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ORIGINAL CONTRIBUTION

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Solubilizing interactions of octylphenol surfactants with liposomes modeling the stratum corneum lipid composition

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Abstract The interaction of a series of polyethoxylated octylphenols (ethylene oxide units average 8.5–20.0) with liposomes modeling the stratum corneum (SC) lipid composition (40% ceramides, 25% cholesterol, 25% palmitic acid and 10% of cholesteryl sulfate) was investigated. 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 free concentration for each surfactant tested was always similar to its critical micelle concentration (CMC) indicates that the liposome solubilization was mainly ruled by the formation of mixed micelles. The Re and K parameters for liposome saturation fell as the surfactant HLB increased. Thus, at this interaction step the higher the surfactant HLB, the higher the ability of these surfactants to saturate SC liposomes and the lower their degree of partitioning into liposomes. However, the maximum solubilizing ability was achieved at intermediate HLB values. Thus, the octylphenols with 20 and 12.5 ethylene oxide units showed, respectively, the highest power of saturation and solubilization of SC structures in terms of the total surfactant amounts needed to produce these effects. Different trends in the interaction of these surfactants with SC liposomes were observed when comparing the Re and K parameters with those reported for PC ones. Thus, whereas the SC liposomes were more resistant to the surfactant action, the affinity of these surfactants with these bilayer structures was higher in all cases.

Key words Stratum corneum lipid liposomes – octylphenol surfactant series – liposome solubilization – interaction stratum corneum liposomes/octylphenol surfactants – static light scattering changes – effective surfactant to lipid molar ratios – surfactant partition coefficients

Abbreviations SC, stratum corneum; Cer, ceramides type III; Chol, cholesterol; PA, palmitic acid; Chol-sulf, cholesteryl sulfate; OP-8.5EO, octylphenol with an average of 8.5 ethylene oxide units; OP-9.5EO, octylphenol with an average of 9.5 ethylene oxide units; OP-12.5EO, octylphenol with an average of 12.5 ethylene oxide units; OP-15.0EO, octylphenol with an average of 15.0 ethylene oxide units; OP-20.0EO, octylphenol with an average of 20.0 ethylene oxide units; PIPES, piperazine-1,4 bis(2-ethanesulphonic acid); 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; 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; $S_{W,SAT}$, surfactant concentration in the aqueous medium for liposome saturation; $S_{W,SOL}$, surfactant concentration in the aqueous medium for liposome solubilization; CMC, critical micellar concentration; HLB, hydrophilic–lipophilic balance.

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Introduction

The permeability barrier of the skin, which prevents penetration of substances from the environment is localized in the horny layer (stratum corneum), which is a compact mass of metabolically inactive cells, embedded in an intracellular matrix of non-polar continuous lamellar lipid layers [1, 2]. In all cellular and intracellular membranes, such bilayer-forming lipids consist predominantly of phospholipids. However, stratum corneum (SC) has been shown to be virtually devoid of phospholipids, as a result of which its ability to form bilayers has proved to be somewhat surprising [3–5]. In order to find out whether SC lipids could form bilayers, Wertz et al. [6], Wertz [7] and Abraham et al. [8] prepared liposomes from lipid mixtures approximating the composition of SC lipids at physiological pH and investigated the interaction of these liposomes with the anionic surfactant sodium dodecyl sulfate to study its deleterious effect on human skin [9]. Furthermore, Blume et al. reported the permeability of the skin to phospholipid vesicles [10].

Polyethoxylated octylphenols have, because of their properties as good solubilization agents for membrane proteins, been subject of a number of studies, in particular that containing 10 ethylene oxide units in its molecular structure (Triton X-100) [11–15]. A number of studies have been devoted to the understanding of the principles governing the interaction of these surfactants with simplified membrane models as phospholid bilayers [16–19]. 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 [20], who postulated that the effective surfactant/lipid molar ratio (Re) producing saturation and solubilization of bilayer structures depends on the surfactant critical micelle concentration (CMC) and on the bilayer/aqueous medium distribution coefficients (K).

In previous papers, we studied the interactions of a series of octylphenols, in particular, Triton X-100 and its mixtures with sodium dodecyl sulfate, with PC liposomes [21–24]. We also investigated the formation and characterization of liposomes formed by mixtures of four commercially available synthetic lipids approximating the composition of stratum corneum [25, 26]. In the present work, we seek to extend these investigations by characterizing the Re and K parameters of a series of octylphenols when interacted with SC liposomes. Comparison of this information with that reported for the interaction of these surfactants with PC liposomes could be useful in order to establish a criterion for the evaluation of their activity in different biological membranes.

Materials and methods

Polyethoxylated octylphenols with different average in ethylene oxide units (8.5, 9.5, 12.5, 15.0 and 20.0) and an active matter of 100% were supplied by Tenneco España S.A. These products are hereafter referred to as OP-8.5EO, OP-9.5EO, OP-12.5EO, OP-15.0EO and OP-20.0EO. Piperazine-1,4 bis(2-ethanesulphonic acid) (PIPES) was obtained from Merck. PIPES buffer was prepared as 10 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) (reagent grade) were supplied by Sigma Chemical Co. Cholesteryl sulfate (Chol-sulf) was prepared by reaction of cholesterol with excess chlorosulphonic acid in pyridine and purified chromatographically. The molecular weight of ceramide type III used in the lipid mixture was determined by low-resolution fast atom bombardment mass spectrometry using a Fisons VG Augo Spec Q (Manchester, U.K.) with a caesium gun operating at 20 Kv. From this analysis a molecular weight of 671 g was obtained for the majority compound of the ceremides type III used (Sigma). This value was nearly equal to the molecular weight of ceramide 3 (667 g) calculated from the structure of this compound reported by Wertz [7], despite the fact that the ceramide type III used was a mixture of ceramides of different chain length (purity approximately 99%). As a consequence, we used the molecular weight obtained to calculate the molarity of the SC 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.

Preparation and stability of SC liposomes

We previously reported the formation and characterization of liposomes formed by a mixture of lipids modeling the composition of the SC (40% Cer, 25% Chol, 25% PA and 10% Chol-sulf) [25], which were prepared following the method described by Wertz et al. [6]. Individual lipids were dissolved in chloroform/methanol (2:1) and appropriate volumes were combined to obtain the aforementioned mixture. Lipid mixture was then placed in a culture tube and the solvent was removed with a stream of nitrogen and then under high vacuum at room temperature. Lipid mixture aqueous dispersions were prepared by suspension in PIPES buffer. The lipids were left to hydrate for 30 min under nitrogen with occasional shaking. The suspensions were then sonicated in a bath sonicator (514 ECT Selecta) at 60 °C for about 15 min until the suspensions

became clear. Vesicles of about 200 nm 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 annealed at the same temperature for 30 min and incubated at 25 °C under nitrogen atmosphere. The range of SC lipid concentration in the liposome suspensions studied was 0.5–5.0 mM.

The vesicle size distribution and the polydispersity index of SC lipid liposomes after preparation was determined by dynamic light-scattering measurements using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV; Malvern, England). The studies were made by particle number measurement [22]. The sample was adjusted to the appropriate concentration range with PIPES buffer and the measurements were taken at 25 °C at a reading angle of 90°.

Lipid analysis and phase transition temperature of SC lipid mixture

The lipid composition and concentration of SC liposomes after preparation were determined using thin-layer chromatography coupled to an automated flame ionization detection system (Iatroscan MK-5, Iatron Lab, Inc. Tokyo, Japan) [25, 27].

In order to find out whether all the components of the SC lipid mixture formed liposomes, vesicular dispersions were analyzed for these lipids [27]. The dispersions were then spun at 140,000 g at 25 °C for 4 h to remove the vesicles [28]. The supernatants were tested again for these components. No lipids were detected in any of the supernatants.

Analyses of proton magnetic resonance (^{1}H NMR) were carried out at temperatures ranging from 25 °C to 90 °C to determine the phase transition temperature of the SC lipid mixture forming liposomes. The ^{1}H NMR spectra were recorded on a Varian Unity of 300 MHz. (Palo Alto, California, U.S.A.). The NMR spectra were measured at intervals of 5 °C. The line widths of the CH_2 band at 1.3 ppm were measured and 1024 scans were accumulated in each measurement. The different line widths were plotted versus the temperature, and inflexion point of the curve was taken as a phase transition temperature, which showed a value of 55–56 °C.

Parameters involved in the interaction of surfactants with SC liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger [29] for bile salt/lecithin systems, Lichtenberg [20] and Almog et al. [28] have shown that for 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

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

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

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

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 [20] and Almog [28] for different surfactant lipid mixtures over wide ranges of Re values. Given that the lipid concentration range used in SC liposomes is similar to that used by Almog to test his equilibrium partition model, the K parameter has been determined using this equation. The solubilization of SC liposomes was characterized by two parameters termed Re_{SAT} and Re_{SOL}, according to the nomenclature adopted by Lichtenberg [20] corresponding to the Re ratios at which static light-scattering starts to decrease with respect to the original value and shows no further decrease. These parameters corresponded to the surfactant/lipid molar ratios at which the surfactant: (a) saturated liposomes and (b) led to a complete solubilization of these structures.

SC liposomes were adjusted to the appropriate lipid concentration. Equal volumes of the appropriate surfactant solutions were added to the 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 [19, 30]. Static light scattering measurements were made with a spectrofluorophotometer Shimadzu RF-540 (Kioto Japan) with both monochromators adjusted to 500 nm at 25 °C [21]. 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 SC lipid concentration

(L), which can be described by the equations

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

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

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 the Eq. (3).

Results and discussion

Stability of SC liposomes

The vesicle size distribution of SC liposomes after preparation varied little (monomodal distribution of about 200 nm) and the polydispersity index was in all cases lower than 0.1 indicating that the liposomes showed a homogeneous size distribution in all cases. The vesicle size after the addition of equal volumes of PIPES buffer and equilibration for 24 h at 25 °C showed in all cases values similar to those obtained after preparation, with a slight rise in the polydispersity index (between 0.12 and 0.14). Hence, SC liposome preparations appeared to be reasonably stable in the absence of surfactants under the experimental conditions used.

Parameters involved in the surfactant/liposomes interaction

The ability of the SC lipids to form bilayers has been reported by Wertz et al. [6], who demonstrated that these lipids form liposomes when hydrated at 80 °C. The Cer type III used in this work is composed primarily of simple sphingosines linked to largely monounsaturated fatty acids. It, therefore, has a much lower bulk-melting temperature than SC ceramides, which contain only saturated fatty acids including hydroxyacids. In the preliminary experiments, we determined the suitable sonication temperature of the lipid mixture investigated by preparing liposomes at temperatures approximating its phase transition temperature (55–56 °C). It was found that temperatures exceeding the phase transition temperature by more than 10 °C caused noticeable alterations in Cer and Chol-sulf. As a consequence, lipid mixture was sonicated at 60 °C.

To determine the Re and S_W parameters, a systematic investigation of static light-scattering variations in SC liposomes caused by the addition of the octylphenols investigated was carried out for various lipid concentrations. The curves obtained for OP-9.5EO (SC lipid concentrations ranging from 0.5 to 5.0 mM) are given in Fig. 1. The addition of surfactant led to an initial increase and a subsequent fall in the static light-scattering intensity of the system until achieving a low constant value, which corresponds to the complete bilayer solubilization. The curves obtained for the other octylphenols investigated showed similar trends (results not shown). This static light-scattering behavior is in accordance with that reported for the

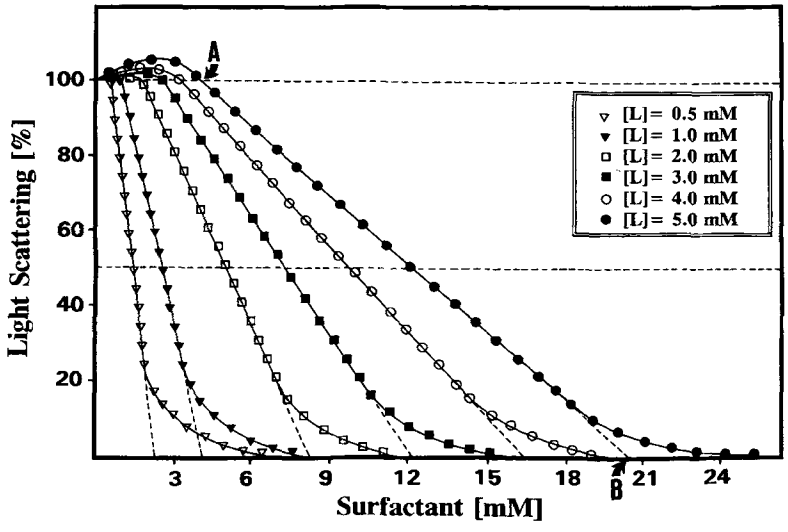


Fig. 1 Percentage changes in static light-scattering of SC liposomes, the bilayer lipid concentration ranging from 0.5 to 5.0 mM, versus OP-9.5EO surfactant concentration.
(▽) [L] = 0.5 mM,
(▼) [L] = 1.0 mM,
(□) [L] = 2.0 mM,
(■) [L] = 3.0 mM,
(○) [L] = 4.0 mM,
(●) [L] = 5.0 mM

Table 1 Surfactant to lipid molar ratios (Re), partition coefficients (K) and surfactant concentrations in the aqueous medium (S_W) resulting in the interaction of octylphenol surfactants with SC liposomes. The critical micelle concentration and the theoretical HLB number of each surfactant tested are also included together with the regression coefficients (r^2) of the straight lines obtained

	CMC [mM]	HLB (Theoretical)	$S_{W,SAT}$ [mM]	$S_{W,SOL}$ [mM]	Re_{SAT} mole/mole	Re_{SOL} mole/mole	K_{SAT} [mM^{-1}]	K_{SOL} [mM^{-1}]	r^2 [SAT]	r^2 [SOL]
OP-8.5EO	0.14	12.9	0.14	0.15	0.96	5.75	3.50	5.67	0.995	0.994
OP-9.5EO	0.15	13.4	0.15	0.16	0.79	4.05	2.94	5.01	0.992	0.998
OP-12.5EO	0.18	14.6	0.18	0.19	0.56	2.90	1.99	3.91	0.996	0.994
OP-15.0EO	0.22	15.2	0.21	0.23	0.52	3.15	1.62	3.30	0.998	0.997
OP-20.0EO	0.26	16.1	0.25	0.27	0.51	5.25	1.35	3.11	0.992	0.996

interaction of these surfactant with neutral or electrically charged PC liposomes [21].

The surfactant concentrations producing 100% (S_{SAT}) and 0% (S_{SOL}) of static light-scattering in the system were obtained for each lipid concentration by graphical methods. The arrows A and B (curve for SC conc. 5.0 mM, Fig. 1) correspond to these parameters. When plotting the surfactant concentrations thus obtained versus lipid concentration, curves were obtained in which an acceptable linear relationship was established in each case. The straight lines obtained corresponded to the Eqs. (4) and (5) from which the Re and S_W parameters were determined. The results obtained including the regression coefficients (r^2) of the straight lines, the experimental CMC value [21] and the theoretical HLB number of each surfactant tested are given in Table 1.

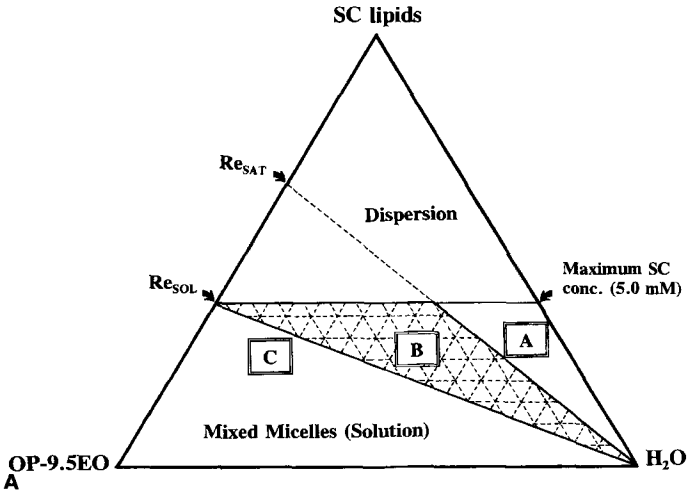
Figure 2A shows a Gibbs triangle for a OP-9.5EO/SC lipids/water system based on the aforementioned static light-scattering variation (SC conc. 0.5–5.0 mM). The triangle is built for a constant relative concentration of water of 99% due to the high water percentage of these systems and the SC lipid concentration used. When studying the relative concentration of SC lipids/OP-9.5EO (left side of the triangle) it may be observed that the extrapolation of the lines corresponding to 100% and 0% of static light-scattering gives the corresponding Re_{SAT} and Re_{SAT} parameters. In addition, this triangle shows three clearly defined domains: (A) area corresponding to the liposome suspension (progressive formation of surfactant/SC mixed vesicles in equilibrium with pure SC lipid vesicles); (B) area in which a progressive decrease in the static light-scattering of the system is observed (progressive formation of surfactant/SC mixed micelles in equilibrium with surfactant/SC mixed vesicles) and (C) area corresponding to the mixed micelles (micellar solution). A schematic drawing of the sequential structures proposed for the lipid-surfactant complexes corresponding to these three zones is shown in Fig. 2B.

The free surfactant concentrations ($S_{W,SAT}$, $S_{W,SOL}$, Table 1) for each surfactant tested were always comparable to the surfactant CMCs although showing slightly reduced values with respect to those reported for the interaction of these surfactants with PC liposomes [21]. This finding extends 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 this solubilization process was mainly ruled by the formation of mixed micelles [20].

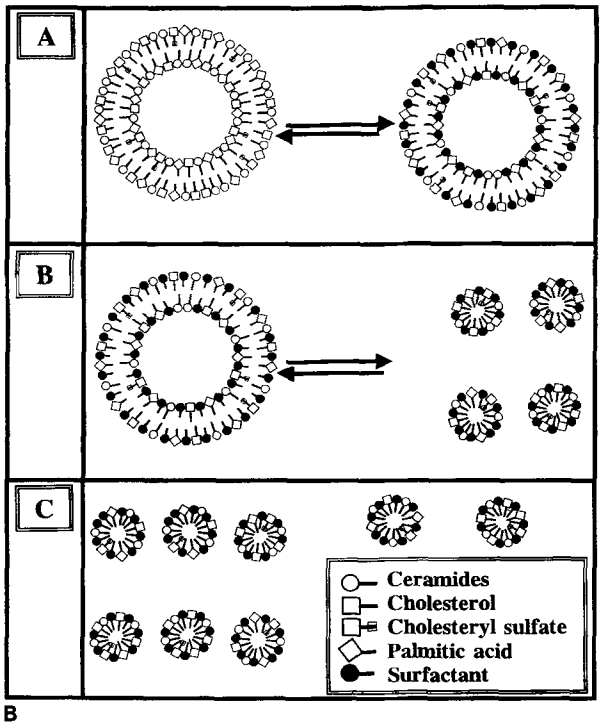
The Re values clearly increased from saturation to complete solubilization of SC liposomes, regardless of the number of ethylene oxide units of the surfactant tested. In addition, the higher the surfactant hydrophilic tail, the lower the Re_{SAT} parameter. However, the Re_{SOL} showed a minimum for OP-12.5EO. Given that the surfactant capacity to saturate or solubilize bilayers in inversely related to the Re parameter, the maximum activity for bilayer saturation corresponded to the OP-20.0EO, whereas that for complete bilayer solubilization corresponded to the OP-12.5EO (lowest Re values).

Comparison of the Re values obtained with those reported for the interaction of the same surfactants with neutral and electrically charged PC liposomes reveals that the ability of these surfactants to saturate or solubilize SC liposomes was lesser (higher Re values) than that reported for the PC ones in all the cases [21], although showing similar tendencies with respect to the influence of the surfactant hydrophilic tail. As a consequence, SC liposomes exhibited more resistance to the surfactant perturbations than PC ones at the interaction levels investigated. These differences could be explained, bearing in mind, the more hydrophilic nature of PC which could facilitate the initial 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 surfactants-PC polar heads and the subsequent bilayer solubilization via mixed micelle formation [31].

The K-parameters (Table 1) also increased from bilayer saturation to complete solubilization of these bilayer structures regardless of the number of ethylene oxide units



A



B

Fig. 2A Gibbs triangle for a OP-9.5EO/SC lipids/water system (99% of water in weight) the SC lipid concentration ranging from 0.5 to 5.0 mM. **B** Schematic drawing of the sequential structures proposed for the lipid-surfactant complexes during vesicle solubilization corresponding to the Gibbs triangle shown in Fig. 2A

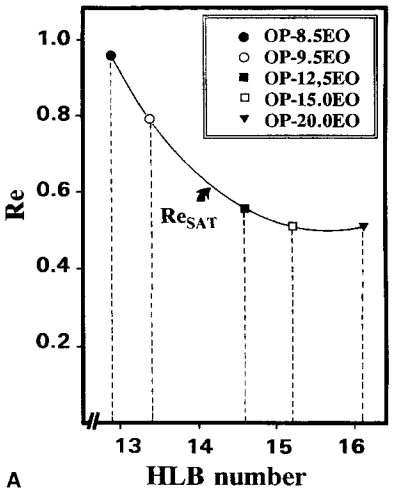
of the surfactant tested. This means that the affinity of surfactant molecules with the lipids building SC liposomes was greater in bilayer solubilization (micellization process) than during the previous step of bilayer saturation (formation of mixed liposomes). In addition, the increase in the surfactant hydrophilic tail also resulted in a fall in K both for bilayer saturation (K_{SAT}) and solubilization (K_{SOL}). As a consequence, the degree of partitioning of these surfactants into SC liposomes (or affinity with these structures) decreased as their hydrophilic moiety increased. Thus, the OP-8.5EO molecules had the highest affinity with SC bilayers (maximum K values), whereas the OP-20.0EO showed the lowest (minimum K values).

Comparison of the K_{SAT} and K_{SOL} values for each surfactant tested also reveals that the higher the number of ethylene oxide units in the surfactant, the higher the quotient between both parameters (K_{SOL}/K_{SAT}). Thus, at the interaction level for complete solubilization the degree of partitioning of these surfactants into SC liposomes relatively increased with respect to that for saturation as their number of ethylene oxide units rose (K_{SOL}/K_{SAT} value from 1.62 for OP-8.5EO to 2.30 for OP-20.0EO). As a consequence, we may assume that the increase of the surfactant hydrophilic tail in addition to reduce the partitioning of surfactant molecules into bilayers also resulted in a relative increase in their ability to be associated with the lipid molecules building SC liposomes to form mixed micelles. Possibly, the first-order phase transition from mixed liposomes into mixed micelles appears to be relatively favored by the increasing surfactant hydrophilic moiety.

Similarly, comparison of the present K values with those calculated from the Re and S_W parameters reported for the interaction of these surfactants with neutral PC liposomes (applying Eq. (3)) shows that the degree of partitioning of these surfactants into SC bilayers (or bilayer affinity) was always greater (higher K values) than that exhibited for PC ones. However, the influence of the surfactant hydrophilic moiety on this affinity was also similar in both cases, in spite of the different compositions and properties of these two bilayer structures [21].

In general terms, different trends in the interaction of these surfactants with SC liposomes may be observed when comparing the present parameters with those reported for PC liposomes. Thus, whereas the SC liposomes appeared to be more resistant to the action of surfactant monomers, the partitioning of surfactant into SC structures was always greater than that reported for PC ones. Thus, although a greater number of surfactant molecules was needed to produce the same alterations in SC liposomes, these molecules showed an increased affinity with these bilayer structures. However, a similar influence of the surfactant hydrophilic tail on the Re and K parameters was observed for both cases.

If the Re and K values obtained for each surfactant tested are plotted as a function of its theoretical HLB number (hydrophilic–lipophilic balance) the graphs shown in Figs. 3A and B and Fig. 4 are obtained. A decrease in Re_{SAT} occurred as the surfactant HLB number rose (or its number of ethylene oxide units increased) (Fig. 3A), this



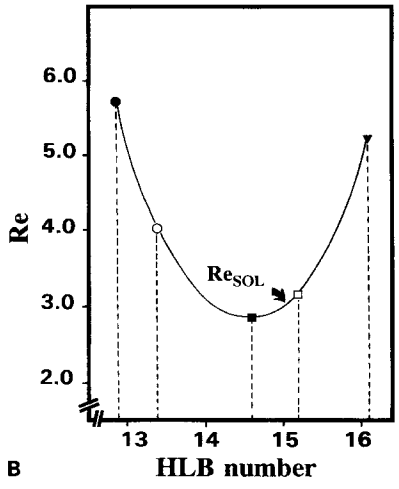


Fig. 3A Effective surfactant/lipid molar ratio Re_{SAT} for (●) OP-8.5EO, (○) OP-9.5EO, (■) OP-12.5EO, (□) OP-15.0EO and (▼) OP-20.0EO surfactants versus the surfactant HLB number for SC liposomes. **B** Effective surfactant/lipid molar ratio Re_{SOL} for (●) OP-8.5EO, (○) OP-9.5EO, (■) OP-12.5EO, (□) OP-15.0EO and (▼) OP-20.0EO surfactants versus the surfactant HLB number for SC liposomes

fall being more pronounced at low HLB values. As for Re_{SOL} (Fig. 3B), this parameter exhibited a minimum when the surfactant HLB was approximately 14.5–14.7, which corresponded to the OP-12.5EO.

The rise in the surfactant HLB also resulted in a fall in K both for bilayer saturation (K_{SAT}) and solubilization (K_{SOL}) (Fig. 4). As a consequence, in this HLB range the degree of partitioning of these surfactants into SC liposomes (or affinity with these bilayer structures) drastically decreased as the surfactant HLB increased, i.e., the surfactant hydrophilic moiety increased.

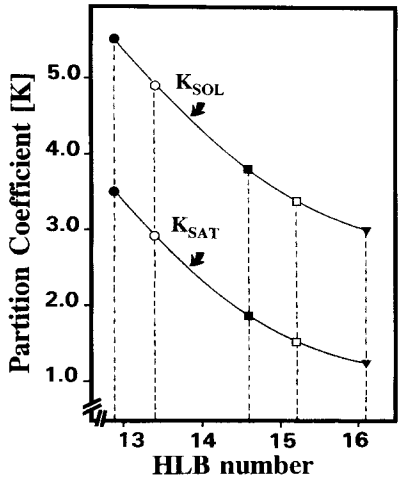


Fig. 4 Partition coefficients K_{SAT} and K_{SOL} for (●) OP-8.5EO, (○) OP-9.5EO, (■) OP-12.5EO, (□) OP-15.0EO and (▼) OP-20.0EO surfactants versus the surfactant HLB number for SC liposomes

As a consequence, two opposite trends may be observed in the saturation of SC bilayers by these surfactants when comparing the variation of Re and K versus the surfactant HLB number. The rise in the HLB number led during the formation of mixed liposomes to a progressive increase in the surfactant ability to saturate liposomes and inversely in a progressive decrease in its affinity with these structures. However, in the interaction step for complete solubilization of liposomes the rise in the surfactant HLB in addition to cause a progressive decrease in the surfactant affinity with SC bilayers showed the maximum solubilizing capacity of SC liposomes at intermediate HLB values (HLB number of about 14.6).

Figure 5 shows for each surfactant tested the total surfactant concentration (mM) needed to saturate (S_{SAT}, Eq. (4)) and solubilize (S_{SOL}, Eq. (5)) SC liposomes versus the surfactant HLB (lipid conc. 5.0 mM). It is noteworthy that, although the lowest surfactant concentration for liposome saturation corresponded to the maximum HLB (OP-20.0EO), that for the OP-12.5EO (14.5–14.7) exhibited the lowest value for SC bilayer solubilization. Thus, the overall balance of the surfactant activity on SC liposomes shows that OP-20.0EO and the OP-12.5EO had, respectively, the highest power with respect to the saturation and solubilization of these bilayer structures. Bearing in mind these findings we may assume that the HLB of these surfactants plays an important role both in the incorporation of surfactant monomers into SC bilayers and in the subsequent association of these monomers with the molecules building SC liposomes, which led to the

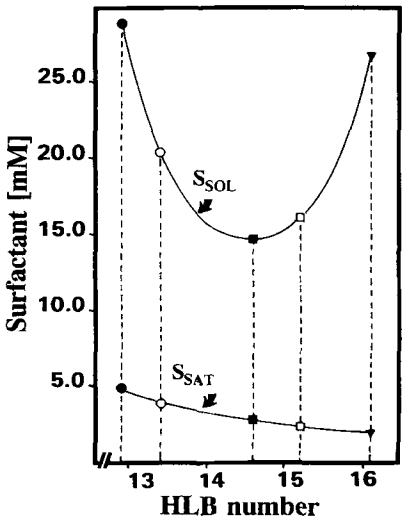


Fig. 5 Plots of the total surfactant concentrations S_{SAT} and S_{SOL} for (●) OP-8.5EO, (○) OP-9.5EO, (■) OP-12.5EO, (□) OP-15.0EO and (▼) OP-20.0EO surfactants versus the surfactant HLB number for SC liposomes, the bilayer lipid concentration remaining constant (5.0 mM)

solubilization of these bilayer structures via mixed micelle formation.

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 comparison of the present Re and K parameters with those reported for the same surfactants with PC liposomes may be useful in establishing a criterion for the evaluation of the activity of these surfactants in a simplified membrane model devoid of phospholipids. Our approach may be also useful in the study of the specific surfactant interactions with SC structured lipids, given the growing use of these non-ionic surfactants in pharmacological applications.

Conclusions

From these findings we conclude that in the interactions of the octylphenols investigated with SC liposomes the Re and K parameters for bilayer saturation decreased as the surfactant HLB increased. Thus, at this interaction step, the higher the surfactant HLB, the higher the ability of these surfactants to saturate SC liposomes and the lower their degree of partitioning into liposomes. However, the maximum solubilizing ability was achieved at intermediate HLB values. Thus, the octylphenols with 20 and 12.5 ethylene oxide units showed, respectively, the highest power of saturation and solubilization of SC structures in terms of the total surfactant amounts needed to produce these effects. Furthermore, the solubilization process for each surfactant tested was mainly ruled by the formation of mixed micelles. Different trends in the interaction of these surfactants with SC liposomes were observed with respect to those reported for PC ones. Thus, whereas SC liposomes were more resistant to the surfactant action, the affinity of these surfactants with these bilayer structures was higher in all cases.

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