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Influence of ceramides in the solubilization of stratum corneum lipid liposomes by C_{12}-betaine/sodium dodecyl sulfate mixtures

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Abstract

The solubilization of liposomes modeling the stratum corneum (SC) lipid composition and those obtained varying the proportion of ceramides by means of dodecyl betaine (C_{12}-Bet)/sodium dodecyl sulfate (SDS) mixtures was studied. The surfactant/lipid molar ratios (Re) and the bilayer/aqueous phase partition coefficients (K) were determined by monitoring the changes in the static light scattering of the system during solubilization. The fact that the free surfactant concentration was always similar to its critical micelle concentration (CMC) indicates that the liposome solubilization was mainly ruled by the formation of mixed micelles. The mole fraction of the zwitterionic component ($X_{zwitter}$) of 0.4 showed the lowest ability to saturate or solubilize liposomes, although exhibiting the highest degree of partitioning into liposomes. This $X_{zwitter}$ corresponded to the highest derivation of the CMCs of these mixtures (negative synergism) and to the highest reduction in the skin irritation with respect to the anionic component. Higher and lower proportion of ceramides in the mixture led to a fall and to a rise in both the activity and the partitioning of a specific surfactant mixture ($X_{zwitter} = 0.4$). This finding could be related to the recently reported dependences of the level of ceramides in skin and function barrier abnormalities. Comparison of the present Re and K values with those reported for phosphatidylcholine (PC) liposomes shows that, although SC liposomes were more resistant to the action of surfactant mixtures, the surfactant partitioning into SC bilayers was similar to that reported for PC ones in all cases. © 1999 Elsevier Science B.V. All rights reserved.

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

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1. Introduction

One of the key functions of stratum corneum (SC) lipids is to maintain the permeability barrier of the skin (Elias, 1981; Grubauer et al., 1989). It has been established that the perturbations in the organized structure of these lipids affect the skin barrier function (Harada et al., 1992; Lavrijsen et al., 1995). Changes in the lipid composition are associated with different skin symptoms. Thus, there is a marked decrease in ceramide level in patients with atopic dermatitis, suggesting that an insufficiency of this lipid is an etiological factor in atopic dry and barrier-disrupted skin (Holleran et al., 1991; Murata et al., 1996; Ponec et al., 1997). However, a physico-chemical confirmation of this barrier abnormality using a simplified membrane model such as liposomes is still lacking.

The interaction of surfactants with liposomes has been extensively studied (Polozava et al., 1995; Inoue, 1996; Partearroyo et al., 1996; Silvander et al., 1996). This process leads to the breakdown of lamellar structures and to the formation of lipid–surfactant mixed micelles. A significant contribution has been made by Lichtenberg et al. (1985), who postulated that the effective surfactant/lipid molar ratio (Re) producing solubilization of liposomes depends on the surfactant critical micelle concentration (CMC) and on the bilayer/aqueous medium distribution coefficients.

In previous studies, Wertz and co-workers (Wertz et al., 1986; Wertz, 1992) and Downing et al. (1993) prepared liposomes from lipid mixtures approximating the SC composition and studied their interaction with sodium dodecyl sulfate (SDS) to determine the deleterious effect of this surfactant on human skin.

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).

We studied the formation of liposomes using a mixture of four lipids modeling the composition of SC and the sublytic interactions of dodecyl betaine (C_{12}-Bet), SDS, and mixtures of these surfactants with SC liposomes (de la Maza et al., 1995; de la Maza and Parra, 1996; de la Maza et al., 1997, 1998). In the present work we seek to extend these investigations by characterizing the Re and K parameters of C_{12}-Bet/SDS mixtures when saturated and solubilized SC liposomes, as well as the influence of proportion of ceramides on these parameters. This information may be useful to evaluate the activity changes of these surfactant mixtures with respect to that of the anionic component on a simplified SC membrane model and to clarify the aforementioned correlation between the presence of ceramides in skin and the abnormalities in the barrier function.

2. Materials and methods

N-Dodecyl-*N*,*N*-dimethylbetaine (C_{12}-Bet) was prepared by Albright and Wilson (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). 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 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 (St Louis, MO). Cholesteryl sulfate (Chol-sulf) was prepared by reaction of cholesterol with excess chlorosulfonic acid in pyridine and purified chromatographically.

The molecular weight of ceramide type III used in the lipid mixtures 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 SC composition (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). This method was also used to prepare liposomes varying the percentage of Cer (from 30 to 50%), the relative proportions of the other lipids remaining constant (see Table 1). After preparation liposomes were annealed at 60°C for 30 min and incubated at 25°C under N_2 atmosphere. The final volumes of liposomes were adjusted with PIPES buffer to provide a final lipid concentration ranging from 0.5 to 5.0 mM. The lipid composition of liposomes after preparation was determined by thin-layer chromatography coupled to an automated flame ionization detection system (Iatroscan MK-5, Iatron Lab., Tokyo, Japan) (Ackman et al., 1990; de la Maza et al., 1995).

Table 1
Liposome lipid composition corresponding to the experiments, in which the percentage of ceramides varied from 30 to 50% and the relative proportion of the other lipids remained constant

Expt. no.	Lipid composition (%)			
	Cer	Chol	PA	Chol-sulf
1	30	29.16	29.16	11.66
2	35	27.08	27.08	10.83
3	45	22.91	22.91	9.16
4	50	20.83	20.83	8.33

In order to find out whether all the components of the lipid mixtures formed liposomes, vesicular dispersions were analyzed for these lipids (Ackman et al., 1990). The dispersions were then spun at $140\,000 \times g$ 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 temperatures (PTT) of the lipid mixtures forming liposomes were determined by proton magnetic resonance (^{1}H NMR), showing values ranging from 55 to 59°C.

The vesicle size distribution and 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 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 con-

centration in the aqueous medium (mM). For $L \gg S_B$, the definition of K, as given by Schurtenberger, applies:

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

where Re is the effective molar ratio of surfactant to lipid in the bilayers ($Re = S_B/L$). Under any other conditions, Eq. (2) 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 et al. (1985) and Almog et al. (1990) for different surfactant lipid mixtures over a wide ranges of Re values. Given that the range of lipid concentrations used is similar to that used by Almog et al. to test their equilibrium partition model, the K parameter has been determined using this equation.

The solubilization of liposomes was characterized by two parameters termed Re_{SAT} and Re_{SOL} (according to the nomenclature adopted by Lichtenberg et al. (1985)), corresponding to the Re ratios at which static light-scattering (SLS) 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 liposome solubilization.

Equal volumes of surfactant solutions were added to the liposome suspensions and the resulting mixtures were left to equilibrate for 24 h at 25°C. The final surfactant concentration (mM) was calculated from each mixture. This time was chosen as the optimum period needed to achieve a complete equilibrium surfactant/liposome in the lipid concentration range used (Ruiz et al., 1988; Partearroyo et al., 1996). The temperature of 25°C was selected by the following reasons: (i) the reasonable stability of the SC liposomes in these conditions; (ii) similar experimental conditions to those used to study the interaction of these surfactants with PC liposomes; (iii) these experimental conditions are generally used in 'in vivo' tests to study the interaction of alkyl sulfates with skin (Wilheim et al., 1991a,b; Schäfer-Korting, 1992). SLS measurements were made at 25°C using a Shimadzu RF-540 spectrofluorophotometer (Kyoto, Japan) with both monochromators adjusted to 500 nm (de la Maza and Parra, 1997). The assays were carried out in triplicate and the results given are the average of those obtained.

The determination of Re and S_W parameters was carried out on the basis of the linear dependence existing between the surfactant concentrations required to saturate and solubilize liposomes and the lipid concentration (L), which can be described by the equations:

$$S_{SAT} = S_{W,\,SAT} + Re_{SAT} \cdot [L] \quad (4)$$

$$S_{SOL} = S_{W,\,SOL} + Re_{SOL} \cdot [L] \quad (5)$$

where S_{SAT} and S_{SOL} are the total surfactant concentrations. The surfactant/lipid molar ratios Re_{SAT} and Re_{SOL} and the aqueous concentration of surfactant $S_{W,\,SAT}$ and $S_{W,\,SOL}$ are in each curve, respectively, the slope and the ordinate at the origin (zero lipid concentration). The K_{SAT} and K_{SOL} parameters (bilayer/aqueous phase surfactant partition coefficient for saturation and complete liposome solubilization) were determined from Eq. (3).

3. Results and discussion

We previously reported the critical micelle concentrations (CMC) of the C_{12}-Bet/SDS mixtures investigated (de la Maza and Parra, 1995). The values for each $X_{zwitter}$ are given in Table 2.

The vesicle size distribution after preparation varied very little, showing in all cases a similar value of about 200 nm (PI lower than 0.1), thereby indicating that the size distribution was very homogeneous. The size of vesicles after the addition of equal volumes of PIPES buffer and equilibration for 24 h always showed values similar to those obtained after preparation with a slight PI increase (between 0.12 and 0.15). Hence, liposomes were reasonably stable in the absence of surfactants under the experimental conditions used.

Table 2
Surfactant to lipid molar ratios (Re), partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction of C_{12}-Bet/SDS mixtures with SC lipid liposomes at different mole fractions of the zwitterionic surfactant[a]

$X_{zwitter}$	CMC (mM)	$S_{W,SAT}$ (mM)	$S_{W,SOL}$ (mM)	Re_{SAT} (mol/mol)	Re_{SOL} (mol/mol)	K_{SAT} (mM^{-1})	K_{SOL} (mM^{-1})	r^2 (SAT)	r^2 (SOL)
0	0.500	0.460	0.500	1.45	3.08	1.28	1.50	0.992	0.994
0.2	0.220	0.216	0.220	6.08	8.70	3.97	4.07	0.994	0.995
0.4	0.160	0.157	0.160	7.02	10.02	5.57	5.68	0.997	0.993
0.6	0.210	0.206	0.210	6.70	9.30	4.22	4.30	0.999	0.995
0.8	0.410	0.406	0.410	5.00	6.55	2.05	2.12	0.991	0.998
1.0	1.250	1.230	1.260	0.80	1.84	0.36	0.514	0.997	0.995

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

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

We previously studied the validity of the equilibrium partition model proposed by Lichtenberg et al. (1985) and Almog et al. (1990) based on Eq. (1) for the surfactant/liposome systems 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 $1/S_W$ at K (Lichtenberg et al., 1985). To test the validity of this model, liposomes were mixed with varying subsolubilizing concentrations of the surfactant mixtures studied (S_T). The resultant surfactant-containing vesicles were then spun at 140 000 × g at 25°C for 4 h to remove the vesicles. No lipids were detected in the supernatants (Ackman et al., 1990). 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 lipid 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 ($r^2 = 0.991$, 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 the surfactant/liposome systems investigated. This model also proved to be valid for the interaction of a specific surfactant mixture ($X_{zwitter} = 0.4$) with liposomes, varying the proportion of Cer from 30 to 50%, the relative proportions of the other lipids remaining constant.

To determine the Re and S_W parameters in liposomes modeling the SC lipid composition (40% Cer, 25% Chol, 25% PA and 10% Chol-sulf) due to the action of C_{12}-Bet/SDS mixtures, a systematic investigation of SLS changes was carried out for various lipid concentrations (from 0.5 to 5.0 mM) at different $X_{zwitter}$. The SLS curves obtained for $X_{zwitter} = 0.4$ are given in Fig. 1.

The addition of surfactant led to an initial increase and a subsequent fall in the SLS intensity of the system until a low constant value was achieved. The curves obtained for the different $X_{zwitter}$ showed similar trends to those exhibited by 0.4 (results not shown). This SLS behaviour is similar to that reported for the interaction of the same surfactant mixtures with PC liposomes (de la Maza and Parra, 1995), although showing in all cases a more pronounced initial SLS increase.

The surfactant concentrations producing 100% (S_{SAT}) and 0% (S_{SOL}) of SLS were obtained for each lipid concentration by graphical methods. The arrows A and B (curve for 5.0 mM lipid concentration, Fig. 1) correspond to these parameters. When plotting the S_{SAT} and S_{SOL} values thus obtained for each $X_{zwitter}$ versus the lipid concentration, the curves shown in Fig. 2A, B were obtained, respectively, in which an acceptable linear relationship was established in all cases. The straight lines obtained corresponded to Eq. (4) and Eq. (5) from which Re and S_W were determined. The results obtained for each surfactant mixture, including the regression coefficients of the straight lines (r^2) and the surfactant

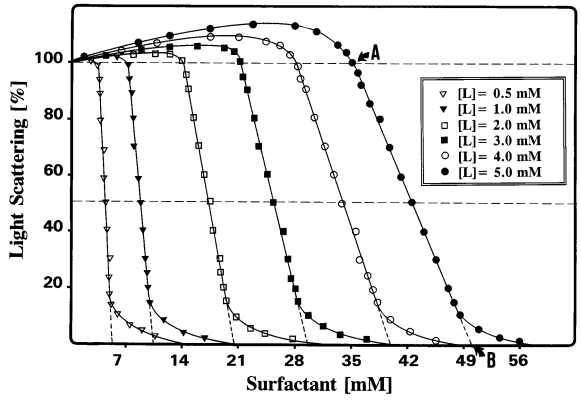

Fig. 1. Percentage changes in static light-scattering of SC liposomes (lipid concentration from 0.5 to 5.0 mM), induced by the presence of increasing amounts of a C_{12}-Bet/SDS mixture at the mole fraction of the zwitterionic surfactant of 0.4. (▽) $[L] = 0.5$ mM, (▼) $[L] = 1.0$ mM, (□) $[L] = 2.0$ mM, (■) $[L] = 3.0$ mM, (○) $[L] = 4.0$ mM and (●) $[L] = 5.0$ mM.

critical micelle concentrations in PIPES buffer (de la Maza and Parra 1995) are given in Table 2.

The free surfactant concentrations ($S_{W,SAT}$, $S_{W,SOL}$) were always comparable to the CMCs of the surfactant mixtures, also showing similar values to those reported for the interaction of these mixtures with PC liposomes (de la Maza and Parra 1995). These findings extend to the SC liposomes investigated; the generally admitted assumption for PC ones that the free surfactant concentration must reach its CMC for solubilization starts to occur and indicates that liposome solubilization was mainly ruled by formation of mixed micelles (Lichtenberg et al., 1985; de la Maza and Parra 1995). 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 liposome saturation and solubilization occurred.

The Re and K values increased as the mole fraction of the zwitterionic surfactant rose, showing a maximum for $X_{zwitter} = 0.4$. Given that the surfactant ability to saturate and solubilize liposomes is inversely related to the Re parameter, the minimum activity at these two interaction levels corresponded to the $X_{zwitter} = 0.4$. However this mole fraction corresponded to the highest degree of partitioning into liposomes or maximum affinity with these bilayer structures (maximum K values). The variation of Re_{SAT} and Re_{SOL} versus $X_{zwitter}$ is plotted in Fig. 3. From these findings we may assume that an inverse association may also be established at the $X_{zwitter}$ of 0.4 between the affinity of these surfactant mixtures with SC liposomes and their ability to saturate or solubilize these bilayer structures. It is noteworthy that this $X_{zwitter}$ corresponded to the higher negative derivation of the CMCs of these mixtures with respect to the ideal behaviour (negative surfactant synergism) and to the highest reduction in the skin irritation with respect to that of the anionic component in the mixture (Rhein and Simion, 1991). This information corroborates the suitability of the simplified membrane model used to study the effect caused by C_{12}-Bet/SDS mixtures on human skin.

Comparison of the present Re and K values with those reported for the interaction of these surfactant mixtures with PC liposomes shows that the ability of these mixtures to saturate or solubilize SC liposomes was lesser (higher Re values) than that reported for PC ones in all cases, although their degree of partitioning into these two bilayers was similar in all cases (similar K values) (de la Maza and Parra, 1995). The increased resistance of SC liposomes to the surfactant perturbations (with respect to that of PC ones) could

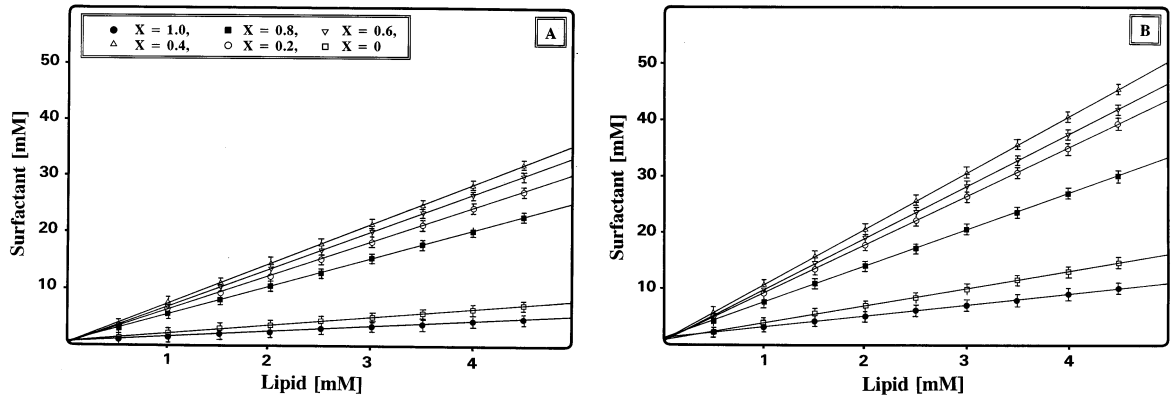

Fig. 2. (A) Surfactant concentrations resulting in saturation (100% SLS) of SC liposomes versus lipid concentration and due to the action of C_{12}-Bet/SDS mixtures at different mole fractions of the zwitterionic surfactant ($X_{zwitter}$). (●) $X = 1.0$, (■) $X = 0.8$, (▽) $X = 0.6$, (△) $X = 0.4$, (○) $X = 0.2$ and (□) $X = 0$. (B) Surfactant concentrations resulting in complete solubilization (0% SLS) of SC liposomes versus liposome lipid concentration and due to the action of C_{12}-Bet/SDS mixtures at different mole fractions of the zwitterionic surfactant ($X_{zwitter}$). (●) $X = 1.0$, (■) $X = 0.8$, (▽) $X = 0.6$, (△) $X = 0.4$, (○) $X = 0.2$ and (□) $X = 0$.

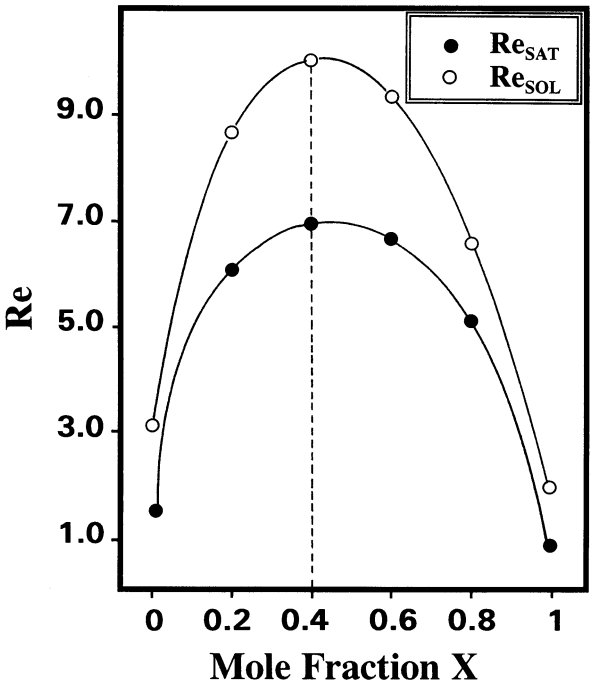

Fig. 3. Variation of the surfactant to lipid molar ratios (Re_{SAT} and Re_{SOL}) in SC liposomes for C_{12}-Bet/SDS mixtures versus the mole fraction of the zwitterionic surfactant ($X_{zwitter}$). (●) Re_{SAT} and (○) Re_{SOL}.

be explained bearing in mind the more hydrophilic nature of PC, which could facilitate the association of surfactant molecules with PC bilayer structures, either through the hydrophilic holes created by the surfactants on the PC polar heads or via formation of short-lived complexes of surfactants–PC polar heads and subsequent bilayer solubilization (Lasic, 1993). The different gel–liquid crystal-phase transition temperatures of lipids building these two liposome structures could also affect both the positional organization of lipid molecules and their polar heads, as well as their mobility (SC and PC vesicles in 'gel state' and 'fluid state', respectively at 25°C), also affecting the aforementioned processes.

A systematic study based on SLS variations of liposomes varying the proportion of ceramides and due to the action of C_{12}-Bet/SDS was carried out to clarify the dependences of the level of ceramides in skin lipids on function barrier abnormalities. To this end, the percentage of Cer in liposomes varied from 30 to 50% (lower and higher percentages than those in SC lipids) and the relative proportion of the other lipids remained constant. The C_{12}-Bet/SDS mixture used was $X_{zwitter} = 0.4$.

Table 3 shows the solubilizing parameters for the liposomes formed at the lipid compositions given in Table 1, including the regression coefficients of the straight lines. It is noteworthy that the increase in the percentage of Cer in liposomes resulted in a clear increase in the Re parameters (increased resistance of liposomes to the surfactant action) and in a decrease in the partitioning of the surfactant mixture into these bilayer structures (decrease in K parameters). These variations are plotted in Fig. 4a, b, respectively. It may be seem that the increase in Re parameters was more pronounced at low ceramide proportions (up to 40%), whereas the decrease in K was more noticeable at high ceramide concentrations, specially for K_{SAT}. Thus, the higher the proportion of Cer in liposomes the lower the ability of the surfactant mixture ($X_{zwitter} = 0{,}4$) to saturate and solubilize

Table 3
Surfactant to lipid molar ratios (Re), partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction of a C_{12}-Bet/SDS mixture ($X_{zwitter} = 0.4$) with SC liposomes varying their lipid composition (Table 1)

Expt. no	$S_{W,SAT}$ (mM)	$S_{W,SOL}$ (mM)	Re_{SAT} (mol/mol)	Re_{SOL} (mol/mol)	K_{SAT} (mM^{-1})	K_{SOL} (mM^{-1})	r^2 (SAT)	r^2 (SOL)
1	0.152	0.155	5.90	9.01	5.63	5.81	0.991	0.997
2	0.155	0.157	6.45	9.52	5.58	5.76	0.998	0.995
3	0.160	0.164	7.60	10.51	5.52	5.56	0.993	0.996
4	0.167	0.169	7.80	10.60	5.30	5.40	0.994	0.992

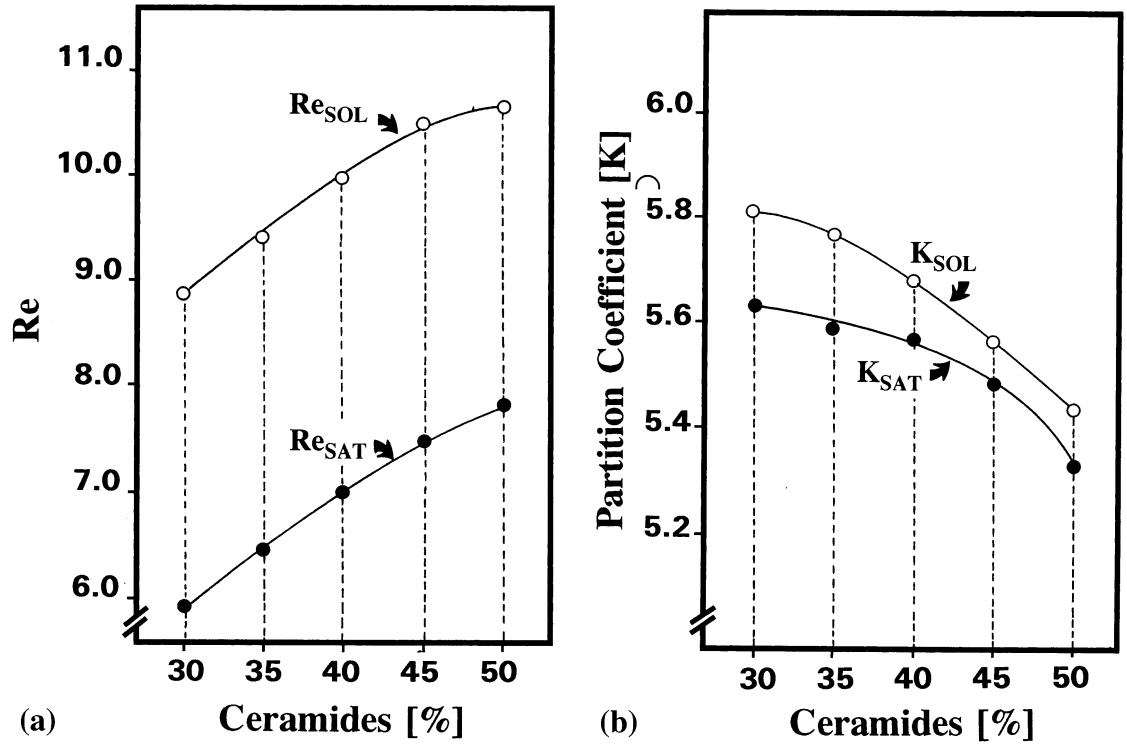

Fig. 4. (a) Effective surfactant to lipid molar ratios (Re_{SAT} and Re_{SOL}) in SC liposomes for a C_{12}-Bet/SDS mixture ($X_{zwitter} = 0.4$) versus the percentage of ceramides in liposomes. (●) Re_{SAT} and (○) Re_{SOL}. (b) Partition coefficients (K_{SAT} and K_{SOL}) in SC liposomes for a C_{12}-Bet/SDS mixture ($X_{zwitter} = 0.4$) versus the percentage of ceramides in liposomes. (●) K_{SAT} and (○) K_{SOL}.

liposomes, and the lower its affinity with these bilayer structures. Hence, higher and lower proportions of Cer than those in the SC lipids led to a fall and to a rise in both the activity and the affinity of the surfactant mixture used ($X_{zwitter} = 0.4$) in the saturation and solubilization of SC liposomes.

As for the free surfactant concentrations (S_W), the increase in the percentage of Cer in liposomes resulted in a slight increase in both the $S_{W,SAT}$ and the $S_{W,SOL}$, although these parameters always showed similar values to that of the surfactant mixture CMC (0.160 mM, see Table 2).

From these findings we may assume that the proportion of Cer in SC lipid liposomes plays an important role, both in the resistance of these liposomes to be saturated and solubilized by the surfactant mixture used and in the surfactant affinity with these bilayer structures. These findings are in agreement and complement the recent studies on the dependences of the level of ceramides in skin and function barrier abnormalities (Murata et al., 1996; Ponec et al., 1997). We are aware of the fact that the lipids used in this work are not exactly the same as those existing in the stratum corneum. Nevertheless, the simplified membrane model used has shown to be useful in establishing a correlation between the level of ceramides in liposomes and the resistance of these bilayer structures against the action of a specific surfactant mixture. Comparison of the Re and K parameters with those reported for PC liposomes may be useful to evaluate the activity of these mixtures in simplified membrane models of different lipid composition and to compare the resistance of these two membranes against these surfactant mixtures.

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