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Optimizing the ratio of horseradish peroxidase and glucose oxidase on a bienzyme electrode: comparison of a theoretical and experimental approach

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Abstract

This study compares the behaviour of an electrochemical enzyme biosensor with a theoretical analysis based on a mathematical model and numerical simulation. The biosensor is based on a bi-enzyme channelling configuration, employing the enzymes glucose oxidase and horseradish peroxidase, with direct electron transfer of horseradish peroxidase at a conducting polymer electrode. This was modelled by a system of partial differential equations and boundary conditions representing convective and diffusive transport of the substrates glucose and hydrogen peroxide, as well as reaction kinetics of the bienzyme electrode. The main parameter investigated was the ratio of the two immobilised enzymes, with the aim of maximising the amperometric signal amplitude. Experimentally, it was found that the optimum ratio of enzymes on the electrode was 1:1. A theoretical model consistent with this outcome suggests that the kinetic rates of horseradish peroxidase were greatly reduced in this configuration.

Keywords: Biosensor; horseradish peroxidase/glucose oxidase electrode; mathematical and computational modelling; Michaelis-Menten kinetics;

1 Introduction

Mathematical modelling is a powerful technique for predicting the behaviour of complex systems which are based on well-defined underlying principles, such as sensors and biosensors. For example, an electrochemical enzyme biosensor may be represented by electron transfer processes at the electrode interface, the reaction kinetics of the enzymes and substrates involved, as well as convection and diffusion mechanisms employed for the transport of substrates and products. Biosensor design (which incorporates multiple parameters and potential interactions) is an extremely complex process which is traditionally carried out in an empirical fashion and often leads to less than optimal output. Using mathematical modelling in parallel with experimental techniques can offer clearer insights into critical design parameters and therefore the potential of significant improvements in biosensor performance.

This study investigates a model biosensor system which consists of two enzymes immobilised onto an electrode modified with the conducting polymer polyaniline/polyvinylsulphonate (PANI/PVS). The first enzyme, glucose oxidase (GOX), acts as the source of the substrate for the second enzyme, horseradish peroxidase (HRP), producing hydrogen peroxide from the oxidation of glucose to gluconolactone. Horseradish peroxidase is in direct electronic communication with the electrode via the conducting polymer thus bringing about the electrocatalytic reduction of hydrogen peroxide, which can be measured amperometrically at moderate reducing potentials such as -100mV (vs. Ag/AgCl). Cascade schemes, where an enzyme is catalytically linked to another enzyme, can produce signal amplification and therefore increase the biosensor efficiency.

One important criterion for the performance of this bi-enzyme system is the ratio of the two enzymes present on the electrode surface. HRP and GOX have very different

kinetic characteristics which have been studied extensively. HRP has very fast reaction kinetics and substrate turnover rates and so has been widely used as a reporter enzyme in many assay systems although its substrate, hydrogen peroxide, is relatively unstable. By comparison, GOX is significantly slower. However, it uses the highly useful substrate, glucose, which is not inhibitory at high concentrations (mM) and also produces hydrogen peroxide as a product. These substrates and products are produced at different rates and are subjected to different diffusional processes. Obtaining the optimum performance of the sensor response will, in one instance at least, depend on the correct ratio of these two components.

Several simplifying assumptions are made about the system. Firstly, it is assumed that the immobilisation mechanisms of the two enzymes are equally efficient on the sensor surface under the conditions employed. Secondly, immobilisation of HRP and GOX is assumed to produce a geometrically close-packed spherical monolayer which is spatially homogeneous. Thirdly, it is assumed that the electron transfer process is 100% efficient, since this parameter only affects the magnitude of the signals, and not their relative responses.

This paper aims to investigate experimentally the optimum ratio of these two enzymes on the electrochemical biosensor. In addition, a mathematical model was set up to represent the behaviour of the system. Numerical simulations of this model were used to investigate a range of parameters and their effects on the biosensor response. Finally, a discussion was given as to how the experimental response of the biosensor could be interpreted within the suggested modelling paradigm.

2 Experimental methods

2.1 Materials

Aniline was purchased from Aldrich (13, 293-4), vacuum distilled and stored frozen under nitrogen. Glucose and polyvinylsulphonate (PVS, 27, 842-4) were purchased from Aldrich. Horseradish peroxidase (HRP, 250U/mg) and glucose oxidase (GOX, 270 U/mg) were purchased from Biozyme Laboratories. 30% (v/v) hydrogen peroxide solution was purchased from Merck. Silver/silver chloride (Ag/AgCl) electrodes were purchased from Bioanalytical Systems Ltd (Cheshire, UK). The platinum mesh (29, 809-3) was purchased from Aldrich.

2.2 Buffers

All electrochemical measurements were carried out in phosphate buffered saline (PBS), (0.1M phosphate, 0.137M NaCl and 2.7mM KCl), ph 6.8. All biochemicals were prepared in PBS.

2.3 Instrumentation

Screen-printed carbon-paste electrodes (7 mm^2) were produced using an automated DEK 248 machine (Weymouth, UK). Electrode modification and protein immobilisation were performed on a CH1000 electrochemical analyser with CH1000 software, using either cyclic voltammetry or time-based amperometric modes. An Ag/AgCl pseudo reference electrode and a platinum mesh auxiliary electrode were used for bulk electrochemical experiments. Electrochemical flow cells were used according to [5]. These were composed of polycarbonate and designed to house the screen printed electrodes. The flow cell incorporated internal Ag/AgCl reference and platinum wire auxiliary electrodes. The

cell volume was 26μl. A peristaltic pump (Gilson Miniplus 3) was used to perform flow-injection analysis at the set flow rate of 400μl/min.

2.4 Screen printed electrode modification with PANI/PVS

Electrodes were placed in 10ml of 0.2M H_2SO_4, prior to the polymerisation of aniline. A platinum mesh auxiliary and a silver/silver chloride reference electrode were used. Electrodes were cleaned and activated using cyclic voltammetry between -1200 and 1500mV versus Ag/AgCl electrode at scan rate of 100mV/s, sensitivity of 10^{-3}A over one cycle. A mixture of 7.8ml 1M HCl, 186μl aniline and 2ml PVS was degassed under nitrogen for 10 min. Aniline was polymerised on the surface of the working electrode using 20 voltammetric cycles between -500 and 1100mV versus Ag/AgCl electrode at 100mV/s, and sensitivity of 10^{-4}A.

2.5 Immobilisation of enzyme

Following polymerisation of aniline, the electrode was transferred to a 2ml batch cell. The surface of the polymer was reduced in 2ml of PBS (degassed for 10 min under nitrogen or argon) at -500mV vs Ag/AgCl, sample interval of 500ms, over 600s at a sensitivity of 1 times 10^{-4} A/V. Mixtures of HRP and GOX at different molar ratios were prepared in PBS prior to use. Very quickly after reduction was complete, PBS buffer was removed from the cell and replaced with the protein solution, not under stirring or degassing. Oxidation was then performed immediately at +700mV vs Ag/AgCl. The protein solution was carefully recovered from the cell and restored for later use.

3 Experimental results

Experiments were carried out with the aim to build a bienzyme-based biosensor for glucose analysis. Horseradish peroxidase (HRP) and glucose oxidase (GOX) were immobilised together in one single step on a polyaniline/polyvinylsulphonate (PANI/PVS) modified screen-printed carbon paste electrode. Different solutions containing the two enzymes were prepared at the ratio HRP/GOX from 1:7 to 7:1, maintaining a total concentration of 0.8 mg/ml, and used to immobilise the enzymes on the electrode. The immobilisation was performed by immersing the electrode into the enzymes solution and applying a static potential of 0.7 V for 25 min. Due to the ability of polyaniline to bind biomolecules, the two enzymes resulted electrostatically adsorbed on the electrode surface and because of the nature of this immobilisation, it can be assumed that the distribution of the enzyme molecules over the surface was equal in ratio to that of the solution used. After the immobilisation, the electrode was inserted in a flow-cell and using a peristaltic pump, an amperometric flow-injection analysis was carried out. A

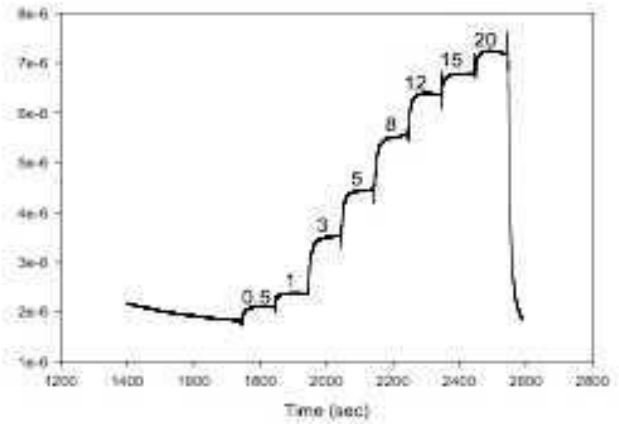

Figure 1: Amperometric responses of a HRP/GOX (mass ratio 2:6) bienzyme electrode to a range of glucose concentrations between 0.5 - 20 mM at -0.1V vs Ag/AgCl.

PBS buffer solution (pH 6.8) was firstly passed over the electrode surface until a steady current signal was recorded at constant potential of −0.1V. Glucose standard solutions at concentrations between 0.5 and 20 mM were then passed over the electrode and the signals recorded. Figure 1 shows a typical amperogram recorded after passing the glucose solutions. The sensitivities of the electrodes were compared using the slope of the

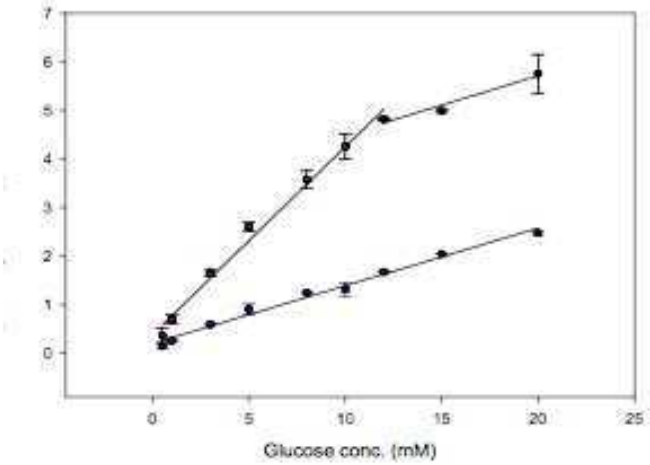

Figure 2: Glucose calibration curves for the bienzyme electrode yielding the highest and lowest sensitivities. The curve with the highest slope was achieved using the molar ratio HRP/GOX of 1:1 and the curve with the lowest slope was achieved using the molar ratio HRP/GOX of 26:1 (−0.1V vs Ag/AgCl).

glucose calibration curves. The mass ratios HRP/GOX in the solutions used for the immobilisation can be more conveniently expressed as molar ratios in order to visualise approximately the relative molecular distribution on the electrode surface of the two enzymes. Figure 2 shows the calibration curves achieved with the best electrode configuration (HRP 0.2, GOX 0.6 mg/ml, which is a molar ratio HRP/GOX of 1:1) and with the worst (HRP 0.7, GOX 0.1 mg/ml, which is a molar ratio HRP/GOX of 26:1).

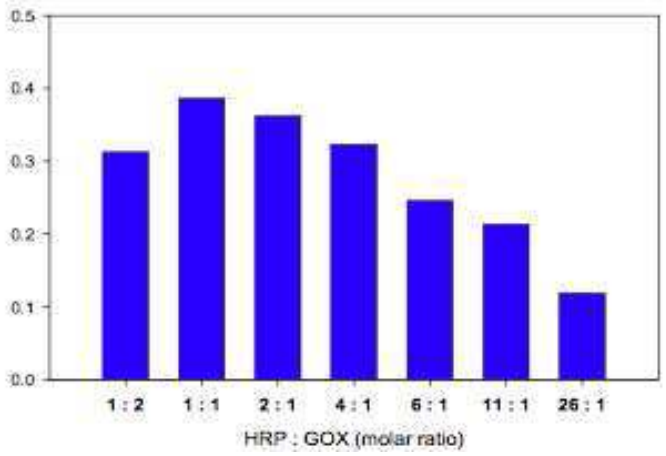

Figure 3: Comparison of HRP/GOX ratio and sensitivity to glucose. The electrode prepared immobilising HRP and GOX at the molar ratio of 1:1 (HRP 0.2, GOX 0.6 mg/ml) yielded the highest catalytic signals and the highest sensitivity (-0.1V vs Ag/AgCl). The glucose concentration used in this experiment was 20mM.

Figure 3 shows a comparison between all the sensitivities of the electrodes with the different molar ratios HRP/GOX. It can be clearly seen that the electrode prepared with HRP/GOX at a molar ratio of 1:1 yielded the highest sensitivity.

The GOX adopted in the experiment has an activity of 270 U/mg protein, and HRP 250 U/mg protein. Expressing the two activities in U/mol protein, it is 1.7 for GOX and 5.7 for HRP. Thus, HRP is approximately 3 times more active than GOX. Considering the difference in activity between the two enzymes, a platform with GOX in excess with respect to HRP was expected to be the most efficient. The fact that the platform with HRP and GOX present at molar ratio of 1:1 produced the highest signals, suggests that other phenomena occur and contribute to generate the response. Diffusion of the reactants in solution over the electrode surface to reach the enzymes is certainly an important factor to be considered. Also, the activity of HRP may be reduced as

a consequence of its immobilisation on the electrode surface and its reliance on direct electron transfer. The numerical simulations presented in the following section seem to agree strongly with this latter hypothesis.

4 Mathematical model formulation

The mathematical model is based on the existence of a convection layer, where the glucose concentration is maintained constant, and a diffusion layer. The two enzymes form a monolayer on the electrode so all reactions can be assumed to take place at the lower boundary of the diffusion domain. For computational simplicity, the flow effects are not explicitly modeled and the existence of the convective zone is only reflected in the boundary conditions imposed at the top of the diffusion layer. The equations are therefore one-dimensional, where the spatial variable x measures the distance from the electrode. (See Figure 4.)

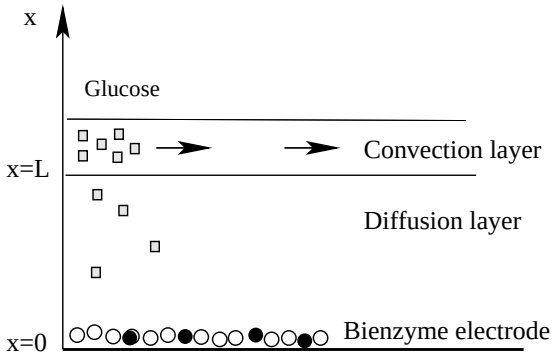

Figure 4: Experimental set-up

A cascade reaction takes place at the electrode. Glucose oxidase catalyses the oxidation reaction of glucose to gluconic acid, with production of H_2O_2. HRP is oxidised by hydrogen peroxide and then subsequently reduced by electrons provided by the elec-

trode, as shown in the following abbreviated reaction. (See, for example, [4], [3].)

$$\beta\text{-D-glucose} + O_2 + H_2O \overset{\text{glucose}}{\underset{\text{oxidase}}{\longrightarrow}} \text{gluconic acid} + H_2O_2 \tag{1}$$

$$H_2O_2 + \text{HRP} \longrightarrow \text{Compound} + H_2O \tag{2}$$

$$\text{Compound} + 2\text{e}^- + H^+ \longrightarrow \text{HRP} + H_2O \tag{3}$$

The two reactions are modeled by standard Michaelis-Menten equations. This simple scheme has been used extensively for modelling glucose-glucose oxidase kinetics (see, for example, [2]) and it was also shown to be appropriate for the case of immobilised HRP in [7]. For the purpose of our comparative analysis, using similar kinetics for the two consecutive reactions is a necessary simplifying assumption. The kinetic scheme is thus given by the equations (4)-(5) below

$$E_1 + S_1 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C_1 \xrightarrow{k_2} E_1 + S_2 \tag{4}$$

$$E_2 + S_2 \underset{k_{-3}}{\overset{k_3}{\rightleftarrows}} C_2 \xrightarrow{k_4} E_2 + P, \tag{5}$$

where we have used the following notation

$E_1(t) =$ first enzyme (Glucose Oxidase) concentration,

$E_2(t) =$ second enzyme (Horseradish peroxidase) concentration

$S_1(x,t) =$ first substrate (Glucose)

$S_2(x,t) =$ second substrate (Hydrogen Peroxide)

$C_1(t) =$ first complex

$C_2(t) =$ second complex

$P(x,t) =$ final product.

We now write down the differential equations governing the behaviour of the relevant chemical species. The two substrates, glucose and hydrogen peroxide are free to diffuse

throughout the domain at all times during the experiment. This is reflected by the diffusion equations

$$\frac{\partial S_1}{\partial t} = D_1 \frac{\partial^2 S_1}{\partial x^2}, \qquad 0 \le x \le L, \quad t \ge 0 \tag{6}$$

$$\frac{\partial S_2}{\partial t} = D_2 \frac{\partial^2 S_2}{\partial x^2}, \qquad 0 \le x \le L, \quad t \ge 0. \tag{7}$$

At the diffusion layer boundary ($x = L$) the concentration of glucose is maintained constant by the injected flow, while hydrogen peroxide is assumed to be constantly flushed away. The resulting boundary conditions are given below,

$$S_1(L,t) = S_0, \qquad S_2(L,t) = 0, \qquad t \ge 0. \tag{8}$$

At the electrode surface ($x = 0$) the boundary conditions express the fact that the diffusive flux is equal to the reaction rate,

$$D_1 \frac{\partial S_1}{\partial x} = k_1 E_1 S_1 - k_{-1} C_1, \tag{9}$$

$$D_2 \frac{\partial S_2}{\partial x} = k_3 E_2 S_2 - (k_2 + k_{-3})\, C_1. \tag{10}$$

In addition, the following evolution equations describe the kinetics of the enzyme-substrate reactions, according to the Michaelis–Menten scheme (4)-(5), taking place at the electrode.

$$\frac{dE_1}{dt} = -k_1 E_1 S_1 + (k_{-1} + k_2)\, C_1, \tag{11}$$

$$\frac{dE_2}{dt} = -k_3 E_2 S_2 + (k_4 + k_{-3})\, C_2, \tag{12}$$

$$\frac{dC_1}{dt} = k_1 E_1 S_1 - (k_2 + k_{-1})\, C_1, \tag{13}$$

$$\frac{dC_2}{dt} = k_3 E_2 S_2 - (k_4 + k_{-3})\, C_2, \tag{14}$$

Finally, we specify the initial conditions

$$S_1(x,0) = S_0(x), \qquad S_2(x,0) = 0, \qquad P(x,0) = 0,$$

$$E_1(0) = \xi e, \qquad E_2(0) = e, \qquad C_1(0) = 0, \qquad C_2(0) = 0. \tag{15}$$

For computational simplicity, the initial glucose profile is given by the following step function

$$S_0(x) = \begin{cases} S_0, \text{ if } x = L, \\ 0, \text{if } x \neq L, \end{cases}$$

where S_0 is the constant concentration present in the injection flow.

The purpose of this study is to determine the ratio of GOX to HRP on the electrode (denoted in the initial conditions (15) by ξ) which maximizes the amplitude of the measured signal, subject to the additional constraint that $\xi e + e = E_0$, where E_0 is the total amount of enzyme present on the electrode. The current measured at the electrode is given by the electron transfer rate in (3) which, in the simplified scheme (5), can be assumed proportional to the rate of formation of final product, P. This will be calculated from the equation

$$\frac{dP}{dt} = k_4 C_2 \tag{16}$$

once the evolution of $C_2(x,t)$ is determined by solving the system of equations (6)–(15).

5 Numerical simulations

The numerical integration of the partial differential equations and boundary conditions (6)–(15) was implemented in C, using a uniform time step of 10^{-5} and 100 spatial grid points. A standard implicit Crank-Nicolson scheme was employed in order to avoid instability restrictions and backward and forward difference displacements were used for the time derivatives. The graphics were produced using IDL (Interactive Data Language). The table in Figure 5 summarizes the values of all physical constants used in the numerical simulations. The purpose of these simulations was to find which of the parameters used has the greatest influence on the optimal bienzyme ratio and hence to

determine the suitability of this model as well as improve it for future applications.

Description	*Constant*	*Value*
Diffusion layer depth (m)	L	2×10^{-4}
Diffusion constants (m^2/s)		
Glucose	D_1	6.7×10^{-10}
Hydrogen peroxide	D_2	8.8×10^{-10}
Reaction rate constants	k_1	$10^2 - 5 \times 10^4$
(m^3/mol·s)	k_3	$10^2 - 5 \times 10^4$
(s^{-1})	k_{-1}	10^{-1}
	k_{-3}	10^{-1}
	k_2	10
	k_4	$1 - 200$
Initial concentrations:		
total enzyme (mol/m^2)	E_0	10^{-5}
glucose (mol/m^3=mM)	S_0	$0.5 - 20$

Figure 5: Typical values for constants

The experimental analysis involves the conversion of an enzyme concentration to a mass of enzyme immobilised on the electrode surface. Previous work in [8] established the experimental conditions necessary for the formation of a monolayer deposition of enzyme on the conducting polymer-modified electrode and calculated the coverage in this instance to be of the order of 10^{-5} mol/m^2. Exact values of the kinetic constants $k_{\pm 1}$, k_2, $k_{\pm 3}$ and k_4 were not rigourously derived for this study and they are generally

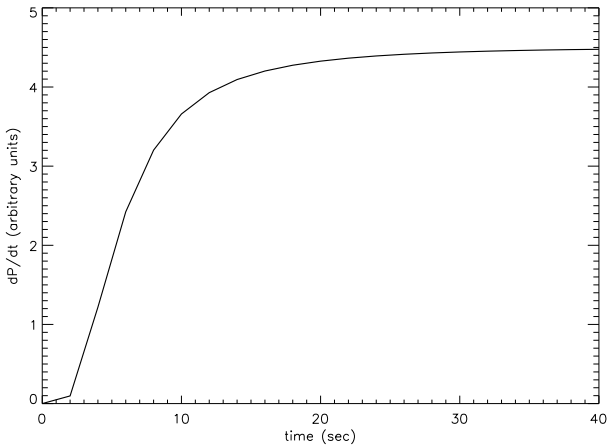

Figure 6: Time evolution of product rate formation in (16)

hard to determine. The orders of magnitude for these parameters were chosen within the accepted ranges for enzyme-substrate kinetics and moreover, it is the relative size of the rate constants for the two reactions that matters most for this study.

The time evolution of $\frac{dP}{dt}$ (the rate of formation of final product) on the electrode, which we took as a measure of the amperometric signal, is plotted in Figure 6. The steady state (obtained after approximately 40 seconds, which is of the same order of magnitude as the recorded experimental value of 100 seconds) is recorded as the current value and used for future parameter iterations. The first set of numerical simulations was conducted with a view to assessing the effect of varying the glucose concentration on the current response and the optimal GOX:HRP ratio. The integration of equations (6)–(15), as described above, was repeated for different values of the initial glucose concentration S_0 (100 values were chosen between 0.5mM and 20mM) and GOX:HRP molar ratio on the electrode, ξ (we used 60 values between 0.1 and 6). The kinetic constants were assumed to be the same for both reactions, as given by Table 5 (with $k_1 = k_3 = 10^2 \mathrm{m}^3/\mathrm{mol}\cdot\mathrm{s}$). Figure 7 shows the dependence of the current response on

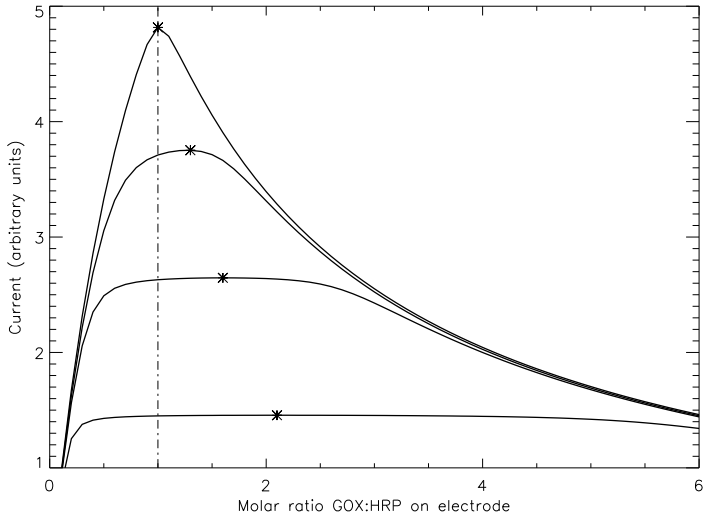

Figure 7: Dependence of current on ξ (electrode GOX:HRP ratio) for different initial glucose concentrations, s_0. From bottom to top the curves correspond to s_0 =1mM, 5mM, 10mM and 20mM. The position of the maximum current value is indicated on each curve.

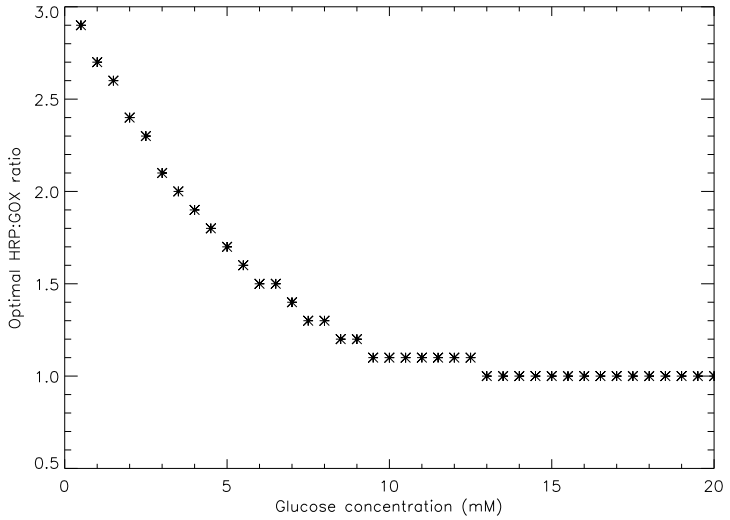

Figure 8: Dependence of optimal GOX:HRP ratio on glucose concentration

the molar ratio of the immobilized enzymes, ξ, for different values of the initial glucose concentration, S_0. (The lower curve is obtained for $S_0 = 1$mM and the upper one for $S_0 = 20$mM.) The optimal ratios (the values which yield the highest current) for various glucose concentrations are then plotted in Figure 8. From these graphs we note that, at low glucose concentrations, varying the ratio of the immobilized enzymes has a small effect on the electrode sensitivity. As the glucose concentration increases, the optimal ratio value becomes more pronounced and converges to 1.

The second set of numerical simulations has the purpose of establishing how the relative speed of the two reactions (oxidation of glucose by glucose oxidase and subsequent reduction of hydrogen peroxide, in the presence of HRP) affects the current magnitude and optimal bienzyme ratio. The results below were obtained for a glucose concentration value of $s_0 = 10$mM (although the value $s_0 = 20$mM produced similar output). In what follows, the biosensor efficiency constant, k_a, is chosen as an indicator of the kinetic behaviour of a chemical reaction (see, for example, [6], [7]). This parameter is, in general, defined as

$$k_a = \frac{k_{cat}}{K_M}$$

where K_M is the Michaelis constant of the enzyme-substrate reaction and k_{cat} is the catalytic turnover number (which is equal to k_2 in the case of the first reaction and k_4 for the second). The number k_a is believed to be a good measure of catalytic efficiency when $S_0 < K_M$ that is, when the enzyme is not saturated with substrate. (See, for example, [1].) The ultimate limit on the value of k_{cat}/K_M is set by the complex formation rate constant (given here by the kinetic parameters k_1 and k_3). For the GOX and HRP reactions in our case, the efficiency constants are

$$k_a^1 = \frac{k_2}{K_M^1}, \qquad k_a^2 = \frac{k_4}{K_M^2},$$

respectively, where

$$K_M^1 = \frac{k_2 + k_{-1}}{k_1} \quad \text{and} \quad K_M^2 = \frac{k_4 + k_{-3}}{k_2}.$$

The numerical simulations presented below study the effects of varying the kinetic characteristics associated with each step of the two reactions. First, the ratio k_a^2/k_a^1 was varied by selecting different values for k_3 and k_1 and keeping all the other kinetic parameters constant. It was expected that, for high values of k_3, the fast rate of the first step of the second reaction would prevent the diffusion of hydrogen peroxide (the product of the first reaction) away from the electrode, thus increasing the efficiency of the biosensor. However, it was observed that as we increased k_3 relative to k_1, the current value quickly becomes stationary, the optimal bienzyme ratio converges again to 1 and there are no noticeable changes in the channelling efficiency. (See Figures 9 and 10.) One possible explanation is that, given the choice of parameters in this experiment, the rate-limiting steps for the two reactions (corresponding to the dissociation of complex into final product) were equal and this was the essential factor characterising the total relative speed.

Secondly, the ratio of the turnover numbers, k_4/k_2, was used as a measure of the relative speed of the two consecutive reactions. According to Table 5, we chose $k_1 = k_3 = 10^3 \text{m}^3/\text{mol}\cdot\text{ s}$, $k_2 = 10^2 \text{s}^{-1}$, $k_{-1} = k_{-3} = 10^{-1} \text{s}^{-1}$ and varied k_4 from 1 to 200s^{-1} (using a total of 50 values). This time, we observed a significant change in the optimal ratio of immobilized enzyme as k_4 increases. Figure 11 shows the current as a function of the molar ratio GOX:HRP, for different values of k_4/k_2 ranging from 0.5 to 8. It is interesting to note that the second curve, which corresponds to $k_4 = k_2$, indicates that the highest sensitivity is obtained for a molar ratio GOX:HRP of 1. The dependence of the optimal bienzyme ratio on k_4/k_2 is plotted in Figure 12. We note that the graph

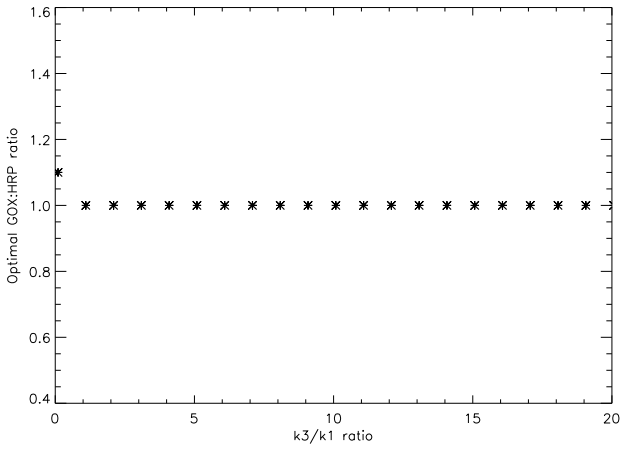

Figure 9: Dependence of optimal GOX:HRP ratio on k_3/k_1 ratio.

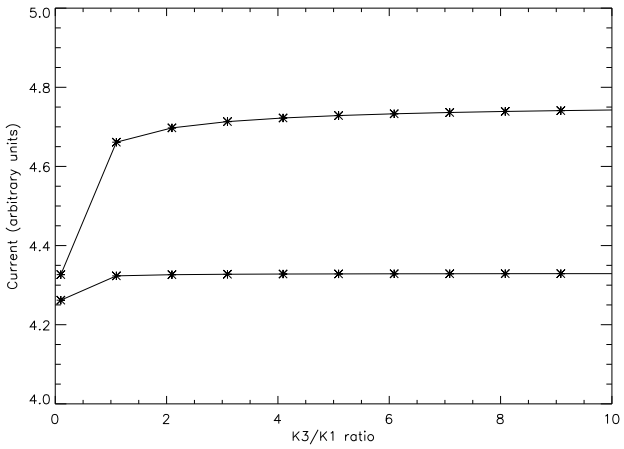

Figure 10: Dependence of current on k_3/k_1 ratio for different values of ξ. The upper curve is obtained for $\xi = 1$ (the optimal GOX:HRP ratio) while the lower curve has $\xi = 2$.

is almost linear for small values of k_4/k_2 and that, if k_4/k_2 is less (or greater) than one then the optimal GOX:HRP ratio is also less (respectively, greater) than one, hence the enzyme corresponding to the slower reaction should predominate in order to ensure maximum biosensor efficiency.

To conclude, the numerical simulations presented here show that, when the two consecutive reactions are assumed to be equally fast, the optimal ratio of immobilised enzymes converges to 1 as the glucose concentration increases. Moreover, the results obtained by fixing the glucose concentration and varying the kinetic rates of the GOX and HRP reactions strongly suggest that an optimal ratio GOX:HRP of 1 is associated with the two consecutive reactions proceeding at the same speed.

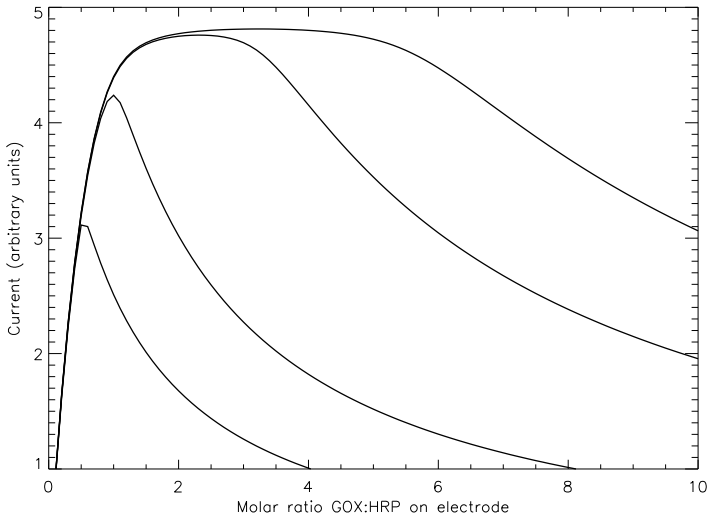

Figure 11: Dependence of current on ξ (electrode GOX:HRP ratio) for different k_4/k_2 values. The lower curve corresponds to $k_4/k_2 = 0.5$ and the upper curve $k_4/k_2 = 8$.

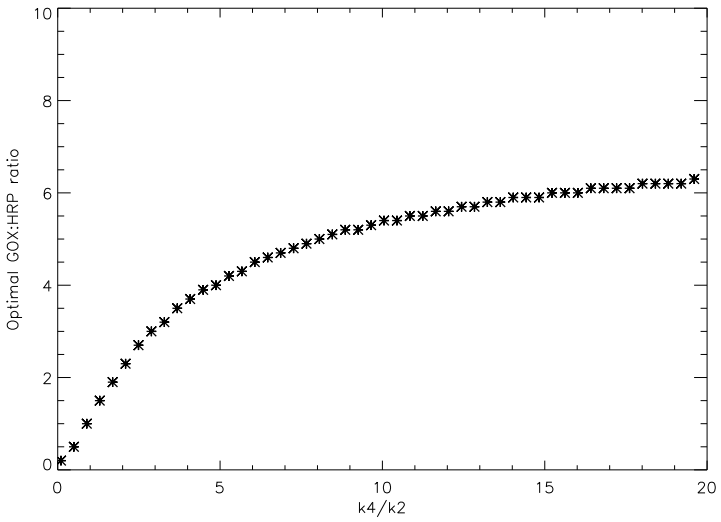

Figure 12: Dependence of optimal GOX:HRP ratio on k_4/k_2 ratio.

6 Conclusions

Mathematical modelling has been used to investigate the processes occurring within an electrochemical enzyme biosensor. The experimental results show that a system employing equal molar ratios of the two enzymes yields the optimal sensor response, which was contrary to what was expected, as the enzyme horseradish peroxidase has a higher activity than glucose oxidase. By contrast, numerical simulations suggest that an optimal ratio GOX:HRP of 1 is associated with the two enzyme reactions proceeding at the same speed. Since the mathematical model on which the simulations are based uses kinetic rate constants for the immobilised enzymes, while the specific activities quoted in the experimental work refer to the enzymes in the PBS solution, it is reasonable to conclude that these conditions might be brought about by a reduction in the actual activity of immobilised HRP. This could be due to the efficiency of electron transfer to the enzyme active site from the conducting polymer surface, which is affected by the

random orientation of enzyme on the surface, possibly making much of the immobilised material completely inactive.

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8 Biographies

Dana Mackey obtained her PhD in Applied Mathematics from University College Dublin, in 1999 and became an Assistant Lecturer in the School of Mathematical Sciences at Dublin Institute of Technology in 2005. Her current research interests include nonlinear dynamical systems and differential equations and modelling of problems arising in industrial settings. She has been collaborating with researchers in the National Centre for Sensor Research for over a year, on mathematical and computational modelling of biosensor devices.

Anthony Killard received his primary degree in Microbiology at Trinity College, Dublin in 1993 and his PhD in Biotechnology at Dublin City University. He is currently a Senior Researcher at the Biomedical Diagnostics Institute, within the National Centre for Sensor Research. His main research interests are in the area of electrochemical sensing, with special interest in electrochemical immunosensing.

Adriano Ambrosi received the Laurea degree in Analytical Chemistry in 2003 from the University of Rome "La Sapienza". At present he is working on a Ph.D. degree in Chemical Sciences at Dublin City University. His research has focused on development

and characterisation of amperometric immunosensor platforms.

Malcolm Smyth is a Professor of Chemistry at Dublin City University and has served as Dean of the Faculty of Science and Health since 1995. He was a founding member of the Sensor Research Group at DCU since 1988, which has now culminated in the formation of the internationally renowned National Centre for Sensor Research (NCSR). He obtained his BSc degree in Biochemistry from The Queen's University of Belfast in 1972, and his PhD in Analytical Chemistry from the University of London in 1976. His research interests are centred mainly in the fields of electroanalysis and separation science, with particular emphasis on electrochemical detection for HPLC and CE, modified electrodes and electrochemical biosensors.

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