Spectroscopy 16 (2002) 343–350
IOS Press

Use of synchrotron radiation SAXS to study the first steps of the interaction between sodium dodecyl sulfate and charged liposomes

O. López [a,*], M. Cócera [a], R. Pons [a], H. Amenitsch [b], J. Caelles [a], J.L. Parra [a], L. Coderch [a] and A. de la Maza [a]

[a] *Departamento de Tecnología de Tensioactivos, Instituto de Investigaciones Químicas y Ambientales de Barcelona (I.I.Q.A.B.), Consejo Superior de Investigaciones Científicas (C.S.I.C.), Calle Jorge Girona 18-26, 08034 Barcelona, Spain*

[b] *IBR, Austrian Academy of Sciences, ELETTRA, Sincrotrone Trieste, S.S. 14 Km 163.5, Bassovizza, 34012 Trieste, Italy*

Abstract. The technique of time resolved small angle X-ray scattering (SAXS) using a synchrotron radiation source was used to study the structural transformations as well as the kinetic associated with the first steps of the solubilization of liposomes induced by the anionic surfactant sodium dodecyl sulfate (SDS). Neutral and electrically charged (anionic and cationic) liposomes were used to investigate the effect of the electrostatic charges on these initial steps. The mechanism that induces the solubilization process consisted in an adsorption of surfactant on the bilayers and a desorption of mixed micelles from the liposomes surface to the aqueous medium. Regardless of the type of charge of the liposome the time needed for the desorption of the first mixed micelles was shorter than that for the complete adsorption of the surfactant on the liposomes surface. The present work demonstrates that the adsorption of the SDS molecules on liposomes was slower when the charges of surfactant and lipids were the same. As for the release of mixed micelles from the surface of these liposomes, this process was slower when the charges of surfactant and lipids were opposite.

Keywords: Liposome solubilization, sodium dodecyl sulfate surfactant, electrostatic charges in liposome, kinetic of solubilization, small angle X-ray scattering, stopped flow-time resolved

Abreviations

CMC, critical micellar concentration; PA, phosphatidic acid; PC, phosphatidylcholine; SA, stearylamine; SAXS, small angle X-ray scattering; SDS, sodium dodecyl sulfate; TRIS, tris(hydroximethyl)-aminomethane.

1. Introduction

It is well known that surfactants solubilize lipid membranes that may contains ionic lipids. Thus, the study of the charge effect on the liposome solubilization is essential to the understanding of biological

*Corresponding author. Tel.: +34 93 400 61 61; Fax: +34 93 204 59 04.

processes in which charges are involved [1,2]. The effect of the electrostatic charge is difficult to evaluate due to the lack of techniques with experimental timescale short enough, especially at a subsolubilizing level of the interaction liposome–surfactant. The same problem exists in the study of the kinetics associated with this process of solubilization. Several works based on spectroscopy techniques have reported decisive aspects of the dynamic of this process as a whole [3,4] however data about the kinetic associated with the fast initial steps of the solubilization are lacking. Thus, the develop and application of techniques using synchrotron radiation, which can provide high time resolution are increasingly important.

In previous papers we investigated the solubilization of liposomes by surfactants from a structural viewpoint [5,6] that raised a number of questions about the kinetic. Dynamic light scattering (DLS) and freeze fracture electron microscopy (FFEM) techniques were performed to investigate this kinetic aspect [7,8], and more recent papers have attempted to deep in this topic [9,10]. However, the initial steps of solubilization were too rapid to be properly detected. In this work, we seek to describe the effect of the electrostatic charge on the initial steps of the liposome solubilization induced by the sodium dodecyl sulfate surfactant (SDS) from mechanistic and kinetic viewpoints. To this end, the technique of small angle X-ray scattering (SAXS) for time resolved using a stopped flow cell and synchrotron radiation was used. This technique has demonstrated to be a very good method to study the phase behaviour of lipid–surfactant systems and to monitor very fast biological processes [11,12]. Thus, the use of this sensitive methodology opens up new possibilities for studying processes with very fast kinetics.

2. Materials and methods

Phosphatidylcholine (PC) was obtained from Lipoid GmbH (Ludwigshafen, Germany). The anionic lipid phosphatidic acid (PA) and the cationic lipid stearylamine (SA) were purchased by Sigma Chemicals Co. (St. Louis, MO). Tris(hydroxymethyl)-aminomethane (TRIS) was obtained from Merck (Darmstad, Germany). TRIS buffer was prepared as 5.0 mM TRIS adjusted to pH 7.4 with HCl, containing 100 mM of NaCl. The anionic surfactant sodium dodecyl sulfate (SDS) was obtained from Sigma Chemicals Co. The surface tensions of buffered solutions containing increasing amounts of SDS were measured by the ring method using a Krüss tensiometer. The surfactant critical micelle concentration (CMC) was determined from the abrupt change in the slope of the surface tension values versus surfactant concentration [13] showing a value of 0.75 mM. Reagent grade organic solvents were purchased from Merck. Polycarbonate membranes and membrane holders were purchased from Nucleopore (Pleasanton, CA).

2.1. Liposome preparation

A lipidic film was formed by removing the organic solvent by rotary evaporation from a chloroform containing the lipid solution. Liposomes of a defined size (about 200 nm) were prepared by extrusion of large vesicles (through 800–200 nm polycarbonate membranes) [14] previously obtained by hydration of the lipidic film with TRIS buffer. Neutral liposomes were formed only by PC. In order to obtain liposomes with anionic or cationic character a percentage of 5% (w/w) with respect to the total lipid amount of PA and SA were added.

2.2. Stopped-flow experiments and SAXS analyses

Buffered solutions containing SDS were added to neutral, anionic or cationic liposomes (total conc. 30 mM lipid and 240 mM SDS). The interaction between the liposomes and the SDS was monitored by

obtaining X-ray scattering curves every 1 sec for 300 sec. Based on a previous study [7,8] we assume that the first steps of the solubilization takes place during the first minutes of interaction of liposomes and surfactant. For this reason we limit the study of the systems to the first five minutes. The experiments were performed on a stopped-flow cell on a Bio-Logic Stopped Flow Module (SMF), which was fully computer-controlled. The stopped-flow portion of the apparatus comprised four pneumatically driven feed syringes, which were thermostated using a recirculating water bath to $25.0 \pm 0.1^{\circ}$C. The solutions were driven from these syringes through a mixing cell to a quartz capillary observation cell. The time-resolved X-ray diffraction data were collected at the SAXS beam line at the Synchrotron radiation source Elettra (Trieste, Italty) containing 1024 channels and 2 GeV electron storage ring. The SAXS measurements were performed with a resolution of 1–140 nm in real-space, with acquisition times of 1 sec in time resolved measurements and 100 sec for static measurements. The radiation wavelength was 1.542 Å. The data were collected on a linear position-sensitive Gabriel detector, which enabled simultaneous detection of the whole resolution range. The scattering intensities were plotted as a function of the scattering vector q defined as $q = (4\pi \sin\theta)/\lambda$, in which θ is the scattering angle and λ the wavelength. The positions of the diffraction peaks are directly related to the repeat distance of the molecular structure, as described by Braggs law $2d \sin\theta = n\lambda$, in which n and d are the order of the diffraction peak and the repeat distance, respectively. In the case of a lamellar structure, the various peaks are located at equidistant positions, then $q_n = 2\pi n/d$, q_n being the position of the nth order reflection. A simulation program allow us to attribute the diffraction peaks to the different structures presents in the study.

3. Results and discussion

3.1. SAXS experiments

Three types of unilamellar liposomes (lipid conc. 30 mM) were used: neutral (PC liposomes), anionic (PC/PA liposomes) and cationic liposomes (PC/SA liposomes). These liposomes and pure SDS micelar solutions (conc. 240 mM) were analyzed by SAXS. In the same way, mixtures of SDS and neutral, anionic or cationic liposomes at the same concentrations were studied five minutes after mixing, when the first steps of solubilization had occurred [7]. The recorded X-ray scattering patterns for the three types of liposomes and for the SDS micellar solution are shown in Fig. 1. It may be seen one diffraction peak on each scattering curve at q values of 0.094, 0.094 and 0.087 Å^{-1}, for the PC, PC/PA, PC/SA liposomes, respectively. The goodness in the fit with the simulation model led us to attribute these peaks to lamellar phases with spacings of 67, 67 and 72 Å, which were associated with the thickness of the bilayer of the PC, PC/PA and PC/SA liposomes, respectively. These data are in agreement with the usual width described previously for phospholipid bilayers and specifically with the results reported by other authors, in which diffraction techniques were used for measuring this parameter [15]. The fact that this value was similar for the three types of liposome indicates that the electrostatic charge did not affect the thickness of the bilayer. Figure 1 also shows the scattering curves of the SDS micellar solution, which presents a peak located at $q = 0.145$ Å^{-1}, for a spacing of 43 Å. Data obtained in previous papers about the diameter of the SDS micelles (40 Å) and the good fit with the simulation of a spherical model allowed us to attribute this spacing to the diameter of the pure SDS micelles [6].

A scheme summary with the different particles present in the three liposome–surfactant systems studied (PC-SDS, PC/PA-SDS, PC/SA-SDS) after the most representative incubation times is shown in the Fig. 2 from these data different periods of time associated with the first steps of solubilization could

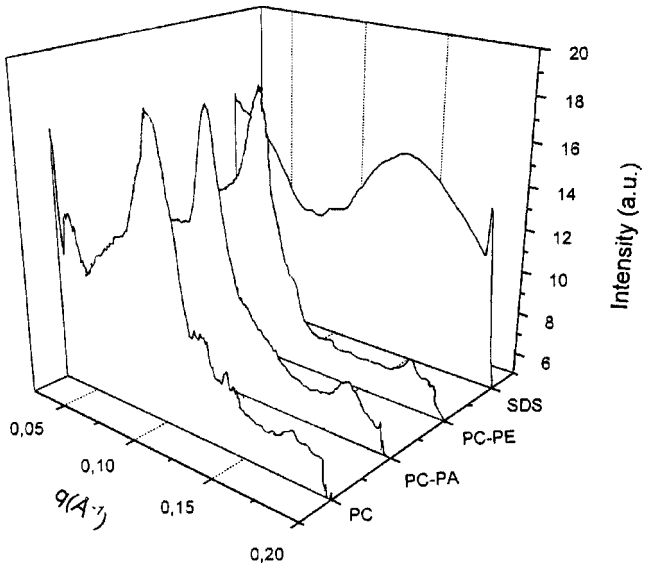

Fig. 1. X-ray scattering curves for the neutral (PC), anionic (PC/PA) and cationic (PC/SA) liposomes, as well as for the pure SDS micelles.

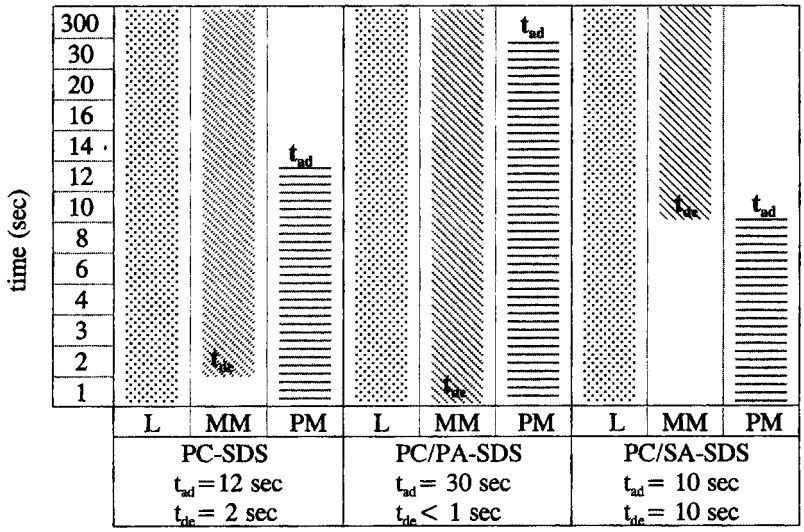

Fig. 2. Scheme summary showing the most representative times after mixing PC, PC/SA and PC/PA liposomes with SDS. The three columns indicate the different particles (L: liposomes; MM: mixed micelles and PM: pure SDS micelles) present in the three systems. The values corresponding to the t_{ad} and to the t_{de} for the three systems are also shown in this scheme.

be established. The adsorption time (t_{ad}) was the time needed for the complete adsorption of surfactant on the liposome surface, and the desorption time (t_{de}) was that needed for the detach of the first mixed micelles from the liposome surface. The t_{ad} and t_{de} values for the three systems are also shown in Fig. 2. The scattering curves for the three liposome–surfactant mixtures, PC-SDS, PC/PA-SDS, PC/SA-SDS, 300 sec after mixing showed peaks at q values of 0.118, 0.118 and 0.114 $Å^{-1}$, respectively (spacings of 53, 53 and 55 Å), which were associated with the diameter of the mixed micelles (Figs 3, 4 and 5).

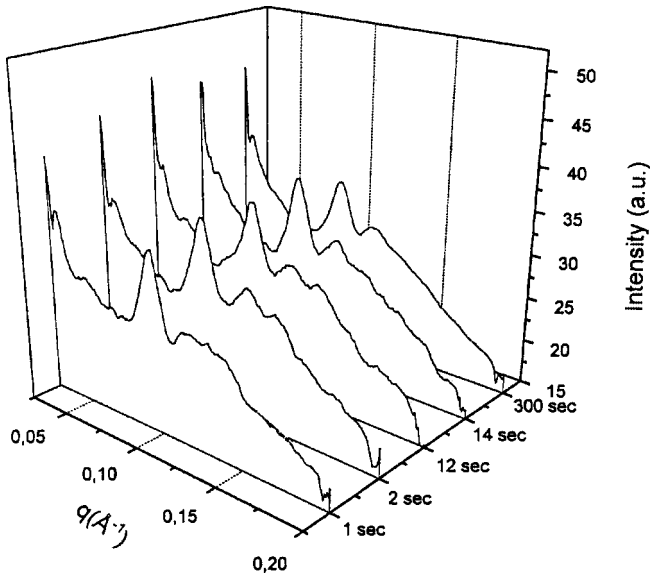

Fig. 3. X-ray scattering patterns for the system neutral liposome (PC) and SDS after different times of mixing, these times represent the most relevant stages at which changes in number and position of the diffraction peaks were detected.

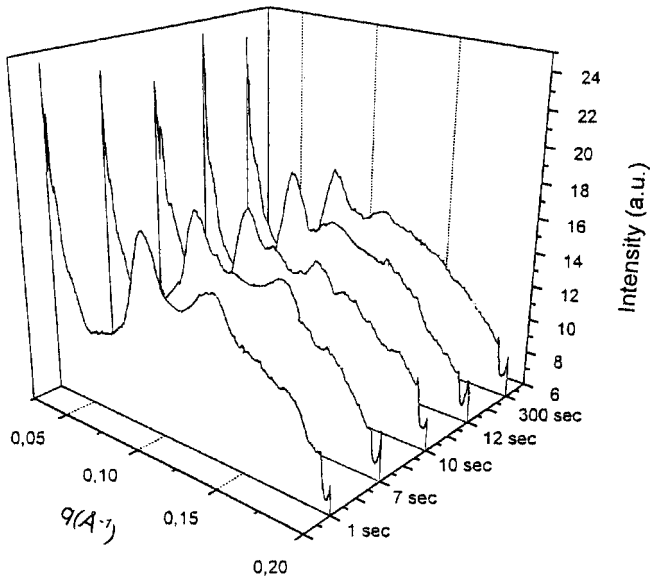

Fig. 4. X-ray scattering patterns for the system anionic liposome (PC/PA) and SDS after different times of mixing, these times represent the most relevant stages at which changes in number and position of the diffraction peaks were detected.

Figure 3 plots the X-ray scattering curves for the system PC-SDS (neutral liposomes and anionic surfactant) after different times from mixing. These times represent the most relevant stages at which changes in the number and position of the diffraction peaks were detected. The scattering curve one sec after mixing shows two peaks, at q values of 0.094 $Å^{-1}$ (for a spacing of 67 Å) and 0.145 $Å^{-1}$ (for a spacing of 43 Å). The first peak was associated with the thickness of the bilayer and coincides with

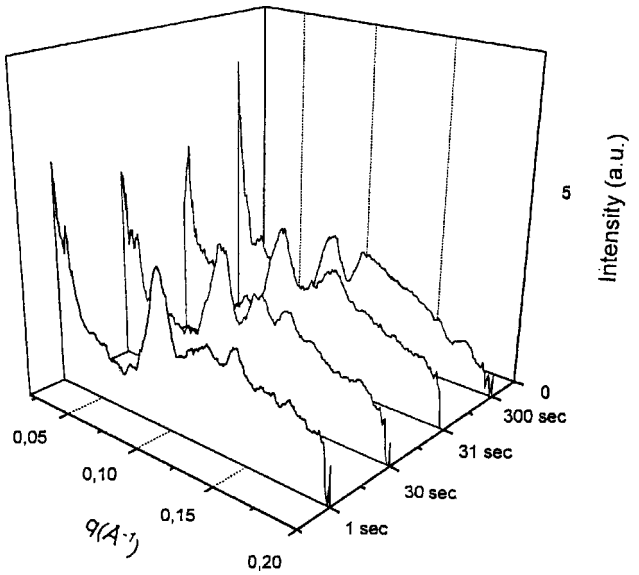

Fig. 5. X-ray scattering patterns for the system cationic liposome (PC/SA) and SDS after different times of mixing, these times represent the most relevant stages at which changes in number and position of the diffraction peaks were detected.

the q value of the Fig. 1 (PC). The second peak was attributed to the diameter of pure SDS micelles, since its position is very similar to that obtained for the pure micelles (Fig. 1, SDS). A third peak was detected two seconds after mixing at $q = 0.118$ Å^{-1} (spacing 53 Å). We attributed this peak to the presence of mixed micelles that were almost spherical given the good fit with the spherical model and in accordance with the size of the mixed micelles detected by DLS in a previous work [6]. These three peaks remained in the system until 12 sec after mixing liposomes and SDS. From this time only two peaks were present in the spectra (Fig. 2). All through the experiment the relative intensity of the peak for the spacing of 67 Å (liposome) decreased with time whereas that of 53 Å (mixed micelles) increased indicating the solubilization of the liposomes by mixed micelles formation. The time needed for the complete adsorption of surfactant on the liposome surface (t_{ad}) was 12 sec given that around this period of time the peak for the pure micelles tended to disappear (Fig. 2). The peak associated with the mixed micelles was detected from 2 sec after mixing. As a consequence, we consider this time as that needed for the first mixed micelles starting to desorpt (t_{de}, Fig. 2). Figure 4 plots the scattering curves for different times after mixing PC/PA liposomes and SDS (anionic liposome and anionic surfactant). It can be seen that until 30 sec three peaks were observed in the spectra corresponding to the thickness of liposome bilayer at $q = 0.094$ Å^{-1} (spacing 67 Å), to the pure SDS micelles at $q = 0.145$ Å^{-1} (spacing 43 Å) and to the mixed micelles at $q = 0.118$ Å^{-1} (spacing 53 Å). From 31 sec until the end of the experiment (300 sec) only two peaks were present, those associated with the liposome bilayer and with the mixed micelle diameter. Thus, it is interesting to note that the t_{ad} was in this case 30 sec (Fig. 2). The mixed micelles were detected from the first second, that is, the t_{de} was lesser than 1 sec (Fig. 2). As in the case of the neutral liposome (Fig. 3), the intensity of the peak corresponding to the thickness of liposome decreased in intensity through the experiment indicating the progressive solubilization of the bilayer by action of the SDS. Regarding to the experiments performed with the cationic liposomes and the SDS (system PC/SA-SDS) the different scattering curves are depicted in Fig. 5. Here, it can be seen that for times lower than 10 sec only two peaks were detected in the scattering curves, those associated with

the liposome ($q = 0.087$ Å^{-1}, spacing 72 Å) and with the pure surfactant micelles ($q = 0.145$ Å^{-1}, spacing 43 Å). X-ray scattering data collected at times above 10 sec showed also two peaks, that for the liposomes and that corresponding to the mixed micelles ($q = 0.1184$ Å^{-1}, spacing 55 Å^{-1}). Thus, all these data indicate that, for this system, both the t_{ad} and the t_{de} were 10 sec (Fig. 2).

These results indicate that although the t_{ad} and the t_{de} were different depending on the charges present in each system, the mechanism involved in the saturation of vesicles by SDS was similar in all cases. This mechanism consists in a surfactant adsorption on the liposome surface and a mixed micelles desorption from the liposome surface to the medium. Although it is reasonable to think that the adsorption process is previous to the desorption one, the t_{ad} and t_{de} values indicate that these processes were simultaneous at least for the system PC/PA-SDS and in a minor degree for the PC-SDS system ($t_{de} < t_{ad}$). The PC/SA-SDS system was the only that required a complete adsorption of the SDS molecules on the liposomes for starting the desorption process ($t_{de} = t_{ad}$).

It is interesting to note that both t_{ad} and t_{de} depended on the electrostatic charge of the species involved in the systems, that is, the electrostatic charge associated with the surfactant and with the lipids. Thus, our results indicate that the shortest t_{ad} corresponds to the system composed by PC/SA liposomes and SDS. In other words, the adsorption process is faster when the electrostatic charges of the lipid and surfactant are opposite. In connection with this, for the system PC/PA-SDS (lipid and surfactant with the same type of charge) the process of surfactant adsorption resulted the slowest ($t_{ad} = 30$ sec). As regards to the system in which neutral lipids were used to form the liposomes, the t_{ad} showed an intermediate value (12 sec). These results indicate an important role of the electrostatic charge on the kinetic of surfactant adsorption. In agreement with this, similar results were found by other authors in different processes also related to the adsorption of molecules on ionic liposomes [16]. Concerning to the desorption of the mixed micelles from the liposome surface it may be seen that, regardless of the electrostatic charges, the desorption process was generally faster than the adsorption one, i.e., $t_{de} \leqslant t_{ad}$. This fact could be related to the hermodynamic associated with the liposome solubilization. In this sense other authors described a strong dependence of the thermodynamic on processes involving lipid/surfactant mixtures [17]. As regards to the effect of the electrostatic charges on the desorption of mixed micelles from the liposome surface, the scheme of Fig. 2 shows that this process is faster when the liposomes and the surfactant have the same type of charge (negative charge). However for the mixtures in which the surfactant and the lipids are oppositely charged the desorption process became slower. For the system with neutral liposomes (formed by PC-SDS) the t_{de} was more similar to that with equally charged species (PC/PA-SDS) than to that with oppositely charged species (PC/SA-SDS). Thus, we can assume that the presence of opposite charges slows down the process of mixed micelle desorption. The findings reported in the present work underline the importance of the electrostatic charge on the kinetics of the first steps of liposome solubilization by surfactants, i.e., of surfactant adsorption and mixed micelle desorption processes.

Acknowledgements

The authors thank the staff of the Small-Angle Scattering beamline, synchrotron ELETTRA, Italy for skillful assistance. We also thank Pedro González from Transtechnics, S.L., for providing financial support. The Company ORTEVE, Barcelona, Spain is thanked for the provision of the lipids (Lipoid S-100). This work was supported by funds from the European Community (Project HPRI-CT-1999-00033).

References

[1] C.R. Miller, B. Bondurant, S.D. McLean, K.A. McGovern and F. O'Brien, Liposome-cell interactions in vitro: effect of liposome surface charge on the binding and endocytosis of conventional and sterically stabilized liposomes, *Biochemistry* **37** (1998), 1287–12 883.

[2] V.L. Sukhorukov, M. Kürschner, S. Dilsky, T. Lisec, B. Wagner, W.A. Schenk, R. Benz and U. Zimmermann, Phloretin-induced changes of lipophilic ion transport across the plasma membrane of mammalian cells, *Biophys. J.* **81** (2001), 1006–1013.

[3] N. Kazanci, N. Toyran, P.I. Haris and F. Severcan, Vitamin D2 at high and low concentrations exert opposing effects on molecular order and dynamics of dipalmitoyl phosphatidylcholine membranes, *Spectroscopy* **15** (2001), 47–55.

[4] A. Sáez-Cirión, A. Alonso, F.M. Goñi, T.P. McMullen, R.N. McElhaney and E.A. Rivas, Equilibrium and kinetic studies of the solubilization of phospholipid–cholesterol bilayers by C12E8. The influence of the lipid phase structure, *Langmuir* **16** (2000), 1960–1968.

[5] O. López, A. de la Maza, L. Coderch, C. López-Iglesias, E. Wehrli and J.L. Parra, Direct formation of mixed micelles in the solubilization of phospholipid liposomes by Triton X-100, *FEBS Lett.* **426** (1998), 314–318.

[6] O. López, M. Cócera, E. Wehrli, J.L. Parra and A. de la Maza, Solubilization of liposomes by sodium dodecyl sulfate: new mechanism based on the direct formation of mixed micelles in the of phospholipid by Triton X-100, *Arch. Biochem. Biophys.* **367** (1999), 153–160.

[7] O. López, M. Cócera, R. Pons, N. Azemar and A. de la Maza, Kinetic studies of liposome solubilization by sodium dodecyl sulfate based on a dynamic light scattering technique, *Langmuir* **14** (1998), 4671–4674.

[8] O. López, M. Cócera, R. Pons, N. Azemar, C. López-Iglesias, E. Wehrli, J.L. Parra and A de la Maza, Use of a dynamic light scattering technique to study the kinetics of liposome solubilization by Triton X-100, *Langmuir* **15** (1999), 4678–4681.

[9] M. Cócera, O. López, J. Estelrich, J.L. Parra and A. de la Maza, Kinetic and structural aspects of the adsorption of sodium dodecyl sulfate on phosphatidylcholine liposomes, *Langmuir* **16** (2000), 4068–4071.

[10] O. López, M. Cócera, L. Coderch, J.L. Parra, L. Barsukov and A. de la Maza, Octyl glucoside-mediated solubilization and reconstitution of liposomes: structural and kinetic aspects, *J. Phys. Chem. B* **105** (2001), 9879–9886.

[11] P. Laggner and M. Kriechbaum, Phospholipid phase transitions: kinetics and structural mechanisms, *Chem. Phys. Lipids* **57** (1991), 121–145.

[12] J. Woenckhaus, R Köhling, P. Thiyagarajan, K.C. Littrell, S. Seifert, C.A. Royer and R. Winter, Pressure-jump small-angle X-ray scattering detected kinetics of staphylococcal nucleasa folding, *Biophys. J.* **80** (2001), 1518–1523.

[13] K. Lunkenheimer and D. Wantke, Determination of the surface tension of surfactant solution applying the method of Lecomte du Noüy (ring tensiometer), *Colloid Polym. Sci.* **259** (1981), 354–366.

[14] V. Dorovska-Taran, R. Wich and P. Walde, A 1H nuclear magnetic resonance method for investigating the phospholipase D-catalyzed hydrolysis of phosphatidylcholine in liposomes, *Anal. Biochem.* **240** (1996), 37–47.

[15] N. Skalko, J. Bouwstra, F. Spies, M. Stuart, P.M. Frederik and G. Gregoriadis, Morphological observations on liposomes bearing covalently bound protein: Studies with freeze-fracture and cryo electron microscopy and small angle X-ray scattering techniques, *Biochim. Biophys. Acta* **1370** (1998), 151–160.

[16] H. Matsiu and S. Pan, Distribution of DNA in cationic liposome complexes probed by Raman microscopy, *Langmuir* **17** (2001), 571–573.

[17] E. Opatowski, D. Lichtenberg and M. Kozlov, The heat of transfer of lipid and surfactant from vesicles into micelles in mixtures of phospholipid and surfactant, *Biophys. J.* **73** (1997), 1458–1467.