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MODELING OF ELECTROCHEMICALLY SYNTHESIZED THIN FILMS OF CONDUCTING POLYMER FOR THE IMMOBILIZATION OF ENZYME



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ABSTRACT

The model is simplified representation of the real world. The simple model for conducting polymer based biosensor system is presented in the present research work. Now days, electrically conducting polymers have received great attention as a class of advanced material due to their remarkable attributes and new application area in various fields of life. In this work, efforts have been made on the description of mathematical modeling of steady-state analysis of mediated amperometric system.

Keywords: *Mathematical modeling, conducting polymer, flux*

Introduction

Computer stimulation is 'experiment' using computer to realize meaningful result. It is less costly, less time consuming and much safer than actual physical experiment. Simulation is done in two steps. The first step is to build reliable model. Then simulation is performed as the second step with carefully chosen input and parameter. When the input and parameter is not appropriate, model and simulation will give misleading and erroneous result. Simulation helps in selecting suitable input set and design parameter [1]. The advantage of using electrochemically deposited polymers is their ease of preparation and the uniformity of the prepared films.

Provision of a formal theoretical description relies upon the available mathematical tools. Since, in considering biosensors, a chemical change will generally be involved. This will most commonly be based upon differential calculus, the mathematics of change. Further, with parameters changing both with time and with distance from the surface at which the

reaction takes place, problems in differential calculus will often be encountered. The purpose of the solution of these differential equation systems will be to provide a description of the behavior of the system in a more manageable. Frequently algebraic form, predictive studies and experimental data analysis can be readily performed. Theoretical analysis will involve the relatively straightforward process of linking together the different components of the system rather than starting completely afresh [2].

Enzymes are biological catalysts and in the presence of these enzymes biological reaction gets accelerated. These are the specialized group of proteins having specific three dimensional active structure and active sites. An enzyme remains active at optimum condition of temperature, pH, substrate concentration, and enzyme concentration, inhibitors etc. These are various parameters, which are used to measure the rate of enzyme reaction, which involves substrate, which get converted into product with the help of

enzyme. Therefore, the rate of reaction can be measured or determined by either measuring the rate at which substrate is consumed or at the rate at which the product is formed [3].

The specific recognition characteristics of enzyme antibodies and general receptor systems are employed to perform a variety of functions within the organisms from which they are isolated, catalysis, defence, communication and control being the primary ones. This specific recognition is based upon chemical binding of the one component to its complementary partner, this being the target analyte and the biological component of the biosensor respectively. This chemical binding can be very strong indeed and the binding process is frequently very rapid. Whatever the normal function of a biological component within an organism, the basic principle behind its capacity for specific recognition will be the same and, for our current purpose, we need concern ourselves only with the fundamentals of the binding process as it relates to biosensors [4].

Immobilized enzymes are used in many applications both in synthetic and in analytical chemistry [5]. Since, the works of Clark and Lyons, hundreds of paper have been published regarding the design of practical amperometric enzyme electrodes for the analysis of clinically important metabolites and industrial monitoring. Among these reports, two kinds of problems are always the main subject. One is the immobilization of enzyme. Different immobilization methods and materials have been developed including adsorption, cross-linking, self assembly methods and conducting or non-conducting polymers as well as different types of gels. The aim of all these efforts was to obtain an easy handling method with cheap and stable material, which can retain high biological activity of the enzyme. However, a good biosensor depends not only on a good immobilization method but also on a sensitive transducer. This is the other focus in the development of amperometric enzyme electrode [6].

In the present research work, efforts have been made on the description of mathematical modeling of steady-state analysis of mediated amperometric system. This model is presented for electrochemically-polymerized thin films of conducting polymers which

can be used for the immobilization of enzyme on an inert electrode. The simplification of this system allows us to set all fluxes as equal. This flux must also equate with the current for generation of the oxidized mediator at the electrode. By varying substrate concentration (S_{∞}), we have observed the changes in the observed flux ($1/j_{obs}$). The characteristics of the observed flux and the interference effects on its performance have been predicted.

Model Building

Researchers and engineers are usually develops physical models called 'Scale model'. An amperometric enzyme substrate electrode with a PO_2 basic sensor [7], modeling and simulation of a diffusion limited glucose biosensor [8], a coupled two-compartment model for immobilized enzyme electrodes, electrochemical immobilization of enzymes [9], for instance is build to study the measuring range, sensitivity and response time of the sensor. Likewise, amperometric enzyme electrodes [10], electrochemical immobilization of enzyme electrodes [11] is built by researchers and engineers. While these are useful, they are, in most cases, static models. We are often interested in the dynamic performance of system, before building the actual prototype. The physical models are difficult to build and can be expensive.

Most of the models can be structured as three-part system. Some input (substrate) is provided, process (enzyme kinetics and chemical reaction) is done and we get some small output (small signal current). Consider the case of the mediated amperometric device. This is an example of considerable interest in biosensor research. In this type of model, input may be substrate (glucose) and mediator is oxygen. The process starts by defining the various elementary steps involved and their rates. The system consists of an electrode, which monitor and provides the measurement signal. The electrolyte layer, which contains the mediator and the enzyme, an outer membrane which serves to control diffusional mass transport to the electrolyte layer as well as to physically constrain the electrolyte close to the sensing electrode.

The steady state analysis of the mediated amperometric system illustrating the various transport and kinetic processes is shown in Fig.1. Here, the

substrate (S) and oxidized form of the enzyme (E_O) reacts with each other and we get enzyme substrate complex ($E_O S$). The next step is that this complex ($E_O S$) gives the product (P) and reduced form of the enzyme (E_R). By allowing a particular rate of O_2 , we get, oxidized form of enzyme (E_O) and reduced form of mediator (M_R) and at the end at the electrode, reduced form of the mediator gets converted into oxidized form by releasing $2e^-$ (electrons). This gives the current at the electrode. All these reactions have particular rate. These rate constants are known as enzyme kinetics rate constants. They are governed by Michaelis-Menten constant (K_M).

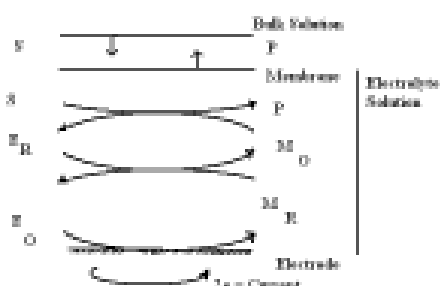


Figure 1 Steady-state analysis of mediated amperometric system.

To begin with models, they are built with a few algebraic equations and a few variables. They may provide a general description of the system. The mathematical model for conducting polymer based amperometric sensor can be built on the basis of material used for the electrode, enzyme used, method of immobilization used, conducting polymer used, size of the sensor which material it sense and what are its area of applications. More complex models would include the more than one enzyme immobilized, rate of electron transfer, rates of various enzyme kinetic reactions and environmental conditions etc. One of the major decisions of a model builder is, then, to choose the variables to be included in the model and to exclude “more complex” or less important variables. A model can grow with addition of variables based on interactions with the users.

Mathematical Structure

Models are built with inter-related set of equations. These equations may be algebraic or differential equations and with logical statements for constraints and bounds. Consider the case of steady-state analysis of mediated amperometric system. It is a probabilistic model. By straightforward algebraic manipulation, an expression describing the output signal in terms of the various kinetic parameters characterizing the system can be derived and the substrate concentration, which it is the purpose of the device to measure. The reaction sequence upon which the system is based can be written as follows.



The relation 1 and 2 represents the oxidation of substrate to product, according to Michaelis-Menten kinetics by the oxidized (E_O) enzyme to give the reduced form of the enzyme (E_R). The relation 3 represents the reoxidation of the reduced enzyme by oxidized mediator (M_O), giving rise to the reduced mediator (M_R). The relation 4 represents the reoxidation of the mediator at an electrode which gives rise to the current upon which the measurement is based. Rates of formation and disappearance of $E_O S$ -complex can be written as,

$$k_1 = \frac{V_f}{[E][S]} \quad \text{and} \quad k_{-1} = \frac{V_d - k_2[E_O S]}{[E_O S]}$$

Where, V_f is the rate of formation and V_d is the rate of disappearance of the bimolecular $E_O S$ -complex.

The steady state assumptions

Consider homogeneous solution enzyme kinetics. A steady state is established in which the rates of different steps occurring in the electrolyte layer are

balanced with one another and with the mass transport of reactant and product to and from it. The assumption requires that the sensing reaction cause negligible change in the bulk solution concentrations of reactant and product. The approximation is valid, provided that the electrolyte layer sufficiently thin so that diffusion times across it are short and that the diffusion rates are relatively rapid as compared with the enzyme reaction rates. We assume that there is an excess of mediator and that the electrode generates M_0 from M_R sufficiently rapidly that the concentration of M_0 is effectively that of the total mediator $[M]$. This condition can be arranged in practice by ensuring that the reoxidation occurs rapidly at the electrode, the electrolyte layer is thin so that the diffusional mass transport of mediator across it to the electrode is also rapid and the mediator concentration is sufficiently in excess of the enzyme concentration.

Estimation of flux of the substrate

Using steady state assumptions, a rate equation for the intermediate can be written as,

$$d[E_0S]/dt = k_1[E][S] - k_{-1}[E_0S] - k_2[E_0S] = 0 \quad [5]$$

As the total concentration of enzyme $[E_T]$, at all times will be the sum of concentrations in free and complexed forms $[E] + [E_0S]$. Now, putting $[E] = [E_T] - [E_0S]$ in the above equation we get,

$$d[E_0S]/dt = k_1[E_T][S] - (k_{-1}[S] + k_{-1} + k_2)[E_0S] = 0 \quad [6]$$

Solving this equation, for $[E_0S]$, we get,

$$[E_0S] = \frac{[E_T][S]}{[S] + (k_{-1} + k_2/k_1)}$$

Where, $K_M = (k_{-1} + k_2/k_1)$, the Michaelis constant.

$$[E_0S] = \frac{[E_T][S]}{[S] + K_M} \quad [7]$$

Putting this into the rate expression for decomposition of the complex to form the product gives the rate (v) of product formation,

$$v = k_2[E_0S] \\ v = k_2[E_T][S] / K_M + [S]$$

$$\frac{1}{j} = \frac{K_M}{k_2[E_T]([S]_{\infty} - j/k_0)} + \frac{1}{k_2[E_T]} + \frac{1}{k_0 M [E_T]} \quad [8]$$

In the above expression each term shows a

different dependence upon the three concentration variables at our control, that of the substrate, enzyme and mediator (O_2). To test the model experimentally, we keep two of these concentrations (enzyme and mediator) constant change the third and investigate the effects on the observed flux. Then, by taking double reciprocal plots and measuring slopes and intercepts from them, we can predict results of simulation about the characteristic rate for each step. When the conducting polymer film is sufficiently thin and there is no concentration polarization of either enzyme (S) or oxygen (O_2) within the film, then in steady state we can write,

$$\frac{1}{j_s} = \frac{K_M}{k_{cat} K_S S_{\infty} E_0 l} + \frac{1}{k_{cat} E_0 l} \quad [9]$$

By rearranging this equation, we get,

$$j_s = \frac{k_{cat} K_S S_{\infty} E_0 l}{K_S S_{\infty} + K_M} \quad [10]$$

This equation describes the enzyme kinetics. And

$$\frac{1}{j_s} = \frac{1}{k_{cat} K_S S_{\infty} E_0 l}$$

This equation represents the contribution to the rate control by the reaction of reduced enzyme with the mediator to generate the oxidized enzyme. Equating equations 10 and 11 and solve them, we get,

$$j_s = \frac{k_{cat} K_S S_{\infty} k_{cat} K_A a_v E_T l}{k_{cat} K_S S_{\infty} + k_{cat} K_A a_v ([K_S S_{\infty} + K_M])}$$

At low substrate concentration, when the term $(k_{cat} K_A a_v K_M)$ in the denominator is dominant, $j_s \propto S_{\infty}$. As the concentration of substrate is increased, either the term $(K_A a_v K_S s_v)$ or the term $(k_{cat} K_S S_v)$ becomes dominant and j_s becomes independent of the substrate concentration. This can occur for two reasons. When the term $(k_{cat} K_A a_v K_S s_v)$ is dominant, then j_s is limited by the saturated enzyme kinetics for the consumption of substrate. When the term $(k_{cat} K_S S_v)$ is dominant, then j_s is limited by the rate of reoxidation of the enzyme by

reaction with the mediator, under these circumstances j_s depends on a_v .

Mathematical Model

The flux of the substrate j_s reacting within the film is not necessarily the same as the flux of reduced mediator detected at the electrode j_{obs} . This happens due to some of the mediator will be lost to the bulk solution. The precise amount will depend on the efficiency of mass transport of hydrogen peroxide (H_2O_2) away from the electrode. When this is very efficient, then the concentration of hydrogen peroxide (H_2O_2) held at zero. At the outside of the film, $j_{obs} = (j_s / 2)$. It is assumed that, the experiment is carried out at the rotating disk electrode; the mass transport of hydrogen peroxide (H_2O_2) away from the electrode can be controlled. We can show that,

$$j_{obs} = (j_s / \acute{a}) \tag{13}$$

$$\acute{a} = \frac{(1 + D_{E, red} l / K_B D_B X_D)}{(1 + D_{E, red} l / 2 K_B D_B X_D)}$$

Note that, $1 < \acute{a} < 2$, as expected. By combining equations 12 and 14, we obtain the expression for the observed current.

$$i = nFA j_s$$

Again, $j_{obs} = j_s \acute{a}$, we get,

Again, $j_{obs} = j_s \acute{a}$, we get,

$$\frac{i}{j_{obs}} = \frac{nFA \acute{a}}{1} = \frac{1}{\acute{a}}$$

$$\frac{i}{j_{obs}} = \frac{nFA \acute{a}}{1} = \frac{k_{cat} K_S S_0 + k K_A \alpha_0 (K_S S_0 + K_M)}{k_{cat} K_S S_0 + k K_A \alpha_0 E_1}$$

Rearrange the terms in the above equation we get,

$$\frac{i}{j_{obs}} = \frac{nFA \acute{a}}{1} = \frac{K_M}{k_{cat} K_S S_0 + k K_A \alpha_0 E_1} + \frac{1}{k_{cat} E_1} + \frac{1}{k K_A \alpha_0 E_1} \tag{15}$$

$$\frac{1}{j_{obs}} = \frac{K_M}{\alpha k_{cat} K_S E_1} + \frac{1}{S_0} + \frac{1 + k_{cat}/k K_A \alpha_0}{\alpha k_{cat} E_1} \times K_S \tag{16}$$

Now, by measuring the current for the enzyme coated electrode as a function of the concentrations of substrate, mediator and of the enzyme loading in

the film, it is possible to estimate the kinetics of the reactions of the immobilized enzyme. This is an operational characteristic of value in a biosensor. By varying the enzyme concentration $[E_T]$, membrane thickness and k_D , one may investigate the contributions of the rates of the two different steps to the overall rate. In equation 16, each term shows a different dependence upon the three concentration variables at our control that of the substrate, enzyme and mediator.

Conclusion

We have described the mathematical model of the steady state mechanism of amperometric system of electrochemically synthesized thin films of conducting polymer on which enzyme was assumed to be immobilized. The flux from which the measured signal obtained is directly proportional to substrate concentration. This is an operational characteristic of value in an enzyme electrode. One may use mathematical model for the development of conducting polymer based mediated amperometric system.

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