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Mathematical Modeling of Biosensors

An Introduction for Chemists and Mathematicians

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Mathematical Modeling of Biosensors

An Introduction for Chemists and Mathematicians

Volume Authors: Romas Baronas · Feliksas Ivanauskas
Juozas Kulys

Springer

Chemical sensors and biosensors are becoming more and more indispensable tools in life science, medicine, chemistry and biotechnology. The series covers exciting sensor-related aspects of chemistry, biochemistry, thin film and interface techniques, physics, including opto-electronics, measurement sciences and signal processing. The single volumes of the series focus on selected topics and will be edited by selected volume editors. The Springer Series on Chemical Sensors and Biosensors aims to publish state-of-the-art articles that can serve as invaluable tools for both practitioners and researchers active in this highly interdisciplinary field. The carefully edited collection of papers in each volume will give continuous inspiration for new research and will point to existing new trends and brand new applications.

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Preface

Biosensors are analytical devices in which specific recognition of the chemical substances is performed by biological material. The biological material that serves as recognition element is used in combination with a transducer. The transducer transforms concentration of substrate or product to electrical signal that is amplified and further processed. The biosensors may utilize enzymes, antibodies, nucleic acids, organelles, plant and animal tissue, whole organism or organs. Biosensors containing biological catalysts (enzymes) are called catalytical biosensors. These type of biosensors are the most abundant, and they found the largest application in medicine, ecology, and environmental monitoring.

The action of catalytical biosensors is associated with substrate diffusion into biocatalytical membrane and it conversion to a product. The modeling of biosensors involves solving the diffusion equations for substrate and product with a term containing a rate of biocatalytical transformation of substrate. The complications of modeling arise due to solving of partially differential equations with non-linear biocatalytical term and with complex boundary and initial conditions.

The book starts with the modeling biosensors by analytical solution of partial differential equations. Historically this method was used to describe fundamental features of biosensors action though it is limited by substrate concentration, and is applicable for simple biocatalytical processes. Using this method the action of biosensors was analyzed at critical concentrations of substrate and enzyme activity. The substrates conversion in single and multienzyme membranes was studied. The different schemes of substrates conversion which found practical application for biosensors construction were analyzed. The biosensors dynamics was considered at the simplest scheme of biocatalyzer action.

The other part of the book covers digital modeling of biosensors. The biosensors based on amperometric as well as potentiometric transducers are considered. The action of biosensors containing single and multienzymes were modeled using the finite difference technique at nonstationary and steady state. Special emphasis was placed to model biosensors utilizing a complex biocatalytical conversion and biosensors with multipart transducers geometry and biocatalytical membranes structure.

The final part of the book is dedicated to the basic concepts of the theory of the difference schemes for the digital solving of linear diffusion equations which are basis for biosensors modeling.

The book can be recommended for the master and doctoral studies as well as for special studies of biosensors modeling. The Part 3 can also be used for independent study of digital solution of differential equations.

The book was prepared for the period of students teaching by R. Baronas and F. Ivanauskas at Vilnius University and by J. Kulys at Vilnius Gediminas Technical University. The authors acknowledge particular universities for the support of the manuscript preparation. The contribution of the coauthors of the cited publications is highly appreciated.

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Contents

Part II Numerical Modeling of Biosensors

Part III Numerical Methods for Reaction–Diffusion Equations

Introduction

The action of biocatalytical biosensors can be modeled with partial differential equations (PDE) of substrates and products diffusion and conversion in biocatalytical membranes. This book deals with biosensors modeling using analytical and digital solution of the PDE. The intrinsic logics of the book is to evaluate critical parameters and conditions that determinate the biosensors response. Since the analytical solutions of PDE describing biosensors action is possible at limited conditions the modeling of complex biosensor action are performed using digital solution of PDE.

The first part of the book is dedicated to the modeling biosensors by analytical solution of partial differential equations. First chapter of Part I contains tutorial introduction of kinetics of biocatalytical reactions, transducer function of biosensors and a general scheme of biosensor action. In second chapter of Part I the modeling biosensors at steady state and internal diffusion limitation is considered with special contribution to varies schemes of enzymes action. Third chapter of Part I concerns the modeling of biosensors at steady state and external diffusion limitations. The action of biosensor containing single enzyme, biosensors with multienzyme and biosensor utilizing non Michaelis–Menten enzyme kinetics was analyzed. Fourth chapter of Part I contains results of modeling biosensors utilizing microbial cells acting as specific biocatalytical or unspecific biochemical oxygen demand microreactor. The main task of fifth chapter of Part I is to analyze limited cases of biosensors modeling at nonstationary state at some critical concentrations of substrate when analytical solution of PDE was performed. The non stationary response of amperometric as well as potentiometric biosensor was analyzed.

At the end of the first part advantages and disadvantages of analytical modeling of biosensors are shown. The largest advantage of aproximal analytical solution is a possibility to get analytical solution of PDE. The disadvantages include limited concentration interval of reactive components, not applicable to biosensors with complex biocatalytical schemes, very complex solution of non stationary state, lack of analytical solution for complex initial and boundary conditions.

In the second part of the book the corresponding reaction–diffusion problems are solved using digital modeling. The solving PDE was performed using the finite difference technique. First chapter of Part II covers mathematical models with nonlinear reaction kinetics. The biosensors are assumed to be flat electrodes

having a mono-layer of an enzyme applied onto the electrode surface. Coupling the enzyme-catalyzed reaction in the enzyme layer (enzyme membrane) with the one-dimensional-in-space diffusion, the mathematical models are described by the non-stationary reaction-diffusion equations. The biosensors based on amperometric as well as potentiometric transducers are considered. The batch and the injection modes of the biosensor operation are modeled in this chapter. The biosensors utilizing the amplification by the conjugated electrochemical and the enzymatic substrates conversion are also investigated. This chapter ends with the modeling of the biosensors with the substrate as well as the product inhibition. The initial boundary value problems are solved numerically by applying the finite difference technique.

Second chapter of Part II deals with the mathematical models of two types of amperometric multi-enzyme biosensors. One type of the biosensors utilizes enzymatic reactions assuming no interaction between the analyzed substrates and the reaction products. The mathematical model of such biosensors is to simulate the biosensor response to a mixture of compounds (substrates). The second type of the biosensors utilizes the enzymatic reaction followed by a cyclic product conversion. Two kinds of the product regeneration in the two-enzyme biosensors are analyzed: enzymatic and electrochemical.

Third chapter of Part II covers multi-layer mathematical models. The biosensors acting in slightly-stirred buffer solutions are described by two-compartment mathematical models. The biosensor operation is analyzed with a special emphasis to the Nernst diffusion layer. This chapter also discusses the multienzyme systems, were the enzymes are immobilized separately in different active layers packed in a sandwich like multi-layer arrangement. The effect of the diffusion limitation to the substrate is investigated when inert outer membranes are applied to stabilize the enzyme layer and to prolong the calibration curve of the biosensor. This chapter also presents the mathematical models of the amperometric biosensor based on the chemically modified electrode as well as of the peroxidase-based optical biosensor.

Fourth chapter of Part II considers modeling of biosensors for which a two-dimensional-in space domain is required to describe mathematically the biosensor action. Firstly, an amperometric biosensor based on a carbon paste electrode encrusted with a single microreactor is considered. Then, an analytical system based on an array of enzyme microreactors immobilized on a single electrode is investigated. Carbon paste porous electrodes are also investigated by applying a plate-gap model. The last section of the this chapter focuses on the modeling of a practical amperometric biosensor containing the selective and the perforated membranes. The perforated membrane is analyzed with a special emphasis to the geometry of the membrane perforation.

Contemporary numerical methods for solving problems of the mathematical chemistry are gaining increasing popularity. The aim of first chapter of Part III is to introduce the reader with the relevant facts about the basic concepts of the theory of the difference schemes for the linear diffusion equations. The linear diffusion equations play an important and crucial role in most models of a biosensor theory. The most popular simple and together effective difference schemes for the linear diffusion equations are presented here. This method is being frequently used in solving

applied problems not only by professional mathematicians, but also by laymen. The concepts presented below are of a primary nature and are sufficient for the solution of the problems of the biosensor. In this book the notations of [222] are mainly applied. The many aspects of the numerical methods for the solution of the partial differential equations are presented in [5, 12, 187, 216].

The difference schemes are extensively applied to the solution of a biosensor problems in second chapter of Part III. This chapter is devoted to various difference approximations of the reaction–diffusion equations. The difference technique, developed in a previous chapter, is employed for the construction of the difference schemes. The main subject of investigation is the system of two nonlinear reaction–diffusion equations in one and two dimensional in space cases.

Part I
Analytical Modeling of Biosensors

Abstract This is part one of the book Mathematical Modeling of Biosensors. The part is dedicated to the modeling biosensors by analytical solution of partial differential equations. This part contains tutorial introduction to kinetics of biocatalytical reactions, transducer function of biosensors and a general scheme of biosensor action. A special emphasis is placed to the modeling biosensors at steady state and internal or external diffusion limitation with special contribution to varies schemes of enzymes action, the modeling of biosensors utilizing microbial cells acting as specific biocatalytical or unspecific biochemical oxygen demand microreactor and the modeling biosensors at nonstationary state at some critical concentrations of substrate when analytical solution of PDE is performed.

Keywords Biocatalyzer · Biosensor · Diffusion · Kinetics · PDE

Biosensor Action

Contents

1 Kinetics of Biocatalytical Reactions

The biosensors contain immobilized enzymes or other biological catalysts [128, 131, 258]. The biocatalyst catalyzes the conversion of the substrate to the product. Biological catalysts (enzymes) show high activity and specificity. The activity of enzymes may exceed the rate of chemically catalyzed reaction by a factor $4.6 \times 10^5 - 1.4 \times 10^{17}$ [58]. The enzymatic activity of the enzymes depends on many factors, i.e. the free energy of reaction, the substrate docking in the active center of enzyme, the proton tunneling and other factors [88, 124, 132, 143].

The general principles of catalytic activity of enzymes are known, but particular factors that determine high enzyme activity are often not established [178]. The specificity of enzymes depends on the enzyme type [65, 81]. There are enzymes which catalyze the conversion of just one substrate. Other enzymes show broad substrates specificity. Oxidoreductases, i.e. enzymes that catalyze electron transfer, may catalyze, for example, the oxidation or reduction of many substrates. To characterize the substrates with diverse activity a slang expression "good substrate" and "bad substrate" is used.

The following scheme of biocatalyzer action was postulated by Henri in 1902 [109]:

$$\mathrm{E} + \mathrm{S} \rightleftarrows \mathrm{ES} \rightarrow \mathrm{E} + \mathrm{P}\,, \tag{1}$$

where E, S, ES and P correspond to the enzyme, the substrate, the enzyme–substrates complex and the product, respectively. In biochemistry the concentrations are expressed as $\mathrm{mol/dm^3} \equiv \mathrm{M}$, whereas in models the concentrations of components are typically expressed in $\mathrm{mol/cm^3}$.

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Michaelis and Menten confirmed this scheme of enzymes action using acetate to keep pH of solution [185]. Following the scheme (1) the change of concentration of each component can be expressed by ordinary differential equation (ODE):

$$\begin{aligned}
\frac{\mathrm{d}E}{\mathrm{d}t} &= -k_1 E S + k_{-1} E_S + k_2 E_S, \\
\frac{\mathrm{d}S}{\mathrm{d}t} &= -k_1 E S + k_{-1} E_S, \\
\frac{\mathrm{d}E_S}{\mathrm{d}t} &= k_1 E S - k_{-1} E_S - k_2 E_S, \\
\frac{\mathrm{d}P}{\mathrm{d}t} &= k_2 E_S,
\end{aligned} \tag{2}$$

where t is time, E, S, E_S, P correspond to the concentrations of the enzyme, the substrate, the enzyme–substrates complex and the product, respectively, and kinetic constants k_1, k_{-1} and k_2 correspond to the respective reactions: the enzyme substrate interaction, the reverse enzyme substrate decomposition and the product formation.

To solve the system of ODE (2), Briggs and Haldane applied quasi-steady state approach (QSS) to E_S which means that $\mathrm{d}E_S/\mathrm{d}t \approx 0$ [55]. The calculated "initial rate" of the steady state reaction rate was expressed (S is equal to the initial concentration S_0):

$$V(S) = -\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{V_{max} S}{K_M + S}, \tag{3}$$

where $V_{max} = k_2 E$ is the maximal enzymatic rate, and $K_M = (k_{-1} + k_2)/k_1$, and it is called the Michaelis constant. The Michaelis constant is the concentration of the substrate at which half the maximum velocity of an enzyme-catalyzed reaction is achieved [55,185]. Typical values of constants of the enzymes which are used for the biosensor preparation are: $k_1 = 10^6 - 10^8\,\mathrm{M^{-1}s^{-1}}$ and $k_{-1} \approx k_2 = 100 - 1{,}000\,\mathrm{s^{-1}}$.

Calculations show that during the enzymes action the quasi-steady state is established during $4.0 - 0.1$ ms at enzyme and substrate concentration 10^{-8} M and 10^{-3} M, respectively. It is sufficient to establish a quasi-steady state in the membranes of biosensors with the thickness more than 2×10^{-4} cm since the thickness δ_d of the effective diffusion layer calculated using the Cottrell equation is [19]:

$$\delta_d = \sqrt{\pi D t}, \tag{4}$$

where the diffusion coefficient D for low molecular weight molecules is about $3 \times 10^{-6}\,\mathrm{cm^2/s}$.

For a more complex biocatalytical process, the establishment of the quasi-steady state requires much longer period of time. It was shown, for example, that for the synergistic reactions, involving cyclic mediators conversion, the time of the QSS establishing can be as large as 180 s [147]. Therefore the expression for the "initial rate" is no longer valid, and the modeling should include the rates of all individual reactions.

2 Transducer Function

The purpose of the transducer is to convert the biochemical recognition into an electronic signal. The transducer is a device that responds selectively to the substrate, the product, the mediator or other compound the concentration of which is related to the analyte under determination [128, 131]. The transducer should show high selectivity since the biosensor selectivity depends on the specificity of the biocatalytical process and the selectivity of the transducer.

The transducers include amperometric and ion-selective electrodes, optical systems and other physical devices realizing different physical phenomena. The biocatalytical membrane is located at close proximity to transducer. There are two fundamental categories of transducers in respect of their response. The transducer of the first type, i.e. the amperometric electrode, is monitoring faradaic current which arises when the electrons are transferred between the substrates, the product or the enzyme active center and an electrode. As a result of electrochemical reaction the concentration of oxidized (reduced) compound at surface of the transducer drops down. The transducers of the second type, i.e. ion-selective electrodes, optical fibber, do not perturb the concentration of the determining compound at the surface. The difference between the transducer types produces different boundary conditions for the modeling of the biosensors.

The boundary condition for the first category of transducer can be written

$$P = 0 \quad \text{or} \quad S = 0 \quad \text{at} \quad x = 0, \tag{5}$$

where x stands for space, P and S are the concentrations of the product and the substrate at the transducer surface, respectively. This boundary condition means that the kinetics of electron transfer is fast, and the potential of the transducer is high enough to keep a current at diffusion limiting condition. If the kinetics of electron transfer is slow then the transducer current depends on the electrode potential and is obtained from the Butler–Volmer expression [56]. The modeling of biosensors at this type of boundary conditions has not been performed due to this uncommon state for the real biosensors.

The boundary condition of the transducer of the second category is

$$\frac{\mathrm{d}P}{\mathrm{d}x} = 0 \quad \text{or} \quad \frac{\mathrm{d}S}{\mathrm{d}x} = 0 \quad \text{at} \quad x = 0, \tag{6}$$

For the ion-selective electrodes this corresponds to the Nerstian boundary condition [56]. For the optical transducer and other transducers this condition means non-leakage (zero flux) of the product or the substrate on the boundary between the transducer and the biocatalytical membrane.

3 Scheme of Biosensor Action

The biosensor produces a signal when the analyte under determination diffuses from the bulk solution into the biocatalytical membrane. The biocatalyst catalyzes the substrate conversion to the product, which is determined by the transducer. The concentration change of S and P is associated with the diffusion and the enzymatic reaction. Following Fick the compounds concentration change in the biocatalytical membrane can be written

$$\begin{aligned} \frac{\partial S}{\partial t} &= D_e \frac{\partial^2 S}{\partial x^2} - V(S), \\ \frac{\partial P}{\partial t} &= D_e \frac{\partial^2 P}{\partial x^2} + V(S), \quad x \in (0,\, d), \quad t > 0. \end{aligned} \tag{7}$$

where x and t stand for space and time, respectively, $S(x,t)$ is the concentration of the substrate, $P(x,t)$ is the concentration of the reaction product, d is the thickness of the enzyme membrane, D_e is the diffusion coefficient of compounds in the enzyme membrane, that is typically used the same for the substrate, the product and the mediator.

The solution of (7) at corresponding initial and boundary conditions produces the concentration change of S and P in time and membrane thickness.

For the first type of transducers the response R of biosensor can be written

$$R(t) = C_1 \left.\frac{\partial P}{\partial x}\right|_{x=0}, \tag{8}$$

and for the second type of transducers

$$R(t) = C_2 P(0,t), \tag{9}$$

or

$$R(t) = C_3 \log P(0,t), \tag{10}$$

where C_1, C_2, C_3 are the appropriate constants.

The logarithmic dependence is characteristic of ion-selective electrodes, whereas for optical and other transducers linear dependence between the response and the concentration is realized.

Simple analytical solution of (7) is impossible even for the simplest initial and boundary conditions due to the hyperbolic function of the enzymatic rate dependence on the substrate concentration (3). Therefore the description of biosensors action is divided into the simplest cases for which analytical solutions still exist. This approach was used widely, especially at the beginning of the development of biosensors, to recognize the principles of the biosensors action. The aproximal analytical solution gives information about the critical cases. They are also useful to test the correctness of numerical calculations found at initial and boundary limiting conditions.

When the concentration S_0 to be measured is very small in comparison with the Michaelis constant K_M,

$$\forall x, t: \; x \in [0, \, d], t > 0: \quad 0 < S(x,t) < S_0 \ll K_M, \tag{11}$$

the nonlinear function $V(S)$ simplifies to that of the first order,

$$V(S) = \frac{V_{max} S}{S + K_M} \approx \frac{V_{max}}{K_M} S. \tag{12}$$

Practically, the enzyme reaction can be considered first-order when the concentration of the detected species is below one-fifth of K_M, i.e. $S_0 < 0.25 K_M$, [99]. This case is rather typical for the biosensors with a high enzyme loading factor.

The nonlinear reaction–diffusion system (7) reduces to a linear one,

$$\begin{aligned} \frac{\partial S}{\partial t} &= D_e \frac{\partial^2 S}{\partial x^2} - kS, \\ \frac{\partial P}{\partial t} &= D_e \frac{\partial^2 P}{\partial x^2} + kS, \quad x \in (0, \, d), \quad t > 0. \end{aligned} \tag{13}$$

where k is the first-order reaction constant (linear enzyme kinetic coefficient),

$$k = \frac{V_{max}}{K_M}. \tag{14}$$

Analytical solutions are typically made at steady state and external and internal diffusion limiting conditions.

The steady state (stationary) conditions mean that

$$\frac{\partial S}{\partial t} = 0, \quad \frac{\partial P}{\partial t} = 0. \tag{15}$$

The external diffusion limitation indicates that the substrates transport through the diffusion (stagnant) layer [134] is a rate limiting process. At internal diffusion limitation the substrates diffusion through external diffusion layer is fast and the process is limited by the diffusion inside an enzyme membrane. The disadvantage of these approximate solutions is an error at the boundaries between the different approximate treatments. It is helpful to illustrate this approach by reference to a trivial problem of the substrate conversion in the biocatalytical membrane of the biosensor and at the concentration of the substrate less than K_M. The calculated profile of the substrate concentration at the steady state or stationary conditions is shown in Fig. 1.

It is possible to identify an abrupt of concentration change of the substrate at the boundary of biocatalytical membrane/stagnant layer as well as at the boundary stagnant layer/bulk solution. This comes from approximate solutions at the boundaries

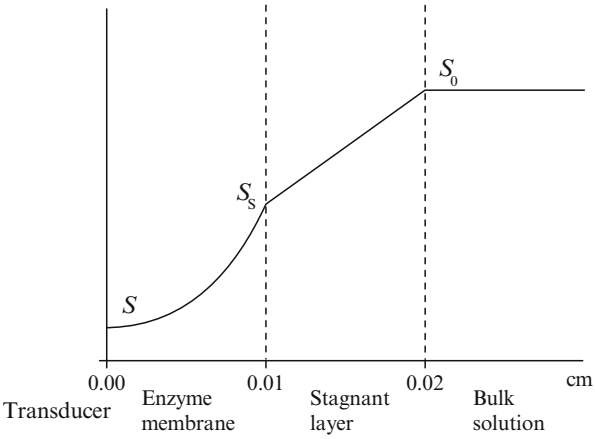

Fig. 1 The substrate concentration profile in a biosensor at steady state conditions. The concentration profile was calculated with the boundary conditions $\partial S/\partial x = 0$ at $x = 0$ and $S = S_0$ at $x \geq d + \delta$. The diffusion coefficient (D_0) in a stagnant layer is 3×10^{-6} cm^2/s, in membrane is $D_e = 10^{-6}$ cm^2/s, $V_{\max} = 5 \times 10^{-7}$ mol/cm^{3}s, $K_M = 10^{-5}$ mol/cm^3, $d = \delta = 0.01$ cm, $S_0 = 10^{-6}$ mol/cm^3

during different approximate treatments. The change of the steady state concentration of the substrate in membrane can be calculated as

$$\frac{S}{S_s} = \frac{\cosh(\alpha x)}{\cosh(\alpha d)}, \tag{16}$$

where S, S_s is substrate concentration at transducer surface and at the boundary of membrane and stagnant solution, respectively,

$$\alpha^2 = \frac{V_{max}}{K_M D_e}. \tag{17}$$

On the other hand, at the steady state a substrate flux through the boundary of stagnant layer/bulk solution is equal to the flux through the boundary of biocatalytical membrane/stagnant layer:

$$D_0 \frac{S_0 - S_s}{\delta} = D_e \left.\frac{\partial S}{\partial x}\right|_{x=d} = D_e \alpha \tanh(\alpha d) S_s. \tag{18}$$

A combination of these two solutions (16), (18) produces the concentration profile of the substrate in the biocatalytical membrane and the stagnant layer (Fig. 1). It is possible to notice that the greatest error of calculations is at $x = d$ and $x = d + \delta$. However, at the limiting (the internal or the external diffusion limitation) cases the two expressions produce very good approximations to the full equation. Therefore, the modeling of the biosensors at two limiting cases was used to solve different biosensors problems.

Modeling Biosensors at Steady State and Internal Diffusion Limitations

Contents

1 Biosensors Containing Single Enzyme

The most popular glucose biosensor is based on glucose oxidase (GO) that catalyzes β-D-glucose oxidation with oxygen [261, 273],

$$\beta\text{-D-glucose} + O_2 \overset{\text{D-glucose oxidase}}{\longrightarrow} \text{D-glucono-}\delta\text{-lactone} + H_2O_2 \qquad (1)$$

The hydrogen peroxide produced is oxidized on platinum electrode acting as a transducer. One of the first tasks of modeling of this type of the biosensors was devoted to evaluate the dependence of biosensors response on enzymatic parameters [128]. The action of the biosensors was analyzed at the internal diffusion limiting conditions and at the steady state conditions.

The biosensor response (the current density) was calculated as

$$i(t) = n_e D_e F \left.\frac{\partial P}{\partial x}\right|_{x=0}, \qquad (2)$$

where n_e – the number of electrons (for hydrogen peroxide $n = 2$), F – the Faraday number, D_e – the diffusion coefficient of the substrate and the product in the biocatalytical membrane.

R. Baronas et al., *Mathematical Modeling of Biosensors*, Springer Series on Chemical Sensors and Biosensors 9, DOI 10.1007/978-90-481-3243-0_2,

To calculate the biosensor response the change of the product concentration at the transducer surface as indicated in (2) was evaluated. Solving (Chapter 1, eq. 13) with the boundary conditions $\partial S/\partial x = 0$, $P = 0$ at $x = 0$, $S = S_0$, $P = 0$ at $x \geq d$ and at $S_0 \ll K_M$ (the concentration of oxygen is taken in access) produces the stationary biosensor response (the steady state current density) as:

$$I = n_e F D_e \frac{S_0}{d} \left(1 - \frac{1}{\cosh(\alpha d)}\right), \tag{3}$$

The solution shows that the biosensor response is a linear function of the substrate concentration. The sensitivity of the biosensors expressed as $\mathrm{d}I/\mathrm{d}S_0$ does not depend on the enzyme activity if the diffusion module (αd) is larger than 1 since

$$1 - \frac{1}{\cosh(\alpha d)} \approx 1. \tag{4}$$

At $\alpha d < 1$ the approximal solution of (13) is

$$I \approx n_e F D_e S_0 \frac{\alpha^2 d}{2} = n_e F S_0 \frac{V_{max} d}{2K_M}. \tag{5}$$

In this case the biosensor sensitivity is determined by the enzyme parameters V_{max} and K_M.

The inactivation of an enzyme following the diffusion module change from ($\alpha d > 1$) to ($\alpha d < 1$) produces a wrong interpretation of an enzyme stability in the biocatalytical membranes. Simple calculations show that if the enzyme inactivates in a solution following the first order reaction with a rate constant $k_{in} = 0.1\,\mathrm{h}^{-1}$ the half-time (τ) of enzyme inactivation is 6.9 h ($\tau = \ln(2)/k_{in}$). If the same enzyme is used for the biocatalytical membranes preparation, and at the beginning of activation the diffusion module of the biosensors is, for example 100 ($d = 0.01$ cm), the response of biosensor will decrease two times only after 87 h.

2 Biosensors Containing Multienzymes

2.1 Consecutive Substrates Conversion

The analysis of the biosensor action containing consecutive substrate conversion with two enzymes has also been analyzed at internal diffusion limitation and steady state conditions [130]. These consecutive reactions occur in the bienzyme electrode based on immobilized D-glucose oxidase and peroxidase. Under the action of D-glucose oxidase (1), D-glucose is oxidized with the production of hydrogen

peroxide. During the second stage, hydrogen peroxide is reduced by ferrocyanide ion (6). This reaction is catalyzed by peroxidase

$$H_2O_2 + 2Fe(CN)_6^{4-} + 2H^+ \longrightarrow 2Fe(CN)_6^{3-} + 2H_2O \tag{6}$$

Under the stationary conditions at excess concentrations of oxygen and ferrocyanide, when reactions (1) and (6) are the first order, the change of concentration within the bienzyme membrane is described by the system of the following equations:

$$\begin{aligned}
\frac{d^2S}{dx^2} &= \frac{V_{max}S}{K_M D_e} = \alpha_1^2 S\,, \\
\frac{d^2P_1}{dx^2} &= -\frac{V_{max}S}{K_M D_e} + \frac{V'_{max}P_1}{K'_M D_e} = -\alpha_1^2 S + \alpha_2^2 P_1\,, \\
\frac{d^2P_2}{dx^2} &= -2\frac{V'_{max}P_1}{K'_M D_e} = -2\alpha_2^2 P_1\,,
\end{aligned} \tag{7}$$

where S, P_1 and P_2 are the concentrations of glucose, hydrogen peroxide and ferricyanide, respectively; D_e – the diffusion coefficients of S, P_1 and P_2, which are taken equal; V_{max}, V'_{max}, K_M and K'_M are the corresponding parameters of the enzyme reactions (1) and (6).

The solution of the system (7) taking into consideration the boundary conditions $S = S_0$, $P_1 = 0$, $P_2 = 0$ at $x \geq d$ and $dS/dx = dP_1/dx = 0$, $P_2 = 0$ at $x = 0$ gives the dependence of electrode current density I on the kinetic and diffusive parameters ($\alpha_1 \neq \alpha_2$):

$$\begin{aligned}
I &= FD_e \left.\frac{dP_2}{dx}\right|_{x=0} \\
&= \frac{2FD_e\alpha_1^2}{d(\alpha_2^2 - \alpha_1^2)} \left[\left(1 - \frac{1}{\cosh \alpha_2 d}\right) - \frac{\alpha_2^2}{\alpha_1^2}\left(1 - \frac{1}{\cosh \alpha_1 d}\right)\right] S_0\,.
\end{aligned} \tag{8}$$

Hence, it follows that the current of the bienzyme electrode is proportional to the substrate (glucose) concentration. The current is determined by means of diffusion module $\alpha_1 d$ and $\alpha_2 d$. When the rate of enzyme reaction is great ($\alpha_1 d > 1$ and $\alpha_2 d > 1$) the response reaches its maximal value and is determined by the substrate diffusion,

$$I = \frac{2FD_e}{d} S_0 \tag{9}$$

When the activity of peroxidase is considerably greater than the activity of D-glucose oxidase ($\alpha_2 d > \alpha_1 d > 1$) the biosensor response is determined by the D-glucose oxidase parameters

$$I = \frac{2FD_e}{d}\left(1 - \frac{1}{\cosh \alpha_1 d}\right) S_0\,. \tag{10}$$

Under the kinetic control ($\alpha_1 d \ll 1$), (10) is transformed to:

$$I = \frac{F V_{max} d}{K_M} S_0 \,. \tag{11}$$

Due to high molecular activity of the peroxidase, these bienzyme biosensors operate in the mode controlled by the D-glucose oxidase reaction. Sensitivity, as well as the stability of electrodes are close to that of mono-enzyme D-glucose electrode.

The modeling of trienzyme biosensor utilizing consecutive substrates conversion with three enzymes was completed at internal diffusion limitation and steady state conditions [145]. The example of successful application of three enzymes might be sensitive to the creatinine biosensor [248]. In the membrane of this biosensor creatininase (E_1) hydrolyses creatinine (S) to creatine (P_1). The creatine is further hydrolyzed with creatinase (E_2) to sarcosine (P_2). The oxidation of sarcosine with sarcosine oxidase (E_3) produces hydrogen peroxide (P_3) that is determined amperometrically:

$$\mathrm{S} \xrightarrow{\mathrm{E_1}} \mathrm{P_1} \xrightarrow{\mathrm{E_2}} \mathrm{P_2} \xrightarrow{\mathrm{E_3}} \mathrm{P_3} \tag{12}$$

The rate of each reaction ($V_i(S)$) can be characterized by the standard enzyme parameters $V_{max}^{(i)}$ and $K_M^{(i)}$, where $i = 1, 2$ and 3, for E_1, E_2 and E_3 catalyed process, respectively. At concentration of S, P_1 and P_2 less than the Michaelis–Menten constants ($K_M^{(i)}$), $V_1 = V_{max}^{(1)} S / K_M^{(1)}$, $V_2 = V_{max}^{(2)} P_1 / K_M^{(2)}$, $V_3 = V_{max}^{(3)} P_2 / K_M^{(3)}$.

At substrates concentration less than $K_M^{(i)}$ and at a constant diffusion coefficients the diffusion equations and the enzymatic conversions take a form

$$\begin{aligned}
\frac{1}{D_e}\frac{\partial S}{\partial t} &= \frac{\partial^2 S}{\partial x^2} - \alpha_1^2 S \,, \\
\frac{1}{D_e}\frac{\partial P_1}{\partial t} &= \frac{\partial^2 P_1}{\partial x^2} + \alpha_1^2 S - \alpha_2^2 P_1 \,, \\
\frac{1}{D_e}\frac{\partial P_2}{\partial t} &= \frac{\partial^2 P_2}{\partial x^2} + \alpha_2^2 P_1 - \alpha_3^2 P_2 \,, \\
\frac{1}{D_e}\frac{\partial P_3}{\partial t} &= \frac{\partial^2 P_3}{\partial x^2} + \alpha_3^2 P_2 \,,
\end{aligned} \tag{13}$$

where D_e – the diffusion coefficient of all compounds in the enzyme membrane, $\alpha_i = (V_{max(i)}^{(i)} / K_M^{(i)} D_e)^{1/2}$, $i = 1, 2, 3$.

The biosensor response (the current density) was calculated as

$$i(t) = 2FD_e \left.\frac{\partial P_3}{\partial x}\right|_{x=0} . \tag{14}$$

The solution of (13) was found at the steady state conditions ($\partial S/\partial t = \partial P_1/\partial t = \partial P_2/\partial t = \partial P_3/\partial t = 0$) with the boundary conditions: $S = S_0$, $P_1 = 0$, $P_2 = 0$, $P_3 = 0$ at $x \geq d$, $\partial S/\partial x = 0$, $\partial P_1/\partial x = 0$, $\partial P_2/\partial x = 0$, $P_3 = 0$ at $x = 0$, where d – the membrane thickness.

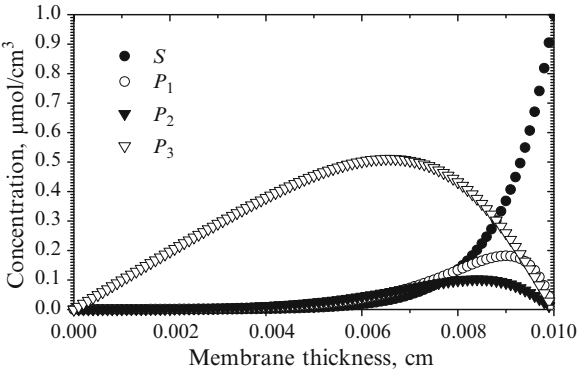

Fig. 1 The profile of compounds concentration in trienzyme membrane of the biosensor. For calculations $S_0 = 10^{-6}$ mol/cm^3, $\alpha_1 d = 10.0$, $\alpha_2 d = 10.1$, $\alpha_3 d = 10.3$ and $d = 0.01$ cm were used

Calculations show that a significant concentration of products in a membrane is produced if all the diffusion modules ($\alpha_i d$) are larger than 1 (Fig. 1).

To prove the correctness of the calculations the distribution of compounds in a membrane was also determined at the boundary condition $\partial P_3/\partial x = 0 (x = 0)$. In this case the sum of the compounds was equal to S_0 at all x values. At $\alpha_1 \neq \alpha_2 \neq \alpha_3$ the expression of three enzyme biosensors response (the current density) is

$$I = 2FD_e \left[\frac{\alpha_2^2 \alpha_3^2}{(\alpha_2^2 - \alpha_1^2)(\alpha_3^2 - \alpha_1^2)} (1 - \cosh(\alpha_1 d)) - \frac{\alpha_1^2 \alpha_3^2}{(\alpha_2^2 - \alpha_1^2)(\alpha_3^2 - \alpha_2^2)} (1 - \cosh(\alpha_2 d)) + \frac{\alpha_1^2 \alpha_2^2}{(\alpha_3^2 - \alpha_1^2)(\alpha_3^2 - \alpha_2^2)} (1 - \cosh(\alpha_3 d)) \right] \frac{S_0}{d} . \quad (15)$$

It is impossible in practice to achieve equal values of the diffusion modules for all enzymes. Therefore the biosensor response has not been derived at $\alpha_1 = \alpha_2 = \alpha_3$.

The dependence of the response of the biosensor on the diffusion module of the least active enzymes E_1 and E_2 is shown in Fig. 2. It is easy to notice that the response is very small, still diffusion modules are less than 1. The maximal biosensor response of 6×10^{-5}A/cm^2 is achieved when the diffusion modules are greater than 10.

Experiments show that among three immobilized enzymes the lowest stability demonstrates creatininase (E_1). The model permits to predict sensitivity change of the biosensor during the enzyme inactivation. If the inactivation follows exponential decay, for example, with half-time 2 days, and at the beginning the biosensor contains large catalytic activities ($\alpha_i d \approx 10$), the response decreases just 34.7 % during 10 days (Fig. 3). The apparent half-time of biosensor inactivation increases up to 11.6 days. In fact, this biosensor can be used even longer, i.e. during 15 days with permanent calibration.

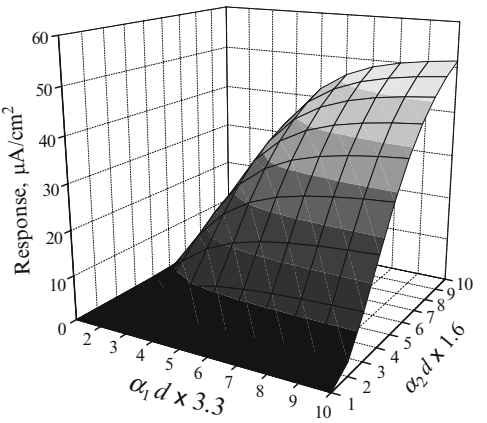

Fig. 2 The dependence of the biosensor response on the diffusion modules $\alpha_1 d$ and $\alpha_2 d$. For calculations $S_0 = 10^{-6}$ mol/cm^3 and $\alpha_3 d = 10.3$ were used

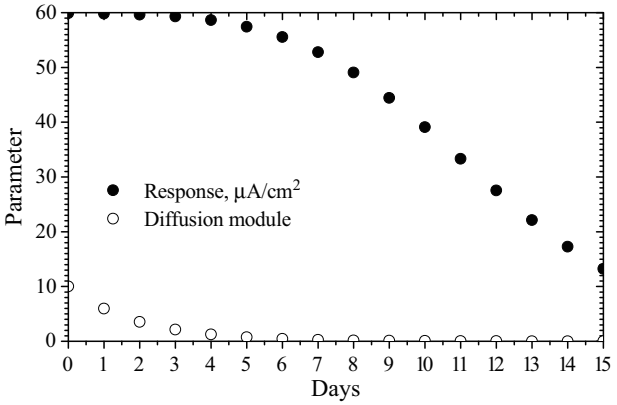

Fig. 3 The changes of the biosensor response and the diffusion module of trienzyme biosensor during the enzyme inactivation. The half-time of enzyme inactivation is 2 days, $S_0 = 10^{-6}$ mol/cm^3, $\alpha_1 d = 10.0$, $\alpha_2 d = 10.1$, $\alpha_3 d = 10.3$ and $d = 0.01$ cm were used

2.2 Parallel Substrates Conversion

In the presence of adenosine triphosphate (ATP), D-glucose oxidation is paralleled by the reaction of glucose phosphorylation in the bienzyme biosensors, the catalytic membrane of which is made of D-glucose oxidase and hexokinase [130],

$$\text{D-glucose} + \text{ATP} \overset{\text{hexokinase}}{\longrightarrow} \text{D-glucose-6-phosphate} + \text{ADP} \tag{16}$$

The increase in ATP concentration leads to the decrease of the response occurring as a result of the D-glucose oxidase reaction (1). For the calculation of the dependence of the biosensor response it is assumed that the oxidation and phosphorylation are first-order for D-glucose and ATP, respectively. The solution of equations of

diffusion and enzyme reactions with boundary conditions: $S = S_0$, $S_1 = S_{10}$, $P_1 = 0$ at $x \geq d$, and $\mathrm{d}S/\mathrm{d}x = \mathrm{d}S_1/\mathrm{d}x = 0$, $P_1 = 0$ at $x = 0$ gives the dependence of the density I of the biosensor current on the D-glucose (S_0) and ATP (S_{10}) concentration ($\alpha_1 \neq \alpha_2$),

$$I = 2FD_e \left.\frac{\mathrm{d}P_1}{\mathrm{d}x}\right|_{x=0} = \frac{2FD_e}{d}\left[S_0\left(1 - \frac{1}{\cosh \alpha_1 d}\right) - S_{10}\left(\frac{\alpha_2^2}{\alpha_2^2 - \alpha_1^2}\left(1 - \frac{1}{\cosh \alpha_1 d}\right) - \frac{\alpha_1^2}{\alpha_2^2 - \alpha_1^2}\left(1 - \frac{1}{\cosh \alpha_2 d}\right)\right)\right], \quad (17)$$

where $\alpha_2 = (V_{max}/K_M D_e)^{1/2}$ is related to hexokinase reaction.

Thus, it follows that at a high rate of both enzyme reactions ($\alpha_1 d > 1$ and $\alpha_2 d > 1$) the density of the biosensor current is determined by the difference between the D-glucose and ATP concentrations,

$$I = \frac{2FD_e}{d}(S_0 - S_{10}) , \quad (18)$$

i.e. the decrease in the biosensors current is proportional to the concentration of coenzyme (ATP),

$$\Delta I = \frac{2FD}{d} S_{10} . \quad (19)$$

If the reaction of D-glucose oxidase proceeds rapidly ($\alpha_1 d > 1$), and the phosphorylation is at low rate ($\alpha_2 d < 1$), then

$$\Delta I = \frac{FD_e V'_{max} d}{K'_M} S_{10} . \quad (20)$$

The sensitivity of such a biosensor is directly proportional to the activity of hexokinase. The experimental results indicate that the action of the ATP electrode is determined by the activity of this enzyme [130]. The hexokinase inactivation results in a quick loss of the biosensors sensitivity.

2.3 Biosensors Utilizing Cyclic Substrates Conversion

Rich biocatalytical possibilities permit to construct different systems utilizing the cyclic substrates conversion. The cyclic conversion substrates in an enzyme membrane may considerably increase the sensitivity of the biosensor.

$$\mathrm{S} \xrightarrow{\alpha_1} \mathrm{P_1} \xrightarrow{\alpha_2} \mathrm{S} + \mathrm{P_2} \quad (21)$$

where $\alpha_1 d$ and $\alpha_2 d$ are the diffusion modules of the corresponding reactions.

If P_2 is considered to be an electrode-active compound, the biosensors response is

$$I = n_e F D_e \left.\frac{\mathrm{d}P_2}{\mathrm{d}x}\right|_{x=0} = \frac{n_e F D_e}{d} \frac{\alpha_1^2 \alpha_2^2}{\alpha_1^2 + \alpha_2^2} \left[\frac{d^2}{2} - \frac{1}{\alpha_1^2 + \alpha_2^2} \left(1 - \frac{1}{\cosh(\sqrt{\alpha_1^2 + \alpha_2^2}\, d)} \right) \right] S_0 \quad (22)$$

where the boundary conditions are: $S = S_0$, $P_1 = 0$, $P_2 = 0$, when $x \geq d$; $\mathrm{d}S/\mathrm{d}x = \mathrm{d}P_1/\mathrm{d}x = 0$, $P_2 = 0$, when $x = 0$.

Two important conclusions can be drawn from Eq. 22:

(i) Under the kinetic control of the first or second reaction ($\alpha_1 d < 1$ or $\alpha_2 d < 1$) the amplification of the signal does not take place.

(ii) At a high enzymatic activity ($\alpha_1 d > 1$ and $\alpha_2 d > 1$) the response of the biosensor increases by a value which is directly proportional to the square of the membrane thickness:

$$I = I_d \frac{\alpha_1^2 \alpha_2^2 d^2}{2(\alpha_1^2 + \alpha_2^2)}, \quad (23)$$

where I_d corresponds to the diffusion controlled response of the biosensors containing single enzyme

$$I_d = n_e F D_e \frac{S_0}{d}. \quad (24)$$

The amplification is rapidly increased by the rise of the enzymatic activity. For example, at $\alpha_1 d = \alpha_2 d = 4$, the 4-fold increase of the sensitivity occurs. At $\alpha_1 d = \alpha_2 d = 10$, the amplification enlarges 25-fold.

The possibility of a considerable increase in the sensitivity of the biosensors by means of chemical amplification was demonstrated using alcohol dehydrogenase with cyclic coenzyme (NAD) conversion and in other biocatalytical systems [136].

Biocatalytical systems may utilize other substrates conversion in addition to cyclic conversion. A biosensor showing submicromolar sensitivity to hydrogen peroxide was developed utilizing fungal peroxidase and pyrroloquinoline quinone-dependent glucose dehydrogenase. High sensitivity of the biosensor was achieved by triggering the initiator conversion with peroxidase following the signal amplification by cyclic conversion of the mediator formed [149].

3 Biosensors Utilizing Synergistic Substrates Conversion

Biocatalytical reactions in biocatalytical membranes may be conjugated with chemical conversions. These synergistic reactions allow to generate high sensitive biosensors. High sensitive biosensors for heterocyclic compounds determination were built

using the oxidases-catalyzed hexacyanoferrate(III) reduction [146]. The detection limit of some heterocyclic compounds determination was 2×10^{-10} mol/cm^3. The sensitivity of the biosensors was 300–10,000 times larger in comparison to the determination of hexacyanoferrate(III).

The steady state current of the biosensor was calculated using the synergistic scheme of oxidases action. Following the scheme the oxidized glucose oxidase (GO_{ox}) is reduced with glucose ant reduction of hexacyanoferrate(III) (Fer) is catalyzed by reduced glucose oxidase (GO_{red}),

$$GO_{ox} + \text{D-glucose} \xrightarrow{k_{red}} GO_{red} + P \tag{25}$$

$$GO_{red} + 2Fer \xrightarrow{k_f} GO_{ox} + 2Fer_{red} \tag{26}$$

In the presence of heterocyclic compounds that act as mediators (M) they are oxidized with hexacyanoferrate(III) to cation radicals. The cation radical (M_{ox}) formed reacts with reduced oxidase. The reduced mediator (M_{red}) is further oxidized with hexacyanoferrate(III),

$$GO_{red} + 2M_{ox} \xrightarrow{k_{ox}} GO_{ox} + 2M_{red} \tag{27}$$

$$M_{red} + Fer \xrightarrow{k_{exc}} M_{ox} + Fer_{red} \tag{28}$$

Chemical reaction (28) increases Fer_{red} production rate, therefore the rate of overall process is larger than the reactions (26) and (27).

Since the electrochemically active compound is hexacyanoferrate(II) (Fer_{red}), the steady state response can be calculated like the current density of the biosensor with a chemical amplification [136]:

$$I = \frac{FD_e}{d}\frac{\alpha_1^2\alpha_2^2}{\beta^2}\left(\frac{d^2}{2} - \frac{1}{\beta^2}\left(1 - \frac{1}{\cosh(\beta d)}\right)\right)M_0\,, \tag{29}$$

where $\alpha_1 d$ and $\alpha_2 d$ are the diffusion modules, $\beta^2 = \alpha_1^2 + \alpha_2^2$, d is the enzyme membrane thickness, M_0 corresponds to the total mediator. The diffusion modules were calculated as $\alpha_1 d = (k_{ox}E_0/D_e)^{1/2}d$, $\alpha_2 d = (k_{exc}Fer/D_e)^{1/2}d$, where E_0 and Fer stand for the total enzyme and the hexacyanoferrate(III) concentrations, respectively, k_{exc} is the bimolecular electron exchange constant between the mediator and hexacyanoferrate (III).

The density I_0 of the steady state current of the biosensor in the absence of the mediator was calculated as

$$I_0 = \frac{FD_e}{d}\left(1 - \frac{1}{\cosh(\gamma d)}\right)Fer\,, \tag{30}$$

where $\gamma = (k_f E_0/D_e)^{1/2}$, k_f is the constant of the reaction of the hexacyanoferrate(III) with the reduced glucose oxidase.

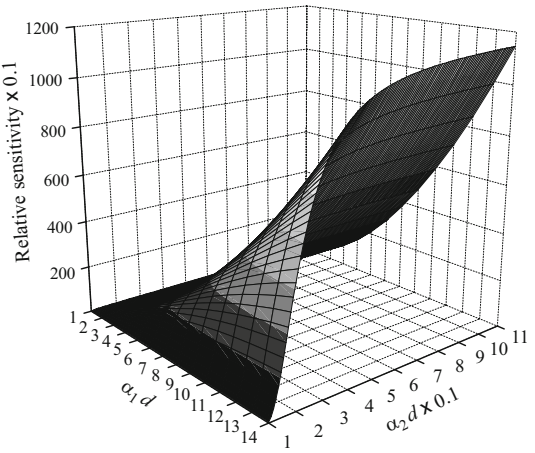

Fig. 4 The dependence of the biosensor relative sensitivity S_r on the diffusion modules $\alpha_1 d$ and $\alpha_2 d$

The analysis of the dependence of the relative sensitivity ($S_r = I/I_0$) on the diffusion modules reveals that the S_r is larger than 1 if $\alpha_1 d$ and $\alpha_2 d$ are larger than 0.5 (Fig. 4).

At $\alpha_1 d = \alpha_2 d = 1$, the relative sensitivity S_r of the biosensor is 12.9. It increases if both diffusion modules are larger than 1. The calculations show that for the biosensor containing 1.3×10^{-7} mol/cm^3 of glucose oxidase $\alpha_1 d$ is 14.5 and $\alpha_2 d$ is 113.5. In contrast, the diffusion module (γd) of the biosensor acting with the pure hexacyanoferrate(III) is 0.13 due to the low constant of the reduced enzyme. Since γd is less than 1 it means that the biosensor acts in a kinetic regime. It is easy to notice that at $\alpha_2 d > \alpha_1 d > 1$ and $\gamma d < 1$ the relative sensitivity equals

$$\frac{I}{I_0} = \frac{k_{ox}}{k_f}\,. \tag{31}$$

The comparison of calculated and experimentally determined values reveals that the calculated relative sensitivity of the biosensors is about three times larger than the experimentally determined. This deference can be caused by the limited stability of the oxidized heterocyclic compounds, uncounted parallel reaction of reduced enzyme with oxygen and external diffusion limitation of hexacyanoferrate(III) and glucose.

4 Biosensors Based on Chemically Modified Electrodes

Chemically modified electrodes (CME) are produced by modifying carbon electrodes with redox active component (mediator) which reacts with an enzyme [138]. For modification of electrodes adsorption or covalent immobilization of the mediator is used. The peculiarities of CME based biosensors modeling arise due to the mediator location on the electrode that produces special boundary conditions.