Investigation of the Adsorption of PEG1500-12-Acyloxystearate Surfactants onto Phospholipid Bilayers: An Ellipsometry and Cryo-TEM Study

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ABSTRACT In this article we present a study of a new class of surfactants denoted as PEG1500-12-acyloxystearates, which have potential use as pharmaceutical solubilizers. These amphiphilic molecules present interesting properties with regard to cell damage effects. PEG1500-12-acyloxystearates with C_{14} to C_{16} acyloxy chains cause little or no damage to red blood and intestinal cells, whereas the surfactants with shorter chains, from C_8 to C_{12}, induce measurable damage. To start unraveling the reason why there is this rather marked dependence of the cell damage effect on surfactant chain length, we have carried out systematic studies of adsorption properties of the surfactants onto phospholipid bilayers by means of ellipsometry. The rate of incorporation of the surfactants in the lipid membrane decreases with increasing length of the acyloxy chain. Cryo-TEM images strengthen the ellipsometry results by showing that the dissolution of the phospholipid bilayer is slower for the surfactants of the series having longer chains.

INTRODUCTION

The use of micelles and liposomes as drug delivery carriers has led to interest in designing such systems that are able to circulate in the bloodstream for several hours. To meet this goal, sterically stabilized colloidal assemblies have been successfully designed to reduce the detection and subsequent uptake of the aggregates by the immune system (1,2). The steric stabilization is usually obtained by grafting a polymer to a lipid-like molecule. Because of its specific biological inertness, poly(ethylene glycol) polymer (PEG) is the most widely used polymer for this purpose. Liposomes and micelles with a PEG-modified surface have an extended blood circulation time because they avoid contact with opsonins, proteins found in the reticuloendothelial system (RES) (3).

We have recently reported a novel class of PEG-derivative surfactants (4,5): PEG1500-12-acyloxystearates (see Fig. 1) consisting of a hydrophilic PEG head, and a branched hydrocarbon chain as hydrophobic moiety. The physicochemical characterization of PEG1500-12-acyloxystearate micelles has shown that these novel molecules behave to a large extent as nonionic poly(ethylene glycol) surfactants with extended PEG headgroups in aqueous solutions. Studies of their hemolytic activity reveal that PEG1500-12-acyloxystearates with C_{14} to C_{16} acyloxy chains do not damage red blood cells and intestinal cells to any appreciable extent, whereas the surfactants with shorter chains, from C_8 to C_{12}, have an increasing damaging effect when the acyloxy chain length is decreased (4). The fact that the PEG1500-12-acyloxystearates with a longer acyloxy chain do not affect both types of cells is most likely a result of differences in the rates with which adsorption of these surfactants occurs onto the cell membrane (6). Even though a number of studies exist on PEG surfactants at the liquid-solid interface (7,8), there are few studies in the literature relating hemolytic activity with interfacial behavior (9). Access to such information is fundamental in the process of developing novel classes of pharmaceutical solubilizers based on colloidal assemblies (10,11) because a necessary demand on such vehicles is that they can be administered without any danger.

In this work, we have studied the adsorption process of PEG1500-12-acyloxystearate surfactants from an aqueous solution to a lipid bilayer adsorbed on silica or on hydrophobized silica by means of ellipsometry. The goal has been to obtain a better understanding of the different hemolytic activities by studying the mechanisms of interaction with a phospholipid double layer as a model of a cell membrane for all the surfactants of the series. Furthermore, cryogenic-transmission electron microscopy (cryo-TEM) measurements were carried out. These were designed on the basis of the results of the ellipsometry study with the aim of obtaining direct images of the morphological changes after the solubilization of phospholipid membranes by these surfactants.

MATERIALS AND METHODS

Materials

The five nonionic PEG1500-12-acyloxystearate surfactants used in this work were PEG1500$C_{18}C_8$, PEG1500$C_{18}C_{10}$, PEG1500$C_{18}C_{12}$, PEG1500$C_{18}C_{14}$, and PEG1500$C_{18}C_{16}$ (for structures, see Fig. 1). These surfactants were synthesized by Fredrick Viklund (Royal Institute of Technology, Department of Biochemistry, Stockholm, Sweden) and used without further purification (4).

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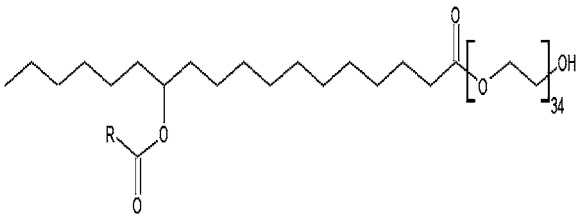

R	Name	Abbreviation
Octanoyl	PEG1500 mono-12-octanoyloxy-stearate	$PEG1500C_{18}C_8$
Decanoyl	PEG1500 mono-12-caproyloxy-stearate	$PEG1500C_{18}C_{10}$
Lauroyl	PEG1500 mono-12-lauroyloxy-stearate	$PEG1500C_{18}C_{12}$
Myristoyl	PEG1500 mono-12-myristoyloxy-stearate	$PEG1500C_{18}C_{14}$
Palmitoyl	PEG1500 mono-12-palmitoyloxy-stearate	$PEG1500C_{18}C_{16}$

FIGURE 1 Structures of the PEG1500-12-acyloxystearate surfactants.

Dioleolyphosphatidylcholine (DOPC) was purchased from Avanti Polar Lipids (Alabaster, AL). The molecular mass is 786 g/mol, and the purity of DOPC was >99% with respect to both its dioleyl chain length and the headgroup composition (data from the supplier). The surfactant used to solubilize the DOPC was *n*-dodecyl-β-D-maltoside (DDM), purchased from Sigma-Aldrich (St. Louis, MO) (purity >98%, molecular mass = 510.6 g/mol). All other chemicals, *N*-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES) and sodium chloride (NaCl), were commercially available from Sigma-Aldrich. Water purified by a Milli-Q system (Millipore, Bedford, MA) was used in all measurements.

Sample preparation

The mixed micellar solution of DOPC and DDM, used to produce the lipid bilayer at the silica-water interface, was prepared by dissolving the surfactant and the lipid in water in a molar ratio of 1:6. The PEG1500-12-acyloxystearate surfactant solutions were prepared by dissolution in aqueous buffer solutions at physiological pH (10 mM HEPES, 100 mM NaCl). DOPC vesicles used in the cryo-TEM studies were obtained by dissolving the lipid in a buffer solution and then extruding the resulting solution 11 times through a polycarbonate membrane with a pore size diameter of 100 nm to obtain large and unilamellar vesicles with a uniform size distribution.

Surface preparation

The silicon wafers (p-type, boron-doped, resistivity 1–20 Ω·cm) were thermally oxidized in an oxygen atmosphere at 920°C for ~1 h, followed by annealing and cooling in argon flow. This procedure produces a SiO_2 layer ~30 nm thick. The wafers were cut into slides and then cleaned first for 5 min in a boiling mixture (1:1:5 by volume) of 25% NH_4OH, 30% H_2O_2, and H_2O and then for 10 min in a boiling mixture (1:1:5 by volume) of 32% HCl, 30% H_2O_2, and H_2O. They were subsequently rinsed with water and stored in absolute ethanol until use. Before use, the surfaces were dried under vacuum (0.01 mbar) and then cleaned in a plasma cleaner (Harrick Scientific, Pleasantville, NY, model PDC-3XG) for 5 min. The deposition of the phospholipid onto the surface followed a protocol described in the literature (12) (see also below). The hydrophobized surfaces were obtained following the procedure described above and then exposing the surfaces to a low-pressure atmosphere of dimethyloctylchlorosilane for 24 h at room temperature. The surfaces were then sonicated first in ethanol and then in tetrahydrofuran and stored in ethanol. Before use, the surfaces were dried under vacuum (0.01 mbar).

Ellipsometry

The number of adsorbed surfactant layers at the silica surfaces as well as their thickness were monitored by means of in situ null ellipsometry using an automated Rudolph Research thin-film ellipsometer, type 43603-200E. The optical properties of the silica surfaces, i.e., the refractive index of the silicon and the oxide layer as well as the oxide layer thickness, were characterized at the beginning of each experiment by measuring the ellipsometric angles, Ψ and Δ, in two different ambient media, air and buffer solution, as described earlier (13). To prevent any air film sticking to the hydrophobic surfaces, ethanol was pumped through the ellipsometric cuvette before the aqueous solution was added. After characterization of the surface, known amounts of the samples were injected into the cuvette, which originally contained 5 mL of the buffer solution, and the ellipsometric angles were recorded continuously until plateau values were reached. All measurements were performed with a light-source wavelength of $\lambda = 401.5$ nm and with an angle of incidence of ~68.5°, under agitation with a magnetic stirrer at ~300 rpm in a temperature-controlled cuvette (25 ± 0.1°C) equipped with plastic tubes that allowed continuous rinsing with HEPES solution. The time resolution of the instrument is 2–3 s, which allows us to follow the kinetics of the adsorption processes. The obtained values of Ψ and Δ were used as input into a four-layer optical model, which assumes isotropic media and planar interfaces. The model yields the mean refractive index, n_f, and the ellipsometric thickness, d_f, of the adsorbed layer through a numerical procedure described elsewhere (14). The adsorbed amount, Γ, was then calculated from n_f and d_f using the formula

$$\Gamma = d_f(n_f - n_0)/dn/dc, \quad (1)$$

where n_0 is the refractive index of the bulk solution and dn/dc is the refractive index increment as a function of the bulk concentration, which was taken to be 0.15 g/cm^3 (12). For typical experimental conditions, the errors in Ψ and Δ are normally distributed with standard deviations of 0.001° and 0.002°, respectively. At small surface coverages, $\Gamma < 0.5$ mg/m^2, the relative errors in refractive index and thickness are rather high, whereas for large adsorbed amounts, $\Gamma > 2$ mg/m^2, the errors rapidly decrease to values below 5%. The errors in the adsorbed amount are typically rather low because the errors in n_f and d_f are covariant.

Cryo-transmission electron microscopy

Cryo-TEM images were carried out at the Center for Chemistry and Chemical Engineering in Lund, Sweden, on a Philips CM120 BioTWIN Cryo electron microscope operating at 120 kV. A small drop of the sample solution was applied on a copper EM grid with a holey carbon film, and excess solution was blotted with a filter paper, leaving a thin sample film spanning the holes in the carbon film. Sample preparation was carried out in a controlled environment vitrification system to avoid water evaporation and to ensure cryofixation of the specimen at a controlled temperature (25°C) (15).

RESULTS AND DISCUSSIONS

Ellipsometry

The goal of this work has been to investigate the process of adsorption of a series of novel nonionic surfactants onto a phospholipid bilayer deposited at the silica-water interface.

For reference, we have also performed adsorption studies onto hydrophobized silica.

Phospholipid surfaces

Ellipsometry measurements were carried out in the presence of a lipid bilayer at the silica-water interface to get insight into the mechanism and kinetics of interaction of the PEG1500-12-acyloxystearate surfactants with the phospholipids of a cell membrane. The approach used for depositing the phospholipid layer at the solid support was originally developed by Tiberg et al. (12). This produces stable and reproducible lipid layers by coadsorption from a mixed micellar solution of DOPC lipids and DDM surfactants. The latter shows no affinity for the surface and so can easily be removed from the surface by a series of rinsing steps. By first adsorbing from solutions with high lipid and surfactant concentrations and then, in succession, rinsing and readsorbing two times from solutions with lower total bulk concentration, a dense bilayer structure was deposited onto the hydrophilic surface.

Fig. 2 *A* shows the time dependence of the adsorbed amount and layer thickness for PEG1500$C_{18}C_{12}$. At time $t = 0$, the first injection of lipid-DDM solution into the cuvette was carried out, which was followed by successive addition and rinsing cycles until a lipid bilayer of thickness 46 ± 3 Å was formed. The equilibrium surface excess concentration of lipids was $\sim$4.3 mg/m^2, giving an average area per lipid molecule in the two layers of 62 ± 3 Å^2. The physical characteristics of the adsorbed bilayer were in good agreement with data reported in the literature. Once the lipid bilayer was deposited and stable with time, the PEG1500$C_{18}C_{12}$ solution was injected at $t \approx 170$ min, yielding a bulk concentration in the ellipsometric cuvette of 0.5 μM, which is above the critical micellar concentration (cmc) of the surfactant. The adsorption then reached a plateau in terms of adsorbed amount, the value of which was not altered when additional surfactant was added (up to a total concentration of 200 μM). The ellipsometric cuvette was then left overnight to permit investigation of possible long-term effects. The profile of the optical properties remained unaltered for more than 12 h. Finally, rinsing with HEPES buffer solution produced no detectable desorption.

The steady-state value for surface excess of the surfactant was $\sim$0.6 mg/m^2. It should be noted that this number is the increase in adsorbed amount above the amount of the phospholipid bilayer and thus less precise then the latter value (see Table 1). The increase in the thickness of the adsorbed layer on addition of the surfactant was low and can be estimated to be $\sim$10 Å. This is because the surfactant penetrates with the branched-chain tail into the lipid bilayer, and moreover, the polyoxyethylene chain, oriented toward the aqueous solution, is highly hydrated, and thus its optical contrast is low. For these reasons there is only a minor thickening of the lipid bilayer observed when the acyloxy surfactant is incorporated in the bilayer. Comparing the Γ_{max} in the absence and presence of surfactant, we find a DOPC/PEG1500$C_{18}C_{12}$ molar ratio of $>$10; that is, there are more than 10 lipids in the bilayer per PEG-surfactant. The amount of adsorbed PEG1500$C_{18}C_{12}$ on the phospholipid bilayer is dictated by need to hydrate the polyoxyethylene chain, which hinders the surfactant molecules from packing close at the surface and thus leads to a low adsorbed amount.

In Figs. 2, *B* and *C*, we report the time dependence of the adsorbed amount and layer thickness for PEG1500$C_{18}C_8$ and PEG1500$C_{18}C_{14}$, respectively. These display higher and lower hemolytic activities than PEG1500$C_{18}C_{12}$. The variation of the ellipsometric parameters, Γ and d_f, show similar trends to those observed for PEG1500$C_{18}C_{12}$. The surface excess concentration values at equilibrium conditions are roughly the same for all of the surfactants of the series; they

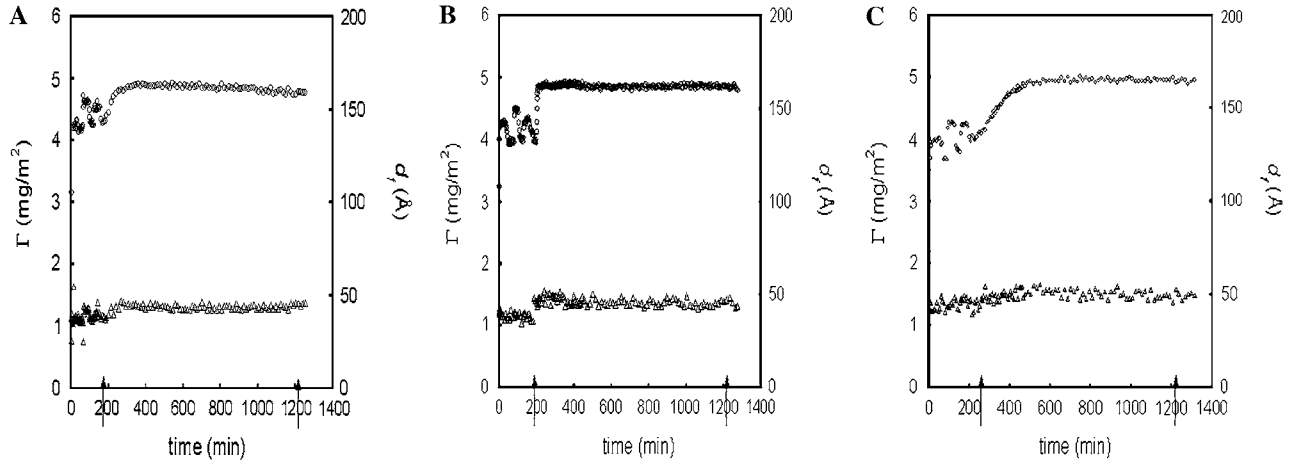

FIGURE 2 (*A*) Adsorbed amount of surfactant (*circles*) and adsorbed layer thickness (*triangles*) as a function of time for PEG1500$C_{18}C_{12}$ at phospholipid surfaces. The arrows refer to the moment when 0.5 μM of the surfactant solution was added into the cuvette and when the surface, after additional surfactant injections reaching a total concentration of 200 μM, was rinsed with buffer solution. (*B*) As in *A*, but for PEG1500$C_{18}C_8$. (*C*) As in *A* but for PEG1500$C_{18}C_{14}$.

TABLE 1 Equilibrium surface excess concentration, initial adsorption rate, and calculated area per surfactant at the phospholipid surfaces

Systems	Γ (mg/m^2)	dΓ/dt (mol/m^{2}s)	A (Å^2/molecule)
PEG1500$C_{18}C_8$	0.9 ± 0.1	(2.3 ± 0.1) × 10^{-9}	350 ± 40
PEG1500$C_{18}C_{10}$	0.7 ± 0.1	(4.6 ± 0.2) × 10^{-10}	440 ± 60
PEG1500$C_{18}C_{12}$	0.6 ± 0.2	(8.4 ± 0.3) × 10^{-11}	570 ± 190
PEG1500$C_{18}C_{14}$	0.9 ± 0.1	(3.0 ± 0.1) × 10^{-11}	350 ± 40
PEG1500$C_{18}C_{16}$	0.7 ± 0.1	(4.6 ± 0.1) × 10^{-12}	500 ± 70

are summarized in Table 1. The main difference in the adsorption process for the surfactants of the series is found in the initial adsorption rate of the surfactants: it takes a half-hour to reach the plateau for PEG1500$C_{18}C_8$, several hundred minutes to reach the plateau for PEG1500$C_{18}C_{14}$, and over 20 h for PEG1500$C_{18}C_{16}$. The results for all the surfactants are summarized in Fig. 3, where we show the rate of adsorption after the injection of the surfactant into the cuvette (which time is defined as $t = 0$ in the graph). The excess surface concentrations, initial rates of adsorption, and calculated areas per molecule for the surfactant are summarized in Table 1.

Analysis of the adsorption rates

It is clear from Fig. 3 that the rate of adsorption onto a DOPC bilayer becomes considerably slower as the size of the hydrophobe of the surfactant is increased (see also Table 1). If the rates are normalized to the lowest value, the relation among the rates of adsorption is ~1:6:16:90:450, i.e., the addition of 2 CH_2 groups leads to a slowing down of the adsorption by roughly a factor of 5. The only other surfactant property that shows a similar dependence on the size of the hydrophobe is the cmc value. For the related surfactant C_xE_5, the cmc changes by roughly a factor of 10 on changing the hydrocarbon chain by 2 CH_2 groups (16). Thus, it seems likely that it is the concentration of nonmicellized free surfactant that governs the initial adsorption. That the decrease in adsorption rate shows a milder dependence on the addition of CH_2 groups is probably because the acyloxystearates carry two hydrocarbon chains; intramolecular contacts between the two chains then lessen the hydrophobic penalty of exposing the hydrophobe to water.

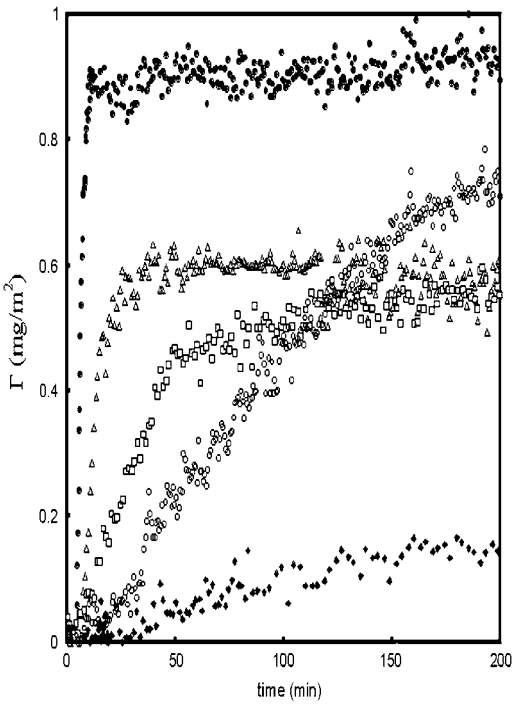

FIGURE 3 Adsorbed amount of surfactant at phospholipid surfaces as a function of time for the different surfactants of the series studied: PEG1500$C_{18}C_8$ (*solid circles*), PEG1500$C_{18}C_{10}$ (*triangles*), PEG1500$C_{18}C_{12}$ (*squares*), PEG1500$C_{18}C_{14}$ (*open circles*), and PEG1500$C_{18}C_{16}$ (*diamonds*).

Attempts at measuring the cmc values by means of the dependence of the pyrene fluorescence intensity ratio I1/I3 on surfactant concentration yielded values of ~1 μM for all surfactants. This is clearly unreasonable because, as noted above for members of the C_xE_5 family, cmc values depend strongly on the hydrocarbon chain length. This is because pyrene has a low but finite solubility in water. Work is under way to resolve this issue, using alternative fluorescent probes.

In conclusion, we suggest that it is the concentration of free surfactant that governs the rate of adsorption onto the DOPC bilayer. Single surfactant molecules are incorporated into the bilayer. No micelles are adsorbed because of the steric repulsion between the micelle with its ''protective'' layer of PEG-chains and the bilayer. It is not unreasonable that there is also a barrier preventing the surfactant from adsorbing onto the bilayer. One contributing factor to such a barrier is given by the bulky PEG part of the surfactant. However, this effect should be approximately equal in magnitude for all the surfactants, as it is controlled by the size of the PEG part. Another possible factor is constituted by a hydrophobic mismatch between the surfactant and the lipid bilayer. If such a mismatch were important, it would give rise to a significant difference in the surface excess concentration, which is an equilibrium property. No such significant differences are observed (see Table 1). Therefore, we expect effects caused by hydrophobic mismatch to be unimportant in the initial adsorption rate.

Tiberg et al. studied the adsorption kinetics of nonionic surfactants of the C_xE_y type at the silica-water interface (17). In their description of the process, the adsorption is controlled by the diffusion of monomers and micelles, if present, from solution to the interface through a stagnant layer in the stirred adsorption cell. The rate of this mass transport is proportional to the concentration gradient between the bulk solution and the adsorbed layer and corresponds to the value of the slope, $d\Gamma/dt$, of the initial linear part of the adsorption isotherm curves (cf. Figs. 2 and 3). Tiberg et al. present detailed equations for this case. Attempts at applying these equations have failed, perhaps

because of the barrier for the incorporation of the surfactant into the DOPC bilayer described above.

Hydrophobized surfaces

Ellipsometry measurements were also carried out on hydrophobized surfaces at the silica-water interface. On hydrophobic surfaces, the surfactants adsorb with their hydrophobic tails in contact with the surface and their hydrophilic heads in contact with the solution. In all the experiments the surfactant solution was added at $t = 0$, leading to a bulk concentration in the ellipsometric cuvette of 0.5 μM, and adsorption was then followed until equilibrium conditions were established between the adsorbed layer and the bulk solution. A constant plateau was reached, the value of which was not altered when additional injections of surfactant were made up to a total concentration of 200 μM. The steady-state values for the surface excess concentration were approximately the same for all the surfactants (see Table 2). The low values of amount of adsorbed surfactant result, as mentioned above, from the need to hydrate the PEG chain. As a consequence, the molecules are not able to pack closely at the surface. As in the presence of the lipid bilayer, the rate of the adsorption process is strongly dependent on the size of the acyloxystearate tail.

Fig. 4 gives the kinetics of adsorption onto hydrophobic surfaces for all the surfactants of the series. This figure shows clearly that there are two regimes where the surface excess increases with different rates (18). There is an initial rapid increase followed by a slower rate of adsorption. The presence of two linear regimes indicates that there are two different species involved in the adsorption process. In the first rapid regime, there is adsorption of only a few single surfactants onto the surface, which act as sites for placing distorted micelles on the surface. Thus, in the first regime, the mass transport is given by micelles and is approximately equal for all surfactants because the concentration of micelles is roughly the same for all surfactants (the cmc values are low). In the second regime, the surface is covered with micelles so that micelles can no longer adsorb because of steric effects, and thus, further adsorption occurs by single surfactant adsorption, again with a considerable barrier for adsorption. This is confirmed by the values of the slopes in the second regime, which are similar to those in the DOPC case.

TABLE 2 Equilibrium surface excess concentration, initial adsorption rate, and calculated area per surfactant at hydrophobic silica surfaces

Systems	Γ (mg/m^2)	dΓ/dt (mol/m^{2}s)	A (Å^2/molecule)
PEG1500$C_{18}C_8$	2.0 ± 0.1	(1.7 ± 0.1) × 10^{-9}	160 ± 10
PEG1500$C_{18}C_{10}$	1.9 ± 0.1	(5.0 ± 0.1) × 10^{-10}	170 ± 10
PEG1500$C_{18}C_{12}$	1.7 ± 0.2	(1.0 ± 0.1) × 10^{-10}	190 ± 20
PEG1500$C_{18}C_{14}$	1.9 ± 0.1	(4.5 ± 0.1) × 10^{-11}	180 ± 10
PEG1500$C_{18}C_{16}$	1.8 ± 0.1	(8.1 ± 0.1) × 10^{-12}	190 ± 10

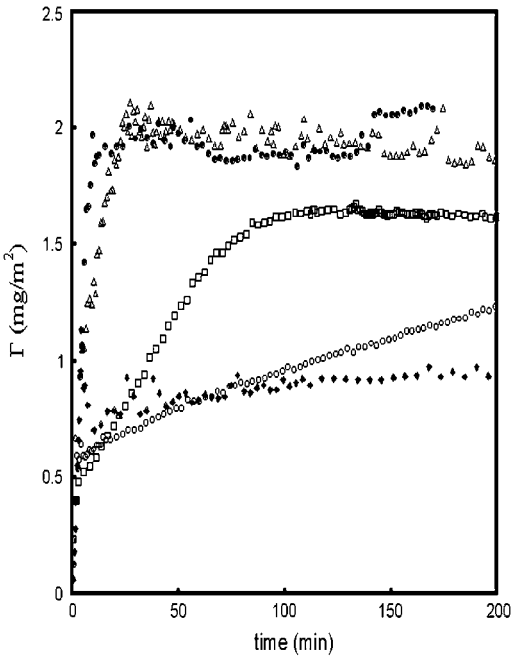

FIGURE 4 Adsorbed amount of surfactant as a function of time for all the different surfactants of the series studied on hydrophobized silica surfaces. Same symbols as in Fig. 3.

Cryo-TEM

Cryo-TEM measurements were performed to obtain direct images with time of the interaction of PEG1500-12-acyloxystearate surfactants with DOPC liposomes. The surfactants selected for this study were PEG1500$C_{18}C_8$, PEG1500$C_{18}C_{12}$, and PEG1500$C_{18}C_{16}$. In terms of properties, these surfactants span the range from showing measurable hemolytic effect to being practically not hemolytic. The surfactant/lipid molar ratio 4:1 was chosen in agreement with the results of a previous turbidity and cryo-TEM study (6). Cryo-TEM images were collected in all three cases after 30 min from the injection of PEG-surfactant micellar solution into a sample of DOPC liposomes, and then at different times, which were chosen on the basis of the ellipsometry results.

The images obtained after 30 min show the different hemolytic activities of the surfactants. For PEG1500$C_{18}C_8$ the carbon grid appeared quite void of liposomes (see Fig. 5, *A* and *B*); the addition of the surfactant produced a dissolution of the bilayer lipid membranes within 30 min. The images were dominated by the presence of dark spots, corresponding to small micelles with a size of 4–6 nm, the limit of size resolution of our cryo-setup. These micelles are mixed micelles formed by the lipid and the surfactant. The few vesicular aggregates present appeared flattened and irregularly curved, surrounded by a micellar background. For PEG1500$C_{18}C_{12}$, cryo-TEM micrographs presented several bilayer structures, polydisperse in size and curvature (Fig. 5, *C* and *D*). Most of them contained several bilayers and were larger compared with the original size of 100 nm. These

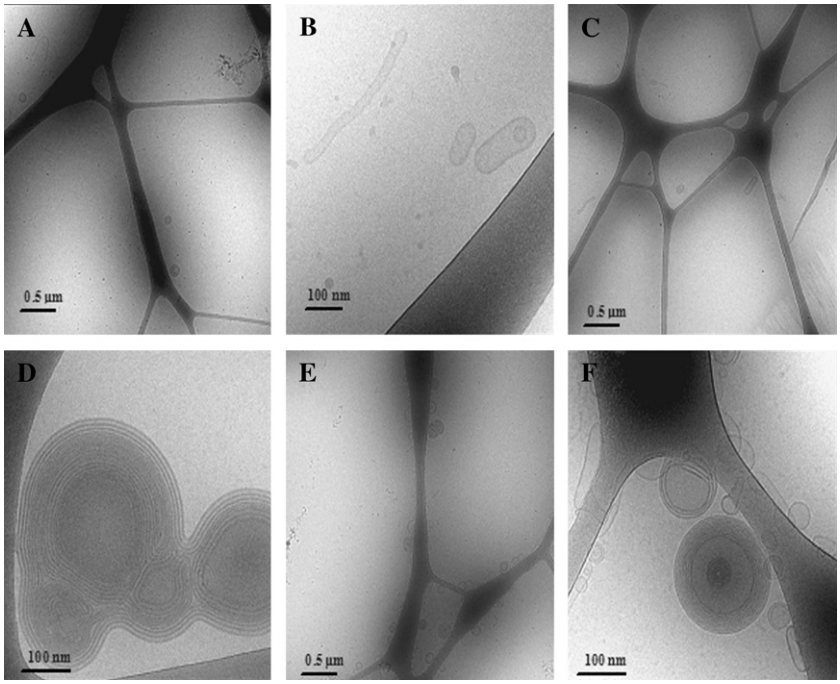

FIGURE 5 Cryo-TEM images obtained a half-hour after addition to DOPC liposomes of a PEG1500$C_{18}C_8$ micellar solution (*A* and *B*), PEG1500$C_{18}C_{12}$ (*C* and *D*), and PEG1500$C_{18}C_{16}$ (*E* and *F*).

structures are typical transitional structures observed in the process of solubilization of lipid bilayers by nonionic surfactants (19). As in the previous case, vesicles were surrounded by a micellar background. Images collected for PEG1500$C_{18}C_{16}$ contained many oligolamellar liposomes, with a diameter range of 100–150 nm (Fig. 5, *E*, and *F*), indicating a slower dissolution of the lipid bilayer compared with the previous two cases. Cryo-TEM measurements were also run for PEG1500$C_{18}C_{12}$ 2 h after the injection (see Fig. 6, *A* and *B*). The images show the dissolution of bilayer membranes present in the previous images collected for this sample; the few bilayer structures present were deformed and elongated as in the case of PEG1500$C_{18}C_8$ and were surrounded by a micellar background. Thus, similar results for both surfactants were obtained, but after different times. In the case of PEG1500$C_{18}C_{16}$, the dissolution of the lipid membranes occurred after 24 h (Fig. 7 *A*) from the time of the surfactant injection. Fig. 7 *B* shows the breaking up of a lipid membrane, which is one of the intermediate stages characterizing the dissolution process of the lipid membranes.

CONCLUSIONS

The aim of this study has been to obtain a comprehensive picture of the mechanism and kinetics of interactions of a novel class of PEG-derivative surfactants, which are good candidates as carriers of active compounds in the context of drug delivery, with human cell membranes. To achieve this, we have investigated by use of null ellipsometry the surfactant adsorption onto a silica surface in the presence of a deposited lipid bilayer and on hydrophobized silica. In parallel to trends presented in previous studies (6) on hemo-

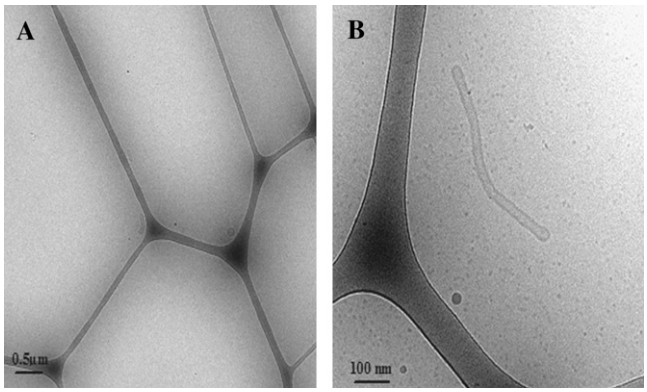

FIGURE 6 Cryo-TEM images obtained 2 h after addition of a PEG1500$C_{18}C_{12}$ micellar solution to DOPC liposomes.

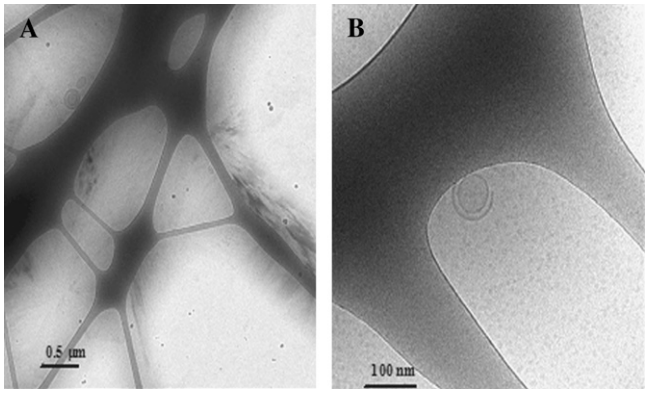

FIGURE 7 Cryo-TEM images obtained 24 h after addition of a PEG1500$C_{18}C_{16}$ micellar solution to DOPC liposomes.

lytic activity of these surfactants, the results presented here have demonstrated that the initial adsorption kinetics into the cell membrane varies considerably with the length of the acyloxy chain. For nonionic surfactants of the C_xE_y type, cmc values vary strongly with the length of the alkyl chain, and the same should hold for the surfactants investigated here. These facts taken together suggest that the alkoxystearates adsorb in the form of free, nonmicellized surfactants and that micelles are not involved. Thus, it is the low cmc value that is the main cause of the low hemolytic activity of PEG1500$C_{18}C_{16}$. There is probably also a barrier for the adsorption of alkoxystearates into the bilayer because of the bulky PEG headgroup and of the hydrophobic mismatch between the surfactant and the lipid bilayer. However, the first effect should be roughly equal for the members of the alcoxystearate family, as the headgroup has the same size for all members, whereas the latter effect is assumed to be unimportant because no significant differences in the surface excess concentration values were observed.

Cryo-TEM images obtained after injection of PEG1500-12-acyloxystearate micelles into samples containing DOPC liposomes have confirmed the conclusions drawn from ellipsometry measurements. The dissolution of lipid membranes is faster for the surfactants of the series with shorter chains and becomes progressively slower on increasing the length of the chain.

To the best of our knowledge, this is the first attempt to investigate hemolytic activity of amphiphilic molecules using ellipsometry to study their interfacial behavior.

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