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How low does the oxygen concentration go within a sandwich-type amperometric biosensor?

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ABSTRACT

Software for the automatic non-linear least squares fit of chronoamperometric responses corresponding to sandwich-type amperometric biosensors has been developed. The so-called Simplex algorithm computes a minimum value for the difference between experimental and theoretical data. The latter consider a numerical model based on a ping-pong reaction mechanism corresponding to an oxidase enzyme that has been immobilized between diffusion membranes.

The results obtained from the simulation of a first-generation lactate biosensor in presence of 0.1 mM substrate indicate that the concentration of O_2 would decrease only 0.1% with regards to its bulk value. Besides, the concentration of this natural mediator would remain practically unchanged during a typical calibration curve. This is because the rather high diffusion coefficient of O_2 and its regeneration at the electrode surface minimize the concentration changes of this species. In addition, it was found that the thicknesses of polycarbonate membranes and the enzymatic matrix have average values of 13 μm and 20 μm, respectively. However, these membranes might exhibit smaller thickness depending on the time provided for the crosslinking reaction. In this regard, if this reaction is slow enough, the enzymatic matrix would be able to diffuse through the pores of polycarbonate membranes and they will appear to be thinner than expected. This effect may compromise the response-time and the reproducibility of this kind of biosensors.

1. Introduction

The high specificity of enzymes enabled the development of biosensors, which are devices that can recognize specific substrates in samples with very complex matrixes [1–6]. The enzymatic reaction is typically detected by electrochemical or spectroscopical transducers [4–12]. A widely used detection strategy corresponds to the amperometric biosensors that use an oxidoreductase enzyme for changing the oxidation state of the substrate [1–6]. The reaction of several oxidases such as glucose oxidase or lactate oxidase can be represented by the following ping-pong mechanism [1,2,13]:

$$E_r + S \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} E_rS \overset{k_2}{\rightarrow} E_o + R \quad (1)$$

$$E_o + M \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} E_oM \overset{k_4}{\rightarrow} E_r + P \quad (2)$$

where E_r and E_o are the reduced and oxidized forms of the enzyme, M is the mediator, while R and P are products of the enzymatic reaction. The species E_rS and E_oM are intermediate complexes of the enzyme with S and M, respectively. From the analysis of Eqs. (1) and (2) it is possible to obtain the expression that describes the velocity of an enzymatic reaction according to a ping-pong scheme [13–18]:

$$v = \frac{v_{max}}{1 + K_S/C_S + K_M/C_M} \quad (3)$$

In this expression $v_{max} = C_E k_2 k_4 \ [(k_2 + k_4)]^{-1} = C_E k_{cat}$, $K_S = k_4 (k_{-1} + k_2) \ [(k_2 + k_4)k_1]^{-1}$, and $K_M = k_2 \ (k_{-3} + k_4) \ [(k_2 + k_4)k_3]^{-1}$. The constants K_S and K_M are usually called Michaelis' constants for S and M, C_E is the total concentration of the enzyme, and the variables C_M and C_S indicate the concentrations for M and S, respectively.

The electrochemical step for most amperometric biosensors can be represented by the following reaction:

$$P \overset{-ne^-}{\rightarrow} M \quad (4)$$

where the enzymatic product P is electrochemically oxidized to regenerate M. If the species P and M correspond to H_2O_2 and O_2, the device is denominated a first-generation biosensor [5,16]. However, if this natural redox mediator is replaced by another artificial electron carrier such as metallic complexes, then it is named

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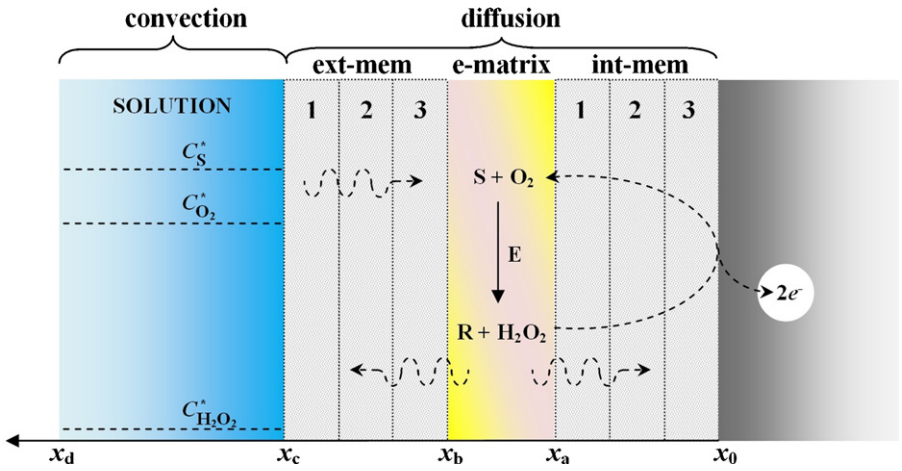

Fig. 1. Scheme of a sandwich-type amperometric biosensor. The numbers indicate different diffusion membranes.

second-generation biosensor [5,17]. Although oxygen is cheaper and more stable than any other artificial mediator, the oxidation of H_2O_2 requires a rather high potential at which other interfering species can react [5,13,17,19]. In addition, the concentration of oxygen is low and it is believed that it might compromise the stoichiometry of the enzymatic reaction [17–20]. Since most interfering species are negatively charged at pH 7, the first drawback can be minimized by coating the electrode surface with Nafion [5,21]. The second problem could be solved by using a membrane that tailors the diffusion of oxygen with regards to other reagents [5,13]. Both strategies have been implemented for optimizing sandwich-type amperometric biosensors, which are typically devices of first-generation [5,13]. Nevertheless, it is difficult or even impossible to measure the concentration profile of O_2 into the E-matrix, without disturbing the normal function of the sensor. Actually, it is also hard to assess several other variables of the sensor and for this reason, most advances on the area are based on a number of hypothesis that intend to explain why a calibration curve provides better or worse result than others.

The aim of this work is to apply a recently developed model to fit experimental chronoamperometric transients [13]. This procedure would let us estimate the concentration profiles of involved species within a sandwich-type amperometric biosensor as well as other parameters such as the thickness of the membrane and the E-matrix.

2. The model

The boundary conditions of this model are related to a sandwich-type amperometric biosensor, where the enzyme is confined within the E-matrix such as the scheme of Fig. 1. It is considered that, before the addition of the substrate, the concentrations of E and M are constants and the values of C_S and C_P are equal to zero. The species P can be rapidly re-oxidized at the electrode surface, and the effects associated with the migration of species can be neglected [22,23]. More details about the model can be consulted in a previous manuscript [13].

The equations used for the evaluation of concentration profiles are the following [13].

For $(x_c \geq x > x_b)$ and $(x_a \geq x > x_0)$:

$$(C_i)_j^{t+1} = (C_i)_j^t + \frac{D_i \delta t}{\delta x^2}\left[(C_i)_{j-1}^t - 2(C_i)_j^t + (C_i)_{j+1}^t\right] \quad (5)$$

For $(x_b \geq x > x_a)$:

$$(C_i)_j^{t+1} = (C_i)_j^t + \frac{D_i \delta t}{\delta x^2}\left[(C_i)_{j-1}^t - 2(C_i)_j^t + (C_i)_{j+1}^t\right] \pm \frac{v_{max}}{1 + K_S/(C_i)_j^t + K_M/(C_i)_j^t} \quad (6)$$

The thickness of most real sandwich-type biosensors (Δx) goes between 10 and 100 μm, where the x-axis is normal to the electrode surface and δx corresponds to the grid-size [19,22,24]. The subindex i represents some of the involved species, while j corresponds to a given position within the membrane [13]. The sign minus is used to evaluate the concentration profiles of S and M while the sign plus is employed for the case of species P and R. At the electrode surface ($x = 0$):

$$\Psi(\tau) = \frac{I(\tau)}{n_e F A C_S^*}\frac{\tau}{\delta x} = \frac{D_P \delta t N}{\delta x^2}\frac{(C_P)_1^t}{C_S^*} \quad (7)$$

In the last expression, $\Psi(\tau)$ corresponds to the dimensionless current at the time $\tau = N\Delta t$. The dimensionless diffusion of the mediator was fixed as: $D_M \delta t/\delta x^2 = 0.40$ [13]. Eq. (7) provides the theoretical transients that need to be fitted to experimental profiles.

The fit of experimental data with theoretical curves requires an algorithm that automatically minimizes the difference between every calculated and measured data point [25–29]. The Simplex algorithm calculates the goodness of fit (f^2) by the following expression of minimum-squares [27]:

$$f^2 = \sum_{t=1}^{\Omega}\left[\frac{I_{exp,t} - I_{cal,t}}{I_{exp,\Omega}}\right]^2 \Omega^{-1}$$

where $I_{exp,\Omega}$ is the experimental limiting-current, $I_{exp,t}$ and $I_{cal,t}$ are the experimental and calculated values of current, and Ω indicates the number of experimental data points. This fitting algorithm requires a set of starting values (seeds) that the researcher has to estimate in order to calculate the first theoretical curve [25–28]. Then the Simplex algorithm automatically changes these values to optimize the fit of the experimental profile, which is in fact a minimum of f^2 [28]. It is critical to provide a good set of seeds, otherwise either the simulated curve will not match the experiment or the out coming results will be meaningless [26,27]. Concerning this last point, it is suggested to compare the results of a set of experimental profiles instead of fitting isolated curves [25–27]. Such comparison helps the researcher to realize about the values of