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ORIGINAL CONTRIBUTION

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# Interaction between phospholipid vesicular structures with long chain zwitterionic surfactants

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**Abstract** Solubilization of different zwitterionic phospholipid vesicles structures such as L-α-phosphatidylcholine (PC) and 1,2-didecanoyl-sn-glycero-3-phosphocholine (DPC) have been studied in aqueous bulk by using zwitterionic surfactant dimethylhexadecylammoniopropanesulfonate (HPS). This has been done by studying the aggregation of HPS in pure water and in the presence of 7–36 μM of fixed concentrations of each lipid with the help of pyrene fluorescence intensity ($I_1/I_3$) measurements. The fluorescence measurements showed that HPS monomers undergo two kinds of aggregation process, which were identified by the three breaks in a plot of pyrene fluorescence versus HPS concentration. The first two breaks, $C_1$ and $C_2$, indicate the onset and completion of vesicle solubilization respectively, upon incorporation of HPS monomers into the vesicles and led to solubilization in the form of mixed micelles. This process was not clearly visible at low lipid concentration. We evaluated the partition coefficient ($K$), which defines the degree of partitioning of surfactant monomers into the vesicles with respect to the aqueous medium. A high $K$ value of HPS-lipid aggregates indicates the stronger interactions between surfactant and lipid vesicles. The $K$ values evaluated for PC and DPC are quite close to each other, which indicates that $K$ values were independent of phospholipid chain length.

**Keywords** Surfactant-lipid interaction · Bulk properties · Mixed micelles · Vesicles · Critical micellar concentration

## Introduction

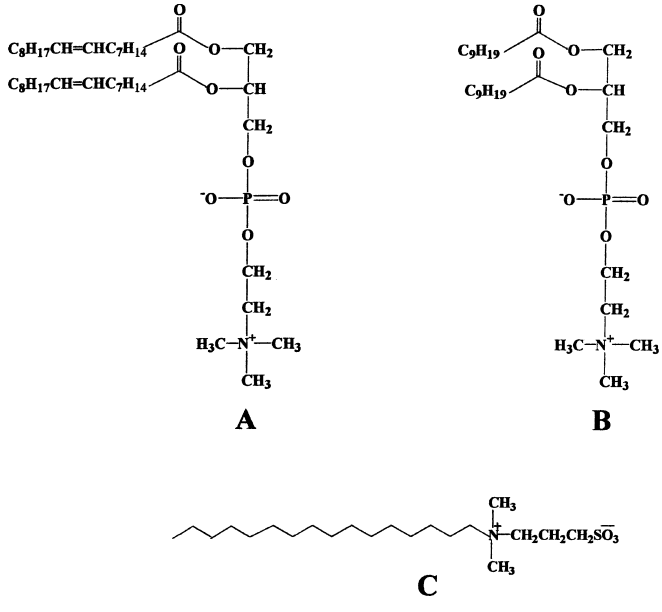
Surfactants are surface-active agent and belong to the category of amphiphilic molecules. They are capable of forming aggregates known as micelles and the concentration at which they form is known as the critical micellar concentration (*CMC*). Phospholipids differ greatly in the relative balance between their hydrophilic and hydrophobic moieties. This is reflected in their behavior in water and provides a basis for their classification [1]. Short chain phospholipids undergo a micelle formation process similar to that of surfactants [2], while phospholipids with longer fatty acid chains form bilayers. These bilayer structures are known as vesicles or liposomes and their size is significantly dependent upon the concentration of phospholipids [3]. They can be used as simplified membrane models for the transport of ions and electrons, and for drug delivery systems [2, 4].

Surfactants are able to solubilize insoluble or sparingly soluble chemical species such as phospholipids in the micelle interior. The interactions between the surfactant and phospholipids lead to the breakdown of lamellar structures into lipid-surfactant mixed micelles [2, 3]. This property of surfactants makes them versatile

agents for the isolation and purification of membrane proteins [1, 2, 3]. It is therefore necessary to understand the overall surfactant-lipid interactions in order to exploit their appropriate uses especially in the solubilization of lipids in an aqueous phase.

A number of studies have been devoted to the understanding of the principles governing the interaction of surfactants with simplified membrane models such as phospholipid bilayers. A significant contribution in this area has been made by Lichtenberg who postulated that the effective surfactant to lipid ratio producing vesicle saturation and solubilization depends upon the surfactant *CMC* and on the bilayer/aqueous medium surfactant distribution coefficients *K* [5, 6]. In a previous work, we explored the lipid-surfactant interactions and concluded that zwitterionic surfactant have stronger interactions with lipids than anionic surfactants of similar hydrocarbon chain but with different hydrophilicities [7]. Johnsson and Edward [8] studied the interactions between non-ionic surfactants and sterically stabilized phosphatidylcholine liposomes by cryogenic transmission electron microscopy and concluded that the bilayer solubilization depends upon the nature of surfactant. Cocera et al. [9] explored the kinetic and structural aspects of the adsorption of sodium dodecyl sulfate on the surface of a bilayer of L-α-phosphatidylcholine (PC) liposomes.

This paper is a continuation of previous work [10] and the aim is to explore the surfactant-lipid interactions by selecting two different kinds of phospholipids (Scheme 1) with identical phosphocholine groups but different double fatty acid chains: (A, Scheme 1) PC, and (B, Scheme 1) 1,2-didecanoyl-sn-glycero-3-phosphocholine (DPC). These phospholipids are expected to exist only in the form of bilayers [10, 11, 12] due to the strong hydrophobic character of their double fatty acid chains. Lipid macromolecules are much more hydrophobic than surfactants like dimethylhexadecylammoniopropanesulfonate (HPS), (C, Scheme 1). Therefore, the aggregation process of HPS has been studied in the presence of micromolar concentrations of each lipid so as to effectively monitor and compare the surfactant aggregation with that in the absence of lipids.




**Scheme 1 A–C** Molecular structure of **A** L-α-phosphatidylcholine (PC), **B** 1,2-Didecanoyl-sn-glycero-3-phosphocholine (DPC), and **C** Dimethylhexadecylammoniopropanesulfonate (HPS)

## Materials and methods

*Materials* PC from egg yolk, pyrene (fluorescence probe), (Nacalai Tesque, Kyoto, Japan), and DPC (Fluka, Buchs, Switzerland), all 99% pure, were kept constantly at −28 °C. HPS (> 99% pure) was procured from Sigma-Aldrich and used as received.

*Sample preparation* The aqueous lipid solutions were prepared by dissolving an appropriate amount of the respective lipid (7–36 μM) in water purified by the Milli-Q Millipore system with ultrasonication using an ultrasonic generator (Model US 600T, Nissei, Tokyo, Japan, with Tip Select (∅) = 7 mm). A clear solution of each lipid was obtained within at least 2 h of sonication in an ice bath, which was expected to contain small vesicles of mono-disperse size in each case. The aggregation process of surfactant in the presence of fixed amounts of each lipid was studied by subsequent addition of surfactant stock solution made with the same lipid into aqueous lipid reference solution. Sufficient time was given to each solution in order to attain complete equilibrium before the measurements.

*Fluorescence measurements* The steady-state pyrene fluorescence measurements were carried out using a Perkin Elmer spectrofluorometer (Model LS 50 B) at an excitation wavelength of 335 nm. The concentration of pyrene used in all the measurements was approximately equal to $10^{-6}$ mol $dm^{-3}$. The ratio of the intensity of pyrene emission i.e. $I_1/I_3$ at 373 and 393 nm,was used for evaluating the polarity of the environment in which pyrene was solubilized.

## Results and discussion

### *CMC*s and linear relationships

Figure 1A shows the plots of *CMC*s of a series of zwitterionic surfactants; dimethyldodecylammoniopropane sulfonate (DPS), dimethyltetradecylammoniopropanesulfonate (TPS), and HPS. The values of DPS and TPS have been taken from our previous work [7, 10]. The *CMC* values of these surfactants are comparable with the literature value [13, 14]. The length of the hydrocarbon chain in a surfactant is a major factor in guiding the *CMC*. The *CMC* decreases linearly with the increase of hydrocarbon tail of surfactant. The relationship between *CMC* and the carbon number ($n_c$) in the alkyl moiety of the surfactants is illustrated in

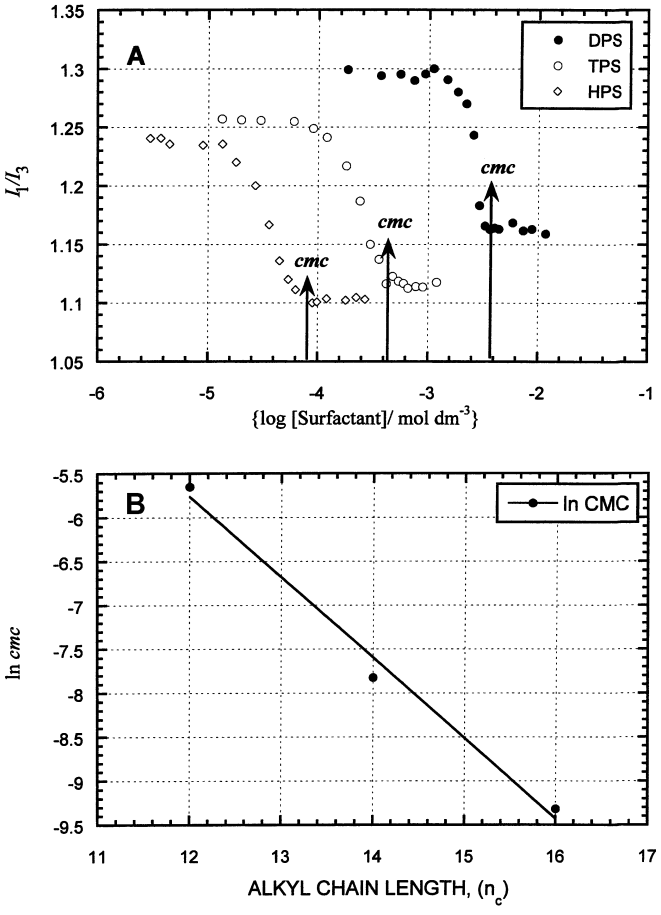



**Fig. 1** **A** Plot of fluorescence intensity ($I_1/I_3$) vs. log of concentration of series of zwitterionic surfactants: dimethyldodecylammoniopropane sulfonate (DPS), dimethyltetradecylammoniopropanesulfonate (TPS), and dimethylhexadecylammoniopropanesulfonate (HPS). **B** Dependence of critical micellar concentration (*CMC*) on alkyl chain length of zwitterionic surfactants

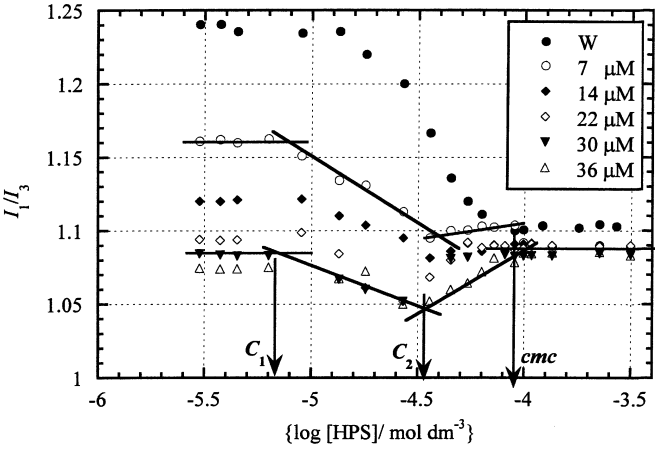



**Fig. 2** Plot of fluorescence intensity ($I_1/I_3$) vs. log of concentration of HPS in pure water and in the presence of L-α-phosphatidylcholine (PC). *W* Water

Fig. 1B. There is a linear variation in *CMC*, which obeys Eq. 1 formerly proposed by Shinoda et al. [15]:

$$\ln CMC = -n_c\omega/kT + \text{constant} \quad (1)$$

where $\omega$, $k$, and $T$ are, respectively, the energy change in transferring one methylene unit from micellar to water environment, the Boltzmann constant, and the absolute temperature.

## Pre-micellar behavior: HPS + PC interactions

The micellar behavior of HPS was studied in fixed concentrations of aqueous PC solutions. A variation in the fluorescence intensity ($I_1/I_3$) data for HPS in 7–36 μM aqueous PC solutions along with that in pure water is shown in Fig. 2. The $I_1/I_3$ variation of pyrene with respect to the increase in the amount of HPS in pure water demonstrates the bulk micellar behavior of surfactant. A significant decrease in the ($I_1/I_3$) values with the increase in the amount of HPS shows the solubilization of pyrene in the interior of micelle, thus indicating micelle formation [16]. The end of the reversed sigmoidal curve gives the value of the *CMC* i.e. 0.09 mM (Fig. 1A).

A comparison with the HPS behavior in aqueous PC shows that HPS-PC has significant interactions, due to which no water-like behavior of HPS was observed even at low lipid concentrations (Fig. 2), and the initial ($I_1/I_3$) value was quite close to that in post-micellar regions of HPS in pure water. This suggests that the hydrophobicity of the pre-micellar HPS-PC is more or less equal to that of micellar HPS in pure water. At high PC concentrations, the association of HPS-PC aggregates becomes stronger, shifting each $I_1/I_3$ curve towards lower values.

A closer inspection of Fig. 2 shows that the $I_1/I_3$ plots in the presence of PC are not qualitatively similar to that in pure water. The curve in pure water shows a single break corresponding to the *CMC* value of HPS in pure water. In the presence of PC, each curve shows two breaks at concentrations $C_1$ and $C_2$. Here, each $I_1/I_3$ curves runs through a minimum, the magnitude of which increases with increasing amounts of PC. The start of this minimum phase can be represented by the onset of vesicle solubilization, $C_1$, which completes at the dip of the minimum, $C_2$, which is the saturation point, i.e. the point where the vesicles become saturated with surfactant. Saturation leads to a significant change in the morphology of vesicles, which finally burst to form mixed micelle [17]. Beyond $C_2$, the process of mixed micellization between the HPS and PC macromolecules as well as independent micellization of HPS will ensue.

The mixed micelle or independent spherical micelles of HPS must have a less hydrophobic environment in

comparison to the more organized vesicle structure. This is the reason that the $I_1/I_3$ value increases slightly beyond $C_2$, because pyrene is now subkected to a less hydrophobic environment, which becomes constant after the third break (Fig. 2). These results are quite similar to those of other studies based on the three stage model [17] for surfactant-vesicle interactions, for example in the case of Triton X-100 and its analogs-lipid [18], alkyltrimethylammonium halide-lipid systems [19, 20], octylglucoside-lipid [21], bile salt surfactants-lipid [22] and sodium alkyl sulfates-lipid [19, 23].

Figure 2 also indicates that even at 7 μM PC, small vesicles exist in the aqueous solution since the value of the intensity ratio before $C_1$ is around 1.16, which is less than the value of HPS in pure water (i.e., 1.24). This value further decreases with the increase in the amount of PC and is 1.07 for 36 μM PC, indicating the presence of large vesicles. This is due to the fact that aggregation of a lipid in an aqueous environment is primarily dependent upon the hydrophobicity of the lipid, and PC is among those lipids that mostly exist in the form of small vesicles in aqueous solutions, even in the micromolar concentration range [17, 18].

## HPS + DPC interactions

The fluorescence behavior of HPS in pure water and in aqueous solutions of 7–36 μM DPC is shown in Fig. 3. A closer look at the bulk properties indicates that the overall variation in the plot of $I_1/I_3$ of HPS (Fig. 3) in 7 and 14 μM DPC is more or less the same as that in pure water, but the values for 14 μM lie slightly lower than those of 7 μM and pure water. Since the $I_1/I_3$ plot preserves water-like behavior at low DPC concentration, it seems that there were few or no interactions between HPS and DPC vesicles present in the solution. However, as the concentration of DPC increased to 22–36 μM, the number of vesicles increased, leading to significant interactions, as shown by clear $C_1$ and $C_2$ breaks which were not visible at low DPC concentrations. The $C_1$ and $C_2$ breaks represent the onset and completion of vesicle solubilization, respectively. The $I_1/I_3$ up to $C_1$ is more or less constant which indicates that HPS monomers were free in the bulk, but the subsequent decrease in the $I_1/I_3$ value between $C_1$ and $C_2$ can be explained by the fact that HPS monomers start accommodating in the DPC vesicular structures. This is an example of HPS at a sublytic concentration, where it has initiated a perturbing action upon the vesicle membranes due to the partitioning between the bilayer and aqueous phase, and is expected to certainly influence the physical properties of the lipid solution with the result that the first break, $C_1$, is observed [19, 20]. This process continues until the saturation point of the vesicle, where the vesicle will no longer be able to accommodate more HPS monomers and it leads to the destruction of the vesicle as demonstrated by the second break, $C_2$ [17]. The entire solubilization process is completed at the *CMC*, where the $I_1/I_3$ value becomes constant.

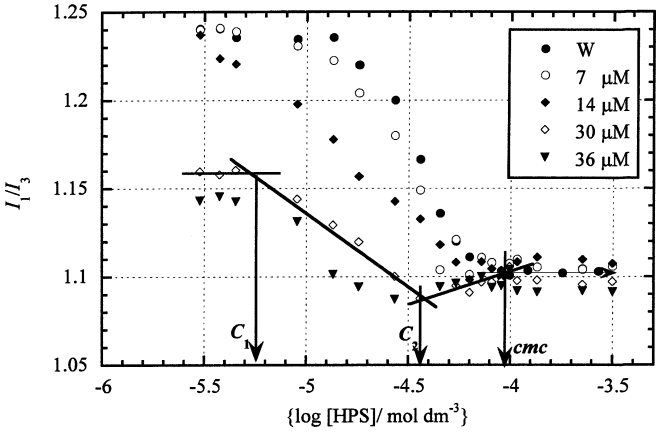



**Fig. 3** Plot of fluorescence intensity ($I_1/I_3$) vs. log of concentration of HPS in pure water and in the presence of 1,2-didecanoyl-sn-glycero-3-phosphocholine (DPC)

## Post-micellar behavior

From the above results, it is possible to understand the independent and mixed micelle formation more precisely. It is suggested by Fig. 2 and Fig. 3 that the third break in the presence of PC and DPC remains slightly less than the *CMC* of HPS in pure water. It could be predominantly due to the more hydrophobic nature of mixed micelles along with the independent micelles of HPS and both kinds of micelle should affect the post-micellar fluorescence behaviors. However, there should be a significant difference between the post-micellar behavior of HPS in pure water and that in the presence of PC and DPC, which is obviously due to the presence of mixed micelles between the HPS and lipid monomers. However, in our case, the $I_1/I_3$ values in the post-micellar region of HPS in pure water and in different amounts of PC and DPC were more or less the same. This indicates that the number of mixed micelles in the post-micellar region is far less than the independent micelles of HPS and hence, no variable concentration effect of the lipid on the post-micellar region was observed. This can also be rationalized by the fact that the concentration range selected for the additive affect of each lipid (7–36 μM) is very much smaller than the concentration of HPS covered in the post micellar region (91–316 μM), and hence the mixed micelle formation is eclipsed by the independent micelle formation in the latter concentration range.

The present results also highlight a significant difference between the influence of sublytic (pre-micellar) and micellar concentrations of HPS on the solubilization of vesicles of both phospholipids. However, in the micellar concentration region of HPS, there is no significant difference between the HPS-PC and HPS-DPC. The $C_1$, $C_2$, and $CMC$ values for HPS-lipid aggregation in the presence of different amounts of PC and DPC are listed in Table 1. First of all, let us understand that the HPS-lipid interactions in the sublytic concentration region of HPS involve the first, $C_1$, and the second, $C_2$, aggregation processes. There is an appreciable decrease in the $I_1/I_3$ value with increasing amounts of each lipid, indicating a stronger interaction between the surfactant and lipid macromolecules. This result suggests that surfactant-vesicle interactions at the sub-solubilizing level must be ruled mainly by the action of the surfactant molecule. These findings are in accordance with the results reported for the overall interaction of $C_8$-Glu and maltoside with lipid liposomes [20, 21, 22].

In the bulk, the fluorescence studies indicate that the solubilization of pyrene in the hydrophobic environment of vesicles increases with increasing amounts of PC from 7 to 36 μM, since the initial $I_1/I_3$ value decreases from 1.16 to less than 1.07 (Fig. 2). The same situation also exists in the case of DPC (Fig. 3). A comparison between the extents of solubilization of pyrene in the respective vesicles at 36 μM of each lipid suggests the following order: HPS-PC > HPS-DPC (Fig. 4), where $I_1/I_3$ values at 36 μM are lower for HPS-PC and higher for HPS-DPC. It demonstrates that the HPS-DPC aggregates are the least available for the solubilization of pyrene. The PC vesicles are expected to be more hydrophobic since PC contains two C-18 carbon chains as compared to DPC's two C-10 chains. Therefore, PC vesicles should be able to accommodate a greater number of pyrene molecules than DPC. This result also suggests that HPS-lipid mixed micelles or aggregates at $C_2$ are much more available for the solubilization of the pyrene molecules rather than those at $C_1$, which suggests that the former are more compact than the latter.

**Table 1** Values of first aggregation process ($C_1$), second aggregation process ($C_2$), and critical micellar concentration, $CMC$ of HPS in HPS + lipid systems from fluorescence measurements. $M$ = mol dm$^{-3}$,L-α-phosphatidylcholine (PC), 1,2-didecanoyl-sn-glycero-3-phosphocholine (DPC)

| Lipid | Concentration (mM) | $C_1$ (mM) | $C_2$ (mM) | $CMC$ (mM) |
|---|---|---|---|---|
| PC | 0.0 | – | – | 0.090 |
| | 0.007 | 0.0079 | 0.037 | 0.064 |
| | 0.014 | 0.0083 | 0.038 | 0.076 |
| | 0.022 | 0.0081 | 0.027 | 0.072 |
| | 0.030 | 0.0063 | 0.027 | 0.068 |
| | 0.036 | 0.0072 | 0.028 | 0.070 |
| | | 0.0076[a] | 0.031[a] | 0.073[a] |
| DPC | 0.0 | – | – | 0.090 |
| | 0.007 | – | – | 0.090 |
| | 0.014 | – | – | 0.080 |
| | 0.022 | 0.0057 | 0.026 | 0.064 |
| | 0.030 | 0.0056 | 0.035 | 0.070 |
| | 0.036 | 0.0058 | 0.027 | 0.070 |
| | | 0.0057[a] | 0.029[a] | 0.077[a] |

[a]Average values

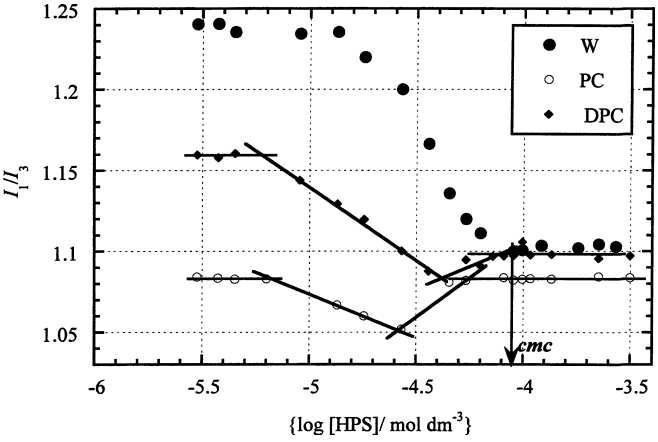



**Fig. 4** Plot of fluorescence intensity ($I_1/I_3$) vs. log of concentration of HPS in pure water and the presence of 30 μM of PC and DPC

The break in $I_1/I_3$ plots at $C_1$ indicates the onset of the penetration of HPS monomers into the lipid vesicles, and $C_2$ indicates completion; therefore, $C_2$-$C_1$ can be equated with the amount of HPS, [$S$], which partitions into the vesicular membrane phase. Thus it is possible to evaluate the mole fraction of surfactant in the lipid bilayer ($x_s$) by using the following relationship:

$$x_s = [S]/([L] + [S]) \quad (2)$$

where [$L$] is the amount of lipid in the form of vesicles which can be taken to be equivalent to the amount of lipid used, since essentially all the lipid is expected to be in the form of vesicles. The partition coefficient ($K$) can be given by the ratio of the mole fraction of the surfactant in the lipid bilayer to aqueous surfactant solution ($C_f$) [17]. The latter can be fixed at the $CMC$ of HPS in pure water (i.e., 0.09 mM).

$$K = x_s/C_f \quad (3)$$

The value of $K$ evaluated for HPS-PC (30 μM) and HPS-DPC (30 μM) aggregates were 5,556 M$^{-1}$ and 5,444 M$^{-1}$ respectively, and were quite close to one another in both cases. However, these values are quite high in comparison to the 280 M$^{-1}$and 2,009 M$^{-1}$ resulting from our previous work on DPS-lipid and TPS-lipid aggregates respectively [7, 10]. The $K$ values evaluated for HPS-lipid aggregates were quite high compared to DPS-lipid and TPS-lipid aggregates, which showed the existence of stronger interactions in HPS-lipid aggregates. This is due to the fact that HPS has a C-16

hydrocarbon chain as opposed to the C-12 and C-14 chains in DPS and TPS, respectively. The increase in hydrocarbon chain length determines the lipophilic character and consequently the affinity of the surfactants for phospholipid vesicles or partition equilibrium. This can also be explained on the basis of structural changes corresponding to the increase in the surfactant alkyl chain length, resulting in a progressive decrease in the surfactant ability to alter the permeability of vesicles or liposomes, and conversely in an abrupt increase in its affinity with this bilayer structure. However, the evolution of high $K$ values in the present study shows that the degree of partitioning of surfactants into bilayers drastically increased as the surfactant alkyl chain length increased or its $CMC$ decreased, which indicates that the affinity of surfactant molecules for lipid bilayers with respect to the aqueous medium appears to be higher as the surfactant alkyl chain length increases [7, 10, 24].

On the other hand, the partition coefficient ($K$) of surfactant between the phospholipid and aqueous phases is related to the standard free energy change associated with the transfer of the surfactant molecule from the bulk phase to the lipid. Similarly, according to the phase separation model of micelle formation, the $CMC$ is related to the standard free energy associated with the micelle formation process [17]. So, it is interesting to see whether or not some correlation exists between log $K$ and $CMC$. Figure 5 shows the plot of log $K$ versus log $CMC$ for the series of zwitterionic surfactants in the presence of DPC. This shows that an almost linear correlation exists between log $K$ and log $CMC$. The $K$ value decreases with increasing $CMC$ or decreasing number of carbons in the hydrocarbon tail of the surfactant. These results show that the driving force responsible for the micelle formation, i.e., the hydrophobic effect, can also be a main factor governing the partioning of surfactants into the vesicle membrane.

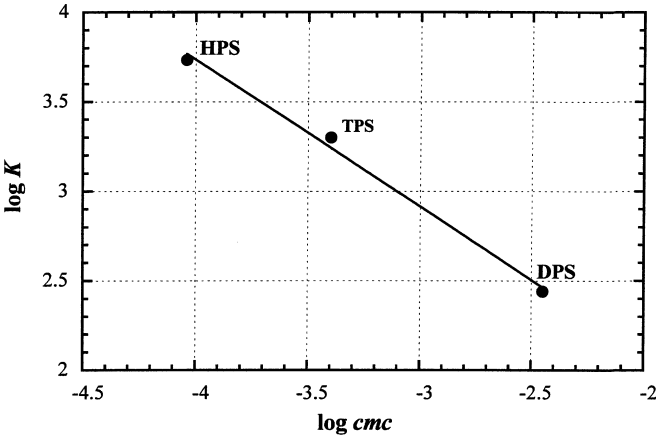



**Fig. 5** Plot of log $K$ vs. log $CMC$ of the series of zwitterionic surfactant in DPC

## Conclusions

Interactions between the binary combination of HPS with PC and DPC in the aqueous bulk were evaluated with the help of pyrene fluorescence ($I_1/I_3$) intensity measurements. The aggregation behavior of HPS was different in aqueous PC and DPC than in pure water. The first and second aggregation processes represent the onset and the completion of vesicle solubilization, which were indicated by $C_1$ and $C_2$ breaks respectively. These interactions were not observed at low lipid concentrations of DPC. The $I_1/I_3$ ratio was lower in the HPS-PC system than in the HPS-DPC. The nature of post-micellar species remains more or less unaffected by the increase in the amount of lipid. This is due to the fact that the concentration range selected for the additive affect of each lipid is, comparatively, very much smaller than the concentration of HPS covered in the post micellar region. A high $K$ value of HPS-lipid aggregates indicates the stronger interactions between surfactant and bilayer assemblies of lipid than those of DPS/TPS-lipid aggregate. This result indicates that the increase in the hydrocarbon chain length determines the hydrophobic character of, and consequently the affinity of the surfactants for, phospholipid vesicles.

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## References

1. Helinius A, Simons K (1975) Biochim Biophys Acta 415:29
2. Lichteunberg D, Robson EA, Dennis EA (1983) Biochem Biophys Acta; Rev Biomemb 285:738
3. Elworthy PH, Florence AT, Macfarlane CB (1968) In: Solubilization of surface active agent. Chapman and Hall, London
4. Le Maire M, Champeil P, Moller JV (2000) Biochim Biophys Acta 1508:86
5. Lichtenberg D (1985) Biochim Biophys Acta 821:470
6. Jones MN (1999) Int J Pharmaceutic 177:137
7. Sehgal P, Doe H, Bakshi MS (2002) J Colloid Interface Sci 252:195
8. Johnsson M, Edwards K (2000) Langmuir 16:8632

9. Cocera M, Lopez O, Estelrich J, Parra JL, De la Maza A (2000) Langmuir 16:4068
10. Sehgal P, Doe H, Doe, Bakshi MS (2003) J Surfactants Detergents 6:31
11. Helinius A, McCaslinDR, Fries E, Tanford C (1979) Methods Enzymol 56:734
12. Tanford C, Reynolds JA (1979) Biochim Biophys Acta 457:133
13. Ribera R, Velazquez (1999) Langmuir 15:6686
14. Brito RM, Vaz WLC (1986) Anal Biochem 152:250
15. Shinoda, K, Arai H, Murata (1971) J Colloid Interface Sci 37:2223
16. Kalyanasundaram K, Thomas JK (1977) J Am Chem Soc 99:2039
17. Inoue T (1996) Interaction of surfactant with phospholipids. In: M Rosoff (ed) Vesicles, Surf Sci Ser, V 62. Dekker Inc, New York pp 151–195
18. Dennis EA (1986) Adv Colloid Interface Sci 26:155
19. Inoue T, Miyakawa K, Shimozawa R (1986) Chem Phys Lipids 42:261
20. Inoue T, Muraoka Y, Fukushima K, Shimozawa R (1988) Chem Phys Lipids 48:189
21. Ueno M (1989) Biochemistry 28:5631
22. Schurtenberger P, Mazer N, King KW (1985) J Phys Chem 89:1042
23. Green RJ, Su TJ, Joy H, Lu JR, (2000) Langmuir 16:5797
24. De la Maza A, Parra JL (1995) J Controlled Release 37:33