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Permeability changes in liposomes modeling the stratum corneum lipid composition due to C_{12}-alkyl betaine/sodium dodecyl sulfate mixtures

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Abstract

The interaction of dodecyl betaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) mixtures at different mole fractions of the zwitterionic component ($X_{zwitter}$) with liposomes modeling the stratum corneum (SC) lipid composition were investigated. Liposomes were formed by a lipid mixture of 40% ceramides, 25% cholesterol, 25% palmitic acid and 10% cholesteryl sulfate. The surfactant/lipid molar ratios (R_e) and the bilayer/aqueous phase partition coefficients (K) were determined at two sublytic levels (50 and 100% CF release) by monitoring the increase in the fluorescence intensity of liposomes due to the 5(6)-carboxyfluorescein (CF) released from the interior of vesicles. The fact that the free surfactant concentrations were always lower than their critical micelle concentrations suggests that the liposomes–surfactant sublytic interactions were mainly ruled by the action of surfactant monomers. At the two interaction levels studied the surfactant mixture for $X_{zwitter} = 0.6$ showed the highest ability to alter the release of the CF entrapped in the interior of vesicles (lowest R_e values), whereas that for $X_{zwitter} = 0.4$ showed the highest degree of partitioning into liposomes or affinity with these structures (highest K values). Different trends in the interaction of these mixtures with SC lipids and phosphatidylcholine (PC) liposomes were observed when comparing the present

Abbreviations: SC, stratum corneum; PC, phosphatidylcholine; Cer, ceramides type III; Chol, cholesterol; PA, palmitic acid; Chol-sulf, cholesteryl sulfate; PIPES, piperazine-1,4 bis(2-ethanesulphonic acid); CF, 5(6)-carboxyfluorescein; SDS, sodium dodecyl sulfate; C_{12}-Bet, *N*-dodecyl-*N*,*N*-dimethylbetaine (dodecyl betaine); $X_{zwitter}$, mole fraction of the zwitterionic component (dodecyl betaine); R_e, effective surfactant/lipid molar ratio; $R_{e,50\%CF}$, effective surfactant/lipid molar ratio for 50% CF release; $R_{e,100\%CF}$, effective surfactant/lipid molar ratio for 100% CF release; K, bilayer/aqueous phase surfactant partition coefficient; $K_{50\%CF}$, bilayer/aqueous phase surfactant partition coefficient for 50% CF release; $K_{100\%CF}$, bilayer/aqueous phase surfactant partition coefficient for 100% CF release; S_W, surfactant concentration in the aqueous medium; $S_{W,50\%CF}$, surfactant concentration in the aqueous medium for 50% CF release; $S_{W,100\%CF}$, surfactant concentration in the aqueous medium for 100% CF release; S_B, surfactant concentration in the bilayers; PI, polydispersity index; CMC, critical micellar concentration; r^2, regression coefficient.

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R_e and K parameters with those reported for PC ones. Thus, whereas SC lipid liposomes were more resistant to the action of C_{12}-Bet/SDS mixtures, the partitioning of these mixtures into SC lipid bilayers was always greater than that reported for PC ones.

Keywords: Dodecyl betaine/sodium dodecyl sulfate mixtures; Stratum corneum lipid liposomes; Stratum corneum lipid liposomes/surfactant interactions; Carboxyfluorescein release; Surfactant/stratum corneum lipids molar ratios; Surfactant partition coefficients

1. Introduction

Zwitterionic surfactants have strong interaction with anionic surfactants in water (Iwasaki et al., 1991). The effect of the micellar solution phase of these mixtures in avoiding or at least reducing the level of anionic/protein interaction has been suggested by several workers as a way of slowing down the irritation potential of the anionic surfactants (García Domínguez et al., 1981; Cooper and Berner, 1985). Thus, a reduction in the skin irritation by anionics has been reported in the presence of amphoteric surfactants (Rhein and Simion, 1991).

Liposomes are aqueous lipid dispersions organized as bilayers which are widely used as simplified membrane models (Lasic, 1993; Sternberg, 1995). A number of studies have been devoted to the understanding of the principles governing the interaction of surfactants with these structures (Kragh-Hansen et al., 1993; Ruiz et al., 1994; Polozava et al., 1995; Inoue, 1996; Partearroyo et al., 1996; Silvander et al., 1996). This interaction leads to the breakdown of lamellar structures and the formation of lipid–surfactant mixed micelles. A significant contribution in this area has been made by Lichtenberg et al. (1985), who postulated that the effective surfactant/lipid molar ratio (R_e) producing solubilization of liposomes depends on the surfactant critical micelle concentration (CMC) and on the bilayer/aqueous medium distribution coefficients (K).

In order to find out whether the lipids building the intercellular matrix of the stratum corneum (SC) could form bilayers, Wertz and Downing (Wertz et al., 1986; Wertz, 1992) prepared liposomes from lipid mixtures approximating the SC composition and studied the interaction of sodium dodecyl sulfate (SDS) with these liposomes to study its deleterious effect on human skin (Downing et al., 1993). Stratum corneum lipid liposomes have been also used as membrane models to study the adsorption of enhancer agents and to compare these results with those obtained in skin studies (Miyajima et al., 1994; Yoneto et al., 1995, 1996; Suhonen et al., 1997).

We previously studied the percutaneous penetration of liposomes using the tape-stripping technique (Coderch et al., 1996). We also investigated the formation of liposomes using a mixture of four commercially available synthetic lipids modeling the composition of stratum corneum lipids (de la Maza et al., 1995) and the sublytic interactions of dodecyl betaine (C_{12}-Bet) and SDS with these liposomes (de la Maza and Parra, 1996; de la Maza et al., 1997).

In the present work we seek to extend these investigations by characterizing the R_e and K parameters of C_{12}-Bet/SDS mixtures when interacted with these SC lipid liposomes. This information may be useful to evaluate the activity changes of these surfactant mixtures with respect to that of the anionic component on a membrane model mimicking the stratum corneum lipid composition.

2. Materials and methods

N-Dodecyl-*N*,*N*-dimethylbetaine (C_{12}-Bet) was prepared by Albright and Wilson, Ltd. (Warley, West Midlands, UK), the active matter was 30% in water and the amino-free content was 0.20%. Sodium dodecyl sulfate (SDS) was obtained from Merck and further purified by a column chromatography (Rosen, 1981). The starting material

5(6)- carboxyfluorescein (CF), was obtained from Eastman Kodak (Rochester, NY) and further purified by column chromatography (Weinstein et al., 1986). Piperazine-1,4-bis(2-ethanesulphonic acid) (PIPES) was obtained from Merck (Darmstadt, Germany). PIPES buffer was prepared as 20 mM PIPES containing 110 mM Na_2SO_4, supplemented with 110 mM CF and adjusted to pH 7.20 with NaOH. Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, CA). Reagent-grade organic solvents, ceramides type III (Cer), cholesterol (Chol) and palmitic acid (PA) were supplied by Sigma Chemical Co. (St. Louis, MO). Cholesteryl sulfate (Chol-sulf) was prepared by reaction of cholesterol with excess chlorosulphonic acid in pyridine and purified chromatographically.

The molecular weight of ceremide type III used in the lipid mixture was determined by low-resolution fast-atom bombardment mass spectrometry using a Fisons VG Auto Spec Q (Manchester, UK) with a caesium gun operating at 20 kV. A molecular weight of 671 g was obtained for the majority compound of the ceremides used (Sigma). This value was used to calculate the molarity of the lipid mixture investigated. The lipids of the highest purity grade available were stored in chloroform/methanol (2:1) under nitrogen at -20°C until use.

2.1. Liposome preparation and characterization

We previously reported the formation of liposomes using a mixture of lipids modeling the composition of the SC (40% Cer, 25% Chol, 25% PA and 10% Chol-sulf) (de la Maza et al., 1995), which was prepared following the method described by Wertz et al. (1986). Individual lipids were dissolved in chloroform/methanol (2:1) and appropriate volumes were combined. The solvent was removed with a stream of nitrogen and then under high vacuum at room temperature. The lipid mixture was suspended in 20 mM PIPES/NaOH buffer, pH 7.20, containing 110 mM Na_2SO_4 and supplemented with 110 mM CF. The lipids were left to hydrate for 30 min under nitrogen with occasional shaking. The suspension was then sonicated in a bath sonicator (514 ECT Selecta) at 60°C for about 15 min until the suspension became clear. Vesicles of about 200 nm size were obtained by extrusion through 800–200-nm polycarbonate membranes at 60°C using a thermobarrel extruder equipped with a thermoregulated cell compartment (Lipex, Biomembranes Inc., Vancouver, Canada). The preparations were then kept at the same temperature for 30 min and incubated at 25°C under nitrogen atmosphere. CF-containing vesicles were freed of unencapsulated dye by passage through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography to study the permeability changes due to the action of surfactants (CF concentration into liposomes 110 mM). The final volumes of liposomes were adjusted with PIPES buffer to provide a final lipid concentration ranging between 0.5 and 5.0 mM.

The lipid composition of SC lipid liposomes after preparation was determined using thin-layer chromatography coupled to an automated flame ionization detection system (Iatroscan MK-5, Iatron Lab. Inc., Tokyo, Japan) (Ackman et al., 1990; de la Maza et al., 1995).

In order to find out whether all the components of the lipid mixture formed liposomes, vesicular dispersions were analyzed for these lipids (Ackman et al., 1990). The dispersions were then spun at $140000 \times g$ at 25°C for 4 h to remove the vesicles (Almog et al., 1990). The supernatants were tested again for these components. No lipids were detected in any of the supernatants.

The phase transition temperature (PTT) of the SC lipid mixture forming liposomes was determined by proton magnetic resonance (^{1}H NMR), showing a value of 55–56°C (de la Maza et al., 1995).

The vesicle size distribution and the polydispersity index (PI) of liposomes after preparation was determined with dynamic light-scattering measurements using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV). Samples were adjusted to the appropriate concentration range with PIPES buffer. Measurements were taken at 25°C at a scattering angle of 90°.

2.2. Parameters involved in the interaction of surfactant mixtures with SC lipid liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger et al. (1985) for bile salt/lecithin systems, Lichtenberg et al. (1985) and Almog et al. (1990) have shown that for a mixing of lipids (at a lipid concentration L (mM)) and surfactant (at a concentration S_T (mM)), in dilute aqueous media, the distribution of surfactant between lipid bilayers and aqueous media obeys a partition coefficient K, given (in mM^{-1}) by

$$K = S_B/[(L + S_B) \cdot S_W] \quad (1)$$

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 $L \gg S_B$, the definition of K, as given by Schurtenberger, applies:

$$K = S_B/(L \cdot S_W) = R_e/S_W \quad (2)$$

where R_e is the effective molar ratio of surfactant to lipid in the bilayers ($R_e = S_B/L$). Under any other conditions, Eq. (2) has to be employed to define K; this yields:

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

This approach is consistent with the experimental data offered by these authors (Lichtenberg et al., 1985; Almog et al., 1990) for different surfactant lipid mixtures over wide ranges of R_e values. Given that the range of lipid concentrations used is similar to that used by Almog to test his equilibrium partition model, the K parameter has been determined using this equation.

The determination of these parameters can be carried out on the basis of the linear dependence existing between the surfactant concentrations required to achieve 50 and 100% CF release and the SC lipid concentration (L), which can be described by the equations:

$$S_{T,50\%CF} = S_{W,50\%CF} + R_{e,50\%CF} \cdot [L] \quad (4)$$

$$S_{T,100\%CF} = S_{W,100\%CF} + R_{e,100\%CF} \cdot [L] \quad (5)$$

where $S_{T,50\%CF}$ and $S_{T,100\%CF}$ are the total surfactant concentrations. The surfactant/lipid molar ratios $R_{e,50\%CF}$ and $R_{e,100\%CF}$ and the aqueous concentration of surfactant $S_{W,50\%CF}$ and $S_{W,100\%CF}$ are in each curve, respectively, the slope and the ordinate at the origin (zero lipid concentration).

2.3. Monitoring the release of CF from liposomes

Changes in the release of the CF trapped into SC lipid vesicles due to the action of the surfactant mixtures investigated were determined quantitatively by monitoring the increase in the fluorescence intensity of the liposomes due to the CF liberated (Weinstein et al., 1986). Fluorescence measurements were made with a spectrofluorophotometer Shimadzu RF-540 (Kyoto Japan). On excitation at 495 nm, a fluorescence maximum emission of CF was obtained at 515.4 nm. The presence of 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 mixture solutions (2.0 ml) were added to these liposomes and the resulting systems were left to equilibrate for 60 min. This interval was chosen as the minimum period of time needed to achieve a constant level of CF release for the lipid concentration range investigated. The experimental determination of this interval is indicated in the Section 3. The fluorescence intensity measurements were taken at 25°C. The percentage of CF released was calculated by means of the equation:

$$\%CF\ release = (I_T - I_o)/(I_\infty - I_o) \cdot 100 \quad (6)$$

where I_o is the initial fluorescence intensity of CF-loaded liposomes in the absence of surfactant, I_T is the fluorescence measured 60 min after adding the surfactant solution to liposomes. I_∞ corresponds to the fluorescence intensity remaining after the complete destruction of liposomes by the addition of Triton X-100 (de la Maza et al., 1995). The assays were carried out in triplicate and the results given are the average of those obtained.

Table 1
Surfactant to lipid molar ratios (R_e), partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction (50 and 100% CF release) of C_{12}-Bet/SDS mixtures versus the mole fraction of the zwitterionic surfactant ($X_{zwitter}$)

$X_{zwitter}$	CMC (mM)	$S_{W,50\%CF}$ (mM)	$S_{W,100\%FC}$ (mM)	$R_{e,50\%CF}$ (mol/mol)	$R_{e,100\%CF}$ (mol/mol)	$K_{50\%CF}$ (mM^{-} 1)	$K_{100\%CF}$ (mM^{-1})	r^2 (50%CF)	r^2 (100%CF)
0	0.500	0.083	0.289	0.350	1.0	3.12	1.73	0.994	0.996
0.2	0.220	0.033	0.121	0.221	0.548	5.49	2.92	0.994	0.995
0.4	0.160	0.020	0.084	0.162	0.348	6.98	3.07	0.997	0.993
0.6	0.210	0.021	0.092	0.154	0.281	6.36	2.38	0.999	0.995
0.8	0.410	0.041	0.180	0.184	0.294	3.79	1.26	0.991	0.998
1.0	1.250	0.418	0.838	0.653	0.756	0.945	0.51	0.997	0.995

The critical micelle concentration of each surfactant mixture tested are also included together with the regression coefficients of the straight lines obtained.

3. Results and discussion

We previously reported the critical micelle concentration (CMC) of the C_{12}-Bet/SDS mixtures (de la Maza and Parra, 1993). These values for each $X_{zwitter}$ are given in Table 1. The characterization of the geometric properties of the liposomes modeling the stratum corneum lipid composition used in the present study was previously reported (de la Maza et al., 1995). This characterization based on the determination of the mean particle size and the internal volume of these vesicles demonstrated that the SC lipid liposomes were formed by unilamellar vesicles. As for the physico-chemical stability of these vesicles, the vesicle size distribution after preparation varied very little showing in all cases a similar value of about 200 nm. The polydispersity index (PI) remained always 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 60 min always showed values similar to those obtained after preparation with a slight PI increase (between 0.10 and 0.12). Hence, liposomes were reasonably stable in the absence of surfactants under the experimental conditions used.

3.1. Interaction of C_{12}-Bet/SDS mixtures with SC lipid liposomes

We first studied the validity of the equilibrium partition model proposed by Lichtenberg and Almog (Lichtenberg et al., 1985; Almog et al., 1990) based on Eq. (1) for the surfactant mixtures investigated. According to these authors this equation may be expressed by: $L/S_B = (1/K)(1/S_W) - 1$. Hence, this validity requires a linear dependence between L/S_B and $1/S_W$; this line should have a slope of $1/K$, intersect with the L/S_B axis at -1 and intersect with the $1/S_W$ axis at K (Lichtenberg et al., 1985). To test the validity of this model, SC lipid liposomes were mixed with varying subsolubilizing concentrations of the surfactant mixtures studied (S_T). The resultant surfactant-containing vesicles were then spun at $140000 \times g$ at 25°C for 4 h to remove the vesicles. No lipids were detected in the supernatants. The concentration of each surfactant component in the supernatants (S_W) was determined by HPLC (Kondoh and Takano, 1986; Pietrzyk et al., 1989) and their concentration in the lipid bilayers was calculated ($S_B = S_T - S_W$). The results of the experiments in which S_B and S_W were measured (at the same range of SC lipids and surfactant concentrations used to determine K) were plotted in terms of the dependence of L/S_B on $1/S_W$. Straight lines were obtained for each surfactant mixture tested ($r^2 = 0.089$, 0.990, 0.988, 0.991, 0.989, and 0.991 for $X_{zwitter} = 1.0$, 0.8, 0.6, 0.4, 0.2 and 0, respectively, which were dependent on L and intersected with the L/S_B axis always at -0.97 ± 0.12. Both the linearity of these dependences and the proximity of the intercept to -1 support the validity of this model to determine K for these surfactants at the two interaction levels investigated.

It is known that, in surfactant/lipid systems, complete equilibrium may take several hours (Lichtenberg et al., 1985; Urbaneja et al., 1990). However, in subsolubilizing interactions a substantial part of the surfactant effect takes place within approx. 30 min after its addition to the liposomes (Ruiz et al., 1988). To determine the time needed to obtain a constant level of CF release of liposomes, a kinetic study of the interaction of C_{12}-Bet/SDS mixtures with SC lipid liposomes was carried out at different $X_{zwitter}$. Liposomes at a lipid concentration of 0.5 mM were treated with 0.2 mM surfactant mixtures and those at a lipid concentration of 5.0 mM were treated with these surfactant mixtures at 1.0 mM concentration. The changes in CF release for each $X_{zwitter}$ were studied as a function of time. The results obtained are given in Fig. 1A and Fig. 1B, respectively. About 60 min was needed to achieve a maximum level of CF release in all cases. Hence, CF release changes were studied 60 min after addition of surfactants to liposomes at 25°C. This finding contrasts with that reported for the interaction of these mixtures with PC liposomes (de la Maza and Parra, 1993), where the time needed to obtain a maximum level of CF release was always lower (about 40 min).

The fact that CF release curves versus time exhibited plateaux for different $X_{zwitter}$ (in accor-

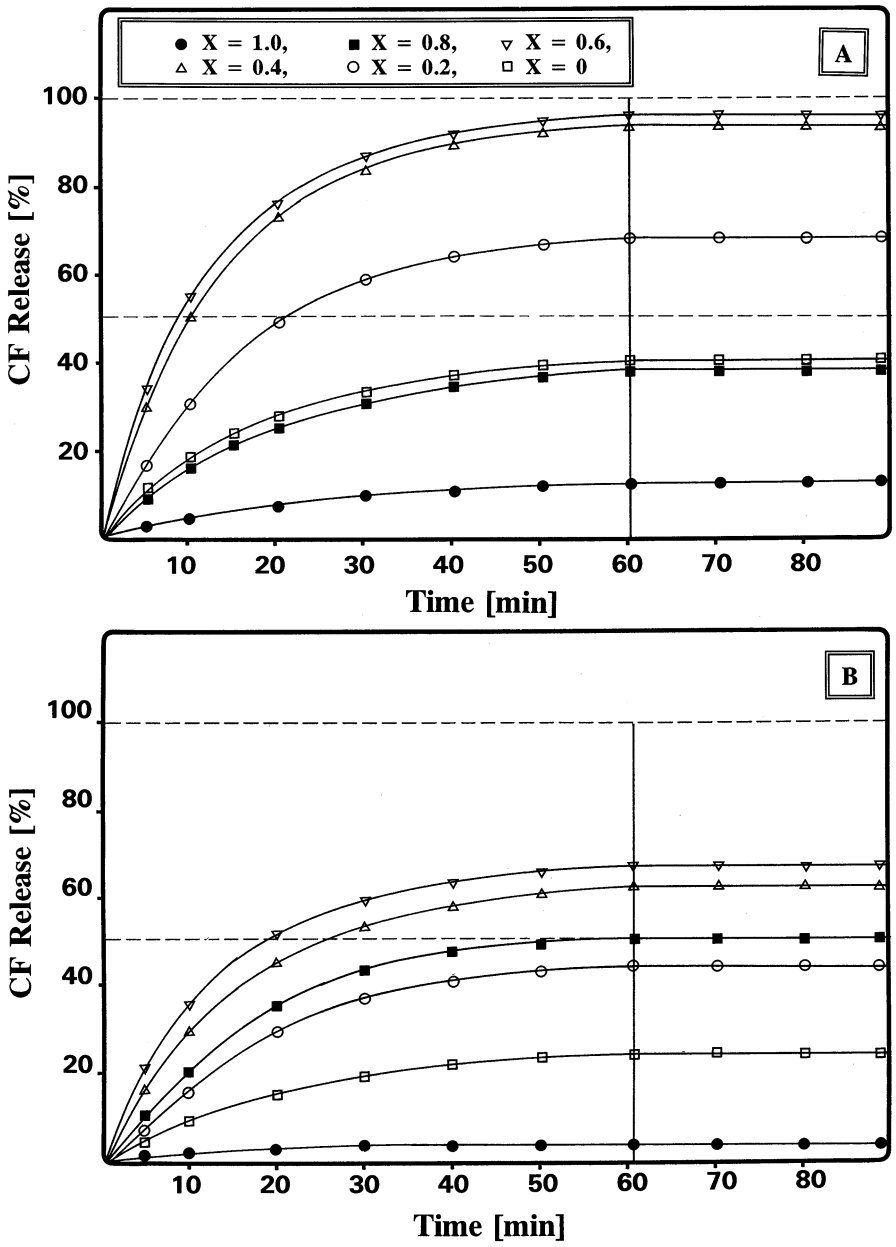

Fig. 1. (A) Time curves of the release of CF trapped into SC liposomes (lipid concentration, 0.5 mM) caused by the addition of a constant concentration of C_{12}-Bet/SDS surfactant mixtures (0.2 mM) at different mole fractions of the zwitterionic surfactant ($X_{zwitter}$). $X = 1.0$ (●), $X = 0.8$ (■), $X = 0.6$ (▽), $X = 0.4$ (△), $X = 0.2$ (○), $X = 0$ (□). (B) Time curves of the release of CF trapped into SC liposomes (lipid concentration, 5.0 mM) caused by the addition of a constant concentration of C_{12}-Bet/SDS surfactant mixtures (1.0 mM) at different mole fractions of the zwitterionic surfactant ($X_{zwitter}$). $X = 1.0$ (●), $X = 0.8$ (■), $X = 0.6$ (▽), $X = 0.4$ (△), $X = 0.2$ (○), $X = 0$ (□).

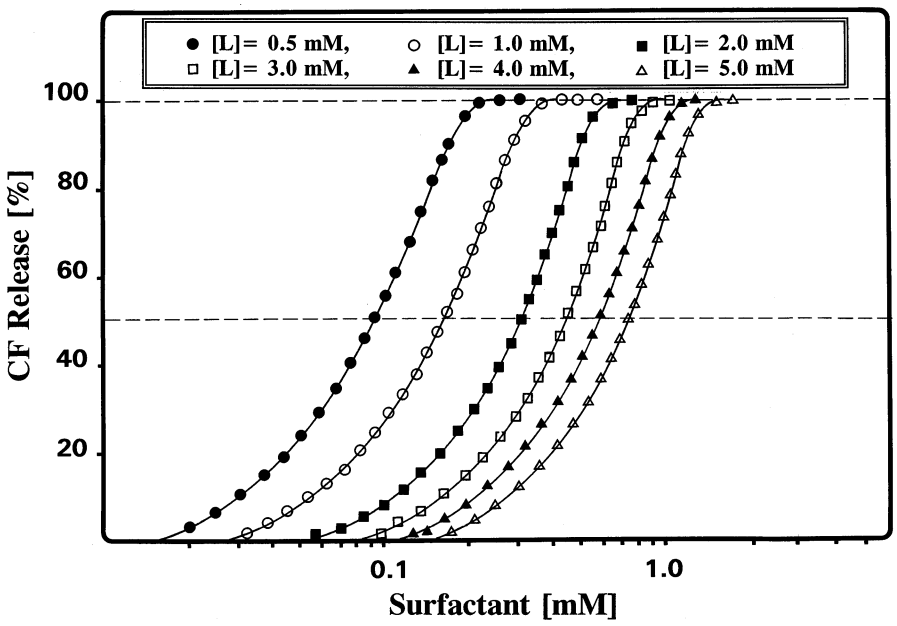

Fig. 2. Percentage changes in CF release of SC liposomes (lipid concentration ranging from 0.5 to 5.0 mM), induced by the presence of increasing concentrations of C_{12}-Bet/SDS surfactant mixtures at the mole fraction of the zwitterionic surfactant of 0.6. Lipid concentrations: 0.5 mM (●), 1.0 mM (○), 2.0 mM (■), 3.0 mM (□), 4.0 mM (▲), 5.0 mM (△).

dance with the results reported for PC liposomes) may be attributable to the continuous release of CF through holes or channels created in the membrane. The incorporation of surfactant monomers to membranes may directly induce the formation of hydrophilic pores in these structures or merely stabilize transient holes, in agreement with the concept of transient channels suggested by Edwards and Almgren (1990, 1992). The differences in the surfactant-induced CF release kinetics in both PC and SC lipid liposomes may be related to the different PTT of lipids building these two bilayer structures, which affects the positional organization of lipids and their polar heads as well as their mobility. The more hydrophilic nature of PC may also facilitate the formation of hydrophilic pores due to the action of surfactants on the PC polar heads and the subsequent permeation of CF through these created holes (Lasic, 1993). The spontaneous release of the CF encapsulated into SC lipid liposomes in the absence of surfactant in this period of time was negligible.

To determine the R_e and S_W parameters, a systematic investigation of CF release changes in SC lipid liposomes was carried out for various lipid concentrations (from 0.5 to 5.0 mM). Liposome aliquots were mixed with equal volumes of surfactant solutions at different $X_{zwitter}$ and the final surfactant concentration (mM) was calculated for each mixture. Changes in the CF release were determined 60 min after surfactant addition at 25°C. The assays were also carried out in triplicate and the results given are the average of those obtained. The curves obtained for $X_{zwitter} = 0.6$ are given in Fig. 2. The C_{12}-Bet/SDS concentrations resulting in 50 and 100% of CF release for each $X_{zwitter}$ tested were plotted versus the lipid concentration of liposomes. An acceptable linear relationship was established in each case. These results are plotted in Fig. 3A (50% CF release) and Fig. 3B (100% CF release), respectively. The error bars are S.D. and represent the error of three experiments. The straight lines obtained corresponded to Eq. (4) and Eq. (5) from which R_e and S_W were determined. These values including the regression coefficients (r^2) of the straight lines obtained are given in Table 1.

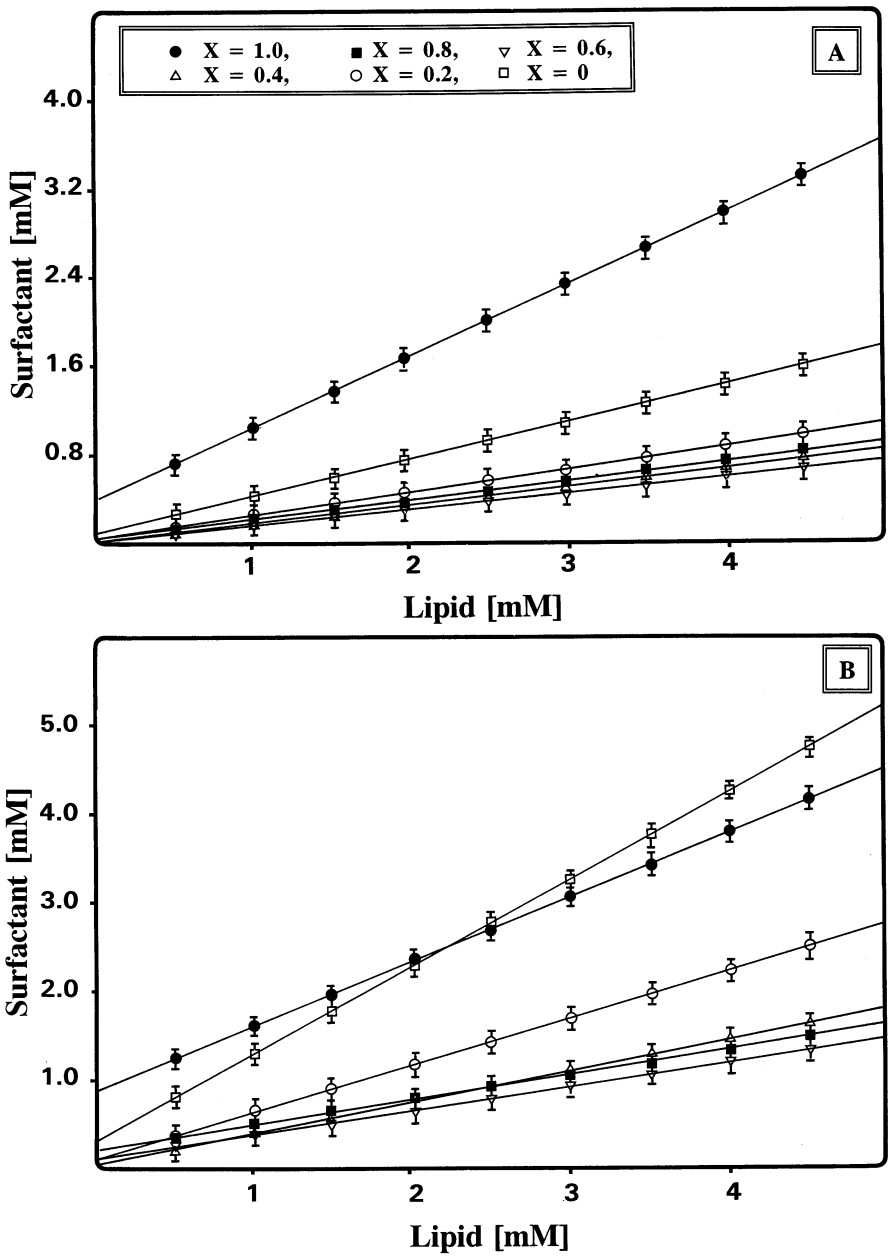

Fig. 3. (A) Surfactant concentrations for different mole fractions of the zwitterionic surfactant ($X_{zwitter}$) resulting in 50% CF release versus lipid concentration of liposomes. $X = 1.0$ (●), $X = 0.8$ (■), $X = 0.6$ (▽), $X = 0.4$ (△), $X = 0.2$ (○), $X = 0$ (□). (B) Surfactant concentrations for different mole fractions of the zwitterionic surfactant ($X_{zwitter}$) resulting in 100% CF release versus lipid concentration of liposomes. $X = 1.0$ (●), $X = 0.8$ (■), $X = 0.6$ (▽), $X = 0.4$ (△), $X = 0.2$ (○), $X = 0$ (□).

The S_W values increased as the CF release percentage rose, although showing always smaller values than those reported for the CMCs of the surfactant mixtures (de la Maza and Parra, 1993) (see Table 1). This finding suggests that surfactant–liposome interaction was mainly ruled by

the action of surfactant monomers, in agreement with those reported for sublytic interactions of these surfactant mixtures with PC liposomes (de la Maza and Parra, 1993). Furthermore, S_W showed the lowest values at the same mole fraction ($X_{zwitter} = 0.4$), at which a minimum in the CMC of these mixtures took place. Thus, the lower the surfactant mixture CMC the lower the S_W concentration at which 50 and 100% CF release of liposomes occurred.

The R_e parameter showed the lowest values for $X_{zwitter} = 0.6$. Given that the surfactant capacity to interact with liposomes is inversely related to the R_e parameter, the maximum activity both at 50 and 100% CF release corresponded to the $X_{zwitter} = 0.6$.

Comparison of the R_e values with those reported for the interaction of these surfactant mixtures with PC liposomes (de la Maza and Parra, 1993) shows that the ability of these mixtures to alter the permeability of SC lipid bilayers (at 50% CF release) was lower (higher R_e values) than that reported for PC bilayers in all cases, although showing similar tendencies with respect to the influence of the $X_{zwitter}$. Thus, SC lipid liposomes showed more resistance to the surfactant perturbations than PC ones. This different behaviour may be explained bearing in mind the more hydrophilic nature of PC, which could facilitate the permeation of surfactant molecules in PC liposomes, either through the hydrophilic holes created or via formation of short-lived complexes of surfactants–PC polar heads (Lasic, 1993).

The K parameters (Table 1) show that the surfactant molecules for $X_{zwitter} = 0.4$ had the highest degree of partitioning into liposomes or maximum affinity with these bilayer structures (maximum K values). The fact that these surfactant mixtures showed, at 100% CF release, lower K values than those for 50% may be explained assuming that at low R_e possibly only the outer vesicle leaflet was available for interaction with surfactant molecules, the binding of additional molecules to bilayers being hampered at slightly higher R_e values. These findings are in agreement with those reported by Schubert et al. (1986) for sodium cholate/PC liposomes and with our investigations involving the interaction of different nonionic surfactants with PC liposomes (de la Maza and Parra, 1994, 1997).

Comparison of the present K values with those reported for the interaction of the same surfactant mixtures with PC liposomes (de la Maza and Parra, 1993) shows that the degree of partitioning

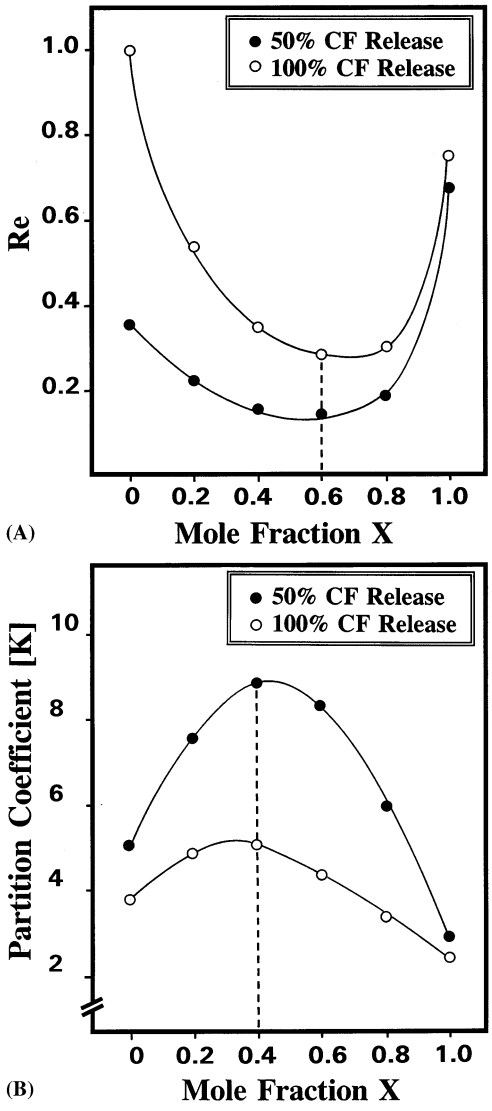

Fig. 4. (A) Surfactant to lipid molar ratios (R_e) for C_{12}-Bet/SDS mixtures at 50 and 100% CF release versus the mole fraction of the zwitterionic surfactant ($X_{zwitter}$): (●) 50% CF release and (○) 100% CF release. (B) Partition coefficients (K) for C_{12}-Bet/SDS mixtures at 50 and 100% CF release versus the mole fraction of the zwitterionic surfactant ($X_{zwitter}$): (●) 50% CF release and (○) 100% CF release.

of these mixtures into SC lipid bilayers was always greater (higher K values) than that for PC ones. The increased degree of partitioning of these mixtures into SC lipid bilayers may be explained bearing in mind the different chain length of lipid building these structures, degree of saturation and nature of polar heads.

If the R_e and K values obtained are plotted as a function of the $X_{zwitter}$, the graphs shown in Fig. 4A,B are obtained. The curves showed that a R_e minimum and a K maximun were obtained when $X_{zwitter}$ was 0.6 and 0.4, respectively. Thus, a positive association may also be established in the $X_{zwitter}$ range 0.4–0.6 between the affinity of these surfactant mixtures with SC lipid liposomes and their ability to alter the permeability of these bilayer structures. This $X_{zwitter}$ range corresponded to the higher negative derivation of the CMC values of these mixtures with respect to the ideal behaviour (negative surfactant synergism, see Table 1).

In general terms, different trends in the interaction of these surfactant mixtures with SC lipids and PC liposomes may be observed when comparing the corresponding R_e and K parameters (de la Maza and Parra, 1993). Thus, whereas SC lipid liposomes were more resistant to the action of surfactant monomers the partitioning of these mixtures into SC lipid structures was always greater than that for PC ones. Thus, although a greater number of surfactant molecules was needed to produce alterations in SC lipid bilayers, these molecules showed increased affinity with these structures. However, a similar influence of the $X_{zwitter}$ in the R_e and K parameters could be observed for both bilayered structures, in spite of their different lipid compositions and physicochemical characteristics.

We are aware of the fact that the lipids used in this work are not exactly the same as those existing in the intercellular spaces of stratum corneum and that this tissue shows a complex structure, in which proteins building corneocytes and corneocyte envelopes play an important role in skin barrier function. Nevertheless, the simplified SC lipid membrane model used has shown to be useful in comparing the activity of these surfactant mixtures in two simplified membrane models of different lipid composition (skin membrane lipids and phospholipid membrane) in order to establish a criterion for the evaluation of the activity of these surfactant mixtures.

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