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Journal of Applied Electrochemistry

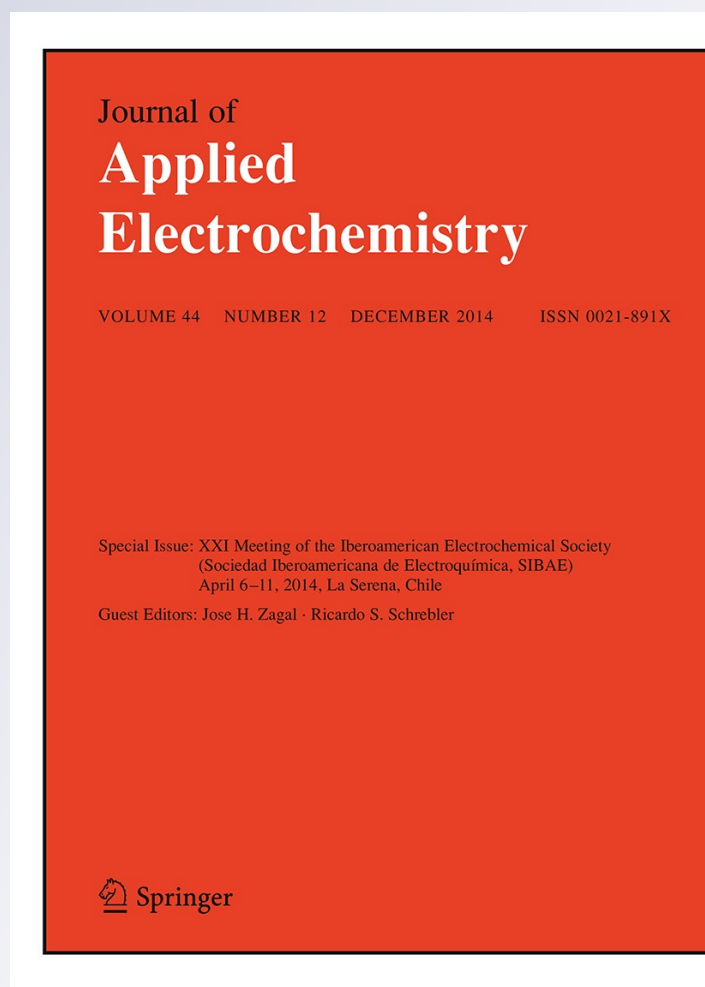
ISSN 0021-891X

Volume 44

Number 12

J Appl Electrochem (2014) 44:1381-1392

DOI 10.1007/s10800-014-0747-2



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Electrochemical behaviour of cationic polyelectrolytes at a polarized liquid/liquid interface

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Received: 31 July 2014 / Accepted: 11 September 2014 / Published online: 21 September 2014
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Abstract The behaviours of several cationic polyelectrolytes (chitosan; polyquaternium-4; diethylaminoethyl dextran; polyquaternium-10, and aminoacrylmethacrylate copolymer, Eudragit[®] E100) at a polarised liquid/liquid interface are analysed and compared in the present paper. Based on the analysis of the voltammetric results, it was possible to determine some of the relationships between polymer structure and the tendency to adsorb at the interface. It was also possible to determine the substitution degree of a polymer comprising a chitosan main chain modified with glycidyltrimethylammonium chloride, and the values obtained are in agreement with those calculated using other methodologies. Finally, we report a comparative study concerning the effect of cationic (chitosan) and anionic (dextran sulphate) polyelectrolytes on the compactness of a phospholipid film.

Keywords Polyelectrolytes · Liquid/liquid interfaces · Phospholipid films · Polymer adsorption · Electrochemistry

1 Introduction

Polymeric materials are widely known for their versatility and their many applications in the fields of medicine and biotechnology as well as in the pharmaceutical, food, and cosmetics industries. Thus, they have been used in surgical devices (implants, prostheses, artificial organs, etc.), in controlled drug release systems with different administration routes, as supports for immobilised enzymes in biosensors, as bio-adhesives, in the preparation of different foods, and in many cosmetic formulations [1]. Particularly, when the polymer chains possess a net charge, as is the case with cationic polyelectrolytes, their most important uses are related to their ability to interact electrostatically with oppositely charged surfaces such as surfactants, micelles, or membrane components such as phospholipids. Thus, it has been shown that cationic polymers such as chitosan and Eudragit E100 cause lysis of membranes through electrostatic interactions with the polar groups of biological membrane components, exerting an antiviral and antifungal effect [2–4]. These applications are enhanced by the fact that these polymers are biodegradable and not toxic. Moreover, they are muco-adhesive and biocompatible; therefore, they can serve as an excellent matrix for controlled release pharmaceutical preparations.

The study of the physicochemical properties of polyelectrolytes and their interactions with oppositely charged molecules of different nature is an important aspect in relation to the above applications. In this way, the behaviour of polyelectrolytes at interfaces has gained great importance and has been studied by several authors due to their relevance in the study of macromolecules as well as their numerous applications, mainly those related with their pharmaceutical uses in topical preparations. In such preparations, polyelectrolytes exhibit long residence times at

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the application site and adequate mechanical properties. For these reasons, the study of surface properties of these substances on diverse interfaces employed for modelling biological membranes, including solid/liquid, air/water and liquid/liquid interfaces, has been a topic of growing interest.

The application of electrochemical methods to an interface formed by two immiscible electrolyte solutions (aqueous phase/organic phase) allows for the study of the transfer of charged species from one phase to the other, giving rise to a faradaic current increase [5]. These studies provide kinetic information about the transfer process and may also be used to develop methods for quantitative analysis of the transferred species or to calculate partition coefficient values of charged molecules [6, 7]. It is also possible to study the interfacial behaviour of amphiphathic neutral or charged species adsorbed at the interface and their interaction with other substances in solution [8].

The first study of polyelectrolyte adsorption at liquid/liquid interfaces was carried out by M.D. Osborne et al., who reported Nafion[®] adsorption with two different adsorbed forms corresponding to a morphological difference associated with the adsorption in the mixed solvent layer [9]. Later, the interfacial behaviour of several polyelectrolytes and their interactions with different ions was studied at liquid/liquid interfaces by different authors [8, 10–15]. Special attention has been devoted to the investigation of biopolymer adsorption [16, 17] such as that of the polysaccharide heparin [18–22] and the proteins haemoglobin [23, 24], myoglobin [25], insulin [26], protamine [27–30], melittin [31], cytochrome c, and ribonuclease A [30, 32]. Another research topic is focused on the study of the interactions between polyelectrolytes and membrane components, such as phospholipid molecules adsorbed at liquid/liquid interfaces [33, 34].

The present paper reports different relevant aspects of the study of polyelectrolytes at polarised liquid/liquid interfaces. First, in Sect. 3.1, we present a comparison of the interfacial behaviour and transfer mechanism of several cationic polyelectrolytes, including chitosan (CHI), polyquaternium-4 (PQ4, Celquat[®] L200) [35], diethylaminoethyl dextran (DEAE-D), polyquaternium-10 (PQ10, Celquat[®] SC230), and aminoacrylmethacrylate copolymer (Eudragit[®] E100) [36], correlating the results with the differences in their chemical structure. Secondly, in Sect. 3.2, we report a methodology to determine the substitution degree in a chitosan chain modified with glycidyltrimethylammonium chloride (GTMAC), a quaternary ammonium group, which confers a permanent positive charge to the polymer. This modification allows high solubility of the polymer in a wide pH range, improving CHI applications mainly at neutral or high pH values. Finally, in Sect. 3.3, we compare the effect of a cationic (CHI) or an

anionic (dextran sulphate, DS) polyelectrolyte on the permeability and the compactness of a distearoyl phosphatidyl glycerol (DSPG) film formed at liquid/liquid or air/water interfaces [37, 38]. The importance of these studies is based not only on the wide application of both polymers in pharmaceutical preparations for topical use but also in the similarity between DS and glycosaminoglycans (GAGs), which 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. GAGs can be bound to proteins, forming proteoglycans and are very important in the process that involves the capture of positive ions and drugs due to their high negative charge [39]. Additionally, GAGs occupy almost all extracellular spaces, form a mechanical support for tissues, and facilitate the diffusion of hydrophilic molecules as well as cell migration [40].

2 Experimental

2.1 Materials and electrochemical cells

The base electrolyte solutions were 1.0×10^{-2} M LiCl (Merck p.a.) in ultrapure water (MilliQ RiOs 16, Millipore) and 1.0×10^{-2} M tetraphenylarsonium dicarbollylcobaltate (TPhAsDCC) or 1.0×10^{-2} M tetrapentylammonium tetrakis (4-chlorophenyl) borate (TPnATCIPhB) in 1,2-dichloroethane (DCE, Dorwil p.a.). TPhAsDCC and TPnATCIPhB were prepared by metathesis of tetraphenylarsonium chloride (TPhAsCl, Sigma) and sodium dicarbollylcobaltate (NaDCC, Strem Chemicals) or tetrapentylammonium bromide (TPnABr, Fluka) and potassium tetrakis (4-chlorophenyl) borate (KTCIPhB, Sigma-Aldrich), respectively. The precipitates were recrystallised from water:acetone mixtures and subsequently dried in an oven at 30 °C for 2 days.

The polyelectrolytes CHI, with an average molecular weight (MW) of 400 kDa (Sigma-Aldrich); diethylaminoethyl dextran (DEAE-D), with a MW of 50 kDa (Carbomer); cationic hydroxyethylcellulose (PQ10 SC230), with a MW of 600–2,000 kDa (The National Starch and Chemical Co.); cationic hydroxyethylcellulose dimethyl diallylammonium chloride copolymer (PQ4) with a MW of 100 kDa (The National Starch and Chemical Co.); and aminoacrylmethacrylate copolymer (Eudragit[®] E100) with a MW of 150 kDa (Etilfarma S.A) were used without further purification. These polyelectrolytes were dissolved in the aqueous phase at concentrations between 0.100 and 0.050 % w/v.

To prepare the polymer CHI-GTMAC, CHI (500 mg) was dispersed in distilled water (10 mL), and then GTMAC was added at a molar ratio of 3:1 (moles of GTMAC per mol of amine groups in CHI). The reaction was performed under nitrogen purging at 70 °C for 10 h.

After the reaction, the solution was poured into acetone/ethanol (50/50, v/v). The product was filtered and dried under vacuum. CHI-GTMAC was subsequently characterised by Fourier transform infrared (FT-IR) spectra, employing a Nicolet Avatar 360 FT-IR spectrometer, and ^1H NMR, employing a Bruker 500 MHz spectrometer. The results obtained from these techniques confirm a covalent union between GTMAC and CHI.

The pH value determines the amount of protonated groups in all of the polyelectrolytes analysed. Thus, the pH of the aqueous phase was varied between 2.00 and 8.50 by addition of HCl (Merck p.a.) or LiOH (Merck p.a.) with the aim of analysing the effect of the charge on the interfacial behaviour of these polymers.

To study the interaction between DSPG films and the polyelectrolytes CHI and DS, pure dextran sulphate sodium salt (Sigma-Aldrich, MW \approx 5000 Da, 17 % sulphur) or pure chitosan (Sigma-Aldrich, MW: 50–190 kDa, >75 % deacetylated) were added to the aqueous phase (w) in a concentration range of 0–1.00 % w/v. DSPG (analytical grade, Sigma-Aldrich), was dissolved in 1:2 methanol:chloroform to prepare a 1 mM solution. To form the film, 50 μL of DSPG solution was injected using a Hamilton microsyringe at the liquid/liquid interface after both phases were brought into contact in the electrochemical cell. A time period of 60 min was required, after the injection of the lipid solution, to obtain an invariant and reproducible voltammetric response, indicating that a stable lipid film had been formed. Thus, all experiments were performed after this equilibration time at room temperature (25 ± 1 °C). The temperature was controlled with a temperature/humidity monitor.

All experiments were carried out in a four-electrode system using a conventional glass cell of 0.18 cm^2 . In both cases, two platinum wires were used as counter-electrodes, and the reference electrodes were Ag/AgCl, which were located in opposing phases and controlled the applied potential across the liquid–liquid interface. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 1.0×10^{-2} M TPhAsCl or 1.0×10^{-2} M TPnABr + 1.0×10^{-2} M LiCl.

2.2 Methods

2.2.1 Cyclic voltammetry

Cyclic voltammetry and potential pulse experiments were carried out using a four-electrode potentiostat, which automatically eliminates the IR drop by means of a periodic current-interruption technique. A wave potential generator (PPR1 Hi-Teck Instruments, UK) and a 10 bit Computer Boards acquisition card connected to a personal computer were also employed.

The potential values (E) reported in the voltammograms are the applied potentials between the two Ag/AgCl reference electrodes, which are related to the Galvani potential difference ($\Delta_o^w \phi$) across the interface by $E = (\Delta_o^w \phi) + \Delta E_{\text{ref}}$, in which ΔE_{ref} depends on the reference electrodes and the reference solutions employed.

2.2.2 Surface pressure/molecular area isotherms

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

The aqueous subphase, contained within a Teflon trough (364 mm \times 75 mm effective film area), was 1.0×10^{-2} M LiCl with or without the polymers at different concentrations.

To prepare DSPG monolayers at the air/water interface, 30 μL of DSPG solution in 1:2 methanol:chloroform (0.40 mg mL^{-1}) was carefully spread at the surface with a Hamilton micro-syringe. Before spreading the DSPG solution, the subphase surface was cleaned by sweeping it with the Teflon barriers; subsequently, surface contaminants were removed by suction from the interface. The cleanliness of the surface was checked by recording an isotherm in the absence of DSPG and verifying a surface pressure value lower than 0.20 mN/m. After spreading, the solvent was allowed to evaporate 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 the lateral surface pressure (π) was measured automatically. The pressure/area isotherms for DSPG were recorded in the presence or absence of CHI or DS in the subphase, with the aim of evaluating the effect of these polyelectrolytes on the monolayer structure.

All experiments were performed at 25 ± 1 °C using a thermostat (HAAKE G.). At least two compression isotherms were registered under each condition. Results were obtained with a typical area and collapse pressure error of $\pm 2 \text{ \AA}^2$ and $\pm 1 \text{ mN m}^{-1}$, respectively.

Moreover, pressure/area isotherms were measured for all of the polyelectrolytes studied (CHI, DEAE-D, PQ10 SC230, PQ4, and Eudragit[®] E100). In these cases, solutions of each polymer at a concentration of 0.025 % w/v in 1:3 water:methanol were prepared, and a volume of 35 μL was carefully injected at the surface of the subphase.

2.2.3 Brewster angle microscopy (BAM)

With the aim of analysing the effect of CHI and DS on the thickness of the DSPG monolayer at the air/water interface, BAM experiments were carried out using an EP3 imaging ellipsometer (Accurion, Goettingen, Germany) with a 20 \times

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 DSPG solution as described in the previous section. Images were registered 10 min after the injection of DSPG solution simultaneously with the surface pressure/molecular area isotherm.

The optical thickness (h) was calculated from the BAM images obtained after the BAM equipment was calibrated. The grey level of each section of the micrograph was then converted to reflected light intensity (R_p), 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 , which is much smaller than the incident light wavelength λ ($\lambda = 532$ nm) [41], leading to the following:

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

In Eq. (1), n_1 , n and n_2 are the air, film, and subphase refractive indices, respectively, and θ_B is the Brewster angle.

The refractive index used for DSPG monolayers in the absence of chitosan was 1.45 because this is the value reported for condensed films [42]. As detailed below, when chitosan was present in the subphase, the DSPG film became more expanded. Subsequently, the refractive index is expected to decrease [43]. Because the refractive index at this condition was unknown, we determined the monolayer thicknesses using 1.42 (index for liquid expanded phases) and 1.45 (index for liquid condensed phases) [43]; in this way, the whole range of possible height values could be evaluated. 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).

3 Results and discussion

3.1 Comparative interfacial behaviour of cationic polyelectrolytes

To compare the interfacial behaviour of polymers and to correlate these behaviours with their chemical structure, voltammograms of different cationic polyelectrolytes were performed at liquid/liquid interfaces.

Figure 1 shows the voltammetric profiles obtained for CHI, diethylaminoethyl dextran (DEAE-D), cationic hydroxyethylcellulose (PQ10 SC230), and cationic hydroxyethylcellulose dimethyl diallylammonium chloride copolymer (PQ4) at a concentration equal to 0.100 % w/v

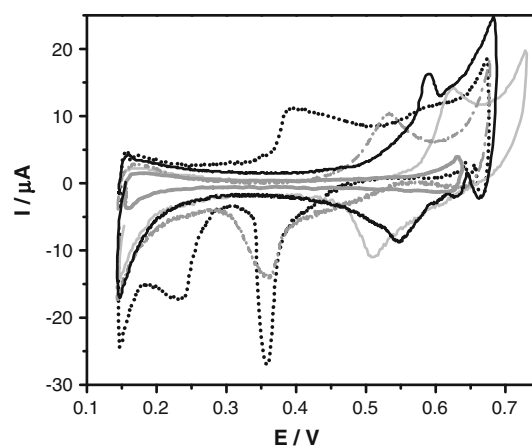


Fig. 1 Voltammograms of cationic polymers: (dotted line) PQ4, (black line) PQ10, (light gray) DEAE-D, (dashed dotted line) Eudragit® E100, (thick gray) CHI. Aqueous phase: 1.0×10^{-2} M LiCl + polymer: 0.100 % w/v (PQ4, PQ10, DEAE-D, CHI) or 0.05 % w/v (Eudragit® E100). Organic phase: 1.0×10^{-2} M DCCTPAs in DCE. $v = 0.025$ Vs $^{-1}$

and aminoacrylmethacrylate copolymer (Eudragit® E100) at concentration equal to 0.050 % w/v.

When CHI is present in the aqueous phase, no transfer or adsorption processes are observed. However, CHI acts as carrier for the DCC^- anion, as previously reported [13].

The voltammogram obtained for the polymer PQ10 SC230 shows a current peak at $E_{p_1}^+ = 0.590$ V during the positive scan and the corresponding negative process at $E_{p_1}^- = 0.550$ V in the reverse sweep. This profile reveals an enhancement of the positive peak current with respect to the negative current. When voltammograms at different sweep rates were recorded, the enhancement of the positive peak current was increased with an increasing sweep rate. This behaviour is explained by taking into account that polymer diffusion and weak adsorption are involved in the transfer process from the aqueous to the organic phase, at $E_{p_1}^+$ [14].

DEAE-D polymer is transferred by a diffusion-controlled mechanism at $E_{p_1}^+ = 0.625$ V because I_p is linear with $v^{1/2}$. Nevertheless, for high DCC^- concentrations in the organic phase (>0.080 M), a linear dependence of I_p with v was observed, indicating an interfacial adsorption process (data not shown). Thus, it can be established that DEAE-D polymer is transferred from the aqueous to the organic phase by a mixed mechanism involving weak adsorption and diffusion depending on the concentration of the anion present in the organic phase.

The transfer of Eudragit® E100 occurs at $E_{p_1}^+ = 0.530$ V, which is a potential value lower than those corresponding to DEAE-D and PQ10. This result is due to the higher hydrophobicity and surface activity of this polymer. On the other hand, although the current values for the

transfer of all of the polymers appear to be similar, the concentration of Eudragit® E100 in the aqueous phase was one-half of that corresponding to the other polymers. This result can be explained by taking into account the fact that E100 has a greater charge in its structure at pH 3.00. As reported previously [36], this polymer is transferred from the aqueous to the organic phase during the first positive sweep through a coupled mechanism of adsorption/diffusion control. Once E100 is transferred to the organic side, it adsorbs at the interface via the formation of ion pairs with the anion DCC^- , and a desorption process at $E_{p1}^- = 0.360 \text{ V}$ takes place during the negative sweep. On the other hand, a second process in the positive sweep appears during the second and successive potential scans that is attributable to the transfer of some species that were not present during the first cycle, whereas the pre-existing peak at $E_{p1}^+ = 0.530 \text{ V}$ remains unchanged with successive sweeps. It was concluded that this type of species could arise due to conformational changes in E100 molecules, due to the adsorption–desorption process, or due to DCC^- ion retention at the positive sites of the polyelectrolyte after the desorption process. This new conformation of the polymer favours its spontaneous adsorption at the interface producing a pre-peak from the second cycle that is attributable to the transfer of these adsorbed species (data not shown, Ref. [36]). This process is favoured by increasing the number of cycles and decreasing the pH. Even in this case, a single peak is observed in the reverse scan, which is attributable to desorption of the polymer.

The voltammetric response of the polymer PQ4 is markedly different from previous polyelectrolytes. Firstly, the transfer occurs at the lowest potential value, and the current involved in this process corresponds to the transfer of the anion DCC^- , which is present in the organic phase, to the aqueous side of the interface, which is favoured by the presence of PQ4. Furthermore, the sharp current peak observed at 0.350 V in the reverse scan corresponds to desorption of the anion that was previously adsorbed on the positive groups of PQ4 present at the interface. The second desorption process at lower potential values ($E_{p1}^- = 0.230 \text{ V}$) corresponds to desorption of the polymer adsorbed at the organic side. These conclusions were deduced from the analysis of the voltammetric charge obtained after applying potential pulses at different positive potential values and during different times as well as by spectroelectrochemistry experiments [35].

A summary of the proposed mechanisms for the interfacial transfer of each polyelectrolyte (P^{z+}) along with its chemical structure is shown in Table 1. From this scheme, the following considerations arise regarding the relationship between the chemical structure of the polymer and the corresponding interfacial behaviour:

- When the charged groups of the polymer are directly attached to the monomers in the chain, as in the case of CHI, the interfacial behaviour is poor, and no transfer processes are observed at the polarised liquid/liquid interface.
- A higher surface activity, and thus an increased level of interaction with the counterion, is observed for polyelectrolytes with charged groups that are more distant from the main chain of the polymer ($\text{DEAE-D} < \text{PQ10} < \text{PQ4}$).
- The greater the hydrophobicity of the monomer chain, the higher the surface activity. Such is the case of the Eudragit® E100 polymer, which has a main chain composed of esters of acrylic and methacrylic acid that is more hydrophobic than cellulose.
- Adsorption at the aqueous or the organic side of the interface depends on the amount of charged groups on the graft chain in the cellulose structure. The results obtained indicate that the increase in charge prevents the transfer of the adsorbed species to the organic phase, which can only occur when their charged groups are neutralised by the anion present in the organic electrolyte (PQ4 requires DCC^- to be transferred to the aqueous phase to allow insertion of the segments into the organic phase).
- When the chain of monomers is branched (DEAE-D), the direct transfer mechanism with diffusional control predominates over the adsorption.

With the aim of analysing the behaviour of these polymers at an air/water interface, adsorption isotherms were performed using $\text{LiCl } 1.00 \times 10^{-2} \text{ M}$ as subphase in all cases to maintain the same conditions as those employed in the electrochemical experiments at the liquid/liquid interface. The formation of stable monolayers of polymeric compounds depends on the attraction forces exerted by the subphase on these molecules. At low concentrations, the polymers form insoluble monomolecular films at the air/water interface such that the non-polar segments predominate in the hydrophobic phase, while the polar segments are in contact with water. The polymers may have different orientations at the interface, and the number of segments in contact with the surface will depend on the molecular flexibility of the chain and on the affinity for the dissolution medium. The configurations of a flexible polymer in the two-dimensional plane of the interface can result in three types of orientations: trains, loops, and tails. When a polymer monolayer is fully extended with each of the monomeric units present at the interface, it occupies a maximum molecular area, and most of the segments are in the standard configuration (“trains”). When the monolayer is compressed, the residues that are in contact with the surface begin to adopt different conformations at the

Table 1 Chemical structures of the polymers and their transfer mechanisms at liquid/liquid interfaces

Chemical structure	Transfer reactions
<p>CHI</p>	$P_{(w)}^{z+} \rightarrow P_{(o)}^{z+}$ $P_{(w)}^{z+} \rightarrow P_{(ads)}^{z+}$
<p>PQ10</p>	$P_{(w, dis, ads)}^{z+} + nDCC_{(o)}^{-} \rightarrow P(DCC)_{n(o, dis)}^{(z-n)+}$
<p>PQ4</p>	<p>At low potential values</p> $P_{(w, ads)}^{z+} + nDCC_{(o)}^{-} \rightarrow P(DCC)_{n(w, ads)}^{(z-n)+}$ <p>At high potential values</p> $P(DCC)_{n(w, ads)}^{(z-n)+} \rightarrow P(DCC)_{n(w, ads)}^{(z-n)+}$
<p>E100</p> <p>R = CH₂, C₄H₉</p>	<p>First cycle</p> $P_{(w, ads, dis)}^{z+} + nDCC_{(o)}^{-} \rightarrow P(DCC)_{n(o, ads)}^{(z-n)+} \rightarrow P(DCC)_{n(o, ads)}^{(z-n)+} \rightarrow P(DCC)_{n(o, ads)}^{(z-x)+}$ <p>Second cycle</p> $P(DCC)_{n(o, ads)}^{(z-x)+} \rightarrow P(DCC)_{n(o, ads)}^{(z-x)+}$
<p>DEAE-D</p>	<p>At low DCC_(o)⁻ concentrations</p> $P_{(w, dis)}^{z+} + nDCC_{(o)}^{-} \rightarrow P(DCC)_{n(o, ads)}^{(z-n)+}$ <p>At high DCC_(o)⁻ concentrations</p> $P_{(w, dis, ads)}^{z+} + nDCC_{(o)}^{-} \rightarrow P(DCC)_{n(o, dis)}^{(z-n)+}$

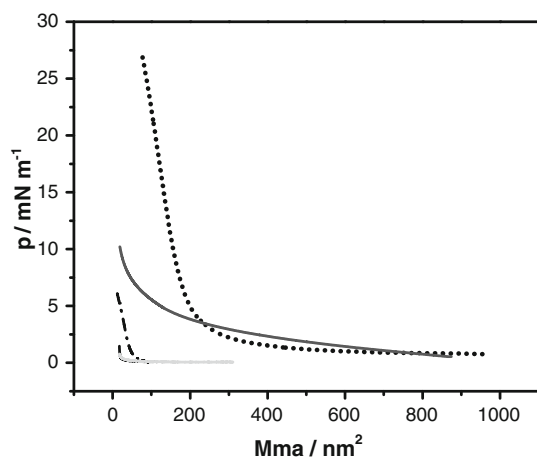


Fig. 2 Surface pressure/mean molecular area (Mma/nm^2) isotherms recorded for different polymers: (dotted line) E100, (black line) PQ10, (dashed dotted line) PQ4, (light gray) CHI, (thick gray) DEAE-D. Subphase: 1.0×10^{-2} M LiCl

interface, existing in segments such as “loop” or “tail” configurations, oriented towards the aqueous subphase. The distribution of these conformations in the polymer depends on its structure, its flexibility, the nature of the subphase, the type of solution used to spread it over the surface, and the state of compression of the monolayer. In general, polymers without polar groups cannot form monolayers. However, excessive electric charges in the molecule lead to loss of the monolayer by dissolution in the subphase.

Figure 2 shows the compression isotherms obtained for Eudragit® E100, PQ10 SC230, PQ4, CHI and DEAE-D. Eudragit® E100 polymer presents a well-defined isotherm, indicating that this polymer has an important surface activity. The maximum pressure reached is approximately 27 mN m^{-1} and corresponds to a predominance of “loop” or “tail” conformations. This is in agreement with the voltammetric results, which indicate that the transfer mechanism at the liquid/liquid interface involves strong adsorption of the polyelectrolyte on the organic side of the interface after the transfer. On the other hand, the compression of PQ10 molecules present at the surface leads to an isotherm with low pressure values (10 mN m^{-1}). Both Eudragit® E100 and PQ10 polymers have a higher surface activity than other polyelectrolytes, and they both exhibited a diffusional transfer mechanism coupled with a previous adsorption stage at the liquid/liquid interface.

DEAE-D and CHI polymers did not adsorb at the air/water interface, and coincidentally, no adsorption processes were evident in the electrochemical studies at the liquid/liquid interface. The voltammetric response obtained for CHI shows that this polymer was not transferred to the organic phase, while diffusion-controlled transfer is evident for the polymer DEAE-D at low anion concentrations

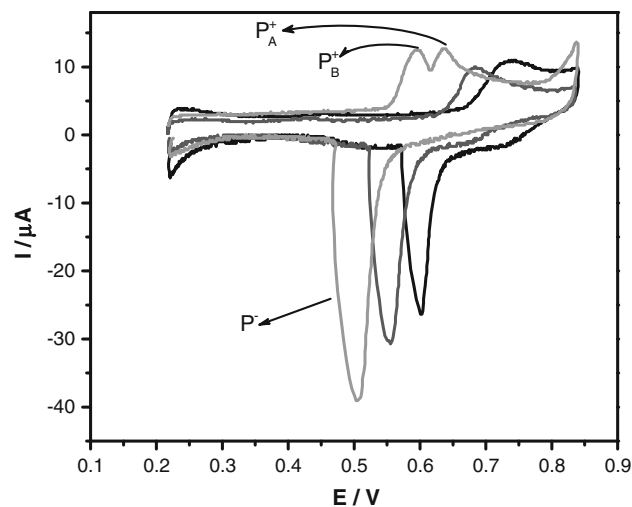


Fig. 3 Cyclic voltammograms for CHI-GTMAC at the water/1,2-dichloroethane interface at pH = (black line) 2.4; (thick gray) 5.5; (light gray) 8.2. Aqueous phase composition: 1.0×10^{-2} M LiCl + 0.02 % w/v CHI-GTMAC. Organic phase composition: 1.0×10^{-2} M TPATCIPhB. $v = 0.100 \text{ V s}^{-1}$

in the organic phase, being the adsorption processes only evident when an excess of the supporting electrolyte was present in the organic phase.

The adsorption isotherm corresponding to the polymer PQ4 reached very low pressure values ($\sim 6 \text{ mN m}^{-1}$), which correspond to a predominance of segments with “train” conformations. Electrochemical studies at liquid/liquid interfaces demonstrated that this polymer adsorbed at the aqueous side of the interface, facilitating the transfer of anions from the organic to the aqueous phase, followed by adsorption and ion pair formation at the interface. However, as the concentration of the organic electrolyte increases, the positive groups of the polymer become neutralised, favouring the penetration and adsorption of the cationic polymer into the organic side.

3.2 Determination of the substitution degree of CHI-GTMAC

The electrochemical behaviour of CHI-GTMAC was characterised by employing cyclic voltammetry at the liquid/liquid interface, analysing the effect of polymer concentration and pH in the aqueous phase. Additionally, potential step experiments followed by negative scans at different sweep rates were carried out.

CHI-GTMAC is a polyelectrolyte that contains primary and secondary amine groups in the chitosan chain and quaternary amine groups in the GTMAC. Thus, the net charge on the polymer depends on the pH of the aqueous phase. With the aim of studying the effect of pH on the electrochemical response, the polymer was added, at

several concentrations, to the aqueous phase at different pH values within the range of 2.3–10.2. Figure 3 shows the voltammetric response obtained for 0.02 % w/v CHI-GTMAC at different pH values. It was found that its behaviour was quite different from that obtained with native CHI, for which no electrochemical response was observed in the potential window employed (see Fig. 1). An asymmetry in the general shape of the voltammetric profiles can be clearly noted. The transfer process in the positive wave corresponds to the transfer of the polymer from the aqueous to the organic side of the interface, where it remains adsorbed via ion pair formation with the anions present in the organic phase. In the reverse scan, a narrow peak is observed, which is characteristic of a desorption process due to the return of the adsorbed polymer to the aqueous phase. It is important to note two interesting points. While the pH increased, the peak potential for the positive process (P_A^+) shifted towards lower values due to the deprotonation of the primary amines of the polymer, which produces greater hydrophobicity. On the other hand, at the highest pH value, a second peak appeared at lower potential values (P_B^+), corresponding to a different transfer process of the polymer to the organic phase. Clearly, this process requires lower free energy than that corresponding to P_A^+ . A linear relationship of I_p^+ with sweep rate was observed for P_B^+ , while the peak current was linear with the square root of the scan rate for P_A^+ at all pH values studied. These results suggest an activation-controlled interfacial adsorption mechanism for P_B^+ and a diffusion process for P_A^+ . Thus, the electrochemical behaviour indicates that at high pH values, CHI-GTMAC can be present not only dissolved in solution but also adsorbed at the aqueous side of the interface prior to the electrochemical transfer.

Once the polarisation is applied to the interface, a mixed transfer mechanism of the polyelectrolyte to the organic side of the interface occurs, involving a direct diffusion transfer of the polymer from the bulk of the aqueous phase and the transfer of the adsorbed CHI-GTMAC molecules from the aqueous to the organic side of the interface. In the reverse scan, a sharp peak can be observed at all pH values studied (P^-), corresponding to desorption of the polymer from the organic side of the interface to the aqueous phase. E_p^- shifts towards more negative values as the pH increases, in accordance with the more hydrophobic nature of the polymer due to the loss of positive charge resulting from the amine deprotonation. More energy is necessary to transfer more hydrophobic polymers to the aqueous phase. Similar behaviour was observed at high CHI-GTMAC concentrations.

In summary, the results allow the following reactions for CHI-GTMAC transfer in the positive scan to be postulated:

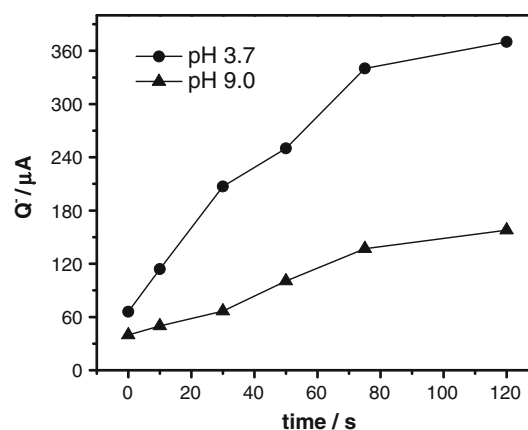
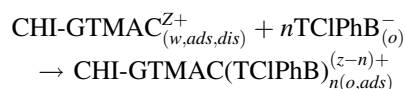
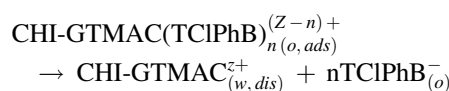


Fig. 4 Plot of $Q(-)$ values vs $\Delta\tau$, calculated from voltammograms obtained after holding the potential at $E = 0.850$ V for different times. pH values: (filled circle) pH 3.7; (filled triangle) pH 9.0



In the reverse scan, the following is postulated:



where the subscripts *ads* or *dis* refer to adsorbed or dissolved CHI-GTMAC molecules.

With the aim of analysing the desorption process, potential steps, followed by negative scans at different sweep rates, were carried out. Steps at $E = 0.850$ V were applied for different times, $\Delta\tau$, prior to the negative scan. From the voltammograms obtained, it is evident that the negative peak current increased with $\Delta\tau$ as a consequence of increases in the amount of polymer adsorbed at the interface. On the other hand, the peak potential was constant with the sweep rate for all $\Delta\tau$ values employed and for all pH values and polymer concentrations studied, which indicates the absence of lateral interactions between the adsorbed molecules. To analyse the desorption charge, $Q(-)$, we selected a low sweep rate value to ensure complete desorption of CHI-GTMAC. Thus, $Q(-)$ values were calculated by integrating the desorption peaks during the negative scans at 0.010 Vs^{-1} after holding the potential at $E = 0.850$ V for different time periods at all pH values studied. The results are shown in Fig. 4, and it can be seen that the charge depends on the pH of the system. At pH 9.0, a plateau is observed corresponding to a $Q(-)$ equal to 150 μC . The same behaviour was observed at pH values higher than 5.3, while at lower pH values (2.4 and 3.7), the saturation charge was equal to 360 μC . Given the polymer charge at the different pH values, the substitution degree of primary amine groups in the CHI chain by GTMAC groups

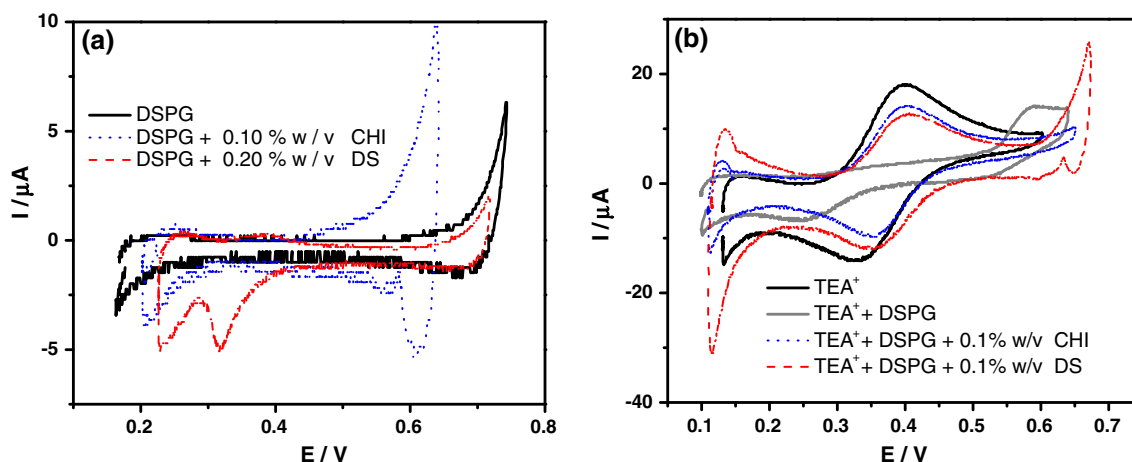


Fig. 5 Cyclic voltammograms recorded in absence (filled rectangle) or presence of 0.10 % w/v DS (dashed dotted line) or 0.10 % w/v CHI (dotted line) after injection of 50 μL of a 1 mM DSPG solution.

can be calculated. At high pH values, the charge obtained depends only on the quaternary amine groups in GTMAC, while at low pH values, the charge depends on both amine groups, i.e., not only the quaternary charged amines in GTMAC but also the primary protonated amines ($\text{pK}_a \approx 6.5$) in CHI, which have not been substituted by GTMAC molecules. Thus, the percentage of substituted amines can be calculated by relating both $Q(-)$ values ($150 \mu\text{C}/360 \mu\text{C}$). After performing this calculation, the percentage obtained was 41 %, which is similar to the values obtained with other methodologies such as acid–base titration (43 % substitution). The small difference observed can be explained considering the effect of ion binding, which can modify the effective charge of the polyelectrolyte in solution. Therefore, this voltammetric technique allows the calculation of the substitution degree of a polymer for reactions involving ionisable groups.

Based on its solubility, CHI-GTMAC is potentially applicable in different therapies and can serve as a carrier for the controlled release of drugs. The methodology presented in this study represents a valuable alternative for evaluating the performance of these materials at interfaces with different hydrophilic/hydrophobic characteristics.

3.3 Interaction of DS and CHI with DSPG

Figure 5a shows the voltammograms obtained 60 min after the injection of DSPG at the liquid/liquid interface in the absence and presence of the polyelectrolyte (PE). In the absence of PE, DSPG adsorbs at the interface [44]. The presence of DS (negatively charged PE) in the aqueous phase modifies the voltammetric response corresponding to DSPG adsorption, as a new process is now evident during the negative scan. The presence of DS and DSPG produce a

Organic phase composition: 1.0×10^{-2} M TPhAsDCC. Aqueous phase composition: 1.0×10^{-2} M LiCl, 2.00 % v/v acetic acid, pH 3.00 with **a** 0 and **b** 5.0×10^{-4} M TEA^+

Table 2 Blocking rate (% BR) of a DSPG film generated at the liquid/liquid interface by the TEA^+ transfer process as function of the polyelectrolyte concentration (DS or CHI)

c (% w/v)	% BR CH	% BR DS
0	100	98
0.05	67	60
0.1	0	14
0.5	0	0
1	0	0

voltammetric response characteristic of an activation-controlled interfacial adsorption–desorption process, in which the negative peak current at $E = 0.300$ V has a linear dependence with the sweep rate and increases as the positive limit of potential increases ([38], data not shown). Under these conditions, an interfacial complex between $\text{DS-Li}^+-\text{DSPG}$ is formed. The function of Li^+ ions is to neutralise the negative charge of DSPG and allow DS adsorption; thus, the interaction between DS and DSPG occurs through a bridge of Li^+ cations.

When CHI (positively charged PE) is present in the aqueous phase, the voltammetric response is similar to that obtained for pure DSPG. In this case, no adsorption–desorption process occurs.

With the aim of evaluating the effect of DS and CHI on the permeability of DSPG film, tetraethylammonium TEA^+ was added to the aqueous phase as a probe ion. Figure 5b shows the voltammetric response of the TEA^+ transfer process through the liquid/liquid interface in the presence and absence of DSPG. The TEA^+ transfer process through the bare interface was reversible and diffusion-controlled, characterised by a positive peak ($E_p^+ = 0.400$ V) and a

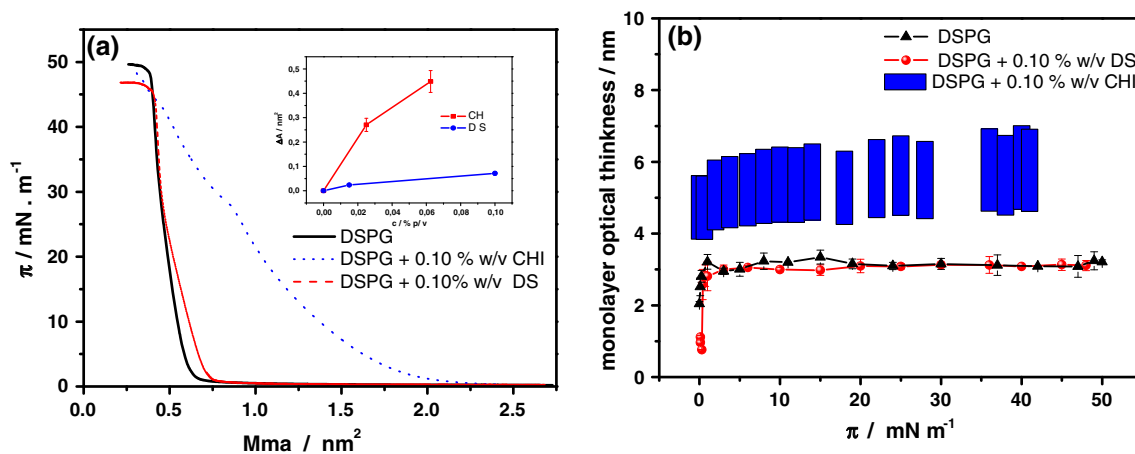


Fig. 6 **a** Surface pressure as a function of the mean molecular area (Mma/nm^2) for the DSPG monolayer at the air/water interface in the absence (black line) or presence of 0.10 % w/v DS (dashed dotted line) or 0.10 % w/v CHI (dotted line). Inset Change in Mma as a function of DS (filled circle) or CHI (filled square) concentration (%)

negative peak ($E_p^- = 0.340$ V). When DS and CHI were present in aqueous phase, in the absence of DSPG, the TEA^+ transfer process was not altered (data not shown). However, if DSPG was adsorbed at the interface, an important decrease in current, and a greater separation of positive and negative peak potential was observed. These changes indicate that DSPG has a blocking effect on TEA^+ transfer [37]. If DSPG film is formed in the presence of DS or CHI, the reversible TEA^+ transfer process is restored. Table 2 shows the blocking rate, which is calculated as follows:

$$\% \text{ BR} = \left(\frac{I_{p_{PE}}^{\text{TEA}^+} - I_{p_{PE}}^{\text{TEA}^+, \text{DSPG}}}{I_{p_{PE}}^{\text{TEA}^+}} \right) \times 100$$

as function of DS or CH concentration. A % BR close to 100 % indicates a completely blocked interface and a % BR equal to 0 % corresponds to an unblocked interface. In both cases, when the PE concentration increases, the % BR decreases, indicating that the presence of PE in the aqueous phase modifies the DSPG film and re-establishes the transfer of TEA^+ . In the case of DS, the formation of the interfacial complex $\text{DS-Li}^+-\text{DSPG}$ disturbs the lipid film, and during the desorption process, some molecules of the lipid can be desorbed, forming an un-blocked interface. When CHI is present, two effects are noted: CHI adsorption at the interface and occupying the position of DSPG molecules leads to the formation of a more expanded DSPG film. Secondly, CHI interacts with DSPG molecules at the interface through hydrophobic and electrostatic interactions, causing the expansion of DSPG film.

To understand the modifications of DSPG films caused by the presence of PE, studies of surface pressure (π) as function of the mean molecular area (Mma) were

w/v). **b** Monolayer optical thickness (nm) as a function of lateral pressure π (mN \cdot m $^{-1}$) for DSPG in the absence (filled triangle) and presence of 0.10 % w/v DS (filled circle) or 0.10 % w/v CHI (filled half block). Subphase composition: 1.0×10^{-2} M LiCl, 2.00 % v/v acetic acid, pH 3.00

performed in the absence and presence of DS or CHI in the subphase (Fig. 6a). When PEs were absent, the isotherm of DSPG shows a change in the slope characteristic of the transition gaseous-liquid condensed phase, with a pressure and area of collapse of 48.0 mN \cdot m $^{-1}$ and 0.38 nm 2 respectively. In the presence of CHI, an expansion of the isotherm is evident (displacement towards higher Mma values), together with a decrease in the collapse pressure (45 mN \cdot m $^{-1}$) and an increase of the collapse area (0.47 nm 2). This effect can be attributed to the electrostatic and hydrophobic interaction between DSPG and CHI. Another important feature is that DSPG film in the presence of CH was liquid-expanded (at 30 mN \cdot m $^{-1}$), which was the opposite of that observed with pure DSPG film. If DS is present in the subphase, the isotherm of DSPG shows slight changes, and the monolayer is still in a liquid-condensed phase (at 30 mN \cdot m $^{-1}$), with a collapse pressure and area of 45 mN \cdot m $^{-1}$ and 0.39 nm 2 , respectively. The inset in Fig. 6a shows the change in Mma as function of the PE concentration; it can be observed that CHI generates more expansion in DSPG film than DS.

Figure 6b shows the optical thickness as function of the surface pressure for DSPG films in the absence and presence of DS or CHI. When CHI was present in the subphase, the thickness of the monolayer was higher than that of pure DSPG. This effect can be attributed to the interaction of CHI with DSPG, even at low pressure. On the other hand, the presence of DS does not modify the thickness of DSPG films at any pressure value. Taken together, these results provide evidence that the $\text{DS-Li}^+-\text{DSPG}$ complex is formed only when a potential is applied to the interface.

In conclusion, the main difference between the interaction of DS with DSPG and the interaction of CHI with DSPG arises from the fact that DS forms an interfacial complex with DSPG mediated by Li^+ ions (DS– Li^+ –DSPG). The formation of this complex requires the application of a potential difference at the interface. On the other hand, the CHI–DSPG interaction occurs spontaneously under all conditions, and these interactions take place at the hydrophobic and electrostatic level.

Acknowledgments Financial support from Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) and Secretaría de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT) is gratefully acknowledged. J.S. Riva and C.I. Cámara thank CONICET for the fellowships awarded. L.M. Yudi and A.V. Juárez are members of the Research Career of CONICET.

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