An Electroactive Versatile Matrix for the Construction of Sensors

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Abstract
The strong interaction between a positively charged electroactive polyelectrolyte (polyallylamine derivatized with an osmium complex) with an anionic surfactant (sodium dodecyl sulfate) produces a composite material soluble in organic solvents. The organic solution of this material can be applied on electrode surfaces (gold, graphite), generating a very stable modified electrode. The modified electrode presents a very efficient charge propagation through the film and it can be used in further modifications. As an example glucose oxidase is adsorbed; in presence of glucose, this electrode presents catalytic currents with similar values compared to others built by self-assembling operations.

Keywords: Redox polyelectrolyte, Surfactant, Modified electrode, Mediator, Glucose oxidase

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1 Introduction

Redox polyelectrolytes have been immobilized on electrode surfaces in different forms; for example as a gel by using a crosslinking the polyelectrolyte with an appropriate bifunctional agent [1–3], or by self-assembling, modifying the electrode with a negatively (or positively) charged species, and then layers of polyelectrolytes with opposite charge are consecutively added [4–6]. In the first case, the reproducibility in the modification is not easily achieved; while in the second one, the production of several layers is a time consuming process.

In this work we present the construction of electrochemical sensor containing a polyelectrolyte (polyallylamine) modified with a polypyridyl osmium complex (PAOs) and a surfactant (sodium dodecyl sulfate, SDS). The osmium modified polyelectrolyte in presence of SDS precipitates, generating a product that is soluble in DMF. This organic solution is applied either on graphite or gold surfaces. The evaporation of these solutions generates an extremely stable and resistant film showing a quasi-reversible electrochemical behavior. Onto this film, glucose oxidase was adsorbed and the osmium complex efficiently acts as a redox mediator between the electrode surface and the enzyme. Simultaneously, the amperometric response was modeled using finite-element software to obtain the cyclic voltammetric responses and the concentration profiles within the film.

2 Experimental

2.1 Reagents and Materials

Sodium dodecyl sulfate (SDS) was from Kodak. Glucose oxidase was from Biozyme. All other reagents were analytical grade. The synthesis of polyallylamine containing a pyridine based osmium complex (PAOs) is described elsewhere [7]; the stoichiometry ratio between osmium complex and allylamine monomer is: 1:15. Graphite screen printing electrodes were constructed as previously described [8]. Gold electrodes were constructed evaporating gold on silicon substrate previously covered with titanium, or gold flags of 1 cm^2 area were used.

2.2 Polyelectrolyte-Surfactant Composite Material

200 μL of 0.18 mM PAOs were mixed with 400 μL of 1 % SDS aqueous solution; immediately, a precipitate was formed that can be easily separated from the remaining solution by centrifugation. The precipitate (PAOs + DS) was analyzed by elementary microanalysis showing a ratio ca. 1:1 between allylamine monomer and dodecyl sulfate (N:S ratio).

2.3 Modified Electrodes

500 μL of DMF were added to 4 mg of the composite material, the solid was completely dissolved after mixing for a few minutes. 2 μL of this solution was applied on a 0.38 cm^2 graphite electrode and left for 1 hour to evaporate. GOx adsorption was carried from a 2 μM GOx solution in a 50 mM phosphate buffer (pH 7, 0.2 M KNO_3) for 1 hour and then the electrode was rinsed with milliQ water.

Electrochemical experiments were carried out using a purpose-built potentiostat (TEQ-02). The system consisted of a working electrode, a platinum mesh counter electrode, and an Ag|AgCl reference electrode. Cyclic voltammetries were carried in a 50 mM phosphate buffer (pH 7, 0.2 M KNO_3) otherwise is stated. Glucose was added from a 0.55 M stock solution in the same buffer.

Film thickness was determined by ellipsometry on gold electrodes using a A Sentech SE 400 rotating-analyzer automatic ellipsometer following the method describe in reference 9.

2.4 Numerical Model

Finite-element software (Comsol Multiphysics 3.4) was used to simulate the cyclic voltammetry experiments and the concentration profiles. The software was executed in a PC Intel Corel Duo, 4 GB RAM, Windows XP operative system, resolving the model with 10600 degrees of freedom and ca. 3.000 nodes, the mesh was refined close the electrode.

The initial conditions are equal to the experimental ones: osmium complex concentration is obtained from the charge and the thickness of the film. GOx concentration was determined by using the experimental results in saturating conditions.

The experimental system was modeled solving Poisson and Fick equations using the finite-element software previously described [10]. The space dimension was set to 2D, the boundary conditions corresponds to linear diffusion to a infinite plane electrode and an electroactive species contained in a 1μm layer; the generated current is calculated by the Butler-Volmer equation:

$$\frac{j}{F} = k_0\left[C_O \exp(\frac{-\alpha F}{RT}(E-E_{eq})) - C_R \exp(\frac{(1-\alpha)F}{RT}(E-E_{eq}))\right]$$

where C_R and C_O are the concentration on the working electrode surface of the osmium reduced and the oxidized species, respectively; k_0 is the standard electron transfer rate constant; $\eta = E - E_{eq}$ is the applied overpotential and E_{eq} is the equilibrium electrode potential. A value of $k_0 = 1\times10^{-4}$ cm s^{-1} was employed. This value arises from the adjustment of the model to experimental cyclic voltammetries without catalysis. Using this value, the model is able to reproduce peak high and peak separation of cyclic voltammetries at different scan rates.

The model describes the general situation of an immobilized redox mediator in a layer at the electrode surface. We related the experimental values for the deposited osmium equal to 3.3×10^{-10} mol with the osmium connected molar concentration equal to 8 mM for a 0.38 cm^2 electrode area and 1 μm film thickness. The rate of the electron hopping process was set to equivalent diffusion coefficient of 1.2×10^{-9} cm^2 s^{-1} [11] and the scan rate was according to experimental conditions.

The model introduces the following catalytical system:
In the film:

$$2Os(III) + GOx(red) \xrightarrow{k} 2Os(II) + GOx(ox)$$

$$Gox(ox) + Glu \xrightarrow{K_{MS}} GOx(ox)Glu \xrightarrow{k_{cat}} GOx(red) + Gluconate$$

At the electrode:

$$Os(III) + e \rightarrow Os(II)$$

where the rate kinetic constants referred to enzyme regeneration and catalysis are $k = 1.5\times10^4$ M^{-1} s^{-1}, $k_{cat} = 700\ s^{-1}$, diffusion coefficients $D_{GOx} = 0$, $D_{Glu} = 6.72\times10^{-6}$ cm^2 s^{-1} were used from [12] and the Michaelis–Menten constant in this environment and wired enzyme concentration arise from the adjustment of the model to the experimental cyclic voltammetries.

3 Results and Discussion

The association of ionic surfactants with oppositely charged polyelectrolytes is a subject of considerable interest due to its importance in biological systems as well as in industrial applications. The main result obtained from numerous studies of systems involving synthetic polyelectrolytes is that the interaction in the pair polyelectrolyte-surfactant is very strong [13].These features encourage us to explore the interactions between PAOs, a electroactive polyelectrolyte containing an osmium complex, with a surfactant with opposite charge to generate a stable modified electrode.

The mixture of SDS and PAOs solutions immediately produced a precipitate; elemental microanalysis was carried out on the obtained solid yielding a stoichiometry ratio between allylamine monomer and dodecyl sulfate ca. 1:1.

The precipitate was easily dissolved in DMF and the solution was applied on any surface and once dried, forming a very stable coating. As an example, 2 μL of this solution was applied onto a graphite electrode and left dry for 1 hour. Then, the electrode is rinsed and immersed in a 50 mM phosphate buffer (pH 7.0). Figure 1 shows cyclic voltammetries at different scan rates applied on the new modified electrode; it can be observed the shape of the voltammogram changes from one of an adsorbed species to one corresponding to a diffusion limited species as the scan rate is increased.

This behavior can be explained by the thickness of the film and the charge propagation rate observed for this new composite material. This is an important difference with self-assembled systems where the system behaves as an adsorbed species at all scan rates. It is interesting to analyze if this behavior is only due to thickness of the film or by the electron hopping efficiency of this new material.

A numerical model was developed using a finite-element software as previously described [10] in which the mediator is assumed to be confined within the film; charge propagation within the film occurs by electron hopping self-exchange between reduced and oxidized forms of the mediator described by a diffusion coefficient D_{eh}. The electrochemistry of thick drop cast films electrodes similar to the one presented in this work shows a D_{eh} in the order of 10^{-11} cm^2 s^{-1} [14], while for thin films its value is in the order of 10^{-9} cm^2 s^{-1} [11,15].Taking into account this two situations, we found that using the diffusion coefficient obtained by Calvo et al.[11] is possible to reproduce with a great accuracy the experimental results (Figure 2), showing an efficient electron hopping behavior and the fact that the observed behavior is a consequence of the thickness of the film. This value was also used for Flexer et al. [12] to simulate a self-assembled system using a similar electroactive polyelectrolyte.

The numerical model also allows describing the concentration profile at different potentials. Figure 3 shows the concentration profile of Os(II) at two different scan rates (10 and 100 mV s^{-1}) when the electrode is at $\eta = 0$ and 0.3 V. When the electrode is scanned at 10 mV s^{-1} the concentration profile is changing as it is expected for a thin film, consuming the whole reduced species at high overpotentials; when the potential is scanned at 100 mV s^{-1} a concentration profile corresponding to a diffusion limited process is observed.

The solution can be applied on gold electrodes, obtaining similar results regarding stability and electrochemical response. Gold electrodes modified with the same amount of the solution per surface unit shows similar voltammograms to those obtained with graphite. This is an interesting feature since the method avoids the use of thiol compounds to generate a charged surface. Another characteristics of the film is its stability even immersed for several days in a buffer solution, the cyclic voltammetry obtained after 14 days immersed in 10mM HEPES buffer (pH 7.05, 0.2 M KNO_3) is practically equal to the obtained the first day.

This electroactive film was exposed to a GOx solution to study its ability to self assembled redox enzymes and its ability to carry out an electron transfer process between the graphite electrode and the enzyme. Figure 4 shows the response of the electrode to different glucose concentrations. Taking into account the electrode surface, a current density close to 100 μA cm^{-2} is observed at high glucose concentrations.

This value can be compared with results previously obtained with this polyelectrolyte using a self assembling technique. In a previous work [5], our group has assembling this polyelectrolyte on a previously thiol modified gold electrodes by using two self assembling sequences as follow: A) $(PAOs/GOx)_n$; B) $(PAOs/SDS/PAOs/GOx)_n$. For the first sequence a catalytic current of 12 μA cm^{-2} and for the second a 90 μA cm^{-2}. The results show the practicality of this new material obtaining current values higher than those obtained in the example A and similar than those obtained in the example B, being in both case $n = 5$.

Another feature of the system is the dynamic range for glucose; as it can be seen in Figure 4, the catalytic current still increases at a concentration of 100 mM glucose. This behavior was analyzed by the proposed numerical model

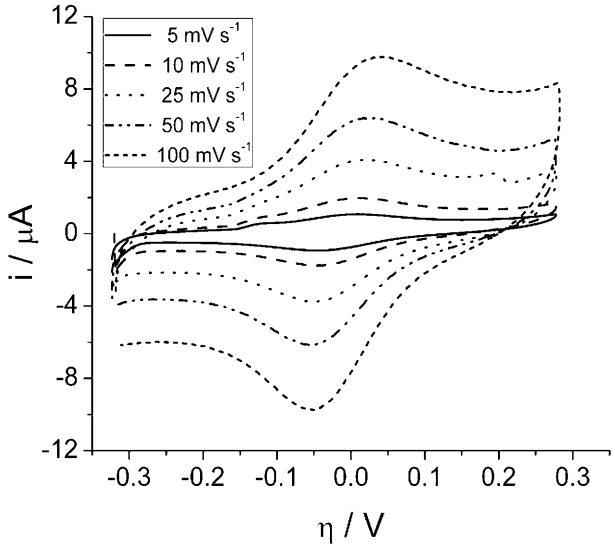

Fig. 1. Cyclic voltammetries at different scan rates for a screen printing graphite electrode.

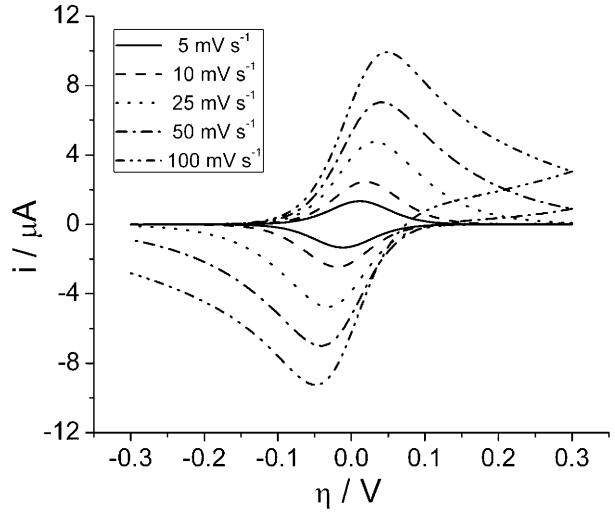

Fig. 2. Digital simulation of the cyclic voltammetries presented in Fig. 1 taking into account the electron diffusion coefficient (D_{eh}) used by Flexer et al. [9] for a polyelectrolyte of similar characteristics.

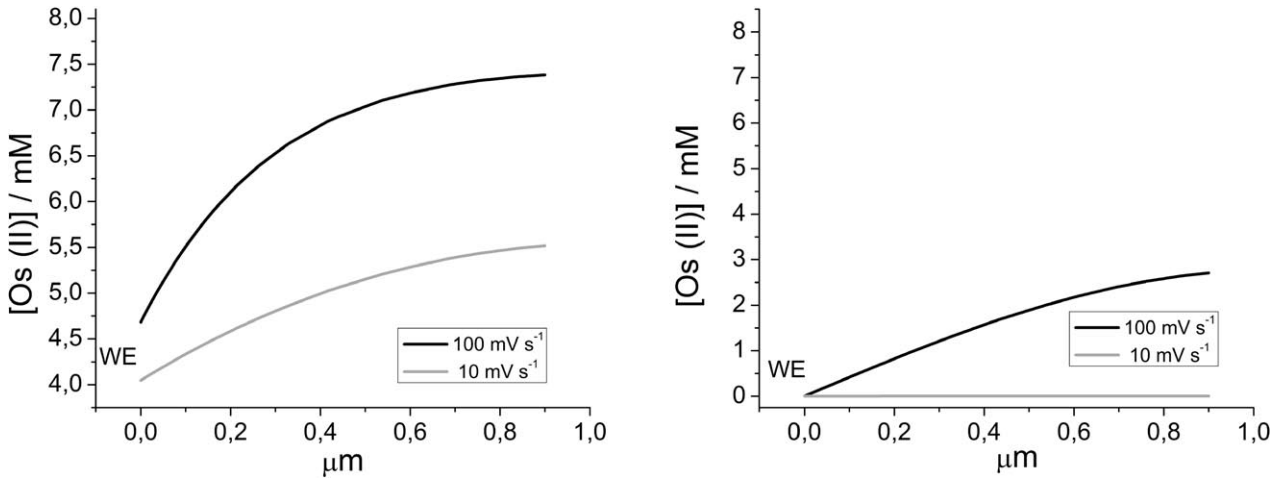

Fig. 3. Digital simulation of the Os(II) concentration throughout the film. Left: at $\eta = 0$ V. Right: at $\eta = 0.3$ V.

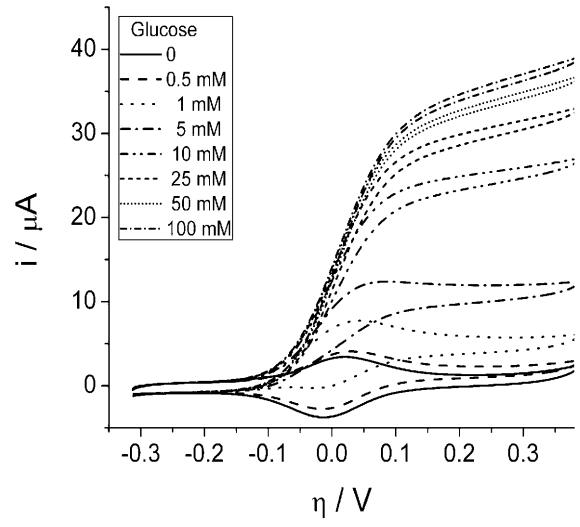

Fig. 4. Response to glucose addition for a graphite/PAOs + DS/GOx electrode immersed in a 50 mM phosphate buffer (pH 7.0, 0.2 M KNO_3) Scan rate: 10 mV s^{-1}.

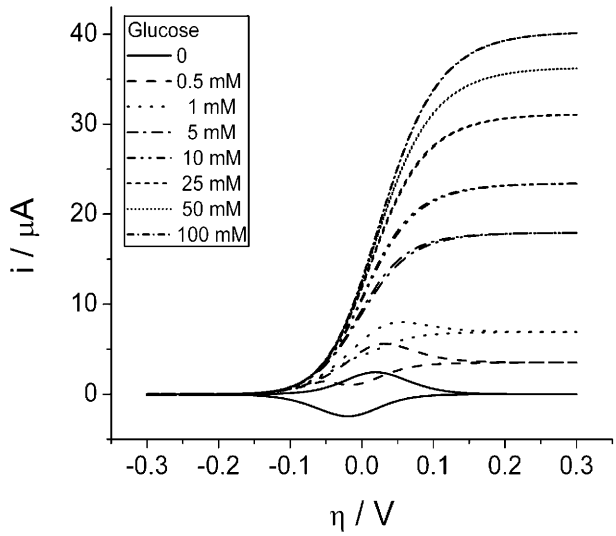

Fig. 5. Digital simulation of the response to the addition of glucose.

using rate coefficient values obtained by Flexer et al. [12] for a self-assembled system. In order to adjust the simulated values with the experimental ones K_{MS} was changed (Figure 5). The value used was 200 mM, a high value compare to those reported in the literature ranging from 50 to 120 mM [7,16]; however, it makes sense with the experimental results since in many works presented in the literature the enzyme is saturated at concentrations lower than 50 mM.

Even though, the electroactive film is able to maintain its electrochemical response immersed in buffer solution for 14 days, the catalytic current decreases around 50% from its original value, suggesting that the lost of activity is mainly due to the typical denaturalizing process that the enzyme suffers when it is exposed to an aqueous solution.

Finally, the thickness of the film can be controlled by applying the solution by spin coating, in this way thinner films are obtained. Figure 6 shows the behavior for this type of electrode in absence and presence of glucose, showing lower currents; however a more reversible behavior is observed and the enzyme is saturated at lower concentrations.

4 Conclusions

The electrolyte-surfactant modified electrodes, compared to others built by self-assembled operations, present the following advantages, it can be applied on different conducting surfaces (graphite, gold) without the need of an anchor molecule (thiols, diazonium salts), it presents excellent electron transfer properties throughout thick films and with other molecules, it is highly stable in time and it can be further modified.

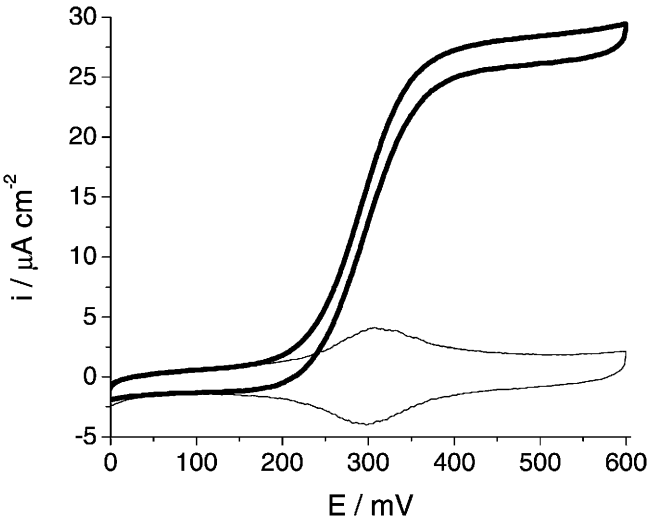

Fig. 6. Voltammograms for a Au/PAOs + DS/GOx where the first layer is applied by spin coating and the enzyme is assembled by immersing the electrode in a GOx solution. Thin line: voltammogram in absence of glucose, thick line: in presence of 50 mM glucose.

Depending on the scan rate, the electrochemical response of the system for a thick layer (1 μm) changes its behavior from an adsorbed species to a diffusion limited one. Regarding the response to glucose, the thickness of the film also produces an important change in the dynamic range of the catalytic current, increasing the concentration at which the enzyme is saturated.

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References

[1] B. A. Gregg, A. Heller, *Anal. Chem.* **1990**, *62*, 258.
[2] E. Cortón, F. Battaglini, *J. Electroanal. Chem.* **2001**, *511*, 1.
[3] J. J. Mitala Jr. , A. C. Michael, *Anal. Chem. Acta* **2006**, *556*, 326.
[4] E. J. Calvo, F. Battaglini, C. Danilowicz, A. Wolosiuk, M. Otero, *Faraday Disc.* **2000**, *116*, 47
[5] M. L. Cortez, A. L. Cukierman, F. Battaglini, *Electrochem. Commun.* **2009**, *11*, 990.
[6] X. Liu, F. Wang, S. Han, L. Shi, G. Xu, *Electroanalysis* **2010**, *22*, 963.
[7] C. Danilowicz, E. Cortón, F. Battaglini, *J. Electroanal. Chem.* **1998**, *445*, 89.
[8] G. Priano, G. González, M. Gunther, F. Battaglini, *Electroanalysis* **2008**, *20*, 91.
[9] V. Flexer, E. S. Forzani, E. J. Calvo, S. J. Ludueña, L. I. Pietrasanta, *Anal. Chem.* **2006**, *78*, 399.
[10] A. S. Peinetti, G. González, F. Battaglini, *Electroanalysis* **2010**, *22*, 1329.
[11] E. J. Calvo; C. Danilowicz, A. Wolosiuk, *J. Am. Chem. Soc.* **2002**, *124*, 2452.
[12] V. Flexer, E. J. Calvo, P. N. Bartlett, *J. Electroanal. Chem.* **2010**, *646*, 24.
[13] N. Vlachy, J. Dolenc, B. Jerman, K. Kogej, *J. Phys. Chem. B* **2006**, *110*, 9061.
[14] R. J. Forster; J. G. Vos, *Electrochim. Acta* **1992**, *37*, 159
[15] A. P. O'Mullane, J. V. Macpherson, P. R. Unwin, J. Cervera-Montesinos, J. A. Manzanares, F. Frehill, J. G. Vos, *J. Phys. Chem. B* **2004**, *108*, 7219.
[16] Q. H. Gibson, B. E. P. Swoboda, V. Massey, *J. Biol. Chem.* **1964**, *239*, 3927.

