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Computer Simulation of Amperometric Biosensor Response to Mixtures of Compounds*

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Abstract. A mathematical model of amperometric biosensors has been developed. The model bases on non-stationary diffusion equations containing a non-linear term related to Michaelis-Menten kinetic of the enzymatic reaction. The model describes the biosensor response to mixtures of multiple compounds in two regimes of analysis: batch and flow injection. Using computer simulation, large amount of biosensor response data were synthesised for calibration of a biosensor array to be used for characterization of wastewater. The computer simulation was carried out using the finite difference technique.

Keywords: reaction-diffusion, modelling, biosensor.

1 Introduction

Biosensors are devices that combine the selectivity and specificity of a biologically active compound with a signal transducer and an electronic amplifier [1–4]. The transducer converts the biochemical signal to an electronic

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signal. The biosensor signal is proportional to the concentration of measured analyte or a group of analytes. The biosensors are classified according to the nature of the physical transducer. Amperometric biosensors measure the current on an indicator electrode due to direct oxidation of the products of the biochemical reaction. In case of the amperometric biosensors the potential at the electrode is held constant while the current flow is measured. The amperometric biosensors are reliable, relatively cheap and highly sensitive for environment, clinical and industrial purposes.

Starting from the publication of Clark and Lyons [1], the amperometric biosensors became one of the popular and perspective trends of biochemistry. The understanding of the kinetic regularities of biosensors is of crucial importance for their design. Mathematical models can explain such regularities. The general features of amperometric response was analyzed in the publications of Mell and Maloy [5,6]. Some later reports were also devoted to the modelling and investigation of the amperometric biosensor response [7–11].

The goal of this investigation is to make a model allowing an effective computer simulation of amperometric biosensor response to a group of analytes (mixtures). The developed model is based on non-stationary diffusion equations [12], containing a non-linear term related to Michaelis-Menten kinetic of the enzymatic reaction. The model allows to simulate the biosensor action in batch and flow injection regimes. In the flow injection analysis the biosensor contacts with the substrate for short time whereas in the batch analysis the biosensor is assumed as immersed in the substrate solution of infinite volume and during long time [13]. The digital simulation of the biosensor response was carried out using the semi-implicit finite difference scheme [14,15].

The developed software was employed to generate multiple biosensor response data for four specific analytes of eight different concentrations. The generated data was then used for the amperometric calibration of a biosensor array [16]. Data needed for a biosensor calibration can be produced by multiple physical experiments. However, computer simulation is much cheaper and faster than the physical experiment. Development of methods of analysis of mixtures with a biosensor array and chemometrics using a multivariate calibration is following [17]. The software for characterisation of wastewater (alarm system) is under development.

2 Mathematical Model

During an enzyme-catalysed reaction

$$\mathrm{S}_k \xrightarrow{\mathrm{E}} \mathrm{P}_k, \qquad k = 1,...,K \tag{1}$$

the mixture of substrates (compounds) (S_k, $k = 1,...,K$) binds to the enzyme (E) to form enzyme-substrate complex. While it is a part of this complex, the substrate S_k is converted to the product (P_k). The rate of the reaction is the rate of appearance of the product. This rate is known to depend upon the concentration of substrate.

Let us consider an amperometric biosensor, which can be treated as enzyme electrode, having a layer of enzyme immobilised onto the surface of the probe. Assuming no interaction between analysed substrates (compounds) of the mixture, the symmetrical geometry of the electrode, homogeneous distribution of immobilised enzyme in the enzyme membrane, and considering one-dimensional diffusion, coupling of enzyme reaction with the diffusion described by Fick's law leads to the following equations:

$$\frac{\partial S^{(k)}}{\partial t} = D_{\mathrm{S}}^{(k)} \frac{\partial^2 S^{(k)}}{\partial x^2} - \frac{V_{\max}^{(k)} S^{(k)}}{K_{\mathrm{M}} + S^{(k)}}, \quad 0 < x < d, \quad 0 < t \le T, \tag{2}$$

$$\frac{\partial P^{(k)}}{\partial t} = D_{\mathrm{P}}^{(k)} \frac{\partial^2 P^{(k)}}{\partial x^2} + \frac{V_{\max}^{(k)} S^{(k)}}{K_{\mathrm{M}} + S^{(k)}}, \quad 0 < x < d, \quad 0 < t \le T, \tag{3}$$

$$k = 1,...,K,$$

where K is the number of compounds, $V_{\max}^{(k)}$ is the maximal enzymatic rate of biosensor attainable with that amount of enzyme, when the enzyme is fully saturated with substrate (compound) S_k, K_{M} is the Michaelis constant, $S^{(k)}$ is the concentration of substrate S_k, $P^{(k)}$ is concentration of the reaction product P_k, d is thickness of the enzyme layer, t is time, T is full time of biosensor operation to be analysed, $D_{\mathrm{S}}^{(k)}$ and $D_{\mathrm{P}}^{(k)}$ are diffusion coefficients of the substrate S_k and product P_k, respectively.

The biosensor operation starts when some substrate appears over the surface of the enzyme layer. This is used in the initial conditions ($t = 0$)

$$S^{(k)}(x,0)=\begin{cases}0, & 0\le x<d,\\ S_0^{(k)}, & x=d,\end{cases} \tag{4}$$

$$P^{(k)}(x,0)=0,\quad 0\le x\le d, \tag{5}$$

$$k=1,\ldots,K,$$

where $S_0^{(k)}$ is the concentration of substrate S_k over the biosensor.

Because of electrode polarisation, the concentration of the reaction product at the electrode surface is being permanently reduced to zero. If the substrate is well-stirred and in powerful motion, then the diffusion layer ($0 < x < d$) will remain at a constant thickness. Consequently, the concentration of substrate as well as product over the enzyme surface (bulk solution/membrane interface) remains constant while the biosensor contact with the substrate. In the flow injection regime the biosensor contacts the substrate for short time only (seconds to tens of seconds) [13]. When the analyte disappears, a buffer solution swills the enzyme surface, reducing the substrate concentration at this surface to zero. Because of substrate (analyte) remaining in the enzyme membrane, the mass diffusion as well as the reaction still continues some time even after the disconnect of the biosensor and substrate. This is used in the boundary conditions ($0 < t \le T$) given by

$$\left.\frac{\partial S^{(k)}}{\partial x}\right|_{x=0}=0, \tag{6}$$

$$S^{(k)}(d,t)=\begin{cases}S_0^{(k)}, & t\le T_{\mathrm{F}},\\ 0, & t>T_{\mathrm{F}},\end{cases} \tag{7}$$

$$P^{(k)}(0,t)=P^{(k)}(d,t)=0, \tag{8}$$

$$k=1,\ldots,K,$$

where T_{F} is the time of flow injection, i.e., the time when analyte is removed from the bulk solution/membrane interface.

In the batch regime of the analysis the modelled biosensor remains as immersed in the substrate all the analysing time. Assuming $T_{\mathrm{F}} = T$ the model expressed by equations (2)–(8) may be accepted for batch analysis as well. In the batch analysis the boundary condition (7) reduces to $S^{(k)}(d,t)=S_0^{(k)}$, $t\le T$.

In computer simulation, discussed below, the initial condition $S^{(k)}(x,0) = \varphi^{(k)}(x)$ was employed instead of (4) to avoid a discontinuity at $x = d$. Here $\varphi^{(k)}$ is a continuous function: $\varphi^{(k)}(x) = 0$, at $0 \le x \le d - \varepsilon$, $\varphi^{(k)}(x)$ monotonous increases at $d - \varepsilon < x \le d$, and $\varphi^{(k)}(d) = S_0^{(k)}$, $k = 1,\ldots,K$. Several different expressions of $\varphi^{(k)}$ as well as values of small ε were employed. Using the similar technique, the discontinuous boundary condition (7) was also reduced to a continuous one. However, notable difference between solutions was not observed. That is why the equations (4) and (7) were used in the simulation.

The current is measured as a response of a biosensor in a physical experiment. The biosensor current depends upon the flux of reaction product at the electrode surface, i.e., at border $x = 0$. Consequently, density $I^{(k)}(t)$ of the biosensor current, as a result of the reaction of the substrate S_k with the product P_k at time t, is proportional to the concentration gradient of the product at the surface of the electrode as described by Faraday's law:

$$I^{(k)}(t) = n_e F D_P^{(k)} \left.\frac{\partial P^{(k)}}{\partial x}\right|_{x=0}, \quad k = 1,\ldots,K, \tag{9}$$

where n_e is a number of electrons involved in a charge transfer at the electrode surface, and F is Faraday constant. In the case, where the biosensor signal equals to the sum of signals of individual analytes, having values of the biosensor current $I^{(k)}(t)$ for all compounds, $k = 1,\ldots,K$, the common density $I^*(t)$ of the biosensor current at time t can be calculated additively

$$I^*(t) = \sum_{k=1}^{K} I^{(k)}(t). \tag{10}$$

2 Solution of the Problem

Let us notice that there is no direct relationship between pairs of the unknown variables $S^{(k_1)}, P^{(k_1)}$ and $S^{(k_2)}, P^{(k_2)}$, when $k_1 \ne k_2$, $k_1, k_2 = 1,\ldots,K$ in equations (2)–(8). Because of this the initial and boundary value problem (2)–(8), which

consists of $7K$ equations can be splitted to K problems, containing only seven equations (2)–(8) at given k, $k = 1,...,K$. The problem (2)–(8), formulated for given k_1 (compound S_{k_1}), can be solved individually and independently from the problem, formulated for another compound S_{k_2}, $k_1,k_2 = 1,...,K$, $k_2 \neq k_1$.

Let us assume the problem (2)–(8) formulation for a single substrate $S = S_k$ and reaction product $P = P_k$, i.e., in a case when $K = 1$. Let V_{max} be the maximal enzymatic rate of the modelled biosensor, S is the concentration of substrate S, and P is concentration of the reaction product P.

The problem (2)–(8), reformulated for substrate S and reaction product P, was solved numerically using the finite difference technique [14,15]. To find a numerical solution of the problem in the domain $[0,d]\times[0,T]$ we introduced an uniform discrete grid $\omega_h \times \omega_\tau$, where

$$\begin{aligned} &\omega_h = \{x_i : x_i = ih, \quad i = 0,...,N_1; \quad hN_1 = d\}, \\ &\omega_\tau = \{t_j : t_j = j\tau, \quad j = 0,...,N_F,...,N_2; \quad \tau N_F = T_F, \quad \tau N_2 = T\}. \end{aligned} \tag{11}$$

Let us assume the following

$$S_i^j = S(x_i,t_j), \quad P_i^j = P(x_i,t_j), \quad i = 0,...,N_1; \quad j = 0,...,N_2. \tag{12}$$

A semi-implicit linear finite difference scheme has been built as a result of the difference approximation. The initial conditions (4) and (5) we approximated as follows

$$\begin{aligned} &S_i^0 = 0, \quad i = 0,...,N_1 - 1; \quad S_{N_1}^0 = S_0, \\ &P_i^0 = 0, \quad i = 0,...,N_1. \end{aligned} \tag{13}$$

Differential equations (2), (3) were approximated by the scheme

$$\frac{S_i^{j+1} - S_i^j}{\tau} = D_S \frac{S_{i+1}^{j+1} - 2S_i^{j+1} + S_{i-1}^{j+1}}{h^2} - \frac{V_{max} S_i^{j+1}}{K_M + S_i^j}, \tag{14}$$

$$\frac{P_i^{j+1} - P_i^j}{\tau} = D_P \frac{P_{i+1}^{j+1} - 2P_i^{j+1} + P_{i-1}^{j+1}}{h^2} + \frac{V_{max} S_i^{j+1}}{K_M + S_i^{j+1}}, \tag{15}$$

$$i = 1,...,N_1 - 1, \quad j = 1,...,N_2 - 1.$$

The boundary conditions (6)–(8) were approximated as follows:

$$S_0^j = S_1^j, \quad j = 1,...,N_2,$$
$$S_N^j = S_0, \quad j = 1,...,N_F, \tag{16}$$
$$S_N^j = 0, \quad j = N_F + 1,...,N_2,$$
$$P_0^j = 0, \quad P_{N_1}^j = 0, \quad j = 1,...,N_2. \tag{17}$$

Equations (13) allow to calculate a solution of the problem on the layer $t = t_0 = 0$. When a solution on a layer t_j has been calculated, a solution on the next layer $t = t_{j+1}$ can be calculated in two steps:

1) calculate values of $S_i^{j+1}, i = 0,...,N_1$, solving the system of linear equations (14), (16);
2) calculate values of $P_i^{j+1}, i = 0,...,N_1$, solving the system of linear equations (15), (17) using values of S_i^{j+1}, which have been calculated in step 1.

The systems of linear algebraic equations can be solved efficiently in both steps above because of the tridiagonality of the matrices of the systems.

Having numerical solution of the problem, the density of biosensor current at time $t = t_j$ is calculated by

$$I(t_j) = n_e F D_P \left(P_1^j - P_0^j\right)/h, \quad j = 0,...,N_2. \tag{18}$$

In the common case of K compounds, having responses of the biosensor to each compound individually, equation (10) allows to calculate the common biosensor response to the mixture of K compounds. To obtain values $I^*(t_j)$, $j = 0,...,N_2$, of the common biosensor current, it is required:

a) to run computer simulation K times to obtain values $I^{(k)}(t_j)$ of the biosensor current using (18) for each compound of the mixture, $k = 1,...,K$; $j = 0,..., N_2$;
b) to calculate the common biosensor current as defined in (10).

In step (a) only values of the following parameters: $D_S^{(k)}$, $D_P^{(k)}$, $V_{max}^{(k)}$ and $S_0^{(k)}$ vary when one computer simulation changes the next one. This procedure of computation is valid for both regimes of analysis: batch and flow injection.

3 Data Synthesis

The developed computer simulation software was employed to generate data for a calibration of an amperometric biosensor. The biosensor was calibrated for mixtures of four ($K = 4$) compounds. Each compound of eight ($M = 8$) different concentrations was employed in the calibration to have the biosensor response to a wide range of substrate concentrations. Because of this it was required to solve the problem (2)–(8) for given compound S_k numerically $K \times M = 4 \times 8 = 32$ times at 4 different values of the maximal enzymatic rate $V_{\max}^{(k)}$ and 8 values of the substrate concentration $S_0^{(k)}$.

The following values of the parameters were assumed constant in the all numerical experiments:

$$\begin{aligned} &D_{\mathrm{S}}^{(k)} = D_{\mathrm{P}}^{(k)} = 3 \times 10^{-6}\,\mathrm{cm}^2/\mathrm{s}, \quad k = 1,\ldots,K, \\ &K_{\mathrm{M}} = 1 \times 10^{-7}\,\mathrm{mol/cm}^3, \quad n_{\mathrm{e}} = 2, \quad d = 0.02\,\mathrm{cm}. \end{aligned} \tag{19}$$

Each compound of the mixture was characterized by the individual maximal enzymatic rate $V_{\max}^{(k)}$:

$$V_{\max}^{(k)} = 10^{k-11}\,\mathrm{mol/cm}^3\mathrm{s}, \quad k = 1,\ldots,K. \tag{20}$$

The following values of the concentration $S_0^{(k)}$ of each of K substrates $S_1,\ldots,S_K$ of the mixture were employed:

$$\begin{aligned} &S_0^{(k)} \in \left\{ S_{0,m} : S_{0,m} = \alpha_m \times S_0, \ m = 1,\ldots,M \right\}, \ k = 1,\ldots,K, \\ &S_0 = 10^{-8}\,\mathrm{mol/cm}^3, \ M = 8, \\ &\alpha_1 = 1, \ \alpha_2 = 2, \ \alpha_3 = 4, \ \alpha_4 = 8, \ \alpha_5 = 12, \ \alpha_6 = 16, \ \alpha_7 = 32, \ \alpha_8 = 64. \end{aligned} \tag{21}$$

Two parameters: T_{F} and T depend on the regime of analysis. In flow injection analysis due to the disappearance of the current, time T was considerably less than in batch analysis. We employed T = 300 s, $T_{\mathrm{F}} = T$ in batch analysis, while T = 100 s, T_{F} = 10 s in flow injection one.

Only values of two parameters: $V_{\max}^{(k)}$ and $S_0^{(k)}$ varied when one computer simulation changes the next one. In addition, every computer simulation was

repeated twice to simulate biosensor response in batch as well as flow injection regime at different values of T_F and T.

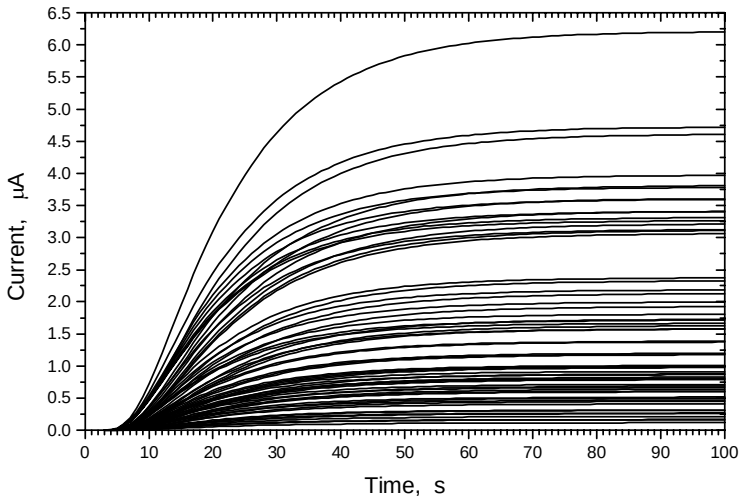

Fig. 1. Every 64th biosensor response curve of full factorial of MK responses at K = 4 values of the maximal enzymatic rate and M = 8 substrate concentrations in batch analysis

Let $I_m^{(k)}(t_j)$ be a value of density $I^{(k)}(t_j)$ of the biosensor current at concentration $S_0^{(k)} = S_{0,m}^{(k)}$ of substrate S_k, $m = 1,...,M$; $j = 1,...,N_2$; $k = 1,...,K$. Having M numerical solutions (M sets of biosensor response values) $I_m^{(k)}(t_j)$, $j = 1,...,N_2$, for each $k = 1,...,K$ (in total $K \times M$ solutions), the full factorial $I_{m_1,...,m_K}(t_j)$ of $M^K = 8^4 = 4096$ solutions can be produced additively:

$$I^*_{m_1,...,m_K}(t_j) = \sum_{k=1}^{K} I_{m_k}^{(k)}(t_j), \quad m_1,...,m_K = 1,...,M; \quad j = 1,...., N_2 . \qquad (22)$$

The simulated biosensor response data was passed to a chemometric analysis. During computer simulation, values of the biosensor current were stored in a file every second of simulation. Only L values of $I_m^{(k)}(t_l)$, $t_l = l\text{-}1$ (s), $l = 1,...,L$; $L = T + 1$ for each $k = 1,...,K$ and $m = 1,...,M$ were produced as a result of computer simulation of the biosensor response (in total $K \times M \times L$ values). Later, using an additional simple utility of summation, a matrix $M^K \times L = 4096 \times L$

of the biosensor response data were produced following (22) and stored in a file which was an input file for chemometric analysis. This was repeated for batch as well as flow injection regimes.

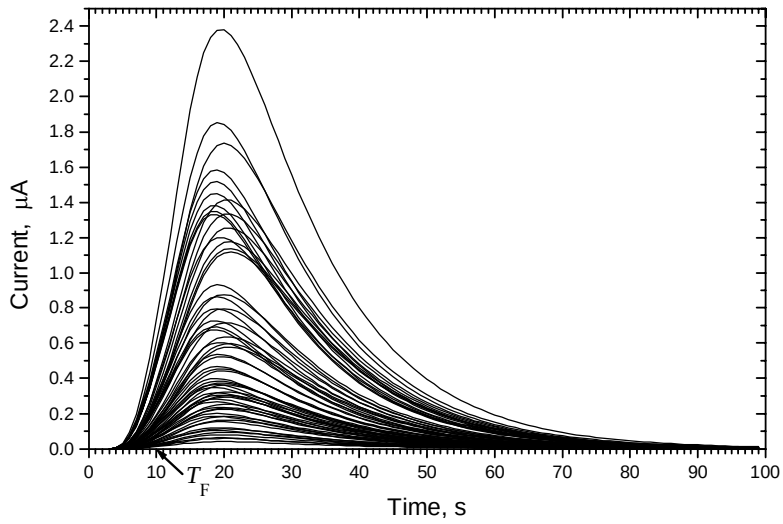

Fig. 2. Every 64th biosensor response curve of full factorial of M^K responses at $K = 4$ values of the maximal enzymatic rate and $M = 8$ substrate concentrations in flow injection analysis

Results of the calculation are depicted in figure 1 and 2. Figure 1 shows every 64th of full factorial of M^K simulated biosensor responses for $K = 4$ values of the maximal enzymatic rate and $M = 8$ substrate concentrations in a case of batch analysis. Figure 2 presents generated biosensor responses in flow injection analysis. Evolution of biosensor current is depicted for the first 100 seconds of biosensor action only because of petty change of the biosensor current at greater values of time t.

The calculation showed, that the maximal biosensor current increases with increase of maximal enzymatic rate V_{max}. The time of the maximal biosensor current decreases with increase of V_{max}. This property is valid for both regimes of analysis: batch and flow injection. In batch analysis the maximal biosensor current is the steady-state current. Figure 2 shows, that the current function $I^*(t)$ is not monotonous in flow injection analysis. The time of maximal current

occurs noticeably later after the time T_F = 10 s of analyte removing. The time when the current starts to decrease varies between 19 and 24 s.

4 Conclusions

The mathematical model (2)–(8) of amperometric biosensors can be used to investigate regularities of the biosensor response to mixtures in batch and flow injection analysis.

If K is a number of mixture compounds and M is a number of different concentrations of each compound, then the result of $K \times M$ computer simulations can be successfully used to generate biosensor response data for full factorial of mixtures (M^K samples) in the cases, where the biosensor signal equals to the sum of signals of individual analytes.

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