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Direct formation of mixed micelles in the solubilization of phospholipid liposomes by Triton X-100

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Abstract The vesicle to micelle transition which results in the interaction of the Triton X-100 surfactant with phosphatidylcholine vesicles was studied by means of dynamic light scattering (at different reading angles) and by freeze-fracture electron microscopy techniques. Vesicle solubilization was produced by the direct formation of mixed micelles without the formation of complex intermediate aggregates. Thus, vesicle to micelle transformation was mainly governed by the progressive formation of mixed micelles within the bilayer. A subsequent separation of these micelles from the liposome surface (vesicle perforation by the formation of surfactant-stabilized holes on the vesicle surface) led to a complete solubilization of liposomes.

Key words: Freeze-fracture electron microscopy; Dynamic light scattering variation; Triton X-100; Phosphatidylcholine liposome; Liposome solubilization; Vesicle-micelle structural transition

1. Introduction

The vesicle to micelle transformation induced by the addition of surfactants to phospholipid vesicles is currently attracting much interest [1–5]. This process leads to solubilization of lipid vesicles and it represents a good model for the solubilization of cell membranes. Vesicle to micelle transformation is also of interest for the problems it raises concerning the packing of phospholipids and surfactants into mixed aggregates. The nonionic surfactant Triton X-100 (T_{X-100}) has, given its properties as a good solubilization agent of membrane proteins, been the subject of a number of studies [6–9].

In earlier papers we studied the interaction of different surfactants with phosphatidylcholine (PC) liposomes [10–14]. In the present work we seek to characterize topologically in detail the vesicle-micelle transition involved in the interaction of T_{X-100} with PC liposomes. To this end, dynamic light scattering technique (DLS), and freeze-fracture applied to transmission electron microscopy (FFEM) were employed. The use of these two specific techniques for measuring and visualizing small and large particles in the same sample open up new possibilities in the study of the vesicle to micelle transition.

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Abbreviations: T_{X-100}, Triton X-100; PC, phosphatidylcholine; DLS, dynamic light scattering; FFEM, freeze-fracture electron microscopy; HD, hydrodynamic diameter; PI, polydispersity index; Re_{SAT}, surfactant/lipid molar ratio for bilayer saturation; Re_{SOL}, surfactant/lipid molar ratio for bilayer solubilization

2. Materials and methods

PC was purified from egg lecithin (Merck, Darmstadt, Germany) by the method of Singleton [15] and was shown to be pure by TLC. Triton X-100 (octhylphenol polyethoxilated with 10 units of ethylene oxide and active matter of 100%) was purchased from Rohm and Haas (Lyon, France). Tris(hydroximethyl)-aminomethane (TRIS buffer) obtained from Merck was prepared as 5.0 mM TRIS buffer adjusted to pH 7.4 with HCl, containing 100 mM of NaCl. Liposomes of a defined size (about 200 nm) were prepared by extrusion of large unilamellar vesicles (through 800–200 nm polycarbonate membranes) previously obtained by reverse phase evaporation [16]. Surfactant solutions were added to the liposomes (PC concentration 3.5 mM) and the resulting mixtures were left to equilibrate for 24 h [11].

The hydrodynamic diameter (HD) and polydispersity index (PI) of the liposomes after preparation and the particles formed during the interaction of T_{X-100} with these bilayer structures were determined by means of a DLS technique using a photon correlator spectrometer (Malvern Autosizer 4700c PS/MV) equipped with an Ar laser source (wavelength 488 nm). Quartz cuvettes were filled with the samples and all the experiments were thermostatically controlled. All the experiments were performed at 90° and some of them were carried out using other angles (60° and 120°). The analysis of the data thus obtained was performed using the version of the program CONTIN provided by Malvern Instruments, England.

Freeze-fracture electron microscopy (FFEM) study, was done according to the procedure described by Egelhaaf et al. [17]. About 1 μl of suspension was sandwiched between two copper platelets using a 400-mesh gold grid as spacer. Then the samples were frozen in a propano-jet at −180°C and fractured at −150°C and 2×10^{-7} mbar in a Balzers BAF 300 freeze-fracturing apparatus (BAL-TEC, Liechtenstein). The replicas were obtained by unidirectional shadowing with 2 nm of Pt/C and 20 nm of C, and they were floated on distilled water and examined in a Philips EM 301 electron microscopy at 80 kV.

3. Results and discussion

The variations of DLS values (at a reading angle of 90°) for small and large particles vs. total T_{X-100} concentration are plotted in Fig. 1. The data for ten representative surfactant/PC systems are also given in Table 1. Size distribution curves of micellar T_{X-100} solutions before mixing with liposomes showed a peak at 10 nm (monomodal distribution, PI, 0.122) and for pure liposomes a monomodal distribution was also obtained with an HD of 160 nm and a PI of 0.134 (point 1).

Three relevant transition steps were detected in Fig. 1: First, lower surfactant concentration than 2.2 mM led to a slight increase in the size of vesicles (surfactant-lipid mixed vesicles) reaching a maximum HD (peak at 200 nm) at point 2. This growth is attributable to the incorporation of surfactant monomers in PC vesicles, in agreement with other authors [1,2,4,9]. The maximum HD corresponded to the satu-

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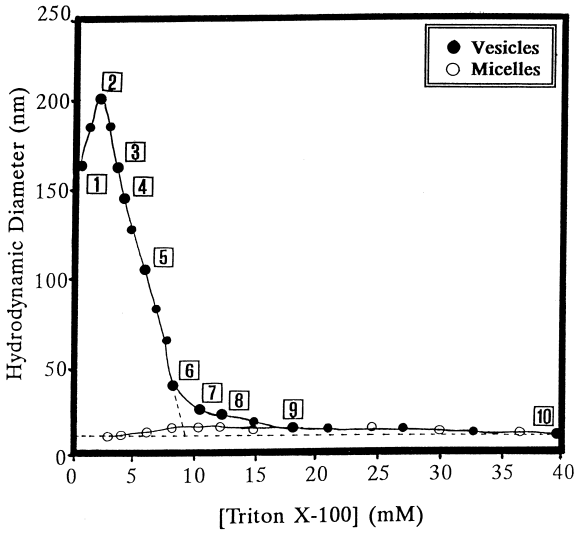


Fig. 1. Variation in the particle size (nm) corresponding to the vesicles (closed circles) and micelles (open circles) in the surfactant/PC systems versus the surfactant concentration (mM). The PC concentration in liposomes was 3.5 mM.

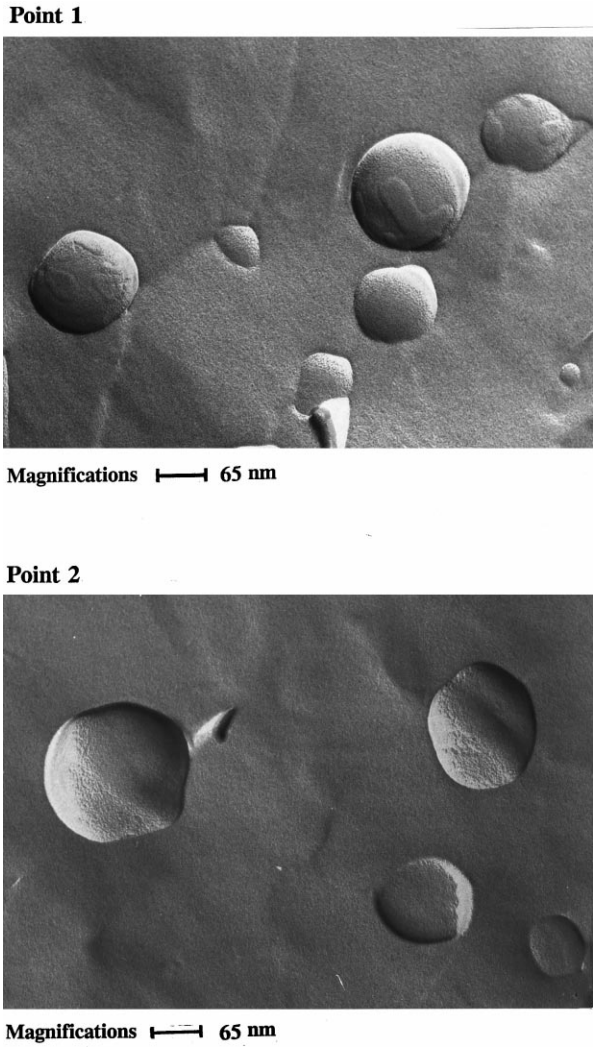


Fig. 2. FFEM micrographs of seven surfactant/PC systems corresponding to the points 1, 2, 3, 5, 6, 8 and 10 of Fig. 1. Point 11 corresponds to a sample that contains pure T_{X-100} (10 mM). Structures are marked as follows: mixed micelles with arrows and vesicle fragments with arrowheads.

ration of bilayers, in accordance with our earlier studies for this system [11] (surfactant/lipid molar ratio for bilayer saturation, Re_{SAT}, defined by Lichtenberg [18]).

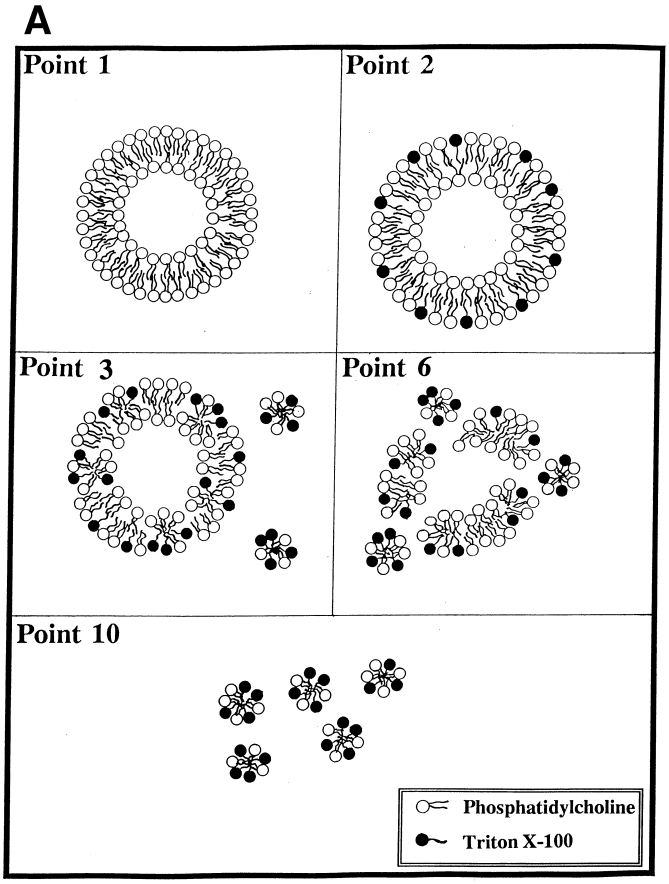
Second, when the T_{X-100} concentration exceeded 3.3 mM (point 3), a new peak in the size distribution curve appeared (11.8 nm) for the formation of surfactant-lipid mixed micelles. Increasing surfactant amounts led to a progressive increase in intensity in this new peak and a decrease in that for mixed vesicles. Thus, from points 3 to 8 the size of small and large particles slightly rose and fell respectively as the T_{X-100} amount in the system rose. Furthermore, a considerable rise in the proportion of small particles took place. Thus, a coexistence domain is depicted in this transition stage from mixed vesicles into mixed micelles without the formation of intermediate complex aggregates.

The determination by graphical methods of the T_{X-100} concentration for complete liposome solubilization (smallest particles, Re_{SOL}, [18]) showed a value of 9.5 mM, which is in accordance with that reported for this system [11]. It is noteworthy that particles of two different sizes (mixed vesicles and mixed micelles) were still detected at this interaction step (see Fig. 1). This coexistence indicates that a higher surfactant concentration than that reported using turbidity measurements [11] was needed for complete liposome solubilization.

Table 1
DLS data for a scattering angle of 90° corresponding to different steps of the interaction of T_{X-100} with PC liposomes (3.5 mM PC)

Sample	Surfactant concentration (mM)	Curve distribution (particle number)					Average mean (nm)	Polydispersity index
		Type	1st peak		2nd peak			
			nm	%	nm	%		
1	–	M	–	–	160	100	160	0.134
2	2.2	M	–	–	200	100	200	0.207
3	3.3	B	11.8	5.2	163	94.8	155.1	0.321
4	4.1	B	12.7	13.7	145.5	86.3	127.3	0.330
5	6.2	B	15.4	12.6	105.3	87.4	93.9	0.321
6	8.6	B	16.9	14.2	39.8	85.8	36.5	0.327
7	10.5	B	17.1	40.5	27.5	59.5	23.2	0.330
8	12.3	B	17.0	88.4	24.6	11.6	17.9	0.336
9	18.1	M	17.0	100	–	–	17.1	0.237
10	40	M	10	100	–	–	10	0.145

The ten samples are numbered in accordance with the surfactant/PC systems indicated in Fig. 1.
M, monomodal; B, bimodal.



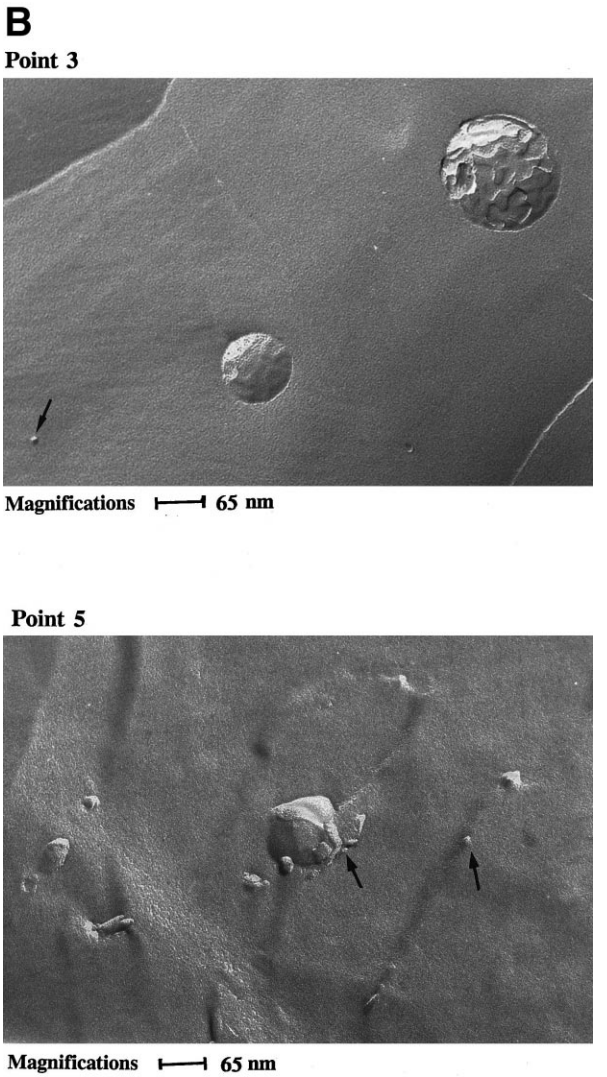


Fig. 3. Representative cartoons corresponding to the structures proposed for the sequential states of the lipid-surfactant interactions during the vesicle to micelle transformation. The draws are numbered in accordance with the surfactant/PC systems indicated in Fig. 1 and corresponding to the pictures of Fig. 2.

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Finally, higher T_{X-100} concentration than 18.1 mM showed a monomodal distribution curve for mixed micelles (point 9 in Table 1). A slight fall in the particle size was noted for the highest surfactant concentration (40 mM, HD 10 nm), which was attributed to the progressive enrichment in T_{X-100} of the mixed micelles formed.

FFEM technique was suitable for topological studies of the aggregates formed in this process without introducing artifacts due to changes in temperature or dehydration. Fig. 2 shows seven TEM pictures corresponding to the most representative points given in Fig. 1.

Particle sizes measured in the micrographs were tested to belong to the log normal distributions obtained by DLS (Table 1) using the Student *t* statistics. On the assumption that no differences in particle size were induced by DLS and FFEM techniques no significant differences between particle size in the micrographs and in the distribution curves were detected, with a probability level always higher than 10%. This percentage means that no evidence of significant differences in particle size obtained with these two techniques can be considered statistically.

For pure liposomes (point 1) TEM picture shows some vesicles with a diameter of about 160 nm. At 2.2 mM T_{X-100} concentration (point 2) large vesicles (about 200 nm) were formed. Picture for point 3 shows vesicles with clear signs of local disintegration ('in situ' perforation). It is noteworthy that this point shows the presence of small particles (arrows) corresponding to surfactant/PC mixed micelles (in accordance with the DLS data). The picture of point 5 also shows the vesicle disintegration and the formation of mixed micelles without intermediate complex aggregates. Although in earlier studies we did not detect small particles at this interaction level (negative staining TEM [11]), the use of FFEM allowed us to visualize these structures, thus corroborating at this step the coexistence of mixed vesicles and mixed micelles. This finding may be regarded as a new approach in the study of this interaction. The picture of point 6 shows the coexistence of mixed micelles (arrows) and vesicle fragments (arrowheads) without intermediate aggregates (in accordance with the data of Table 1). Point 8 shows an increasing number of small particles together with some large structures, whereas the micrograph for the highest T_{X-100} concentration (point 10) shows only the presence of small particles (mixed micelles, Table 1). The micrograph of point 11 (Fig. 2) corresponds to a sample that contains pure T_{X-100} (10 mM). This image has been included in order to compare the differences in the back-

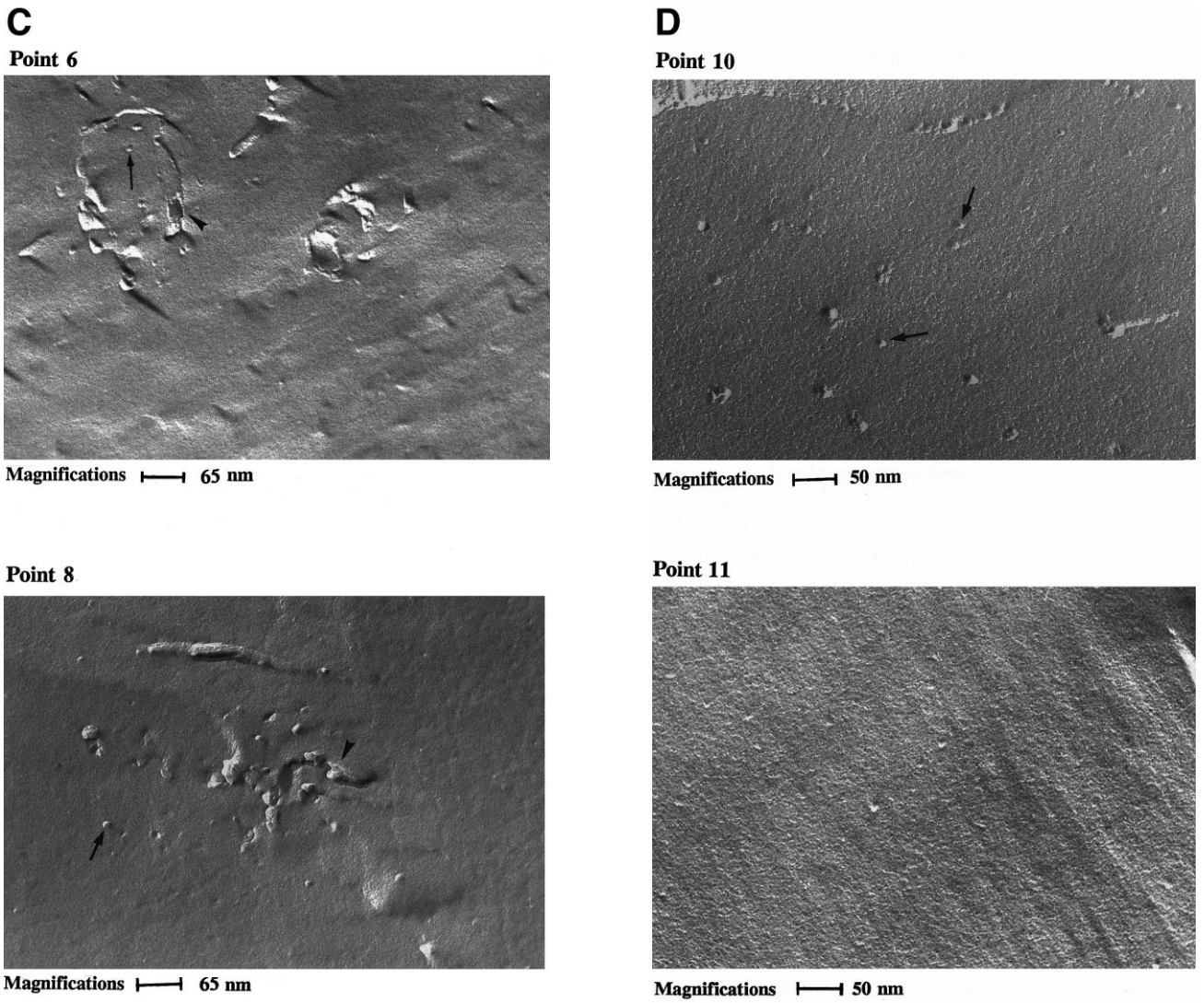

Fig. 3. (*continued*)

ground of the pictures containing increasing T_{X-100} concentrations.

In order to study the shape of the particles formed, DLS experiments were carried out at scattering angles of 60° and 120° for the main systems (points 4, 5, 7, and 9). The results obtained are given in Table 2. These experiments confirmed the angular dependence of the PI and the HD as reported by Egelhaaf et al. [19], as well as the presence of two particle sizes for points 4, 5 and 7 and only one for point 9. These data also reveal that the size for the large mixed vesicles did not change with the scattering angle thus indicating the spherical shape of these bilayer structures. The size of the small particles (mixed micelles) for points 4 and 5 shows variation with the scattering angle, whereas for points 7 and 9 these size variations were negligible. These data indicate that the shape of mixed micelles was elongated before becoming spherical when the

Table 2
DLS data for scattering angle of 60° and 120° corresponding to the more complex samples (4, 5, 7 and 9) in the interaction of T_{X-100} with PC liposomes (3.5 mM PC)

Sample	60°				120°			
	1st peak		2nd peak		1st peak		2nd peak	
	nm	%	nm	%	nm	%	nm	%
4	22.2	11.7	135.6	88.3	14.2	25.2	150.1	74.8
5	14.3	57.4	102.1	42.6	18.4	79.2	107.6	20.8
7	16.1	76.7	31.1	23.3	16.8	78.6	30.6	21.4
9	16.7	100	–	–	17.8	100	–	–

The samples are also numbered in accordance with Fig. 1.

surfactant proportion in the system increased. This finding correlates with that reported by Egelhaaf et al., who described for the interaction of bile salts with lecithin the evolution of vesicles to large worm-like micelles and finally to small spherical micelles [19].

A scheme is proposed for this solubilization process (Fig. 3). As the surfactant molecules are added they are distributed into the lamellar phase of liposomes up to a critical concentration (growth in the vesicle size) (point 2). At this point mixed micelles start to form within the saturated PC bilayer (FFEM image shows vesicles with hole-like formations) and some of them are liberated from the vesicles (point 3). Finally, the number of the micelles detached from the vesicles increase (point 6) up to the complete formation of mixed micelles (point 10). The points marked in each cartoon correspond to the micrographs of Fig. 2.

The use of DSL equipped with an Ar laser source (useful for measuring micelles) employing different angles and FFEM technique (suitable for structural studies without artifacts) allows us to present a new vesicle to micelle transformation mechanism which is more simple than that previously proposed [11]. The new mechanism is based on the fact that no intermediate aggregates between vesicles and micelles were formed during solubilization in contrast to the generally admitted assumption that different aggregates existed between these two structures during solubilization [4]. It is interesting to note that although the shape of the vesicles remained spherical that for the mixed micelles depended on the surfactant concentration in the system. Thus, we propose a liposome solubilization process which is mainly governed by a local disintegration of vesicles ('in situ' bilayers perforation). The formation of mixed micelles within the bilayer and the subsequent separation of these micelles (initially elongated) from the liposome surface led to the formation of surfactant-stabilized holes on the vesicle surface. The end of this process was the complete solubilization of liposomes without the formation of complex structures.

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