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Mathematical Modeling of the Amperometric Response to Glucose of Glucose Oxidase Films Deposited by AC-Electrophoresis

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Abstract

Previous work illustrated that glucose oxidase (GOx) could be deposited on conducting substrates using asymmetrical alternating current electrophoretic deposition (AC-EPD) to form thick enzyme layers suitable for the manufacturing of highly active biosensors. Here, we modeled the amperometric response of GOx layers to glucose as a function of the thickness of the enzyme layer. The model is based on reaction-diffusion equations with irreversible first-order catalytic reactions. The numerical results displayed qualitative and reasonable quantitative agreement with the experimental data obtained for oxidation currents due to glucose, which increase with the enzyme layer thickness.

Keywords: Alternating Current Electrophoretic Deposition, Glucose Oxidase, Modeling

1. Introduction

Immobilization of enzymes is used in biosensors to detect the concentration of a specific analyte as a result of the biological recognition between the analyte and the immobilized enzyme [1-6]. Thick enzyme layers have been fabricated via cross linking with glutaraldehyde [7-9], entrapment in polymers or gels and carbon paste mixing [10-12] and, by electrochemical deposition [13, 14]. The purpose of these approaches is to immobilize enzymes in their active state. Recently, we reported that enzymes such as glucose oxidase, glutamate oxidase and β-galactosidase can be deposited using AC-EPD to yield thick, active enzyme layers which were used for the development of highly sensitive biosensors [15-18]. In this regard, it is worth noting that among the advantages of the AC-EPD technique over the existing deposition methodologies we quote the ease of the manufacturing process as well as the high reproducibility due to the automated deposition procedure.

Mathematical models and solutions of enzyme electrodes prepared by various processes such as immobilization in dispersed carbon nanotubes, electropolymerization and encapsulation in membranes have been reported [19-22]. In this note, the steady state amperometric currents of glucose oxidase layers deposited by AC-EPD is modeled as a function of the layer thickness and compared to experimental values.

2. Theoretical Model

A schematic representation of the enzyme modified electrode on which a uniform GOx layer is deposited is illustrated in **Figure 1**.

The amperometric response to glucose is due to the enzymatic conversion of glucose to hydrogen peroxide and D-glucono-1,5-lactone:

$$\beta-\mathrm{D}-\mathrm{glucose}+\mathrm{O_2}+\ \mathrm{H_2O}\xrightarrow[\mathrm{k}]{\mathrm{Gox}}\mathrm{D}-\mathrm{glucono}-1,5-\mathrm{lactone}\ +\ \mathrm{H_2O_2}$$

This reaction model shows that for every molecule of glucose that reacts, one molecule of hydrogen peroxide is formed.

As the transport of glucose inside the enzyme film occurs by diffusion, we assume that the concentration c_1 of glucose inside the film is governed by the one-dimensional reaction-diffusion equation:

$$D_1^e\frac{\mathrm{d}^2c_1}{\mathrm{d}x^2}-kc_1=0\ \text{for}\ 0\le x\le\delta \qquad (1)$$

where D_1^e is the effective diffusion coefficient of glucose ($[\mathrm{m^2\cdot s^{-1}}]$), k is the forward reaction rate constant

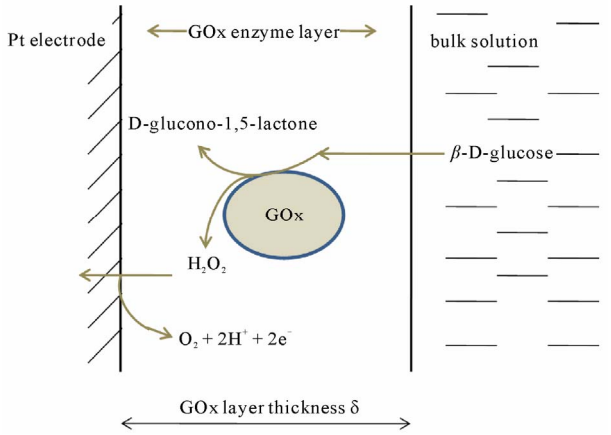

Figure 1. Schematic representation of the electrocatalytic oxidation of glucose inside the GOx enzyme layer deposited by AC-EPD on a platinum electrode.

([s^{-1}]) of the reaction between glucose and oxygen, x is the normal distance to the electrode and δ is the thickness of the enzyme film ([m]). In Equation (1), we assume that the reaction rate of glucose is first order in the glucose concentration (kc_1). Based on the experimental evidence of **Figure 5** of Reference [23], this is a valid assumption for the glucose concentrations used in this work. Equation (1) also assumes that the reaction is irreversible or that the backward reaction proceeds at a much smaller rate and that the oxygen concentration is high enough as experimentally observed for the enzyme electrode [16]. Equation (1) is subject to the following boundary conditions:

$$\frac{\mathrm{d}c_1}{\mathrm{d}x} = 0 \text{ at } x = 0 \tag{2}$$

$$-D_1^e \frac{\mathrm{d}c_1}{\mathrm{d}x} = h_1\left(c_1 - c_1^\infty\right) \text{ at } x = \delta \tag{3}$$

where c_1^∞ is the glucose concentration in the bulk (in this work 5 mol·m^{-3}). The first boundary condition states that glucose itself is not electrochemically active and hence does not oxidize or reduce on the surface of the electrode (x = 0). This is indeed the case at the applied potential of +0.6 V vs. AgCl/Ag. The second boundary condition states that, at the enzyme film-electrolyte interface, the mass transport by diffusion in the film equals the convective transport in the electrolyte, with h_1 the convection coefficient of glucose (m·s^{-1}).

The enzymatically generated hydrogen peroxide is subsequently electrochemically oxidized on the surface of the platinum electrode:

$$H_2O_2 \xrightarrow{\text{Pt}} O_2 + 2H^+ + 2e^-$$

Hence, the concentration c_2 of H_2O_2 inside the enzyme film is governed by the following one-dimensional reaction-diffusion equation:

$$D_2^e \frac{\mathrm{d}^2 c_2}{\mathrm{d}x^2} + kc_1 = 0 \text{ for } 0 \le x \le \delta \tag{4}$$

with D_2^e the effective diffusion coefficient of H_2O_2. This equation is subject to the following boundary conditions:

$$c_2 = 0 \text{ at } x = 0 \tag{5}$$

$$-D_2^e \frac{\mathrm{d}c_2}{\mathrm{d}x} = h_2\left(c_2 - c_2^\infty\right) \text{ at } x = \delta \tag{6}$$

where c_2^∞ is the concentration of H_2O_2 in the bulk (in this work 0 mol·m^{-3}). The first boundary condition states that at the applied potential (+0.6 V vs. Ag/AgCl), hydrogen peroxide is mass transport limited inside the enzyme film and its concentration becomes zero on the surface of the electrode. The second boundary condition is identical to Equation (3). Equations (1) through (6) can be solved analytically for c_2 (see Appendix). The current density i_2 (Am^{-2}) due to the oxidation of hydrogen peroxide is proportional to the concentration gradient of hydrogen peroxide on the surface of the electrode according to:

$$i_2 = -nFD_2^e \frac{\mathrm{d}c_2}{\mathrm{d}x} \text{ at } x = 0 \tag{7}$$

where n is the number of the electrons exchanged in the oxidation of one molecule of H_2O_2 (2 in this case) and F is Faraday's constant. This results in the following closed-form expression for the current density:

$$i_2 = -\frac{nFD_1^e kc_1^\infty}{D_2^e + h_2\delta} \cdot \frac{D_2^e\sqrt{k/D_1^e}\sinh\sqrt{k/D_1^e}\,\delta + h_2\left(\cosh\sqrt{k/D_1^e}\,\delta - 1\right)}{\cosh\sqrt{k/D_1^e}\,\delta + \frac{\sqrt{k/D_1^e}}{h_1}\sinh\sqrt{k/D_1^e}\,\delta} \tag{8}$$

3. Results and Discussion

The amperometric response of the GOx film was calculated using Equation (8) based on values for the diffusion coefficients found in literature and listed in **Table 1**. In accordance with Reference [24] on the diffusion of organic solutes in biofilms (which we believe to be similar to the enzyme films of this work), we assumed that the ratio of the effective diffusion coefficient inside the enzyme film to the diffusion coefficient in water equals 0.25. The convection coefficients for glucose and hydrogen peroxide were estimated assuming:

$$h_i = D_i/t \tag{9}$$

Table 1. Diffusion coefficients in water at 25˚C.

Solute	D_i ($m^2 \cdot s^{-1}$)	Reference
Glucose	$6.7 \cdot 10^{-10}$	[25]
Hydrogen peroxide	$1.3 \cdot 10^{-9}$ (20˚C)	[26]

where t represents the diffusion layer thickness in the unstirred glucose solution, which is assumed to be 100 μm.

The amperometric response of the GOx film on a Pt disk electrode with a diameter of 1 mm (surface area ≈ 0.78 mm^2) to a glucose concentration of 5 mM is plotted in **Figure 2** as a function of the thickness of the enzyme layer for various values of the reaction rate constant k. As can be seen, the current due to glucose strongly increases as the thickness of the enzyme layer increases, in accordance with our experiments [15-16]. **Figure 2** also displays that when the film reaches a certain thickness (value depending on the rate constant), the current levels off and reaches a maximum. This behavior can be understood as follows: when the film is very thin, almost no glucose is converted to hydrogen peroxide and the current is small. As the film thickness increases, more and more glucose reacts to hydrogen peroxide and the current increases. At a certain thickness, all of the glucose that enters the film is converted to hydrogen peroxide and the current reaches a maximum. When the film becomes thicker, the current drops as part of the hydrogen peroxide that is formed in the film diffuses out before it reacts at the electrode. In our previous experimental study, it was found that the thickness of the enzyme layers after 10, 20 and 30 minutes deposition time are respectively 3, 7 and 11 μm and the corresponding amperometric responses to 5 mM glucose are 665, 1254 and 1804 nA [15], shown as triangles in **Figure 2**. **Figure 2** reveals that the absolute values of the simulated currents are in reasonable agreement with the experimental values assuming a value of order 1 for the enzymatic conversion rate k. When the enzymatic reaction rate k takes on a value of 1 s^{-1}, the model deviates from the experimental values by −10, +13 and +33% for films of resp. 3, 7 μm and 11 μm thickness.

4. Conclusions

The amperometric current response of glucose oxidase layers deposited by AC-EPD to glucose was modeled as a function of the thickness of the enzyme layer. The model is based on reaction-diffusion equations with irreversible first-order reactions. The numerical results are qualitatively and quantitatively in reasonable agreement with the experimental data.

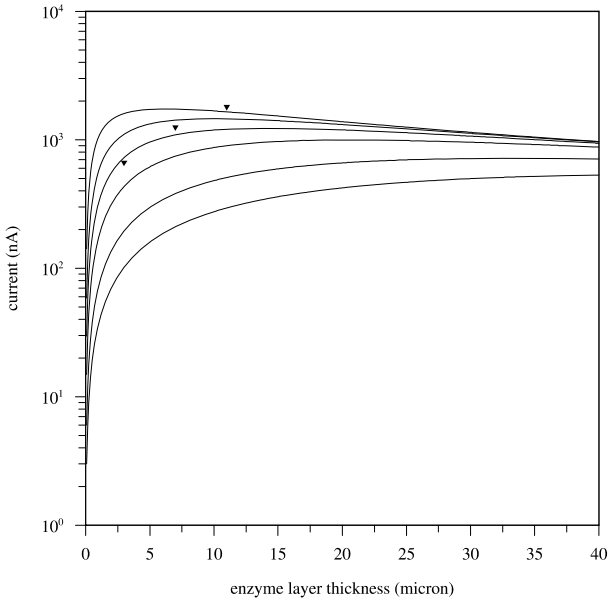

Figure 2. Simulated currents due to 5 mM glucose on a Pt disk electrode with a diameter of 1 mm (≈ 0.78 mm^2 surface area) as a function of the enzyme thickness for various values of the reaction rate constant k. From bottom to top, the rate constant k equals 0.1, 0.2, 0.5, 1, 2 and 5 s^{-1}. The experimental data points are shown as triangles.

5. Acknowledgements

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Appendix: Derivation of Equation (8)

Equation (1) is a second order differential equation with constant coefficients for which the general solution is given by:

$$c_1 = A\cosh\sqrt{k/D_1^e}\,x + B\sinh\sqrt{k/D_1^e}\,x$$

with A and B two integration constants, whose value can be determined from the boundary conditions (2) and (3). Equation (2) yields B = 0 while Equation (3) yields:

$$A = \frac{c_1^\infty}{\cosh\sqrt{k/D_1^e}\,\delta + \frac{\sqrt{k/D_1^e}}{h_1}\sinh\sqrt{k/D_1^e}\,\delta}$$

yielding the following solution for c_1:

$$c_1 = c_1^\infty \frac{\cosh\sqrt{k/D_1^e}\,x}{\cosh\sqrt{k/D_1^e}\,\delta + \frac{\sqrt{k/D_1^e}}{h_1}\sinh\sqrt{k/D_1^e}\,\delta}$$

With the solution of c_1, the general solution of Equation (4) can be found:

$$c_2 = -A\frac{D_1^e}{D_2^e}\cosh\sqrt{k/D_1^e}\,x + Cx + D$$

The two integration constants C and D which can be found from the boundary conditions (5) and (6), result in the following solution for c_2:

$$c_2 = \frac{D_1^e}{D_2^e}A\left(1-\cosh\sqrt{\frac{k}{D_1^e}}x\right) + \frac{D_1^e A}{D_2^e + h_2\delta} \cdot\left[\sqrt{\frac{k}{D_1^e}}\sinh\sqrt{\frac{k}{D_1^e}}\delta + \frac{h_2}{D_2^e}\left(\cosh\sqrt{\frac{k}{D_1^e}}\delta - 1\right)\right]x$$

from which Equation (8) can be derived by differentiation according to Equation (7).

