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The effect of the functionalization and molecular weight of cationic dextran polyelectrolytes on their electrochemical behavior at the water/1,2-dichloroethane interface

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The electrochemical behaviour of several cationic dextran polyelectrolytes, aminodextran (AD), cationic dextran (CD) and diethylaminoethyl dextran (DEAE-D), at the polarized water/1,2-dichloroethane interface was studied. An investigation into the influence of the scan rate, concentration, pH, the nature and the concentration of the anion present in the organic electrolyte and the effect of the polymer molecular weight is presented. Different responses were obtained and explained considering the structural difference between these species, mainly the position of the positive charge in the macromolecule. The AD polymer is not transferred to the organic phase, regardless of molecular weight, while CD and DEAE-D are transferred from the aqueous to the organic phase at $E = 0.650$ V, independent of the polymer concentration and of the molecular weight. The shape of the voltammograms corresponding to DEAE-D transfer as well as the magnitude of the peak currents and the peak potential values were all dependent on the pH of the aqueous phase solution and on the nature and concentration of the anion present in the organic electrolyte. Based on this dependence, we postulated a mixed mechanism, which involves the transfer of dissolved and adsorbed DEAE-D molecules.

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1. Introduction

Dextran is a complex, branched polysaccharide made of many glucose molecules with $\alpha 1 \rightarrow 6$ glycosidic linkages, with branches beginning from alpha-1,4 linkage (as well as alpha-1,2 and alpha-1,3 linkages). The chains are of variable length, and therefore, the molecular weights range from 10 to 150 kDa. Dextran polymers are structurally diverse and are characterized according to the percentage, nature and distribution of their bonds. These polymers are synthesized from sucrose by many species of the genera *Leuconostoc*, *Lactobacillus* and *Streptococcus*.¹

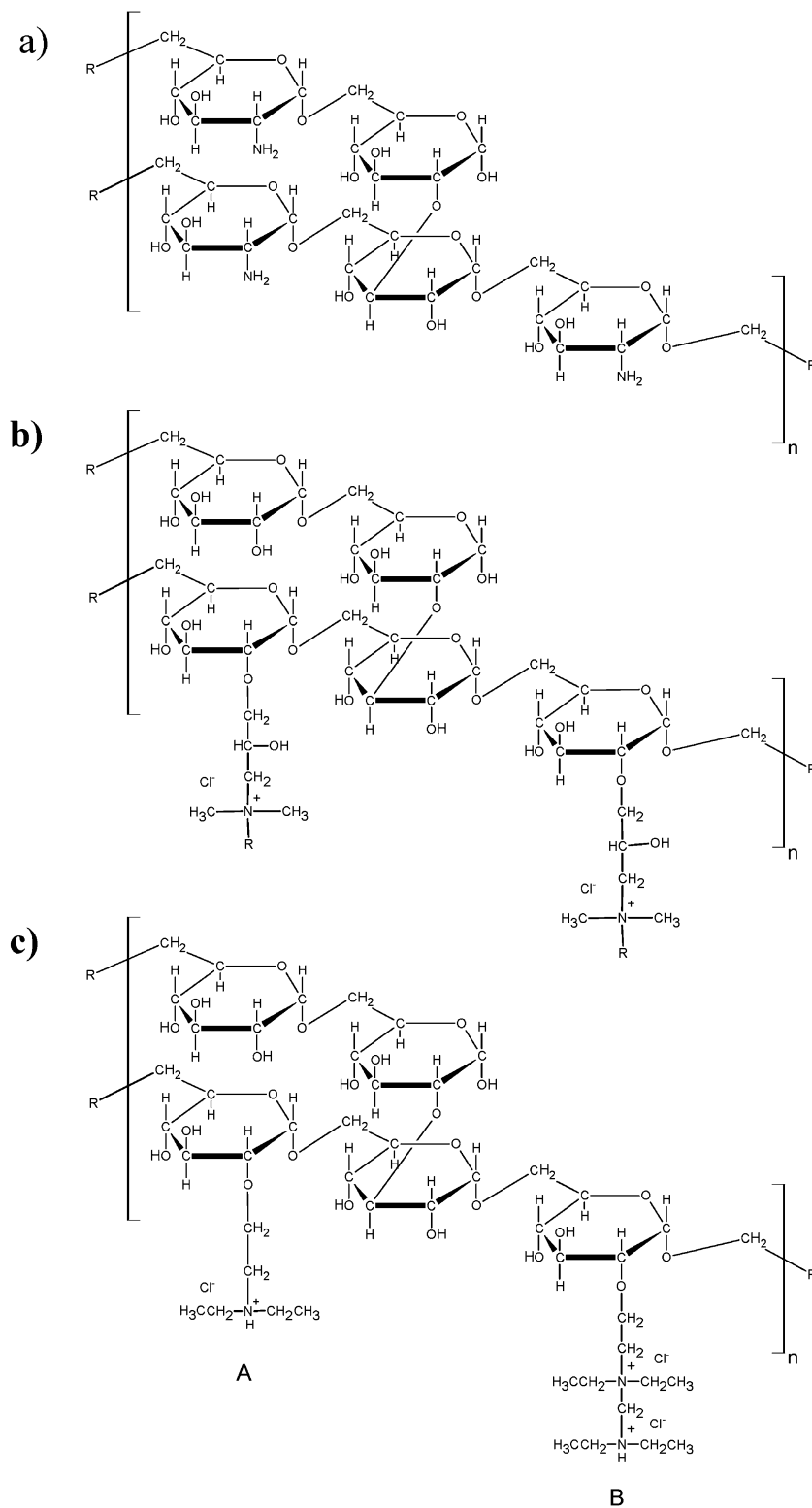
The versatile use of dextran products is due to their favorable properties, such as water solubility and biocompatibility. Moreover, these compounds are biodegradable and stable for more than 5 years.

The applications of these compounds as adjuvants, emulsifiers, carriers, and stabilizers² in food, medicine, pharmaceutical and chemical industries have been reported. Furthermore, in the

crosslinked form, these polymers have been used in some size-exclusion chromatography matrices, for the separation and purification of biomolecules with molecular weights in the range between 0.7 and 200 kDa, an example is sephadex.³

The hydroxyl groups present in dextran offer many sites for derivatization, and the resulting functionalized glycoconjugates represent a largely unexplored class of biocompatible and environmentally safe compounds. Amino dextran (AD), Scheme 1a, is functionalized with primary amine groups directly bonded to the main chain of the cellulose structure. At low pH values these amine groups are protonated resulting in a positively charged polyelectrolyte. Cationic dextran (CD), Scheme 1b, is substituted with an aliphatic chain, which contains a quaternary amine as a terminal group, conferring permanent positive charge to the polymer, independent of the pH value. Diethylaminoethyl dextran (DEAE-D), Scheme 1c, is a chemically derivatized dextran produced by the reaction of diethylaminoethyl chloride with standard dextran. It is a polycationic derivative and the degree of substitution corresponds to approximately 1 DEAE substituent per 3 glucose units (0.33 mol DEAE per 1 mol glucose).⁴ DEAE-D contains three kinds of charged amine groups. One of them (2-(diethyl amino) ethyl), (A) consists of a tertiary amine group with a pK_a of 9.5. The other two moieties are present in the branch 2-[[2-(diethyl amino) ethyl]-diethyl ammonium] ethyl, (B) which contains a tertiary amine

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Scheme 1 Molecular structure of (a) AD, (b) CD and (c) DEAE-D.

group with pK_a 5.7 and a quaternary amine group with permanent positive charge, independent of pH. The pH value and the ionic strength determine the charge and the conformational state of DEAE-D.⁵ DEAE-D has been widely used as a non-viral vector for

gene delivery, as it binds and interacts with the negatively charged DNA molecules and causes nucleic acid uptake by the cell.⁶

The medicine and pharmaceutical uses of these materials involve interactions with biomolecular components of biomembranes.

With the aim of contributing to the knowledge underlying these applications, this paper reports the interfacial behavior of these polymeric molecules on polarized liquid/liquid interfaces. The contribution is based on the similarity of the water-organic solvent interface to that generated at the cell membrane in the extracellular environment.

Electrochemistry at the interface between two immiscible electrolyte solutions (ITIES) is an analytical method based on the measurement of charge transfer across the interface. The voltammetric behavior of biological macromolecules at the ITIES has been studied by different authors as a means to detect such molecules and evaluate their behaviors.⁷⁻¹³

Much attention has been paid to the investigation of proteins, like protamine,^{14,15} haemoglobin (Hb),^{16,17} bovine pancreatic insulin,¹⁸ hen-egg-white lysozyme,^{19,20} myoglobin,^{21,22} melitin,²³ cytochrome c and ribonuclease A,²⁴ catechol amine, like dopamine,²⁵⁻²⁷ and polysaccharides, like heparin,²⁸⁻³⁴ chitosan,³⁵ polyquaternium-10,³⁶ polyquaternium-4³⁷ and Eudragit E100.³⁸ The study of hydrophilic proteins at the ITIES has shown that they adsorb at the interface. Cyclic voltammetric studies have also shown that the adsorbed protein interacts with the anion of the electrolyte salt dissolved in the organic phase¹⁹⁻²² and the electrochemical signal depends on the nature and the concentration of both, the protein and the anion.²⁰ Particularly, regarding the study of polyelectrolytes, polymers and surfactants at liquid/liquid interfaces, this methodology has been successfully employed to elucidate the interaction of the cationic polyelectrolyte polyquaternium-10 with anionic surfactants of different chain length and head group;³⁶ to characterize the behavior and interaction with phospholipid monolayers of chitosan,³⁹ dextran sulphate⁴⁰ and nanostructures composed of dextran sulfate/ruthenium nanoparticles;⁴¹ to sense the anticoagulant heparin (a multi-charged polysaccharide) and its antidote protamine (a charged polypeptide) in aqueous/PVC plasticized membranes *via* amperometric²⁸⁻³⁴ and potentiometric methods;⁴²⁻⁴⁵ to study the counterion binding to the protamine polyion in aqueous solution;¹⁵ to study the behavior of proteins, DNA and their complexes;^{46,47} and to characterize the interaction between humic acids and herbicides,⁴⁸ the association of pharmaceutical drugs with neutral or charged sites in hydrogels and membranes,^{49,50} the transfer process of dendritic molecules⁵¹ and the formation of a conductive polymer using nanostructured composites.⁵²

In the present study, cyclic voltammetry is used to characterize the electrochemical behavior of CD, DEAE-D and AD at the polarized aqueous/1,2-dichloroethane interface, with the aim of correlating the nature of substituents in the chain with the interfacial behavior of the resulting polyelectrolyte. An investigation into the influence of the scan rate, concentration, pH, the nature and the concentration of the anion present in the electrolyte of the organic phase and into the effect of the polymer molecular weight is presented.

2. Experimental

All experiments were performed in a four-electrode system using a conventional glass cell of 0.18 cm² 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 tetraphenylarsoniumchloride (TPhAsCl) (Sigma) or 1.0×10^{-2} M tetrapentylammonium bromide (TPnABr) (Fluka) and 1.0×10^{-2} M LiCl (Merck p.a.).

The base electrolyte solutions were 1.0×10^{-2} M LiCl (Merck p.a.) in ultrapure water (MilliQRiOs 16, Millipore) and tetraphenylarsoniumdicarbollycobaltate (TPhAsDCC) at concentration values in the range between 1.0×10^{-3} M and 1.0×10^{-1} M or 1.0×10^{-2} M tetrapentylammoniumtetrakis (4-chlorophenyl) borate (TPnATCIPhB) in 1,2-dichloroethane (DCE, Dorwil p.a.). TPhAsDCC and TPnATCIPhB were prepared by the metathesis of TPhAsCl (Sigma) and sodium dicarbollycobaltate (NaDCC, Strem Chemicals) or TPnABr (Fluka) and potassium tetrakis (4-chlorophenyl) borate (KTClPhB), respectively. The method involves separately dissolving each salt in ultrapure water, and then mixing the solutions. The precipitate obtained was filtered, rinsed thoroughly with ultrapure water and then recrystallized from acetone:water 1:2 mixtures to ensure a higher purity. After 24 hours, the precipitate was filtered and washed, and then dried in an oven at 30 °C for two days.

The dextran cationic polymer (CD), molecular weight 40 000 g mol⁻¹, aminodextran (AD) of molecular weights 5000, 40 000 and 250 000 g mol⁻¹, and diethylaminoethyl dextran (DEAE-D) of molecular weight 50 000 to 2 000 000 g mol⁻¹ were obtained from Carbomer Inc., and used without further purification.

The polyelectrolytes were added to the aqueous phase at concentrations between 0.025% w/v and 0.200% w/v. The pH was varied from 1.90 to 11.68 by addition of HCl or LiOH (Merck p.a.) to determine the amount of protonated amine groups in the different polymer molecules.

The electrochemical cell used is as follows:

Ag	AgCl	TPhAsCl 10 mM or TPnABr 10 mM + LiCl 10mM (w')	TPhAsDCC 1-100 mM or TPnATCIPhB 10 mM (o)	LiCl 10 mM + Dextran x % w/v (w)	AgCl	Ag
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Cyclic voltammetry was performed using a four-electrode potentiostat, which automatically eliminates the IR drop by means of a periodic current-interruption technique.⁵³ 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, the Ag|AgCl electrode of the aqueous phase being positive with respect to the electrode present in the organic phase, during the positive scan. The potential values E are related to the Galvani potential difference ($\Delta_{\text{o}}^{\text{w}}\phi$) across the interface by,

$$E = (\Delta_{\text{o}}^{\text{w}}\phi) + \Delta E_{\text{ref}}$$

where ΔE_{ref} depends on the reference electrodes and the reference solutions employed. In the present case, potential values reported, E , include the term $\Delta_{\text{o}}^{\text{w}}\phi_{\text{lr,TPHAs}^+}^{\text{o}}$ or $\Delta_{\text{o}}^{\text{w}}\phi_{\text{lr,TPnA}^+}^{\text{o}}$, corresponding to the standard potential transfer of the reference cations TPHAs^+ or TPnA^+ , respectively.

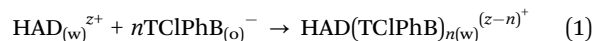
3. Results and discussion

3.1 Voltammetric behavior of AD, CD and DEAE-D at the water/1,2-dichloroethane interface

Fig. 1a shows the voltammetric response of aminodextran (AD) of different molecular weights: 5000, 40 000 and 250 000 g mol^{-1} . This polymer has about 0.1 mmol of primary amine per gram of dextran and, as discussed in Introduction, the primary amines directly bonded to the main chain of the polymer structure can be protonated at low pH values, conferring positive charge to the polymer. The structures of these polymers are quite similar to that of chitosan, with the difference that aminodextran is a branched polymer while chitosan is a linear one. As can be noted in the figure, no transfer process is observed in the presence of 0.100% w/v AD, at pH 3.05, for any molecular weight. However,

it can be seen that the presence of aminodextran in the aqueous phase decreases the potential window at the positive end, producing an enhancement of the transfer current of the anion TCIPhB^- from the organic to the aqueous phase.

A possible explanation for this result is the consideration that the positively charged polymer, AD, acts as an anion carrier for TCIPhB^- , according to the following facilitated mechanism:



as already reported for the polyelectrolyte chitosan.³⁴ It can also be observed in Fig. 1a that the increase in current is independent of the molecular weight of AD. Comparing this enhancement effect in the presence of TPnATCIPhB or TPHAsDCC as organic electrolytes, it was found that the first one produces a higher increase in the current (Fig. 1b), pointing to a larger value of the equilibrium constant for the formation of $\text{HAD}(\text{TCIPhB})_{n(\text{w})}^{(z-n)^+}$ from $\text{HAD}_{(\text{w})}^{z^+}$ and $n\text{TCIPhB}_{(\text{o})}^-$ (eqn (1)) compared to the corresponding $\text{HAD}(\text{DCC})_{n(\text{w})}^{(z-n)^+}$, in accordance with the predicted variation of $\Delta_{\text{o}}^{\text{w}}\phi$ with the equilibrium constant for the formation of an ionic pair or a complex through a facilitated transfer mechanism.⁵⁴

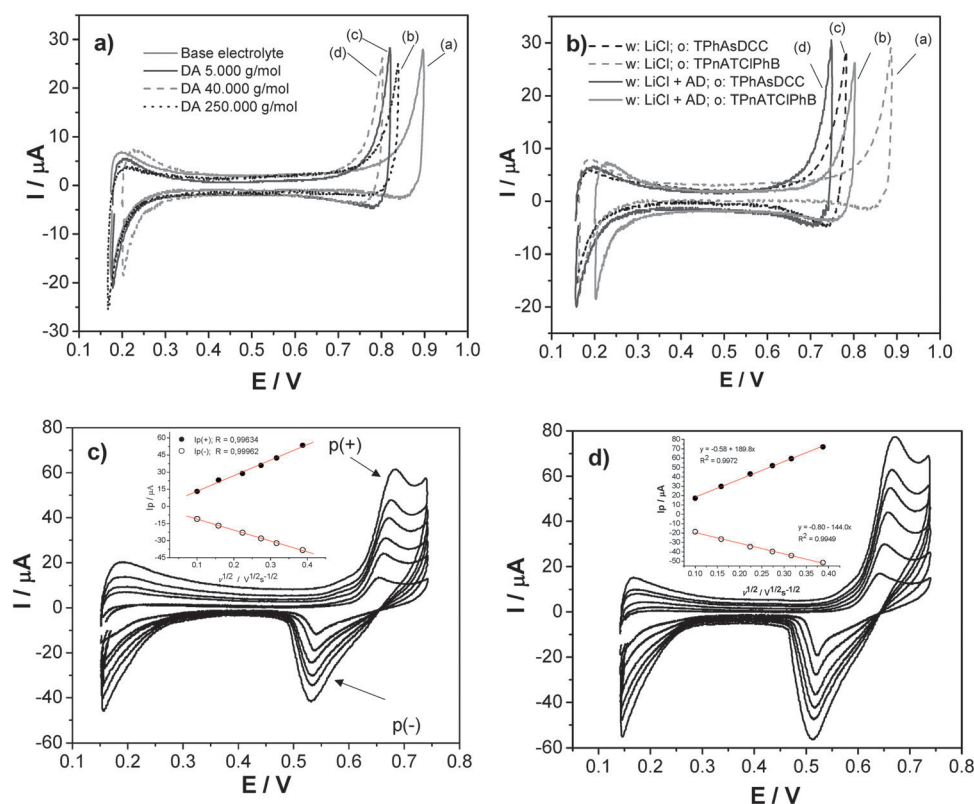


Fig. 1 (a) Cyclic voltammogram corresponding to the base electrolytes in the absence (a) and in the presence (b–d) of AD. Aqueous phase: $\text{LiCl } 1.0 \times 10^{-2} \text{ M} + 0.10\% \text{ w/v}$ of (b) AD 250 000 g mol^{-1} ; (c) AD 5000 g mol^{-1} , or (d) 40 000 g mol^{-1} . Organic phase: $1.0 \times 10^{-2} \text{ M TPnATCIPhB}$. $\nu = 0.050 \text{ V s}^{-1}$, $\text{pH} = 3.00$. (b) Cyclic voltammogram corresponding to the base electrolytes in the absence (a and c) and in the presence (b and d) of AD of 40 000 g mol^{-1} . Aqueous phase: $\text{LiCl } 1.0 \times 10^{-2} \text{ M} + 0.10\% \text{ w/v}$ of AD (b and d). Organic phase: $1.0 \times 10^{-2} \text{ M TPnATCIPhB}$ (a and b), or $1.0 \times 10^{-2} \text{ M TPHAsDCC}$ (c and d). $\nu = 0.050 \text{ V s}^{-1}$, $\text{pH} = 3.00$. (c) Cyclic voltammograms for cationic dextran, CD. Aqueous phase: $1.0 \times 10^{-2} \text{ M LiCl} + \text{CD } 0.20\% \text{ w/v}$. Organic phase: $1.0 \times 10^{-2} \text{ M TPHAsDCC}$. Inset: plot of I_p , positive and negative, as a function of $\nu^{1/2}$. (d) Cyclic voltammograms of DEAE-D. Aqueous phase: $1.0 \times 10^{-2} \text{ M LiCl} + \text{DEAE-D } 0.20\% \text{ w/v}$. Organic phase: $1.0 \times 10^{-2} \text{ M TPHAsDCC}$. Inset: plot of I_p , positive and negative, as a function of $\nu^{1/2}$. (c and d) Scan rates: 0.010, 0.025, 0.050, 0.075, 0.100, and 0.150 V s^{-1} .

Fig. 1c shows the voltammetric response of cationic dextran (CD) at different scan rates, ν . In this case CD was dissolved in the aqueous phase at a concentration of 0.200% w/v, nevertheless similar results were obtained at any concentration within the range 0.025 to 0.200% w/v. A forward current peak, $p(+)$, at $E_p^+ = 0.650$ V and the corresponding backward process, $p(-)$, at $E_p^- = 0.530$ V can be observed. The positive and negative peak currents, $I_p(+)$ and $I_p(-)$, are linear with $\nu^{1/2}$ in the whole range of sweep rates analyzed (shown in the inset) indicating a diffusion controlled transfer mechanism. Potential transfer values, E_p^+ and E_p^- , were independent of ν and CD concentration, while $I_p(+)$ and $I_p(-)$ varied linearly with CD concentration (data not shown). This behavior was independent of pH, as expected considering that CD is substituted with quaternary amines which confer permanent positive charge to the polymer, independent of the pH value.

A quite similar behaviour was observed for the polymer DEAE-D, of molecular weight $50\,000\text{ g mol}^{-1}$, (Fig. 1d) in the concentration range 0.025 to 0.200% w/v. Nevertheless, in this case both the peak current and potential values were dependent on pH due to the fact that DEAE-D contains tertiary amine groups in addition to the quaternary amine group with permanent positive charge. This dependence on pH is described in Section 3.2.

The differences observed in the electrochemical behaviour of the three polymeric structures could be explained assuming that the adsorption process depends on the localization of positive charge in the polymeric structure: the positive charge in AD is given by protonated primary amine groups directly bonded to the main chain of the cellulose structure, while in CD and DEAE-D the positive charge is separated from the cellulose structure by an aliphatic chain, this charge distribution would probably favour adsorption.

3.2. Effect of pH on the voltammetric behavior of DEAE-D

As described in Introduction, the molecular structure of the polymer DEAE-D contains three kinds of charged amine groups with different pK_a values. One of them, the (2-(diethyl amino) ethyl), (A) group, is a tertiary amine group, with a pK_a value of 9.5. The other two groups are present in the 2-[[2-(diethyl

amino)ethyl]-diethyl ammonium] ethyl branch, (B) which contains a tertiary amine with pK_a 5.7 and a quaternary amine group with permanent positive charge. The pH value of the aqueous phase determines the charge on this polyelectrolyte.⁵⁵

With the aim of analyzing the effect of pH on the electrochemical response of DEAE-D, 0.100% w/v of the polymer was added to the aqueous phase at different pH values within the range 1.92–11.68. The voltammograms obtained are shown in Fig. 2a, where a gradual decrease in peak currents and a shift of peak potentials towards more negative values with increasing pH value can be observed, in accordance with the decrease of polymer charge.

For simplicity, in the explanation below, we will call the polymer form H_3D^{3+} in which the monomers units have both tertiary amine groups protonated in addition to the permanent positive charge on the quaternary amine group, so each unit in the polymer has charge 3+. This species predominates at pH values lower than 3.7 (two units under pK_a 5.7). The species H_2D^{2+} prevails at around 7.7 and denotes the polymer form in which only one of the tertiary amines is protonated, while the species HD^+ is the major species at pH above 11.5 and refers to monomers with only one positive charge provided by the quaternary amine group.

Fig. 2b shows the variation of the positive peak current, $I_p(+)$, as a function of pH. At low pH values, $pH \leq 3.7$, where the species H_3D^{3+} predominates, the current is constant and equal to $15.2\ \mu\text{A}$. Above $pH = 4$, $I_p(+)$ decreases with increasing pH, due to the occurrence of species with lower charge. If we assume that the current value $I_p(+)$ = $15.2\ \mu\text{A}$ corresponds to the transfer of the species H_3D^{3+} , then, considering the dependence of $I_p(+)$ on $z^{3/2}$ (z = charge of the transferred species) for a reversible diffusional process, a value equal to $8.3\ \mu\text{A}$ for $I_p(+)$ corresponding to the species H_2D^{2+} , and $2.9\ \mu\text{A}$ for HD^+ would be expected. Nevertheless the results shown in Fig. 2b indicate $I_p(+)$ values equal to 10.5 or $6.5\ \mu\text{A}$ at pH 7.7 (predominance of H_2D^{2+}) or 11.5 (prevalence of HD^+), respectively. This apparent discrepancy could be explained considering that, at low pH values, the species H_3D^{3+} would be partially neutralized by ion pair association with counter-ions present in the aqueous phase, leading to a decrease of transfer current.

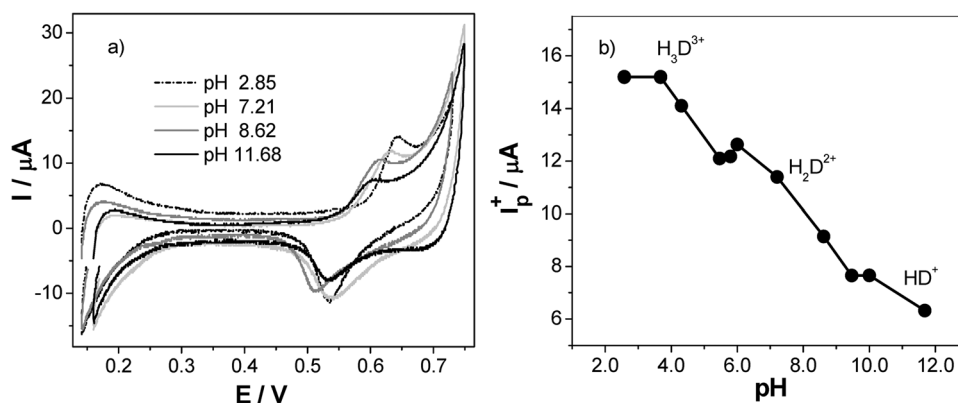


Fig. 2 (a) Effect of pH on the voltammetric response. Aqueous phase: 1.0×10^{-2} M LiCl + DEAE-D 0.10% w/v. Organic phase: 1.0×10^{-2} M TPhAsDCC. $\nu = 0.050\text{ V s}^{-1}$. (b) I_p vs. pH, for DEAE-D 0.10% w/v.

The dependence of the electrochemical response on pH suggests that transfer of the polymer from the aqueous to the organic phase followed by complex formation with the organic anion occurs in this case, since the increase of pH results in a decrease of the net charge of the polymer, which leads, on one hand, to a decrease of transfer currents, proportional to the charge of the transferred species and, on the other hand, to a decrease in peak potential values associated with the increased hydrophobicity of the polymer as the net charge is reduced. The processes observed could not be attributed to the facilitated transfer of the organic anion by the polymer in the aqueous phase, since, if this were the mechanism, an increase in E_p^+ values with pH would be expected.

3.3. Effect of molecular weight on the voltammetric behavior of DEAE-D

We analyzed the effect of polymer molecular weight, comparing the voltammetric response obtained using DEAE-D of molecular weights $50\,000\text{ g mol}^{-1}$ and $2\,000\,000\text{ g mol}^{-1}$. The results are shown in Fig. 3. The voltammograms obtained in both cases are similar despite the differences in the molecular weights, which indicate that the polymer is transferred as a single molecule, and should be considered as a chain of individual segments with charge $1+$, $2+$ or $3+$, depending on pH, rather than a polycation carrying the charge $z+$.¹²

Fig. 3 also includes the i/E profile of the polymer CD for comparison and it is evident that these three dextran polymers have the same electrochemical response.

3.4. Effect of the anion present in the organic electrolyte on the voltammetric behavior of DEAE-D

Previous studies indicated that the degree of hydrophobicity of the anion present in the electrolyte of the organic phase affects the voltammetric response of several biomolecules.²² Fig. 4 shows the voltammograms obtained for DEAE-D of molecular weight $50\,000\text{ g mol}^{-1}$, at a concentration 0.100% w/v, added to an aqueous phase of $1.0 \times 10^{-2}\text{ M LiCl}$, in contact with an

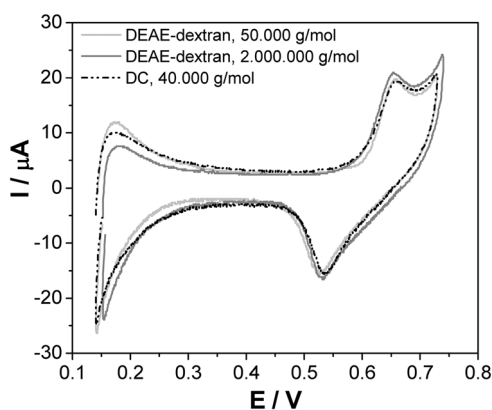


Fig. 3 Effect of the molecular weight on the voltammetric response. Aqueous phase: $1.0 \times 10^{-2}\text{ M LiCl}$ + DEAE-dextran 0.10% w/v; — 50 000 g mol^{-1} , — 2 000 000 g mol^{-1} , - - - CD 40 000 g mol^{-1} . Organic phase: $1.0 \times 10^{-2}\text{ M TPhAsDCC}$. $\nu = 0.050\text{ V s}^{-1}$.

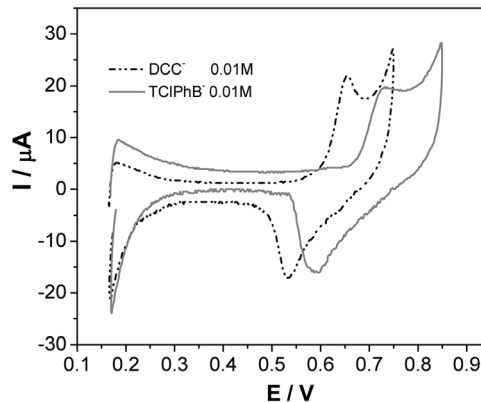


Fig. 4 The effect of the organic anion on the cyclic voltammetry response of DEAE-dextran: CV response with (---) DCC^- , and (—) TClPhB^- acting as the organic anion. Aqueous phase: $1.0 \times 10^{-2}\text{ M LiCl}$ + DEAE-dextran 0.10% w/v. Organic phase: (---) $1.0 \times 10^{-2}\text{ M TPhAsDCC}$ or (—) $1.0 \times 10^{-2}\text{ M TPnATClPhB}$. $\nu = 0.050\text{ V s}^{-1}$.

organic phase containing either TPhAsDCC or TPnATClPhB . The general voltammetric features are similar, in terms of their shape, peak currents, charges and ΔE_p for both anions, but the transfer takes place at different potential values. A slight increase in peak potentials for the forward and reverse process is observed in the presence of the more hydrophobic anion, TClPhB^- , in agreement with previous studies.¹⁷ This behavior indicates that the polymer transfer requires a certain amount of the organic anion at the interface, demonstrating that the transfer process of positively charged DEAE-D, from the aqueous to the organic phase, is coupled to ion pair formation between the anion of the organic salt and the positive amine groups in DEAE-D. Under the conditions used in experiments shown in Fig. 4, the formation/dissociation of the ion pair is a reversible process, as suggested by the similar values of peak currents for the positive and negative scans.

The above observation is similar to that made by Trojanek *et al.*¹⁴ for protamine 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 protamine polycation and the organic electrolyte anion, and found that the stability of the ion pair decreases with increasing size of the anion. Correlating this prior information to our results, we can explain the differences observed in peak potential values.

3.5. Effect of DCC^- concentration on the voltammetric behavior of DEAE-D

To determine the effect of the organic anion concentration on the electrochemical response, TPhAsDCC was used as an organic electrolyte at different concentrations in the range $5.0 \times 10^{-3}\text{ M}$ to $1.0 \times 10^{-1}\text{ M}$, keeping constant the concentration of the polymer and the base electrolyte in the aqueous phase. As shown in Fig. 5, the voltammetric response of the polymer depends strongly on the concentration of the supporting electrolyte in the organic phase. In the positive scan, the transfer current increases with the concentration of DCC^- , while other

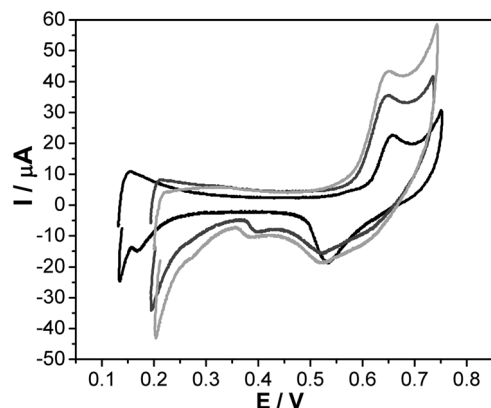


Fig. 5 Cyclic voltammety of DEAE-D of $50\,000\text{ g mol}^{-1}$, at different concentrations of the organic anion in the organic phase. Aqueous phase: $1.0 \times 10^{-2}\text{ M LiCl} + \text{DEAE-dextran } 0.010\% \text{ w/v}$. Organic phase: (—) $5.0 \times 10^{-3}\text{ M}$, (—) $6.0 \times 10^{-2}\text{ M}$, or (—) $8.0 \times 10^{-2}\text{ M TPhAsDCC}$. $v = 0.050\text{ V s}^{-1}$.

modifications are observed in the reverse scan, defining two transfer processes. The increase in the current during the positive sweep could be related to a facilitated transfer mechanism of the polymer, according to:



However, in this case the transfer potential should decrease with increasing concentration of the organic anion, but as seen in Fig. 5, the transfer potential is constant at any DCC^{-} concentration.

Other possible explanations for the disproportion observed between $I_p(+)$ and $I_p(-)$ at high DCC^{-} concentrations arise from considering that two processes are associated with the transfer of the polymer: weak adsorption of DEAE-D at the interface, coupled to a diffusional process or the occurrence of an electrochemical/chemical mechanism. In the latter case the potential should change with the sweep rate, which does not occur at any concentration of the polymer, or the organic electrolyte.

Therefore, it is determined that DEAE-D is transferred from the aqueous phase to the organic phase by a mixed mechanism of weak adsorption and diffusional transfer, depending on DCC^{-} concentration in the organic phase. It is expected that a pure diffusional process predominates at low concentrations of DCC^{-} , as the adsorption process of positive charge at the interface requires an equivalent negative charge accumulation at the organic side, which is favored by increasing anion concentration.

Fig. 6a shows the variation of peak current with DCC^{-} concentration. At low values, the diffusional transfer of polymer is favored, and $I_p(+)$ remains constant regardless of the concentration of DCC^{-} , whereas at high anion concentrations, weak adsorption of the polymer at the aqueous side of the interface takes place prior to the transfer step. In the global electrochemical process the transfer of the adsorbed polymer is coupled to the transfer of molecules diffusing from the bulk solution, causing an increase in the current during the positive sweep, reaching a constant value, at DCC^{-} concentrations higher than 0.08 M , due to saturation of interfacial sites. This explanation is

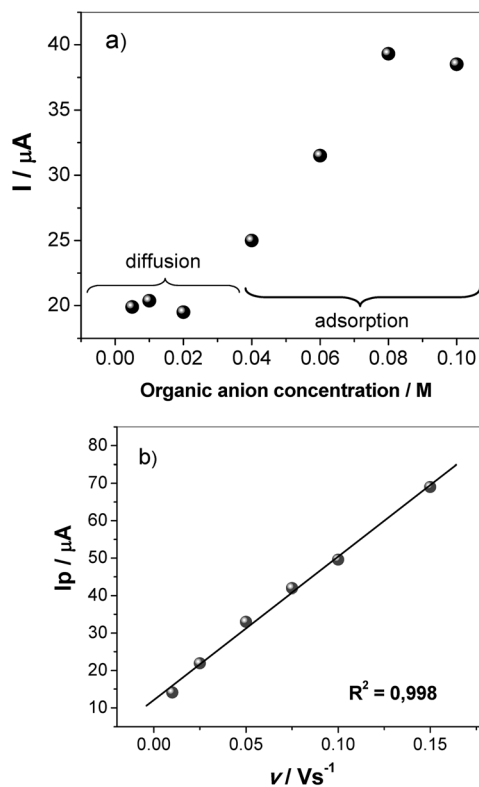


Fig. 6 (a) DEAE-D transfer peak current intensity as a function of organic anion concentration. Aqueous phase: $\text{LiCl } 1.0 \times 10^{-2}\text{ M} + \text{DEAE-dextran } 50\,000\text{ g mol}^{-1}, 0.010\% \text{ w/v}$. Organic phase: $\text{TPhAsDCC } 5.0 \times 10^{-3} - 0.10\text{ M}$. $v = 0.050\text{ V s}^{-1}$. (b) Current response to v for cyclic voltammety of DEAE-D of $50\,000\text{ g mol}^{-1}$, at high concentration of the organic anion in the organic phase. Aqueous phase: $1.0 \times 10^{-2}\text{ M LiCl} + \text{DEAE-dextran } 0.010\% \text{ w/v}$. Organic phase: 0.080 M TPhAsDCC . $v = 0.050\text{ V s}^{-1}$.

confirmed by the linear relationship observed between $I_p(+)$ and the sweep rate, Fig. 6b, when the anion concentration in the organic phase is high enough to favor the adsorption process with respect to diffusion. The two peaks observed on the negative sweep in Fig. 5 can be explained by considering the desorption of two species with different interfacial stability.

4. Conclusions

Studies of the electrochemical behaviour of different dextran polymers, aminodextran (AD), cationic dextran (CD), and diethylaminoethyl dextran (DEAE-D), at liquid/liquid interfaces have been undertaken. The main structural difference between these species is the position of the positive charge in the macromolecule: in the case of AD the positive charge is given by protonated primary amine groups directly bonded to the main chain of the cellulose structure, while in CD and DEAE-D the positive charge is separated from the cellulose structure by an aliphatic chain. As a consequence of these structural differences the AD polymer is not transferred to the organic phase, regardless of molecular weight, while CD and DEAE-D are transferred from the aqueous to the organic phase at $E = 0.650\text{ V}$, independent of the polymer concentration and of the molecular weight. The shape of the voltammograms corresponding to DEAE-D transfer as well

as the magnitude of the peak currents and the peak potentials values were all dependent on the pH of the aqueous phase solution and on the nature and concentration of the anion present in the organic electrolyte. Based on this dependence, we postulate a mixed mechanism, which involves the transfer of dissolved and adsorbed DEAE-D molecules. At low anion concentrations, diffusional transfer of the polymer from the aqueous to the organic phase prevails, whereas at higher anion concentrations there is a previous step of weak adsorption of the polymer at the aqueous side of the interface, and the electrochemical transfer of dextran diffusing from bulk aqueous solution coupled to the transfer of the adsorbed fraction occurs, producing an appreciable increase of the current in the positive sweep.

The results presented in this paper provide further knowledge of the electrochemical behavior of macromolecules at immiscible liquid/liquid interfaces demonstrating that electrochemical techniques are suitable for characterizing the effect of a set of experimental variables (pH, electrolyte nature, ionic strength, molecular weight, *etc.*) on the interfacial behavior of polyelectrolytes.

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References

- 1 P. Salou, P. Loubiere and A. Pareilleux, Growth and energetics of *Leuconostoc* during cometabolism of glucose with citrate or fructose, *Appl. Environ. Microbiol.*, 1994, **60**, 1459–1466.
- 2 A. N. Belder, *Dextran, Handbook*, Amersham Biosciences, 2003.
- 3 T. R. Neyestani, Isolation of α -lactalbumin, β -lactoglobulin, and bovine serum albumin from cow's milk using gel filtration and anion-exchange chromatography including evaluation of their antigenicity, *Protein Expression Purif.*, 2003, **29**, 202–208.
- 4 Z. Didem, J. Icoz and L. Kokini, Probing the boundaries of miscibility in model carbohydrates consisting of chemically derivatized dextrans using DSC and FTIR spectroscopy, *Carbohydr. Polym.*, 2007, **68**, 68–76.
- 5 C. Demirbilek and C. ÖzdemirDinç, Synthesis of diethylaminoethyl dextran hydrogel and its heavy metal ion adsorption characteristics, *Carbohydr. Polym.*, 2012, **90**, 1159–1167.
- 6 F. L. Graham and A. J. van der Eb, A new technique for the assay of infectivity of human adenovirus 5 DNA, *Virology*, 1973, **52**, 456–467.
- 7 R. A. W. Dryfe, The electrified liquid–liquid interface, *Adv. Chem. Phys.*, 2009, **141**, 153–215.
- 8 Z. Samec, E. Samcova and H. H. Girault, Ion amperometry at the interface between two immiscible electrolyte solutions in view of realizing the amperometric ion-selective electrode, *Talanta*, 2004, **63**, 21–32.
- 9 S. O'Sullivan, E. Alvarez de Eulate, Y. H. Yuen, E. Helmerhorst and D. W. M. Arrigan, Stripping voltammetric detection of insulin at liquid–liquid microinterfaces in the presence of bovine albumin, *Analyst*, 2013, **138**, 6192–6196.
- 10 P. Vanysek and L. B. Ramirez, Interface between two immiscible liquid electrolytes: A Review, *J. Chil. Chem. Soc.*, 2008, **53**, 1455–1463.
- 11 M. D. Scanlon, J. Strutwolf and D. W. M. Arrigan, Voltammetric behaviour of biological macromolecules at arrays of aqueous|organogel micro-interfaces, *Phys. Chem. Chem. Phys.*, 2010, **12**, 10040–10047.
- 12 S. Ulmeanu, H. J. Lee and H. H. Girault, Voltammetric characterisation of polyelectrolyte adsorption/transfer at the water/1,2-DCE interface, *Electrochem. Commun.*, 2001, **3**, 539–543.
- 13 G. Herzog and D. W. M. Arrigan, Electrochemical strategies for the label-free detection of amino acids, peptides and proteins, *Analyst*, 2007, **132**, 615–632.
- 14 A. Trojanek, J. Langmaier, E. Samcova and Z. Samec, Counterion binding to protamine polyion at a polarised liquid–liquid interface, *J. Electroanal. Chem.*, 2007, **603**, 235–242.
- 15 Y. Yuan and S. Amemiya, Facilitated Protamine Transfer at Polarized Water/1,2-Dichloroethane Interfaces Studied by Cyclic Voltammetry and Chronoamperometry at Micropipet Electrodes, *Anal. Chem.*, 2004, **76**, 6877–6886.
- 16 G. Herzog, V. Kam and D. W. M. Arrigan, Electrochemical behaviour of haemoglobin at the liquid/liquid interface, *Electrochim. Acta*, 2008, **53**, 7204–7209.
- 17 G. Herzog, W. Moujahid, J. Strutwolf and D. W. M. Arrigan, Interactions of proteins with small ionised molecules: electrochemical adsorption and facilitated ion transfer voltammetry of haemoglobin at the liquid|liquid interface, *Analyst*, 2009, **134**, 1608–1613.
- 18 F. Kivlehan, Y. H. Lanyon and D. W. M. Arrigan, Electrochemical Study of Insulin at the Polarized Liquid–Liquid Interface, *Langmuir*, 2008, **24**, 9876–9882.
- 19 M. D. Scanlon, E. Jennings and D. W. M. Arrigan, Electrochemical behaviour of hen-egg-white lysozyme at the polarised water/1,2-dichloroethane interface, *Phys. Chem. Chem. Phys.*, 2009, **11**, 2272–2280.
- 20 M. D. Scanlon, J. Strutwolf and D. W. M. Arrigan, Voltammetric behaviour of biological macromolecules at arrays of aqueous|organogel micro-interfaces, *Phys. Chem. Chem. Phys.*, 2010, **12**, 10040–10047.
- 21 S. O'Sullivan and D. W. M. Arrigan, Electrochemical behaviour of myoglobin at an array of microscopic liquid–liquid interfaces, *Electrochim. Acta*, 2012, **77**, 71–76.
- 22 J. Perrenoud-Rinuy, P.-F. Brevet and H. H. Girault, Second harmonic generation study of myoglobin and hemoglobin and their protoporphyrin IX chromophore at the water/1,2-dichloroethane interface, *Phys. Chem. Chem. Phys.*, 2002, **4**, 4774–4781.
- 23 M. A. Mendez, Z. Nazemi, I. Uyanik and H. H. Girault, Melittin Adsorption and Lipid Monolayer Disruption at Liquid–Liquid Interfaces, *Langmuir*, 2011, **27**, 13918–13924.
- 24 T. Osakai, Y. Yuguchi, E. Gohara and H. Katano, Direct Label-free Electrochemical Detection of Proteins Using the Polarized Oil/Water Interface, *Langmuir*, 2010, **26**, 11530–11537.

- 25 V. Beni, M. Ghita and D. W. M. Arrigan, Cyclic and pulse voltammetric study of dopamine at the interface between two immiscible electrolyte solutions, *Biosens. Bioelectron.*, 2005, **20**, 2097–2103.
- 26 D. P. Zhan, S. N. Mao, Q. Zhao, Z. Chen, H. Hu, P. Jing, M. Q. Zhang, Z. W. Zhu and Y. H. Shao, Electrochemical Investigation of Dopamine at the Water/1,2-Dichloroethane Interface, *Anal. Chem.*, 2004, **76**, 4128–4136.
- 27 J. A. Ribeiro, I. M. Miranda, F. Silva and C. M. Pereira, Electrochemical study of dopamine and noradrenaline at the water/1,6-dichlorohexane interface, *Phys. Chem. Chem. Phys.*, 2010, **12**, 15190–15194.
- 28 J. Langmaier, E. Samcova and Z. Samec, Potentiometric Sensor for Heparin Polyion: Transient Behavior and Response Mechanism, *Anal. Chem.*, 2007, **79**, 2892–2900.
- 29 Z. Samec, A. Trojanek, J. Langmaier and E. Samcova, Cyclic voltammetry of biopolymer heparin at PVC plasticized liquid membrane, *Electrochem. Commun.*, 2003, **5**, 867–870.
- 30 P. J. Rodgers, P. Jing, Y. Kim and S. Amemiya, Electrochemical Recognition of Synthetic Heparin Mimetic at Liquid/Liquid Microinterfaces, *J. Am. Chem. Soc.*, 2008, **130**, 7436–7442.
- 31 J. Guo and S. Amemiya, Voltammetric Heparin-Selective Electrode Based on Thin Liquid Membrane with Conducting Polymer-Modified Solid Support, *Anal. Chem.*, 2006, **78**, 6893–6902.
- 32 J. Langmaier, J. Olsak, E. Samcova, Z. Samec and A. Trojanek, Amperometric Sensor for Heparin: Sensing Mechanism and Application in Human Blood Plasma Analysis, *Electroanalysis*, 2006, **18**, 1329–1338.
- 33 J. Langmaier, J. Olsak, E. Samcova, Z. Samec and A. Trojanek, Amperometry of Heparin Polyion Using a Rotating Disk Electrode Coated with a Plasticized PVC Membrane, *Electroanalysis*, 2006, **18**, 115–120.
- 34 Z. Samec, A. Trojanek, J. Langmaier and E. Samcova, Cyclic voltammetry of biopolymer heparin at PVC plasticized liquid membrane, *Electrochem. Commun.*, 2003, **5**, 867–870.
- 35 J. S. Riva, A. V. Juarez, D. M. Beltramo and 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*, 2012, **59**, 39–44.
- 36 J. S. Riva, K. Bierbrauer, D. M. Beltramo and L. M. Yudi, Electrochemical study of the interfacial behavior of cationic polyelectrolytes and their complexation with monovalent anionic surfactants, *Electrochim. Acta*, 2012, **85**, 659–664.
- 37 J. S. Riva, R. Iglesias and L. M. Yudi, Electrochemical adsorption of a cationic cellulosic polymer by ion pair formation at the interface between two immiscible electrolyte solutions, *Electrochim. Acta*, 2013, **107**, 584–591.
- 38 J. S. Riva, D. M. Beltramo and L. M. Yudi, Adsorption-desorption mechanism of a cationic polyelectrolyte based on dimethyl amino ethyl poly methacrylates at the water/1,2-dichloroethane interface, *Electrochim. Acta*, 2014, **115**, 370–377.
- 39 C. I. Cámara, M. V. Colqui Quiroga, N. Wilke, A. Gimenez-Kairuz and L. M. Yudi, Effect of chitosan on distearoylphosphatidylglycerol films at air/water and liquid/liquid interfaces, *Electrochim. Acta*, 2013, **94**, 124–133.
- 40 C. I. Cámara and L. M. Yudi, Potential-mediated interaction between dextran sulfate and negatively charged phospholipids films at air/water and liquid/liquid interfaces, *Electrochim. Acta*, 2013, **113**, 644–652.
- 41 H. A. Santos, V. García-Morales, L. Murtomäki, J. A. Manzanares and K. Kontturi, Preparation of nanostructures composed of dextran sulfate/ruthenium nanoparticles and their interaction with phospholipid monolayers at a liquid-liquid interface, *J. Electroanal. Chem.*, 2007, **599**, 194–202.
- 42 B. Fu, E. Bakker, J. H. Yun, V. C. Yang and M. E. Meyerhoff, Response Mechanism of Polymer Membrane-Based Potentiometric Polyion Sensors, *Anal. Chem.*, 1994, **66**, 2250–2259.
- 43 S. C. Ma, V. C. Yang and M. E. Meyerhoff, Electrochemical sensor for heparin: further characterization and bioanalytical applications, *Anal. Chem.*, 1992, **64**, 694–697.
- 44 A. Shvarev and E. Bakker, Reversible Electrochemical Detection of Non electroactive Polyions, *J. Am. Chem. Soc.*, 2003, **125**, 11192–11193.
- 45 A. Shvarev and E. Bakker, Response Characteristics of a Reversible Electrochemical Sensor for the Polyion Protamine, *Anal. Chem.*, 2005, **77**, 5221–5228.
- 46 R. A. Hartvig, M. A. Méndez, M. Van de Weert, L. Jorgensen, J. Ostergaard, H. H. Girault and H. Jensen, Interfacial Complexes between a Protein and Lipophilic Ions at an Oil-Water Interface, *Anal. Chem.*, 2010, **82**, 7699–7705.
- 47 F. Kivlehan, M. Leifoix, H. A. Moynihan, D. Thompson, V. I. Ogurtsov, G. Herzog and D. W. M. Arrigan, Interaction of acridine-calix[4]arene with DNA at the electrified liquid|liquid interface, *Electrochim. Acta*, 2010, **55**, 3348–3354.
- 48 J. S. Riva, A. V. Juarez and L. M. Yudi, Prometrine-Humic Acids Interactions Studied at a Water/1,2-Dichloroethane Interface, *Electroanalysis*, 2010, **22**, 413–418.
- 49 A. V. Juarez, L. M. Yudi, C. I. Alvarez Igarzabal and M. C. Strumia, Cation transfer across a hydrogel/organic phase: Effect of cation size, hydrophobicity and acid-base properties, *Electrochim. Acta*, 2010, **55**, 2409–2413.
- 50 H. L. T. Ho and R. A. W. Dryfe, Transport of Neutral and Ionic Solutes: The Gel/Electrode and Gel/Electrolyte Interfaces, *Langmuir*, 2009, **25**, 12757–12765.
- 51 M. Calderon, L. M. A. Monzón, M. Martinelli, A. V. Juarez, M. C. Strumia and L. M. Yudi, Electrochemical Study of a Dendritic Family at the Water/1,2-Dichloroethane Interface, *Langmuir*, 2008, **24**, 6343–6350.
- 52 M. Gniadek, M. Donten and Z. Stojek, Electroless formation of conductive polymer-metal nanostructured composites at boundary of two immiscible solvents. Morphology and properties, *Electrochim. Acta*, 2010, **55**, 7737–7744.
- 53 A. M. Baruzzi and J. Uhlken, Current interruption potentiostat for elimination of the IR drop in four-electrode systems, *J. Electroanal. Chem.*, 1990, **282**, 267–273.
- 54 F. Reymond, P. A. Carrupt and H. H. Girault, Facilitated ion transfer reactions across oil|water interfaces. Part I. Algebraic development and calculation of cyclic voltammetry experiments for successive complex formation, *J. Electroanal. Chem.*, 1998, **449**, 49–65.
- 55 T. Heinze, T. Liebert, B. Heublein and S. Hornig, Functional Polymers Based on Dextran, *Adv. Polym. Sci.*, 2006, **205**, 199–291.