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ORIGINAL CONTRIBUTION

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Alterations in phospholipid bilayers caused by sodium dodecyl sulphate/ triton X-100 mixed systems

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Abstract The mechanisms governing the subsolubilizing and solubilizing interaction of sodium dodecyl sulphate (SDS)/Triton X-100 mixtures and phosphatidylcholine liposomes were investigated. Permeability alterations were detected as a change in 5(6)-carboxyfluorescein (CF) released from the interior of vesicles and bilayer solubilization as a decrease in the static light-scattered by liposome suspensions. Three parameters were described as the effective surfactant/lipid molar ratios (*Re*) at which the surfactant system a) resulted in 50% of CF release ($Re_{50\%CF}$); b) saturated the liposomes (Re_{SAT}); c) led to a complete solubilization of these structures (Re_{SOL}). From these parameters the corresponding surfactant partition coefficients $K_{50\%CF}$, K_{SAT} and K_{SOL} were determined. The free surfactant concentrations S_W were lower than the mixed surfactant CMCs at subsolubilizing level, whereas they remained similar to these values during saturation and solubilization of bilayers in all cases. Although the *Re* increased as the mole fraction of the SDS rose (X_{SDS}), the *K* parameters showed a maximum at X_{SDS} values of about 0.6, 0.4 and 0.2 for $K_{50\%CF}$, K_{SAT} and K_{SOL} respectively. Thus, the higher the surfactant contribution in surfactant/lipid system, the lower the X_{SDS} at which a maximum bilayer/water partitioning of mixed surfactant systems added took place and, consequently, the lower the influence of the SDS in this maximum bilayer/water partitioning.

Key words Phospholipid bilayers – sodium dodecyl sulphate/Triton X-100 mixed systems – permeability alterations and solubilization – carboxyfluorescein release – static light scattering –surfactant/ phospholipid molar ratios and partition coefficients

Abbreviations PC, Phosphatidylcholine; PIPES, piperazine-1,4 bis(2-ethanesulphonic acid); SDS, sodium dodecyl sulphate; X_{SDS}, mole fraction of sodium dodecyl sulphate in the mixed system; CF, 5(6)-carboxyfluorescein; *Re*, effective surfactant/lipid molar ratio; $Re_{50\%CF}$, effective surfactant/lipid molar ratio for 50% CF release; Re_{SAT}, effective surfactant/lipid molar ratio for bilayer saturation; Re_{SOL}, effective surfactant/lipid molar ratio for bilayer solubilization; S_W, surfactant concentration in the aqueous medium; $S_{W,50\%CF}$, surfactant concentration in the aqueous medium for 50% CF release; $S_{W,SAT}$, surfactant concentration in the aqueous medium for bilayer saturation; $S_{W,SOL}$, surfactant concentration in the aqueous medium for bilayer solubilization; S_B, surfactant concentration in the bilayers; *K*, bilayer/aqueous phase surfactant partition coefficient; $K_{50\%CF}$, bilayer/aqueous phase surfactant partition coefficient for 50% CF release; K_{SAT}, bilayer/aqueous phase surfactant partition coefficient for bilayer saturation; K_{SOL}, bilayer/aqueous phase surfactant partition coefficient for bilayer solubilization; PL, phospholipid; TLC-FID, thin-layer chromatography/flame ionization detection system; PI, polydispersity index; CMC, critical micellar concentration; r^2, regression coefficient.

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Introduction

The interaction of the anionic surfactant sodium dodecyl sulphate (SDS) with skin induces structural changes in the epidermal surfaces [1–3] and in the stratum corneum transcutaneous permeability barrier [4, 5]. The nonionic surfactant Triton X-100 has been the subject of a number of studies given its properties as a good solubilization agent of membrane proteins [6–9]. Mixtures of these surfactants in aqueous solution show strong deviation from ideality [10–13]. The change in the physico chemical properties of mixed micelles and their additional stability arise from the charge separation of the ionic head groups, which affects the mean aggregation numbers of these micelles [14, 15]. From the biological viewpoint these mixed systems are less injurious to corneal and epithelial tissue than pure anionic surfactant [16].

A number of investigations have been devoted to the understanding of the principles governing the interaction of SDS and Triton X-100 surfactants with simplified membrane models such as phospholipid or stratum corneum lipid bilayers when these surfactants interacted individually with these structures [17–23]. This interaction in excess water leads to the breakdown of lamellar structures and to the formation of lipid-surfactant mixed micelles systems. A significant contribution to these investigations has been made by Lichtenberg [24], who postulated that the critical effective surfactant/lipid ratio (*Re*) producing saturation and solubilization depends on the surfactant critical micellar concentration (CMC) and on the bilayer/aqueous medium distribution coefficients (K) rather than on the nature of the surfactants.

In recent papers, we studied some parameters implicated in the individual interaction of SDS and polyethoxilated octylphenols with different number of ethylene oxide units with unilamellar phosphatidylcholine liposomes [25–27]. In the present work, we seek to extend these investigations by characterizing the subsolubilizing and solubilizing alterations caused by mixtures of SDS and Triton X-100 also in unilamellar phosphatidylcholine liposomes. Knowledge of the partition of mixtures of both surfactants between lipid bilayers and the aqueous phase could be useful in improving our understanding of the synergism existing between these two surfactants and in establishing a criterion for the evaluation of their activity in biological membranes.

Materials and methods

Phosphatidylcholine (PC) was purified from egg lecithin (Merck, Darmstadt, Germany) according to the method of Singleton [28] and was shown to be pure by thin-layer chromatography (TLC). The nonionic surfactant Triton X-100 (octylphenol polyethoxilated with 10 units of ethylene oxide and active matter of 100%) was purchased from Rohm and Haas (Lyon, France). The anionic surfactant sodium dodecyl sulphate (SDS) was purchased from Merck and further purified by a column chromatographic method [29]. Piperazine-1,4 bis(2-ethanesulphonic acid) (PIPES buffer) obtained from Merck was prepared as 10 mM PIPES adjusted to pH 7.20 with NaOH, containing 110 mM Na_2SO_4. Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, CA). The starting material 5(6)-carboxyfluorescein, (CF) was obtained from Eastman Kodak (Rochester, NY) and further purified by a column chromatographic method [30].

Unilamellar liposomes of a defined size (about 200 nm) were prepared by extrusion of large unilamellar vesicles previously obtained by reverse phase evaporation [31]. To study the bilayer permeability changes, vesicles containing CF were freed of unencapsulated fluorescent dye by passage through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography. The range of phospholipid concentration in liposomes was 0.5–5.0 mM. The phospholipid concentration of liposomes was determined using thin-layer chromatography (TLC) coupled to an automated ionization detection (FID) system (Iatroscan MK-5, Iatron Lab. Inc. Tokyo, Japan) [32].

To determine the distribution of surfactants (single surfactants or mixtures) between the lipid phase and the aqueous media, equilibrated surfactant/PC mixed vesicular dispersions (containing subsolubilizing surfactant concentrations) were analyzed for PC [32]. The dispersions were then spun at 140 000 g at 20 °C for 4 h to remove the vesicles [33]. The supernatants of all the mixed dispersions were tested again for PC and surfactants. Surfactant analyses were carried out by spectrophotometric methods [34]. All the samples were assayed in quadruplicate. No PC was detected in any of the supernatants.

The vesicle size distribution and polydispersity index of liposomes were determined with a photon correlator spectrometer (Malvern Autosizer 4700 c PS/MV). The studies were made by particle number measurement. Samples were adjusted to the appropriate concentration range and the measurements were taken at 25 °C and with a lecture angle of 90°.

Surface tensions of buffered solutions containing mixtures of SDS/Triton X-100 at different mole fractions of the anionic surfactant (X_{SDS}) were measured by the ring method [35] using a Krüss tensiometer (processor tensiometer K-12). The critical micelle concentration (CMC) values for single surfactants and mixed surfactant systems

in PIPES buffer were determined from the abrupt change in the slope of the surface tension values versus surfactant concentration.

In the analysis of the equilibrium partition model proposed by Schurtenberger [36] for bile salt/lecithin systems, Lichtenberg [24] and Almog et al. [33] have shown that for a mixing of lipids (at a PL phospholipid concentration (mM)) and surfactant (at a S_T concentration (mM)), in dilute aqueous media, the distribution of surfactant between lipid phase and aqueous media obeys a partition coefficient K, given (in mM^{-1}) by

$$K = S_B/[(PL + S_B)S_W] , \quad (1)$$

where S_B is the concentration of surfactant in the bilayers (mM) and S_W is the surfactant concentration in the aqueous medium (mM). For $PL \gg S_B$, the definition of K, as given by Schurtenberger, applies:

$$K = S_B/(PLS_W) = Re/S_W , \quad (2)$$

where Re is the effective molar ratio of surfactant to phospholipid in bilayers: ($Re = S_B/PL$). Under any other conditions, Eq. (1) has to be employed to define K; this yields:

$$K = Re/[S_W(1 + Re)] . \quad (3)$$

This approach is consistent with the experimental data offered by Lichtenberg [24] and Almog [33] for different surfactant phospholipid mixtures over wide ranges of Re values. Given that the range of phospholipid concentrations used in our investigation is similar to that used by Almog to test his equilibrium partition model, the K parameter has been determined using this equation.

The permeability alterations caused by the mixtures of SDS/Triton X-100 at different X_{SDS} values were determined by monitoring the increase in the fluorescence intensity of the liposome suspensions due to the CF released from the interior of vesicles to the bulk aqueous phase [30]. Fluorescence measurements were made with a Shimadzu RF-540 spectrofluorophotometer. On excitation at 495 nm, a fluorescence maximum emission of CF was obtained at 515.4 nm. The fluorescence intensity measurements were taken at 25 °C. The percentage of CF released was calculated by means of the equation:

$$\%\text{CF release} = \frac{I_T - I_0}{I_\infty - I_0} \cdot 100 , \quad (4)$$

where I_0 is the initial fluorescence intensity of CF-loaded liposome suspension in the absence of surfactant, I_T is the fluorescence intensity measured 40 minutes after adding the surfactant solution to a liposome suspensions. This interval was chosen as the minimum period of time needed to achieve a constant level of CF release for the lipid concentration range used (0.5–5.0 mM). The experimental determination of this interval of time is indicated in the "Results and Discussion Section". I_∞ corresponds to the fluorescence intensity remaining after the complete destruction of liposomes by the addition of Triton X-100 aqueous solution [30].

With regard to liposome solubilization, it has been previously demonstrated that static light-scattering constituted a very convenient technique for the quantitative study of the bilayer solubilization by surfactants [17, 37, 38]. Accordingly, the solubilizing perturbation produced by the surfactant mixtures in PC liposomes was monitored using this technique. The overall solubilization can be mainly characterized by two parameters termed Re_{SAT} and Re_{SOL}, according to the nomenclature adopted by Lichtenberg [24] corresponding to the Re ratios at which light-scattering starts to decrease with respect to the original value and shows no further decrease. These parameters corresponded to the surfactant/lipid molar ratios at which the surfactant: a) saturated liposomes and b) led to a complete solubilization of these structures.

Liposomes were adjusted to the adequate lipid concentration (from 1.0 to 10.0 mM). Equal volumes of the adequate surfactant solutions were added to these liposomes and the resulting mixtures were left to equilibrate for 24 h. This time was chosen as the optimum period needed to achieve a complete equilibrium surfactant/liposomes for the lipid concentration range used [17, 38]. Light-scattering measurements were made using the spectrofluorophotometer at 25 °C with both monochromators adjusted to 500 nm. The assays were carried out in triplicate and the results given are the average of those obtained.

The determination of the Re, S_W and K parameters can be carried out on the basis of the linear dependence existing between the surfactant concentrations required to achieve the 50% of CF release (Eq. (5)), to saturate the bilayer (Eq. (6)), or to achieve the complete solubilization of liposome structures via mixed micelles formation (Eq. (7)) and the phospholipid concentration in liposomes which can be described by the equations:

$$S_{T,50\%CF} = S_{W,50\%CF} + Re_{50\%CF} \cdot PL \quad (5)$$

$$S_{T,SAT} = S_{W,SAT} + Re_{SAT} \cdot PL \quad (6)$$

$$S_{T,SOL} = S_{W,SOL} + Re_{SOL} \cdot PL , \quad (7)$$

where the Re ($Re_{50\%CF}$, Re_{SAT} and Re_{SOL}) and the aqueous concentration of surfactant S_W ($S_{W,50\%CF}$, $S_{W,SAT}$ and $S_{W,SOL}$) are in each curve respectively the slope and the ordinate at the origin (zero phospholipid concentration).

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Results and discussion

Mean vesicle size and stability of liposome suspensions

The mean vesicle size of liposome suspensions after preparation (phospholipid concentration ranging from 0.5 to 5.0 mM) varied little (around 200 nm). The polydispersity index (PI), was in all cases lower than 0.1 indicating that the liposome suspensions showed a homogeneous size distribution in all cases. The size of vesicles after the addition of equal volumes of PIPES buffer and equilibration for 24 h at 25 °C showed in all cases values similar to those obtained after preparation, with a slight increase in the PI (between 0.12 and 0.14). Hence, the liposome preparations appeared to be reasonably stable in the absence of surfactants under the experimental conditions used in solubilization studies.

Critical micelle concentration (CMC)

Figure 1 shows the variation of the surface tensions as a function of total surfactant concentration for the mixed surfactant systems at different X_{SDS}. The surface tensions decrease as the total surfactant concentration increases showing in each case a characteristic change in the slope, which corresponds to the CMC of the system. The CMC values are given in Table 1. When plotting the CMC values against the X_{SDS} curves are obtained (Fig. 2) in which the CMC values increase with increase of the mole fraction of the anionic surfactant.

Assuming that the thermodynamics of the micellation process for these systems obey the ideal solution theory, when monomer and micelles are in equilibrium in the system, the CMC values would fall on the line predicted by the relationship [39]:

$$\frac{1}{C_{12}} = \frac{X}{C_1} + \frac{1-X}{C_2}, \qquad (8)$$

where C_{12} is the CMC for the mixed micelle system of surfactants 1 (SDS) and the surfactant 2 (Triton X-100); C_1 is the CMC of the surfactant 1; C_2 is the CMC of the surfactant 2, and X is the mole fraction of surfactant 1 in the mixture. The theoretical CMC values for each molar ratio thus calculated are also indicated in the upper curve of Fig. 2. For all mixtures studied, the CMC values of the mixed surfactant systems are lower than those predicted by Eq. (8). This means that the mixed micelle formation shows a negative deviation with respect to the ideal behavior.

Interaction of SDS/Triton X-100 with liposomes

In order to determine the time needed to obtain a constant level of CF release of liposomes in the range of the

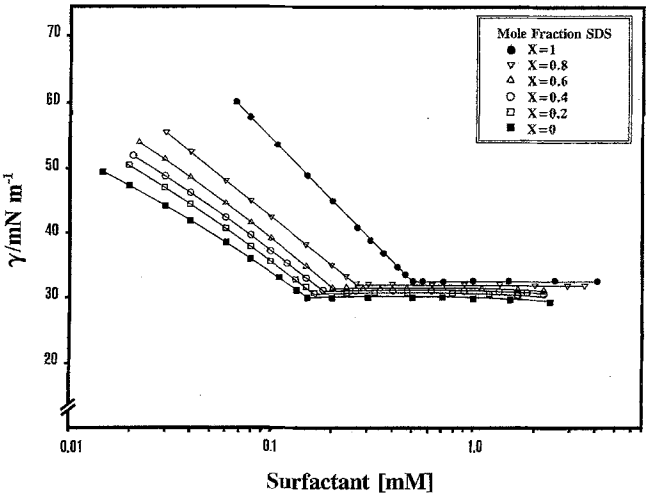

Fig. 1 Variation of the surface tensions versus total surfactant concentration for different mole fractions of anionic surfactant (X_{SDS}) for the SDS/Triton X-100 mixed systems X_{SDS} = 1 (●), 0.8 (▽), 0.6 (△), 0.4 (○), 0.2 (□), 0 (■)

Table 1 Surfactant to phospholipid molar ratios, partition coefficients and surfactant concentrations in the aqueous medium corresponding to the 50% CF release, saturation and complete solubilization of lipid bilayers resulting in the interaction of SDS/Triton X-100 mixed systems at different X_{SDS} with PC liposomes. The CMC of the surfactant mixed systems and the regression coefficients of the straight lines obtained are also indicated

X_{SDS}	CMC (mM)	$S_{W,50\%CF}$	$S_{W,SAT}$	$S_{W,SOL}$	$Re_{50\%CF}$	Re_{SAT}	Re_{SOL}	$K_{50\%CF}$	K_{SAT}	K_{SOL}	r^2 (50%CF)	r^2 (sat)	r^2 (sol)
0	0.15	0.041	0.16	0.18	0.150	0.64	2.60	3.18	2.43	4.01	0.992	0.996	0.995
0.2	0.16	0.040	0.16	0.17	0.172	0.72	2.63	3.67	2.61	4.26	0.994	0.991	0.995
0.4	0.175	0.040	0.17	0.18	0.180	0.80	2.64	3.81	2.61	4.03	0.993	0.997	0.991
0.6	0.20	0.042	0.20	0.21	0.20	0.90	2.68	3.96	2.36	3.46	0.998	0.994	0.993
0.8	0.26	0.058	0.26	0.28	0.245	0.96	2.69	3.40	1.88	2.60	0.997	0.990	0.994
1.0	0.50	0.083	0.50	0.53	0.25	1.10	2.70	2.41	1.04	1.37	0.992	0.998	0.991

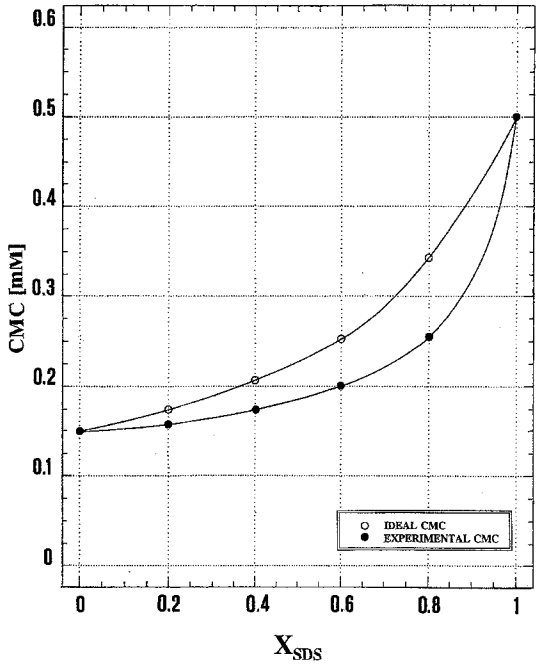

Fig. 2 Relationship between the experimental CMC values (mM) (●) and different mole fractions of the anionic surfactant for the SDS/Triton X-100 mixed systems. The CMC values theoretical calculated for each molar ratio have been also indicated in the upper curve (○)

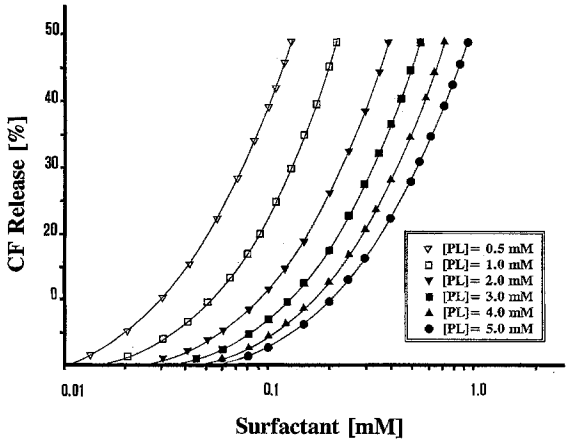

Fig. 3 Percentage changes in CF release of unilamellar liposomes (lipid bilayer concentration ranging from 0.5 to 5.0 mM), induced by the presence of increasing concentrations of SDS/Triton X-100 mixed surfactant system for the mole fraction of the anionic surfactant of 0.4. [PL] = 0.5 mM (▽), [PL] = 1.0 mM (□), [PL] = 2.0 mM (▼), [PL] = 3.0 mM (■), [PL] = 4.0 mM (▲), [PL] = 5.0 mM (●)

phospholipid concentration investigated (0.5 and 5.0 mM), a kinetic study of the interaction of surfactant mixtures at different X_{SDS} with liposomes was carried out. Liposome suspensions were treated with surfactants at subsolubilizing concentrations, and subsequent changes in permeability were studied as a function of time. The permeability kinetics were similar for each system tested: about 40 min was needed to achieve a constant level of CF release. Hence, changes in permeability were studied 40 min after addition of surfactant to the liposomes at 25 °C. The CF release of liposome suspensions in the absence of surfactant 40 min after preparation was negligible.

To determine $K_{50\%CF}$ of surfactant mixtures in liposomes, a systematic investigation of bilayer permeability alterations was carried out. Changes in the CF released were determined 40 min after surfactant addition at 25 °C. The results obtained for $X_{SDS} = 0.4$ are plotted in Fig. 3. The surfactant concentrations resulting in 50% of CF release were graphically obtained and plotted versus the phospholipid concentration. An acceptable linear relationship was established in each case. The straight lines obtained correspond to the aforementioned Eq. 5 from which the Re and K parameters were determined. The results obtained for different X_{SDS} including the free surfactant concentration S_W and the regression coefficient of the straight lines are given in Table 1.

Different trends in the evolution of Re and K parameter were observed as the X_{SDS} increased. Thus, whereas $Re_{50\%CF}$ progressively increased, the $K_{50\%CF}$ values showed a maximum approximately for $X_{SDS} = 0.6$. Furthermore, $S_{W,50\%CF}$ increased as the X_{SDS} rose specially for X_{SDS} values higher than 0.6. Bearing in mind the CMCs experimentally obtained for the different X_{SDS} (Table 1), $S_{W,50\%CF}$ showed always lower values than the corresponding CMCs, thereby confirming that permeability alterations were determined by the action of surfactant monomer.

In accordance with the procedure described by Urbaneja et al., the solubilizing interaction of surfactant mixtures and liposomes was studied through the changes in the static light scattered by these systems 24 h after the addition of surfactant [17, 38]. Figure 4 shows the solubilization curves of liposome suspensions (lipid concentration 0.5 mM–5.0 mM) arising from the addition of increasing amounts of surfactant mixed systems at $X_{SDS} = 0.4$. At low surfactant concentration an initial increase in the light-scattering was observed in all cases due to the incorporation of surfactant molecules into bilayers. Increasing amounts of surfactant led to a fall in the scattered intensity until a low constant value for bilayer solubilization was reached. The surfactant concentrations producing 100% and 0% of light-scattering were obtained for each lipid concentration by graphical methods. Plotting the surfactant concentration versus the lipid concentration, curves were obtained in which an acceptable linear relationship was also established in each case. The Re_{SAT}, Re_{SOL}, K_{SAT} and K_{SOL} parameters were determined from

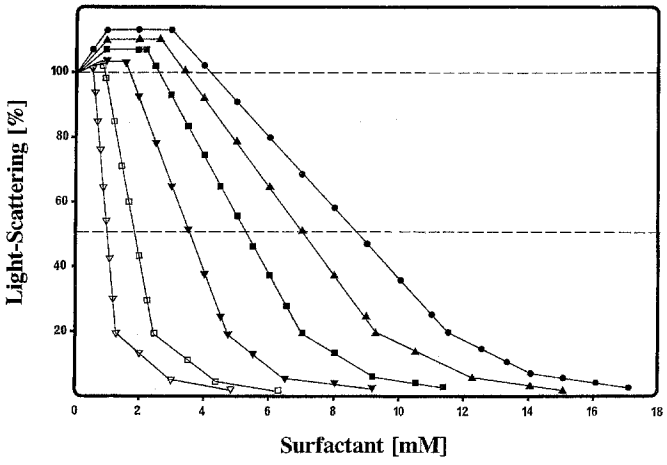

Fig. 4 Percentage changes in static light-scattering of unilamellar liposomes, (lipid concentration ranging between 0.5 and 5.0 mM), induced by the presence of increasing concentrations of SDS/Triton X-100 mixed surfactant system for the mole fraction of the anionic surfactant of 0.4. [PL] = 0.5 mM (▽), [PL] = 1.0 mM (□), [PL] = 2.0 mM (▼), [PL] = 3.0 mM (■), [PL] = 4.0 mM (▲), [PL] = 5.0 mM (●)

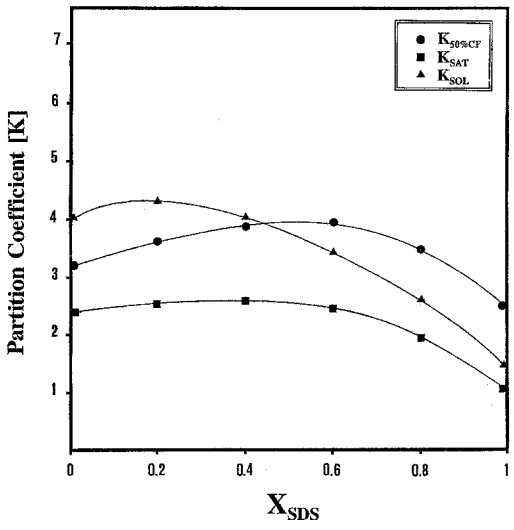

Fig. 5 Relation between the $K_{50\%CF}$ (●), K_{SAT} (■) and K_{SOL} (▲) and the mole fraction of the anionic surfactant X_{SDS} for the SDS/Triton X-100 mixed systems

these straight lines (Eqs. (6, 7)). The results obtained for each X_{SDS} are also given in Table 1.

From these data it should be noted that the *Re* parameter progressively increased as the X_{SDS} rose. Given that the ability of surfactant to saturate or solubilize liposomes is inversely related to the *Re* values, the higher the X_{SDS} the lower its ability for saturation and solubilization of these structures. Furthermore, despite the increasing tendency of *Re*, the *K* values showed a maximum for X_{SDS} between 0.2 and 0.4, both parameters showing a progressive increase from bilayer saturation to complete solubilization of bilayers regardless of the X_{SDS}. From these findings, we may assume that an increasing partition equilibrium governs both the incorporation of surfactant molecules into the lipid bilayers and the subsequent association of the surfactant molecules with the lipid building liposomes to form mixed micelles. Thus, the affinity of surfactant molecules for lipids appears to be greater in the bilayer solubilization (micellization process) than during the previous step of bilayer saturation. The fact that the free surfactant concentration ($S_{W,SAT}$, $S_{W,SOL}$) was always comparable to the CMCs of surfactant mixtures supports the generally admitted assumption that the concentration of free surfactant must reach the CMC for solubilization to occur [24]. These findings emphasize the influence of the negative synergism of SDS/Triton X-100 mixed micelles on the aqueous surfactant concentration needed to saturate or solubilize PC liposomes.

Figure 5 shows the variation in the *K* parameters at subsolubilizing and solubilizing levels versus the X_{SDS}. It is interesting to note that $K_{50\%CF}$ showed a maximum approximately at $X_{SDS} = 0.6$, whereas K_{SAT} and K_{SOL} showed a maximum at X_{SDS} values about of 0.4 and 0.2 respectively. Thus, the higher the surfactant contribution in the surfactant/lipid system, the lower the X_{SDS} at which the maximum partition of surfactant molecules between the lipid and aqueous phase took place. The influence of SDS in this partition appears to be more significant at the sublitic level, whereas the influence of Triton X-100 seems to be greater during saturation and solubilization of liposomes via formation of mixed micelles.

Systematic analysis of the free surfactant (S_W) for different surfactant/liposome systems at subsolubilizing level was carried out in order to confirm these findings. Thus, equilibrated surfactant/PC mixed vesicular dispersions corresponding to the 50% of CF release and 100% of light scattering (bilayer saturation) at different X_{SDS} were analyzed for PC [32]. The dispersions were then spun at 140 000 g at 20 °C for 4 h to remove the vesicles [33]. The supernatants of all the mixed dispersions ($S_{W,50\%CF}$ and $S_{W,SAT}$) were tested again for PC and surfactants (single surfactant or mixtures). A similar pattern was observed for various PC concentrations (0.5 mM–5.0 mM): up to the surfactant concentration for bilayer saturation ($S_{T,SAT}$), no PC became solubilized. These findings are in agreement with the result reported by Almog et al. for octyl glucoside/PC systems [33] and confirm the fact that the surfactant concentration must reach the $S_{T,SAT}$ for PC solubilization to occur [24].

From the surfactant analyses it is noteworthy that the free SDS and Triton X-100 concentrations for

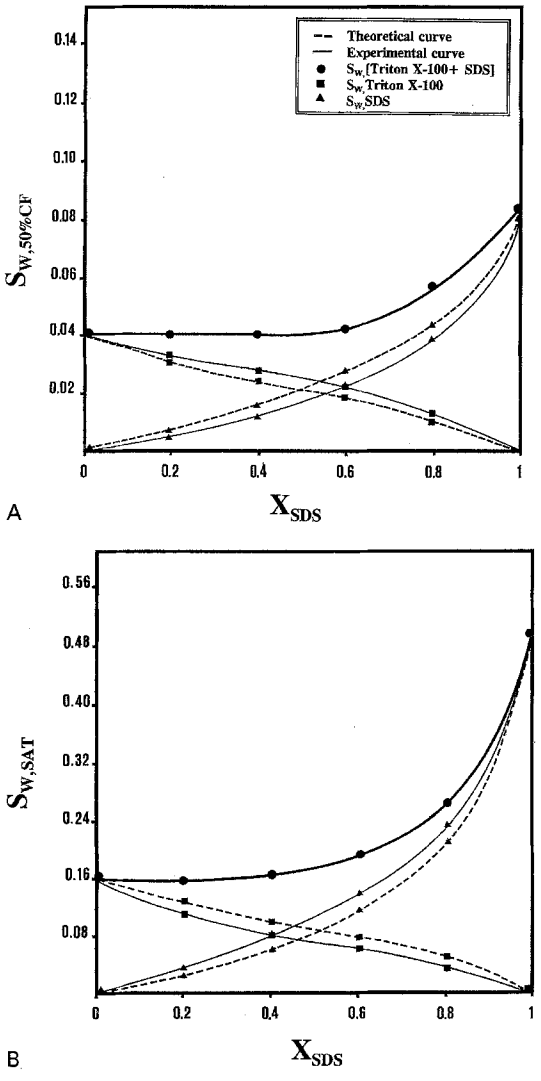

Fig. 6 Variation of $S_{W,50\%CF}$ for surfactant mixtures (●) and single surfactants (Triton X-100) (■), and SDS (▲)) versus X_{SDS} (Fig. 6-A). Variation of $S_{W,SAT}$ for surfactant mixtures (●) and single surfactants (Triton X-100 (■), and SDS (▲)) versus X_{SDS} (Fig. 6-B). The theoretical values of $S_{W,50\%CF}$ and $S_{W,SAT}$ for single surfactants and for each X_{SDS} are also indicated (discontinuous lines)

$S_{W,50\%CF}$ showed, respectively, lower and higher values than those theoretically predicted (Fig. 6-A), whereas their concentrations for $S_{W,SAT}$, showed inversely higher and lower values than those predicted (Fig. 6-B). The theoretical free surfactant concentrations were calculated for each X_{SDS} from the $S_{W,50\%CF}$ and the $S_{W,SAT}$ values respectively and are indicated in Fig. 6 (discontinuous lines).

On the basis of these findings we may assume that in the step corresponding to the 50% of CF release, the SDS and the Triton X-100 exhibited respectively higher and lower affinities with bilayers than theoretically predicted, whereas in the step for bilayer saturation the affinities of these surfactants with bilayers showed the opposite tendency.

Comparison of Figs. 5 and 6 confirms the greater influence of the anionic surfactant SDS at sublytic interaction level, whereas the nonionic Triton X-100 appears to be more active both in the saturation and subsequent solubilization of lipid bilayers via mixed micelle formation. This selective behavior in the surfactant partitioning may be also correlated with the results previously reported by Dubin et al. [10], who argued, in terms of head group surfactant areas at low ionic strength, that mixed micelles become smaller with increasing X_{SDS}, thereby producing strong repulsion effects. The maximum K_{SOL} at low X_{SDS} emphasizes the low influence of the electrostatic forces in the complete solubilization of liposomes via lipid/surfactant mixed micelle formation.

The fact that $K_{50\%CF}$ showed higher values than K_{SAT} suggest, as previously described by Shubert et al. [40] for sodium cholate/liposome interactions, that at low *Re* only the outer vesicle leaflet was available for the added (mixed surfactant systems until saturation of this monolayer (maximum *K*). The presence of additional amounts of surfactant (increase in *Re*) resulted in an increased rate of surfactant flip-flop, thus making the inner monolayer available for interaction with added surfactant.

In the light of our results, the X_{SDS} appears to be the governing parameter regulating the physico-chemical properties of these binary surfactant systems. The selective control in the lipid/water partitioning of these mixed surfactant systems both at sublytic and lytic levels, which depends on the relative proportion of each surfactant in the mixture, opens up new avenues in the potential application of these mixtures in biological and technological domains.

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