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SHORT COMMUNICATION

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Micellar behavior of short chains phospholipids binary mixture in an aqueous medium: interfacial and fluorescence study

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Abstract The mixed micellar behavior of short chain zwitterionic phospholipids 1,2-dioctanoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-diheptanoyl-sn-glycero-3-phosphocholine (DHPC) have been studied at the air-water interface and in the aqueous bulk phase via interfacial tension and pyrene fluorescence (I_1/I_3) intensity measurements, respectively. The critical micellar concentration (*cmc*) and the thermodynamic parameters such as the free energy of micellization have been evaluated. The interfacial study reveals that a mixed monolayer is formed at the air-water interface by the adsorption of both of the phospholipids. This has been confirmed by evaluating interfacial parameters; the maximum surface excess (Γ_{max}) or the minimum area per molecule (A_{min}) of those monomers. Their mixing behaviors have also been expressed in terms of the regular solution interaction parameter, β, in the mixed micelle. The β parameters are not significantly different from each other, and very close to zero; in other words they are consistent with ideal behavior. The equilibrium distribution of components between the micelle and the aqueous bulk was evaluated by Motomura's formulation. The micellar mole fraction evaluated from regular solution theory and Motomura's formulation also lies close to that corresponding to ideal behavior.

Keywords DOPC · DHPC · Mixed micelles · Interfacial · Fluorescence behaviors

Introduction

A lot of work has been done in order to understand the behavior of surfactant mixtures [1, 2, 3]. Mixtures of two or more surfactants have desired properties for potential industrial applications such as surface activity, detergency, wetting, spreading, and foaming. In the various combinations of nonionic, ionic, and zwitterionic surfactants, it is known that the nonionic mixtures often show ideal behaviors [4, 5] while the other surfactant mixtures usually show nonideality resulting from the synergistic (attractive) or antagonistic (repulsive) interactions between the surfactant monomers [6, 7, 8, 9, 10, 11, 12, 13, 14]. The relevant theories of Clint [15], Motumura [16], Rubingh [17] and Blackschtein [18] have been extensively used to analyze and compare the experimental results to reveal the synergistic (attractive) and antagonistic (repulsive) behaviors of different binary combinations.

Phospholipids are naturally occurring surface active compounds, and are also known as amphiphiles due to the zwitterionic nature of the polar head group and the non-polar hydrophobic tail. Phospholipid molecules with short hydrocarbon chains form spherical, disk-like, or cylindrical micelles in water similar to conventional amphiphiles [19]. Long chains of phospholipids also

exist in the form of bilayers in order to prevent the contact of the hydrocarbon tails with water. Phospholipid monolayers at the air-water interface resemble the monolayers formed by conventional amphiphiles such as fatty acids, but they have one important difference – they have two hydrocarbon chains per head group, and the head groups can not approach each other as close as they can in the monolayers formed by amphiphiles with a single alkyl chain [20].

In the present study, we have explored the aggregation behavior of short chain phospholipids such as 1,2-dioctanoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-diheptanoyl-sn-glycero-3-phosphocholine (DHPC). The theories of Clint, of regulation solutions, and of Motomura have been used to evaluate the *cmc*, composition and interaction parameter of the mixed aggregates. The choice of double *C*-8 and *C*-7 chains of phospholipid was made because of their ability to form micelles in an aqueous medium whereas long fatty acid chains of phospholipids exist in the form of bilayer assemblies. Studies on these binary mixtures have not been previously carried out.

Experimental

Materials

Phospholipids 1,2-dioctanoyl-sn-glycero-3phosphocholine (DOPC) and 1,2-diheptanoyl-sn-glycero-3phosphocholine (DHPC), of more than 99% purity, were purchased from Avanti Polar Lipids, Inc., USA and used as received. Pyrene was purchased from Nacalai Tesque, Japan. All solutions were prepared in Milli-Q water having specific conductance 2 μS cm^{-1}.

Methods

The interfacial tension was measured with the help of an Interfacial Tensiometer Model PD-X (pendant drop method) from Kyowa Interface Science Co. Ltd., Japan. A drop of sample was obtained using a syringe of 1 cm^3, which was fitted with a teflon needle of 22 gauge. The readings of interfacial tension were taken until a constant reading was obtained. The instrument was calibrated with pure water. All measurements were taken at 25.00 ± 0.01°C.

The steady-state pyrene fluorescence measurements were carried out with the help of a Perkin Elmer Spectrofluorometer (Model LS 50 B) at the excitation wavelength of 335 nm. The concentration of pyrene used in all the measurements was approximately 10^{-6} mol dm^{-3}. The ratio of the intensities of the first and third vibronic (I_1/I_3) was used to evaluate the polarity of the environment in which the pyrene was solubilized.

Results and discussion

Critical Micelle Concentration (*cmc*)

The characteristic features of all the measurements shows distinct breaks recognized as the *cmc*'s of the surfactants. The plots of interfacial tension (γ) are shown in Fig. 1 and the values of *cmc* are summarized in Table 1. The plots of the fluorescence intensity ratio (I_1/I_3) and the corresponding values of the *cmc* are shown in Fig. 2 and Table 2, respectively. The I_1/I_3 variation of pyrene demonstrates the bulk micellar properties of surfactants. A significant decrease in the

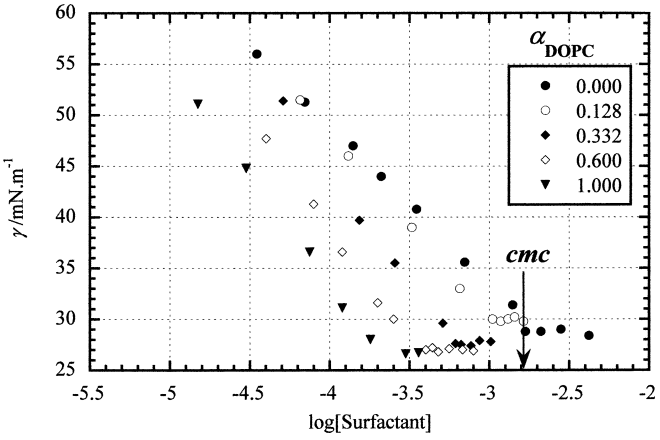

Fig. 1 Plot of interfacial tension (γ) vs log *C* of DOPC + DHPC mixtures in pure water

Table 1 Parameters for DOPC and DHPC mixed systems obtained from interfacial study

α_{DOPC}	*cmc** (mmol dm^{-3})	*cmc* (mmol dm^{-3})	$x_{1,i}$	$\bar{x}_1^m$	x_1	β
0.000	1.70	1.70	0.00	0.00	0.00	
0.128	0.97	1.01	0.49	0.49	0.51	0.21
0.332	0.58	0.61	0.77	0.76	0.76	0.95
0.600	0.38	0.35	0.91	0.86	0.91	-1.20
0.801	0.30	0.28	0.95	0.96	0.97	-1.74
1.000	0.25	0.25	1.00	1.00	1.00	
						Average -0.45

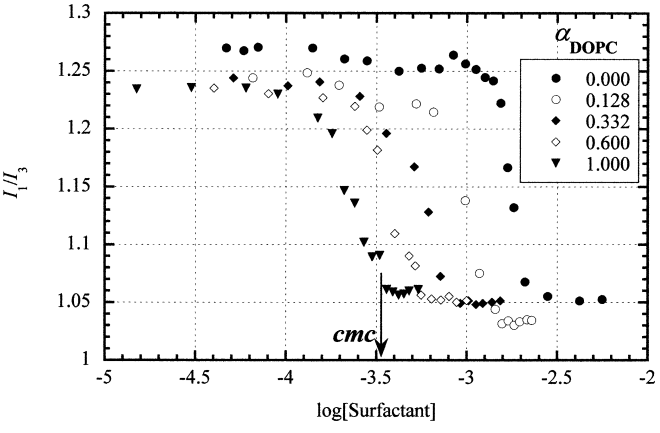

Fig. 2 Plot of fluorescence intensity (I_1/I_3) vs log *C* of DOPC + DHPC mixtures in pure water

Table 2 Parameters for DOPC and DHPC mixed systems obtained from fluorescence study

α_{DOPC}	*cmc** (mmol dm^{-3})	*cmc* (mmol dm^{-3})	$x_{1,i}$	$\bar{x}_1^m$	x_1	β
0.000	2.10	2.10	0.00	0.00	0.00	
0.128	1.30	1.41	0.46	0.43	0.42	0.53
0.332	0.81	0.79	0.74	0.73	0.74	-0.23
0.600	0.53	0.50	0.88	0.85	0.86	-0.88
0.801	0.43	0.42	0.95	0.99	0.96	-1.59
1.000	0.36	0.36	1.00	1.00	1.00	
					Average	-0.54

Table 3 Interfacial thermodynamic parameters for DOPC and DHPC mixed system

α_{DOPC}	$\Gamma_{max}\times10^6$ (mol m^{-2})	A_{min} (nm^2)	π_{cmc} (mNm^{-1})	$-\Delta G^o_m$ (kJ mol^{-1})
0.000	2.76	0.60	43.2	25.7
0.128	3.14	0.53	42.0	27.0
0.332	3.78	0.44	44.4	28.2
0.600	3.94	0.42	45.0	29.6
0.801	5.30	0.31	46.0	30.0
1.000	3.79	0.44	46.4	30.4

I_1/I_3 values with the increase in the concentration of surfactants shows the solubilization of the pyrene probe into the interior of the micelles. The end of the reversed sigmoidal curve gives the value of *cmc*. The *cmc* values obtained from the interfacial tension and the fluorescence intensity are slightly different from each other. Small method dependent variations are apparent [3, 21]. The *cmc* values have also been compared with the literature values [22].

Adsorption at the air-water interface

Phospholipids with short fatty acid chains orient at the air-water interface and decrease the surface tension like surfactants. The interfacial adsorption per unit area at various concentrations can be calculated with the help of the Gibbs adsorption equation. The surface pressure π ($\pi =$ surface tension of pure water (γ_0) – surface tension of surfactant solution (γ)) is given by the following equation:

$$d\pi = \sum \Gamma_i RTd \ln a_i \tag{1}$$

where Γ_i and a_i are the surface excess and the activity of the ith component at temperature T, respectively. For a constant composition of the surfactant mixture with total concentration C,

$$\Gamma = \frac{1}{2.303RT} \cdot \left[d\pi (d \log C + d \log \gamma)^{-1} \right] \tag{2}$$

In the case of dilute surfactant solution, the activity coefficient, $d\log\gamma$, was not considered in the calculations.

The maximum surface excess (Γ_{max}) and the minimum area per molecule of a surface active compound (A_{min}) can be estimated from the relation:

$$\Gamma_{max} = \frac{1}{2.303RT} \cdot \lim_{C \to cmc} \left[d\pi (d \log C)^{-1} \right] \tag{3}$$

and

$$A_{min} = 10^{18}/N\Gamma_{max} \tag{4}$$

where N is the Avogadro number. The values of Γ_{max} and A_{min} have been calculated for the pure and binary combinations of DOPC and DHPC from the least square slopes of π vs log C plot. The results are presented in Table 3. Fig. 1 indicates that DOPC (*C*-8) is more surface active than DHPC (*C*-7), since its γ values are lying lower than those of DHPC. On other hand, it is revealed from Table 3 that DOPC has more surface excess than DHPC. That is, DOPC molecules are probably arranged more compactly at the interface than those of DHPC because of larger attractive interactions. Further, it is evident from Table 3 that Γ_{max} for the mixtures gradually increases with an increase in the mole fraction of DOPC. Similar results have been reported for cetylpyridiniumchloride/Tween-40 binary mixtures [23]. On other hand, it is obvious that A_{min} values follow the reverse trend. Assuming ideal mixing, ideal surface excess and minimum area per molecule can be calculated (not shown in Table 3 [21]). The ideal values are not much different from the experimental values. This behavior shows that mixing of DOPC and DHPC molecules is close to ideal for the whole mole fraction range. Phillips et al. reported that for PC's with long chains or those unsaturated with ester chains, a difference of more than two CH_2 groups per chain led to immiscibility [24].

Thermodynamics of micellization

The standard free energy of micellization (ΔG^o_m) for DOPC, DHPC, and their binary mixtures was calculated by applying the equation [25]:

$$\Delta G^o_m = RT \ln X_{cmc} \tag{5}$$

Unless the *cmc* is very high, X_{cmc} is almost equal to cmc/ω, where ω is the molar concentration of solvent and X is the mole fraction of surfactant. Using the *cmc* values, the ΔG^o_m values for the pure and mixed systems have been computed and listed in Table 3. It has been observed that all the ΔG^o_m values are negative; that is, mixed micelle formation process is energetically favorable. The ΔG^o_m value of DOPC is more negative than that of DHPC, because DOPC with *C*-8 double chains is

more hydrophobic than DHPC with *C*-7 double chains. Further, it has been observed in the mixture that the ΔG^{o}_{m} values become more negative with an increase in the amount DOPC. This behavior suggests the addition of DOPC molecules stabilize the mixed micelle.

Micelle formation

The mixed *cmc* values for DOPC and DHPC are shown in Fig. 3 and Tables 1 and 2. In order to explore whether the mixed micelles show ideal behavior or not, we obtained quantitative information about the micellar aggregate formation by using the pseudo phase separation model [15, 16]. The model relates the ideal mixed *cmc** with the experimental *cmc* of the pure components by using the following equations:

$$C_1 = x_1 cmc_1 = \alpha_1 cmc^* \tag{6}$$

and

$$C_2 = (1 - x_1) cmc_2 = (1 - \alpha_1) cmc^* \tag{7}$$

Equation 8 can be obtained after combining equations 6 and 7

$$\frac{1}{cmc^*} = \frac{\alpha_1}{cmc_1} + \frac{(1 - \alpha_1)}{cmc_2} \tag{8}$$

α_1 and x_1 are the mole fractions of surfactant 1 (DOPC) in the total mixed solute and in the mixed micelle; respectively. The cmc_1, cmc_2, C_1, and C_2 are the critical micellar concentrations and the overall concentrations in the micelle formation of components 1 and 2, respectively. The *cmc** values calculated using equation 8 have also been listed in Tables 1 and 2. The mixed *cmc* values of Tables 1 and 2 are plotted in Fig. 3 along with the ideal *cmc**. Figure 3 shows the experimental *cmc* values are close to the ideal *cmc** values over the whole mixing range.

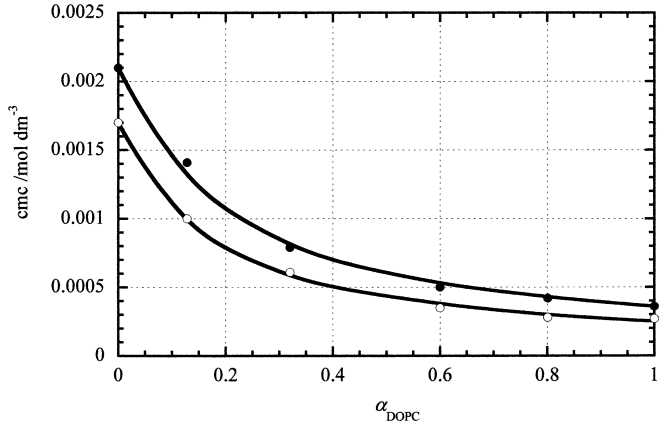

Fig. 3 Plot of mixed critical micelle concentration (*cmc*) vs α_{DOPC} for DOPC + DHPC mixtures in pure water. Predicted *cmc** is the solid line, experimental *cmc* data are white circles (for interfacial data) and black circles (for fluorescence data)

Since the mixing behavior of present binary mixtures of phospholipid shows almost ideal behavior, these results have been further confirmed using the regular solution approximation [17]. x_1 can be iteratively computed from the following equation:

$$\frac{x_1^2 \ln (cmc\alpha_1 / cmc_1 x_1)}{(1 - x_1)^2 \ln (cmc(1 - \alpha_1)/cmc_2(1 - x_1))} = 1 \tag{9}$$

The x_1 values are listed in Tables 1 and 2. From the x_1 values, the interaction parameter, β, can be computed using the following equation:

$$\beta = \ln \left(\frac{cmc\alpha_1}{cmc_1 x_1} \right) / (1 - x_1)^2 \tag{10}$$

The negative and positive β values suggest attractive and repulsive interactions between two surfactants in a mixed micelle, respectively. For ideal mixing (no interaction), the β value should be close to zero. The β values computed for the DOPC + DHPC are given in Tables 1 and 2. The average β values calculated from the interfacial and fluorescence studies are -0.45 and -0.54 respectively. These values are not so significant as to demonstrate a significant non- ideal behavior. In the literature, small β values have been reported for the mixtures of cationic/cationic, non-ionic/non-ionic, and zwitterionic/non-ionic [26], but β values for phospholipid binary mixtures are not available.

In order to further confirm the present results, the formulation of Motomura and Aratono [1] was applied. The composition of the mixed micelles can be determined using the following equation:

$$\bar{x}_2^m = \bar{x}_2 - (\bar{x}_1 \alpha_2 / \overline{cmc})(\partial \overline{cmc} / \partial \bar{x}_2)_{T,P} \tag{11}$$

and

$$\overline{cmc} = (v_1 x_1 + v_2 x_2) cmc \tag{12}$$

where $\bar{x}_2^m$ and $\bar{x}_2$ are the mole fractions in the micelle and in the bulk phase of component 2 (DHPC) respectively. $\bar{x}_2$ is given by

$$\bar{x}_2 = v_2 \alpha_2 / (v_1 \alpha_1 + v_2 \alpha_2) \tag{13}$$

where v_1 and v_2 are the number of ions produced by the surfactant upon dissociation, but in our case, no ion is produced since both components are zwitterionic in nature.

The $x_{1,i}$, $\bar{x}_1^m$and x_1 values are given in Tables 1 and 2, where $x_{1,i}$ is the micellar mole fraction in the ideal state which is obtained from Equations 6 and 8. The above values for DOPC have also been plotted in Fig. 4 (fluorescence data only). It has been observed that $\bar{x}_1^m$and x_1

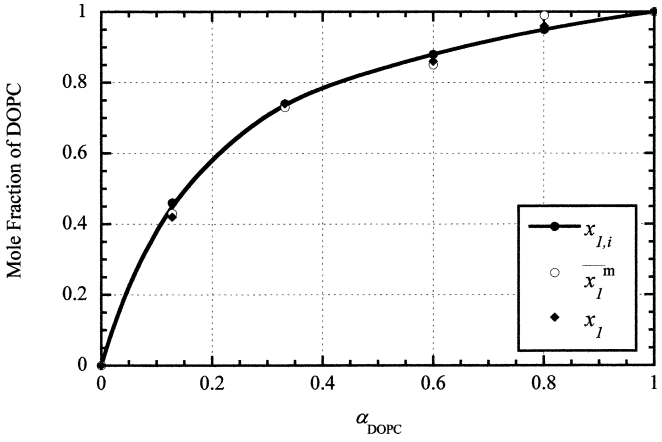

Fig. 4 Plot of micellar mole fraction (x_1) and micellar mole fraction in the ideal state ($x_{1,i}$) obtained by the regular solution theory and the micelle mole fraction ($\bar{x}_1^m$) according to the Motomura and Aratono [1] of DOPC vs α_{DOPC} for DOPC + DHPC mixtures in pure water (fluorescence data)

is not significantly different from those of $x_{1,i}$ over the whole mole fraction range. A similar trend has been observed from the interfacial study. This result also demonstrates that the mixed micelles of DOPC and DHPC show nearly ideal behavior.

Conclusions

Mixed micelle formation of DOPC and DHPC has been studied in an aqueous medium by interfacial and fluorescence measurements. The maximum surface excess increases with an increase in the mole fraction of DOPC. The $\Delta G^{o}{}_{m}$ values are negative; that is, micelle formation process is energetically favorable. Experimental *cmc* values lie very close to the ideal *cmc** ones which indicates the ideal mixing of DOPC and DHPC. The mixed micellar mole fractions over the whole mixing range are also almost identical to their ideal values. The average values of interaction parameters β for the mixed micelle show small negative values, indicating the ideal behavior of mixed micelle formation between both of the amphiphilic components.

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References

1. Motomura K, Aratono M (1993) In: Ogino K, Abe M (eds) Mixed surfactant systems. Marcel Dekker, New York, p100
2. Holland P, Rubingh D (1991) In: Holland P, Rubingh D (eds) Cationic surfactants, Vol 37. Marcel Dekker, New York, Ch 4
3. Moroi Y (1992) Micelles, theoretical and applied aspects. Plenum, New York
4. Hua X, Rosen MJ (1982) J Colloid Interface Sci 90:213
5. Warr GG, Grisier F, Healey T (1983) J Phys Chem 87:122
6. Jonsson B, Lindman B, Holmberg K, Kronberg B (eds) (1998) Surfactants and polymers in aqueous solutions. Wiley, New York, p125
7. Rosen MJ, Zhu ZH, Gao T (1993) J Colloid Interface Sci 157:254
8. Penfold J, Staples E, Thompson L, Tucker I, Hines J, Thomas RK, Lu JR, Warren NJ (1999) J Phys Chem B 103:5204
9. Cummins PG, Penfold J, Staples E (1992) Langmuir 8:31
10. Picullel L, Lindman B (1992) Adv Colloid Interface Sci 41:149
11. Bakshi MS, Sachar S, Mahajan M, Kaur I, Kaur G, Singh N, Sehgal P, Doe H (2002) Colloid Polym Sci 280:990
12. Trenier C, Makayssi A (1992) Langmuir 8:794
13. Tsujii K, Saito N, Takeuchi T (1980) J Phys Chem 84:2287
14. Bakshi MS, Kaur R, Kaur I, Mahajan RK, Sehgal P, Doe H (2003) Colloid Polym Sci 281:716
15. Clint JH (1975) J Chem Soc, Faraday Trans 71:1327
16. Motomura K, Yamanaka M, Aranato M (1984) Colloid Polym Sci 262:948
17. Rubingh DN (1979) In: Mittal KL (ed) Solution chemistry of surfactants, Vol 1. Plenum, New York, p 337
18. Sarmoria C, Puvvada S, Blankschtein D (1992) Langmuir 8:2690
19. Cevc G, Seddon JM (1993) In: Cevc G (ed) Phospholipids handbook. Marcel Dekker, New York, p 555
20. Tanford C (1980) The hydrophobic effect: formation of micelles and biological membranes. Wiley, New York
21. Moulik SP, Haque Md. E, Jana PK, Das AR (1996) J Phys Chem 100:701
22. Lange RK (1999) In: Lange RK (ed) Surfactants; a practical handbook. Hanser, Munich, p 20
23. Ghosh S (2001) J Colloid Interface Sci 244:128
24. Phillips MC, Ladbrooke BD, Chapman D (1970) Biochim Biophys Acta-Biomembrane 196:35
25. Rosen MJ (1989) Surfactants and interfacial phenomenon, 2nd edn. Wiley, New York, p111
26. Holland PM, Rubingh DN (eds) (1992) Mixed surfactant systems, ACS Symposium Series 501. ACS, Washington, p 40