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Modelling glucose dehydrogenase-based amperometric biosensor utilizing synergistic substrates conversion

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ABSTRACT

A scheme of a glucose dehydrogenase-based bioelectrocatalytical system where ferricyanide is converted to ferrocyanide in the presence of highly reactive organic electron transfer compounds is investigated digitally. Glucose dehydrogenase (GDH) is highly reactive towards organic electron acceptors compared to the low reactivity of GDH with inorganic electron acceptors. In this bioelectrocatalytical scheme, the enzyme efficiently catalyzes biocatalytical conversion of an organic mediator, which is followed by the fast chemical cross reaction of the product in the presence of ferricyanide excess. By changing input parameters a special emphasis was directed towards the influence of the kinetic constants and reagents concentration on the sensitivity of the bioelectrode. The digital simulation of the system confirmed that the high sensitivity of the bioelectrode achieved in the presence of organic mediators is due to the synergistic substrates conversion demonstrated experimentally. in (*Electroanalysis*, 2012 (24) 273-277).

1. Introduction

Biosensors are sensitive, reliable and relatively cheap devices capable to resolve a large number of analytical problems and challenges in very diverse areas such as clinical diagnostics, drug discovery, agriculture and food safety, environmental monitoring, etc. [1,2]. The catalytical biosensors are based on enzyme-catalyzed conversion of analytes. The response of these biosensors is determined by enzymatic activity and mass transfer in biocatalytic membranes [3]. The output current of amperometric biosensors (or amperometric bioelectrodes) is produced by means of oxidation or reduction of enzymatic reaction products. The high sensitivity and selectivity towards a number of different analytes is achieved through the specificity of the incorporated enzyme and the selectivity of a transducer [4,5]. The measured current is usually proportional to the concentration of the target analyte, which allows the determination of the analyte concentration using a preestablished calibration curve [6,7].

The abundance of enzymatic catalytic processes allows employing many different schemes of bioelectrocatalysis. In the simplest scheme the substrates are converted to the products following the Michaelis-Menten scheme [8,9]. The more complex bioelectrocatalytical schemes include cyclic, consecutive multistep and parallel conversions [3]. In the synergistic scheme, an enzyme catalyzes the parallel conversion of substrates into the products, with the concomitant cross reaction of the substrates and the products [10–13]. In this scheme, the rate of substrates conversion exceeds the rate of individual substrate conversion, so the total effect is greater than the sum of the two reactions. The synergistic schemes of the substrates conversion are of particular interest due to applying them to producing highly sensitive bioelectrodes and powerful biofuel cells [14]. The synergistic substrates conversion has been demonstrated using glucose oxidase, carbohydrate oxidase and laccase [15–18].

For the development of highly specific and sensitive biosensors with the optimal performance a number of characteristics should be considered [1,4]. A digital model of a biosensor could facilitate the improvement of the productivity as well as the efficiency of the biosensor design [19–21]. Mathematical models have been widely used to fine-tune the analytical characteristics of the biosensors [22–26]. Biosensors utilizing a few synergistic schemes of substrates conversion have been mathematically modelled at steady state conditions [17,27] and at transient conditions [28,29]. A laccase based biosensor was investigated with a special emphasis to the influence of the species concentrations on the synergy of the simultaneous substrates conversion [28], and the effect of the chemical amplification was investigated for biosensors used for heterocyclic compounds determination [29].

The task of this investigation was to investigate the impact of the synergistic effect on the biosensor response and the sensitivity at different concentrations of glucose, which provides a strong

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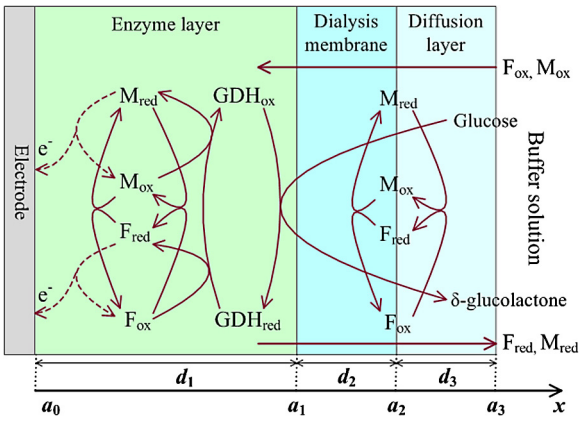

Fig. 1. The schematic view of the GDH amperometric biosensor. The thicknesses of the layers are indicated next to the boundaries of the layers.

insight on the prospect of practical application of the synergistic effect when developing glucose dehydrogenase biosensors. A computational model was developed for simulating of the glucose dehydrogenase-based bioelectrode action [18], and the influence of the physical and kinetic parameters on the biosensor sensitivity was investigated for different glucose concentrations. The developed model is based on non-stationary non-linear reaction-diffusion equations [30]. The modeling biosensor comprises three compartments, an enzyme layer, a dialysis membrane and an outer diffusion layer. The digital simulation was carried out using the finite difference technique [30,31]. By mimicking the experimental set-up, the developed model confirmed and qualitatively explained the synergistic effect in the GDH bioelectrode [18].

2. Mathematical modeling

2.1. Reaction scheme of the biosensor

The GDH biosensor being modelled is assumed to be composed of a graphite electrode covered with the nylon net (160 mesh, the thread thickness 100μm) and the enzyme solution. The enzyme layer is separated from the bulk solution by means of the dialysis membrane. The diffusion layer where the flux of the substances takes place is considered also. The schematic view of the modelled biosensor is presented in Fig. 1.

The scheme of GDH action is rather complex. The effect of substrate inhibition and cooperativity on the electrochemical response of GDH at steady state conditions was investigated in [38]. For simplification of modelling, a ping pong scheme of mediators oxidation at high glucose concentration describing GDH action was used as described in [39].

The scheme of the GDH-based bioelectrocatalytical system involves GDH reaction (1a) with glucose followed by the reduced GDH oxidation (1b) with ferricyanide and (1c) with the oxidized mediator as well as a cross reaction (1d) of the ferricyanide and the reduced mediator,

$$GDH_{ox} + \text{glucose} \xrightarrow{k_{red}} GDH_{red} + P, \tag{1a}$$

$$GDH_{red} + 2F_{ox} \xrightarrow{k_f} GDH_{ox} + 2F_{red}, \tag{1b}$$

$$GDH_{red} + 2M_{ox} \xrightarrow{k_{ox}} GDH_{ox} + 2M_{red}, \tag{1c}$$

$$F_{ox} + M_{red} \underset{k_{ex(r)}}{\overset{k_{ex(d)}}{\rightleftarrows}} F_{red} + M_{ox}, \tag{1d}$$

where GDH_{ox} and GDH_{red} are the oxidized and reduced glucose dehydrogenase, P - reaction product (δ-glucolactone), F_{ox} and F_{red} are ferricyanide and ferrocyanide, M_{ox} and M_{red} stand for the oxidized and reduced mediators, respectively. The reaction rate constants k_{red}, k_f and k_{ox} correspond to the respective biocatalytical process, $k_{ex(d)}$ and $k_{ex(r)}$ belong to the electron exchange reactions.

The biocatalytical current is produced during the oxidation of ferrocyanide and reduced mediator on the electrode surface as both, the mediators and hexacyanoferrates, are the redox active compounds,

$$F_{red} - e^- \longrightarrow F_{ox}, \tag{2a}$$

$$M_{red} - e^- \longrightarrow M_{ox}. \tag{2b}$$

In terms of substrates and products the reaction scheme (2.1) and (2.1) can be rewritten as follows:

$$E_{ox} + G \xrightarrow{k_1} E_{red} + P, \tag{3a}$$

$$E_{red} + 2S_1 \xrightarrow{k_2} E_{ox} + 2P_1, \tag{3b}$$

$$E_{red} + 2S_2 \xrightarrow{k_3} E_{ox} + 2P_2, \tag{3c}$$

$$S_1 + P_2 \underset{k_5}{\overset{k_4}{\rightleftarrows}} P_1 + S_2, \tag{3d}$$

$$P_1 - e^- \longrightarrow S_1, \tag{4a}$$

$$P_2 - e^- \longrightarrow S_2, \tag{4b}$$

where E_{red} and E_{ox} correspond to the reduced and oxidized GDH, S_1 and S_2 are the substrates - ferricyanide and oxidized mediator, P_1, P_2 stand for the products (ferrocyanide and reduced mediator) of the reactions.

2.2. Mathematical model

The mathematical model of the biosensor in a one-dimensional domain involves the following regions [18]:

1) An enzyme-loaded nylon net ($a_0 < x < a_1$, see Fig. 1). Due to the relatively small volume of the nylon net in comparison with the volume of the enzyme, the enzyme-loaded mesh is assumed as a periodic media, and the homogenisation process is applied to the enzyme-loaded mesh. In the enzyme layer the enzymatic reactions (3a)-(3c), the cross reaction (3d) and the mass transport by the diffusion of all compounds take place.
2) A dialysis membrane ($a_1 < x < a_2$), where only the reactions (3d) and the mass transport of low molecular weight compounds (substrates, products and glucose) take place.
3) An outer diffusion limiting region ($a_2 < x < a_3$), where the cross reaction (3d) and the mass transport by the diffusion take place. This layer is modelled according to the Nernst approach.
4) A convective region ($x > a_3$), where the analyte concentration is maintained constant.

These assumptions lead to a three compartment model. The homogenised enzyme layer corresponds to the first compartment of the mathematical model. The dialysis membrane and the outer diffusion are the next two compartments.

2.2.1. Governing equations

Assuming the symmetrical geometry of the biosensor and homogeneous distribution of the immobilized enzyme, the mass transport and the reaction kinetics in the enzyme layer can be described by the following system of the reaction-diffusion equations ($a_0 < x < a_1$, $t > 0$):

$$\frac{\partial E_{red}}{\partial t} = D_{E_{red}} \frac{\partial^2 E_{red}}{\partial x^2} + k_1 E_{ox} G_1 - 2k_2 E_{red} S_{1,1} - 2k_3 E_{red} S_{2,1}, \tag{5a}$$