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Electro-oxidation of glucose at an increased current density at a reducing potential

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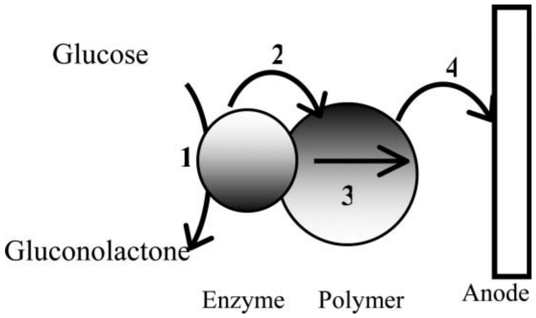
Glucose was electro-oxidized at -0.1 V *vs.* Ag/AgCl and at 1.5 mA cm^{-2} current density in a physiological buffer solution at 37 °C.

We described earlier a glucose electrooxidizing anode made by "wiring" glucose oxidase (GOx) with poly(4-vinylpyridine)-[Os(*N*,*N*′-dimethyl-2,2′-biimidazole)$_3$]$^{2+/3+}$, comprising 10.7 wt% of osmium.[1] The unique features of this "wire" were its 13-atom long flexible tethers, binding the redox centers to the backbone, and the reducing redox potential of the dialkylated biimidazole complex of Os$^{2+/3+}$, -0.2 V *vs.* Ag/AgCl. The long tethers increased the frequency of effective electron transferring collisions between reduced and oxidized osmium centers and thereby the apparent electron diffusion coefficient, D_{app}, which reached in the crosslinked redox hydrogel 5.8×10^{-6} cm^2 s^{-1}, an order of magnitude higher than in earlier redox hydrogels.[2,3] The effective collection of the electrons from the glucose-reduced GOx allowed poising of the anode at a potential as reducing as -0.10 V *vs.* Ag/AgCl, only 0.26 V positive to the redox potential of the FAD/FADH$_2$ cofactor in GOx at pH 7.2, where it reached a limiting current density of glucose electrooxidation at 1.15 mA cm^{-2}.

To build a faster glucose anode and to decrease the overpotential for glucose electrooxidation, we synthesized two new redox polymers with respectively 12.8 wt% and 20 wt% of osmium. The synthetic strategy for the polymers resembled that reported.[1] The NH$_2$-spacer modified tris(*N*,*N*′-dialkylated 2,2′-biimidazole)-Os$^{2+/3+}$ was condensed with 6-bromohexanoic acid-quaternized poly(4-vinylpyridine) to form the amide. The (tethered complex/pyridine/carboxyl-pentylpyridinium) ratios were for polymer ***I*** 10.7/85/4.3, for polymer ***II*** 12.8/85/2.2, and for polymer ***III*** 20/75/5.

As illustrated in Scheme 1, there are four electron transfer steps in the "wired"-GOx catalyzed electrooxidation of glucose. As discussed in the case of the O$_2$ cathode,[4] at steady state the current associated with each step is necessarily the same. Thus, the currents flowing from glucose to the enzyme molecules (1), from the enzyme molecules to the polymer (2), through the redox polymer (3), and from the polymer to the anode (4) are all identical. The limit of each of these currents is defined by the polymer/enzyme ratio. The limits of (3) and (4) increase upon increasing the polymer/enzyme mass ratio, unless the electrostatic adduct between the polycationic polymer and the polyanionic enzyme precipitates. The polymer/enzyme mass ratio can be increased if the specific activity of the enzyme is higher and less enzyme mass is required. If the apparent electron diffusion coefficient of the polymer is higher and less polymer mass is required, the ratio can be decreased.



Scheme 1 The electrooxidation of glucose. Electrons are transferred from glucose to the enzyme (1), from the enzyme to the polymer (2), through the polymer (3) and from the polymer to the electrode (4).

Blauch and Saveant[5,6] developed a bounded diffusion model to predict D_{app} for polymers when the displacement of the redox centers is rapid and extensive. According to the model

$$D_{\text{app}} = 1/6\, k_{\text{ex}}\, (\delta^2 + 3\lambda^2)\, C_{\text{RT}} \tag{1}$$

where k_{ex} is the solution-phase self exchange rate of the redox species, δ is the characteristic electron hopping distance, λ is the distance across which the tethered redox center can actually move and C_{RT} is the concentration of the redox species. The model suggested that D_{app} could scale in redox hydrogels with the concentration of redox species assuming λ remains constant over the C_{RT} range examined. In pratice however, decreases in D_{app} with increasing C_{RT}, or constant D_{app} values, regardless of C_{RT}, or more complicated profiles of D_{app} *vs.* C_{RT} have been observed, especially in cases where multiply charged redox species were incorporated in the polymer matrix.[6–9]

Fig. 1 represents the optimal composition (polymer/enzyme/crosslinker) obtained for the bioelectrocatalysts made with polymers ***I*** (solid circles), ***II*** (open circles) and ***III*** (asterisks).

Increasing the osmium wt% in the redox polymer allowed a decrease in the polymer/enzyme weight ratio from 1.5 for the polymer loaded with 10.7 wt% of Os, to 1 for the polymer loaded with 12 wt% of Os, and to 0.5 for the polymer loaded with 20 wt%

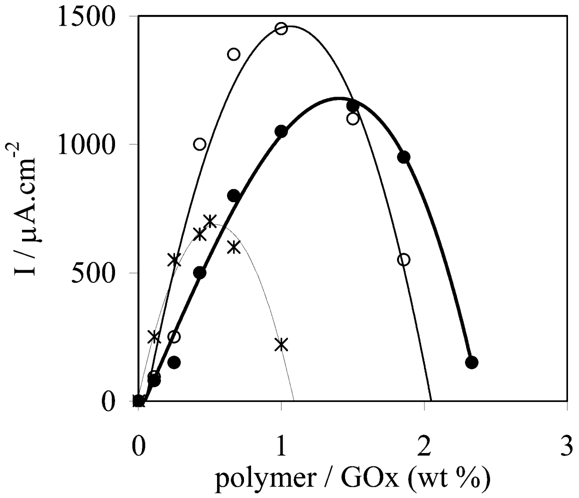


Fig. 1 Dependence of the glucose electrooxidation current density on the polymer/GOx weight percentage ratio when the bioelectrocatalyst is made with polymer ***I*** (solid circles), polymer ***II*** (open circles), and polymer ***III*** (asterisks). Electrode poised at +0 V *vs.* Ag/AgCl in a 20 mM phosphate buffer, 37 °C, 1 mV s^{-1} scan rate, 200 rpm, under 1 atm N$_2$, 15 mM glucose.

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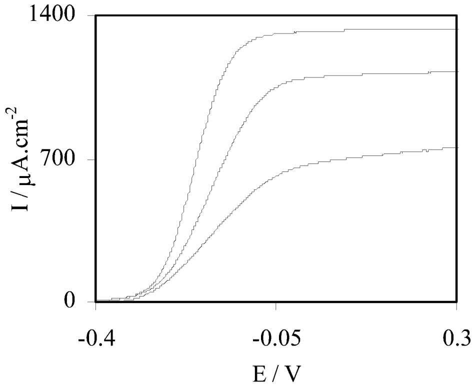

Fig. 2 Polarization of 7 μm diameter, 2 cm long carbon fiber anodes, modified with polymer ***I*** (center), polymer ***II*** (top), or polymer ***III*** (bottom) at the GOx/polymers/PEGDGE ratios indicated in the text. Quiescent solution, under argon, 37.5 °C, PBS buffer, 15 mM glucose, 1 mV s^{-1}.

of Os. The 0.5 ratio could not be exceeded for polymer ***III***, because of precipitation of the electrostatic adduct. The precipitation, associated with a low current density, is attributed to formation of a charge-neutral electrostatic adduct between the polycationic polymer and the anionic (pI = 4.1) enzyme. In polymer ***II***, with the Os wt% increased by 1.3%, the polymer-to-enzyme ratio could be reduced by as much as 33%, and the limiting current density increased by 20% to 1.5 mA cm^{-2}. At this time, it is not clear whether the cause of the increase is tighter electrostatic coupling of the enzyme to the polymer, or enhanced electron diffusion.

The polarization of the miniature carbon fiber anodes (7 μm diameter, 2 cm long) in a quiescent pH 7.2, 0.1 M NaCl, 20 mM phosphate, 15 mM glucose solution under air at 37.5 °C in PBS, modified with 1 wt% PEGDGE and containing either 45 wt% glucose oxidase–54 wt% polymer ***II*** (top curve), 39.5 wt% glucose oxidase–59.5 wt% polymer ***I*** (center curve) or 69 wt% glucose oxidase–30 wt% polymer ***III*** (bottom curve) is seen in Fig. 2.

Fig. 2 shows that the carbon fiber anodes made by wiring GOx with polymers ***I*** and ***II*** reach, respectively, limiting current densities of 1.15 mA cm^{-2} and 1.3 mA cm^{-2} already at −0.1 V *vs.* Ag/AgCl, only 0.26 V positive the redox potential of the FAD/FADH$_2$ cofactor in GOx at pH 7.2.[10] For both, the threshold for glucose electrooxidation is −0.36 V *vs.* Ag/AgCl, the redox potential of the FAD/FADH$_2$ centers of GOx. The limiting current density of glucose electrooxidation of the electrode made with polymer ***III*** is, however, only 0.65 mA cm^{-2} at +0.1 V *vs.* Ag/AgCl, well below the 1.30 mA cm^{-2} limiting current density of the electrode made with polymer ***II***.

The hydrated films of crosslinked polymer ***II*** constitute not only the best electron conducting hydrogel to date, but also the best "wires" of glucose oxidase (GOx). Even though the redox potential of the hydrogel formed upon crosslinking polymer ***II*** with poly(ethylene glycol) diglycidyl ether (PEGDGE) is as reducing as −195 mV *vs.* Ag/AgCl, the polymer efficiently oxidizes the FADH$_2$ centers of glucose oxidase.

As earlier shown, the long tethers not only increase the apparent diffusivity of electrons, but also facilitate electron transfer from the reduced (FADH$_2$) reaction centers of glucose oxidase to the redox polymer. Engineering of the polymer and the higher density of active redox sites also led to an adhering, tough, better electrode-covering film, with the glucose flux limited current density increased by 20%.

In combination with a reported cathode, enabling the rapid four-electron electroreduction of molecular oxygen to water at pH 7,[4,11,12] the described glucose anode is likely to form the basis for a compartment-less, miniature glucose/O$_2$ biofuel cell, operating at a voltage exceeding +0.58 V *vs.* Ag/AgCl, in a physiological buffer solution at 37 °C.[13–15]

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