DTAB, TTAB, and HTAB indicates the facilitation of mixed micellization due to additional hydrophobicity provided by the DLPE component. The facilitation of the mixed micellization further increases with the increase in hydrophobicity of monomeric cationic surfactant. This trend has not been observed in the mixed micelles of DLPE + dimeric surfactant. The mixed micellization process becomes less facilitated with the increase in hydrophobicity of the dimeric component. The latter has been explained on the basis of steric hindrances generated by dimeric head group as well as twin tails. In the mixed micelle state, the increase in [DLPE] leads to a clear structure transition from spherical to rod shape micelles especially beyond [DLPE] $\approx 7 \times 10^{-4}$ mol dm^{-3}. Hence, the latter concentration is responsible for structure transition and above this concentration the DLPE molecules cannot stay in spherical mixed micellar arrangement.

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