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Mixed polyelectrolyte coatings on glassy carbon electrodes: Ion-exchange, permselectivity properties and analytical application of poly-L-lysine–poly(sodium 4-styrenesulfonate)-coated mercury film electrodes for the detection of trace metals

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Abstract

The present work describes the preparation, optimization and characterization of mixed polyelectrolyte coatings of poly-L-lysine (PLL) and poly(sodium 4-styrenesulfonate) (PSS) for the modification of thin mercury film electrodes (MFEs). The novel-modified electrodes were applied in the direct analysis of trace metals in estuarine waters by square-wave anodic stripping voltammetry (SWASV). The effects of the coating morphology and thickness and also of the monomeric molar ratio PLL/PSS on the cation-exchange ability of the PLL–PSS polyelectrolyte coatings onto glassy carbon (GC) were evaluated using target cationic species such as dopamine (DA) or lead cation. Further, the semi-permeability of the PLL–PSS-coated electrodes based both on electrostatic interactions and on molecular size leads to an improved anti-fouling ability against several tensioactive species. The analytical usefulness of the PLL–PSS-mixed polyelectrolyte coatings on thin mercury film electrodes is demonstrated via SWASV measurements of trace metals (lead, copper and cadmium at the low nanomolar level; accumulation time of 180 s) in estuarine waters containing moderate levels of dissolved organic matter, resulting in a fast and direct methodology requiring no sample pretreatment.

Keywords: Poly-L-lysine; Poly(sodium 4-styrenesulfonate); Thin mercury film electrodes; Anodic stripping voltammetry; Anti-fouling; Trace metals; Estuarine waters

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1. Introduction

Mercury film electrodes (MFEs) have been already widely used combined with anodic stripping voltammetry (ASV) for the determination of heavy metals [1–5]. However, with the conventional MFE, obtaining reliable data in complex samples is often complicated due to the fouling of the electrode surface by adsorbable species that hinder or distort the voltammetric signals. Polymer-modified electrodes can minimize this effect and hold a great promise for increasing the selectivity and, eventually the sensitivity of the voltammetric measurements [6,7]. The polymer coatings also improve the mechanical stability of thin mercury films plated on glassy carbon (GC) surfaces. Nafion is the most commonly employed ion-exchange polymer for the determination of metal cations [6]. However, when the preparation of nafion-modified electrodes involves solvent evaporation procedures lack of reproducibility is often reported [8,9]. Aditionally, for relatively thick coatings (e.g. ∼μm), diffusion restrictions usually occur and the sensitivity of the ASV determinations is diminished relatively to the uncoated MFE [7–9]. Poly(sodium 4-styrenesulfonate) (PSS) is also a cation exchanger which was applied in electrode modification for voltammetric measurements of trace metals in estuarine water samples [7,10], and in the preparation of layer-by-layer assembled structures for sensing devices [11–13]. Very stable and reproducible PSS-coated thin MFE (TMFE) could be easily prepared by the solvent evaporation method [7,10]. In what concerns electrode modification, poly-L-lysine (PLL), a cationic polyaminoacid, was used for the in situ coating of the mercury drop electrode [14] and for coating pyrolytic graphite or glassy carbon electrodes for anion detection [15–17]. On the other hand, mixed coatings of PLL and PSS, containing

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immobilized enzymes were applied as amperometric biosensors [18,19]. However, no full characterization of PLL–PSS-modified glassy carbon electrodes has yet been reported, namely regarding its utilization coupled to MFE for trace metal determinations. Previous work [7,10] has shown that PSS coatings on a thin MFE do not preclude diffusion of heavy metal cations through the polyion layer and, in fact, significant improvements on the stripping peak currents for lead and copper were observed, compared to the uncoated TMFE. However, the anti-fouling ability of such coatings was not as high as it would be desirable, especially for low molecular mass cationic surfactants such as the linear chain hyamine (HYA). Hence, in the present work, the performance towards the ASV determinations of modified thin mercury film electrodes coated with a mixed layer of the polyions PLL and PSS was evaluated. The main goal is in terms of the analytical application, the study of the anti-fouling capabilities of such mixed polycationic–polyanionic layers. The PLL–PSS coatings on glassy carbon were also examined by scanning electron microscopy (SEM). Furthermore, the ability against several tensioactive compounds was evaluated and the ionic exchange and permselectivity properties of the PLL–PSS coating were also assessed. The PLL–PSS-coated mercury film electrodes were applied to the direct determination of trace metals (Cd, Pb and Cu) in estuarine water samples having moderate levels of dissolved organic matter.

2. Experimental

2.1. Apparatus

All the voltammetric measurements were performed with a BAS 100B/W electrochemical analyser (Bioanalytical Systems) connected to a Cell Stand BAS-C2. The working electrode was a TMFE plated onto a glassy carbon disc (BAS, MF-2012), the auxiliary electrode was a Pt wire and the reference electrode was Ag/AgCl (sat. KCl). A combined glass electrode (Orion 9104SC) connected to a pH meter (Cole Parmer, Model 05669-20) was used for pH measurements. Dissolved organic carbon (DOC) determinations were made by an automated procedure (using a 4-channel Alliance auto-analyser-segmented flow analyser, model Evolution II) and were based on the discolouring of a buffered solution of phenolphthalein due to the carbon dioxide produced by oxidation of the dissolved organic matter. The colour intensity was measured at 560 nm and the calibration curve method was applied [20].

Scanning electron microscopy was conducted at a S-4100 HITACHI system.

2.2. Reagents and solutions

Poly(sodium 4-styrenesulfonate), (molecular weight, 70,000 and degree of sulfonation 1.0) and poly-L-lysine hydrobromide (molecular weight, 72,000–84,000) were purchased from Sigma–Aldrich and used without further purification. All chemicals were of analytical reagent grade and all solutions were prepared with ultra-pure water (18.2 MΩ cm, Milli-Q systems, Millipore-waters). Sodium chloride (Merck, suprapur), hydrochloric acid, 37% (Fluka, trace select) and 1000 ppm AA-spectrosol metal ion standards (BDH) were also used. Stock solutions of PSS (25 and 50 mM in the monomer units) and of PLL (20 and 40 mM in the monomer units) were prepared in phosphate buffer solution (pH 7.0, ionic strength 0.14 M) and stored at 4 °C. Potassium ferricyanide, dopamine (DA), L(+)-ascorbic acid (AA) and uric acid (UA), all 1 mM, were also prepared in phosphate buffer solution. Stock surfactant solutions of Triton X-100, sodium dodecyl sulphate (SDS), agar, poly(allylaminehydrochloride) (PAAHC), hyamine and bovine serum albumine (BSA) (all 0.1%) were stored at 4 °C. Biohit Proline pipettes equipped with disposable tips were used for appropriate dilutions. All measurements were carried out at room temperature (18–20 °C).

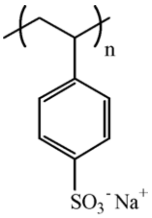
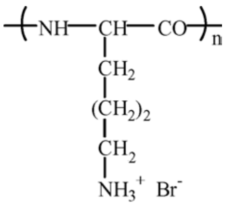
Schematic representations of the two polymers used for electrode modification are displayed in Table 1. PSS (pK_a = 1) [21] is a water soluble, thermally stable polyelectrolyte [22] having both hydrophilic parts (sulfonate groups and their counterions) and hydrophobic parts (hydrocarbon backbone and phenyl groups). In aqueous solution PSS is a linear polyanion [23,24] but, a highly coiled structure is obtained whenever the deposition proceeds from solutions of high ionic strength e.g. 0.1–2 M [24,25]. The deposition of the protonated PLL (from aqueous solutions of pH < 10.5) onto carbon-like electrodes leads to three-dimensional layers [17].

2.3. Electrode preparation

Prior to coating, the glassy carbon electrode was conditioned following a reported polishing/cleaning procedure [10]. Coatings of different loadings were obtained by the droplet evaporation method [10] placing on the electrode surface 3–10 μL of a PLL solution (20 or 40 mM in monomeric units), followed by 6 μL of a PSS solution (25 or 50 mM in monomeric units). Then the solvent was allowed to evaporate in a stream of warm air (∼60 °C). The electrochemically active surface area of the glassy carbon electrode, 6.42 mm^2 was measured by cronoamperometry (in 1.02×10^{-3} M ferricyanide/0.5 M KCl solution; four polishing experiments, each one with four replicate determinations) [7].

Thin mercury films were ex situ plated in a solution containing 0.12 mM mercury(II) nitrate in 0.01 M nitric acid (pH ca. 1.9) by electrodeposition through the mixed polymer coating at −1.3 V for 20 s whilst the solution was rotated with a

Table 1
Chemical structures of the polyelectrolytes used for electrode coating

Polystyrene sulfonate (PSS)	Poly-L-lysine hydrobromide (PLL)
Monomer mass 206.20	Monomer mass 209.08
	

magnetic stirrer (BAS-C2 stand, position 3). The conventional mercury film electrode was obtained in a similar fashion except for the polymer coating steps. The present methodology of ex situ deposition of the mercury film at a high cathodic overpotential in acidic solution has proven to produce relatively stable and homogeneous mercury films of closely packed Hg microdroplets [4,26]. Although an improved procedure for the deposition of Hg films in the presence of thiocyanate ion has been recommended recently [4,27] it was considered unsuitable for the present purpose due to the unknown effect of the presence on an additional anion, SCN^-, on the properties of the mixed polymer coating.

When required, the polymer layer was wiped off with a wet tissue and the GC surface was re-conditioned as mentioned above.

2.4. Voltammetric procedures

The ASV experiments for the examination of the analytical performance of the PLL–PSS/TMFE were carried out in 10 mL NaCl 0.5 M solutions spiked with lead(II) as a reference metal ion (otherwise stated the cell concentration of lead(II) was 6.00×10^{-8} mol dm^{-3}). The deposition step lasted 20 s at −0.8 V whilst the solution was rotated with a magnetic stirrer (BAS-C2, position 3). After a 5 s quiescent time the stripping step was initiated at −0.8 V and ended at −0.15 V to prevent the stripping of the mercury film. The instrumental parameters used in the square-wave anodic stripping voltammetry (SWASV) experiments were as follows: frequency 50 Hz, modulation amplitude 25 mV and step potential 5 mV.

For the ASV determination of heavy metals in estuarine water samples (A and B), 10 mL of a fresh sample filtered through a 0.45 μm membrane filter (Millipore) was pipetted into the voltammetric cell and the SWASV measurements were carried out promptly, with an accumulation step of 3 min at −0.8 V (rotation rate; BAS-C2 position 3). The SW parameters were the same as described above. Both the conventional TMFE and the PLL–PSS-coated TMFE were used as working electrodes. The quantification of the trace metals was done by the standard addition method. The samples presented moderate ionic strength (salinities of 17.3 and 7.3‰ for samples A and B, respectively) and reasonable high dissolved organic matter contents (DOC of 4 and 10 ppm for samples A and B, respectively).

All peak currents and electric charges quoted are mean values of six replicate measurements, except for the data regarding the application to estuarine waters where three replicates were obtained. All solutions were purged with nitrogen for 5 min prior to the voltammetric experiments.

3. Results

3.1. Performance of the PLL–PSS-coated TMFE towards the ASV determination of lead

Previous work [7,10] has shown that a thin mercury film can be ex situ plated on a glassy carbon electrode coated with a layer of the cation-exchange polymer PSS. At the best experimental conditions (PSS loading of 23.4 nmol mm^{-2}, i.e., 4.8 μg mm^{-2}) there were no diffusion restrictions to the mass transport of heavy metal cations through the polymer layer and, in fact, significant improvements on the stripping peak currents for lead and copper were observed, compared to the uncoated TMFE. The actual polymer loading used for electrode modification is an important experimental parameter because it affects both the morphology and the compactness of the coating, which, in turn, influence its permselectivity and mass transport properties. In order to evaluate how the PLL (cationic)–PSS (anionic) loading influence the ASV response of lead, experiments were carried out using four different mixed coatings with increasing PLL loadings (9.3, 18.7, 31.2 and 62.4 nmol mm^{-2}) keeping the PSS loading constant at 23.4 nmol mm^{-2} (c.f. Table 2).

Firstly, the modified electrodes were examined in terms of the features of the thin mercury films. The values of the deposited mercury charge (calculated by electronic integration of the linear sweep stripping peak of mercury [4]) are displayed in Table 3 together with reference values for the PSS-coated TMFE and for the bare TMFE.

The data shows the effect of the increasing molar ratio PLL/PSS, i.e., of the increasing relative amount of positively charged ionic sites at the mixed polyelectrolyte layer. In fact, as the molar ratio PLL/PSS increases, the amount of electric charge of deposited mercury decreases gradually, illustrating the effect of the repulsive electrostatic interactions, limiting the transport of the cationic mercury ion within the mixed polymer. The data also show that for the PLL–PSS electrode with the molar ratio of 1.3, the charge of deposited mercury is close to that obtained for the bare TMFE, whereas for the highest molar ratio PLL/PSS that value is rather low, highlighting the repulsive electrostatic effects due to the high amount of positive ion-exchange sites within the mixed polyelectrolyte coating. Additionally, as the mass loading increases, diffusion restrictions might also occur. Nevertheless, all the PLL–PSS electrodes presented good repeatability (expressed as low R.S.D. values).

Fig. 1A and B shows the SWASV peak current of lead, $I_{p(Pb)}$, as well as the corresponding SW voltammograms obtained for the various PLL–PSS/TMFE, as a function of the molar ratio (values for the PSS/TMFE and for the bare TMFE are also represented as reference values). It turns out clear the effect of increasing the ratio of positive/negative ionic exchange sites on the accumulation/detection of lead. For the PLL–PSS electrode with the ratio PLL/PSS of 1.3 (total molar loading 54.6 nmol mm^{-2}) the value of $I_{p(Pb)}$ is close to the that for the uncoated TMFE. Interestingly, the existence of a moderate excess (ca. 30%) of

Table 2
Loading characteristics of the mixed polyelectrolyte PLL–PSS used for electrode coating

Loadings in monomeric units (nmol mm^{-2})		Molar ratio (PLL/PSS)	Total mass loading (μg mm^{-2})
PLL	PSS		
9.3	23.4	0.4	6.8
18.7	23.4	0.8	8.7
31.2	23.4	1.3	11.3
62.4	23.4	2.7	17.8

Table 3
Electric charge of deposited mercury for the TMFE ex situ prepared through the mixed PLL–PSS coatings[a]

	PLL–PSS mass loading[b] (μg mm^{-2})				PSS coating (23.4 nmol mm^{-2})	TMFE
	6.8 (0.4)	8.7 (0.8)	11.3 (1.3)	17.8 (2.7)	–	–
Q_{Hg} (μC)	40.81	35.49	28.00	19.21	47.24	31.23
R.S.D. (%)	4.2	3.6	4.5	5.5	3.34	7.56

Reference values for PSS/TMFE and uncoated TMFE are also displayed [7].

[a] Five replicate measurements of the charge under the voltammetric stripping peak of the deposited mercury obtained by LSV at 250 mV s^{-1}.

[b] Molar ratios PLL/PSS in brackets.

positive sites in the coating layer does not preclude an effective ASV determination of lead—eventually, at these conditions, there are no restrictions to the mass transport of lead throughout the rather thick PLL–PSS polyelectrolyte layer. The SEM examination of the surface morphology of the PLL–PSS coatings revealed a rather three-dimensional structure with spread cavities, some of those with micrometer dimensions (see Fig. 2). This type of structure may, in fact, facilitate the ionic transport towards the electrode surface, regardless of the layer thickness (the estimate thickness value for a 23.4 nmol mm^{-2} PSS electrode is ca. 6 μm [7]; the present PLL–PSS electrodes shall be thicker, although the possibility of an inter-penetration of the individual layers cannot be discharged).

Additionally, there is a small deviation of the SWASV peak potentials of lead towards more negative values as the molar ratio increases, i.e., E_p decreases from −535 to −555 mV. This may be related to the effect of the strengthening of electrostatic repulsion and/or to the increase of the coating thickness. On the other hand, the peak-width-at-half-height of the lead SWASV signal remained unaltered indicating that no significant kinetic constrains to the ionic exchange process shall occur.

Another consideration arising from the data presented in Fig. 1 is that, considering merely the electrostatic interactions within the mixed PLL–PSS layer, the electrode with the PLL/PSS ratio of 1.3 should restrict the accumulation of the lead cations producing SWASV peak current intensities lower than those for a bare TMFE. However, such was not the case. An eventual alteration in the ionic exchange mechanism, involving the additional accumulation of negatively charged lead–chloro complexes (e.g. $[\mathrm{PbCl_3}]^-$; log β_3 1.9 [28]) which may exist in the electrolyte solution (NaCl 0.5 M) might provide an explanation.

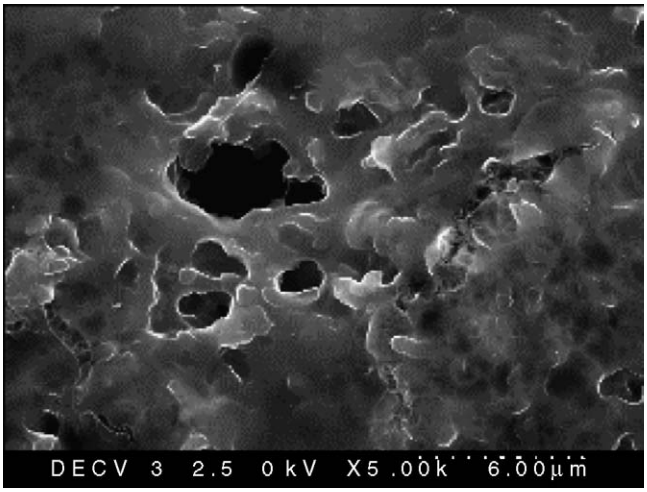


Fig. 2. Scanning electron micrograph (×5000) of a PLL–PSS-mixed coating on a GC electrode (ratio PLL/PSS: 1.3; total mass loading: 11.3 μg mm^{-2}).

The electrode with the PLL/PSS molar ratio of 1.3 was also tested in terms of its analytical performance towards the SWASV determination of lead and the results were compared with those for the uncoated TMFE. The calibration curve data were (concentration range 2.00×10^{-8} to 10.0×10^{-8} mol dm^{-3}; t_{acc} 20 s at −0.8 V): slope 8.3 ± 0.1 A mol^{-1} dm^3 ($r = 0.9997$, $N = 5$, $p < 0.05$) and 8.9 ± 0.6 A mol^{-1} dm^3 ($r = 0.9998$, $N = 5$, $p < 0.05$)

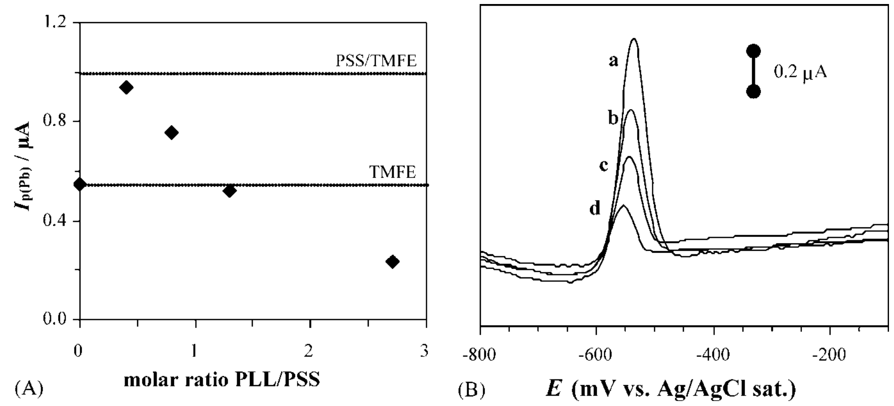


Fig. 1. Effect of the molar ratio PLL/PSS on the SWASV peak current of lead (A) and on the corresponding square-wave voltammograms and (B) molar ratios: (a) 0.4, (b) 0.8, (c) 1.3, (d) 2.7. Deposition time: 20 s at −0.8 V. SW parameters: $a = 25$ mV, $f = 50$ Hz. Concentration of lead, 6.00×10^{-8} M. Thin mercury films ex situ plated (charge values in Table 3). Reference values for the bare TMFE and a PSS/TMFE also included for comparison [7].

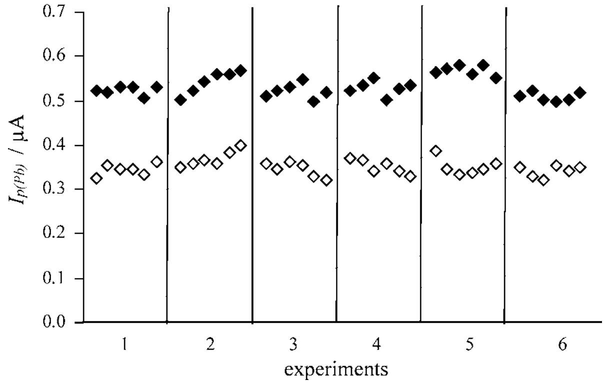


Fig. 3. SWASV peak current for repeated measurements ($N = 6$) in six test solutions of the same composition (6.00×10^{-8} M Pb^{2+} in 0.5 M NaCl) with six different PLL–PSS/TMFE electrodes with the same PLL/PSS molar ratio (1.3) and two different mass loadings; 11.3 µg mm^{-2} (black diamonds) and 22.8 µg mm^{-2} (white diamonds). Other experimental conditions as in Fig. 1.

for the PLL–PSS/TMFE and for the TMFE, respectively. The limits of detection were identical, i.e., ca. 0.3 nM (3σ). Hence, the sensitivity of both electrodes is similar. The R.S.D. values ranged between 1.0 and 2.5% for the PLL–PSS/TMFE and 3.1 and 4.7% for TMFE showing that the repeatibility of the SWASV signals is better for the polyelectrolyte-modified electrode.

Additional experiments were done in order to statistically evaluate the repeatibility of the SWASV signals and the reproducibility of the preparation method of the PLL–PSS-coated thin mercury film electrodes. Two type of electrodes, with the same PLL/PSS ratio (1.3) but different mass loadings (11.3 and 22.8 µg mm^{-2}) were examined. Fig. 3 shows the lead SWASV peak current, $I_{p(Pb)}$, for six different PLL–PSS electrodes of each type, in six test solutions of the same composition (6.00×10^{-8} M lead(II) in NaCl 0.5 M). For each experiment six repeated measurements of I_p were performed. Lower $I_{p(Pb)}$ values were obtained for the electrode with the thicker coating (ca. 66% lower), indicating that diffusion restrictions are now operative. Slightly better results in respect to the repeatibility and reproducibility were obtained using the PLL–PSS thinner coating (11.3 µg mm^{-2}). Table 4 presents the median, the range and the variance within samples and between samples for both types of electrodes. It can be concluded that the preparation procedure of the mixed polyelectrolyte-coated electrodes is reproducible, likewise the case of TMFE coated with only a single layer of PSS (in this case the reproducibility, based on σ_b^2, was 0.004 for similar experimental conditions) [10].

Further, the statistic evaluation of the results show that the repeatibility of the voltammetric signal at the PLL–PSS electrodes (molar ratio 1.3) does not depend of the coating thickness, within the studied range. Though, the sensitivity to lead is diminished for the thicker electrodes, most probably due to mass transport restrictions (the thickness shall be approximately twice that of the thinner electrodes).

Table 5
Variation of peak currents (in percentages) for three PLL–PSS-coated GC electrodes with different loadings, relative to the bare GC electrode

Substance	PLL–PSS loading (µg mm^{-2})[a]		
	11.3 (1.3)	17.8 (2.7)	22.8 (1.3)
DA	+14	−77	−62
UA	−32	−37	−53
AA	−8.6	−17	−81
$[Fe(CN)_6]^{3-}$	−100	−100	−100

[a] PLL/PSS molar ratio in brackets.

3.2. *Electrochemistry of electroactive molecular species at a PLL–PSS-modified glassy carbon electrode*

Additional experiments at PLL–PSS-coated electrodes with different mass loadings (11.3, 14.1 and 18.9 µg mm^{-2}) were carried out in order to obtain more information on the ionic exchange and permselectivity properties of the PSS coating. In this set of experiments no mercury film was plated. The selected electroactive molecular species were ions of different molecular weight and charges, $[Fe(CN)_6]^{3-}$ (M_W 212), L(+)-ascorbic acid (M_W 176) and uric acid (M_W 168) and dopamine (M_W 192). At the working pH of 7.0, L(+)-ascorbic acid ($pK_a = 4.10$) and uric acid ($pK_a = 5.4$) are both anionic and dopamine ($pK_b = 8.87$) is cationic. All four species are electroactive at the glassy carbon electrode within the potential window [0 V; +1 V]. Table 5 shows the relative variation of the peak current values obtained with the three different PLL–PSS electrodes compared to the values obtained at the bare glassy carbon electrode.

For all PLL–PSS coatings used, no peaks were detected for $[Fe(CN)_6]^{3-/4-}$ (100% exclusion). The exclusion of this anion certainly has an electrostatic nature due to the high charge of the ferricyanide, despite the PLL–PSS electrode with 17.8 µg mm^{-2} having a high content of the anion exchanger PLL. On the other hand, for ascorbate and urate anions, decreasing peak currents were observed for thicker coatings with the same molar ratio (1.3) indicating that besides the electrostatic exclusion, diffusion restrictions must be operative. For the PLL–PSS electrode with 17.8 µg mm^{-2} the effect of the higher content of the anion exchanger PLL is now clearly observed: although being thicker than the PLL–PSS electrode with 11.3 µg mm^{-2}, the relatively small decrease in the oxidation peak currents for UA and for AA means that now the anion exchanger PLL has an effect by accu-

Table 4
Statistical characterization of the results obtained with PLL–PSS-coated thin mercury electrodes of different total mass loadings (11.3 and 22.8 µg mm^{-2}) and same PLL/PSS loading molar ratio (1.3)

PLL–PSS loading (µg mm^{-2})	Median (µA)	Range (µA)	Repeatibility[a] based on σ_w^2	Reproducibility[a] based on σ_b^2
11.3	0.521	0.084	0.0003	0.0025
22.8	0.346	0.079	0.0004	0.0038

[a] σ_w^2 estimates the repeatibility of the measurements and σ_b^2 represents the reproducibility between samples [29].

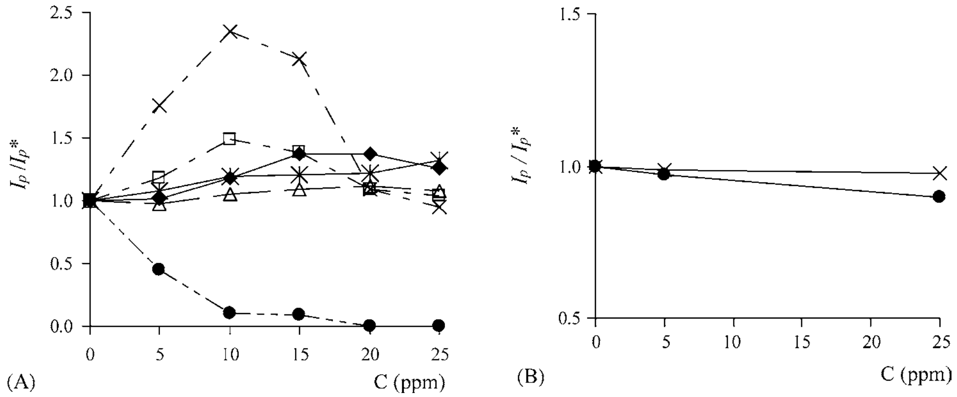
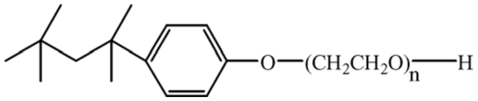
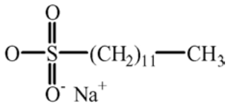
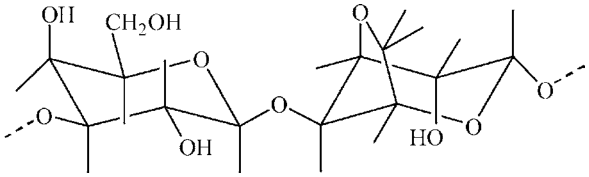
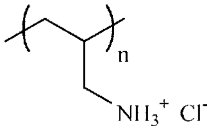
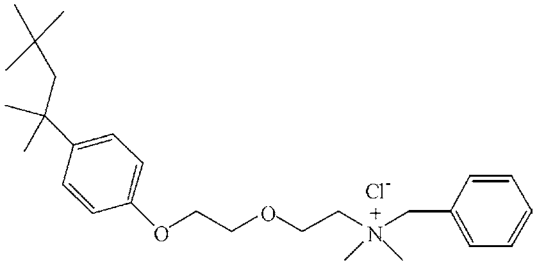


Fig. 4. Effect of surfactants on the SW stripping peak current of lead, I_p, expressed as a normalized current with respect to the value obtained without surfactant, I_p^*, at the PLL–PSS/TMFE with a molar ratio 1.3 and different thicknesses: (A) 11.3 μg mm^{-2} and (B) 22.8 μg mm^{-2}. Triton X-100: cross; SDS: squares; agar: triangles; PAAHC: star; BSA: diamonds and HYA: circles. Experimental conditions as in Fig. 1.

mulating ascorbate and urate anions. Hence, these anions were not totally rejected by any of the PLL–PSS coatings in opposition to published results for enzyme incorporating PLL–PSS membranes [18,19] to which a molecular cut-off mass of 110 was assigned. For cationic dopamine some incorporation was observed using the thinner PLL–PSS film (PLL/PSS molar ratio 1.3). However, for the other PLL–PSS electrodes the dopamine peak current decreased. The larger decrease was observed for the PLL–PSS film with 17.8 μg mm^{-2} (molar ratio 2.7) highlighting the effect of the relative increase in the number of positive-charged groups due to PLL. For the thicker coating, a high exclusion is still observed in spite of the lower molar ratio (which is again 1.3) and this fact surely is related to mass transport restrictions.

It may be concluded that the PLL–PSS coatings are charge selective and permselective to molecular size in some extent. The

Table 6
Chemical structures of the surface-active compounds used

Tensioactive	Structure
Triton X-100 M_W 646 non-ionic	
SDS M_W 288 anionic	
Agar M_W 5000–30,000 non-ionic	
PAAHC M_W 15,000 cationic	
HYA M_W 448 cationic	
BSA M_W 68,000 anionic	Globular protein with 585 residues in a single chain

incorporation of an organic cation-like dopamine is more dependent on the actual thickness and molar ratio PLL/PSS compared with the studied case of lead cation (c.f. Section 3.2).

3.3. Anti-fouling properties of the PLL–PSS-coated TMFE

The anti-fouling ability of the PLL–PSS/TMFE against several surfactants was evaluated regarding the SWASV analysis of lead. Triton X-100 and SDS are detergents, agar serve as a model for polysaccharides present in natural waters, PAAHC is a high molecular cationic polymer whereas HYA presents a relatively low M_W and BSA is a globular protein of high M_W. Some characteristics of these surfactants are displayed in Table 6. Fig. 4A presents the variations of the SWASV peak height of lead in the presence of increasing amounts of different surfactants at the PLL–PSS/TMFE with a mass loading of 11.3 μg mm^{-2} (molar ratio 1.3).

The cationic surfactant HYA was not excluded in spite of having a molecular weight higher than that of dopamine or ferricyanide. In fact, HYA produced the total fouling of the PLL–PSS-coated electrode for concentrations >20 ppm. A similar behaviour has been observed at PSS-coated TMFE [7], and an explanation based on the conformational features of HYA was advanced. Conversely, the high molecular weight cationic PAAHC did not have a significant effect on $I_{p(Pb)}$. For the non-ionic detergent Triton X-100 there was a large increase of the ASV peak current of lead with the addition of that species. Only for 20 ppm, $I_{p(Pb)}$ dropped to a value close to the observed without Triton X-100. Similar enhancements of the lead SWASV signal at Nafion and PSS-modified mercury film electrodes were already reported [9,10]. For BSA and SDS, there were slight enhancements of $I_{p(Pb)}$; these effects might be due to some accumulation of lead complexes with those anionic species at the solution/coating interface.

On the other hand, for a thicker PLL–PSS coating (mass loading: 22.8 μg mm^{-2}) the performance of the PLL–PSS-coated TMFE was not limited by the presence of the HYA (c.f. Fig. 4B); the high film thickness certainly was responsible for the diffusion restriction to the transport of this cationic surfactant within the mixed polyelectrolyte layer. Additionally, for Triton X-100 the enhancements in $I_{p(Pb)}$ were not observed. These results demonstrate the suitable ability of the PLL–PSS/TMFE against several surfactants, especially for thicker coatings (22.8 μg mm^{-2}).

A medium term stability test was performed for the thick PLL–PSS-coated TMFE (22.8 μg mm^{-2}): the glassy carbon electrode was coated on the first day of the week and the mercury film was ex situ plated and electrochemically removed at the end of the SWASV scan. The PLL–PSS coating was maintained and the modified electrode was kept overnight in water. In each subsequent day, a new mercury film was ex situ plated and the SWASV measurements were repeated. The test was made for lead (80.0 nM in 0.5 M NaCl solution). On the seventh day the peak intensity of lead decreased ca. 6.0%, comparing with the results obtained on the first day. The R.S.D. values were maintained in the range 2.5–3.7% all along the week. Hence, the present PLL–PSS coatings have a reasonable mechanical resistance and chemical stability, as well as good anti-fouling ability.

4. Determination of heavy metals in estuarine waters samples

The applicability of the PLL–PSS-coated TMFE (11.3 μg mm^{-2}; PLL/PSS ratio 1.3) was tested in the SWASV

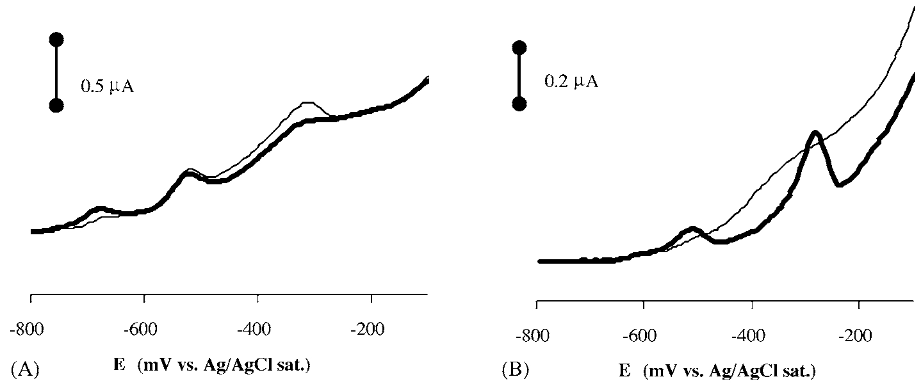


Fig. 5. SW anodic stripping voltammograms of two estuarine waters (samples A and B) at the uncoated TMFE (thin line) and at the PLL–PSS/TMFE (thick line). PLL–PSS loading: 11.3 μg mm^{-2} (PLL/PSS molar ratio 1.3); SW parameters: $a = 25$ mV, $f = 50$ Hz and deposition time 180 s at −0.8 V.

Table 7
SWASV determinations in estuarine water samples at the uncoated TMFE and at the PLL–PSS-coated TMFE

Electrode	Sample A (nM)			Sample B (nM)	
	Cu	Pb	Cd	Cu	Pb
TMFE	2.69 ± 0.09	3.74 ± 0.08	nd	nd	nd
PLL–PSS/TMFE	2.65 ± 0.03	3.84 ± 0.04	1.53 ± 0.05	2.65 ± 0.07	1.53 ± 0.05

See text and Fig. 5 for experimental conditions, nd: not detected.

determination of trace metals in estuarine water samples containing moderate levels of dissolved organic matter (DOC values of 4 ppm for sample A and 10 ppm for sample B; c.f. Section 2.4). The results, displayed in Fig. 5 and Table 7 show that the PLL–PSS-coated TMFE can be successfully used to quantify trace metals in samples with relatively high DOC (10 ppm), where the bare mercury film electrode fails. Additionally, for sample A (DOC 4 ppm), the Cu and Pb metal concentrations determined by both methodologies were statistically identical, confirming the accuracy of the PLL–PSS-coated TMFE for this type of application. Though, in this complex matrix the sensitivity to lead at the PLL–PSS/TMFE was significantly higher than that at the bare TMFE: the slope was $9.3 \pm 0.3\ \text{A mol}^{-1}\ \text{dm}^3$ ($r = 0.998, N = 4, p < 0.05$) and $5.8 \pm 0.2\ \text{A mol}^{-1}\ \text{dm}^3$ ($r = 0.997$, $N = 4$, $p < 0.05$) for the PLL–PSS/TMFE and for the TMFE, respectively. Note that it was the sensitivity at the classic TMFE that decreased to approximately half the value obtained with a simple NaCl solution, highlighting the vulnerability of the TMFE to matrix effects (c.f. results in Section 3.1). These results show the potential of the ex situ PLL–PSS/TMFE for the direct determination of trace levels of heavy metal ions in samples containing surface-active compounds.

5. Conclusions

The utilization of mixed polycation/polyanion (PLL–PSS) coatings onto glassy carbon electrodes allows the production of stable thin mercury films for ASV determinations, ensuring a suitable sensitivity and interesting anti-fouling features. These coatings present a heterogeneous three-dimensionally structured morphology, with random distributed holes, some of micrometer dimensions. However, the TMFE coated with PLL–PSS with a loading of $11.3\ \mu\text{g}\ \text{mm}^{-2}$ (PLL/PSS molar ratio 1.3) showed good reproducibility and repeatibility, meaning that the preparation procedure leads to stable and reproducible coatings with good voltammetric performance. For that mass loading the incorporation/accumulation of lead within the mixed polyion layer was somewhat restricted (certainly due to the presence of the positive NH_3^+ groups from PLL), although the SWASV peak currents were not significantly lower than those for a bare TMFE. An eventual alteration in the ionic exchange mechanism, involving the additional accumulation of negatively charged lead–chloro complexes was advanced as a possible explanation. Compared with the case of lead(II), the incorporation of the organic cation dopamine depended markedly on the layer thickness meaning that the mixed coatings are permselective to molecular size. In fact, the PLL–PSS coatings presented interesting anti-fouling properties especially those with a higher thickness. The applicability of the PLL–PSS/TMFE in the direct SWASV determination of trace heavy metals was demonstrated by analysing cadmium, lead and copper at the low nanomolar level, in estuarine waters containing moderate amounts of dissolved organic matter, where the uncoated TMFE failed due to fouling.

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