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Potential-mediated interaction between dextran sulfate and negatively charged phospholipids films at air/water and liquid/liquid interfaces

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ABSTRACT

The effect of dextran sulfate (DS) on distearoyl phosphatidyl glycerol (DSPG) and distearoyl phosphatidic acid (DSPA) films formed in the presence of Ca_2Cl, LiCl or KCl as aqueous electrolytes, was analyzed by cyclic voltammetry, surface pressure-area and surface potential-area isotherms and Brewster angle microscopy. Experiments of cyclic voltammetry at the water/1,2-dichloroethane interface showed an interfacial adsorption/desorption process in the presence of DSPG, or DSPA, DS and LiCl. It is suggested that this process could correspond to the formation of an interfacial complex DS–Li^+–DSPG or DS–Li^+–DSPA at positive potentials with respect to the potential of zero charge (pzc) and its desorption from the interface towards the aqueous phase, by complex dissociation, at potentials below pzc. The voltammetric analysis of tetraethylammonium (TEA^+) cation transfer, from the aqueous to the organic phase, evidenced a blocking effect of DSPG and DSPA films on that process, whose magnitude depends on both the nature of the phospholipids and the cation forming the aqueous electrolyte. The structure and permeability of those films were altered by the presence of DS, by formation of the complex DS–cation–phospholipid, which produces an expansion of the monolayer, whose extent depends on the cation present in water, and the polar head group of the phospholipid. Electrochemical experiments were completed with compression isotherms for DSPG and DSPA monolayers both in the absence and in the presence of DS. In this case, as opposed to the voltammetric results, a negligible effect of DS was observed in the isotherms. This apparent contradiction could be explained considering the effect of polarization, which enables the accumulation of cations at the interface, favouring the formation of the DS–cation–phospholipid complex which is responsible for the increase in the film permeability. Similarly, the thickness values for phospholipid films obtained from the Brewster angle microscopy experiments were the same either in the absence or in the presence of DS at any lateral pressure value, which is a direct evidence of lack of interaction between DS and DSPA or DSPG molecules at open circuit. Therefore, it was concluded that the formation of the complex DS–cation–phosholipid, responsible for the increase in permeability observed in voltammetric experiments, must be attributed to the polarization of the interface. The application of high positive potentials at the interface promotes cations accumulation, which neutralize the negative charge in phosphate groups and in DS and allows their interaction.

1. Introduction

Dextran sulfate (DS, Scheme 1) is a linear polysaccharide, with a high permanent negative charge due to the sulfate groups present in the monomers. In the last decade there has been a growing interest in the study of this polyelectrolyte because of its use as an antiatherosclerotic drug [1], as a potent agent against HIV infection [2] and in gene delivery system [3–5].

Moreover, its similarity with glycosaminoglycans (GAGs), enhances the interest in studying the interaction between DS and phospholipids. GAGs are one of the main components of the extracellular matrix of many tissues and can also be found inside or on the surface of cells, they can be bounded to proteins forming the proteoglycans [6]. GAGs are very important in the process that involves the capture of positive ions and drugs, due to their high negative charge [7]. Besides GAGs occupy almost all extracellular spaces and form a mechanical support for tissues and facilitate the diffusion of hydrophilic molecules and cell migration [8]. They are also important because of their functional modulation of the extracellular matrix and their influence on the development and repair of the central nervous system [9].

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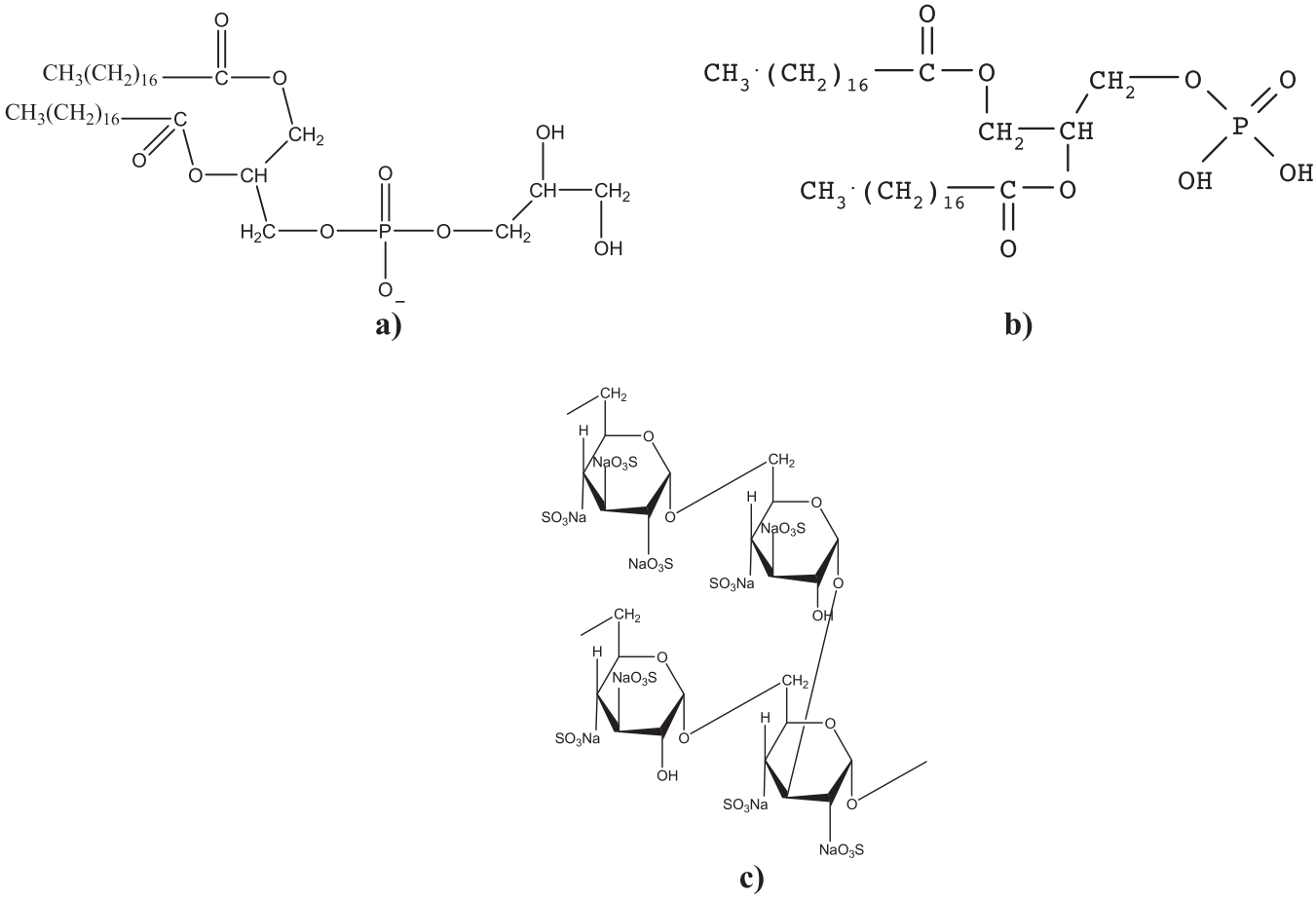


Scheme 1. Molecular structure of: (a) DSPG, (b) DSPA and (c) dextran sulfate.

There are many studies related to the interaction between DS and monolayers, bilayers and vesicles of amphiphilic molecules [10–12]. They evidenced that the interaction between DS and amphiphilic molecules occurs via the formation of cation bridges. Hugerth and Sundelöf [10] researched into the selectivity of dextran sulfate – counterion in the interaction with amitriptyline. They found out that dextran sulfate–amitriptyline adsorption depends on the counterion and follows the sequence $Li^+ > Na^+ > K^+ > Rb^+$, which has been explained considering the alkaline cation hydration.

Besides, Zschorining et al. investigated the M^{2+} (M: Ca, Mg and Mn)-mediated interaction of dextran sulfate (of different molecular weights) with phospholipid vesicles, finding that the binding produces vesicle aggregation which strongly depends on the cation nature ($Ca^{2+} > Mn^{2+} > Mg^{2+}$) and on the molecular weight of DS (500 > 40 > 8 > 1 kDa).

Huster et al. [13] investigated the effect of Ca^{2+} mediated DS adsorption on the organization of monolayers and bilayers of zwitterionic phospholipids, and they suggest that DS induces changes in the lipid packing, which depend on both Ca^{2+} concentration and DS molecular weight.

Santos et al. [9] studied the interaction between DS of high molecular weight (500 kDa) and negative or zwitterionic phospholipids through Ca^{2+} bridges. They found that DS leads to a more condensed layer of phospholipids, and that such a layer is strongly dependent on the surface pressure and the potential applied in the electrochemical studies. In subsequent studies they introduced gramicidin into the monolayer, finding that the interaction between DS and the hybrid monolayer (lipid–gramicidin), varies according to the chemical nature of the lipid [14]. Finally they also studied the formation of multilayers containing DS [1] and the interaction of nanoparticles composed of DS [15] with phospholipids.

Electrochemical measurements applied to liquid/liquid interfaces modified by different films have been carried out in the last decades with the aim of developing new biomimetic membranes models. In this sense, the adsorption of lipid monolayers [16,17], proteins [18,19], or polyelectrolytes [20] including biopolymers as heparin [21–24], another sulphated polysaccharide, has been studied and the properties of these films have been characterized by cyclic voltammetry, electrochemical impedance spectroscopy, and surface tension measurements. One aspect of special interest has been the study of the interaction or the complex formation between phospholipid monolayers and alkaline or alkaline earth cations [25], or peptides [26], as well as between polyelectrolytes with different ions [27,28] and DNA [29]. All these antecedents demonstrate that electrochemical techniques applied at the interface between two immiscible electrolyte solutions (ITIES) are ideal to follow dynamic changes in the lipid layer compactness and interfacial interactions at a hydrophobic/hydrophilic boundary [30].

In previous papers we have studied the incorporation of anxiolytic drugs into phospholipid monolayers adsorbed at liquid/liquid interfaces [31–35]. The results obtained have contributed to the knowledge of the non-specific interaction between these drugs and biological membranes components, which is particularly important because their accumulation in biomembranes alters the structural properties and leads to side effects. We have also studied the interaction between phospholipids and the positively charged polyelectrolyte chitosan [36], demonstrating that between this polymer and negatively charged phospholipids

there occur both electrostatic and hydrophobic interactions depending on the state of the monolayer. The combination of surface pressure–molecular area measurements and electrochemical experiments employing a test cation such as tetraethyl ammonium (TEA^+) allowed us to evaluate the permeability and the compactness of the monolayer.

In the present paper we study the effect of DS on distearoyl phosphatidyl glycerol (DSPG) and phosphatidic acid (DSPA) films formed at water/1,2-dichloroethane or at air/water interfaces, employing different experimental setups and techniques such as surface pressure–molecular area and surface potential–molecular area isotherms, Brewster angle microscopy and cyclic voltammetry. Special emphasis is placed on the composition of the aqueous phase which contains LiCl, KCl or $CaCl_2$ as base electrolyte.

2. Experimental

2.1. Materials and electrochemical cell

Cyclic voltammetry (CV) performed in a four-electrode system using a conventional glass cell of a 0.16 cm^2 interfacial area, was used to characterize the film. Two platinum wires were employed as counter electrodes and the reference electrodes were Ag/AgCl. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 10.0 mM tetraphenyl arsonium chloride (TPAsCl, Aldrich). Potential values (E) reported in this work are those which include $\Delta\phi^0_{tr,TPAs+} = 0.364$ V for the transfer of the ion $TPAs^+$.

The base electrolyte solutions were 10.0 mM MCl_z ($M^{z+} = Ca^{2+}$, Li^+, K^+) (p.a. grade) in ultra-pure water and 10.0 mM tetraphenyl arsonium dicarbollyl cobaltate (TPAsDCC) in 1,2-dichloroethane (DCE, Dorwill p.a.). TPAsDCC was prepared by metathesis of tetraphenyl arsonium chloride (TPAsCl, Sigma–Aldrich) and sodium dicarbollyl cobaltate (NaDCC, Aldrich p.a.). The pH of the aqueous solution was 3.00, adjusted with 2.00% (v/v) acetic glacial acid (Baker Analyzed). In all experiments 1.00 mL of organic and 4.00 mL of aqueous phase were used to fill the cell.

The electrochemical cell used was as follows:

		TPAsCl 10.0 mM	TPAsDCC 10.0 mM	MCl_n 10.0 mM + Acetic Acid (pH= 3.00		
Ag	AgCl	(w´)	(o)	(w)	AgCl	Ag

Pure dextran sulfate sodium salt (Sigma–Aldrich, Mr ≈ 5000 Da, 17% of sulfur) was added to the aqueous phase (w) in a concentration range 0–1.00% (w/v).

Distearoylphosphatidylglycerol (DSPG) and distearoylphosphatidic acid (DSPA) were of analytical grade (Sigma–Aldrich). A solution 1 mM of DSPG or DSPA in 1:2 methanol:chloroform was prepared. In order to form the lipid film, 50 μL of DSPG or DSPA solutions were injected, using a Hamilton microsyringe, at the liquid/liquid interface after both phases were brought into contact in the electrochemical cell. It was required 60 min, since the injection of the lipid solution, to obtain an invariant and reproducible voltammetric response, indicating that a stable lipid film has been formed. Thus, all experiments were performed after this equilibration time at room temperature (25 ± 1 °C). Temperature was controlled with a temperature/humidity monitor.

2.2. Cyclic voltammetry experiments

Voltammograms were carried out using an aqueous solution of 0.5 mM tetraethylammonium chloride (TEACl, Sigma). TEA^+ was employed as a probe ion, since its transfer, from the aqueous to the organic phase, is reversible and diffusion controlled [33]. The comparison between the voltammetric profiles for TEA^+ before and after injection of DSPG or DSPA, in the absence and in the presence of dextran sulfate dissolved in the aqueous phase, allows us to evaluate the ion permeability of the monolayer.

CV was performed using a four-electrode potentiostat with periodic current interruption for automatic elimination of solution resistance. The voltage was changed from 0.200 to 0.750 V with a potential sweep generator (L y P Electrónica, Argentina). Voltammograms were recorded employing a 10 bit computer board acquisition card connected to a personal computer.

2.3. Langmuir monolayers

2.3.1. Surface pressure–molecular area isotherms

Surface pressure–molecular area isotherms were recorded with a mini-trough II from KSV Instruments Ltd. (Helsinki, Finland). The surface tension was measured by the Wilhelmy plate method with a platinum plate.

The aqueous subphase, contained in a Teflon trough (364 mm × 75 mm effective film area), was 10.0 mM MCl_z ($M^{z+} = Ca^{2+}$, Li^+, K^+), 2.00% (v/v) acetic acid pH = 3.00 with or without dextran sulfate at different concentrations.

To prepare DSPG or DSPA monolayers at the air/water interface, 30 μL of phospholipid solution in 1:2 methanol:chloroform (0.40 mg/mL) was carefully spread on the surface with a Hamilton microsyringe. Before spreading the phospholipid solution, the subphase surface was cleaned by sweeping it with the Teflon barriers and then, any surface contaminant was removed from the interface by suction. Surface cleaning was checked by recording an isotherm in the absence of phospholipids and verifying a surface pressure value lower than 0.20 mN/m. After spreading, evaporation of the solvent was allowed for 10 min, and then the film was compressed with two barriers, one on each side of the trough at a compression speed of 5 mm/min while lateral surface pressure (π) was automatically measured. All experiments were performed at 25 ± 1 °C using a HAAKE G. thermostat. At least two compression isotherms were registered at each condition and results with a typical area and collapse pressure errors of ±0.02 nm^2 and ±1 mN m^{-1} respectively were obtained.

The surface compression modulus κ (mN m^{-1}) was calculated from the compression isotherm as:

$$\kappa = -A \times \left(\frac{\partial \pi}{\partial A}\right)_T \quad (1)$$

where A is the molecular area per molecule and π is the surface pressure in mN m^{-1}. The compression modulus uncertainty was ±10 mN m^{-1}.

2.3.2. Surface potential–molecular area isotherms

The surface potential–molecular area isotherms were measured with a home-made Langmuir balance using an air-ionizing ^{241}Am plate surface electrode and an Ag/AgCl/Cl^- (3 M) reference electrode [37]. The composition of the subphases used in these compression isotherms was the same as those mentioned in Section 2.3.1. In order to prepare DSPG or DSPA monolayers at the air/water interface volumes between 15–25 μL of DSPG in 1:2 methanol:chloroform solution (5.0 mM) were carefully spread on the surface with a Hamilton microsyringe. The experiments were performed 10 min after the injection. The film was compressed with one barrier at a compression speed of 13 cm/min. Lateral pressure was registered simultaneously and the lateral pressure-molecular area isotherms obtained with this equipment were similar to the ones obtained as explained in Section 2.3.1.

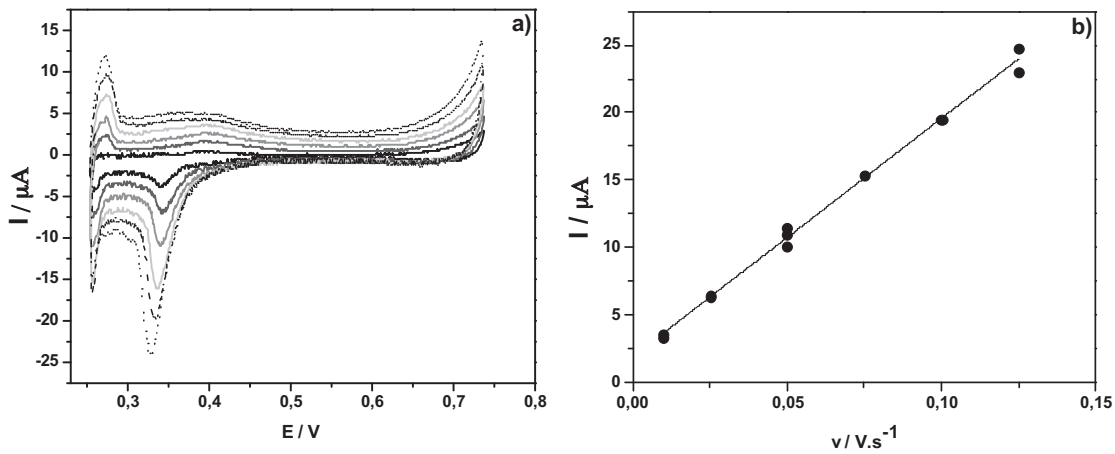


Fig. 1. (a) Cyclic voltamograms recorded in the presence of 0.100% (w/v) DS, 60 min after the injection of 50 μL of 1 mM DSPG solution. v: (▬) 0.010, (▬) 0.025, (▬) 0.050, (▬) 0.075, (–) 0.100, (.....) 0.125 V s^{-1}. Aqueous phase composition: 10.0 mM LiCl, 2.00% (v/v) acetic acid, 0.100% (w/v) DS, pH = 3.00. Organic phase composition: 10.0 mM TPhAsDCC. Potential values, E, reported in this figure are the applied potentials, related to the interfacial potential, $\Delta\phi$, by: $\Delta\phi = E - \Delta\phi^0_{\mathrm{tr,TPAs+}}$ (where $\Delta\phi^0_{\mathrm{tr,TPAs+}} = 0.364$ V). (b) Plot of Ip(–) vs V at Ep = 0.330 V.

2.4. Brewster angle microscopy (BAM)

BAM experiments were carried out using an EP3 Imaging ellipsometer (Acucurion, Goettingen, Germany) with a 20× or a 10× objective. The monolayer was formed in a Langmuir film balance (KSV minitrough, KSV Instruments Ltd., Helsinki, Finland) using the same volumes and phospholipid solutions than those described in Section 2.3.1. Images were registered, simultaneously with the surface pressure–molecular area isotherm, 10 min after injecting the DSPG solution, by a CCD video camera AxioCam HRc (Carl Zeiss, Oberkochen, Germany) commanded through the Axiovision 3.1 software of the Zeiss microscope.

Thickness (h) was calculated from the BAM images taken after calibrating the BAM equipment. The grey level of each section of the micrograph can then be converted to reflected light intensity (Rp), and h was calculated assuming a smooth but thin interface in which the refractive index varies along the normal to the interface on a distance h, much smaller than the incident light wavelength λ (λ = 532 nm) [38], which leads to [39]:

$$h = \frac{\sqrt{Rp}}{\sin(2\theta_B - 90)} \left(\frac{\pi\sqrt{n_1^2 + n_2^2}(n_1^2 - n^2)(n_2^2 - n^2)}{\lambda(n_1^2 - n_2^2)n^2} \right)^{-1} \quad (2)$$

In Eq. (2) n_1, n and n_2 are the air, film and subphase refractive index respectively and θ_B is the Brewster angle.

The refractive index used for DSPG monolayers in the absence and in the presence of DS was 1.45, since this is the value reported for condensed films [40]. The refractive index for the subphases was calculated for each experiment from the experimental Brewster angle $n_2 = \tan\theta_B$, using 1.00 as the refractive index of air) obtaining a value of 1.336 for the subphase of MCl_z (M = Li^+ or Ca^{2+}) 10.0 mM + 2.00% (v/v) acetic acid in the absence and in the presence of DS.

3. Results and discussion

3.1. Cyclic voltammetry

3.1.1. Adsorption of DS on DSPA and DSPG films

Fig. 1a shows the voltammetric profiles obtained when 50 μL of 1 mM DSPG solution were injected to the interface formed between the organic phase and an aqueous solution containing 10.0 mM LiCl and 0.100% (w/v) DS. During the positive sweep several charge transfer processes can be distinguished between 0.300 and 0.450 V while in the negative sweep a well defined current peak, at Ep(–) = 0.330 V, is observed. The general shape of the profile together with the fact that the peak current of the negative process, Ip(–), has a linear dependence on the sweep rate, v, (Fig. 1b) suggest that the transfer of DS could involve an activation-controlled interfacial adsorption/desorption mechanism.

In order to characterize this adsorption/desorption process, voltammograms reversing the sweep at successively more positive potentials, E_λ, were performed. Fig. 2 shows the resulting i/E profiles, where it can be noticed that the current for the negative process increases with E_λ, indicating that Li^+ cations, transferred to the organic phase at the positive limit of the potential window, probably play a fundamental role in this process through a mechanism involving electrochemical transfer of Li^+ from the aqueous phase to the organic side of the interface, at the region of polar head group of DSPG. In this way, a possible explanation for the results emerges from considering that at high potential values, where the accumulation of Li^+ is enhanced, there occurs either an important

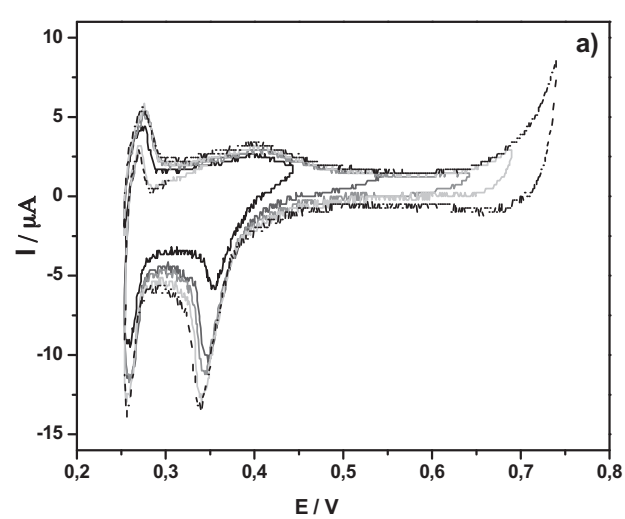
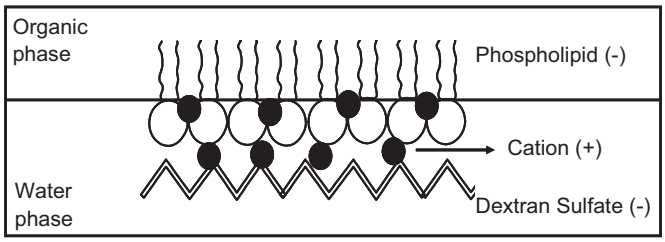


Fig. 2. Voltammograms at different E_λ: (▬) 0.450, (▬) 0.550, (▬) 0.650, (▬) 0.700, (–) 0.750 V, for DSPG film and 0.100% (w/v) DS solution in LiCl 10.0 mM. Aqueous and organic phase compositions are the same than in Fig. 1. v = 0.050 V s^{-1}. Potential values, E, reported in this figure are the applied potentials, related to the interfacial potential, $\Delta\phi$, by: $\Delta\phi = E - \Delta\phi^0_{\mathrm{tr,TPAs+}}$ (where $\Delta\phi^0_{\mathrm{tr,TPAs+}} = 0.364$ V).



Scheme 2. Model for the interaction dextran sulfate–cation–phospholipid.

neutralization of the polyelectrolyte DS near the interface or Li^+ penetration into the head group region of DSPG, as stated by [41], promoting under these conditions the formation of an interfacial DS–Li^+–DSPG complex, as postulated by Santos et al. [9]. This complex remains adsorbed at both sides of the interface, with the more polar zones oriented towards the aqueous phase and the hydrophobic fragments towards the organic one (Scheme 2). The formation of this interfacial complex is thus favoured at positive potentials with respect to the potential of zero charge (pzc). Furthermore, the negative processes could correspond to the desorption, mediated by complex dissociation, from the interface towards the aqueous phase, at potentials below pzc, according to:

$$Li^+_{(w)} + DS^-_{(w)} + DSPG_{(o/w,ads)} \rightleftarrows [DS - Li^+ - DSPG]_{(o/w,ads)} \quad (3)$$

where the forward and the reverse reactions correspond to the processes taking place during the positive and the negative potential sweeps, respectively.

A similar behaviour was observed for the phospholipid DSPA (results not shown) and the formation of the complex $[DS–Li^+–DSPA]_{(o/w,ads)}$ can also be postulated.

Fig. 3 shows the charge values, *Q*, obtained from integrating the negative peak in the voltammograms for DSPA and DSPG, as a function of DS concentration. The general increase in Q values with DS concentration supports the hypothesis of DS participation in the electrochemical process. The change in the slope of both curves indicates saturation of adsorption sites at the interface. It is important to highlight that Q values obtained in the case of DSPA are higher than those obtained for DSPG films due to the smaller size of the DSPA head group, allowing adsorption of a higher amount of species. Considering the saturation *Q* values equal to 12 μC or 24 μC for DSPG or DSPA films and the interfacial area (A = 0.16 cm^2), and assuming the formation of a close-packed monolayer for both complexes, DS–Li^+–DSPG and DS–Li^+–DSPA, an area of 0.214 or 0.107 nm^2/molecule can be calculated for DSPG or DSPA films respectively. These values of mean molecular areas are smaller than those obtained for both phospholipids in Langmuir studies (see Section 3.2.1), even more taking into account that molecular areas of phospholipids molecules at liquid/liquid interfaces are higher than those corresponding at air/water interfaces due to the penetration of solvent molecules and ions into the hydrocarbons tails. Thus, it can be postulated that the stoichiometric relations Li:DSPG or Li:DSPA are higher than 1. On other hand, this result can be interpreted considering the formation of phospholipids aggregates in the film or multilayer.

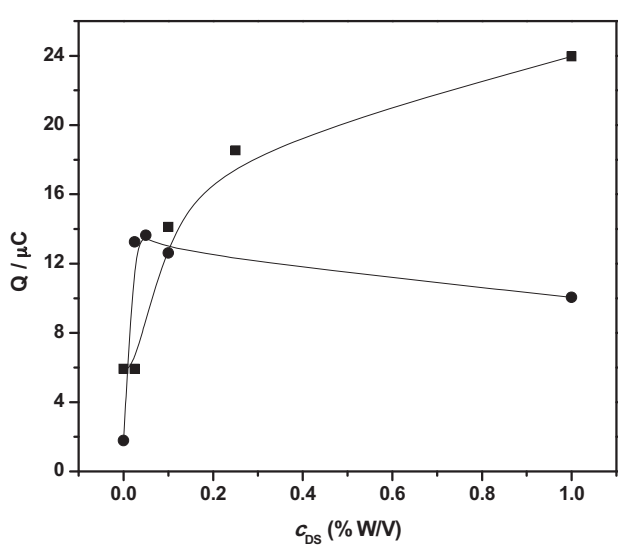


Fig. 3. Plot of charge vs DS concentration for the desorption peak in the presence of (■) DSPA or (●) DSPG films generated as described in Fig. 1. Aqueous phase composition: 1.0×10^{-2} M LiCl, 2.00% (v/v) acetic acid, pH = 3.00. Organic phase composition: 10 mM TPhAsDCC.

3.1.2. *Effect of DS on the permeability of DSPA and DSPG films*

Fig. 4a and b shows the voltammetric responses corresponding to TEA^+ transfer across the bare interface (solid black lines) and in the presence of DSPA or DSPG monolayers respectively, formed 60 min after injecting 50 μL of 1 mM DSPA or DSPG solutions at the interface between the organic and the aqueous phase containing 10.0 mM LiCl and 1.00% (w/v) DS. Solid black lines correspond to the very well known reversible diffusion controlled behaviour of TEA^+ transfer process across the bare liquid/liquid interface. A forward current peak at Ep = 0.380 V and the corresponding backward process with a peak to peak separation ΔEp = 0.060 V can be observed. The peak current, Ip, is linear with $v^{1/2}$ over the whole range of sweep rates analyzed (not shown). If this response is compared with that obtained when the phospholipid molecules are present at the interface in the absence of DS (light grey lines), important differences between DSPA and DSPG can be noticed. On the one hand, the presence of DSPA films (Fig. 4a) nearly blocks TEA^+ transfer process, showing the strong compactness of this film. In this case, the TEA^+ reversible transfer process observed at E = 0.380 V completely disappears and a new process is evident at higher potential values ($Ep \cong$ 0.600 V) without the corresponding negative peak. It has been demonstrated in a previous paper [35] that the positive peak at Ep = 0.600 V corresponds to the TEA^+ adsorption at the negative polar head groups of DSPA. Besides, the presence of DSPG films (Fig. 4b) produces an important decrease in the current and a separation of positive and negative peak potentials, as previously reported [36]. These changes evince a blocking effect of the layer on TEA^+ transfer, since it can be assumed that the transfer potential shift is due to the increase in Gibbs energy on transfer caused by the work of permeation of species across the film.

The blocking effects observed for both phospholipid films, DSPA and DSPG, decrease in the presence of DS in the aqueous phase (Fig. 4a and b, grey lines), indicating that the interfacial complex postulated in Section 3.1.1 could probably produce an expansion of the film. Similar experiments were performed employing KCl and $CaCl_2$ as supporting electrolytes in the aqueous phase. Fig. 5 summarizes the results obtained for the effect of increasing DS concentrations on the permeability of DSPA (a) or DSPG (b) films in the presence of LiCl (▲), KCl (□) or $CaCl_2$ (●). For this purpose a blocking ratio, BR, for each DS concentration value was calculated as:

$$BR = \frac{(I_{DS}^{TEA^+} - I_{DS}^{TEA^+,PL})}{I_{DS}^{TEA^+}} 100 \quad (4)$$

where $I_{DS}^{TEA^+}$ and $I_{DS}^{TEA^+,PL}$ are the peak current values for TEA^+ transfer process for each DS concentration in the absence and in the presence of the phospholipid monolayer respectively. As it can be noted, BR reaches values close to 100% for the DSPA monolayer (Fig. 5a), mainly in the case of $CaCl_2$, without any effect of DS even at high concentration values. When LiCl is present as aqueous electrolyte, a slight decrease in BR, from 100 to 78%, is observed, while no changes

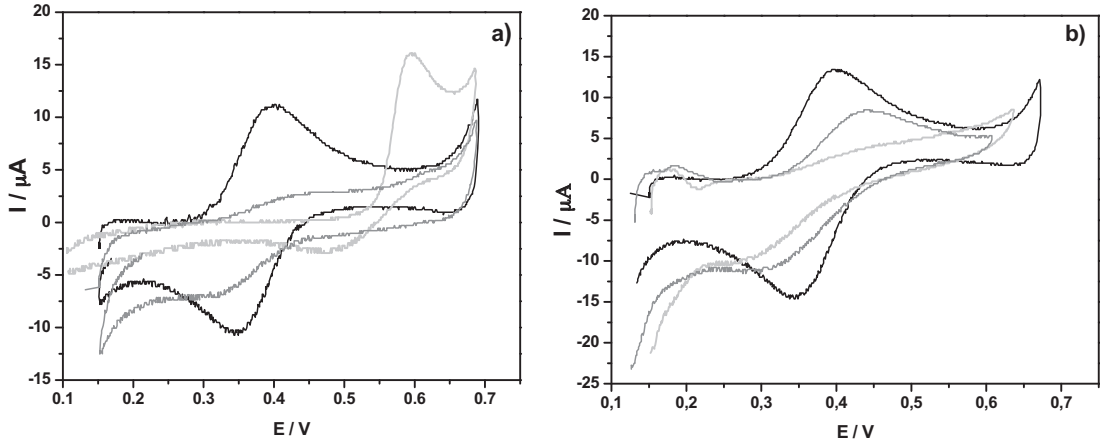


Fig. 4. Voltammograms corresponding to TEA^+ transfer through the bare interface (▬) or 60 min after the injection of 50 μL of 1 mM: (a) DSPA or (b) DSPG solutions at the interface between the organic and the aqueous phase containing (▬) 0 or (▬)1.00% (w/v) DS. Aqueous phase composition: 10.0 mM LiCl, 2.00% (v/v) acetic acid, 0.5 mM TEA^+, pH = 3.00. Organic phase composition: 10.0 mM TPhAsDCC. $\upsilon = 0.050\ V\ s^{-1}$. Potential values, E, reported in this figure are the applied potentials, related to the interfacial potential, $\Delta\phi$, by: $\Delta\phi = E - \Delta\phi^0_{tr,TPAs+}$ (where $\Delta\phi^0_{tr,TPAs+} = 0.364$ V).

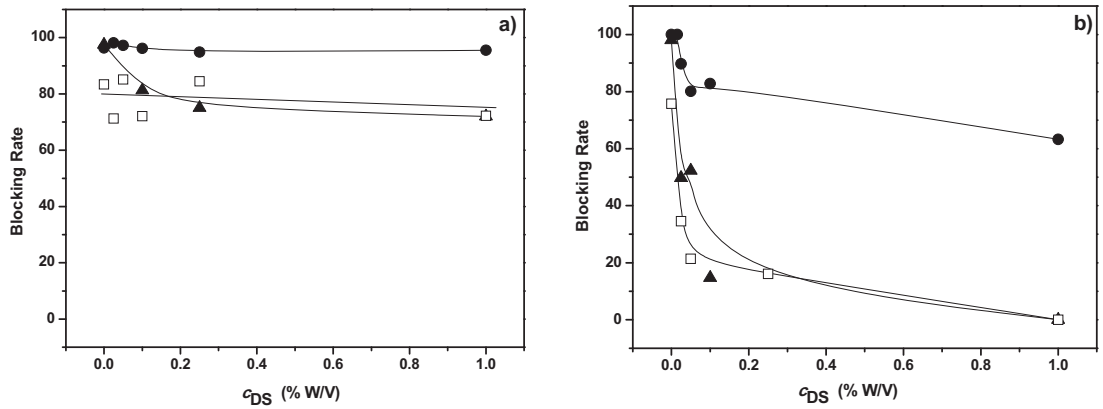


Fig. 5. Plot of blocking rate, BR = $(((I_{DS}^{TEA^+} - I_{DS}^{TEA^+,PL})/I_{DS}^{TEA^+}).100)$ vs DS concentration for (a) DSPA or (b) DSPG films. Aqueous phase composition: 10.0 mM (□) KCl, (●) $CaCl_2$ or (▲) LiCl, 2.00% (v/v) Acetic Acid, 0.5 mM TEA^+, x% (w/v) DS, pH = 3.00. Organic phase composition is the same as in Fig. 1.

occurred in the case of KCl. In contrast, for the DSPG monolayer, a sharp decrease in BR, from 100% to values close to 0%, is evident as DS concentration increases in the case of LiCl or KCl, while a slight change (100 to 75%) in BR is observed when $CaCl_2$ is the aqueous electrolyte. These different voltammetric responses depending on the cation present in the aqueous phase can be explained taking into account that Ca^{2+} cations produce an important structuring effect on DSPG monolayers due to their strong interaction with the partially ionized anionic polar head groups of phospholipids, which diminishes lateral electrostatic repulsions as it has already been reported [41]. Under these conditions, DS is not able to disorganize the film, either because the complex DS–Ca^{2+}–DSPG is

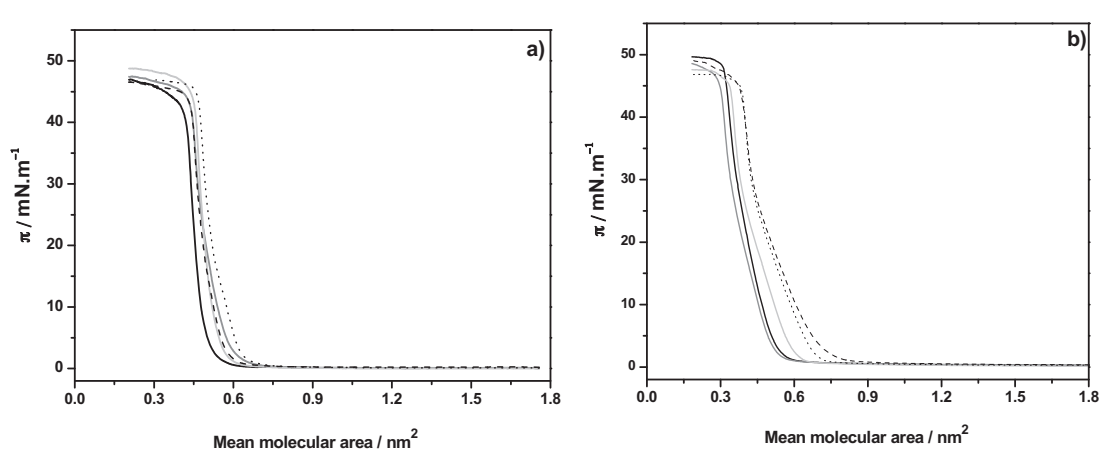


Fig. 6. Surface pressure (π) as a function of the mean molecular area for (a) DSPA and (b) DSPG monolayer at the air/water interface. Subphase composition: 2.00% (v/v) acetic acid, dextran sulfate: (▬) 0, (▬) 0.005, (▬) 0.015, (–) 0.050 and (.....) 0.100% (w/v) in 10.0 mM LiCl. pH = 3.00.

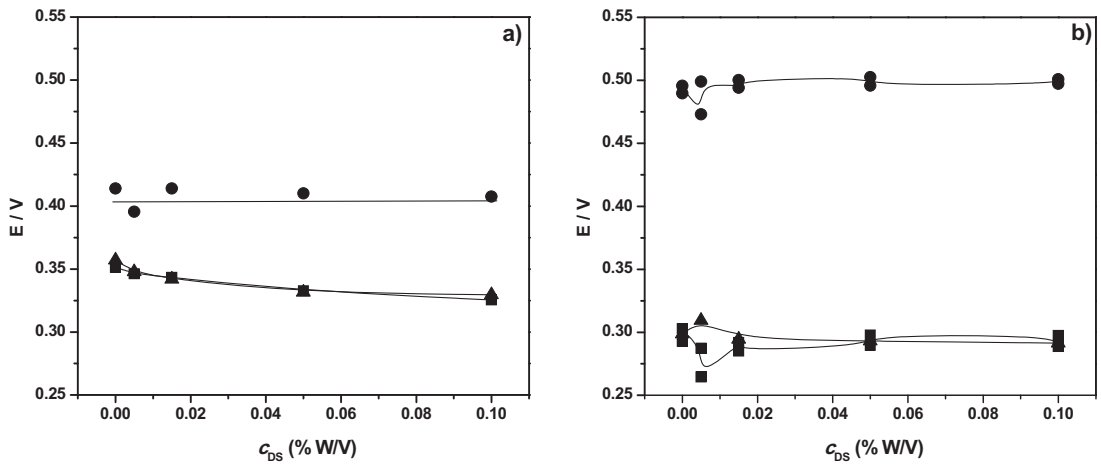


Fig. 7. Surface potential for (a) DSPA and (b) DSPG monolayers as a function of DS concentration at 30 mN m^{-1}. Subphase composition: 2.00% (v/v) acetic acid, 10.0 mM (▲) LiCl, (●) $CaCl_2$ or (■) KCl, *x*% (w/v) DS, pH = 3.00.

not formed or even if it is formed, the monolayer is not expanded. Similar considerations can be applied to the DSPA monolayer.

Summing up, the results shown above indicate that DSPG and DSPA monolayers have a blocking effect on TEA^+ transfer, but its structure and permeability can be altered by the presence of DS, by formation of a DS–cation–phospholipid complex, which can expand the monolayer in greater or lesser extent depending on the cation present in water and the polar head group of the phospholipid.

3.2. Langmuir monolayers

3.2.1. Surface pressure–molecular area isotherms

Fig. 6 shows the surface pressure-area isotherms for DSPA (Fig. 6a) and DSPG (Fig. 6b) monolayers, obtained at 25 °C in the absence and in the presence of different DS concentrations using LiCl in the subphase, as this electrolyte is the one which exhibited the greater changes in voltammetry experiments. The isotherms obtained in the absence of DS (solid line) show a change in the slope that is characteristic of the transition gaseous-liquid condensed phase. The monolayer collapse is evident at surface pressures of 48.7 or 48.0 mN m^{-1}, with mean molecular areas of 0.39 or 0.38 nm^2 for DSPA or DSPG respectively. Almost negligible changes in the surface pressure–area isotherms are observed when the DSPA monolayer is spread on the subphases containing increasing concentrations of DS, while a slight expansion is visible for the DSPG monolayer, denoted by the shift of the isotherms towards larger areas per molecule as DS concentration increases. To better characterize this expansion, the compression modulus values, κ (mN m^{-1}), were calculated for both, DSPA and DSPG monolayers at various DS concentrations. The compression modulus values allow classifying the state of the monolayer as: liquid-expanded (κ = 10–100 mN m^{-1}), liquid-condensed (κ = 100–250 mN m^{-1}) and condensed (κ > 250 mN m^{-1}) [42]. Table 1 shows the values of κ obtained at a constant pressure equal to 30 mN m^{-1} and, as it can be observed, the results indicate that in the absence of DS the state of the monolayer corresponds to a condensed phase for DSPA or a liquid-condensed state for DSPG. These differences arise from the strong cohesive lateral forces exerted between DSPA molecules, due to the small volume of their polar head group, which favour the attraction between the chains leading to a highly compact structure. In the presence of DS, even at the highest concentration value, the monolayers remain the same state, i.e. condensed or liquid-condensed phase for DSPA and DSPG respectively.

The negligible effect of DS observed in the pressure - area isotherms, seems to contradict voltammetric results, which exhibited changes in the monolayer permeability mainly for DSPG. However it is important to consider three fundamental aspects that determine the differences between liquid/liquid and air/water interfaces: the different molecular areas, the penetration of ions and solvent into the hydrocarbon tails in the case of liquid/liquid interfaces and, mainly, the effect of polarization which allows accumulation of Li^+ cations at the interface, favouring the formation of the DS–Li^+–phospholipid complex postulated in Section 3.1.1.

3.2.2. Surface potential–molecular area isotherms

Fig. 7 shows the change in surface potential, *E*, measured at 30 mN m^{-1} for DSPA (Fig. 7a) or DSPG (Fig. 7b) monolayers as a function of DS concentration, in the presence of LiCl, KCl or $CaCl_2$ as electrolytes in the subphases. As concluded in the previous section, no important changes are observed as DS concentration increases, indicating that neither the amount of charge at the surface, nor the orientation of polar head groups is altered. The higher *E* values observed in the presence of $CaCl_2$, can be explained by taking into account the excess of positive charge present at the interface due to Ca^{2+} cations interacting with the polar head groups of phospholipid molecules.

3.3. Brewster angle microscopy (BAM)

Brewster angle microscopy studies were performed in order to confirm the effect of DS on DSPA and DSPG monolayers. Petrov et al. [40] demonstrated that the contribution of the hydrophilic heads to the optical signal is negligible when the state of the monolayer is liquid expanded due to the complete hydration of the polar groups under such conditions. In this case, BAM is able to detect

Table 1
Compression modulus at π = 30 mN m^{-1} for DSPA and DSPG monolayers at different DS concentrations. Subphase composition: 2.00% (v/v) acetic acid and 10.0 mM LiCl.

c_{DS} (%, w/v)	κ (mN m^{-1})	
	DSPA	DSPG
0	319	110
0.005	271	93
0.015	336	96
0.050	310	90
0.100	315	114

κ, compression modulus at π = 30 mN m^{-1}; c_{DS}, dextran sulphate concentration; DSPG, distearoyl phosphatidyl glycerol; DSPA, distearoyl phosphatidic acid.

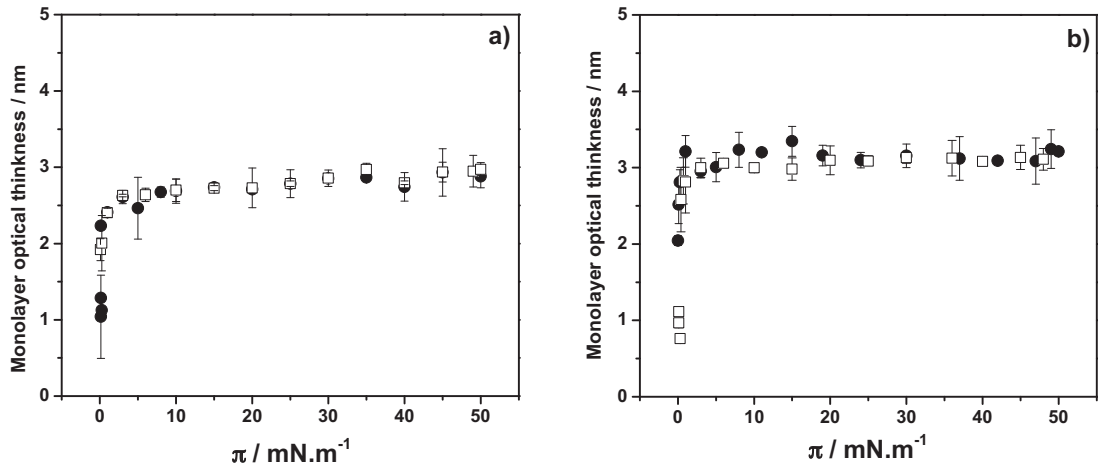


Fig. 8. Monolayer thickness for (a) DSPA and (b) DSPG. Subphase composition: 2.00% (v/v) acetic acid and 10.0 mM LiCl in the absence (●) and in the presence (□) of DS.

only the hydrocarbon tails zone. On the opposite, for condensed monolayers, such as the studied in the present paper, the head groups contribute specifically to the optical characteristics of Langmuir monolayers. They proposed a simple "heads-and-tails" model to relate the optical signal of the monolayer to the polarizability and thickness of the head group and hydrocarbon chain regions. So that, we can state that, under the present experimental conditions, it is possible to evaluate the association between DS and polar head groups, at the layer which resides below the surface, employing BAM. Fig. 8 shows the optical thickness of the DSPA (Fig. 8a) and DSPG (Fig. 8b) monolayers, calculated at several π values from reflected light intensity using Eq. (2), as described in Section 2.4. As it can be clearly noticed, thickness values are the same in the absence and in the presence of DS, at any lateral pressure value, which is a direct evidence that DS does not interact with DSPA or DSPG molecules at open circuit. Thus, as mentioned above, the formation of the complex DS–cation–phosholipid, responsible for the increase in permeability observed in voltammetric experiments, must be attributed to polarization of the interface. The application of high positive potentials at the interface promotes the accumulation of cations, which neutralizes the negative charge in phosphate groups and in DS, thus allowing their interaction.

4. Conclusions

The results obtained in the present paper indicate that DS leads to disintegration of DSPG and DSPA monolayers, the extent of this effect being greater in the presence of Li^+ or K^+ with respect to Ca^{2+} in the aqueous phase. This disintegration of the films is mediated by high positive potentials, which allow the formation of the complex DS–cation–DSPG (or DSPA) at the interface. Dissociation and desorption of this interfacial complex has been characterized by cyclic voltammetry. The results obtained from surface pressure–molecular area isotherms, surface potential–molecular area isotherms and Brewster angle microscopy, have not shown any change in the monolayers structure in the presence of DS, reinforcing the hypothesis that the applied potential participates in the disintegration of the films via the formation of a cation bridge facilitating the approach of DS to the negatively charged DSPG or DSPA monolayers.

Taking these results into account, we propose the model shown in Scheme 2 for the interaction between DSPG or DSPA and DS mediated by the applied potential. In this model we postulate that at high potential values, where the accumulation of cations from the aqueous electrolyte is enhanced, important neutralization of the polyelectrolyte DS near to the interface occurs, or Li^+ can penetrate into the head group region of DSPG or DSPA, promoting the formation of an interfacial DS–cation–DSPG (or DSPA) complex. This complex remains adsorbed at both sides of the interface, with the more polar zones oriented towards the aqueous phase and the hydrophobic fragments towards the organic one. In this way, the negative processes observed in voltammetric experiments in the presence of the monolayer and of DS could correspond to the desorption, mediated by complex dissociation, from the interface towards the aqueous phase.

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