Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Torres, Walter and Guiseppi-Elie, Anthony(2006) 'Simulations of Redox Mediation within Bioactive Hydrogels of Amperometric Biosensors', Journal of Macromolecular Science, Part A, 43: 12, 1923 — 1928
To link to this Article: DOI: 10.1080/10601320600996080
URL: http://dx.doi.org/10.1080/10601320600996080

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry, 43:1923–1928, 2006

ISSN 1060-1325 print/1520-5738 online
DOI: 10.1080/10601320600996080

Simulations of Redox Mediation within Bioactive Hydrogels of Amperometric Biosensors

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Highly hydrated bioactive hydrogels containing immobilized oxidoreductase enzymes and immobilized redox mediators were simulated as the biorecognition layer of amperometric biosensors. The linear dynamic range of the amperometric response of mediated biosensors increases and moves to higher concentration brackets with an increase in the concentration of mediator. This informs the design of biosensors that target the same analyte but possesses several independently addressable electrodes modified with hydrogels that contain different concentrations of mediator. Increases in enzyme concentration increase the linear dynamic range but does not alter the sensitivity of amperometric biosensors. Both sensitivity and linear dynamic range of mediated amperometric enzyme biosensors may be "tuned" by varying the concentrations of the enzyme and the mediator. Simulations effectively guide the initial domains of study of complex systems such as implantable biosensors.

Keywords simulation, enzymes, hydrogels, mediation, biosensors

Introduction

The development of electrochemical biosensors for *in vivo* measurements is an active area of research (1). Critical design issues that influence the performance of these devices are the judicious choice of the biorecognition component (oxidoreductase enzyme) and any electrochemically active species that allow for fast, sensitive, and reliable measurements of the analyte of interest, the optimization of the electrical communication between the sensing species and the underlying electrodes, the efficient co-immobilization of these species and even their spatial distribution within the biorecognition layer. Special attention must also be paid to interference shielding (2) and to the maximization of biosensor biocompatibility, that is, the control of undesirable interactions between the biosensor and the host body initiated by adsorption of proteins and subsequent recruitment and accumulation of cells onto the sensor surface that result in fibrous capsule formation (3, 4).

Our laboratory is currently developing implantable electrochemical biosensors in which enzymes and artificial redox species that mediate electron transfer between the

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enzyme and a metal electrode are co-immobilized within highly hydrophilic hydrogels made from non-cytotoxic polymers such as poly(2-hydroxyethyl methacrylate) p(HEMA). These biorecognition membranes include the co-polymerization of phosphorylcholine (PC) derivatives and poly(ethylene glycol) (PEG) that provide for improved biocompatibility (5) and polypyrrole that provides for interference shielding (6, 7). In all cases, these constituents are covalently tethered to the polymer network. The PC and PEG components are covalently tethered by incorporation as the methacryloyl derivatives and co-polymerized with HEMA. The enzyme and mediator are likewise tethered by "monomerization" reactions with acryloyl(polyethylene glycol) NHS ester that links lysine residues of the enzyme and primary amines of the mediator along highly flexible PEG chains set pendant to the backbone. The polypyrrole is similarly tethered by the use of heterobifunctional crosslinkers such as 2-methacryloyloxyethyl-4-(3-pyrrolyl)butanate (MPB) that allow the formation of a network structure between the poly(HEMA) and poly(pyrrole) (8). Together these polymers comprise "bioactive" (recognition) and "biosmart" (responsive) polymers.

Analytical figures-of-merit that are typically required for bioanalytical biosensors are low detection limits, high sensitivity and high selectivity for the analyte of interest. These must be combined with relatively fast response times and an appropriate range for the linear relation between the analyte concentration and the sensor signal. Computer simulations are now common engineering design tools and here we show their application for one biosensor design aspect, often neglected in the literature, namely the dependence of the linear dynamic range of the amperometric biosensor on the relative concentrations of the co-immobilized enzyme and its redox mediator (9). Here, a simple design is considered, that of co-immobilized mediator and enzyme within a highly hydrophilic hydrogel.

Experimental

ESP (Electrochemical Simulations Package), a freeware program developed by Professor Carlo Nervi at the *University of Torino*, Italy (10), was used to simulate the coupling of the enzyme reactions (1–3) to the electrode reaction (4), where E and M stand for the enzyme and mediator species and the subscripts ox and red stand for the oxidized and reduced forms, respectively:

$$E_{ox} + \text{Substrate} \leftrightarrow \text{ES-Complex} \quad (1)$$

$$\text{ES-Complex} \leftrightarrow E_{red} + \text{Product} \quad (2)$$

$$E_{red} + M_{ox} \leftrightarrow E_{ox} + M_{red} \quad (3)$$

$$M_{ox} + 1e \leftrightarrow M_{red} \quad (4)$$

The polymer hydrogel was modeled as an homogeneous, isotropic medium in which all the participant chemical species undergo semi-infinite linear diffusion. In the real biosensor, however, the enzyme and the mediator are covalently anchored to PEG chains that are themselves pendant to the backbone of the poly(HEMA) network. The enzyme and the mediator diffusion coefficients were set to 1×10^{-9} and 1×10^{-6} cm^2/s, respectively. These are reasonable values since the diffusion coefficient of the haloenzyme of glucose oxidase is 4.9×10^{-7} cm2/s and of ferrocene monocarboxylic acid, a common redox mediator of oxidoreductase enzyme reactions, has a D_{app} of 4.6×10^{-6} cm^2/s. The corresponding substrate and product diffusion coefficients were set 1×10^{-5} cm^2/s each. Kinetics were assumed fast for both the "homogeneous" and

heterogeneous reactions. In this regard, if one assumes that the stationary state for the enzyme-substrate complex holds, reactions (1) and (2) may be combined for the purpose of the simulation (11). The standard potential for the M_{ox}/M_{red} redox couple was taken as zero V. Charging effects at the electrode interface and electrostatic interactions among all chemical species in the system was assumed negligible and thus were neglected. Lateral diffusion within the layer, while highly important for dense membranes, was deemed less important in highly hydrated hydrogel layers and was neglected.

The simulated electrochemical characterization techniques were staircase voltammetry (SCV, the digital version of cyclic voltammetry) and chronoamperometry (CA). For the simulation of CA experiments, a potential step from -0.2 to $+0.3$ V and a sampling time of 5 min were used. For the construction of the current *vs*. substrate concentration graphs, the current was sampled at 3 min (180 s), a time (τ) sufficiently long for the system to reach "quasi-stationary" conditions. The concentration of the E_{ox} species was varied between 0.1 and 0.001 mM, while that of the M_{ox} species was varied between 1 and 10 mM. In turn, the substrate concentration was varied in the range from 0 through 50 mM.

Results and Discussion

Figures 1 and 2 show a series of simulated SCV and CA curves for the mediated enzymatic biosensor as a function of substrate concentration. These figures illustrate the characteristic evolution of the shape of the current *vs*. potential and the current *vs*. time profiles as the system reaches steady state conditions.

At zero mM substrate, the mediator is readily oxidized and reduced supported by semi-infinite linear diffusion to and away from the electrode. However, as the substrate concentration is increased and the enzyme acts on this substrate, the reduced form of the enzyme's cofactor is readily reoxidized by the mediator, itself becoming reduced. The preponderance of the reduced form of the mediator allows for an enhanced oxidation current. Thus, cyclic voltammograms show an increase in anodic current over cathodic current and a shift toward an s-shaped curve. This is however unique to scan

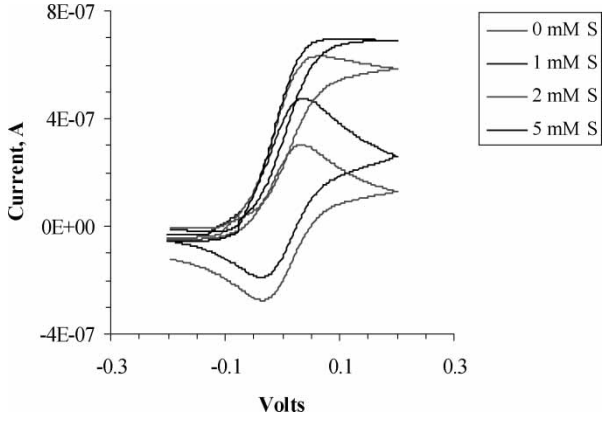

Figure 1. Simulated voltammograms for an enzymatic biosensor (with 1 mM M_{ox} and 0.01 mM E_{ox}) in the presence of several concentrations of the substrate, S = 0, 1, 2, and 5 mM. Scan rate = 20 mV/s.

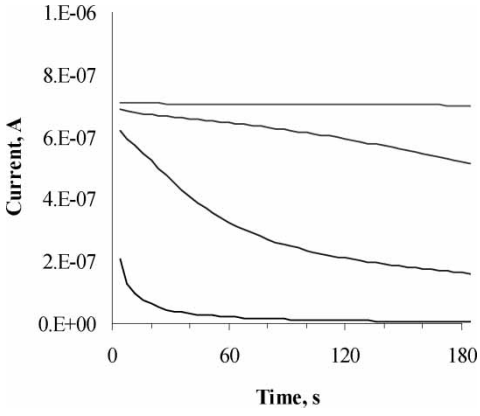

Figure 2. Simulated chronoamperograms for an enzymatic biosensor (with 1 mM M_{ox} and 0.01 mM E_{ox}) in the presence of several concentrations of the substrate S = 0 (lowest curve), S = 2 (middle low), S = 4 (middle high), and S = 10 mM (highest curve), respectively. Potential step from −0.2 to 0.3 V.

rates that are slow compared to the enzyme kinetics. At fast scan rates (compared to the enzyme kinetics) the voltammograms show the familiar shape.

Figure 3 shows the dependence of the simulated chronoamperometric current (sampled at 180 s) as a function of substrate concentration for biosensors in which the concentration of the mediator was changed from 1 to 10 mM while keeping the enzyme concentration constant at 0.01 mM.

The notable features in Figure 3 are the increase in the linear dynamic range of the sensor and the increase of their sensitivity as the concentration of the mediator increases. Thus, for the sensor with the lowest mediator concentration, the response of the sensor is linear over a relatively low range at relatively low substrate concentrations (approximately from 0.5 to 5 mM substrate). Also, for this same sensor, the variation of

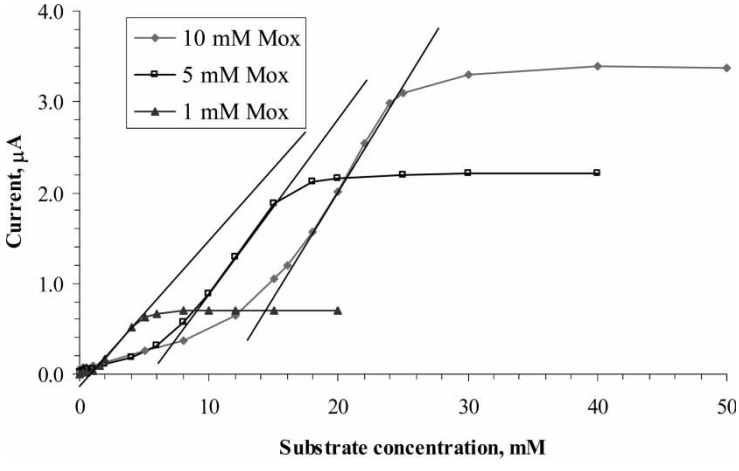

Figure 3. Simulated chronoamperometric response of sensors containing 0.01 mM enzyme and various mediator concentrations. The current is sampled 180 s after applying the potential step.

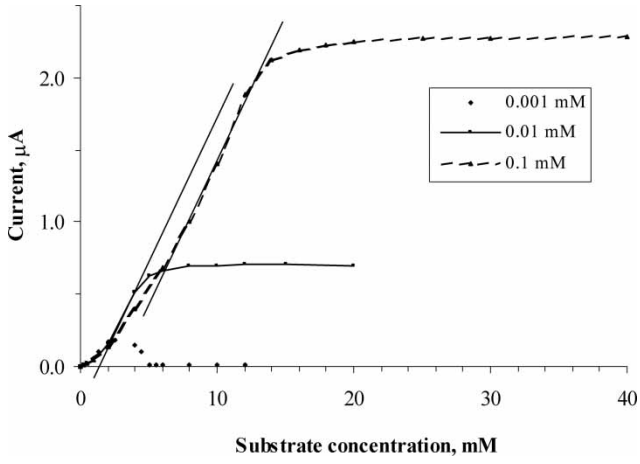

Figure 4. Simulated chronoamperometric response of sensors containing 1 mM mediator at various enzyme concentrations. The current is sampled 180 s after applying the potential step.

the current output per unit of substrate concentration is the lowest of the three sensors in this series. As the concentration of the mediator in the sensor increases, the linear dynamic range of the simulated device moves to a higher substrate concentration bracket. Correspondingly, the sensitivity of the device increases. If one is interested in devices that can measure substrate concentrations both above and below the 1 mM domain, the performance of the sensor might then be improved by using several independently addressable electrodes modified with hydrogels that contain different concentrations of mediator. This is an important insight that guides the design of multi-element array biosensors that may be based on microlithographic fabrication techniques and microarray spotting techniques.

Figure 4 yields information that complements that shown in Figure 3. Here in Figure 4 the concentration of the mediator was kept constant at 1 mM to observe the effect of the variation of enzyme concentration in the range between 0.001 to 0.1 mM.

The striking difference with respect to the data shown in Figure 3 is that a change in the enzyme concentration increases the linear range of the biosensor without appreciably changing the "sensitivity" of the biosensor in the low substrate concentration region. Thus, as the enzyme concentration increases, the range of concentration of substrate over which a linear response may be obtained is likewise increased. However, there is expected to be a limit above which increasing the enzyme concentration is likely to have deleterious effect on other membrane properties. We do not have a satisfactory explanation for the fall of the current for the case of the 0.001 mM enzyme system at substrate concentrations above 4 mM. This may well be an artifact of product inhibition.

The simulated results in the two previous figures suggest that the change of both mediator and enzyme concentrations are useful to "tune" the dynamic linear range and the sensitivity of the device. For example, Figure 5 shows the simulated dose-response curves of two sensors (data from Figures 3 and 4), which exhibit different linear dynamic ranges (1–12 mM and 6–15 mM, respectively) but present similar sensitivities (slopes) over these ranges.

A comparison of the simulated results with data generated from real enzyme biosensors for glucose and lactate is currently underway in our laboratory.

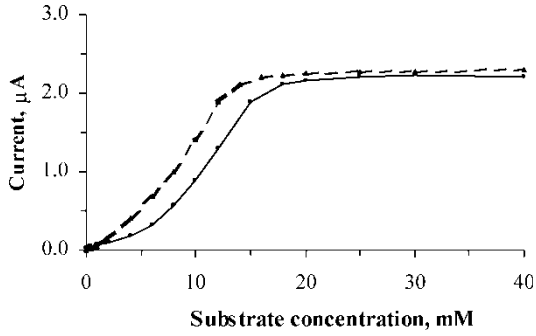

Figure 5. Simulated chronoamperometric response of sensors containing 1 mM mediator +0.1 mM enzyme (dashed line) and 5 mM mediator +0.01 mM enzyme (data from Figures 3 and 4).

Conclusions

Computer simulations of the electrochemical response of amperometric biosensors yield useful information that may be used to "tune" the sensitivity and linear dynamic range of the biosensor by varying the concentrations of the enzyme and the mediator. The authors acknowledge, however, that the simulated system is still simple compared to the environment of the real biosensor. A comparison of the real *vs.* simulated systems is needed.

Acknowledgements

This work was supported by the US Department of Defense (DoDPRMRP) grant PR023081 and by the Consortium of the Clemson University Center for Bioelectronics, Biosensors and Biochips.

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