Full Paper

Modelling of Amperometric Biosensors in the Case of Substrate Inhibition

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Abstract: The response of an amperometric biosensor at mixed enzyme kinetics and diffusion limitations was modelled digitally in the case of substrate inhibition. Digital simulations were carried out using a finite difference technique. Calculations showed complex kinetics of biosensor response. At low enzyme activity and substrate concentration (S_0), the response of the sensor looks like it is limited by a simple substrate diffusion. At substrate concentration comparable to the Michaelis-Menten constant (K_M), the response change shows a maximal value. A sharp response change was indicated at high enzyme activity and high ($4.9 > S_0/K_M > 4.5$) substrate concentration. This was explained by multi-concentration of substrate generation inside the enzyme layer. This conclusion was confirmed by the analytical solution of the simplified biosensor model with external diffusion limitation at steady-state conditions. The complex kinetics of response change produces different calibration graphs for biosensor response at transition and steady state.

Keywords: biosensor, enzyme inhibition, diffusion, modelling

1. Introduction

Catalytic biosensors are sensors that use enzymes which catalyse a specific conversion of analyte [1]. Amperometric biosensors measure the faradic response that arises on a working indicator electrode due to direct electrochemical oxidation or reduction of products of biocatalytic reaction. In

amperometric biosensors, the potential at the electrode is held constant while the response is measured as a current. The response of amperometric biosensors is determined by enzyme activity and mass transport [1]. It is established that if enzyme activity is low in comparison with the mass transfer rate the response is determined by biocatalytic reaction, and the biosensor acts in a "kinetic regime". If the overall process is determined by mass transport through a stagnant (Nernst) layer the biosensor acts in an "external diffusion limitation" regime. If the process is limited by mass transport inside the biocatalytic membrane the biosensor acts in an "internal diffusion limitation" regime. The modelling of biosensors utilizing simple Michaelis-Menten kinetics in different regimes and geometries of electrodes and micro bioreactors was carried out using digital integration [2-16].

Very often the kinetics of enzyme action is much more complicated. Inhibition, activation, allostery and other types of non-Michaelis-Menten kinetics are known for the diversity of enzymes [17]. The simplest scheme of non-Michaelis-Menten kinetics may be, for example, described by adding to the Michaelis-Menten scheme (1) the relationship of the interaction of the enzyme substrate complex (ES) with another substrate molecule (S) (2) followed the generation of non-active complex (ES$_2$):

$$E + S \leftrightarrow ES \rightarrow E + P \tag{1}$$

$$ES + S \leftrightarrow ES_2 \tag{2}$$

For this scheme, the steady-state "initial" rate (V_{st}) shows maximum dependence of V_{st} on substrate concentration.

The modelling of the amperometric biosensor utilizing the enzyme with substrate inhibition has been very recently performed at external diffusion limitation and steady state conditions [18]. The inhibitory constant in these calculations was assumed to be equal to the Michaelis-Menten constant. These simplified calculations revealed a multi-steady response of the biosensor if the diffusion module was much larger than 1 and the substrate bulk concentration was much higher than the Michaelis-Menten parameter [18]. The non-steady state and a more generalised analysis of amperometric biosensor action at substrate inhibition can be performed using digital calculations. This work presents the results of non-steady state biosensor modelling at mixed enzyme kinetics, external and internal diffusion limitation with substrate inhibition.

2. Mathematical Model

It is assumed that substrate (S) conversion to product (P) was catalysed by the enzyme (E) and proceeded according to the scheme (1, 2). The biosensor is considered as an electrode having a layer of enzyme (enzyme membrane) applied to the electrode surface. The model involves three regions: (i) the enzyme layer where enzymatic reaction, as well as mass transport, by diffusion takes place; (ii) the diffusion region where only mass transport by diffusion takes place; (iii) the convective region where the analyte concentration is maintained constant. Assuming the symmetrical geometry of the electrode and homogeneous distribution of immobilized enzyme in the enzyme layer of uniform thickness, the concentration of substrate and product at $t > 0$ can be expressed by the system of reaction-diffusion equations:

$$\frac{\partial S_e}{\partial t} = D_{Se} \frac{\partial^2 S_e}{\partial x^2} - \frac{V_{\max} S_e}{K_M + S_e + S_e^2 / K_i},$$

$$\frac{\partial P_e}{\partial t} = D_{Se} \frac{\partial^2 P_e}{\partial x^2} + \frac{V_{\max} S_e}{K_M + S_e + S_e^2 / K_i}, \quad 0 < x < d, \tag{3}$$

$$\frac{\partial S_b}{\partial t} = D_{Sb} \frac{\partial^2 S_e}{\partial x^2},$$

$$\frac{\partial P_b}{\partial t} = D_{Pb} \frac{\partial^2 P_e}{\partial x^2}, \quad d < x < d + \delta, \tag{4}$$

where x stands for space, t stands for time, $S_e(x, t)$, $S_b(x, t)$ ($P_e(x, t)$, $P_b(x, t)$) are the concentration of the substrate (reaction product) in the enzyme and in the bulk solution, respectively, d is the thickness of the enzyme layer, δ is the thickness of the diffusion layer, $V_{\max}$ is the maximal enzymatic rate, K_M and K_i are Michaelis-Menten and inhibition constants, respectively, D_{Se} and D_{Pe} are the diffusion coefficients of the substrate and the reaction product in the enzyme layer, respectively, D_{Sb} and D_{Pb} are the corresponding diffusion coefficients in the bulk solution.

Let $x = 0$ represent the electrode surface, while $x = d$ – the boundary between the enzyme membrane and the buffer solution. The biosensor starts to respond when some substrate appears in the bulk solution. This is used to define initial conditions ($t = 0$)

$$S_e(x,0) = 0, \quad P_e(x,0) = 0, \quad 0 \le x \le d, \tag{5}$$

$$S_b(x,0) = 0, \quad P_b(x,0) = 0, \quad d \le x < d + \delta, \tag{6}$$

$$S_b(d + \delta, 0) = S_0, \quad P_b(d + \delta, 0) = 0, \tag{7}$$

where S_0 is the concentration of the substrate in the bulk solution.

On the boundary between two regions having different diffusivities, we define the matching conditions ($t > 0$).

$$D_{Se} \frac{\partial S_e}{\partial x}\bigg|_{x=d} = D_{Sb} \frac{\partial S_b}{\partial x}\bigg|_{x=d}, \quad S_e(d,t) = S_b(d,t), \tag{8}$$

$$D_{Pe} \frac{\partial P_e}{\partial x}\bigg|_{x=d} = D_{Pb} \frac{\partial P_b}{\partial x}\bigg|_{x=d}, \quad P_e(d,t) = P_b(d,t). \tag{9}$$

These conditions mean that fluxes of the substrate and product through the stagnant external layer is equal to the corresponding fluxes entering the surface of the enzyme membrane. The partition of the substrate and the product in the membrane vs bulk is assumed to be equal.

In the bulk solution, the concentration of the substrate, as well as of the product, remains constant ($t > 0$),

$$S_b(d + \delta, t) = S_0, \quad P_b(d + \delta, t) = 0. \tag{10}$$

Because of electrode polarization, the concentration of the reaction product at the electrode surface ($x = 0$) is permanently reduced to zero. Since the substrate is electro-inactive substance, this is described by the following boundary conditions ($t > 0$):

$$P_e(0,t) = 0 \,, \tag{11}$$

$$D_{Se} \left. \frac{\partial S_e}{\partial x} \right|_{x=0} = 0 \,. \tag{12}$$

The diffusion layer ($d < x < d + \delta$) may be treated as the Nernst diffusion layer. According to the Nernst approach, a layer of thickness δ remains unchanged with time. Away from it, the solution is in motion and is uniform in concentration.

The concentration S of the substrate and the concentration P of the reaction product can be defined for the entire domain $0 \le x \le d + \delta$ as follows:

$$S(x,t) = \begin{cases} S_e(x,t), & 0 \le x \le d, \ t \ge 0, \\ S_b(x,t), & d < x \le d + \delta, t \ge 0, \end{cases} \tag{13}$$

$$P(x,t) = \begin{cases} P_e(x,t), & 0 \le x \le d, \ t \ge 0, \\ P_b(x,t), & d < x \le d + \delta, t \ge 0. \end{cases} \tag{14}$$

Let us notice that due to the matching conditions (10) and (11) both concentrations, S and P, are continuous in the entire domain: $0 \le x \le d + \delta$.

The measured response is accepted as the current of a biosensor in physical experiments. The current is directly proportional to the flux of the reaction product at the electrode surface, i.e. on the border $x = 0$. Since the total response is also directly proportional to the area of the electrode surface, we normalize the total response with the area of that surface. The density $i(t)$ of the biosensor current at time t can be obtained explicitly from Faraday's and Fick's laws,

$$i(t) = n_e F D_{Pe} \left. \frac{\partial P_e}{\partial x} \right|_{x=0} \,, \tag{15}$$

where n_e is the number of electrons involved in charge transfer at the electrode surface, and F is the Faraday constant, $F = 96485$ C/mol. We assume that the system (3)-(14) approaches a steady state as $t \to \infty$,

$$I = \lim_{t \to \infty} i(t) \,, \tag{16}$$

where I is assumed as the steady-state biosensor response.

A simplified analytical solution of the biosensor response was performed at external diffusion limitation at steady-state conditions assuming that surface enzyme activity is $V_{max}d$. The steady-state surface rate (V_{st}^*) of enzyme activity following the scheme (1, 2) is described by:

$$V_{st}^* = \frac{V_{max} d S_s}{K_M + S_s + S_s^2 / K_i} \tag{17}$$

where S_s is the substrate concentration at $x = d$.

At steady-state conditions, the substrate flux (J_{st}) through the Nernst diffusion layer is equal to the enzymatic rate on the surface:

$$J_{st} = \frac{D_{Se}(S_0 - S_s)}{\delta} = \frac{V_{max} d S_s}{K_M + S_s + S_s^2 / K_i} \,, \quad x = d, \tag{18}$$

S_s at fixed S_0 was calculated solving the cubic equation (18) and using a symbolic processor of *"Mathcad"* (MathSoft Inc., Cambridge, MA). The analytical solution is not presented in the text due to a very bulky expression.

The steady-state biosensor response for this case was calculated:

$$I = n_e F J_{st} \tag{19}$$

The digital modelling of biosensor response was evaluated for different values of the maximal enzymatic rate (V_{max}), substrate concentration (S_0) and the thickness d of the enzyme layer. The following values of the model parameters were constant in all simulations:

$$D_{Se} = D_{Pe} = 100 \mu m^2/s, \quad D_{Sb} = 2D_{Se}, \quad D_{Pb} = 2D_{Pe}, \\ K_M = 0.01M, \quad K_i = 0.1K_M, \quad \delta = 300 \mu m, \quad n_e = 1. \tag{20}$$

3. Results and discussion

The modelling of the amperometric biosensor was performed at 2 critical values of V_{max}. At $V_{max} = 10$ mM/s and $d = 20$ μm, the internal diffusion module calculated as $\Phi = (V_{max}/K_M D_{Se})^{1/2}d$ was 2, and at $d = 10$ μm it was 1. The formal external diffusion module calculated as $\rho = V_{max}d\delta/K_M D_{Sb}$ was 30 and 15 for the respective thickness of the enzyme layer. This means that the biosensor response was strongly limited by diffusion.

At 100 times lower catalytic activity, i.e. at $V_{max} = 100$ μM/s, Φ was 0.2 and 0.1, whereas ρ was 0.3 and 0.15 for $d = 20$ μm and $d = 10$ μm, respectively. The values of diffusion modules less than 1 indicated that the biosensor acted in a kinetic regime.

The digital modelling of the biosensor with low active enzyme indicated a complex character of the transition state (Fig. 1). At low substrate concentration, the kinetic curves resembled the diffusion shape, whereas at S_0/K_M above 0.4 they showed maximal values.

The dependence of the maximal and steady-state responses on the normalised substrate concentration is shown in Fig.2.

The appearance of the maximal response value is associated with enzyme kinetics. The differentiation of the enzyme reaction rate on the bulk substrate concentration gave the maximal value at S_0/K_M=0.316. A similar maximal value can be indicated in the dependence of response on substrate concentration (Fig. 2). The appearance of the maximal response at non-steady conditions (Fig. 1) is associated with change in the substrate concentration in the enzyme layer that agrees with the dependence of the enzyme rate on the substrate concentration. The steady-state response is determined by the stationary substrate concentration and enzyme rate. This response is less than the maximal value due to the inhibition of enzyme activity with the substrate.
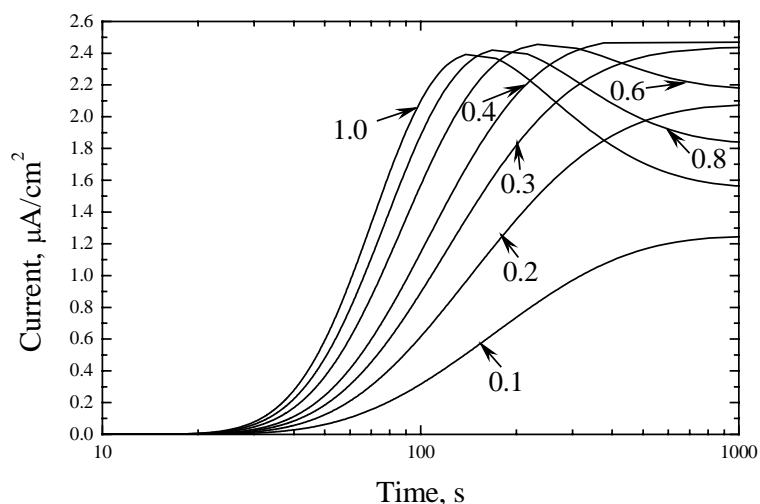
Figure 1. Kinetics of biosensor response at different values of the normalised substrate concentration. Numbers on the curves show S_0/K_M values, $V_{max} = 100$ μM/s, $d = 20$ μm.
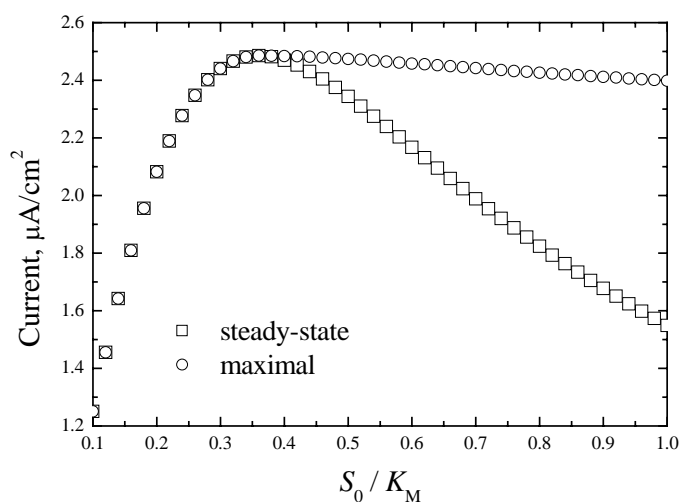

Figure 2. Dependence of the maximal and steady-state response on the normalised substrate concentration. Calculation parameters as in Fig. 1.

At the enzyme layer with a thickness of 10 μm, the response is almost 2 times lower, and the maximal value of the normalised substrate fits more precisely the value 0.316 (data not shown in figures). These results indicate that biosensor response is determined by enzyme kinetics.

When enzyme activity is high, the response change is quite different at different substrate concentrations (Fig. 3). At S_0/K_M less than 4.5, the response monotonically increases and saturates during 3000 s. However, a further small increase in substrate concentration causes a sharp decrease in response for a time of about 1000 s. This time   becomes shorter to 300 s at $S_0/K_M = 4.9$, but the character of response change is similar. Further increase in S_0 changes the character of response kinetics as it is shown for S_0/K_M value 6 (Fig. 3).
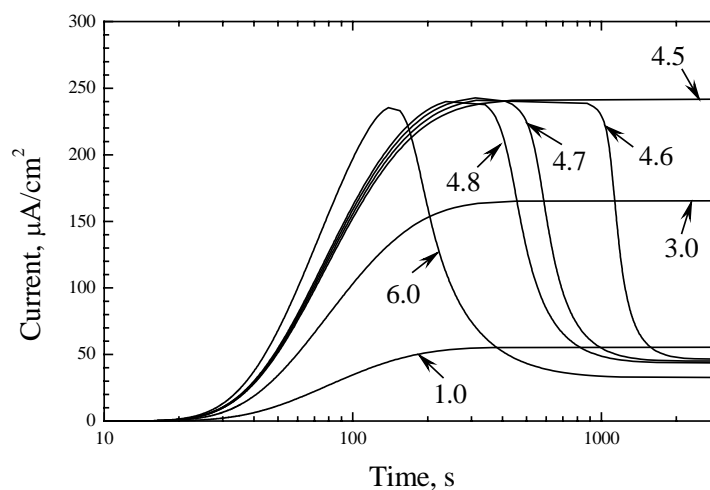
Figure 3. Kinetics of biosensor response at different values of the normalised substrate concentration. Numbers on the curves show S_0/K_M values, $V_{max} = 10$ mM/s, $d = 20$ μm.

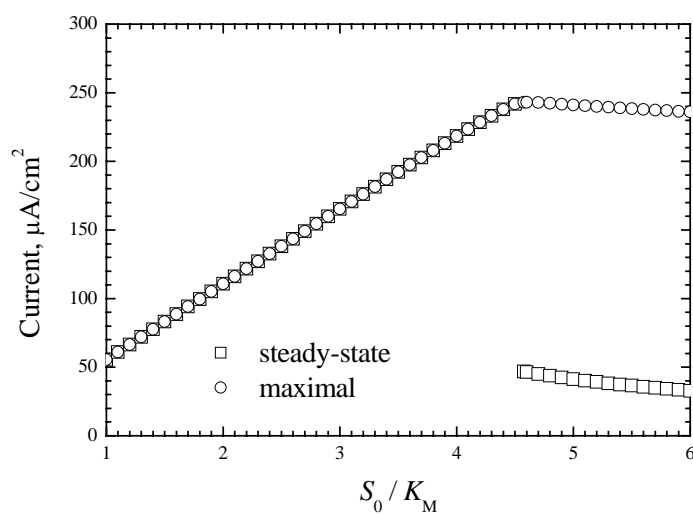These sharp changes in response more precisely demonstrate the data presented in Fig. 4.

Figure 4. Dependence of the maximal and steady-state response on the normalised substrate concentration. Calculation parameters as in Fig. 3.

If the thickness of the enzyme layer is lower ($d = 10$ μm), the effect of a sharp change in response is much less pronounced (Fig.5). Only at S_0/K_M value of about 2.5, some acceleration of response change can be indicated at $t = 1000$ s.
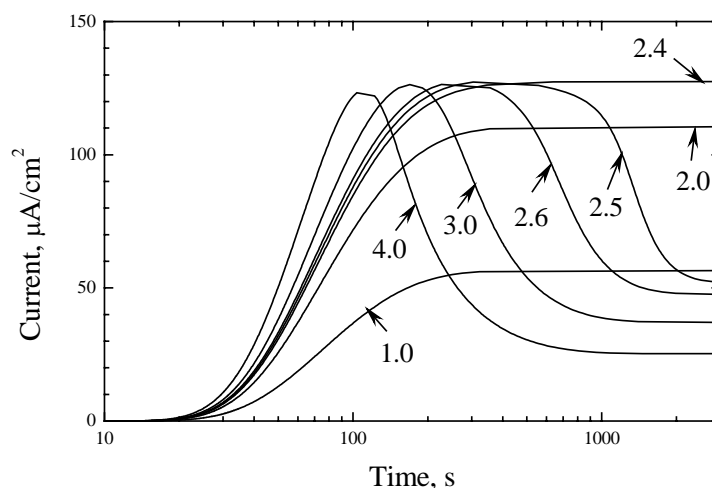
Figure 5. Kinetics of biosensor response at different values of the normalised substrate concentration. Numbers on the curves show S_0/K_M values, $V_{max} = 10$ mM/s, $d = 10$ μm.
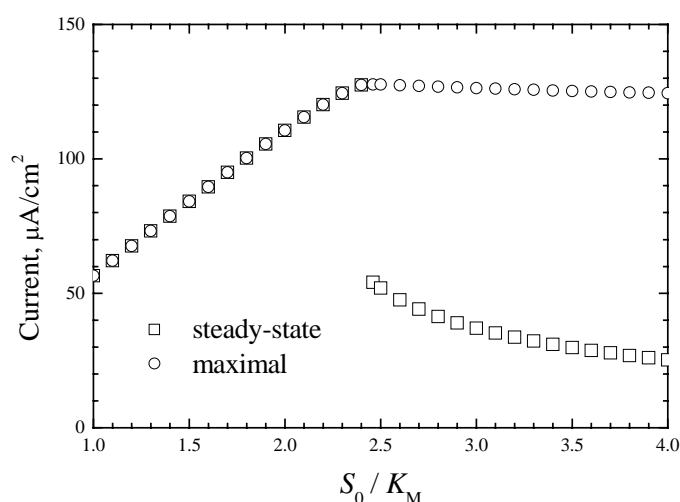

Figure 6.   Dependence of the maximal and steady-state response on the normalised substrate concentration. Calculation parameters as in Fig. 5.

To explain a sharp response change and non-typical calibration curves, the biosensor response was modelled at simplified external diffusion limitation and steady-state conditions (18), (19). If $V_{max} = 10$ mM/s and $d = 20$ μm the analytical solution gave biosensor steady-state responses 62, 185, 50, 48, 47, 46 and 34 μA/cm2 at S_0/K_M 1.0, 3.0, 4.5, 4.6, 4.7, 4.8 and 6.0, respectively. These values, with exception of the value at $S_0/K_M = 4.5$, are very similar to those digitally calculated. The difference of the values at $S_0/K_M = 4.5$ indicates some critical concentration when the simple external diffusion model does not further represents the model of the biosensor. The modelling of simple external diffusion limitation, however, permits to explain this difference, as well as the sharp change in response kinetics (Fig.3) and concentration dependence (Fig. 4).

The solution of the cubic equation (18) produces 3 meanings of S_s at some S_0/K_M. At $V_{max} = 10$ mM/s and $d = 20$ μm these values appear at $3.3 < S_0/K_M < 4.4$. If $d = 10$ μm the zone is more narrow; 3 solutions can be found at $2.2 < S_0/K_M < 2.4$. At low enzyme activity there is always only a single value of S_s. These calculations indicate the zones of S_0/K_M where multi steady-state solution exists. They are close to the critical zones found by digital calculations, and little difference is caused by excluding the internal diffusion in the simplified model.

The presence of zones with multi-concentrations means that at the same bulk substrate concentration 2 stable surface concentrations may exist. At external diffusion limitation, the concentration change was indicated at the vanishing S_0 change [18]. The presence of the enzyme layer with fixed thickness produces different concentration zones inside the enzyme layer. Therefore, the transition is not so sharp. Nevertheless, the kinetics of response change in the multi-concentration zone $(4.9 > S_0/K_M > 4.5)$ are still faster than in the other zones indicating a critical state of the system (Figs. 3 and 4). A more detailed analysis of these processes, possibly, can be evaluated with higher space resolution during the digital modelling. A decrease in enzyme layer thickness decreases diffusion modules and the multi-concentration zone becomes very narrow. For this reason the kinetics of response change (Figs. 5 and 6) are similar to those of all substrate concentrations (Figs. 1 and 2).

4. Conclusions

The modelling of the amperometric biosensor at mixed enzyme kinetics and diffusion limitation revealed complex kinetics of biosensor response. At low enzyme activity and substrate concentration, kinetics looks like a simple substrate diffusion. At substrate concentration comparable to the Michaelis-Menten constant (K_M), the response change shows a maximal value. A sharp response change was indicated at high enzyme activity and high (in comparison with K_M) substrate concentration. This was explained by multi-concentration generation that was confirmed by the analytical solution of a simplified model with external diffusion limitation at steady-state conditions. Digital simulations show that substrate inhibition produces different calibration graphs for biosensor response at transition and steady state.

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