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The application of the relaxation and simplex method to the analysis of data for glucose electrodes based on glucose oxidase immobilised in an osmium redox polymer

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ABSTRACT

Data for a series of fully integrated glucose oxidase, osmium redox polyelectrolyte layers deposited on thiolated gold electrodes by layer-by-layer self assembly was analysed using the relaxation and simplex method described in our earlier work (Flexer et al., 2008) [12]. The layer-by-layer assembly method allows fine control over the film thickness, enzyme loading, osmium and glucose concentrations with good reproducibility from electrode to electrode. In the analysis we combine the use of approximate analytical expressions with digital simulation to fit the data from an extensive set of experiments. The analysis shows a thickness dependence of the fraction of "wired enzyme molecules" and second order enzyme re-oxidation rate constant for thin films (below 300 nm) following changes in the multilayer film structure. For films thicker than 300 nm the kinetic data approach that of a redox hydrogel.

1. Introduction

Amperometric enzyme electrodes find application both in biosensors for environmental and medical diagnostic applications [1], such as blood glucose measurement, and in biofuel cells [2,3]. In many cases coupling of the enzyme catalysed redox reaction to the electrode is achieved using a redox mediator to "wire" the active site of the enzyme to the electrode. This can be achieved using redox hydrogels, a very successful and flexible approach pioneered by Heller's group [4], by entrapping the redox species in a layer at the electrode together with the enzyme [5], or by assembling using a layer-by-layer method a redox polymer and the enzyme [6]. In practical applications in both sensors or biofuel cells the performance of the enzyme electrode, in terms of the current passed at a given enzyme substrate concentration and electrode potential, is important for the application. Thus in a sensor application the amperometric current should, ideally, be linearly dependent on the substrate concentration over the appropriate range, reproducible from electrode to electrode, and stable during use. In a biofuel cell application the enzyme electrode should exhibit fast kinetics for the reaction of the enzyme substrate at a low overpotential, to ensure good energy conversion efficiency, and show good stability in operation.

The practical performance of an amperometric enzyme electrode is determined by the interplay of a number of kinetic processes including mass transport of species within the immobilised enzyme layer, the kinetics of the enzyme catalysed reactions and the kinetics of electrode reactions and charge transport through the film [7,8]. In principle any of these processes could limit the performance of the enzyme electrode and so a detailed understanding of these processes and their interplay is essential to an understanding of the overall performance of the amperometric enzyme electrode. Such an understanding also allows the rational design and optimisation of practical enzyme electrode performance – knowing which step or steps is limiting electrode performance allows one to make systematic changes to improve sensor electrode performance or biofuel cell efficiency or stability.

Despite the significant potential benefits detailed studies of the kinetics of amperometric enzyme electrodes are still relatively rare [8]. There are several factors which contribute to this. First, because of the number of possible rate limiting processes involved a full analysis demands a substantial set of data covering a range of electrode preparation conditions such as enzyme loading, and film thickness. A full analysis cannot be made on the basis of measurements on a single electrode, there is simply not enough information. Second it is often difficult to prepare enzyme electrodes with sufficient reproducibility between electrodes to allow a detailed analysis requiring quantitative comparisons between different electrodes – in effect a full kinetic analysis of an amperometric enzyme electrode requires sufficient reproducibility in fabrication

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between electrodes that one could make enzyme electrodes which could be used in an analytical application without the need for calibration. Nevertheless with care it is possible to achieve the necessary level of reproducibility, see for example the recent work of Barton's group on biofuel cell electrodes [9,10].

In earlier publications we have described a general kinetic model for the membrane-enzyme electrode in which the enzyme and mediator are immobilised in a multilayer at the electrode surface [11] and explained how this can be implemented by combining the relaxation method with automated grid point allocation with the simplex algorithm to fit experimental data [12]. In the present paper we employ this approach for the first time to an extensive set of experimental data and use the method to extract kinetic parameters for a real enzyme electrode. To do this we use electrodes fabricated using layer-by-layer self-assembly of glucose oxidase and an osmium redox polymer (GOx/PAH-Os). This system has the advantages that it has already been physically well characterised using a variety of techniques including QCM, ellipsometry, FT-IR and Raman spectroscopies [13–18], and it exhibits good reproducibility and stability. In addition, layer-by-layer assembly allows us to systematically control the film thickness – an important point when we come to test the applicability of the model.

Based on our previous studies of the GOx/PAH-Os system we know that the film thickness, Os surface concentration and enzyme loading all grow with the number of adsorption steps. The catalytic current varies with the film thickness. It has been established that the redox charge propagation within the film is by electron hopping and the diffusion coefficient has been estimated [15]. We assume that we can approximate the substrate partition coefficient between the solution and the film, K_S, to unity and finally, we assume that, because of the high water content of the films, the glucose diffusion coefficient within the multilayers is almost the same as in pure water [18].

The great advantage of electrostatically self-assembled systems as compared to other enzyme electrodes, such as hydrogels, is that we can design our electrodes at will choosing from a more or less wide spectrum the thickness, enzyme loading and Os concentration and, to a lesser extent, the re-oxidation rate constant. In this way, we are able to test the simulations on electrodes of a wide variety of parameters, and not only on one or two specific cases.

In this paper we present a complete study of the system for different film thickness, glucose concentration and electrode potentials. In the analysis of the experimental data we combine the use of analytical expressions describing the different limiting cases identified in the theory of Pratt and Bartlett [11] with digital simulation by the relaxation method with automatic mesh point allocation combined the simplex optimisation to extract kinetic parameters from experimental data [12].

2. The model

A general kinetic model for an enzyme-membrane electrode has been described previously [11] and for fuller details the reader is referred to the original paper. The model describes the general situation of a redox mediator and redox enzyme immobilised in the same layer at the electrode surface. This is a very common situation exemplified by, for example the popular and successful approach of using a redox hydrogel pioneered by Heller and his colleagues [4].

The reactions occurring in the film can be written

$$A + E_2 \xrightarrow{k} B + E_1 \tag{1}$$

$$E_1 + S \overset{K_{MS}}{\leftrightarrow} ES \xrightarrow{k_{cat}} E_2 + P \tag{2}$$

and at the electrode

$$B \rightarrow A + ne^- \tag{3}$$

where A and B are the oxidised and reduced forms of mediator, E_1 and E_2 are the oxidised and reduced forms of the enzyme, S and P are the substrate and product of the enzymatic reaction and ES is the complex formed between enzyme and substrate.

The substrate undergoes partition between the bulk solution and the film (partition coefficient K_S) and then diffuses within the film with a diffusion coefficient D_S. The mediator is assumed to be confined within the film. Charge propagation within the film can either be by physical diffusion of the mediator within the film described by a diffusion coefficient D_A or, if the mediator is covalently bound within the film, charge propagation can occur by electron hopping self-exchange between the reduced and oxidised forms of the mediator described by a diffusion coefficient D_e. According to the Dahms-Ruff formalism [19,20] these two situations are equivalent with $D_A = D_e$. The enzyme-substrate reaction is described using Michaelis Menten kinetics (Eq. (2)). The oxidised mediator, in the case of an oxidation reaction such as that in the glucose oxidase/glucose electrode, regenerates the oxidised form of the enzyme with a second order rate constant k, according to the conventional "ping-pong" mechanism [21].

In the specific case of the glucose electrode using glucose oxidase (GOx) and an osmium redox mediator the reactions are

$$S + GOx_{ox} \overset{K_{MS}}{\leftrightarrow} S - GOx_{ox} \xrightarrow{k_{cat}} P + GOx_{RED} \tag{4}$$

$$GOx_{RED} + 2PAH - Os(III) \xrightarrow{k} S + GOx_{ox} + 2PAH - Os(II) + 2H^+ \tag{5}$$

$$PAH - Os(II) \rightarrow PAH - Os(III) + e \tag{6}$$

Notice that in Eq. (5) we have included the stoichiometric factor of 2.

The second-order differential equations describing the system in the steady state are

$$D_A \frac{d^2[A]}{dx^2} = \frac{\varsigma k k_{cat}[A][S][E]_{TOT}}{k[A]\ (K_{MS} + [S]) + k_{cat}[S]} \tag{7}$$

$$D_S \frac{d^2[S]}{dx^2} = \frac{k k_{cat}[A][S][E]_{TOT}}{k[A]\ (K_{MS} + [S]) + k_{cat}[S]} \tag{8}$$

where [S] is the concentration of glucose, $[E]_{TOT}$ the concentration of enzyme in all forms, and [A] the concentration of Os(III). In Eq. (7) ζ is the stoichiometric factor (equal to 2) when the enzyme mediator reaction proceeds in two sequential steps both first order in mediator [7]. The concentrations of mediator and substrate vary with position within film. (Eqs. (7) and (8)) are non-linear second-order differential equations and do not have a closed form analytical solution.

We begin by recasting the problem in terms of the following dimensionless variables [7,11]

$$\chi = \frac{x}{L} \tag{9}$$

$$a = \frac{[A]}{[A]_{TOT}} \tag{10}$$

$$s = \frac{[S]}{K_S[S]_\infty} \tag{11}$$

$$\mu = \frac{K_S[S]_\infty}{K_{MS}} \tag{12}$$

$$\kappa = L\sqrt{\left(\frac{k[E]_{TOT}}{D_A}\right)} \tag{13}$$

$$\gamma = \frac{k[A]_{TOT}K_{MS}}{k_{cat}K_S[S]_\infty} \tag{14}$$

$$\eta = \frac{D_S k K_{MS}}{D_A k_{cat}} \tag{15}$$