Chapter 6

Computer-Aided Biosensor Design

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Abstract

Amperometric biosensors are widely used in point-of-care medical devices that help patients control blood glucose and cholesterol levels in an effective and convenient way. On the other hand, computer-aided technologies for biosensor design remain an actively developing field. In this chapter, we present a computational model for biosensor design that uses a reaction-diffusion equation. We have successfully applied this model to simulate cholesterol analysis based on a multienzyme system. Furthermore, we show that this computer-aided approach can be used to optimize biosensor performance. This model can be applied to industry-grade biosensor development and can be easily extended to model multiple types of biosensors for a wide array of clinical applications.

Keywords: biosensor, multienzyme system, reaction-diffusion equation, computer-aided modeling and simulation

1. Introduction

High blood cholesterol is a major risk factor of cardiovascular disease including hypertension, hyperthyroidism, anemia, and coronary artery disease. Many prevention strategies including lifestyle modification, diet, and cholesterol-lowering drugs have been indicated for early prevention and management of the diseases. For adults, the normal cholesterol level is less than 200 mg/dL, and greater than 200 mg/dL is associated with increased risk of disease [1]. For detecting whole blood cholesterol levels and diagnosing cardiovascular disease, a biosensor is a convenient way to monitor cholesterol levels because the device is compact and easy to carry, and the diagnostic results can be obtained in seconds. A biosensor is defined as an analytical device that incorporates a transducer and biologically active material within

it [2, 3]. A biosensor obtains a signal that is proportional to the concentration of the analyte measurement. There are many types of biosensors including potentiometric, optical, and amperometric types [4–6]. The most common type is the amperometric biosensor, which measures the concentration of the analytes based on current. To use a biosensor for cholesterol-level determination, the patient uses a lancet to prick a small nick on the fingertip and apply a small drop of blood (around 10–25 μl) on the reactive area of the test strip. The test strip is coated with immobilized enzymes, which initiate the enzymatic reaction necessary for signal production.

2. Biosensor design

Although enzyme composition is optimized for bulk reagent laboratory testing, the composition does not apply to biosensors used for point-of-care applications. As a result, the enzyme composition needs to be reformulated and optimized for a specific biosensor platform. The enzyme formulation step is often labor intensive and time-consuming and requires tremendous amount of manual testing and calibration to achieve optimal performance. The construction of a mathematical model that can model biosensor kinetics and extract important system parameters to improve the biosensor performance will facilitate a more cost-effective biosensor design. The three most important criteria in biosensor characterization are sensitivity, measurement time, and measurement range [7]. Sensitivity measures the ability of the biosensor to differentiate close analytical values with minimal error and can be quantified based on the slope of the current density versus substrate calibration curve. The measurement time measures how fast the biosensor signal reaches steady state and is able to establish a linear relationship between substrate concentration and current magnitude. The measurement range is the range of the substrate concentration in the linear region of the calibration curve and will often include both the normal and pathological values.

To design a biosensor, a mathematical model can be constructed based on the design specification including input (type of analyte and its concentration), signal output (current density), and system parameters (enzyme compositions). The model can be then validated by comparing its signal output with the real-time biosensor data to test its accuracy. Meanwhile, several assumptions of the model including boundary conditions and initial values can be adjusted based on the specification. Once the model is validated, system parameters such as enzyme compositions and reaction area can be further adjusted to optimize biosensor signal.

2.1. Modeling enzymatic reactions

The chemical reaction of the amperometric biosensor is known as a heterogeneous reaction that occurs at the interface between the membrane and sensing electrode through the immobilized enzymes (**Figure 1**) [8].

As a result, the transient response of the amperometric biosensor can be modeled using a reaction-diffusion equation with the reaction term corresponding to the Michaelis-Menten kinetics of the enzyme reactions [9]:

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$$\frac{\partial[C]}{\partial t} = D\mathrm{e}\frac{\partial^2[C]}{\partial x^2} + R_e \tag{1}$$

where

$$R_e = \pm\frac{V_{\max}[C]}{ks + [C]} \tag{2}$$

Here, $V_{\max}$ is the maximal rate of the enzymatic reaction, which is proportional to the total amount of enzyme loaded on the membrane.

$$V_{\max} = k_{\mathrm{cat}}[Et] \tag{3}$$

The current density of the biosensor can also be calculated based on the flux of the electroactive species at the sensing membrane:

$$i(t) = zFDp\left.\frac{\partial[P]}{\partial x}\right|_{x=0} \tag{4}$$

z is the number of electrons transferred per species, D is the diffusion constant for the electroactive species, and F is the Faraday constant (F = 96485 C/mole).

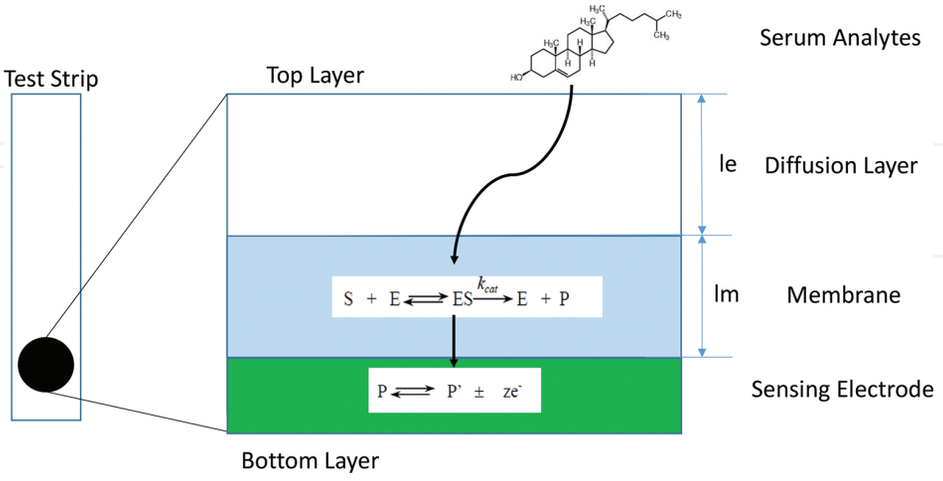

Figure 1. Layout of a biosensor test strip. The test strip consists of three layers: diffusion layer, membrane, and sensing electrode. The serum analytes go from the top layer through the diffusion layer and reach the membrane where enzymatic reactions take place. The sensing electrode converts electrons from the electron-donating species in the reaction to a current signal that can be used to quantify the amount of serum analytes.

2.2. One-enzyme reactions

The physical significance of a diffusion layer accounts for the serum diffusion. Different blood volumes can produce significant variance in the biosensor signal. As a result, volume control becomes an important issue in biosensor design. The reactions that occur at the membrane region are

$$S + E \Leftrightarrow ES \rightarrow E + P \tag{5}$$

$$P \rightarrow P^{'} \pm e - \tag{6}$$

In the first reaction, S represents the applied analyte, and E represents the catalytic enzyme. The enzyme first converts the analyte into an electro-active species P. The second reaction is the electron-donating step in which the electro-active species donate electrons at the membrane electrode. Since the electro-reaction is very fast, the electro-reaction term is ignored in the model. The reactions can be captured using the system reaction-diffusion equation in non-dimensionalized form:

$$\frac{\partial[S]}{\partial T} = \frac{\partial^2[S]}{\partial X^2} - \phi^2 \frac{[S]}{1+[S]} \tag{7}$$

$$\frac{\partial[P]}{\partial T} = D\frac{\partial^2[P]}{\partial X^2} + \phi^2 \frac{[S]}{1+[S]} \tag{8}$$

where $X = \frac{x}{\mathrm{lm}}$, $T = \frac{t\,D}{\mathrm{lm}}$, and lm is the membrane thickness. The behavior of the system equation is dictated by the parameter called the Thiele modulus (φ):

$$\phi = \mathrm{lm}\sqrt{\frac{V_{\max}}{Dks}} \tag{9}$$

The Thiele modulus represents the ratio of the diffusion time ($1/\sqrt{D}$) over reaction time ($1/\sqrt{Ks}$) and is often the reaction characteristic of a particular enzyme in the reaction [10]. For an amperometric biosensor, the Thiele modulus is also directly proportional to the square roots of the enzyme concentration as well as the length of the membrane layer (lm), which is important for reaction control. Another important parameter to characterize an enzymatic diffusion equation is the Biot number:

$$B_i = \frac{\text{lm} / \text{Dm}}{\text{le} / \text{De}} \tag{10}$$

The Biot number measures the ratio of internal diffusion and external diffusion and can be approximated by the ratio of the thickness between the inner membrane (lm) and outer diffusion layer (le) for a constant diffusion constant D. Previous studies indicate that the biosensor achieves the best sensitivity at high Thiele modulus and high Biot number, that is, under the conditions where the reaction is in internal diffusion control with minimal external diffusion. To minimize external serum diffusion, the pipette blood volume can be controlled based on the geometric design of the reaction region.

3. Application

3.1. Enzymatic determination of serum cholesterol

Methods for determining serum cholesterol levels have been developed for prepared aqueous reagents (**Figure 2**) [11]. In the first step, the cholesterol ester is converted to free cholesterol and fatty acids using the cholesterol esterase (CE) enzyme. The free cholesterol is then converted to electro-active species hydrogen peroxide and cholest-4-en-3-one using the cholesterol oxidase (CO) enzyme. Hydroxide is an electron-donating species that can be used to generate an electrical current to quantify the amount of cholesterol in the blood serum. The determination scheme is shown below.

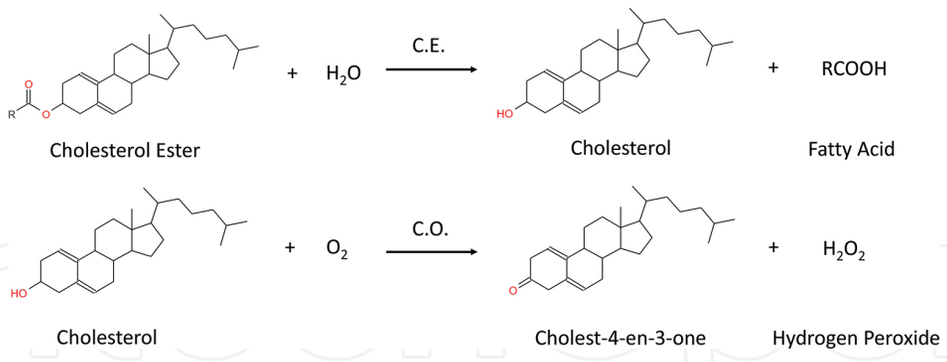

Figure 2. Enzymatic reaction for the quantification of cholesterol. In the first step, cholesterol ester is converted to free cholesterol and fatty acid through hydrolysis by the cholesterol esterase (CE) enzyme. In the second step, free cholesterol is converted to cholest-4-en-3-one and the electron-donating species hydrogen peroxide by the cholesterol oxidase (CO) enzyme.

3.2. Modeling multienzyme systems

To model the biosensor kinetics for cholesterol determination, a multienzyme model is constructed based on the one-enzyme model. Assume the biosensor is operated under internal diffusion control, that is, Biot number is infinite, and the serum diffusion layer thickness is

small compared with the membrane thickness. The biosensor kinetics is simulated only in the membrane region.

3.2.1. Multienzyme reaction-diffusion equation

The conversion of cholesterol ester to hydroxide is a two-step enzymatic reaction and thus can be captured using a multienzyme reaction-diffusion equation. In non-dimensionalized form

$$\frac{\partial[S1]}{\partial T} = \frac{\partial^2[S1]}{\partial X^2} - (\phi 1)^2 \frac{[S2]}{1+[S2]} \tag{11}$$

$$\frac{\partial[S2]}{\partial T} = \frac{\partial^2[S2]}{\partial X^2} - (\phi 2)^2 \frac{[S2]}{1+[S2]} + (\phi 1)^2 \frac{[S1]\left(\frac{Ks1}{Ks2}\right)}{1+[S1]} \tag{12}$$

$$\frac{\partial[P]}{\partial T} = D\frac{\partial^2[P]}{\partial X^2} + (\phi 2)^2 \frac{[S2]}{1+[S2]} \tag{13}$$

[S1] = cholesterol ester, [S2] = cholesterol, and [P] = hydrogen peroxide

$\varphi 1$ = cholesterol esterase Thiele modulus

$\varphi 2$ = cholesterol oxidase Thiele modulus

ks1 (cholesterol esterase catalytic constant) = 1.9×10^{-5} [M]

ks2 (cholesterol oxidase catalytic constant) = 3.5×10^{-4} [M]

V_{max} = 30 u*mg for both enzymes

3.2.2. Boundary and initial conditions

The multienzyme reaction-diffusion equation for cholesterol analysis is a system partial differential equation, and to solve the equation, six boundary conditions and six initial conditions are required.

3.2.2.1. Boundary conditions 1 and 2

Since the cholesterol ester and free cholesterol are nonreactive at the electrode surface, their flux is zero:

$$\left.\frac{\partial[S1]}{\partial x}\right|_{x=0} = \left.\frac{\partial[S2]}{\partial x}\right|_{x=0} = 0 \tag{14}$$

3.2.2.2. Boundary condition 3

Since hydrogen peroxide is consumed very fast, thus there is zero concentration at the electrode surface:

$$P\big|_{x=0} = 0 \tag{15}$$

3.2.2.3. Boundary conditions 4–6

Assume the concentration of cholesterol ester at the top of the membrane maintains constant concentration and the concentration of cholesterol and hydrogen peroxide are zero:

$$S_1\big|_{x=\mathrm{lm}} = S_O \tag{16}$$

$$S_2\big|_{x=\mathrm{lm}} = 0 \tag{17}$$

$$P\big|_{x=\mathrm{lm}} = 0 \tag{18}$$

3.2.3. Initial condition

At the initial time point, there is only cholesterol ester S1 on top of the sensing membrane with a concentration S0. As the reaction has yet to start, there is no cholesterol S2 or hydrogen peroxide P presented in the membrane:

$$\text{At } t = 0 \quad \mathrm{S1} = \mathrm{S0} \quad X \geq 1 \tag{19}$$

$$\mathrm{S2} = 0 \quad 0 \leq X < 1 \tag{20}$$

$$\mathrm{P} = 0 \qquad \forall X \tag{21}$$

3.3. MATLAB simulation

The biosensor performance for multienzyme reaction cholesterol determination is simulated by solving a system of partial differential equations using MATLAB. The system of partial

differential equations is casted into matrix form and solved using the MATLAB function PDEPE by assuming slab geometry. All units are non-dimensionalized. Note that $X = 1$ is the top of the membrane and $X = 0$ is the side of sensing electrode. For the simplified model, the Thiele modulus for both CE and CO is 1.

The solution of the system reaction-diffusion equation for cholesterol analysis is shown in **Figures 3–5**. The concentration profile shows that only 25% of the cholesterol ester has been converted to cholesterol at steady-state time and maintains at a constant concentration. On the other hand, the free cholesterol achieves 100% conversion to hydrogen peroxide. This suggests that the cholesterol ester conversion is the rate-limiting step. Interestingly, the hydrogen peroxide concentration reaches a maximum value toward the middle of the membrane and decreases on both ends, possibly due to higher mean cholesterol ester and cholesterol concentration in the middle region.

The current density plot shows that the current density reaches steady state at time 2T (17 seconds) correlating with the steady concentration of cholesterol ester and cholesterol concentration at this time point (**Figure 6**). Due to low cholesterol ester conversion, the cholesterol concentration at steady state is low, 0.0028 (0.5 μM). The current density plot also shows that the biosensor signal reaches a steady current at 2T (17 seconds) with a current magnitude of 0.0032 (0.64 μA/(cm^2)).

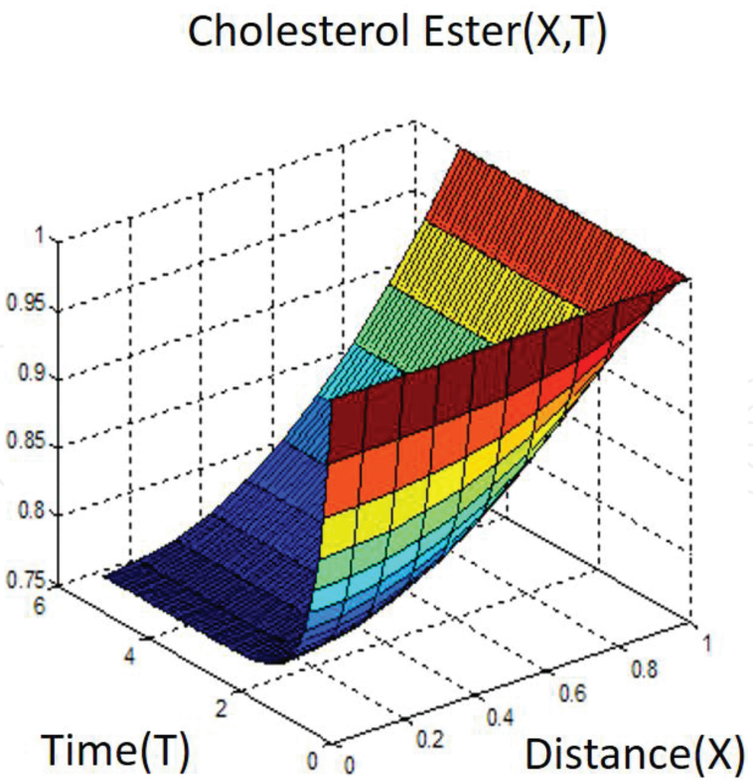

Figure 3. The concentration profile of cholesterol ester. The concentration profile shows that only 25% of the cholesterol ester has been converted to cholesterol at steady-state time and maintains at a constant concentration.

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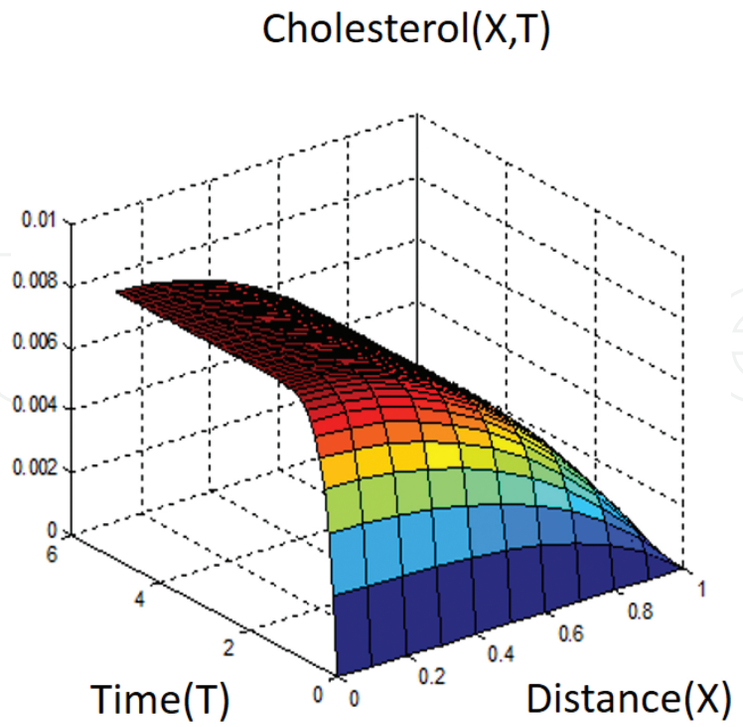

Figure 4. The concentration profile of free cholesterol. The free cholesterol achieves 100% conversion to hydrogen peroxide, suggesting that the cholesterol ester conversion is the rate-limiting step.

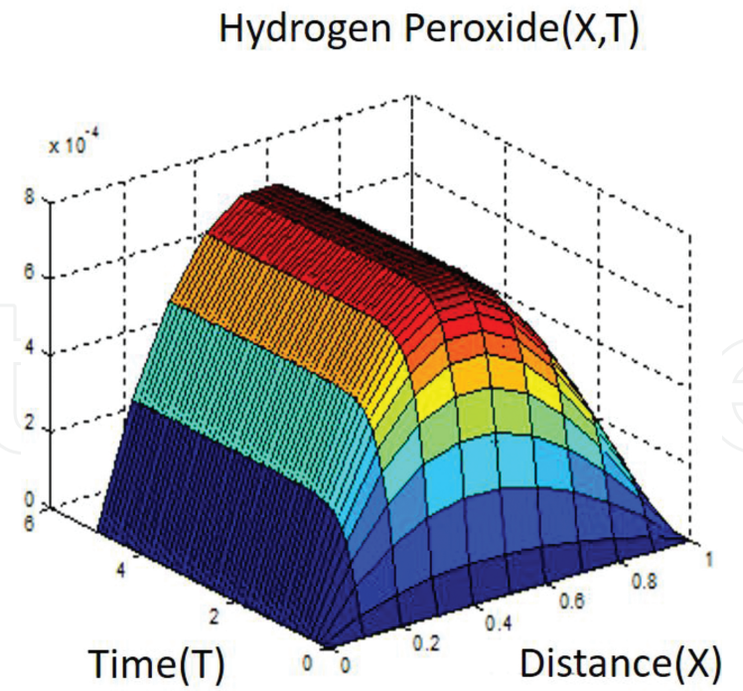

Figure 5. The concentration profile of the reaction product hydrogen peroxide. The hydrogen peroxide concentration reaches a maximum value toward the middle of the membrane and decreases on both ends, possibly due to higher mean cholesterol ester and cholesterol concentration in the middle region.

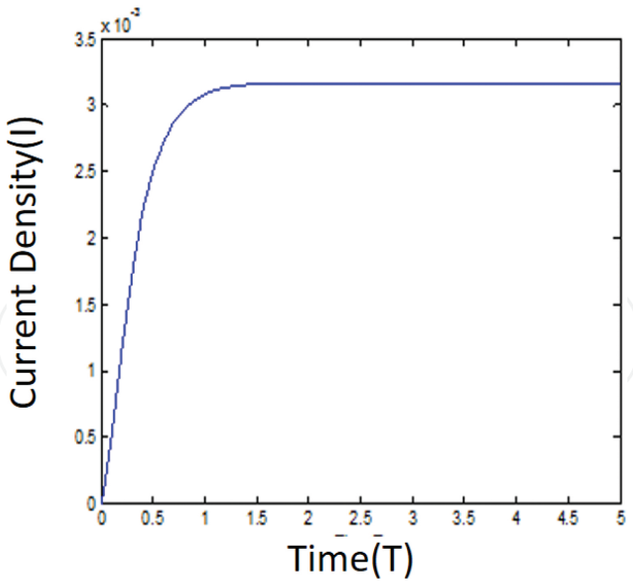

Figure 6. Plot of current density as a function of time derived from hydrogen peroxide reaction profile (**Figure 5**) at $X = 0$.

3.4. Biosensor performance characterization

3.4.1. Substrate-current activity relationship

The current-substrate calibration curve is constructed by simulating the steady-state current at 2T and varying the concentration of cholesterol ester from 1 to 10 S. The plot shows that the biosensor has high sensitivity and is linear at low substrate concentration (<1 S unit) and the current density continues to increase and gradually reaches a plateau at high substrate concentration (> 1 S unit). The maximum current density is 0.064 (12.8 μA/(cm^2)) (**Figure 7**).

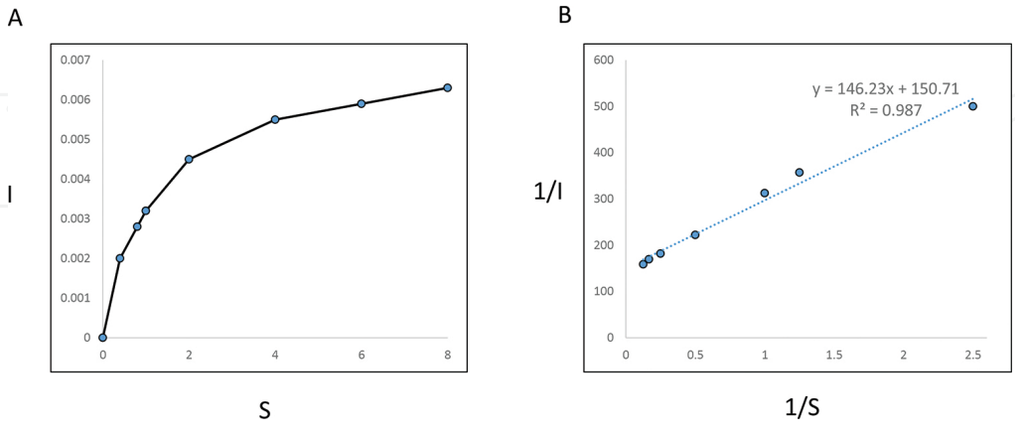

Figure 7. Characterization of biosensor performance using substrate-current activity relationship. (A) The maximum current density is predicted at different substrate concentrations. Note that the substrate-current activity relationship obeys the Michaelis-Menten kinetic equation. (B) Inverse linear plot of the inverse current and substrate showed high linearity ($R^2 = 0.987$).

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To quantitatively model how current density varies with substrate concentration, a mathematical model was constructed. Here, we observed that substrate-current activity relationship obeys the Michaelis-Menten kinetic equation. Specifically,

$$J = \frac{a[S]}{b + [S]} \tag{22}$$

J is the dimensionless current density, $[S]$ is the cholesterol ester substrate concentration, and a and b are undetermined variables. At low substrate concentration ($[S] \ll 1$), the equation can be approximated by a linear equation with the slope proportional to the ratio of a and b:

$$J = \frac{a}{b}[S] \tag{23}$$

At high substrate concentration, the current density is constant, corresponding to maximum current density:

$$J = J_{\max} = a \tag{24}$$

The two variables can be solved by the inverse linear plot:

$$\frac{1}{J} = \frac{b + [S]}{a[S]} = \frac{b}{a}[S] + \frac{1}{a} \tag{25}$$

By curve fitting, we determine $a = 0.0066$ and $b = 0.965$. Thus, we have recovered the substrate-current activity relationship:

$$J = \frac{0.0066[S]}{0.965 + [S]} \tag{26}$$

3.4.2. Sensitivity

The sensitivity of a biosensor signal can be defined as the unit increases in current density per unit increase in substrate concentration at the steady-state condition. High sensitivity is often required to differentiate between normal and pathological readings for a biosensor measurement. From the substrate-current activity relationship, the sensitivity can be expressed as the derivative of current density J with respect to $[S]$. To determine the optimal sensitivity, we calculate the second derivatives of the sensitivity expression at small S concentrations:

$$\frac{d^2J}{dS^2} = \frac{2a[S]}{\left(b+[S]\right)^3} - \frac{2a}{\left(b+[S]\right)^2} \cong \frac{2a}{b+[S]^2}\left(\frac{[S]}{b}-1\right) \tag{27}$$

Thus, we find that the substrate concentration [*S*] at $b = 0.965$ yields the greatest sensitivity based on the current density output.

3.4.3. Measurement time

The measurement time is another important element in biosensor design. For a point-of-care biosensor, it determines how fast the patient can retrieve the test results after the serum has been applied to the test strip. For an amperometric biosensor, the measurement time is determined by how fast the current achieves steady state. In our example, the measurement time is approximated at 2T or 17 seconds.

3.4.4. Measurement range

The measurement range is defined as the substrate concentration range where the substrate-current activity relationship is linear. A broad measurement range is preferable to be able to differentiate normal from pathological condition as well as pathological severity. As the calibration curve often plateaus out at higher substrate concentration, it becomes harder to provide an accurate reading at this region. In our example, the linear region is at $[S] < 2$ (38 μM).

3.5. Biosensor optimization

Since the behavior of the reaction-diffusion equation is controlled by two Thiele moduli φ1 and φ2 of CE and CO, it is possible to optimize biosensor response using these two parameters. From Eqs. (11) and (13), the conversion rate of cholesterol ester and the production rate of hydrogen peroxide are proportional to $\varphi 1^2$ and $\varphi 2^2$, respectively. From the computational simulation, we observed that Thiele modulus 1 = 1 at $S = 1$ only achieved a 25% cholesterol ester conversion rate. By increasing the Thiele moduli 1–5, the system was able to achieve full cholesterol ester conversion at 1T (8.5 seconds). Similarly, by increasing the Thiele moduli 2–5, the steady current attained a maximum value 0.05 (10 μA/(cm^2)). The results showed a 25-fold increase in sensitivity from the original value of 0.007 to 0.05, and the maximum current increased from 0.006 (1.2 μA/(cm^2)) to 0.55 (0.1 mA/(cm^2)) (**Table 1**).

φ1	φ2	Sensitivity (*dJ*/*dS*)	Measurement range (*S*)	Measurement time (*T*)
1	1	0.0068	1	1
5	5	0.053	1	1
8	16	0.05	20	1

Table 1. Biosensor optimization using the Thiele modulus.

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3.6. Biosensor model validation

The biosensor simulation result was compared with real-time experimental data [12]. The biosensor system consisted of a planar gold electrode modified with immobilized enzyme peroxidase, an insoluble mediator, layers of acetate cellulose, and the enzyme cholesterol oxidase in a buffer solution. Furthermore, all the cholesterol in the ester form was pretreated with cholesterol esterase to convert it to free cholesterol. The experiment showed that the linear range is up to 40 μM. We applied the optimization techniques to completely convert and linearize the cholesterol ester calibration up to 2S or 38 μM using a steady-state current. The optimized Thiele moduli were found to be Thiele modulus 1 = 4 and Thiele modulus 2 = 5, respectively. The membrane thickness was chosen at 0.1 um to match the maximum current in the experiment 70 nA and assumed that the reaction area diameter was 5mm. The simulation results showed excellent agreement with the experimental data. The calibration curve showed that the current response was linear up to 50 μM with a sensitivity of 0.5 nA/μM, which was close to the experiment values of the maximum range 40μM and sensitivity 0.6 nA/μM (**Figure 8**).

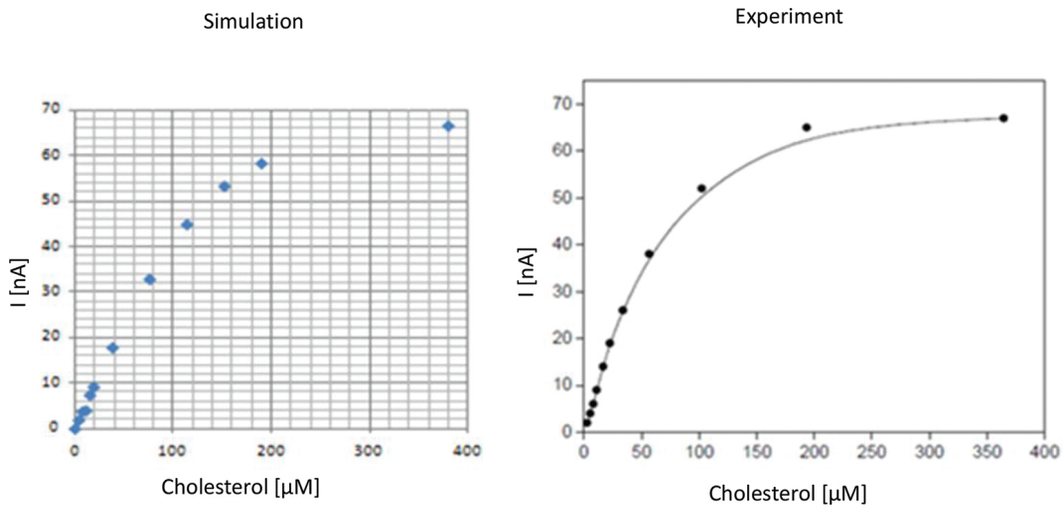

Figure 8. Biosensor model validation. The biosensor current response of cholesterol input predicted by the enzymatic diffusion equation is compared with that derived from the experiment. (A) Biosensor current response by mathematical simulation. Simulation parameters: l = 0.1, cholesterol esterase = 0.029 mg, cholesterol oxidase = 0.21mg, and the Biot number = ∞. (B) Biosensor current response by experimentation. Planar gold electrode modified with immobilized enzyme peroxidase, an insoluble mediator, layers of acetate cellulose, and the enzyme cholesterol oxidase in a buffer solution.

4. Conclusion

We have constructed a mathematical model to simulate biosensor kinetics for cholesterol determination. By monitoring the steady current from the current density plot, the measurement time can be determined, and the current-substrate calibration can be constructed at the steady-state time point. The maximum range is the maximum concentration of cholesterol ester

in the linear region. The characteristic substrate-current activity relationship obeys Michaelis-Menten kinetics, and the equation can be determined by the sensitivity and maximum current density. Through mathematical analysis of system parameters, the optimal enzyme composition is shown to be the minimum amount of enzymes that can achieve total conversion of cholesterol ester as well as produce the highest possible steady-state current. Finally, the model was validated by comparing the results with real-time biosensor data. The free parameters such as membrane thickness or reaction area can be scaled to match specific design criteria or scale-up operation. Several potential improvements for the biosensor model include incorporating a serum diffusion layer by reducing the Biot number to produce a more realistic simulation, generalizing the multienzyme formulation and simulating the biosensor response under different amplification methods. Finally, we anticipated that the computer-aided model could be applied to simulate other multienzyme systems such as glucose or triglyceride determination as well as any other point-of-care diagnostic application.

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