Determination of the substitution degree of modified chitosan by cyclic voltammetry at the water/dichloroethane interface

Ana Agustina Aldana [a], Miriam C. Strumia [a], Lidia M. Yudi [b], Marisa Martinelli [a], Ana Valeria Juarez [b],*

[a] Instituto Multidisciplinario de Biología Vegetal (IMBIV) CONICET-UNC, Departamento de Química Orgánica, Facultad de Ciencias Químicas
[b] Instituto de Investigaciones en Físico Química de Córdoba (INFIQC) CONICET-UNC, Departamento de Físico Química, Facultad de Ciencias Químicas, Ala 1, Pabellón Argentina, Ciudad Universitaria, X5000HUA Córdoba, Argentina

ARTICLE INFO

ABSTRACT

The electrochemical behavior of the cationic polymer chitosan-gtmac (Ch-GTMAC), at the polarized water/1,2-dichloroethane interface was studied. This polymer was obtained by nucleophilic reaction of chitosan (Ch) with glycidyltrimethylammonium chloride groups (GTMAC). The voltammetric response was found to be dependent on the concentration of the polymer, on pH of the aqueous phase, and on the nature of the organic electrolyte. The results suggest a weak adsorption of the polymer coupled to the transfer towards the organic phase. Desorption process is quasi-reversible, and there is not interaction between adsorbents. From the analysis of desorption charge, it was possible to determine the substitution degree of the polymer

1. Introduction

Chitosan, the main derivative of chitin, is an attractive linear aminopolysaccharide, composed primarily of repeating units of β-(1→4)2-amino-2-deoxy-D-glucose (D-glucosamine). As an unique natural alkali polysaccharide, chitosan has many potential applications including drug delivery, artificial skin, reinforced bone nail, absorbable suture and wound dressing [1–9], due to its distinctive properties, such as biodegradability, biocompatibility, non-toxicity, antibacterial activity, wound healing acceleration ability[10–12]. However, compared with collagen and hyaluronic acid, its natural materials counterparts, the widespread use of chitosan has been restricted. The limited utility of chitosan, principally arises from its insolubility at neutral or high pH values. So, many efforts were made to increase the solubility of chitosan [6,13,14], and to enable a broader application in a wide pH range.

In this work, we present the electrochemical behavior of chitosan modified with glycidyltrimethylammonium chloride, GTMAC a quaternary ammonium group, which confers a permanent positive charge to the polymer. This modification allows high solubility in a wide pH range.

In the last years, the interfacial behavior of polyelectrolytes at liquid/liquid interfaces has been studied by different authors. Among these studies, cationic polymers have gain a lot of interest regarding both, their interaction with others molecules, and their interfacial behavior [15–21]. Principal interest has been paid to the study of the adsorption of biopolymers which includes: heparine [22–24], proteins [25,26], insulin [27], haemoglobin [28–30], myoglobin [31], cytochrome C and ribonuclease A, DNA and his complex [28,30,32–34]. Another research topic is that focused on the study of the interactions between polyelectrolytes and membrane components such as phospholipid molecules adsorbed at liquid/liquid interfaces [33,35] or nanoparticles [36].

The aim of the present paper is to study the interfacial behavior of chitosan modified with glycidyltrimethylammonium chloride (GTMAC) applying cyclic voltammetry at a water/1,2-dichloroethane interface and analyzing the effect of pH and concentration of all the species. As mentioned above, this modification allows the solubilization of the polymer at neutral and high pH values. Therefore an important topic is the determination of the degree of substitution resulting from the modification. In this sense we demonstrate that the result obtained from voltammetric experiments enable us to infer the substitution degree of the polymer chain with GTMAC.

2. Experimental

2.1. Materials

Chitosan, Ch (85% DA, LMW, Aldrich); glycidyltrimethylammonium chloride, GTMAC (Sigma Aldrich); tetrapentylammonium bromide, TPnABr (Fluka); tetraphenylarsonium chloride, TPhAsCl
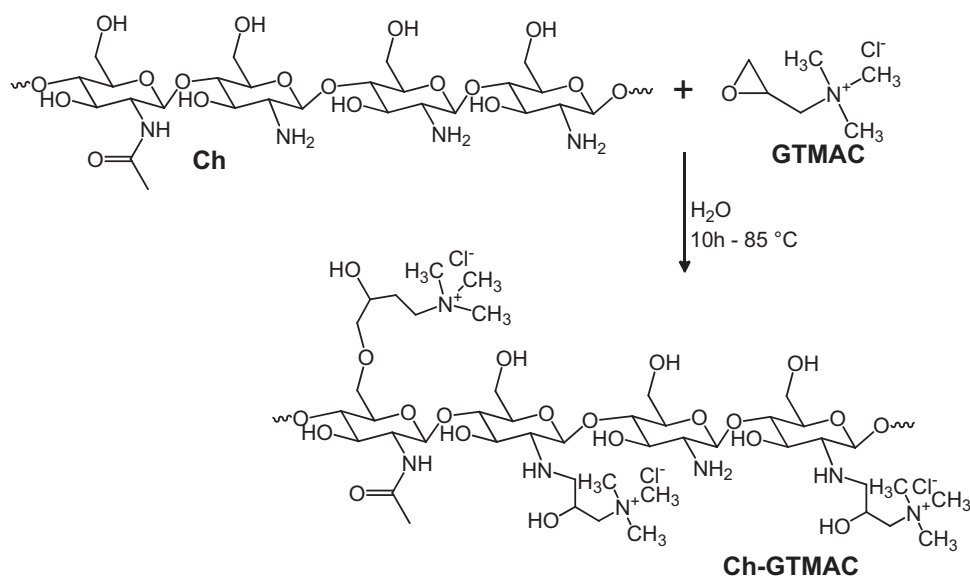
Scheme 1. Synthesis of quaternized chitosan Ch-GTMAC.

(Sigma); sodium dicarbollylcobaltate (NaDCC, Strem Chemicals); potassium tetrakis (4–chlorophenyl) borate (KTClPhB, Aldrich); LiCl (Merck p.a.); HCl (Merck p.a.); LiOH (Merck pa) were used without further purification. Ultrapure water (MilliQ RiOs 16, Millipore) was used to prepared aqueous solutions.

Tetraphenylarsonium dicarbollylcobaltate (TPhAsDCC) and tetrapentylammonium tetrakis (4–chlorophenyl) borate (TPnATClPhB) solutions, in 1,2–dichloroethane (DCE, Dorwil p.a.), were prepared by metathesis of TPhAsCl and NaDCC or TPnABr and KTClPhB, respectively. The precipitates were recrystallized from water: acetone mixtures and then dried in an oven at 30 °C for two days.

2.2. Preparation of the polymer Ch-GTMAC

Chitosan (500 mg) was dispersed in distilled water (10 ml), and then GTMAC was added in a molar ratio 3:1 (moles of GTMAC per each mols of amine group in Ch). 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 in vacuum [37]. The product Ch-GTMAC was characterized by Fourier Transform Infrared (FT-IR) spectra, employing a Nicolet Avatar 360 FT-IR spectrometer and H^1 NMR employing a Bruker 500 MHz spectrometer. The results obtained from these techniques confirm a covalent union between GTMAC and Ch.

2.3. Voltammetric measurements

The voltammetric experiments were performed in a four–electrode system using a conventional glass cell of 0.18 cm^2 interfacial area. Two platinum wires were used 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 1.0×10^{-2} M tetraphenylarsonium chloride or 1.0×10^{-2} M tetrapentylammonium bromide and 1.0×10^{-2} M LiCl.

The base electrolyte solutions were 1.0×10^{-2} M LiCl in ultrapure water and 1.0×10^{-2} M tetraphenylarsonium dicarbollylcobaltate (TPhAsDCC) or 1.0×10^{-2} M tetrapentylammonium tetrakis (4–chlorophenyl) borate (TPnATClPhB) in 1,2–dichloroethane.

Cyclic voltammetry was performed using a four–electrode potentiostat, which automatically eliminates the IR drop by means of a periodic current–interruption technique.[38] A Hi-Teck Instruments waveform–generator 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 ($\Delta_o^w \varphi$) across the interface by,

$E = (\Delta_o^w \varphi) + \Delta E_{ref}$ where ΔE_{ref} depends on the reference electrodes and the reference solutions employed.

For cyclic voltammetry experiments, the polymer Ch-GTMAC was added to the aqueous phase at concentrations between 0.01% w/v and 0.10% w/v. Due to the presence of primary amines in these polymers, pH value determines the amount of protonated groups, so that, the pH of the aqueous phase was varied between 2.4 and 9.6 by addition of HCl or LiOH solutions, with the aim of analyzing the effect of the charge in the interfacial behavior of this polymer.

The electrochemical cell used was as follows:

Ag	AgCl	TPhAsCl or TPnABr + LiCl 1×10^{-2} M (w')	TPhAsDCC or TPnATClPhB 1×10^{-2} M (o)	LiCl 1×10^{-2} M + Ch-GTMAC x % w/v (w)	AgCl	Ag

3. Results and Discussion

3.1. Synthesis and characterization of Ch- GTMAC

Ch-GTMAC was obtained following a reported methodology (Scheme 1) [37]. The amine groups of chitosan react with epoxy group of GTMAC by nucleophilic attack and ring opening. Both, amine and hydroxyl groups of chitosan, can act like nuclephile in the reaction, but the first group is better nucleophilic than the other. The product was characterized by FT-IR spectroscopy and ^{1}H-NMR. The FT-IR spectrum of product shows a new signal at 1484 cm^{-1} corresponding to methyl groups of GTMAC. In addition, the intensity of the band at 1590 cm^{-1}, corresponding to amine groups of Ch, decreases respect to the band of carbonyl groups of polymer. In the ^{1}H-NMR spectrum of the product, the bonding of GTMAC to Ch was evident for the appearing of the characteristic peak at 3.1 ppm corresponding to methyl groups of GTMAC. From
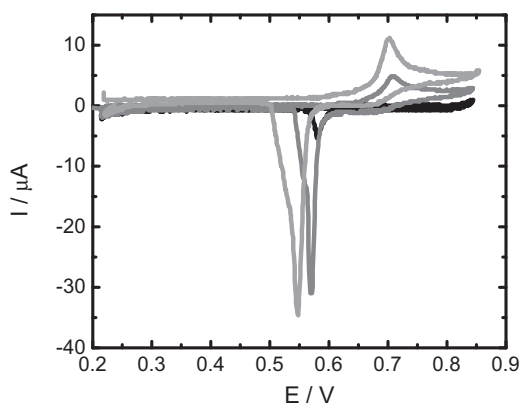
Fig. 1. Cyclic voltammograms for different Ch-GTMAC concentration at the water/1,2-dichloroethane interface. Aqueous phase composition: 1.0×10^{-2} M LiCl + x % w/v Ch-GTMAC: (—) 0.02%; (—) 0.05% and (—) 0.10%. Organic phase composition: 1.0×10^{-2} M TPATClPhB. $v = 0.005$ V s^{-1}, pH = 7.3.

acid-basic titration, 1.99×10^{-3} mol of GTMAC per gram of polymer, was estimated.

3.2. Electrochemical behaviour of Ch-GTMAC

Fig. 1 shows the voltammetric profiles obtained when Ch-GTMAC was present in the aqueous phase at different concentration values and pH 7.20. The behavior is completely different to the obtained with native chitosan, in which case no electrochemical response is observed in the potential window employed (data not shown). It can be clearly noted an asymmetry in the general shape of the voltammetric profiles. 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 anions present in the organic phase (DCC$^-$ or TClPhB$^-$) In the reverse scan a narrow peak is observed, which is characteristic of a desorption process, corresponding to the return of the polymer to the aqueous phase. During the forward scan, the peak potential is almost constant, for all the concentration of the polymer analyzed. On opposite, a peak potential shift, towards more negative values as concentration increases, is evident during the reverse scan. This behavior is associated to different adsorption configuration for the polymer in the organic side of the interface, which depends on the amount of polymer adsorbed. The adsorption process in the organic side is facilitated by the ion pair formation with the anions of electrolyte present in the organic phase, as will be analyzed in the next section.

Fig. 2, shows the voltammograms resulting from reversing the sweep at successively more positive switching potentials, E_λ. As it can be noted, the negative peak current increases with E_λ, reinforcing the hypothesis of adsorption of the polymer in the organic phase. Also, the extent of adsorption depends on the amount of the hydrophobic anion accumulated at the organic side of the interface, which increase with E_λ. Nevertheless, for E_λ values higher than 0.800 V, the peak current for the desorption process remains constant, so a saturation of adsorption sites at the interface can be postulated. Similar behavior was obtained for all Ch-GTMAC concentration and pH values analized.

The value of the ratio i_p^-/i_p^+ is considerably larger than unit, i.e., the ratio expected for a simple diffusion-controlled charge transfer reaction. The enhanced peak current ratio could be ascribed to the coupling of polyelectrolyte transfer with adsorption of this species at the organic side of the interface. During the positive scan the polymer transferred accumulates nears the interface rather than diffuses away into the bulk of the organic phase, and during the reverse scan it is stripped into the aqueous phase. [25,27,39]
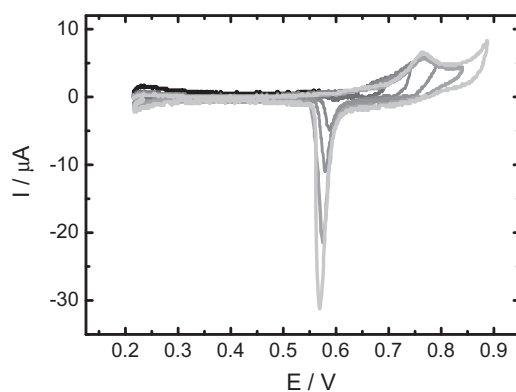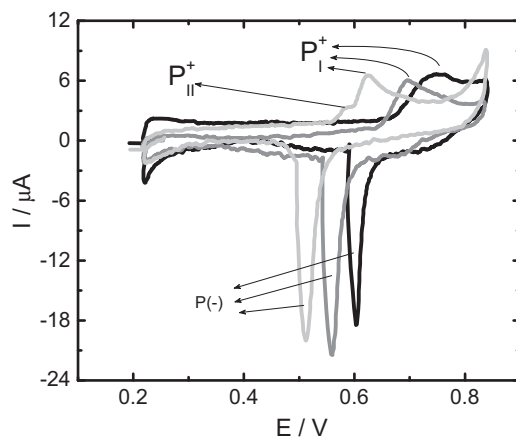

Fig. 2. Cyclic voltammograms for Ch-GTMAC at the water/1,2-dichloroethane interface, reversing the scan at successively more positive potentials, E_λ. Aqueous phase composition: 1.0×10^{-2} M LiCl + 0.050% w/v Ch-GTMAC. Organic phase composition: 1.0×10^{-2} M TPATClPhB. $v = 0.010$ Vs^{-1}, pH 2.4.

3.3. Effect of the pH

The structure of Ch-GTMAC, contains two different positive charges, a primary amine in the chitosan chain and a quaternary amine in the GTMAC group. For this reason the total charge in the polymer depends on the pH of the aqueous phase. With the aim to study the pH effect on the electrochemical response, several concentrations of polymer were added to the aqueous phase at different pH values within the range 2.3 - 10.2. Voltammetric profiles obtained for 0.02% w/v Ch-GTMAC at different pH values are shown in Fig. 3. It's important to remark two interesting points. While pH increase, the peak potential for the main positive process (P^+_I) shifts to lower values, due to the increasing hydrophobicity of the polyelectrolyte by deprotonation of the primary amines. On the other hand, at higher pH values a second peak appears at lower potential values, P^+_{II}, corresponding to a different transfer process of the polymer to the organic phase. The free energy for this process is clearly lower than that corresponding to P^+_I.

The dependence of the peak current (I_p^+) for P^+_I and P^+_{II}, on scan rate was analyzed with the purpose to characterize both process. A linear relationship of I_p^+ with sweep rate was observed for P^+_{II}, while a linear behavior of peak current with the square root of scan rate was obtained for P^+_I at all pH values studied. Figs. 4a and 4b show the I_p^+ dependence with scan rate for both processes at different pH values. These results suggest an activation-controlled
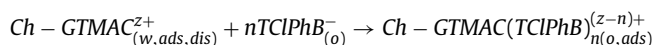

Fig. 3. Cyclic voltammograms for Ch-GTMAC at the water/1,2-dichloroethane interface at pH = (—) 2.4; (—) 5.5; (—) 8.2. Aqueous phase composition: 1.0×10^{-2} M LiCl + 0.02% w/v Ch-GTMAC. Organic phase composition: 1.0×10^{-2} M TPATClPhB. $v = 0.050$ V s^{-1}.
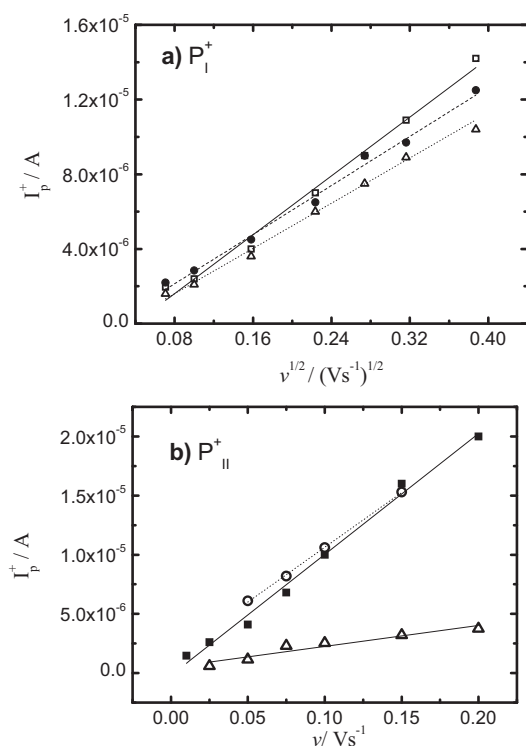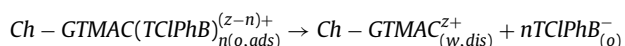
Fig. 4. Values of I_P^+ for the transfer process P^+_I and P^+_{II} with ν or $\nu^{1/2}$ at different pH values: **a)** P^+_I ($\square$) pH 2.4; ($\bullet$) pH 4.5; ($\triangle$) pH 5.5. **b)** P^+_{II} ($\triangle$) pH 5.5; ($\blacksquare$) pH 7.3; ($\bigcirc$) pH 9.6. Aqueous phase composition: 1.0×10^{-2} M LiCl + 0.02% w/v Ch-GTMAC. Organic phase composition: 1.0×10^{-2} M TPATClPhB.

interfacial adsorption mechanism for P^+_{II}, and a diffusion process for P^+_I. The electrochemical behavior observed indicates that, at high pH values, Ch-GTMAC can be present not only dissolved in solution but also adsorbed at the aqueous side of the interface, previous to the electrochemical transfer. Once the polarization is applied to the interface a mixed transfer mechanism of the poly-electrolyte to the organic side of the interface occurs, a direct diffusional transfer of the polymer from the bulk of the aqueous phase, and the transfer of the adsorbed Ch-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, as has been explained in previous section. This statement assumes that all Ch-GTMAC molecules, transferred through processes P^+_I or P^+_{II}, are adsorbed at the organic side of the interface after the transfer. Slight shifts of E_P^- to more negative values take place as the pH increases, in accordance with the higher hydrophobic nature of the polymer due to the loss of positive charge resulting from the amine deprotonation. More hydrophobic is the polymer more energy is necessary to transfer to the aqueous phase. Similar behavior was observed employing high Ch-GTMAC concentration values. However, as the Ch-GTMAC concentration increases, the second process appears at lower pH values (result not shown).
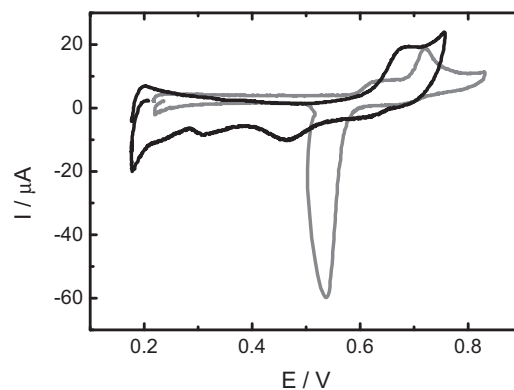
Summing up, the results allow postulating the following reactions for Ch-GTMAC transfer in the positive scan:

$$Ch-GTMAC^{z+}_{(w,ads,dis)} + nTClPhB^-_{(o)} \rightarrow Ch-GTMAC(TClPhB)^{(z-n)+}_{n(o,ads)}$$

And, in the reverse scan:

$$Ch-GTMAC(TClPhB)^{(z-n)+}_{n(o,ads)} \rightarrow Ch-GTMAC^{z+}_{(w,dis)} + nTClPhB^-_{(o)}$$

Were the subscripts *ads* or *dis* refer to adsorbed or dissolved Ch-GTMAC molecules.

Fig. 5. Cyclic voltammograms for Ch-GTMAC at $\nu = 0.050$ Vs^{-1} using different organic electrolytes. Aqueous phase composition: 1.0×10^{-2} M LiCl + 0.05% w/v Ch-GTMAC, pH = 5.5. Organic phase composition: (—) 1.0×10^{-2} M TPhAsDCC; (—) 1.0×10^{-2} M TPATClPhB.

3.4. Effect of the organic electrolyte

Fig. 5 compares the voltammetric profiles obtained for 0.050% w/v Ch-GTMAC in the aqueous phase and two different base electrolytes in the organic phase: TPhAsDCC and TPnATClPhB at a concentration 1.0×10^{-2} M. Both, the forward and reverse profiles change with the nature of the hydrophobic anion in the organic supporting electrolyte. It suggests that the voltammetric response depends on the interaction between the cationic polymer and the hydrophobic anion through the formation of ion pairs in the organic side of the interface.

In contrast with the mechanism proposed in the previous section for the anion TClPhB$^-$, in the case of DCC$^-$, a single process at 0.670 V is observed, which corresponds to a purely diffusional controlled mechanism.

The above asseveration is similar to that concluded by other authors, [27,34] for protamine and insulin transfer at a liquid/liquid interface, who observed that the peak potential depends on the nature of the organic electrolyte. The authors attributed this effect to the ion pair interactions between the polycation and the organic electrolyte anion, and found that the stability of the ion pair decreases with increasing the size of the anion or the hydrophobic character of the anion.

As can be noted in Fig. 5, during the reverse scan, the negative peak observed in presence of TPnATClPhB is sharper than the corresponding for TPhAsDCC, which would indicate a faster desorption process for Ch-GTMAC when TPnATClPhB is used as organic electrolyte, revealing a lower stability of the ion pair formed by the anion TClPhB$^-$ and the amine groups of Ch-GTMAC at the organic side of the interface. On the other hand, for DCC$^-$ anions two processes are observed, which would be attributed to the desorption of two kinds of conformations of the complex formed at the interface. Based on these results we can postulate a stronger interaction between Ch-GTMAC and DCC$^-$ anion compared to TClPhB$^-$ anion. Indeed, the radii of DCC$^-$ and TClPhB$^-$ anions reported in literature are 0.275 and 0.535 nm respectively [40,41], confirming the hypothesis of a lower stability of the ion pair formed by the anion TClPhB$^-$ respect to that formed by DCC$^-$.
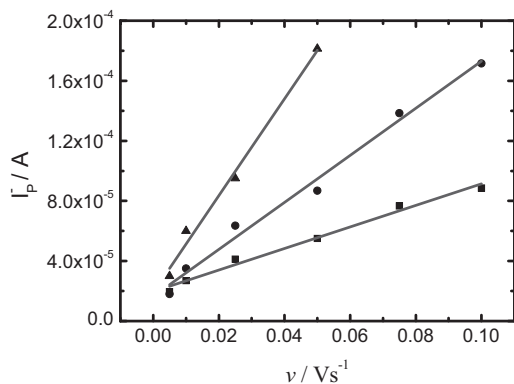
3.5. Potential step followed by negative linear sweep

With the aim of analyzing the desorption process; potential steps followed by negative scans at different sweep rates were carried out. Steps at E = 0.850 V were applied during different times, $\Delta\tau$, previous to the negative scan (see inset Fig. 6). As can be seen in Fig. 6, the peak current increase with $\Delta\tau$, as a consequence of
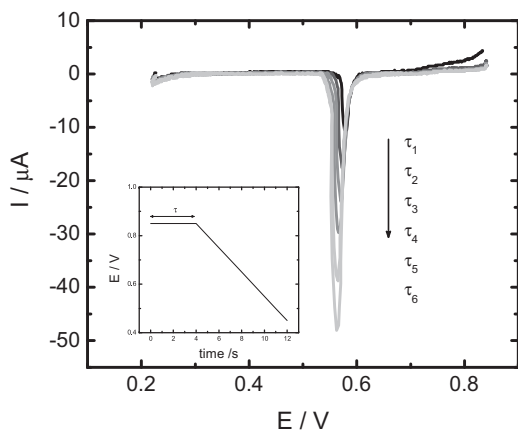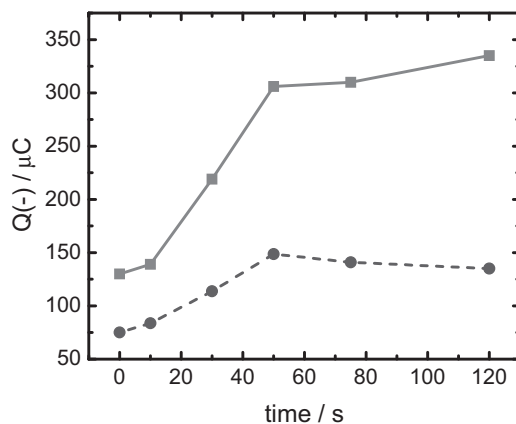
Fig. 6. Negative profiles for Ch-GTMAC at the water/1,2-dichloroethane interface recorded after holding the potential at E = 0.850 V during different pulse times $\Delta\tau$: 0, 10, 30, 50, 75 and 120 s, $\nu_{(-)} = 0.010$ Vs^{-1}. Aqueous phase composition: 1.0×10^{-2} M LiCl + 0.020% w/v Ch-GTMAC, pH 4.5. Organic phase composition: 1.0×10^{-2} M TPAT-ClPhB.

Fig. 8. Plot of Q(-) values vs $\Delta\tau$, calculated from voltammograms in Fig. 6 for the process in the negative sweep, at different pH values: (■) pH 2.4; (●) pH 6.5.

the amount of polymer adsorbed at the interface increases. Fig. 7 shows the lineal dependence of I_P^- with sweep rate, for several $\Delta\tau$, confirming the activation-controlled interfacial adsorption mechanisms proposed. On the other hand, the peak potential is constant with the sweep rate, at all $\Delta\tau$ values employed at all the pH values studied, which indicate no lateral interaction between the adsorbed molecules [42]. To carry out the analysis of charge desorption, Q(-), we selected the conditions of low sweep rate to ensure a complete desorption of Ch-GTMAC. The Q(-) values were calculated integrating the desorption peaks, during the negative scans at 0.010 Vs^{-1}, after holding the potential at E = 0.850 V during different times, at all pH values studied. The results are shown in Fig. 8, as can be seen the charge depends on the pH of the system. At pH= 6.5 a plateau is observed corresponding to Q(-) equal to 125 μC. The same behavior was observed in the range $5.3 \leq$ pH ≤ 9.6. While at lower pH values, 2.4 and 3.7, the saturation charge is equal to 325 μC. As it was discussed in previous sections, the charge of the polymer depends on pH of the aqueous phase. Considering the charge of the polymer at the different pH values, it is possible to calculate a degree of substitution of primary amine groups in the Ch chain for GTMAC. At high pH values, the charge obtained depends only on the quaternary amine groups in GTMAC, while at low pH values the charge depends of both amine groups, not only the quaternary charged amines in GTMAC but also the primary protonated amines (pK$_a \approx$
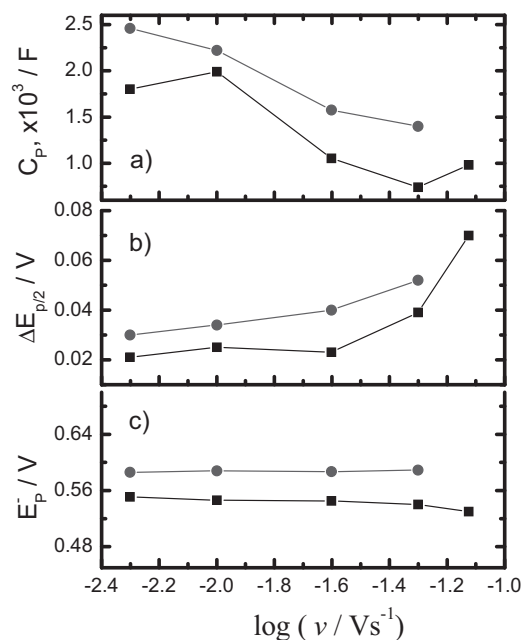
6.5) in Ch, which has not been substituted by GTMAC molecules. So that the percentage of substituted amines can be calculated relating both Q(-) values (125 μC/325 μC). In this way, the percentage obtained was 38.5%, which is similar to the values obtained for other methodology like acid base titration (41% of 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 ionizable groups.

In addition, a characterization of the desorption process was possible analyzing the results obtained at different pulse times and pH values. Fig. 9 shows the variation of E_p^-, the half peak width $\Delta E_{p/2}$ and the pseudo-capacitance, C_P, obtained by the quotient of I_p^- and ν, with log ν. From comparing the results obtained

Fig. 7. Values of Ip (-) for Ch-GTMAC desorption obtained at different τ values. Aqueous phase: 1.0×10^{-2} LiCl M + Ch-GTMAC 0.05% w/v, τ: (■) 0; (●) 10; (▲) 30. Organic phase: 1.0×10^{-2} M TPATClPhB. pH: 4.5.

Fig. 9. Plots of a) C_P; b) $\Delta E_{p/2}$ and c) E_p^- values vs log ν showing the quasireversible behavior of desorption process at different time pulse: (■) 0 and (●) 10 s. Aqueous phase: 1.0×10^{-2} LiCl M + Ch-GTMAC 0.05% w/v. Organic phase: 1.0×10^{-2} M TPATClPhB. pH 5.5.

with theoretical predictions [42], it is possible to conclude that the desorption process is quasireversible.

4. Conclusions

The results obtained in this paper allow us to postulate a mechanism of adsorption/diffusion control for the transfer of the polyelectrolyte Ch-GTMAC at the water/1,2-DCE interface. Important differences were observed when pH of the aqueous phase or concentration of the polymer were changed. The variation of peak current I_p^+ with sweep rate revealed a mixed activation and diffusion control, the first one occurs at high pH values and high polymer concentration, while the diffusion transfer occurs at all the conditions studied. Also, it was possible to postulate the adsorption of transferred molecules at the organic side of the interface facilitated by the ion pair formation with the anion of the supporting electrolyte of the organic solvent. The analysis of the desorption process of the polymer, allowed the determination of a quasi-reversible mechanism, and it is possible to conclude that no lateral interaction, between molecules of polymer adsorbed at the interface, takes place. From the analysis of the charge with pH, it was possible to calculate the degree of substitution of the primary amine groups of Ch with GTMAC groups. In this case we obtain a 38.5% of substitution which is similar to that obtained from acid-based titration. So, this electrochemical method allows inferring in the degree of substitution when ionized groups are involved in the reaction.

The solubility of Ch-GTMAC makes a material potentially applicable in different therapies to be used as carriers for controlled release of drugs. The methodology presented in this study represents a valuable alternative to evaluate the perfomance of these materials in interfaces with different hydrophilic/hydrophobic characteristics.

References

[1] B. Veleirinho, F.V. Berti, P.F. Dias, M. Maraschin, R.M. Ribeiro-do-Valle, J.A. Lopes-da-Silva, Manipulation of chemical composition and architecture of non-biodegradable poly(ethylene terephthalate)/chitosan fibrous scaffolds and their effects on L929 cell behavior, Materials Science and Engineering: C 33 (2013) 37–46.
[2] M.Z. Elsabee, H.F. Naguib, R.E. Morsi, Chitosan based nanofibers, review, Materials Science and Engineering: C 32 (2012) 1711–1726.
[3] A.A. Aldana, R. Toselli, M.C. Strumia, M. Martinelli, Chitosan films modified selectively on one side with dendritic molecules, Journal of Materials Chemistry 22 (2012) 22670.
[4] A.A. Aldana, A. González, M.C. Strumia, M. Martinelli, Preparation and characterization of chitosan/genipin/poly(N-vinyl-2-pyrrolidone) films for controlled release drugs, Mater. Chem. Phys. 134 (2012) 317–324.
[5] S. Balaji, R. Kumar, R. Sripriya, P. Kakkar, D.V. Ramesh, P.N.K. Reddy, P.K. Sehgal, Preparation and comparative characterization of keratin–chitosan and keratin–gelatin composite scaffolds for tissue engineering applications, Materials Science and Engineering: C 32 (2012) 975–982.
[6] M. Garcia-Fuentes, M.J. Alonso, Chitosan-based drug nanocarriers: Where do we stand? Journal of Controlled Release 161 (2012) 496–504.
[7] R. Jayakumar, M. Prabaharan, P.T. Sudheesh Kumar, S.V. Nair, H. Tamura, Biomaterials based on chitin and chitosan in wound dressing applications, Biotech. Adv. 29 (2011) 322–337.
[8] M. Amidi, E. Mastrobattista, W. Jiskoot, W.E. Hennink, Chitosan-based delivery systems for protein therapeutics and antigens☆, Adv. Drug Deliv. Rev. 62 (2010) 59–82.
[9] R.A.A. Muzzarelli, Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone, Carbohydr. Polym. 76 (2009) 167–182.
[10] M. Kong, X.G. Chen, K. Xing, H.J. Park, Antimicrobial properties of chitosan and mode of action: A state of the art review, Int. J. Food Microbiol. 144 (2010) 51–63.
[11] D. Raafat, H.-G. Sahl, Chitosan and its antimicrobial potential–a critical literature survey, Microbial Biotech. 2 (2009) 186–201.
[12] M.N.V. Ravi Kumar, A review of chitin and chitosan applications, React. Funct. Polym. 46 (2000) 1–27.
[13] J. Cho, J. Grant, M. Piquette-Miller, C. Allen, Synthesis and Physicochemical and Dynamic Mechanical Properties of a Water-Soluble Chitosan Derivative as a Biomaterial, Biomacromolecules 7 (2006) 2845–2855.
[14] R. Jayakumar, D. Menon, K. Manzoor, S.V. Nair, H. Tamura, Biomedical applications of chitin and chitosan based nanomaterials—A short review, Carbohydr. Polym. 82 (2010) 227–232.
[15] M.D. Osborne, H.H. Girault, Nafion® adsorption | anion transfer at the interface between two immiscible electrolyte solutions (ITIES), Journal of Electroanalytical Chemistry 370 (1994) 287–293.
[16] L. Zhang, Y. Kitazumi, T. Kakiuchi, Potential-Dependent Adsorption and Transfer of Poly(diallyldialkylammonium) Ions at the Nitrobenzene|Water Interface, Langmuir 27 (2011) 13037–13042.
[17] S. Ulmeanu, H. Jin Lee, H.H. Girault, Voltammetric characterisation of polyelectrolyte adsorption/transfer at the water|1, 2-DCE interface, Electrochemistry Communications 3 (2001) 539–543.
[18] J.S. Riva, K. Bierbrauer, D.M. Beltramo, L.M. Yudi, Electrochemical study of the interfacial behavior of cationic polyelectrolytes and their complexation with monovalent anionic surfactants, Electrochim. Acta 85 (2012) 659–664.
[19] J.S. Riva, R. Iglesias, L.M. Yudi, Electrochemical adsorption of a cationic cellulosic polymer by ion pair formation at the interface between two immiscible electrolyte solutions, Electrochim. Acta 107 (2013) 584–591.
[20] J.S. Riva, A.V. Juarez, D.M. Beltramo, L.M. Yudi, Interaction of Chitosan with mono and di- valent anions in aqueous solution studied by cyclic voltammetry at a water/1,2-dichloroethane interface, Electrochim, Acta 59 (2012) 39–44.
[21] M. Calderon, L.M.A. Monzo'n, M. Martinelli, A.V. Juarez, M.C. Strumia, L.M. Yudi, Electrochemical Study of a Dendritic Family at the Water/1,2-Dichloroethane Interface, Langmuir 24 (2008) 6343–6350.
[22] J. Guo, S. Amemiya, Voltammetric Heparin-Selective Electrode Based on Thin Liquid Membrane with Conducting Polymer-Modified Solid Support, Anal. Chem. 78 (2006) 6893–6902.
[23] P.J. Rodgers, P. Jing, Y. Kim, S. Amemiya, Electrochemical Recognition of Synthetic Heparin Mimetic at Liquid/Liquid Microinterfaces, J. Am. Chem. Soc. 130 (2008) 7436–7442.
[24] P. Jing, Y. Kim, S. Amemiya, Voltammetric Extraction of Heparin and Low-Molecular-Weight Heparin across 1,2-Dichloroethane/Water Interfaces, Langmuir 25 (2009) 13653–13660.
[25] Y. Yuan, S. Amemiya, Facilitated Protamine Transfer at Polarized Water/1,2-Dichloroethane Interfaces Studied by Cyclic Voltammetry and Chronoamperometry at Micropipet Electrodes, Anal. Chem. 76 (2004) 6877–6886.
[26] M. Shinshi, T. Sugihara, T. Osakai, M. Goto, Electrochemical Extraction of Proteins by Reverse Micelle Formation, Langmuir 22 (2006) 5937–5944.
[27] F. Kivlehan, Y.H. Lanyon, D.W.M. Arrigan, Electrochemical Study of Insulin at the Polarized Liquid-Liquid Interface, Langmuir 24 (2008) 9876–9882.
[28] G. Herzog, P. Eichelmann-Daly, D.W.M. Arrigan, Electrochemical behaviour of denatured haemoglobin at the liquid|liquid interface, Electrochem. Commun. 12 (2010) 335–337.
[29] G. Herzog, M.-T. Nolan, D.W.M. Arrigan, Haemoglobin unfolding studies at the liquid–liquid interface, Electrochem. Commun. 13 (2011) 723–725.
[30] G. Herzog, V. Kam, D.W.M. Arrigan, Electrochemical behaviour of haemoglobin at the liquid/liquid interface, Electrochim. Acta 53 (2008) 7204–7209.
[31] S. O'Sullivan, D.W.M. Arrigan, Electrochemical behaviour of myoglobin at an array of microscopic liquid–liquid interfaces, Electrochim. Acta 77 (2012) 71–76.
[32] T. Osakai, Y. Yuguchi, E. Gohara, H. Katano, Direct Label-free Electrochemical Detection of Proteins Using the Polarized Oil/Water Interface, Langmuir 26 (2010) 11530–11537.
[33] R.A. Hartvig, M.A. Méndez, M.v.d.Weert, L. Jorgensen, J. Østergaard, H.H. Girault, H. Jensen, Interfacial Complexes between a Protein and Lipophilic Ions at an Oil-Water Interface, Anal. Chem. 82 (2010) 7699–7705.
[34] F. Kivlehan, M. Lefoix, H.A. Moynihan, D. Thompson, V.I. Ogurtsov, G. Herzog, D.W.M. Arrigan, Interaction of acridine-calix[4] arene with DNA at the electrified liquid|liquid interface, Electrochim. Acta 55 (2010) 3348–3354.
[35] C.J. Slevin, A. Malkia, P. Liljeroth, M. Toiminen, K. Kontturi, Electrochemical Characterization of Polyelectrolyte Multilayers Deposited at Liquid - Liquid Interfaces, Langmuir 19 (2003) 1287–1294.
[36] H.A. Santos, M. Chirea, V. García-Morales, F. Silva, J.A. Manzanares, K. Kontturi, Electrochemical Study of Interfacial Composite Nanostructures: Polyelectrolyte/Gold Nanoparticle Multilayers Assembled on Phospholipid/Dextran Sulfate Monolayers at a Liquid-Liquid Interface, The Journal of Physical Chemistry B 109 (2005) 20105–20114.
[37] J.Y. Kim, J.K. Lee, T.S. Lee, W.H. Park, Synthesis of chitooligosaccharide derivative with quaternary ammonium group and its antimicrobial activity against Streptococcus mutans, Int. J. Biol. Macromol. 32 (2003) 23–27.
[38] A.M. Baruzzi, J. Ühlken, Current interruption potentiostat for elimination of the IR drop in four-electrode systems, Journal of Electroanalytical Chemistry and Interfacial Electrochemistry 282 (1991) 267–273.

[39] R.H. Wopschall, I. Shain, Effects of adsorption of electroactive species in stationary electrode polarography, Analytical Chemistry 39 (1967) 1514–1527.

[40] A. Popov, T. Borisova, Adsorption of Dicarbollylcobaltate(III) Anion {((-(3)-1,2-B9C2H11)2Co(III)-} at the Water/1,2-Dichloroethane Interface. Influence of Counterions' Nature, Journal of Colloid and Interface Science 236 (2001) 20–27.

[41] R. Benz, Structural requirement for the rapid movement of charged molecules across membranes. Experiments with tetraphenylborate analogues, Biophysical Journal 54 (1988) 25–33.

[42] J.O.M. Bockris, U.M.S. Khan, Surface electrochemistry: a molecular level approach, in: Surface electrochemistry: a molecular level approach, Plenum press, New York, 1993.