Changes in Phospholipid Bilayers Caused by Sodium Dodecyl Sulfate/Nonionic Surfactant Mixtures

Alfonso de la Maza* and Jose Luis Parra

Departamento de Tensioactivos, Centro de Investigación y Desarrollo (C.I.D.), Consejo Superior de Investigaciones Científicas (C.S.I.C.), 08034 Barcelona, Spain

ABSTRACT: The interaction of mixtures of sodium dodecyl sulfate (SDS) and oxyethylenated nonylphenol (30 mol of ethylene oxide) [$NP(EO)_{30}$] with phosphatidylcholine liposomes was investigated. Permeability alterations were detected as a change in 5(6)-carboxyfluorescein (CF) released from the interior of vesicles, and bilayer solubilization was measured 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: (i) resulted in 50% CF release ($Re_{50\%CF}$); (ii) saturated the liposomes (Re_{SAT}); (iii) led to complete solubilization of these structures (Re_{SOL}). The corresponding surfactant partition coefficients ($K_{50\%CF}$, K_{SAT}, and K_{SOL}) were determined from these parameters. The free surfactant concentrations S_W were lower than the mixed surfactant critical micellar concentration at subsolubilizing levels, whereas they remained similar to these values during saturation and solubilization of bilayers. Although the *Re* values increased linearly as the mole fraction of the SDS rose (X_{SDS}), the *K* parameters showed maximum values at X_{SDS} 0.6 for $K_{50\%CF}$ and approximately at X_{SDS} 0.2 for K_{SAT} and K_{SOL}, respectively. Thus, the lower the surfactant contribution in the surfactant/lipid system, the higher the X_{SDS} at which the maximum bilayer/water partitioning of added mixed surfactant systems occurred. As a consequence, the influence of SDS in this partition appears to be more significant at the sublytic level (monomeric effect), whereas the influence of $NP(EO)_{30}$ seems to be greater during saturation and solubilization of liposomes *via* formation of mixed micelles.

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KEY WORDS: Carboxyfluorescein release, partition coefficients, permeability alterations and bilayer solubilization, phosphatidylcholine liposomes, sodium dodecyl sulfate/nonylphenol oxyethylenated [$NP(EO)_{30}$] mixtures, static light-scattering, surfactant/phospholipid molar ratios.

The interaction of sodium dodecyl sulfate (SDS) with skin induces structural changes in the epidermal surfaces (1–3) and in the stratum corneum transcutaneous permeability barrier (4,5). Mixtures of this anionic surfactant with oxyethylenated nonylphenols of different oxyethylenation levels in aqueous solution show negative deviation from ideal solution behavior (6–10). These mixtures also exhibit changes in the surface properties that improve the wetting ability of water on hydrophobic surfaces (11,12) and the salinity tolerance with regard to that of the anionic component (13). The change in the physico- chemical properties of mixed micelles and their additional stability arises from the charge separation of the ionic head groups, which affects the mean aggregation numbers of these micelles (8,14).

A number of investigations have been devoted to the understanding of the principles that govern the interaction of SDS with simplified membrane models, such as phospholipid or stratum corneum lipid bilayers, when this surfactant interacted individually with these structures (15–18). This interaction in excess water leads to the breakdown of lamellar structures and to the formation of lipid–surfactant mixed micelle systems. A significant contribution to these investigations has been made by Lichtenberg (19) who postulated that the critical effective surfactant/lipid ratio (*Re*) that produces saturation and solubilization depends on the surfactant critical micellar concentration (CMC) and on the bilayer/aqueous medium distribution coefficients (*K*).

In previous papers, we studied some parameters implicated in the interaction of SDS with phosphatidylcholine (PC) unilamellar liposomes when interacted individually (20,21) or in mixtures with amphoteric or nonionic surfactants (22,23). In the present work, we seek to extend these investigations by characterizing the subsolubilizing alterations caused by mixtures of SDS/oxyethylenated monylphenol (30 mol of ethylene oxide)[$NP(EO)_{30}$] surfactants in phosphatidylcholine liposomes, which do not affect the bilayer architecture and are associated with permeability changes, and the solubilization of these structures *via* mixed micelle formation. Knowledge of the partition of mixtures of these surfactants between lipid bilayers and the aqueous phase could be useful in improving our understanding of the synergism that exists between these two surfactants and in establishing a criterion for the evaluation of their activities with respect to those reported for other surfactant mixtures in biological membranes.

MATERIALS AND METHODS

The nonionic surfactant $NP(EO)_{30}$ was supplied by Tenneco S.A. (Spain) as a 100% active matter product. Triton X-100 was purchased from Rohm and Haas (Lyon, France). The an-

*To whom correspondence should be addressed at Dr. A. de la Maza, Departamento de Tensioactivos, Centro de Investigación y Desarrollo (C.I.D.), Consejo Superior de Investigaciones Científicas (C.S.I.C.), C/Jordi Girona, 18-26, 08034 Barcelona, Spain.

ionic surfactant SDS was purchased from Merck (Darmstadt, Germany) and further purified by a column-chromatographic method (24). PC was purified from egg lecithin (Merck) according to the method of Singleton *et al.* (25) and was shown to be pure by thin-layer chromatography (TLC). Piperazine-1,4 *bis*(2-ethanesulfonic acid) (PIPES buffer), obtained from Merck, was prepared as 20 mM PIPES adjusted to pH 7.20 with NaOH, and contained 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 (26).

Surface tensions of buffered solutions that contained mixtures of SDS/NP(EO)$_{30}$ at different mole fractions of the anionic surfactant (X_{SDS}) were measured by the ring method (27) with a Krüss tensiometer (processor tensiometer K-12; Hamburg, Germany). The 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 vs. surfactant concentration.

Unilamellar liposomes of a defined size (about 200 nm) were prepared by extrusion of large unilamellar vesicles that were previously obtained by reverse-phase evaporation (28,29). 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 by TLC coupled to an automated ionization detection (FID) system (Iatroscan MK-5; Iatron Lab. Inc. Tokyo, Japan) (30).

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

To evaluate the changes caused on lipid bilayers by the mixtures of SDS/NP(EO)$_{30}$ at different mole fractions of the anionic surfactant (X_{SDS}), the effective surfactant/phospholipid molar ratio *Re* in an aggregate (liposome or micelle) is defined as (21):

$$Re = S_B/PL_B \quad [1]$$

where S_B is the concentration of surfactant in the bilayers (mM), ($S_B = S_T - S_W$, where S_T is the total surfactant concentration and S_W the surfactant concentration in the aqueous medium). PL_B is the PC concentration in the bilayers (mM), [$PL_B = PL - PL_{Mon}$, where PL is the total PC concentration and PL_{Mon} the monomeric PC concentration]. PL_{Mon} is negligible due to the low solubility of PC in water.

It is generally accepted that an equilibrium partition of surfactants between bilayer and the aqueous medium governs the incorporation of surfactants into liposomes, thereby producing saturation and solubilization of these structures. In the analysis of the equilibrium partition model proposed by Schurtenberger *et al.* (31) for bile salt/lecithin systems, Lichtenberg (19) and Almog *et al.* (32) have shown that for a mixing of lipids, in dilute aqueous media, the distribution of surfactant between lipid phase and aqueous media (in mM^{-1}) can be described by:

$$K = S_B/[(PL + S_B)S_W] \quad [2]$$

Combining Equations 1 and 2 yields

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

This approach is consistent with the experimental data offered by Lichtenberg (19) and Almog *et al.* (32) 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 et al. (32) to test their equilibrium partition model, the *K* parameter has been determined from this equation.

The permeability alterations caused by the surfactant mixtures 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. Fluorescence measurements were made with a Shimadzu RF-540 spectrofluorophotometer (Kyoto, Japan) at 25°C. On excitation at 495 nm, a fluorescence maximum emission of CF was obtained at 515.4 nm (33). The presence of single surfactants or surfactant mixtures did not affect the direct quenching of the aforementioned spectrofluorophotometric CF signal.

Liposomes were adjusted to the appropriate lipid concentration (from 1.0 to 10.0 mM). Equal volumes of the appropriate surfactant solutions were added to these liposomes, and the fluorescence intensity of the resulting mixtures was measured 40 min after the surfactant addition. 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. The experimental determination of this interval of time is indicated in Results and Discussion section. With regard to liposome solubilization, it has been previously demonstrated that static light-scattering constituted a convenient technique for the quantitative study of bilayer solubilization by surfactants (15,34,35). Accordingly, the solubilizing perturbation produced by the surfactant mixtures in PC liposomes was monitored with this technique. The overall solubilization can be mainly characterized by two parameters, termed Re_{SAT} and Re_{SOL}, that according to the nomenclature adopted by Lichtenberg (19) correspond 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: (i) saturated liposomes and (ii) led to a complete solubilization of these structures.

Liposomes were adjusted to the appropriate lipid concentration (from 1.0 to 10.0 mM). Equal volumes of the appropriate 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–liposome for the lipid concentration range used (15,35). Light-scattering measurements were made in 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 averages.

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 50% CF release (Eq. 4), to saturate the bilayer (Eq. 5), or to achieve complete solubilization of the liposome structures *via* mixed micelles formation (Eq. 6) and the phospholipid concentration in liposomes, which can be described by the equations:

$$S_{T,50\%CF} = S_{W,50\%CF} + Re_{50\%CF} \cdot PL \quad [4]$$

$$S_{T,SAT} = S_{W,SAT} + Re_{SAT} \cdot PL \quad [5]$$

$$S_{T,SOL} = S_{W,SOL} + Re_{SOL} \cdot PL \quad [6]$$

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).

RESULTS AND DISCUSSION

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 PI, defined as a measure of the width of the particle size distribution obtained from the "cumulants analysis", remained always lower than 0.1, indicating that all liposome suspensions showed a homogeneous size distribution. The size of vesicles after addition of equal volumes of PIPES buffer and equilibration for 24 h showed always values that were similar to those obtained soon 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. Furthermore, the interaction of liposomes with surfactant always resulted initially in a slight growth of vesicles, followed by a decrease in the vesicle size due to solubilization of these structures *via* mixed micelle formation. However, no aggregation of vesicles was detected by light-scattering during the interaction process.

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

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 (36):

$$\frac{1}{C_{12}} = \frac{X}{C_1} + \frac{1 - X}{C_2} \quad [7]$$

where C_{12} is the CMC for the mixed micelle system of surfactants 1 (SDS) and 2 [$NP(EO)_{30}$]; C_1 is the CMC of surfactant 1; C_2 is the CMC of surfactant 2, and *X* is the mole fraction of surfactant 1 in the mixture. The theoretical CMC values for each molar ratio thus calculated also are indicated in the upper curve of Figure 2. For all mixtures studied, the CMC values of the mixed surfactant systems were lower than those predicted by Equation 7. This finding confirms that mixed micelle formation showed a negative deviation with respect to ideal behavior.

Interaction of SDS/NP(EO)$_{30}$ with liposome suspensions. To determine the time needed to obtain a constant level of CF release of liposomes in the range of the phospholipid concentration investigated (0.5 and 5.0 mM), a kinetic study of the interaction of mixed surfactant systems at different X_{SDS} was carried out with liposomes. Liposome suspensions were treated with different concentrations of surfactant 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 was negligible 40 min after preparation.

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 Figure 3. The surfactant concentrations that resulted in 50% CF release were graphically obtained and plotted vs. the phospholipid concentration. An acceptable linear relationship was established in each case. The straight lines obtained correspond to aforementioned Equation 4 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 X_{SDS} was increased. Thus, whereas $Re_{50\%CF}$ progressively increased, the $K_{50\%CF}$ values showed a maximum for X_{SDS}= 0.6. Furthermore, the $S_{W,50\%CF}$ values increased as X_{SDS} was increased, this rise being more pronounced for X_{SDS} values ranging between 0.6 and 1.0. The $S_{W50\%CF}$ values were always clearly lower than those corre-

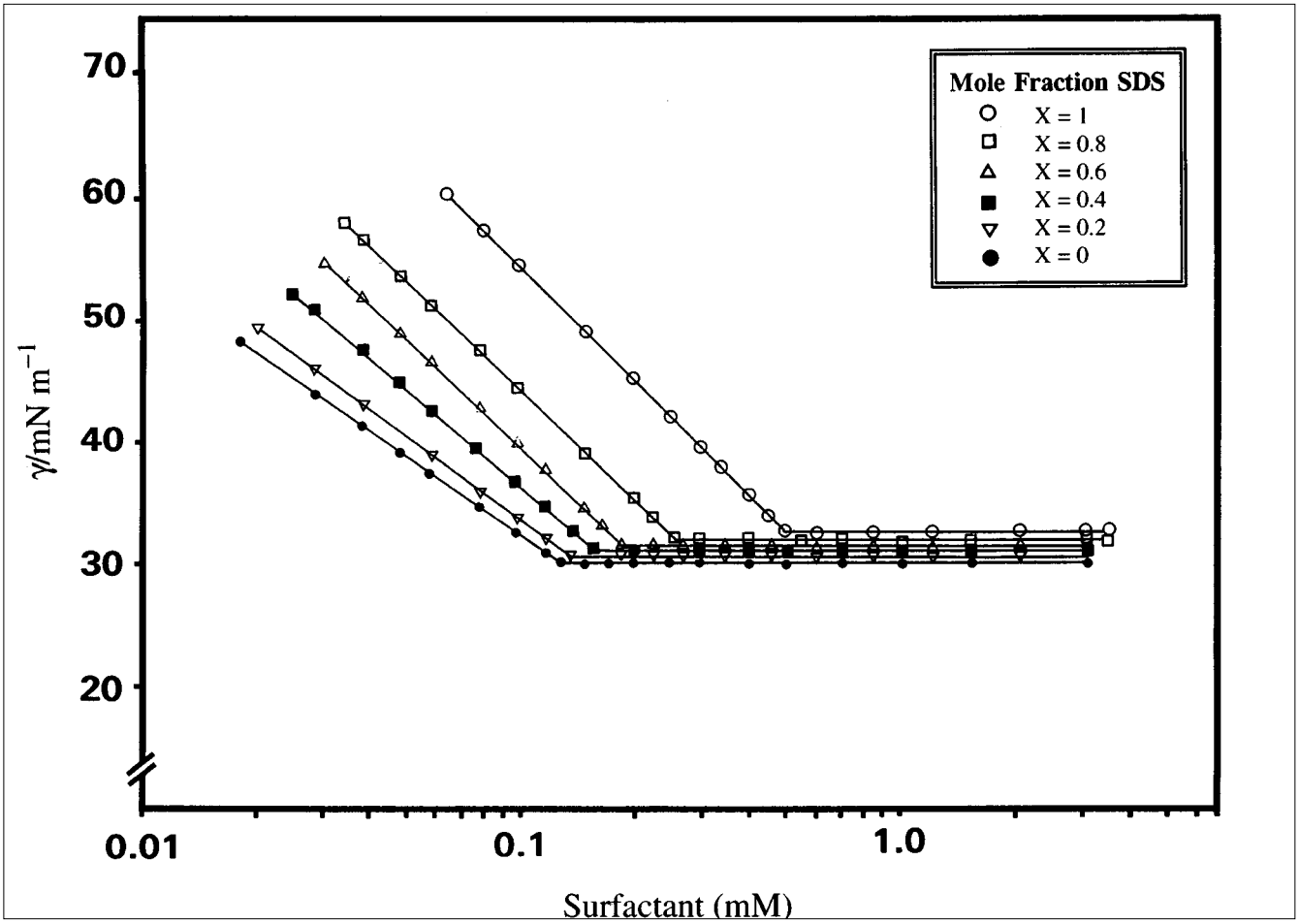

FIG. 1. Variation of the surface tension vs. total surfactant concentration for different mole fractions of anionic surfactant (X_{SDS}) for the SDS/NP(EO)$_{30}$ mixed systems. X_{SDS} = 1 (○), 0.8 (□), 0.6 (Δ), 0.4 (■), 0.2 (▽), 0 (●).

sponding to the CMC experimentally obtained, thereby confirming that permeability alterations were determined by the action of surfactant monomer.

In accordance with the procedure described by Urbaneja *et al.* (15,35), the solubilizing interaction of surfactant mixtures and liposomes was studied through changes in the static light scattered by these systems 24 h after addition of surfactant. Figure 4 shows the solubilization curves of liposome suspensions (lipid concentration 0.5–5.0 mM) arising from the addition of increasing amounts of mixed surfactant systems at X_{SDS} = 0.4. At low surfactant concentration, an initial increase in light-scattering was observed in all cases due to the presence of surfactant molecules in bilayers, until a maximum value was achieved. 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 that produced 100% and 0% light-scattering were obtained for each lipid concentration by graphical methods. Plotting the surfactant concentration vs. 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 these straight lines (Eqs. 5,6). The results obtained for each X_{SDS}, including the regression coefficient of each straight line, are also given in Table 1.

The *Re* and *K* parameters increased progressively from bilayer saturation to complete solubilization of these structures, regardless of 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 liposome-building lipids to form mixed micelles. Thus, the affinity of surfactant molecules for lipids appears to be greater in 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 CMC of surfactant mixtures supports the generally accepted assumption for single surfactants that the concentration of free surfactant must reach the CMC for solubilization to occur (19). These findings emphasize the influ-

TABLE I

Surfactant to Phospholipid Molar Ratios, Partition Coefficients, and Surfactant Concentrations in the Aqueous Medium Corresponding to 50% CF Release, Saturation, and Complete Solubilization of Lipid Bilayers[a]

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	0.130	0.035	0.13	0.15	0.117	0.43	1.47	2.99	2.31	3.96	0.996	0.998	0.994
0.2	0.137	0.035	0.13	0.15	0.151	0.55	1.67	3.75	2.72	4.17	0.997	0.997	0.996
0.4	0.158	0.036	0.15	0.16	0.185	0.68	1.93	4.34	2.69	4.12	0.996	0.995	0.993
0.6	0.188	0.038	0.18	0.20	0.204	0.82	2.22	4.46	2.50	3.45	0.998	0.993	0.997
0.8	0.260	0.050	0.26	0.28	0.238	0.96	2.43	3.84	1.88	2.53	0.996	0.995	0.994
1.0	0.500	0.083	0.50	0.53	0.25	1.10	2.70	2.41	1.04	1.37	0.990	0.996	0.992

[a]Result in the interaction of SDS/NP(EO)$_{30}$ mixed systems at different X_{SDS} with PC liposomes. The experimental CMC values of the surfactant mixed systems and the regression coefficients of the straight lines obtained are also indicated. SDS/NP(EO)$_{30}$, sodium dodecyl sulfate/oxyethylenated nonylphenol (30 mol of ethylene oxide); CF, 5(6)-carboxyfluorescein; PC, phosphatidylcholine; CMC, critical micelle concentration; X_{SDS}, mole fraction of SDS in the mixed system; $S_{W50\%CF}$, surfactant concentration in the aqueous medium for 50% CF release; S_{WSAT}, surfactant concentration in the aqueous medium for bilayer saturation; S_{WSOL}, surfactant concentration in the aqueous medium for bilayer solubilization; $Re_{50\%CF}$, 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; $R_{50\%CF}$ effective surfactant–lipid molar ratio for 50% CF release; $K_{50\%CF}$, 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; r^2, regression coefficient.

ence of the negative synergism of SDS/NP(EO)$_{30}$ mixed micelles on the aqueous surfactant concentration needed to saturate or solubilize PC liposomes.

When plotting the $Re_{50\%CF}$, Re_{SAT}, and Re_{SOL} parameters vs. X_{SDS}, straight lines are obtained (Fig. 5). The *Re* values increased linearly with X_{SDS} in all cases. Given that the ability of surfactant to saturate or solubilize liposomes is inversely related to its *Re* value, the higher the X_{SDS}, the lower is its ability to release 50% CF, saturate and solubilize these structures. Bearing in mind that the slope of these straight lines rose as the level of interaction between the surfactant mixture and liposomes increased (from $Re_{50\%CF}$ to Re_{SOL}), the aforementioned relative fall in the activity of surfactant mixtures vs. X_{SDS} was more pronounced for bilayer solubilization (Re_{SOL}) than for bilayer saturation (Re_{SAT}) and 50% CF release ($Re_{50\%CF}$), respectively.

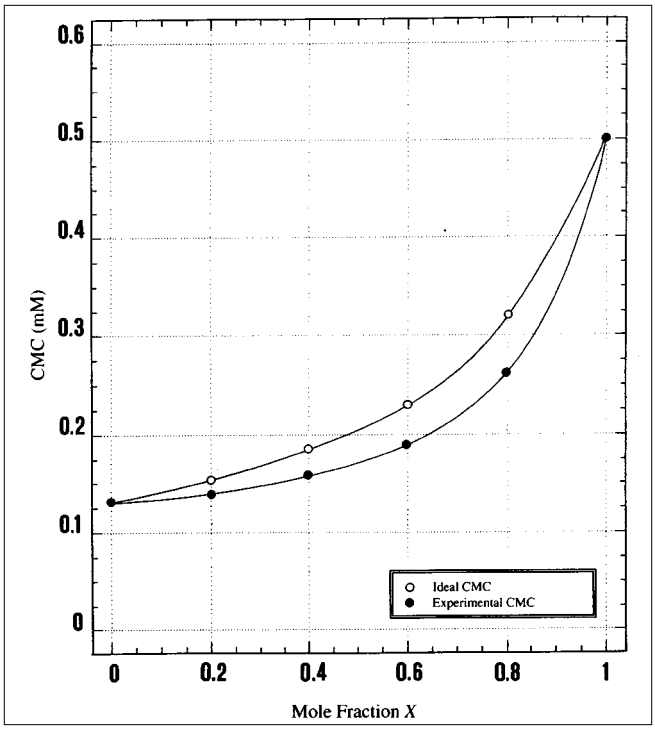

FIG. 2. Relationship between the experimental CMC value (mM) and the mole fraction of the anionic surfactant for the SDS/NP(EO)$_{30}$ mixed systems. (●) The CMC values theoretically calculated for each molar ratio (36) have been also indicated in the upper curve (○).

Figure 6 shows the variation in the *K* parameters at subsolubilizing and solubilizing levels vs. X_{SDS}. $K_{50\%CF}$ showed a maximum approximately at X_{SDS} = 0.6, whereas K_{SAT} and K_{SOL} showed maximum values at X_{SDS} 0.2. Thus, the lower the surfactant contribution in the surfactant–lipid system, the higher the X_{SDS} at which the maximum partition of surfactant molecules between the lipid and aqueous phase took place. As a consequence, the influence of SDS in this partition was more significant at sublytic level (monomeric effect), whereas the influence of NP(EO)$_{30}$ was greater during saturation and solubilization of liposomes *via* formation of mixed micelles. In general terms, the contribution of each component of the mixture in the interaction with PC liposomes appears to be similar to that reported for the interaction of mixtures of SDS/NP(EO)$_{30}$ in its molecular structure (23). However, the assembly properties of these two surfactant mixtures, when interacted with PC molecules (incorporation into bilayers up to bilayer saturation and liposome solubilization *via* mixed micelle formation), showed specific differences in each case (in terms of solubilizing activity and bilayer affinity), which will be discussed below.

The selective behavior obtained in the partitioning of SDS/NP(EO)$_{30}$ mixtures between lipid bilayers and the aqueous phase may be correlated with the findings reported by Scamehorn *et al.* (8), who argued that the reduction of electrostatic repulsion between the ionic surfactant head groups in the mixed micelle, due to the insertion of nonionic hy-

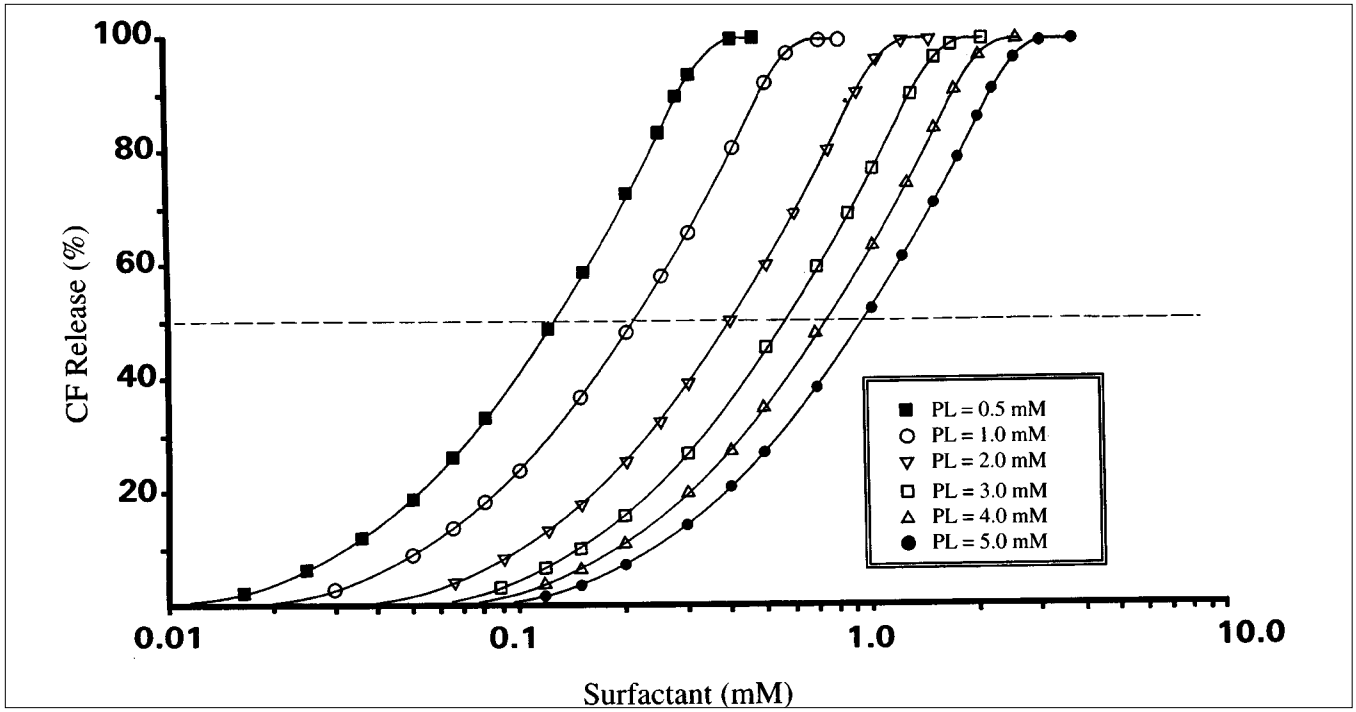

FIG. 3. Percentage changes in CF release of unilamellar liposomes, (lipid bilayer concentration ranging from 0.5 to 5.0 mM), induced by the presence of increasing concentrations of SDS/NP(EO)$_{30}$ mixed surfactant system for the mole fraction of the anionic surfactant of 0.4. [PL]= 0.5 mM (■), [PL]= 1.0 mM (○), [PL]= 2.0 mM (▽), [PL]= 3.0 mM (□), [PL]= 4.0 mM (Δ), [PL]= 5.0 mM (●).

drophilic groups (charge separation), appears to be the source of the nonideality and the cause of enhanced micelle formation. The maximum values of K_{SAT} and K_{SOL} at a low SDS mole fraction (X_{SDS} = 0.2), together with the reduced Re_{SAT} and Re_{SOL} values in this range of mole fractions, emphasize the low influence of the electrostatic forces in the saturation and complete solubilization of liposomes *via* lipid–surfactant mixed micelle formation.

The fact that $K_{50\%CF}$ exhibits higher values than K_{SAT} regardless of X_{SDS} suggests, as previously described by Shubert *et al.* (37) for sodium cholate (37), that, for each surfactant mixture, at low *Re* (subsolubilizing level) only the outer vesicle leaflet was available for the added mixed surfactant system until saturation, the binding of additional surfactant molecules to the bilayer being hampered. Then, the increase in *Re* resulted in an increased rate of surfactant flip-flop, thus making the inner monolayer available for interaction with added surfactant until the complete saturation of bilayers is achieved.

Comparison of these findings with those reported for the interaction of betaine-type zwitterionic surfactant (C_{12}-Betaine)/SDS mixtures (22,38) shows that these mixtures exhibited the lowest ability to produce 50% CF release, saturation, and solubilization of liposomes and the highest affinity with these structures at the X_{SDS} at which the maximum CMC negative deviation with respect to ideal behavior took place (X = 0.4). These findings sharply contrast with the present results in which the role played by SDS and NP(EO)$_{30}$ on the partition coefficients is more significant at the sublytic level (monomeric effect) and during saturation and solubilization of liposomes, respectively. Also, the mixtures investigated in the present work show a clearly increased ability to saturate or solubilize liposomes and a reduced affinity with these structures with respect to that reported for betaine/SDS mixtures, regardless of the mole fraction of the SDS component in the mixture (22).

Different mechanisms of mixed micelle formation have been reported for these surfactant mixtures to explain the negative deviation of their CMC values with respect to ideal behavior. Thus, whereas Iwasaki *et al.* (39) reported for C_{12}-Betaine/SDS mixtures that the association of these surfactants occurred easily by electrostatic attraction between the cationic portion of the betaine and the SDS ion in the mixed micelles, Scamehorn *et al.* (8) argued for SDS/NP(EO)$_{30}$ mixtures that the aforementioned charge separation was the cause of enhanced micelle formation. The fact that the aqueous surfactant concentration needed to saturate ($S_{W,SAT}$) or solubilize ($S_{W,SOL}$) liposomes was always comparable to the CMC of surfactant mixtures suggests that the mechanisms of mixed micelle formation reported for these mixtures could also be correlated with those governing the interaction of these mixtures, with the PC molecules building liposomes, specially during liposome solubilization *via* mixed micelle formation that included PC molecules. The specific amphiphilic characteristics of PC molecules, as well as the practically nonelectrostatic character of this phospholipid in the aqueous buffered medium used (pH 7.20), supports this hypothesis.

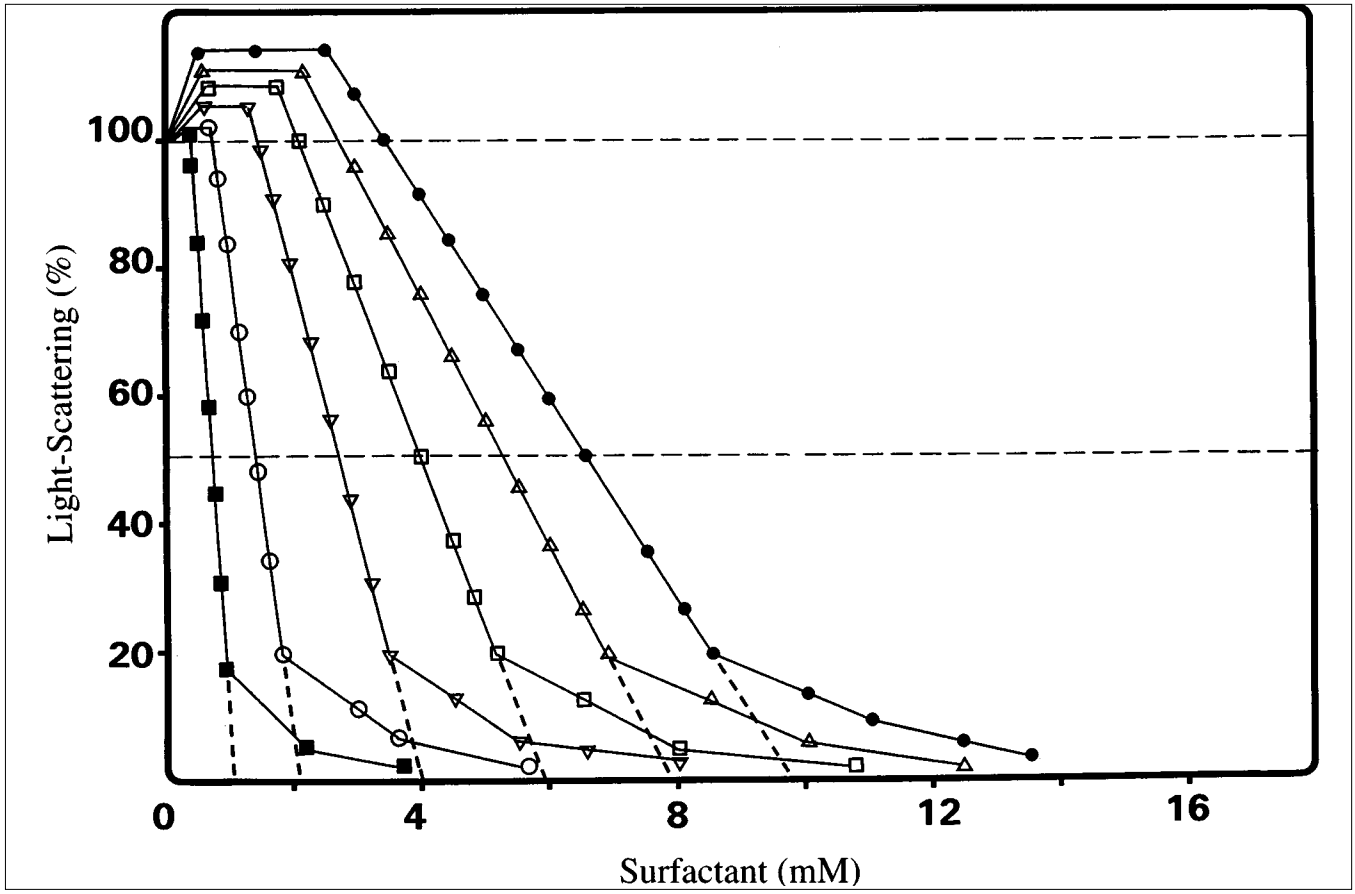

FIG. 4. Percentage change in static light-scattering of unilamellar liposomes, (lipid concentration ranging between 0.5 and 5.0 mM), induced by the presence of increasing concentrations of SDS/NP(EO)$_{30}$ mixed surfactant system for the mole fraction of the anionic surfactant of 0.4. [PL]= 0.5 mM (■), [PL]= 1.0 mM (○), [PL]= 2.0 mM (▽), [PL]= 3.0 mM (□), [PL]= 4.0 mM (△), [PL]= 5.0 mM (●).

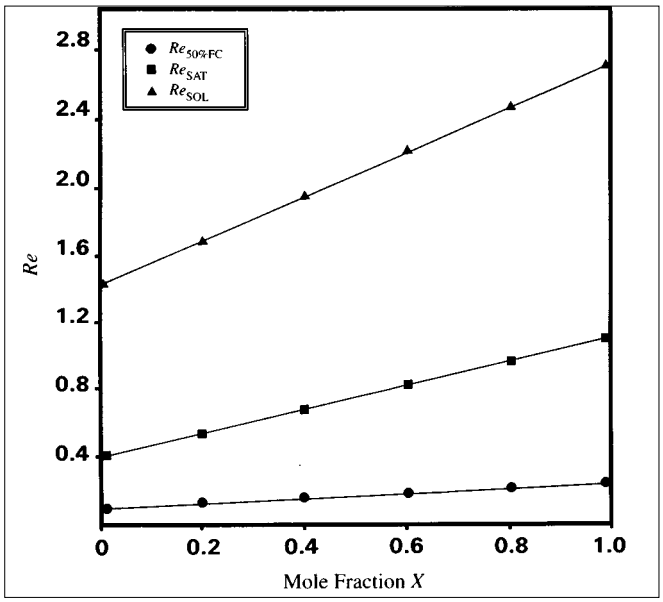

FIG. 5. Variation of $Re_{50\%CF}$ (●), Re_{SAT} (■), and Re_{SOL} (▲) vs. the mole fraction of the anionic surfactant X_{SDS} for the SDS/NP(EO)$_{30}$ mixed systems.

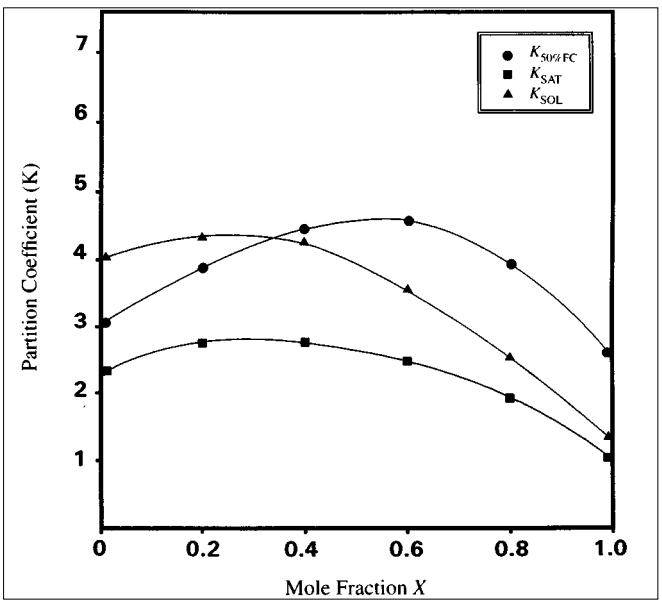

FIG. 6. Variation of $K_{50\%CF}$ (●), K_{SAT} (■), and K_{SOL} (▲) vs. the mole fraction of the anionic surfactant X_{SDS} for the SDS/NP(EO)$_{30}$ mixed systems.

Comparison of the present results with those reported for the interaction of NP(EO)$_{10}$/SDS mixtures with PC liposomes (23) shows that, although the relative contribution of each mixture component on K vs. X_{SDS} appears to be similar (at the interaction levels investigated), the number of EO units present in the structure of the nonionic component affects both the ability of these mixtures to saturate or solubilize liposomes and the level of affinity with these bilayer structures. Thus, although the NP(EO)$_{30}$/SDS mixtures exhibited always slighty higher Re_{SAT} and Re_{SOL} values (slighty lower ability to saturate or solubilize liposomes), their affinities with bilayers (both at sublytic and lytic levels) were drastically reduced (approximately 3–4 times), especially in the X_{SDS} interval ranging between 0.4 and 0.6. However, the ability of these two different mixtures to produce 50% CF release was similar in both cases, regardless of the mole fraction of the anionic component.

In addition to that, the evolution of Re vs. X_{SDS} at the interaction levels investigated shows clear differences. Thus, whereas the Re values for NP(EO)$_{30}$/SDS mixtures show a linear dependency with X_{SDS} (linear increase), those reported for NP(EO)$_{10}$/SDS mixtures at solubilizing level (Re_{SAT} and Re_{SOL}) did not show this linear behavior, especially at high X_{SDS}, where the increase of these parameters was more pronounced. This means that the synergism that exists between the surfactants in these two mixtures at different mole fractions studied clearly affects their ability to saturate and solubilize PC liposomes. The number of EO units on the nonionic component in the mixture, and consequently its hydrophilic–lipophilic balance (HLB), could affect both the incorporation of these mixtures into bilayers up to saturation of these structures and the subsequent process of mixed-micelle formation with the PC molecules from the liposome structure. The insertion of nonionic hydrophilic groups with different EO units between the ionic surfactant head groups in the mixed micelle seems to play an important role in these processes.

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