



Formation of redox-active self-assembled polyelectrolyte–surfactant complexes integrating glucose oxidase on electrodes: Influence of the self-assembly solvent on the signal generation



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ABSTRACT

In this work the effects of the self-assembly solvent on the structure and electrochemical behavior of redox-active polyelectrolyte–surfactant complexes cast on electrode supports from aqueous and DMF solutions are presented. The complex studied is formed by complexation of osmium complex-modified polyallylamine (OsPA) with dodecyl sulfate (DS) surfactants. The structure of the films was characterized by GISAXS, showing that films present a lamellar mesostructure. However, when they are exposed to humid environments, films cast from aqueous solutions (OsPA–DS^{aq}) undergo a structural transition that ultimately leads to the disappearance of the mesostructural order. On the other hand, OsPA–DS films cast from DMF solutions (OsPA–DS^{DMF}) revealed no significant changes upon exposure to humid environments. Both types of films were exposed to glucose oxidase (GOx), showing similar adsorption characteristics. Notwithstanding these similarities in GOx and content, OsPA–DS^{aq} films revealed a more sensitive bioelectrocatalytical response to glucose as compared to OsPA–DS^{DMF} films.

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1. Introduction

Responsive materials represent a broad field in chemical research involving the integration of different building blocks to create devices with the ability to respond to specific stimuli [1,2]. Among the main areas that have received particular attention is the one devoted to electrode modification toward its application to biological and environmental analysis, where materials such as carbon nanotubes [3–6], nanorods [7–9], and nanoparticles [10–13] have shown their great potentials. Another approach is the use of electroactive polyelectrolyte films containing species that can be oxidized and reduced in a reversible way [14,15]. These films coupled to different surfaces can be useful for fundamental studies [16,17] as well as for applications in electroanalytical chemistry [18,19], and fuel cells [20–22]. In the field of biosensors, the combination of these films with enzymes (e.g. oxidases) was accomplished through different strategies such as the formation of polyelectrolyte multilayers (PEM) via layer by layer deposition [23–25], hydrogel formation by mixing the two components in the presence of a hydrophilic crosslinker [26,27] and more recently, by the combination of an electroactive polyelectrolyte with a surfactant, followed by further

adsorption of the enzyme [28,29]. Polyelectrolyte–surfactant complexes are systems able to generate self-assembled structures on a surface resulting from the evaporation of a solution containing their components. This simple process represents an attractive method for the construction of molecular devices [30–32]. They are mainly processed in organic solvents or as colloidal suspensions in aqueous solutions. In this way they can be cast virtually on any surface producing coatings with tuned functional properties. New features can be introduced to these complexes by modifying them with moieties presenting particular properties; for example, we have introduced osmium complexes in polyallylamine (OsPA) that combined with dodecyl sulfate (DS) yield a self-assembled system with excellent electron transfer properties [33]. GISAXS characterization of OsPA–DS films shows the presence of lamellar domains oriented parallel to the substrate that remains structurally stable upon incorporating water in the hydrophilic regions of the film [10].

In most of the cases polyelectrolyte complexes are processed and cast from solutions of organic solvents. To the best of our knowledge no attempt has been made to process electroactive polyelectrolyte–surfactant complexes from aqueous solutions. This would be of great benefit to integrate enzymes and other biological building blocks into the supramolecular architecture during the self-assembly step, especially if we consider that processing the film in organic solvents might affect the stability and activity of the integrated enzymes. In this work we

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show that processing self-assembled OsPA–DS films in aqueous solutions is feasible, but their mesostructural characteristics when exposed to aqueous environments differ markedly from similar films processed in organic solvents. Finally, we demonstrate that this novel “aqueous route” for the fabrication and processing of polyelectrolyte–surfactant films is fully compatible with the integration of glucose oxidase into the functional architecture. To this end, we explored two routes: the first one – a two-step method – by casting the film from an aqueous suspension of a polyelectrolyte–surfactant complex and then exposing it to a GOx solution (OsPA–DS^{aq}/GOx) and the other one – a one-pot method – by casting an aqueous solution containing the polyelectrolyte, the surfactant and the GOx (OsPA–DS–GOx^{aq}). Both films are sensitive to glucose, and their performances were compared to the modified electrode obtained from casting the polyelectrolyte–surfactant complex from an organic solvent (OsPA–DS^{org}/GOx). The sensitivity toward glucose resulted in the following sequence: OsPA–DS^{aq}/GOx > OsPA–DS^{org}/GOx \gg OsPA–DS–GOx^{aq}. The response of these systems is discussed in terms of the structure generated during the casting process affecting the electron transfer process involved in the glucose oxidation.

2. Experimental

2.1. Reagents and materials

Sodium dodecyl sulfate (SDS) and Glucose Oxidase (GOx) were from Sigma-Aldrich, and tris(hydroxymethyl)-aminomethane, D (+) glucose, and dimethylformamide (DMF) were from Carlo Erba. The redox polymer Os(bpy)₂ClpyNHpoly(allylamine) (OsPA) was synthesized as previously reported [34], and the stoichiometry ratio between the osmium complex and allylamine monomer was 1:35. All other reagents were of analytical grade. The QCM-D sensors used were 14 mm diameter quartz crystals with a fundamental resonance frequency of 5 MHz (QSX 301, Q-sense AB, Sweden). For electrochemical experiments, gold electrodes were produced on silicon, coated with 15 nm of Ti, 20 nm of Pd and 200 nm of gold by metal evaporation.

2.2. Synthesis of the OsPA–DS composite materials

200 μ L of 10 mM SDS in Milli-Q water was added to 100 μ L of OsPA (220 mM in amino groups). The mixture immediately generated a precipitate (OsPA–DS), which was easily separated by centrifugation. The precipitate was dissolved in 125 μ L of dimethylformamide (DMF) and sonicated for 15 min to facilitate complete dissolution of the solid. This solution was used for the generation of the OsPA–DS^{org} composite material. On the other hand, the supernatant solution was used for the generation of the OsPA–DS^{aq} composite material.

2.3. Surface modification of electrodes

Gold coated silicon substrates were employed as working electrodes. The first step of the substrate modification was the application of a uniform layer of OsPA–DS (the organic or aqueous complex) solution by spin coating. Afterwards, the electrode was left at room temperature to allow complete evaporation of the solvent.

2.4. Equipment

2.4.1. Electrochemical measurements

Electrochemical experiments were carried out using a potentiostat (TEQ-02, Nanoteq, Buenos Aires, Argentina) and a three-electrode Teflon electrochemical cell equipped with a platinum mesh counter electrode and a Ag|AgCl reference electrode. Unless otherwise stated, all electrochemical experiments were performed at room temperature (ca. 22 °C) in a 0.1 M Tris–HCl, 0.1 M NaCl buffer solution at pH 7.4. Glucose was added from a 1 M stock solution in the same buffer.

2.4.2. Quartz crystal microbalance with dissipation monitoring (QCM-D)

The QCM-D experiments were performed using a Q-Sense instrument (QCM-D, Q-Sense E1, Sweden) equipped with Q-Sense Flow Module (QFM 401). The sensor was an AT-cut piezoelectric quartz crystal covered with two gold electrodes, and for all measurements QSX 301 Gold sensors were used. Samples were perfused using a peristaltic microflow system (ISMATEC, ISM 596D, Switzerland). All experiments were performed in a 0.1 M Tris–HCl, 0.1 M NaCl buffer solution at pH 7.4, in flow mode; the flow rate used in the experiments was 150 μ L/min, and at temperature of 27 °C. The modified crystal (Au/OsPA–DS^{org} or Au/OsPA–DS^{aq}) was equilibrated in buffer and when stable baselines for both frequency and dissipation were achieved, a 1 mg/mL GOx solution (in carrier buffer) was allowed to flow through the system. Once the GOx adsorption achieves a constant value, the flow was changed to the carrier buffer to rinse the unbound sample; in both cases, negligible changes were observed. All QCM-D experiments were processed using the acquisition software QSoft 401.

2.4.3. Contact angle measurements

A KSV Cam200 equipment was used for contact angle measurements. Distilled water was used as the liquid probe in all assays.

2.4.4. Grazing-incidence small-angle X-ray scattering (GISAXS)

GISAXS measurements were performed at the D10A-XRD2 line of Laboratório Nacional de Luz Síncrotron (LNLS, Campinas, Brazil). A monochromatic beam of 7709 eV ($\lambda = 1.608$ Å) was used to perform the experiments. Si(100) wafers were used as support substrates and were pretreated before coating. They were rinsed with acetone and dried with argon blowing before coating.

3. Results and discussion

3.1. Film casting

In previous works [28] we have combined polyallylamine modified with osmium complexes (OsPA) with sodium dodecyl sulfate (SDS) to obtain an electroactive polyelectrolyte–surfactant complex. The mixture of equal amounts of a solution of OsPA (220 mM in amino groups) with a 35 mM SDS solution yields a precipitate that can be easily separated from the solution. When the concentration of SDS is decreased to 10 mM, a small amount of the complex precipitate and no aggregates is observed in the solution. Casting this aqueous solution on silica or gold substrates yields a stable film that cannot be rinsed with water in contrast to films formed from a pure OsPA solution that can be easily removed with water.

3.2. Film structure and meso-organization

GISAXS characterization with an incident angle of 0.27° was carried out to elucidate the formation of mesoscale organized domains and the orientation of these oriented domains with respect to the substrate (Fig. 1). Previous SAXS studies have shown the presence of a narrow scattering peak corresponding to the long period of ca. 3.7 nm. This result is consistent with the formation of lamellar mesostructures in the supramacromolecular assembly. In this work, complementary GISAXS studies on these films show a bright region (highest intensity) in the direction q_z (for $q_y \rightarrow 0$) and the presence of a faint halo. This suggests the presence of lamellar domains mostly oriented parallel to the substrate. Noteworthy, the faint halo GISAXS pattern might also indicate that a small fraction of these domains are multi-oriented. We have hypothesized [35] that the presence of multiorientation in the mesostructure facilitates the electronic connectivity between the redox-active domains and the conducting substrate. In Fig. 1b, it can be observed that the exposition of this system to a humid environment, relative humidity (RH) of 95%, produces a slight change in the halo, that can be explained as slight swelling of the film. On the other hand, when the

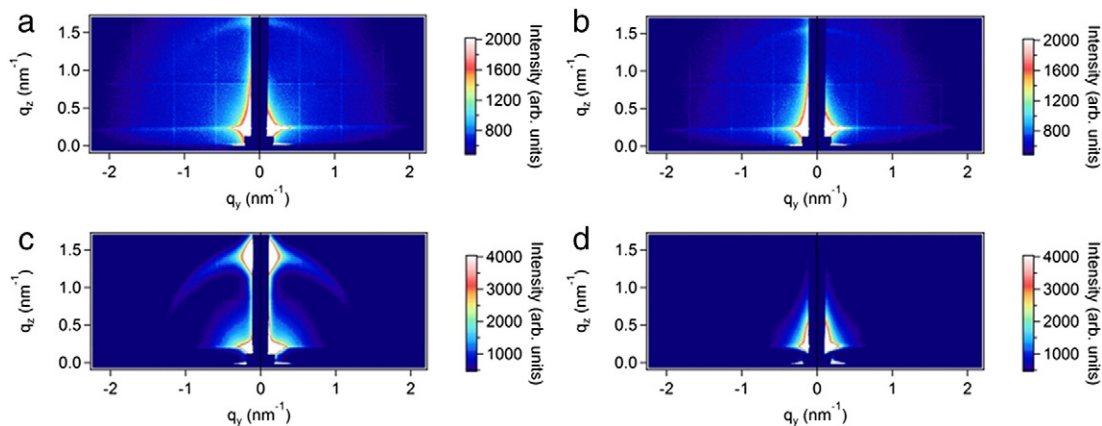


Fig. 1. GISAXS patterns obtained from OsPA-DS^{org} (a and b) and OsPA-DS^{aq} (c and d) films measured at an incident angle of 0.27° under 0% (a and c) and 95% (b and d) relative humidity conditions. Films were spin-coated on Si(100) substrates.

same complex is cast from an aqueous solution (Fig. 1c), the presence of a well-defined lamellar order manifested by the strong halo can be observed, that is lost when the system is exposed to 95% RH conditions (Fig. 1d). This fact clearly reflects that the self-assembly solvent plays a significant role in the meso-organization of the supramolecular films when they are in contact with an aqueous environment.

In a similar vein, water contact angle experiments showed remarkable differences. Contact angles of 90° and 49° were observed for coatings generated from organic and aqueous solutions, respectively. The hydrophobic behavior exhibited by films processed from organic solutions was mainly attributed to organization of the outermost layers exposing aliphatic moieties to the solution [35]. On the other hand, the lower value in the contact angle for the films processed in aqueous solutions suggests that the outer layer is exposing hydrophilic groups to the solution. We should also consider that the hydrophilic moieties (amino and sulfate groups) are able to retain water molecules, thus further increasing the hydrophilic character of the film. In this scenario, the stability of the self-assembled hydrophilic film should mainly be attributed to van der Waals and hydrophobic forces between aliphatic domains within the film. As we will see later, the system is stable in aqueous solutions during electrochemical experiments.

QCM-D has shown to be an efficient technique to understand structural changes in soft materials. By tracking changes in frequency and dissipation it is possible to obtain information regarding mass incorporation (or loss) and changes in the viscoelastic properties of the film. In a previous work we have shown that GOx can be efficiently adsorbed on OsPA-DS^{org} films yielding a sensitive response to glucose [28]. In this work two gold-coated QCM sensors were modified with OsPA-DS^{aq} and OsPA-DS^{org}. Both sensors have nearly the same density of redox centers considering that the charge density is 10.3 and 7.4 $\mu\text{C cm}^{-2}$, for the aqueous and organic systems, respectively. Fig. 2 shows the frequency vs. time (black lines) and dissipation vs. time (gray lines) variations for GOx adsorption on sensors modified with OsPA-DS^{aq} (bold line) and OsPA-DS^{org} (dotted line) nanocomposites, respectively. The change in frequency is practically the same for both samples. However, the dissipation (D) trace reveals significant differences between samples processed in aqueous and organic solvents. The shape of the traces for both systems goes through a minimum that rapidly levels off to a steady value. The decreasing dissipation indicates that the film is changing from a soft state to a more rigid state. We hypothesize that rapid protein adsorption leads to a rather compact film that is formed during the early stages of the process. The magnitude of the decrease in D is significantly more pronounced in films cast from aqueous solutions (gray bold line) as compared with those processed from organic solvents (gray dotted line). This experimental observation implies that OsPA-DS^{aq} films undergo considerable structural changes during the adsorption and uptake of GOx, that ultimately leads to a more rigid film.

3.3. Electrochemical response

The electrochemical response of gold electrodes modified with OsPA-DS^{org} and OsPA-DS^{aq} films is depicted in Fig. 3. Films processed from aqueous solutions exhibit current densities higher than films processed from organic solvents due to the formation of thicker films. In both cases a quasi-reversible behavior is observed (peak separation of 33 and 30 mV for Au/OsPA-DS^{org} and Au/OsPA-DS^{aq}, respectively). As can be observed both types of electrodes display similar characteristics with regard to heterogeneous rate constant and diffusion coefficient for charge transport.

Both systems are able to integrate GOx in order to trigger a catalytic response in the presence of glucose. To compare the response of both electrodes to glucose addition, the density currents (j_{cat}) were normalized to the peak current density (j_p) measured in the absence of glucose. Fig. 4 shows the bioelectrocatalytic behavior of both electrodes, indicating that in the whole concentration range studied the j_{cat}/j_p ratio is higher for films cast from aqueous solutions. This difference is more pronounced at low glucose concentration; for example at 2 mM glucose the j_{cat}/j_p ratio is 14 for OsPA-DS^{aq}/GOx versus 4 for the OsPA-DS^{org}/GOx film whereas this difference is reduced to 26 vs. 16 when both films are exposed to 50 mM glucose. Using the j_{cat}/j_p ratio as a criterion of catalytic efficiency, our system performs better than others using osmium

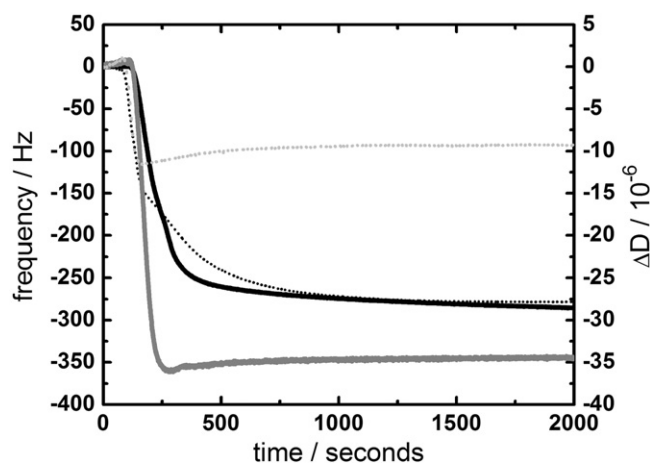


Fig. 2. Changes in frequency and dissipation as a function of time during adsorption of glucose oxidase (GOx) onto modified gold-coated QCM sensors with OsPA-DS^{aq} (bold lines; black line corresponding to frequency changes and gray line corresponding to dissipation changes) and OsPA-DS^{org} (dotted lines; black line corresponding to frequency changes and gray line corresponding to dissipation changes).

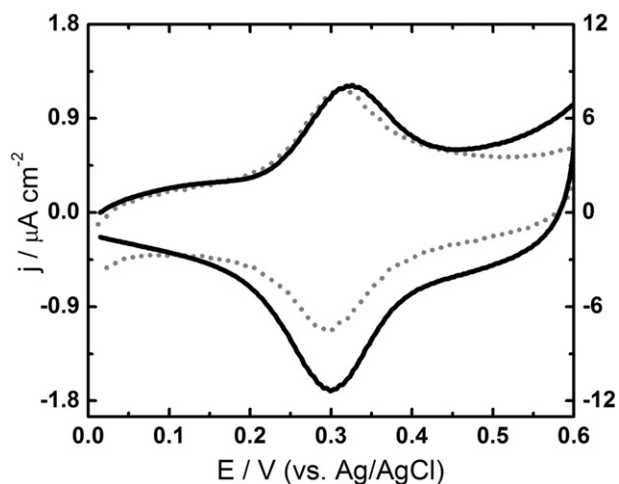


Fig. 3. Cyclic voltammogram at 10 mV s^{-1} for OsPA-DS^{aq} (black line, current scale on the right y-axis), and OsPA-DS^{org} (dotted line, current scale on the left y-axis).

containing polyelectrolytes built either by layer-by-layer self-assembly [36] or hydrogel crosslinking [37,38], and surpassed by systems containing long tethers binding osmium centers to a polymer backbone [39]. Another interesting feature is that the saturation current is reached at lower glucose concentrations in OsPA-DS^{aq} as compared with OsPA-DS^{org} films (inset, Fig. 4). This fact can be explained considering the hydrophilic nature of the film and the degree of disorder that facilitate the interaction between the molecules that participate in the different redox process. First, considering the diffusion of glucose through the film that reacts with the enzyme, OsPA-DS^{aq}/GOx presents a more hydrophilic behavior that ultimately permits the fast diffusion of glucose compared to OsPA-DS^{org}/GOx; the last one is a more structured film with hydrophobic characteristics that may introduce an important limitation in the diffusion of glucose inside the film thus reducing its nominal concentration in the surroundings of the enzyme. The manifestation of the limited diffusion inside the OsPA-DS^{org}/GOx film is given by the way that the current increases, almost linear; whereas in the case of OsPA-DS^{aq}/GOx films the typical Lineweaver–Burk plot can be represented.

Additionally, in the case of polyelectrolyte–surfactant complexes cast from aqueous solutions, the incorporation of GOx could be carried in one step. An important issue to be taken into account is the influence of the surfactant on the enzyme activity; however, it has been demonstrated that SDS does not affect the activity of GOx in concentrations below 10 mM [40], the maximum surfactant concentration used in this work. We accomplished the electrode modification combining OsPA, SDS and

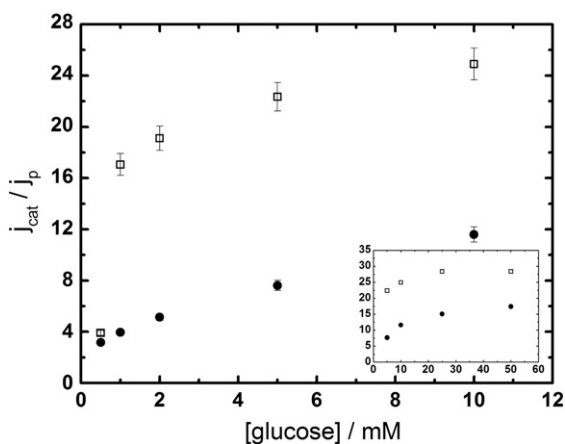


Fig. 4. Current response of OsPA-DS^{aq} (white squares) and OsPA-DS^{org} (black circles) to low glucose concentrations. Inset: the extended plot until 50 mM glucose concentration.

GOx in an aqueous solution in one step. Two strategies were followed: (a) GOx was dissolved in the supernatant, and (b) initial low concentrations of OsPA and SDS were used together with GOx. In all cases the final concentration in GOx was between 0.75 to 1.0 mg/mL. Cyclic voltammetry of electrodes modified in this way presents at the beginning an important decrease in current that after a few cycles is stabilized. This could be understood considering that the concentration of OsPA is higher than the other two components and it is not stable alone. As blank experiment, the combination of OsPA and GOx without SDS was carried out yielding also a film of relative stability and an electrochemical behavior different than the one with SDS (Fig. 5). For the system without SDS the behavior corresponds to adsorbed species with a current peak separation of 50 mV, almost double of that observed in Fig. 3. While, for the system containing SDS, an important change in the behavior is observed, with a greater peak separation and the shape of the voltammogram closer to a diffusive species. These facts suggest that the introduction of GOx in the system from the beginning introduces an important perturbation for the good contact among the osmium centers.

Also, the catalytic behavior is poorer than the other two examples. Again, using the ratio j_{cat}/j_p it is observed that sensitivity to glucose is far less than the other two cases (Fig. 6). From the point of view of sensor construction each system has its pros and cons: for OsPA-DS^{aq}/GOx it can have a very high sensitivity at low concentrations; however, the response saturates at 25 mM; on the other hand OsPA-DS-GOx^{aq} is easier to prepare (one step), but less sensitive. Finally, the one prepared from the organic solvent as the trade-off solution shows a good sensitivity and a good linearity, but an organic solvent should be used and the construction is in two steps.

4. Conclusions

In this work the casting of the same polyelectrolyte–surfactant complex was carried out from two different solvents (water and DMF). After solvent evaporation, both films present a lamellar structure as determined by GISAXS experiments. Thereafter, exposure to 95% humidity revealed significant structural changes in OsPA-DS^{aq} leading to an amorphous layer whereas OsPA-DS^{org} films virtually retain their original lamellar structure. This behavior does not introduce an important effect on the film stability when immersed in aqueous solutions and does not represent an important change in the electrochemical response of the osmium complexes since cyclic voltammetry experiments show the same features in both types of films. However, the contact angle characterization indicated important differences between both films (90° and 49° for OsPA-DS^{org} and OsPA-DS^{aq}, respectively) suggesting

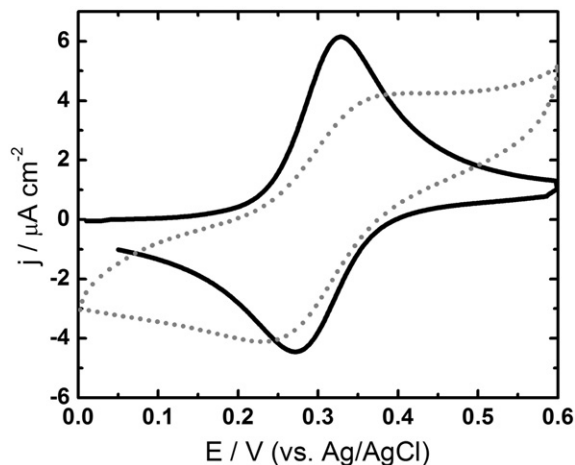


Fig. 5. Cyclic voltammogram at 10 mV s^{-1} for OsPA-DS-GOx^{aq} (black line), and OsPA-GOx (gray dotted line).

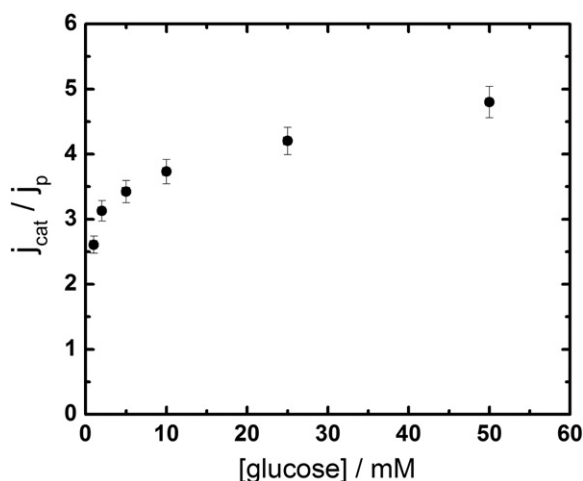


Fig. 6. Current response of OsPA-DS-GOx^{aq} to different glucose concentrations.

that affinity to water and film hydration is strongly affected by the type of solvent used during film processing. To a certain extent, this fact influences the redox connectivity of the electroactivity groups confined within the film. In OsPA-DS^{org} the osmium centers are kept close in a rather rigid environment, while in OsPA-DS^{aq}, the osmium centers are more separated due to the swelling in the presence of water, but in compensation some movement is allowed to maintain an efficient electron transfer hopping.

The incorporation of GOx was followed by QCM-D showing that enzyme integration is feasible in both systems. Changes in dissipation indicated that GOx adsorption leads to more compact films. The most important differences appear when the sensitivity to glucose was tested. At low concentrations, the response to glucose is 3.5 times higher for OsPA-DS^{aq} as compared to OsPA-DS^{org}, while as glucose concentration reaches the saturation condition the response becomes 1.35 times higher. We propose that this behavior is mainly due to the hydrophilic characteristics of OsPA-DS^{aq} that facilitate the permeation of glucose into the film, while in OsPA-DS^{org} its diffusion will be hindered by its hydrophobic characteristics; at high concentration, where the current is limited by the enzyme, this difference becomes smaller. These results show that the casting of an electroactive polyelectrolyte-surfactant complex followed by the adsorption of a redox enzyme produces stimulus-responsive films compatible with the detection of glucose.

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