NUMERICAL SIMULATION OF AMPEROMETRIC BIOSENSORS PERFORMANCES

A. BENYAHIA, and S. BACHA
Laboratoire de génie de l'environnement
Université Abderrahmane Mira de Béjaia.
Route de Targa Ouzemmour 06000 Béjaia, Algeria.
E-mail: hal_ben@yahoo.fr

KEYWORDS:
amperometric biosensor, homogeneous enzyme kinetic, enzyme membrane, diffusion, modelling, simulation.

ABSTRACT:

A mathematical model is used to analyse the transient response of amperometric biosensors has been developed. The model is based on non stationary diffusion equations containing a non linear term related to Michaelis-Menten kinetics of the enzymatic reaction. The numerical simulation is performed with finite volume method and results are compared with known analytic solutions obtained for some restricted parameters value's at steady state.

Numerical results have shown that best performances are obtained for biosensor when the biosensor operate under internal diffusion control and this may be obtained either by a high loading of the membrane with enzyme and/or by ensuring a good stirring of the solution.

INTRODUCTION

Biosensors are defined as analytical devices incorporating a physicochemical transducer or transducing system and biologically active material integrated with it. Biosensors yield a signal witch is proportional to the concentration of a measured analyte or group of analytes.

Amperometric biosensor is one of the most types of biosensors witch typically rely on an enzyme system that catalytically converts electrochemically non-active analyte into products that can be oxidized or reduced at a working electrode. This is electrode is maintained at an appropriate potential with respect to a reference electrode.

Modelling of biosensors is of a crucial importance to understand their behaviour. Biosensor modelling have began with the work of (Gough et al. 1985) which have simulated the performance of a cylindrical biosensor for glucose monitoring at steady state. An other mathematical model have been used for the description of steady state and transient behaviour of a multi-membrane multi-enzyme amperometric biosensor (Jobst et al. 1996), a finite difference scheme was used for the discretization of the model equation. (Caras et al.1985a,b and c) have developed a model to simulate the behaviour of a potentiometric biosensor where only a membrane enzyme layer zone is considered, a method of line was used to solve numerically the model. (Bacha et al. 1995 and 1996) have developed a model that take into account a variety of configuration designs to describe the behaviour of amperometric biosensor for glucose monitoring. A finite volume method is used to solve the model.

Recently (Baronas et al 2003a) have developed a mathematical model to examinate the dynamic response of amperometric biosensors as well in stirred solutions as in non-stirred ones. The authors in another paper examine the influence of the thickness of the membrane on the response of amperometric biosensors (Baronas et al 2003b). The authors use a finite difference scheme to solve the model equations. The same authors (Baronas et al 2002) have already used slightly the same model to study the response of biosensors to a mixture of compounds.

In the present work, we have developed a mathematical model in order to describe and evaluate the performance of amperometric biosensors. The chosen configuration is the most used in the design of nowadays enzymatic biosensor realizations such as the use of polymeric matrices as an enzyme support and the mass production of biosensors by the screen printing technique.

DESCRIPTION OF THE MODEL

The sensors considered in this study consist of a metallic electrode surface, a membrane hold against the surface electrode. According to operating conditions, a diffusion layer adjacent to the outer surface of the membrane is considered.

Consider an enzyme-catalysed reaction

$$S + E \rightleftharpoons ES \xrightarrow{k_{cat}} E + P \qquad (I)$$

Proceedings 16th European Simulation Symposium
György Lipovszki, István Molnár © SCS Press, 2004
ISBN 1-56555-286-5(book) / ISBN 1-84233-106-x(CD)

In this scheme the substrate (S) binds to the enzyme (E) and converts to the product (P).

P is electroactive; it can be oxidized or reduced - whether it is considered as a reductor or an oxidant respectively- electrochemically at a specified potential.

$$P \rightleftharpoons P' \pm ze^- \qquad \text{(II)}$$

Let us assume the symmetrical geometry of the electrode and homogeneous distribution of immobilised enzyme in the membrane. Coupling the enzyme catalysed reaction in enzyme layer with the one-dimensional-in-space diffusion, described by Fick's second law, leads to the following equation:

$$\frac{\partial [C]}{\partial t} = D_c \frac{\partial^2 [C]}{\partial x^2} + R_c \qquad (1)$$

Where $[C]$ is the concentration of any species involved in both enzymatic and electrochemical process, D_c its diffusion constant and R_c is a term related to its production or consumption by the enzyme kinetics.

$$R_c = \pm \frac{v^v_{\max}[C]}{ks + [C]} \qquad (2)$$

$v^v_{\max}$ is the maximal rate of enzymatic reaction which is obtained with a given amount of enzyme $[Et]$:

$$v_{\max} = k_{cat}[Et] \qquad (3)$$

The current density is calculated by estimation the gradient of the electroactive species at the surface of electrode:

$$i(t) = zFD_p \left.\frac{\partial [P]}{\partial x}\right|_{x=0} \qquad (4)$$

z is the number of electrons involved in the electrochemical reaction, D_p is the diffusion constant of the electroactive species and F is the Faraday number (F =96485 C/mole).

The biosensor configuration is given in figure 1 and In order to work with a minimal amount of parameters, mass balance equations of involved species are transformed to dimensionless ones where parameters of reference are the Michaelis-Menten constant ks, the membrane thickness l_m and the substrate diffusion constant D_{sm}.

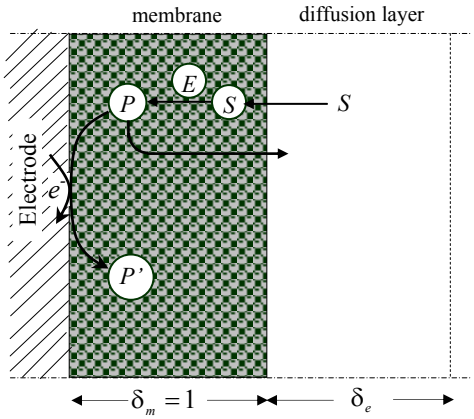

Figure1**:** Scheme of simulated biosensor and build-up

The following table summarize the whole parameters used in this work.

Table 1: Dimension and Dimensionless Parameters

Parameter	**Dimensional**	**Dimensionless**
Time	t (s)	$T = \frac{t\, D_{sm}}{l_m^{\,2}}$
Membrane thickness	l_m (m)	$\delta_m = \frac{l_m}{l_m} = 1$
Diffusion layer thickness	l_e (m)	$\delta_e = \frac{l_e}{l_m}$
Distance from electrode	x (m)	$X = \frac{x}{l_m}$
Substrate concentration	$[S]$ (mol.m^{-3})	$S = \frac{[S]}{ks}$
Product concentration	$[P]$ (mol.m^{-3})	$P = \frac{[P]}{ks}$
Michaelis-Menten constant	ks (mol.m^{-3})	$Ks = \frac{ks}{ks} = 1$
Substrate diffusion coefficient 1- Membrane 2- Diffusion layer	 D_{sm} (m^2.s^{-1}) D_{se} (m^2.s^{-1})	$\overline{D}_{sm} = 1$ $\overline{D}_{se} = \frac{D_{se}}{D_{sm}}$
Product diffusion coefficient 1- Membrane 2- Diffusion layer	 D_{pm} (m^2.s^{-1}) D_{pe} (m^2.s^{-1})	$\overline{D}_{pm} = \frac{D_{pm}}{D_{sm}}$ $\overline{D}_{pe} = \frac{D_{pe}}{D_{sm}}$
Current density	i (A.m^{-2})	$\Psi = \frac{i\, l_m}{zF\, D_{sm} ks}$

By converting equation (1) to dimensionless form, a dimensionless quantity arises in the source term which is similar to the Thiele Modulus. This parameter describes the relative importance of diffusion and reaction in the enzyme layer:

$$\Phi = l_m \sqrt{\frac{v_{\max}}{D_{sm} ks}} \tag{5}$$

When Φ is small, the kinetics are the predominant. In contrast, when the Thiele modulus is large, diffusion limitations are the principal determining factor.

Another dimensionless number, namely the Biot number is needed to express the ratio of the internal mass transfer resistance to the external one is given by:

$$Bi = \frac{l_m / D_{sm}}{l_e / D_{se}} = \frac{D_{se}\, l_m}{D_{sm}\, l_e} \tag{6}$$

Transient Mass Balance Equations:

The governing equations of the model are written in the two regions witch represent the physical domain of both biosensors, namely, the membrane and the diffusion layer in witch mass transfer is occurring only by diffusion. In the bulk solution the transport is carried by convection and the concentration of all species is assumed to remain constant thanks to the stirring of the solution.

In the membrane, the model is described by the two following equations in which, three terms are present, the accumulation term, the diffusion term and the kinetic one.

$$\frac{\partial S}{\partial T} = \frac{\partial^2 S}{\partial X^2} - \Phi^2 \frac{S}{1+S} \tag{7}$$

$$\frac{\partial P}{\partial T} = \overline{D}_{rm} \frac{\partial^2 P}{\partial X^2} + \Phi^2 \frac{S}{1+S} \tag{8}$$

In the diffusion layer, equations are the same as in the membrane except there's no kinetic term within the diffusion layer:

$$\frac{\partial S}{\partial T} = \overline{D}_{se} \frac{\partial^2 S}{\partial X^2} \tag{9}$$

$$\frac{\partial P}{\partial T} = \overline{D}_{pe} \frac{\partial^2 P}{\partial X^2} \tag{10}$$

Boundary Conditions

The substrate is not electroactive, its mass flux density is thus nil at the surface of electrode:

$$\left.\frac{\partial S}{\partial X}\right|_{X=0} = 0. \tag{11}$$

The electrochemical reaction is assumed to be fast enough to ensure a nil concentration at the electrode

$$P\big|_{X=0} = 0. \tag{12}$$

At the membrane-diffusion layer interface the mass flux density of both S and P must be continuous, this is provided by:

$$\left.\frac{\partial S}{\partial X}\right|_{X=1^-} = \overline{D}_{se} \left.\frac{\partial S}{\partial X}\right|_{X=1^+} \tag{13}$$

$$\overline{D}_{pm} \left.\frac{\partial P}{\partial X}\right|_{X=1^-} = \overline{D}_{pe} \left.\frac{\partial P}{\partial X}\right|_{X=1^+} \tag{14}$$

At the diffusion layer-bulk interface, during biosensor operation all concentrations are supposed to be constant.

$$S\big|_{X=1+\delta e} = S_0 \tag{15}$$

$$P\big|_{X=1+\delta e} = 0. \tag{16}$$

The dimensionless current density due to the mass flux of P at the electrode is provided by:

$$\Psi(T) = \overline{D}_{pm} \left.\frac{\partial P}{\partial X}\right|_{X=0} \tag{17}$$

Description of the Numerical Method:

The previous equations governing the dynamic of the biosensors considered as well as boundary conditions are solved by the finite volume method (FVM) which is a simple variant of the well known finite element method (FEM). The FVM use the same weighting function in the entire domain.

$$W(X) = \begin{cases} 1 & X \in [X_W, X_E] \\ 0 & \text{otherwise} \end{cases}$$

The entire physical domain Ω (membrane + diffusion layer) which is divided into subdomains Ω_i (ΔX_i) in which mass balance equations are integrated assuming a piecewise profile of the dependent variable S and P.

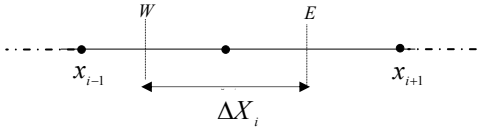

Figure 2: One dimension finite volume discretization scheme.

A great advantage of the finite volume integration is that there's no problem if the space gird is enlarged or reduced in any part of the integrated domain. For example, applying this basis to the governing equation relative to the substrate, this leads to:

$$\int_W^E \frac{\partial S}{\partial T} dX = \int_W^E \frac{\partial^2 S}{\partial X^2} dX - \int_W^E \Phi^2 \frac{S}{1+S} dX$$

After linearization of the non-linear term related to the enzyme kinetic and integration of each term we obtain a system of algebraic equations that has the following form:

$$a_i^n S_{i-1}^{n+1} + b_i^n S_i^{n+1} + c_i^n S_{i+1}^{n+1} = d_i^n$$

Where a_i^n, b_i^n, c_i^n and d_i^n are constant that depend on previous calculated values of *S*..

S_{i-1}^{n+1} S_i^{n+1} and S_{i+1}^{n+1} are the current value of *S* at points $\sum_{k=1}^{i-1} k\Delta X_k$, $\sum_{k=1}^{i} k\Delta X_k$ and $\sum_{k=1}^{i+1} k\Delta X_k$ at time $(n+1)\Delta t$.

Applying the same treatment to the product mass balance equation we obtain finally two systems of algebraic equations to solve simultaneously:

$$\boldsymbol{A}_S^{(n)} * \boldsymbol{S}^{n+1} = \boldsymbol{D}_S^{(n)}$$
$$\boldsymbol{A}_P^{(n)} * \boldsymbol{P}^{n+1} = \boldsymbol{D}_P^{(n)}$$

$\boldsymbol{A}_S^{(n)}$ and $\boldsymbol{A}_p^{(n)}$ are tridiagonal matrixes of a_i^n, b_i^n and c_i^n elements respectively for *S* and *P*.

$\boldsymbol{D}_S^{(n)}$ and $\boldsymbol{D}_p^{(n)}$ are second member vectors respectively for *S* and *P*.

The two systems of algebraic equations are solved numerically by Thomas algorithm. The algorithm is written in Visual Fortran and run on a Pentium III PC (733 kHz) processor.

General Considerations:

One of the most problems encountered in biosensor modelling is the lack of information about numerical value of each parameter and the scope of their variation. Many parameters are not accessible or not known with sufficient accuracy. Explicitly; independent experiments must be carried out before simulation. Nevertheless, this can be avoided if one studies the influence of a group of parameters on biosensor's output in stead of examining the influence of each parameter taken lonely. It is still possible when introducing dimensionless number such as Thiele modulus and Biot number.

RESULTS AND DISCUSSION:

The model presented below is applied to simulate the response of the biosensors versus many parameters such as the amount of the immobilized enzyme, the membrane thickness and the thickness of the diffusion layer witch is estimated by the Levich relation for the rotating electrode:

$$l_e = 1.61\, D^{1/3} \nu^{1/6} \omega^{-1/2}$$

D: diffusion coefficient (m^2/s).
ν: viscosity of the solution equal to $1.02\ 10^{-6}$ m^2/s.
ω: Speed of rotation of the electrode (rad/s).
l_e is inversely proportional to the stirring strength.

Influence of the Internal Resistance on the Signal Magnitude:

In Order to study the effect of the internal resistance, a diffusion layer thickness is taken deliberately to equal to 0. This leads to a value of $Bi = \infty$. The following parameters are taken for all the simulation experiment (Bacha et al. 1996) and (Baronas et al 2003a):

l_m= 50 µm, $D_{sm} = D_{pm} = 3.0 \times 10^{-10}$ m^2/s.
$D_{se} = D_{pe} = 2 \times D_{sm}$.
ks =0.1mol/m^3, $[S_0]$ =0.1 mol/m^3.

Value of v_{max} are taken in such a manner to have a decades of Φ ranging from 0.1 to 7.

Equations (7)-(10) and the corresponding boundary conditions (11)-(16) are solved to simulate the behaviour of the biosensor. Initial conditions are taken according to following assay protocol:

At t=0 S=0 for $0 \le X \prec 1$
S=S_0 $X \ge 1$
P=0 $\forall X$

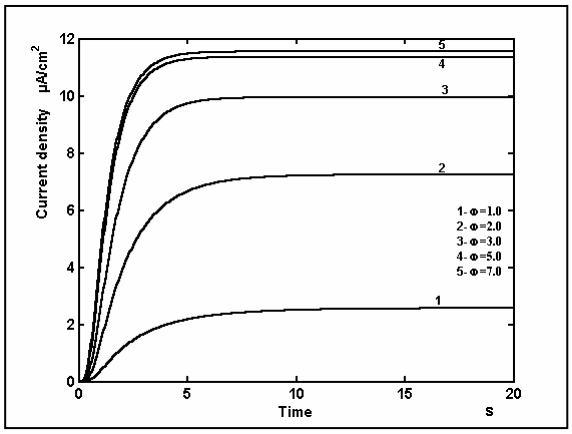

Figure 3a : The dynamics of the biosensor current *i* at va Thiele modulus values ranging from 1 to 7.0
Other parameters: l_m=50µm, $[S_0]$=ks=0.1 mol/m^3 and Bi=∞

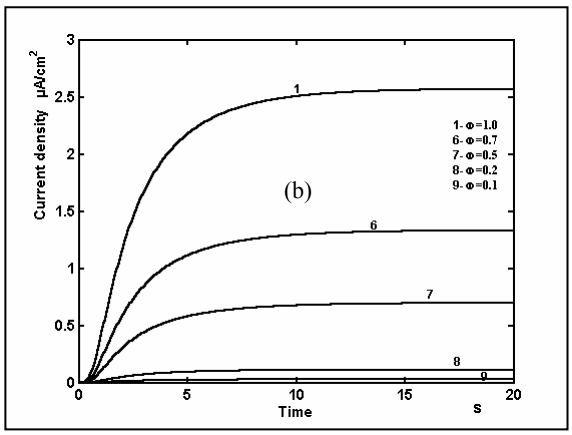

Figure 3b: The dynamics of the biosensor current i at various Thiele modulus values ranging from 0.1 to 1. Other parameters: l_m=50µm, $[S_0]$=ks=0.1 mol/m^3 and Bi= ∞

Figures 3a and 3b show the response to the biosensor for a wide range of Φ value. One can see that the maximal magnitude of the biosensor which corresponds to the steady state current increases with the increase of the amount of enzyme since v_{max} is directly related to the enzyme concentration. All current's curve have an *S* shape and the response reaches 99,99 % (which is considered the response time) of the steady state value as faster as the value of Φ is important.

Response time is less than 5 seconds for a Φ value of 5.0 or beyond (i.e. for a response controlled by diffusion).

Another interesting feature is that the maximum current density does not exceed a limit of 11.56 µA/cm^2 regardless the amount of immobilized enzyme. One can take advantage of that remembering the deactivation of the enzyme during the storing of the biosensor and this may keep the same magnitude as long as the response is controlled by diffusion. Figure 4 illustrate this well, as we can see the maximum

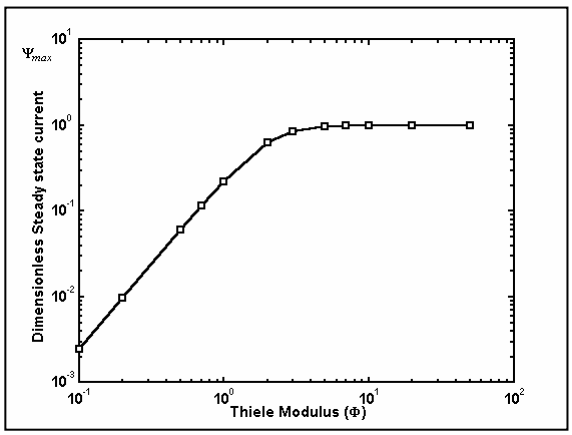

Figure 4: Dimensionless maximal current density versus Thiele modulus**.**
l_m= 50µm, $[S_0]$=ks=0.1 mol/m^3 and Bi= ∞

dimensionless current density Ψ_{max} versus Thiele modulus is linear up to a value of Φ=2. No further increase of Ψ_{max} is obtained beyond this value whatever is the Thiele's modulus value.

Effect of External Resistance to Mass Transfer

To study the effect of external resistance to mass transfer, values of Φ=7 and Φ=1 are taken to perform the simulation. The diffusion layer thickness δ_e is chosen to ensure value of Bi from 0.5 to 50 (i.e. for diffusion layer thickness δ_e varying from 200 to 2µm). Figure 5a show the biosensor response obtained with a value of Φ =7 which correspond to diffusion control. The magnitude of the response increase with the decrease of the diffusion layer thickness. One can see response 7 and 8 in figure 5a obtained respectively for Bi=1 and Bi=0.5 show a peack of 6.6 µA/cm^2 approximately at t=4s, this may be attributed to the excessive thickness of the diffusion layer (100µm for Bi=1 and 200µm for Bi=0.5). This layer was

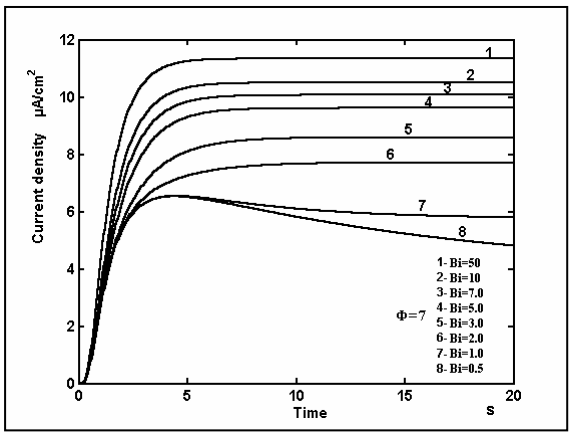

Figure 5a: The dynamics of the biosensor current versus Biot number at Φ=7.
Other parameters: l_m=50µm, $[S_0]$=ks=0.1 mol/m^3

initially loaded with the substrate, which induced fast reaction in the membrane, when most of substrate had been consumed, diffusion from the bulk of the solution was too slow to maintain the high initial reaction rate. The maximum current density at Bi value of 50 is (well stirred solutions) 1.8 times its value at Bi value of 1 (weak stirred ones).

Responses obtained when reaction is the limiting factor (i.e. for Φ=1) in figure 5b does not show any peak. This is well understood because the diffusion rate of the substrate in the diffusion layer has at some extent the same order of magnitude as its consumption in the membrane.

The obtained current densities are ranging from 2.5 to 3 µA/cm^2 and do not exceed the later value despite the wide scope of Bi value. In such situation stirring would decrease the maximal current density (curve n°4 Figure 5b). Biosensor's responses obtained are slower than those

obtained previously with Φ=7.0 and the strength of stirring seems not to have commonly a drastic influence on response magnitudes.

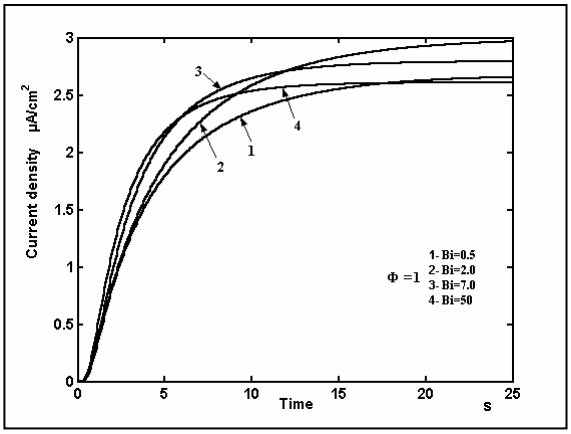

Figure 5b: The dynamics of the biosensor current versus Biot number at Φ=1.
Other parameters: l_m=50μm, $[S_0]$=ks=0.1 mol/m^3

Effect of Internal Resistance on the Linearity of the Biosensor Response

As we have seen in the previous section, best and fast responses are obtained for biosensor operating under diffusion control, investigations are made to check the linearity of the biosensor response versus the amount of enzyme loaded in the membrane. Figure 6 show that the widest interval of concentration in which the relationship with the steady state current density is linear.

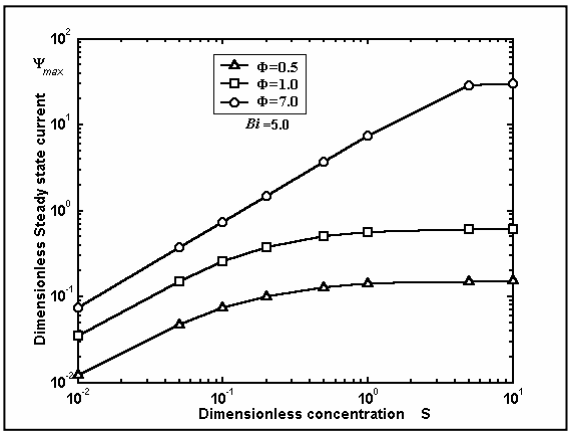

Figure 6: Steady state current density versus dimensionless concentration.

MODEL VALIDATION

The model is validated by comparing numerical solutions obtained at steady state to analytical ones obtained for some limited cases. For example the substrate masse balance equation (7) may be approximated to the following equations:

$$0 = \frac{d^2S}{dX^2} - \Phi^2 S \qquad \text{(a)}$$

Or

$$0 = \frac{d^2S}{dX^2} - \Phi^2 \qquad \text{(b)}$$

According to whether we assume $S >> 1$ (equation a) or $S << 1$ (equation b). Equations a and b are linear ordinary second order differential equations which can be integrated analytically without any problem and provide as well the steady state current density as the steady state substrate or product profiles.

Figure 7 show the comparison between steady state current density obtained by both finite volume method and finite difference method (FD) in one hand to the analytical solution obtained at steady state (equation b) in the other hand in the case S<<1). Comparison shows that the relative error in percent does not reach 0.1% when the number of space points I_{max} exceed 51. This demonstrate the power of the FVM for a relatively little effort of calculation.

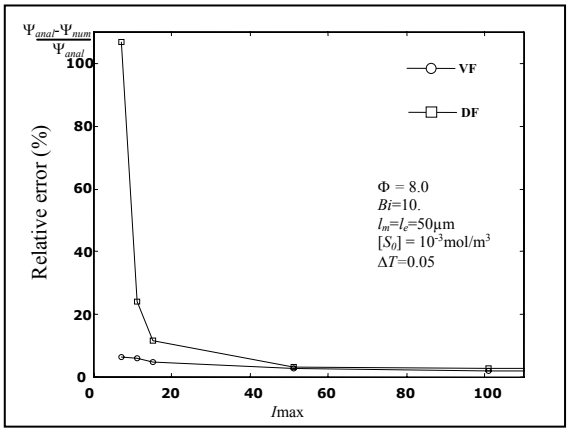

Figure 7: Relative error evolution versus the maximal number of space points.

The comparison of both substrate and product numerical profiles at steady state and analytical ones show that the numerical method (the FVM) is in good agreement all above in the region located near the electrode (see product profile).

CONCLUSION

The described model has shown interesting features in investigating amperometric biosensor performance. It is useful to explain the sensing mechanism and provide a basis for interpretation of the response in order to incorporate further improvements in amperometric biosensor design.

Numerical results demonstrate that best performances are obtained when the biosensor operate under internal

diffusion control. High loading membrane with enzyme will provide, in addition to a maximal and fast signal for a given substrate concentration, a wide range of linearity and may keep somewhat a regular biosensor signal during the storing of the biosensor as long as the biosensor is under diffusion control.

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