Colloid & Polymer Science Colloid Polym Sci 272: 721–730 (1994)

Solubilization of unilamellar phospholipid bilayers by nonionic surfactants

A. de la Maza and J. L. Parra

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

Abstract: The mechanisms governing the solubilization of neutral or electrically charged unilamellar liposomes by a series of octylphenol polyethoxylated surfactants (average of ethylene oxide units between 8.5 and 20.0) were investigated. Solubilization was detected as a decrease in light-scattering of liposome suspensions. To this end, in accordance with the nomenclature adopted by Lichtenberg, three parameters were considered as corresponding to the effective surfactant/lipid molar ratios (*Re*) at which the surfactant (a) saturated the liposomes Re_{sat}; (b) resulted in a 50% solubilization of vesicles $Re_{50\%}$ and (c) led to a total solubilization of liposomes Re_{sol}. These parameters, corresponded to the *Re* at which light scattering starts to decrease, reaches 50% of the original value and shows no further decrease.

It is noteworthy that the Re_{sat} parameter decreases as the EO contents or the surfactant critical micellar concentration (CMC) increases. However, the $Re_{50\%}$ and the Re_{sol} parameters show the lowest values for the surfactant with 12.5 EO units in its molecular structure regardless of the electrical charge of the lipid bilayers. As a consequence, these last parameters are not linearly correlated with the CMC of these surfactants. The CMC values of the surfactant/lipid systems at 0.5 mM lipid concentration corresponded in all cases to the surfactant concentration at which liposomes were saturated by surfactants (S_{sat}).

Key words: Liposomes solubilization – light-scattering changes – surface tension changes – critical micellar concentration – effective surfactant/phospholipid molar ratio

Introduction

The interaction of surfactants with phospholipid bilayers in excess water leads to the breakdown of lamellar structures and the formation of lipid-surfactant mixed micelles [1, 2]. This process is commonly denoted as "solubilization". Many studies have been devoted to the understanding of the principles governing this complex process [3–9]. A significant contribution has been made by Lichtenberg [10], who postulated that the minimum effective surfactant/lipid ratio producing solubilization depends on the surfactant critical micellar concentration (CMC) and on the bilayer/aqueous medium partition coefficients rather than on the nature of the surfactants. In addition, it expresses the need for experimental data on the distribution coefficients at subsolubilizing surfactant concentrations in order to obtain complementary information on the complex phenomenon involved in the surfactant-bilayer solubilization. Accordingly, we carried out studies on the partition coefficients of octylphenol ethoxilated surfactant series [11] in order to determine the main factors involved in the modifications of the permeability of lipid bilayers by these nonionic surfactants.

In the present work, we seek to extend these investigations by characterizing the solubilization of neutral and electrically charged unilamellar lipid bilayers by these surfactants (average of ethylene oxide units between 8.5 and 20.0). In our

case, lipid bilayers consisted of phosphatidylcholine unilamellar liposomes, to which phosphatidic acid or stearylamine was added when required to increase the negative or the positive surface charge respectively. The selection of these surfactants was based on the increasing importance of the octylphenols in biological processes, especially the octylphenol with 9–10 units of ethylene oxide (Triton X-100) [12–18].

Recently, Urbaneja et al. [19] demonstrated that when performed systematically, light-scattering measurements constitute a very convenient technique for the quantitative study of the bilayer solubilization by surfactants. Consequently, in the present work, the vesicle solubilization process was assessed as a decrease in the light-scattered by the liposome/surfactant systems. Likewise, we attempted to compare the solubilizing parameters with the surface tension alterations resulting in the lamellar to micelle transition process involved in this solubilization.

In order to evaluate the light-scattering variations, three parameters were determined as corresponding to the effective surfactant/lipid molar ratios (*Re*) at which the surfactant saturated the liposomes (Re_{sat}), resulted in a 50% solubilization of vesicles ($Re_{50\%}$), and led to a total solubilization of liposomes (Re_{sol}), according to the three-stage model adopted by Lichtenberg [2, 10].

The main aim of this work was to obtain some physicochemical evidence concerning nonionic surfactant-liposome solubilizing interactions in order to determine the influence of the hydrophilic-lipophilic balance of these surfactants in the saturation and solubilization of lipid bilayers. This information also allowed us to establish a criterion for the evaluation of surfactant activity on phospholipid vesicles and to explain why Triton X-100 is so useful in biomembrane studies, among the nonionic octylphenol series.

Experimental

Materials

Phosphatidylcholine (PC) was purified from egg lecithin (Merck) according to the method of Singleton [20] and was shown to be pure by thin-layer chromatography TLC. Phosphatidic acid (PA) from egg yolk lecithin and stearylamine (SA) were purchased from Sigma Chemicals Co. (St. Louis, MO). Lipids were stored in chlorophorm under nitrogen at − 20 °C until use.

T-octylphenol surfactant series with different average ethylene oxide units (8.5, 9.5, 12.5, 15.0, and 20.0) and an active matter of 100% was especially prepared by Tenneco España S.A. These products are hereafter referred to as OP-8.5 EO, OP-9.5 EO, OP-12.5 EO, OP-15.0 EO, and OP-20.0 EO.

Piperazine-1,4 bis (2-ethanesulphonic acid) (PIPES) was obtained from Merck. The buffer used was 20 mM PIPES, adjusted to pH 7.2 with NaOH, supplemented with 110 mM Na_2SO_4. Water was purified by the Milli-Ro system (Millipore). Polycarbonate membranes and membrane holders were purchased from Nucleopore.

Methods

Liposome preparation

Unilamellar liposome vesicles of a defined size (about 100 nm) were prepared by extrusion of large unilamellar vesicles previously obtained by the reverse phase evaporation method [21, 22] based on an early method described by Szoka and Papahadjopoulos [23]. A lipidic film was formed by removing the organic solvent by rotatory evaporation from a chloroform solution of lipids (lipid composition PC 100% or PC/PA, PC/SA 9:1 molar ratio). The lipids were then redissolved in diethyl ether, and PIPES buffer was added to the solution of phospholipids. Gentle sonication led to the formation of a W/O-type emulsion. After evaporation of the ethyl ether under reduced pressure a viscous gel was formed. Elimination of the final traces of the organic solvent at high vacuum transformed the gel into a liposome suspension in which no traces of ether were detectable by NMR. In order to obtain a total elimination of the ether in the liposome suspensions, ether was washed in advance with the PIPES buffer and stored in bottles over water containing bisulfite [24].

Unilamellar vesicles were obtained by extrusion of vesicle suspensions through 800, 400, 200, and 100 nm polycarbonate membranes to achieve a uniform size distribution [25]. The range of phospholipid concentration in liposome suspension studied was 0.5–5.0 mM.

Phosphorus estimation

Phospholipid concentration of the liposome vesicles was determined by the ascorbic acid spectrophotometric method for total phosphorus estimation [26].

Determination of particle size distribution and stability of liposome preparations

Mean size and the polydispersity of the liposome preparations were determined with a Photon correlator spectrometer (Malvern Autosizer 4700c PS/MV). The studies of particle size distribution were made by particle number measurement. Samples were adjusted to the adequate concentration range with PIPES buffer and the measurements were taken at 25 °C and lecture angle 90°.

Liposome solubilization by surfactants

The perturbation produced by the surfactants in the phospholipid bilayers leads to the solubilization of the lipid components via mixed micelle formation [10]. This solubilization results in changes in light-scattering of these systems which depend on the nature of both surfactant and lipid components. This can be monitored by measuring the variations in light scattering during the solubilizing processes [19, 27].

In order to evaluate these variations obtained with the various surfactants and bilayer compositions, the "effective" surfactant/phospholipid molar ratio *Re*, in an aggregate (liposome or micelle) is defined as follows [2]:

$$Re = \frac{[\text{total surfactant}] - [\text{surfactant monomer}]}{[\text{total phospholipid}] - [\text{phospholipid monomer}]}$$

The second term of the denominator is negligible due to the low solubility of phospholipids in water. From a practical viewpoint, the smaller the *Re* value, the stronger the surfactant efficiency, in both saturation and solubilization of liposomes.

The overall solubilization process can be characterized by three parameters termed Re_{sat}, $Re_{50\%}$, and Re_{sol}, according to the nomenclature adopted by Lichtenberg [2, 10] corresponding to the surfactant/lipid molar ratios at which light-scattering starts to decrease, reaches 50% of the original value, and shows no further decrease. These parameters corresponded to the "*Re*" at which surfactant a) saturated liposomes, b) resulted in a 50% solubilization of lipid bilayers, and c) resulted in a complete solubilization of these structures.

The determination of these parameters can be carried out on the basis of the linear dependence existing between the surfactant concentrations required to achieve these parameters and the phospholipid concentration in liposomes. The equations describing the surfactant concentration needed to saturate the bilayers (Eq. (1)), solubilize 50% of liposomes (Eq. (2)) or achieve the complete solubilization of these structures via mixed micelles formation (Eq. (3)) are given as:

$$S_T = S_a + Re_{sat} \cdot [PL] \quad (1)$$

$$S_{T'} = S_b + Re_{50\%} \cdot [PL] \quad (2)$$

$$S_{T''} = S_c + Re_{sol} \cdot [PL] \,, \quad (3)$$

where [PL] is the phospholipid bilayer concentration (mM) and the effective surfactant to phospholipid molar ratios (Re_{sat}, $Re_{50\%}$ and Re_{sol}) and the aqueous concentration of surfactants (S_a, S_b, and S_c) are in each curve respectively the slope and the ordinate at the origin (zero phospholipid concentration).

Liposome suspensions were adjusted to the proper lipid concentration (from 1.0 to 10.0 mM). To these, equal volumes of the appropriate surfactant solutions were added and the resulting mixtures were left to equilibrate for 24 h. Light-scattering measurements were made at 25 °C with a Shimadzu RF-540 spectrofluorophotometer equipped with a thermoregulated cell compartment, with both monochromators adjusted to 500 nm. The assays were carried out in triplicate and the results given are the average of those obtained.

Surface tension measurements

Surface tensions of buffered solutions of single surfactants and liposome/surfactant systems were measured by the ring method [28] using a Krüss tensiometer (processor tensiometer K-12) which determines directly the real surface tension values at equilibrium.

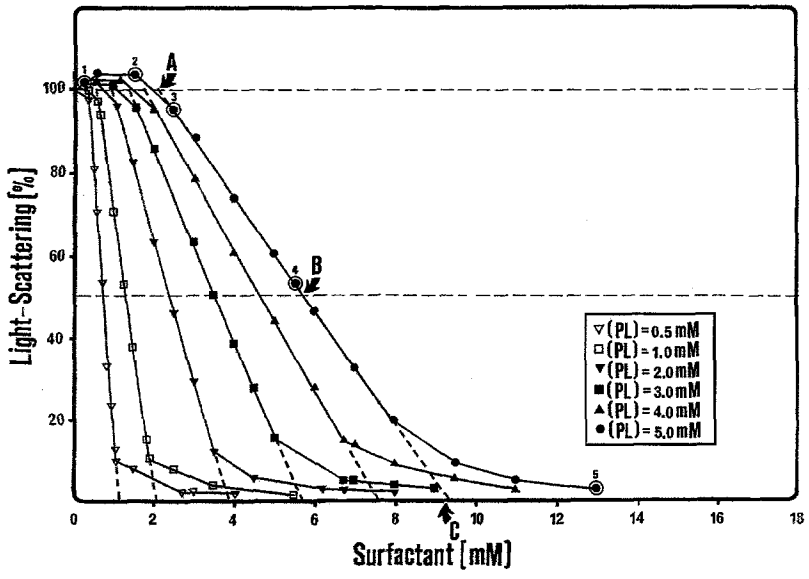

Fig. 1. Percentage change in light-scattering of neutral liposomes, the bilayer lipid concentration ranging between 0.5 and 5.0 mM, versus OP-12.5 EO surfactant concentrations

Critical micellar concentration determination

The critical micelle concentration values for a single surfactant or various liposome/surfactant systems at lipid concentration 0.5 mM in PIPES buffer were determined from the abrupt change in the slope of the surface tension values versus log surfactant concentration.

Results and discussion

Stability of liposome preparations

The particle size distribution after preparation (lipid concentration from 0.5 to 5.0 mM) varied very little, showing in all cases a similar value around 100 nm. Moreover, the polydispersity index of liposomes after preparation was lower than 0.1, which indicated that the size distribution was very homogeneous.

Solubilization studies

It is well known that, in surfactant/phospholipid solubilizing interactions, complete equilibrium needs several hours to occur [13]. In accordance with the procedure described by Urbaneja et al. [19], changes in the light-scattered by these systems were determined 24 h after the addition of surfactants to liposomes at 25 °C. Lipid bilayers consisted of phosphatidylcholine (PC) unilamellar vesicles to which phosphatidic acid (PA) or stearylamine (SA) was added, yielding liposomes with molar ratios PC/PA or PC/SA of 9 : 1 in order to increase the negative or positive charge of the bilayers.

Figure 1 shows the solubilization curves of the neutral liposome preparations (lipid concentration from 0.5 mM to 5.0 mM) obtained by light-scattering variations arising from the addition of different concentrations of OP-12.5 EO. From these curves the surfactant concentration producing the saturation, the half solubilization and the total solubilization of liposomes can be obtained by graphical methods. The arrows A, B, and C (curve 5.0 mM lipid conc.) correspond to these parameters respectively. i.e., the molar surfactant concentration at which light-scattering starts to decrease with respect to the initial value (S_{sat}), reaches 50%, ($S_{50\%}$) and shows no further decrease (S_{sol}).

Plotting these surfactant concentrations previously achieved for each surfactant tested versus phospholipid concentration (PC 100%) curves of Fig. 2 are obtained. An acceptable linear relationship is established in each case. The straight lines obtained correspond to the aforementioned Eqs. (1, 2 and 3). Similar results were obtained when treating electrically charged liposomes (PC/PA or PC/SA 9 : 1 molar ratio) with each surfactant

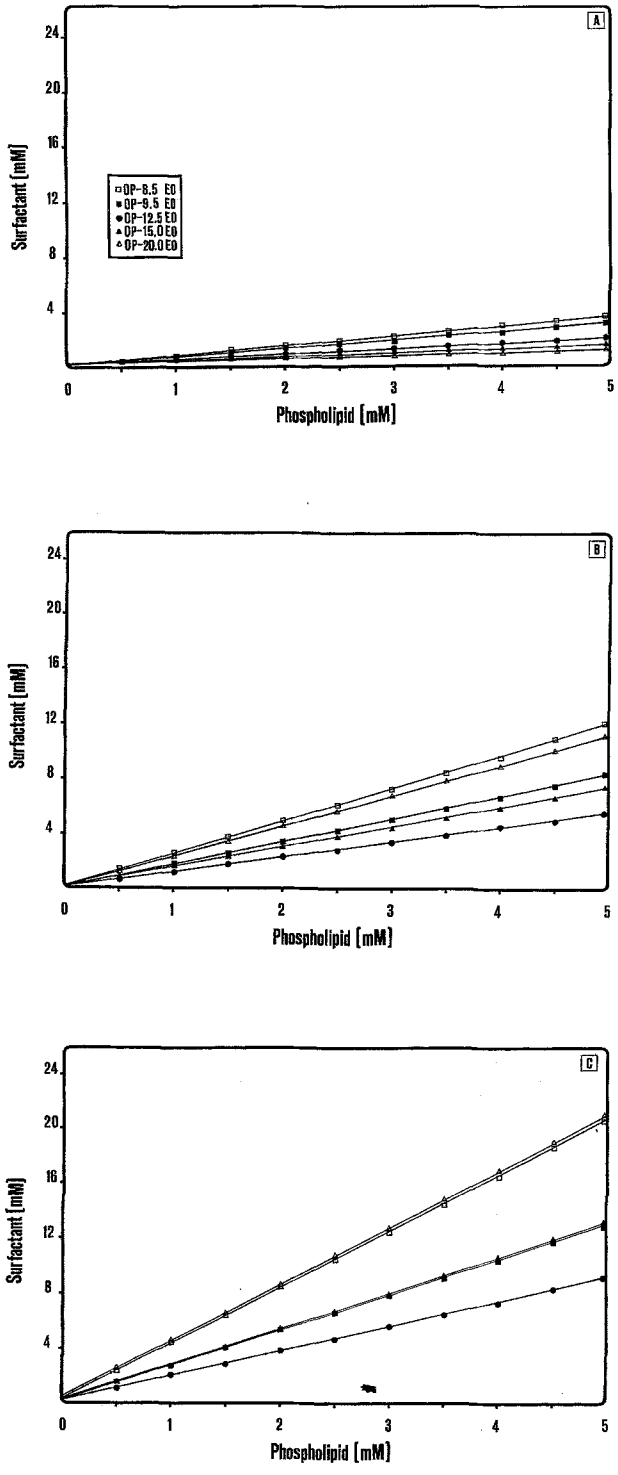

Fig. 2. Plots of the concentration of OP-8.5 EO (□), OP-9.5 EO (■), OP-12.5 EO (●), OP-15.0 EO (▲) and OP-20.0 EO (△) corresponding to the S_{sat} (Fig. 2-A), $S_{50\%}$ (Fig. 2-B) and S_{sol} (Fig. 2-C) values for neutral liposomes, versus bilayer lipid concentration

tested under the same conditions (curves not shown).

The solubilizing parameters obtained, including the regression coefficients of the straight lines (Fig. 2) and the CMC values of the surfactant in the buffered medium are shown in Table 1.

It should be pointed out that in the vast majority of cases, solubilization of liposomes is virtually unaffected by the presence of electric charge (positive or negative) in lipid bilayers, i.e., neutral or electrically charged liposomes are saturated and solubilized at similar surfactant concentrations. These results confirm the small influence of the electrostatic factors in the liposome solubilization by nonionic surfactants and are in agreement with those reported by Urbaneja et al. using electrically charged surfactants [19].

On the other hand, the surfactant concentration in the aqueous medium was always comparable to the critical micellar concentrations of each surfactant tested regardless of the electrical charge of the liposomes. Consequently, our experimental results support the fact that the concentration of free surfactant must reach the CMC for solubilization to occur [10].

As regards the *Re* parameters, it should be noted that as the EO surfactant content increases, the Re_{sat} parameter decreases, whereas the evolution of the $Re_{50\%}$ and Re_{sol} parameters show a minimum for OP-12.5 EO regardless of the electrical charge of liposomes. Thus, OP-12.5 EO appears to be the most effective for liposome solubilization despite the fact that it needs a higher number of molecules (per mol of lipids) to saturate bilayers than OP-15.0 EO and OP-20.0 EO. Moreover, in studies on disruption of mitochondrial membranes by the homologous series of Triton surfactants, Egan et al. [29] reported that a maximum protein and phospholipid extraction occurred at hydrophilic-lipophilic balance (HLB) values between 12.5 and 13.5, which corresponded to the 8.0–9.5 EO units in the surfactant molecular structure. However, our results on liposome suspensions show an increase in the range of EO units with an optimum for 12.5 (theoretical HLB 14.6).

Bearing in mind the influence of the HLB of lipids in the formation and solubilization of bilayers, our results could be explained taking into account that the molecular structure of OP-12.5 EO is more equilibrated in terms of hydrophilic

Table 1. Solubilizing parameters (*Re*) and (*S*) of liposomes (lipid bilayer composition PC 100% or PC/PA, and PC/SA 9:1 molar ratio). The CMC of the surfactants tested and the regression coefficients of the straight lines of Fig. 2 are also included

		Bilayer lipid composition													
		PC:PA (9:1)							Egg PC						
	CMC (mM)	S_a	S_b	S_c	Re_{sat}	$Re_{50\%}$	Re_{sol}	r^2	S_a	S_b	S_c	Re_{sat}	$Re_{50\%}$	Re_{sol}	r^2
OP-8.5 EO	0.14	0.15	0.16	0.17	0.70	2.40	4.10	0.994	0.14	0.15	0.17	0.71	2.45	4.20	0.994
OP-9.5 EO	0.15	0.16	0.17	0.17	0.61	1.60	2.60	0.996	0.16	0.16	0.18	0.62	1.62	2.64	0.992
OP-12.5 EO	0.18	0.18	0.19	0.20	0.38	1.08	1.80	0.997	0.18	0.19	0.21	0.39	1.12	1.84	0.993
OP-15.0 EO	0.22	0.23	0.23	0.25	0.26	1.44	2.60	0.995	0.23	0.24	0.26	0.28	1.47	2.64	0.995
OP-20.0 EO	0.26	0.26	0.28	0.28	0.20	2.20	4.20	0.994	0.27	0.29	0.30	0.22	2.23	4.23	0.994

	PC:SA (9:1)						
	S_a	S_b	S_c	Re_{sat}	$Re_{50\%}$	Re_{sol}	r^2
	0.15	0.16	0.18	0.72	2.46	4.20	0.996
	0.16	0.17	0.19	0.62	1.62	2.62	0.999
	0.18	0.20	0.21	0.39	1.10	1.82	0.997
	0.23	0.25	0.27	0.29	1.45	2.62	0.993
	0.26	0.29	0.31	0.22	2.23	4.24	0.999

lipophilic balance than those of the other octylphenols. This argument is consistent with the most appropriate interaction of this surfactant with the lipid bilayer organization of liposomes.

Figure 3 shows a Gibbs triangle for OP-12.5 EO/Egg PC/Water system considering the light-scattering variations shown in Fig. 1. The triangle is built for a constant relative concentration of water of 99% due to the considerable water percentage of these systems at the lipid concentration range used. When studying the relative concentration of PC/OP-12.5 EO (left side of the triangle) it may be observed that the extrapolation of the lines corresponding to different levels of light-scattering gives the corresponding *Re* parameters for each level. The values thus obtained are in accordance with those calculated from Eqs. (1), (2) and (3). In addition, this triangle shows three clearly defined domains: A) area corresponding to the liposome suspension, B) area in which a progressive decrease of light-scattering is observed (progressive formation of mixed micelles in equilibrium with lipid bilayers), and C) area corresponding to mixed micelles.

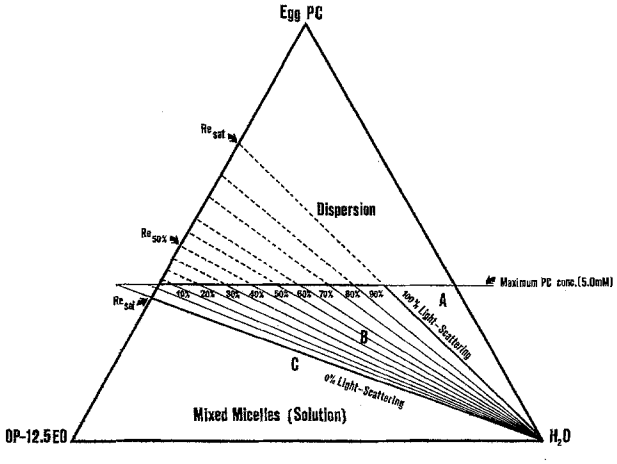

Fig. 3. Gibbs triangle for PO-12.5 EO/Egg PC/Water system (99% of water in weight) in the range of phospholipid concentrations given in Fig. 1

Graphs are obtained plotting the *Re* parameters given in Table 1 versus CMC of surfactants for neutral liposomes (Fig. 4). It may be seen that the Re_{sat} parameter appears to be inversely

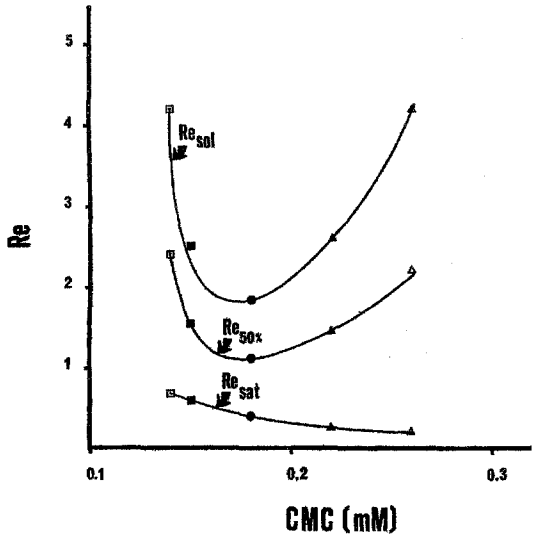

Fig. 4. Effective molar ratios (Re_{sat}, $Re_{50\%}$, Re_{sol}) of the octylphenols tested for neutral liposomes versus CMC of surfactants

correlated with the CMC, whereas the $Re_{50\%}$ and Re_{sol} parameters show a minimum for the OP-2.5 EO surfactant. From these results, in no case may a direct correlation be established between the *Re* parameters and the CMC of the surfactants used.

Surface tension studies

In order to determine the relationship existing between the *Re* parameters and the CMC of the surfactants tested, a systematic investigation was carried out comparing the surface tension values of the single surfactants and surfactant/liposome systems versus surfactant concentration.

Figure 5-A plots the surface tension variations versus octylphenol surfactant concentration in the range of EO units studied, showing the conventional inflexion for their CMC values. Figure 5-B shows the same variation for liposome/surfactant systems (lipid composition PC 100% and

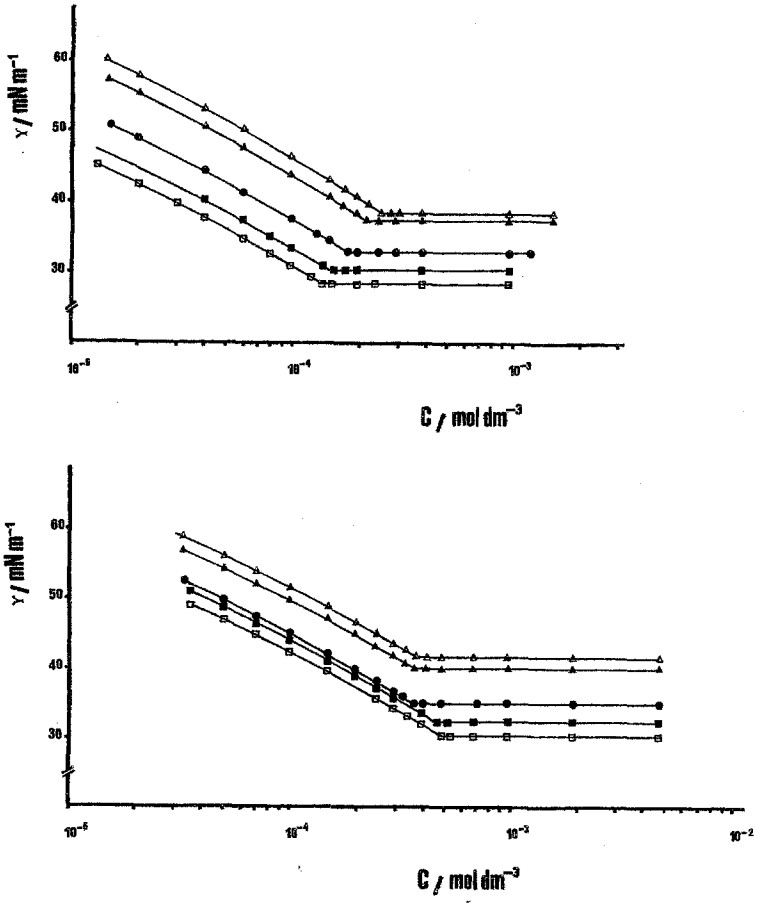

Fig. 5. Plots of the surface tensions of OP-8.5 EO (□), OP-9.5 EO (■), OP-12.5 EO (●), OP-15.0 EO (▲) and OP-20.0 EO (△) (Fig. 5-A) and the surface tensions of liposome/surfactant systems for neutral liposomes (lipid conc. 0.5 mM) and the same surfactants (Fig. 5-B), versus surfactant concentration

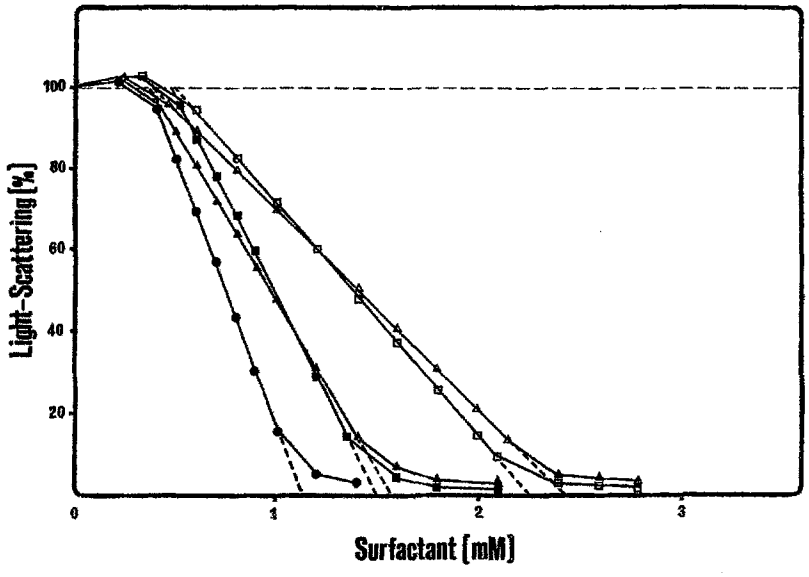

Fig. 6. Percentage changes in light-scattering of neutral liposomes (lipid concentration 0.5 mM), versus surfactant concentration in the presence of OP-8.5 EO (□), OP-9.5 EO (■), OP-12.5 EO (●), OP-15.0 EO (▲) and OP-20.0 EO (△) surfactants

concentration 0.5 mM). We can see here that the surface tensions also decrease as the surfactant concentration increases also with an abrupt inflexion point. This concentration can be regarded as a physico-chemical parameter correlated with the critical micellar concentration of the surfactant/phospholipid binary systems which in the present work is expressed as the CMC_{system}. Similar behavior was observed when treating electrically charged liposomes (PC/PA or PC/SA molar ratio 9:1) with the surfactants in the same conditions.

Comparing the CMC and CMC_{system} values (Figs. 5-A and 5-B), it may be observed that an increasing concentration of lipids in bilayers increases the surfactant concentrations needed to achieve the corresponding CMC_{system}. These displacements can be attributed to the interactions of both components involved in the mixed micelle formation which finally lead to the solubilization of the system. Likewise, in all cases, slightly increased surface tension values in the CMC_{system} are obtained with respect to those corresponding to the single surfactants in their CMC.

Figure 6 shows the solubilization curves of liposome suspensions (lipid composition PC 100%, 0.5 mM) due to the addition of different amounts of the octylphenols tested. When comparing Figs. 5-B and 6, it is interesting to note that the CMC_{system} values corresponded, approximately to the S_{sat} values, i.e., the surfactant concentration producing bilayer saturation of these systems. The results obtained for neutral and electrically charged liposomes are given in Table 2.

Table 2. Surfactant molar concentration corresponding to the S_{sat} values (lipid composition PC 100% or PC/PA and PC/SA 9:1 molar ratio, at 0.5 mM lipid concentration) and the corresponding CMC_{system} values for each surfactant/liposome system

	Bilayer lipid composition					
	PC:PA (9:1)		Egg PC		PC:SA (9:1)	
	S_{sat} (mM)	CMC_{system} (mM)	S_{sat} (mM)	CMC_{system} (mM)	S_{sat} (mM)	CMC_{system} (mM)
OP-8.5 EO	0.50	0.51	0.49	0.50	0.51	0.50
OP-9.5 EO	0.46	0.47	0.47	0.48	0.47	0.49
OP-12.5 EO	0.37	0.39	0.37	0.36	0.37	0.39
OP-15.0 EO	0.36	0.35	0.37	0.38	0.37	0.38
OP-20.0 EO	0.36	0.34	0.38	0.38	0.37	0.35

In the light of this agreement and bearing in mind that Lichtenberg [2] postulated the solubilization of liposomes by surfactants via the formation of mixed micelles between both components, we can assume that the CMC_{system} parameter corresponds to the CMC of mixed micelle formation during solubilizing processes.

Particle size distribution of liposomes during the solubilizing processes

The variations in mean vesicle size distribution of neutral liposomes (5.0 mM lipid concentration) treated with the OP-12.5 EO at different concentrations are plotted in Fig. 7. The five surfactant/liposome systems studied correspond to the five points marked with a circle in Fig. 1 which represent the following *Re* ratios: 1) 0.06; 2) 0.32; 3) 0.5; 4) 1.1; and 5) 2.6.

It should be noted that point 1 shows a single distribution (mean vesicle size about 100 nm and polydispersity index 0.150). Similar tendency is observed in the size distribution curve corresponding to the point 2, in which the vesicle size was slightly increased (about 120 nm and polydispersity index 0.198). This increment is due to the incorporation of surfactant monomers into the vesicle membranes. Curves corresponding to point 3 show a bimodal distribution with an increased polydispersity index (mean vesicle size distribution about 111 nm, and polydispersity index 0.295). It is noteworthy that a sharp distribution curve appears approximately at 40–50 nm which corresponds to a new particle size distribution. Curves corresponding to point 4 showed similar tendencies to those observed in point 3 with an increase in the low particle size distribution (mean particle size about 95 nm and polydispersity index 0.278). Finally, the curves of point 5 again show a single distribution with a mean particle size distribution of about 35 nm and polydispersity index 0.184.

Regarding the results given in Figs. 1 and 7 it is noteworthy that the solubilization of liposomes by octylphenol polyethoxylated leads to the progressive formation of a low particle size distribution which appears to be related to the decrease in the light-scattering of the surfactant/liposome systems during the solubilizing process. The presence of these particles at the end of the process could be considered as an indication of the formation of

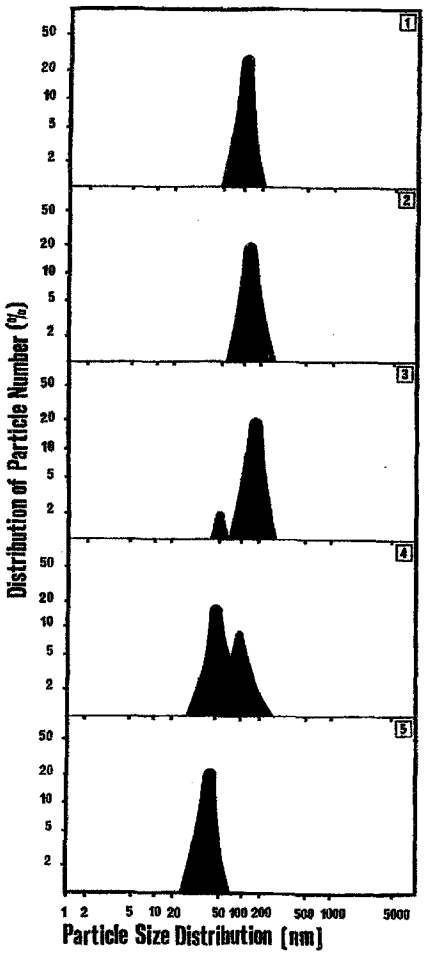

Fig. 7. Mean vesicle size distribution of neutral liposomes (5.0 mM lipid concentration treated with OP-12.5 EO surfactant at different surfactant concentrations during the solubilizing process

surfactant/phospholipid mixed micelles. Similar results were obtained when treating liposomes with all the surfactant tested under the same conditions.

Conclusions

Lichtenberg and his co-workers in their review [10] express the need for experimental data to correlate the surfactant CMC with its solubilizing power. From our results, we conclude that in solubilizing processes of liposomes by different octylphenols the concentration of the monomeric surfactants in equilibrium with the saturated

bilayers (S_a), half the amount of total mixed micelles (S_b) or total mixed micelles (S_c) is always similar to the CMC values of surfactants. Moreover, the most striking result is the observation that the Re_{sat} parameters are inversely correlated with the CMC of the octylphenols tested, whereas the $Re_{50\%}$ and the Re_{sol} values are not directly dependent on these CMC values, showing a minimum for OP-12.5 EO (theoretical HLB 14.6), regardless of the liposome charge. Consequently, the OP-12.5 EO surfactant appears to be the most effective for liposome solubilization despite the fact that it needs a higher number of molecules to saturate bilayers (per mol of phospholipids) than the OP-15 EO and the OP-20 EO surfactants.

Furthermore, the CMC_{system} can be regarded as an interesting value related to the surfactant monomeric concentration needed to initiate the mixed micelle formation. The correlation existing between the CMC_{system} and the S_{sat} surfactant concentration support the validity of the three-stage model for liposome solubilization due to the homologous series of octylphenol polyethoxylated surfactants. In this connection, we suggest that liposome solubilization by surfactants should be studied taking into account the correlation with the CMC of the single surfactants as well as the more specific physico-chemical properties of the new mixed micelles formed between phospholipids present in the bilayer and surfactants during the solubilizing processes.

Acknowledgements

This work was supported by funds from D.G.I.C.Y.T. (Dirección General de Investigación Científica y Técnica) (Prog No PB91-0065), Spain. The authors acknowledge the expert technical assistance of Mr. G. von Knorring.

References

1. Helenius A, Simons K (1975) Biochim Biophys Acta 415:29–79
2. Lichtenberg D, Robson RJ, Dennis EA (1983) Biochim Biophys Acta 737:285–304
3. Ollivon M, Eidelman O, Blumenthal R, Walter A (1988) Biochemistry 27:1695–1703
4. Keren-Zur M, Beigel M, Loyter A (1989) Biochim Biophys Acta 983:253–258
5. da Graça Miguel M, Eidelman O, Ollivon M, Walter A (1989) Biochemistry 28:8921–8928
6. Levy D, Gulik A, Seigneuret M, Rigaud JL (1990) Biochemistry 29:9480–9488
7. Almog S, Litman BJ, Wimley W, Cohen J, Wachtel EJ, Barenholz Y, Ben-Shaul A, Lichtenberg D (1990) Biochemistry 29:4582–4592
8. Edwards K, Almgren M (1991) J Colloid Interface Sci 147:1–21
9. Inoue T, Yamahata T, Shimozawa R (1992) J Colloid Interface Sci 149:345–358
10. Lichtenberg D (1985) Biochim Biophys Acta 821:470–478
11. De la Maza A, Parra JL, García MT, Ribosa I, Sanchez Leal J (1992) J Colloid Interface Sci 148:310–316
12. Ruderman G, Grigera JR, (1986) Biochim Biophys Acta 863:277–281
13. Alonso A, Urbaneja MA, Goñi FM, Carmona FG, Cánovas FG, Gomez-Fernandez J (1987) Biochim Biophys Acta 902:237–246
14. Sappey Marinier D, Letoublon R, Delmau J (1988) J of Lipid Res 29:1237–1243
15. Ruiz J, Goñi FM, Alonso A (1988) Biochim Biophys Acta 937:127–134
16. Hernandez-Borrell J, Pons M, Juarez JC, Estelrich J (1990) J of Microencapsulation 7:255–259
17. Edwards K, Almgren M (1990) Prog Colloid Polym Sci 82:190–197
18. Kamenka N, El-Amrani M, Appell J, Lindheimer M (1991) J Colloid Interface Sci 143:463–471
19. Urbaneja MA, Alonso A, Gonzalez-Mañas JM, Goñi FM, Partearroyo MA, Tribout M, Paredes S (1990) Biochem J 270:305–308
20. Singleton WS, Gray MS, Brown ML, White JL (1965) J Am Oil Chem Soc 42:53–56
21. Paternostre MT, Roux M, Rigaud JL (1988) Biochemistry 27:2668–2677
22. Rigaud JL, Paternostre MT, Bluzat A (1988) Biochemistry 27:2677–2688
23. Szoka F, Papahadjopoulos D (1981) In: Knight CG, (ed), Liposomes: Preparation and Characterization. Elsevier, North-Holland, Amsterdam, Chap 3.
24. Allen TM (1986) In: Gregoriadis G (ed) Liposome Technology. CRC Press, Boca Raton, FL, Vol I Chap 8
25. Mayer LD, Hope MJ, Cullis PR (1986) Biochim Biophys Acta 858:161–168
26. Standard Methods (1976) 14th ed Amer Public Health Ass, Washington, pp 466–484
27. Goñi FM, Urbaneja MA, Arrondo JLR, Alonso A, Durrani AA, Chapman D (1986) Eur J Biochem 160:659–665
28. Lunkenheimer K, Wantke D (1981) Colloid and Polymer Sci 259:354–366
29. Egan RW (1976) J Biol Chem 251:4442–4447

Received May 6, 1993
accepted August 4, 1993

Authors' address:

Dr. A. de la Maza
Departamento de Tensioactivos
Centro de Investigación y Desarrollo (C.I.D.)
Consejo Superior de Investigaciones Científicas (C.S.I.C.)
Calle Jorge Girona, 18–26, 08034 Barcelona, Spain