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## Surfactant presence in a multilayer polyelectrolyte–enzyme system improves its catalytic response

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## ABSTRACT

Self-assembling of poly(allylamine) containing an osmium polypyridil complex (PAOs) alternatively with sodium dodecyl sulfate (SDS) and glucose oxidase (GOx) generates a ternary multilayer system, (PAOs/SDS/PAOs/GOx)<sub>n</sub>. The introduction of this anionic surfactant allows a sensitive increase of the polyelectrolyte and the enzyme uptake at pH 7.0, enhancing its catalytic behavior in presence of glucose more than 5 times, compared to the system (PAOs/GOx)<sub>n</sub> constructed at the same pH. The balance between ionic and hydrophobic interactions in the construction of this system is discussed.

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## 1. Introduction

Polyelectrolyte-surfactant [1] and polycation–polyanion self-assembled systems [2,3] have been extensively studied in past years and are known to exhibit a unique combination of physical properties due to their ionically cross-linked nature. Generally, this type of complexes are not soluble in water and they can be manipulated in two different ways, by subsequent adsorption onto a surface from aqueous solutions (layer-by-layer method), or by solubilizing the precipitated complex in an organic solvent like chloroform. Both methods are very simple; however, the first one is most convenient if work is planned with biomolecules.

Particularly, the presence of a redox-active group in the polyelectrolyte [4,5] or in the surfactant [6,7] opens opportunities in the area of electrocatalysis and analytical chemistry. Calvo and coworkers have produced an important body of work devoted to the study of the electron transfer process between a redox-active weak polyelectrolyte, based on an osmium complex bound to poly(allylamine) (PAOs), and redox enzymes in self-assembled systems [8–11]. These systems show dependence with the pH in the electron transfer process, with a pronounced increase in the current assembling the polyelectrolyte at pH 8 or higher [11]. This effect was explained considering that most of the weak

polyelectrolyte charge is lost at pH 8, and that the system adopts a conformational arrangement of loops and tails, allowing a greater uptake of PAOs and GOx. At lower pH, a severe decrease in current is observed. This result correlates with an increase in charge density in the PAOs, since its primary amino groups are increasingly protonated; therefore, the polyelectrolyte is extended, lying flat on the surface. On the other hand, self-assembled systems can be constructed from a polyelectrolyte and a counter-ion surfactant based on the strong electrostatic interaction of the species, generating a neutral surface with a hydrophobic character [6].

Taking the aforementioned findings into account, the layer by layer construction of a PAOs/GOx system at pH 7.0 was carried out introducing a new step by dipping the surface in an anion surfactant solution. The ternary system was assembled in the following sequence PAOs/surfactant/PAOs/GOx to explore if this configuration can help to improve the interaction between the osmium complex and GOx. Our results show that the introduction of the sodium dodecyl sulfate as surfactant allows the uptake of a higher amount of the enzyme and the polyelectrolyte, improving the catalytic current in presence of glucose compared to previous self-assembled systems.

## 2. Experimental

Sodium 3-mercaptopropyl-sulfonate (MPS), sodium dodecyl sulfate, and octadecyl sulfate were purchased from Aldrich.

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Glucose oxidase was from Biozyme. All other reagents were analytical grade. The synthesis of poly(allylamine) containing a pyridine based osmium complex (PAOs) is described elsewhere [4].

Self-assembly was carried out by immersing the gold electrode subsequently in each corresponding solution. In each step the following conditions were used:

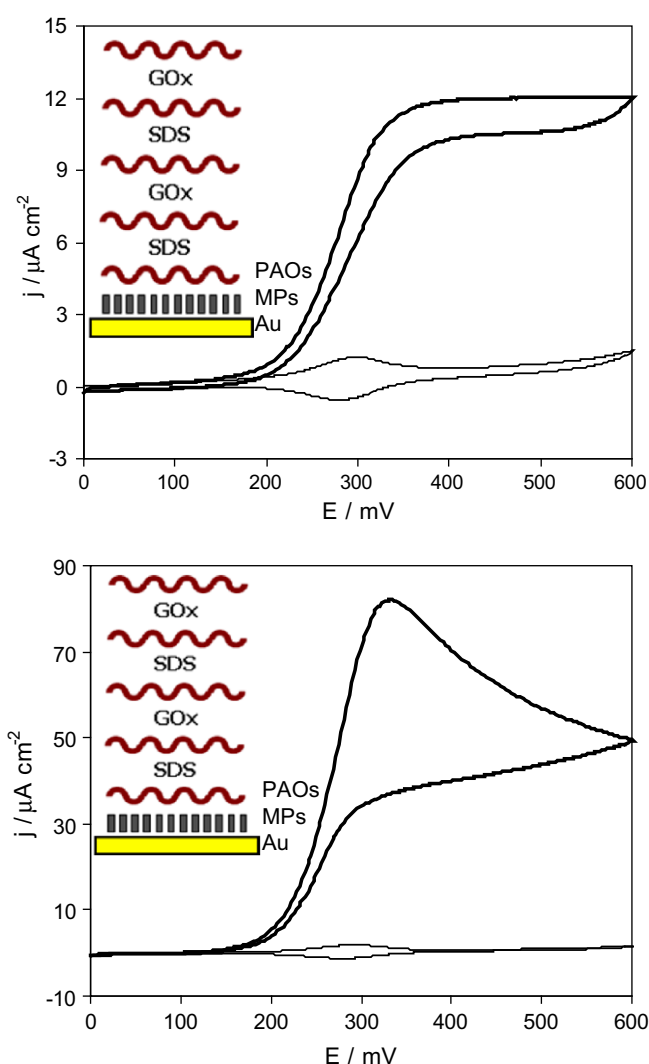
- **Thiol adsorption:** Clean gold electrodes were immersed in a 20 mM 3-mercapto-1-propanesulfonate solution in 10 mM H<sub>2</sub>SO<sub>4</sub> for 60 min.
- **PAOs adsorption:** The modified electrodes were immersed in a 50 mM Tris solution (pH 7.0) containing 0.4% w/v PAOs for 10 min.
- **Sodium dodecylsulfate adsorption:** The electrode was immersed in a 1% SDS solution in water for 10 min.
- **GOx adsorption:** The electrode was immersed in a 1  $\mu$ M GOx solution in 50 mM HEPES buffer (pH 7.0) for 10 min.

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. Quartz crystal microbalance experiments were carried out in a 5Mz QCM200 (Stanford Research Systems). The variation in the resistive parameter of the Butterworth–Van Dyke electrical equivalent circuit that represents the composite quartz crystal resonator loaded with the film,  $\Delta R$ , in all cases is negligible compared to the inductive quartz impedance component,  $\Delta X_L$ . Therefore, the films behave as acoustically thin in the gravimetric regime and the mass uptake was calculated in each case with the Sauerbrey equation [11,12]. Advancing contact angle of water experiments were carried out in a KSV Cam200 optical contact angle meter.

### 3. Results

In order to better understand the changes produced by this new assembling strategy, (PAOs/SDS/PAOs/GOx)<sub>n</sub>, all the experiments were always compared to (PAOs/GOx)<sub>n</sub> self-assembled systems contemporarily constructed. In this way, possible differences due to GOx activity variability were avoided. In Fig. 1, in the insets, schematic representations of the two self-assembled systems are presented. In both cases, the uptake of PAOs and GOx was from solutions at pH 7.0, while SDS was adsorbed from a solution at pH 6.5.

During the self-assembled process, the electroactive behavior of PAOs and the electrocatalytic response in presence of glucose was followed by cyclic voltammetry, as the number of layer increases, the current increases in a similar mode that in previously reported self-assembled systems [11]; however, a higher catalytic current for the SDS containing system was observed since the second assembled GOx layer. Fig. 1 shows the typical electrochemical response observed for both systems on modified gold quartz crystal. The upper figure corresponds to (PAOs/GOx)<sub>n</sub> and the lower one to (PAOs/SDS/PAOs/GOx)<sub>n</sub>, in both cases  $n = 5$ . The voltammogram in absence of glucose (thin line) is practically the same in shape and in current for both systems. However, these features change in presence of 50 mM glucose (bold line); the current magnitudes are very different, and for the system with SDS the maximum catalytic current is more than five times higher than the system without SDS. Also, the shapes of the voltammograms are different. Whereas the system without SDS presents the typical voltammogram for an electrochemical–catalytic chemical (EC') behavior, the one for (PAOs/SDS/PAOs/GOx)<sub>n</sub> shows a more complex feature, that can be attributed to the consumption of the substrate [13]. The response reproducibility of the self-assembled system is very



**Fig. 1.** Cyclic voltammeteries corresponding to: (PAOs/GOx)<sub>n</sub> (top) and (PAOs/SDS/PAOs/GOx)<sub>n</sub> (bottom) systems. Sweep rate: 10 mV s<sup>-1</sup>. Thin line corresponds to the experiment without glucose. Bold line corresponds to the experiment in presence of 50 mM glucose. Inset: a schematic representation of the self-assembled system.

good as far as care is taken regarding the quality of the reagents, specially using a GOx with the same activity and a well preserved MPS, since this chemical decomposes with time. In these conditions, three independent experiments carried out in different days show a standard deviation of 16% for a 5 GOx layer system. The system is very stable because the electrode response is tested after the addition of each layer.

As the construction of these modified electrodes was followed by the quartz crystal microbalance, it is possible to obtain an insight into the compositional differences of both systems. Fig. 2 shows the mass changes after the exposition of the crystal to the different species in chronological order. The gray bars correspond to the (PAOs/SDS/PAOs/GOx)<sub>n</sub> system, while the black bars correspond to the system (PAOs/GOx)<sub>n</sub>. At first glance, it can be observed that the mass uptake for the system containing SDS is greater; after the formation of 5 GOx layers, the incorporation of GOx and PAOs are ca. 3.3 and 2.7 times greater. Another feature to take into account is the fact that, as the number of layers growth, the uptake of both species (PAOs and GOx) increases, suggesting a cooperative mechanism in the adsorption process. Also, it is interesting to point out the differences in the PAOs mass incorporated after GOx or SDS exposition. After GOx, the adsorption of PAOs is

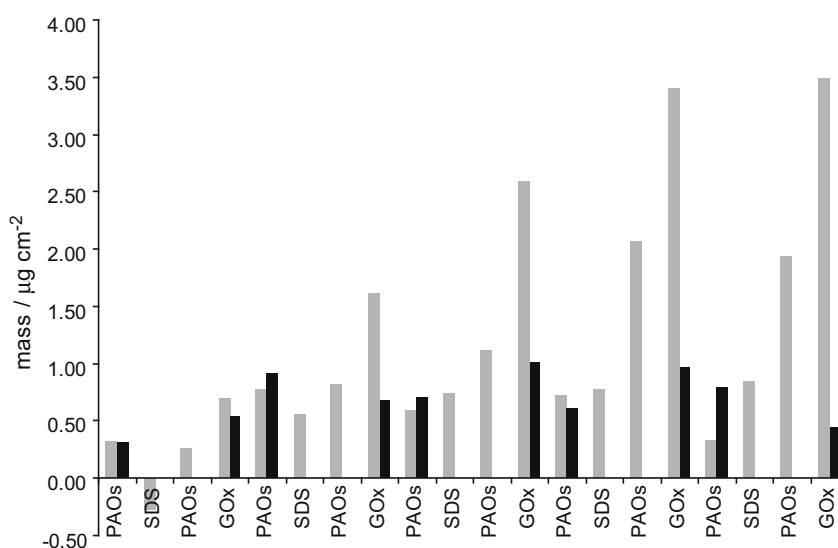


Fig. 2. Mass uptake at pH 7.0 after electrode immersion in the solution depicted in the abscissa.

similar to the system without SDS, while an important increase is observed after SDS.

To better understand the role of charge interactions and hydrophobic forces, the two systems were also analyzed by contact angle measurements after the addition of the third GOx layer. In the case of (PAOs/GOx)<sub>n</sub>, after the uptake of PAOs, the contact angle is 59°, while for GOx is 41°. In the system (PAOs/SDS/PAOs/GOx)<sub>n</sub>, after the uptake of GOx, the contact angle is 45°, practically the same as before. However, the contact angle after the uptake of PAOs is 93°, 114° after SDS uptake, and then 104° for the new layer of PAOs; for the following layer of GOx, the contact angle decreases to 67°.

If the assembly of PAOs with SDS is intended without GOx, once the first layer of PAOs is formed, the subsequent exposition to SDS reduces the current observed due to the osmium complex; in this case, SDS acts as a rinsing solution. This effect can be also observed in the QCM experiment after the formation of the first layer of PAOs (see Fig. 2). The exposition to SDS produces a decrease in the mass of the system, that is recovered after a new exposition to PAOs. Further, after the uptake of GOx and a new layer of PAOs, also SDS is incorporated to the system and helps to increase the GOx and PAOs incorporation. Another way to assemble the system is avoiding a layer of PAOs between SDS and GOx. When this configuration was tested, no further increase in the current response is observed after the first layer, indicating that the direct exposition of GOx to SDS is not an efficient method for the assembling, and prevents the incorporation of PAOs.

Other surfactants were tested. They included sodium deoxycholate (NaDC), a weak anion surfactant, triton X-100, a non-ionic surfactant, and dodecyltrimethylammonium bromide (CTAB), a positively charged surfactant. None of them show improvements respect to the system without surfactant. It is particularly important the case of NaDC, since it presents a negatively charged group at pH 7 and a hydrophobic tail, however its behavior is far from to be similar to the system with SDS. On the other hand, preliminary results using sodium octadecylsulfonate shows similar results to those involving SDS.

The use of SDS was also tested at pH 8. At this pH, in absence of surfactant, the uptake of PAOs and GOx presents an important growth [11]. In presence of 50 mM glucose, the catalytic current observed for (PAOs/SDS/PAOs/GOx)<sub>n</sub> is only twice than the one for (PAOs/GOx)<sub>n</sub>, and again the shape of the voltammograms preserves the behavior observed at pH 7.0.

#### 4. Discussion and conclusions

The interaction of surfactants with heme proteins has been previously reported [14,15]. A surfactant layer casted on the electrode has been used and, due to hydrophobic forces, the protein was then adsorbed, improving its direct electron transfer rate. In our case, a more complex phenomenon is taking place.

Dodecyl sulfate is an anion with a negatively charged head and a hydrophobic tail with a molecular weight of 265 Da. It does not remain efficiently adsorbed onto a PAOs modified electrode, even though a mixture of these species in solution produces a precipitate. On the other hand, it is known that, above pH 6.0, incubation of GOx with SDS yields a cooperative (hydrophobic) binding with retention of activity [16]. Taking all these aspects into account, the effective incorporation of SDS to the self-assembled system, only after the formation of a GOx/PAOs layer, can be explained considering that SDS is attracted toward the surface by the positively charged outer layer of PAOs, followed by its anchorage to the hydrophobic regions of GOx. The increase in the contact angle measurement for the PAOs can be attributed to the fact that the presence of SDS also changes the orientation of the amino charged groups toward the inner layer of GOx, exposing its hydrophobic backbone to the solution. In this way, the important changes observed in the contact angle measurements can be explained. At pH 8, as the PAOs becomes less charged, stronger hydrophobic interaction with GOx are observed [11]. Accordingly, the addition of SDS is a process that competes with PAOs for the hydrophobic regions of GOx; the charge interaction decreases and the increase in catalytic current only duplicates. Further studies are needed to confirm this hypothesis.

The introduction of a negatively charged surfactant in the construction of self-assembled structures comprising a redox-active electrolyte and GOx allows the incorporation of a greater amount of each species. This fact is reflected in the increase of the catalytic current observed in presence of glucose. However, the cyclic voltammograms for the system in absence of glucose are practically equal than for the system without SDS. Modeling of these results is complex since different factors, such as the diffusion of the different species across the film, the electron diffusion, and the thickness of the film, have to be taken into account. At the moment, it can be suggested that the participating redox centers in the electron transfer process are the same in both cases, and that the improvement in the catalytic current can be mainly attributed to

a greater adsorption of GOx and a better interaction between the enzyme and the polyelectrolyte due to the presence of SDS.

The introduction of a third molecule in the self-assembling process of these two macromolecules opens more flexible synthetic routes for the construction of molecular devices; for example, the SDS modification with a redox couple could lead to a further improvement of the electron transfer process between PAOs and GOx.

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### References

- [1] M.S. Jobal, P.A. Chiarelli, *Soft Matter* 3 (2007) 34–46.
- [2] S.S. Shiratori, M.F. Rubner, *Macromolecules* 33 (2000) 4213–4219.
- [3] J. Hodak, R. Etchenique, E.J. Calvo, K. Singhal, P.N. Bartlett, *Langmuir* 13 (1997) 2708–2716.
- [4] C. Danilowicz, E. Cortón, F. Battaglini, *J. Electroanal. Chem.* 445 (1998) 89–94.
- [5] B.A. Gregg, A. Heller, *Anal. Chem.* 62 (1990) 258–263.
- [6] Z. Cheng, B. Ren, M. Gao, X. Liu, Z. Tong, *Macromolecules* 40 (2007) 7638–7643.
- [7] M.E. Hays, N.L. Abbott, *Langmuir* 21 (2005) 12007–12015.
- [8] E.J. Calvo, F. Battaglini, C. Danilowicz, A. Wolosiuk, M. Otero, *Faraday Discuss.* 116 (2000) 47–65.
- [9] E.J. Calvo, C. Danilowicz, A. Wolosiuk, *J. Am. Chem. Soc.* 124 (2002) 2452–2453.
- [10] E.J. Calvo, A. Wolosiuk, *ChemPhysChem* 5 (2004) 235–239.
- [11] V. Flexer, E. Forzani, E.J. Calvo, S.J. Ludueña, L.I. Pietrasanta, *Anal. Chem.* 78 (2006) 399–407.
- [12] G.Z. Sauerbrey, *Z. Phys.* 155 (1959) 206–222.
- [13] V. Flexer, M.V. Ielmini, E.J. Calvo, P.N. Bartlett, *Bioelectrochemistry* 74 (2008) 201–209.
- [14] J. Rusling, *Acc. Chem. Res.* 31 (1998) 363–369.
- [15] Y. Xu, C. Hu, S. Hu, *Bioelectrochemistry* 74 (2009) 254–259.
- [16] M.N. Jones, P. Manley, A. Wilkinson, *Biochem. J.* 203 (1982) 285–291.