

# **Numerical Modelling of an Amperometric Biosensor**

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*by*

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**NATIONAL INSTITUTE OF TECHNOLOGY**  
**ROURKELA**  
**CERTIFICATE**

This is to certify that the thesis entitled "*Numerical Modelling of an Amperometric Biosensor*" submitted by Mr. Nikhil in partial fulfillment of the requirements for the award of Bachelor of Technology Degree in Biotechnology at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by him under my guidance.

To the best of my knowledge the matter embodied in the thesis has not been submitted to any University/Institute for the award of any Degree or Diploma.

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## **ABSTRACT**

Amperometric biosensor is a type of biosensor which measures the change in the current of a working indicator electrode by direct electrochemical oxidation or reduction of the products of a biochemical reaction. In these types of biosensors, the potential at the electrode is made constant during the measurement of current. These are known to be reliable, cheap and highly sensitive for environment, clinical and industrial purposes. These biosensors have plethora of applications in diverse fields; hence mathematical modeling of the same is highly desirable. This can help in prefiguring its various characteristics. A mathematical model is proposed which can study the cyclic conversion of substrate in an amperometric biosensor. The governing parameters for the Michaelis-Menten kinetics of enzymatic reactions are the enzyme kinetic rate and the diffusion rate across the enzymatic layer. Relative influence of these parameters is decided by a non dimensional number called Damkohler number, which is a ratio of the rate of enzymatic reaction to the rate of diffusion. The effect of Damkohler number on the current density, substrate concentration, and product concentration has been studied. It has been observed that when the Damkohler number is low then enzyme kinetics controls the biosensor response whereas when it is high (of the order of 1) the response is under control of diffusion rate. The current density is found to increase with the decrease in Damkohler number and vice versa.

## TABLE OF CONTENTS

	PAGE
➤ <b>CHAPTER 1: INTRODUCTION</b>	
• 1.1 Historical review.....	2
• 1.2 Biosensor.....	3
• 1.3 Enzyme substrate interaction.....	10
• 1.4 Literature review.....	10
➤ <b>CHAPTER 2: MATHEMATICAL MODELING</b>	
• 2.1 Mathematical modeling.....	13
• 2.2 Model description.....	13
• 2.3 Governing Equations.....	15
• 2.4 Governing Equations in Non-Dimensional Form.....	19
➤ <b>CHAPTER 3: RESULTS AND DISCUSSIONS</b>	
• 3.1 Validation.....	19
• 3.2 Dependence of substrate concentration on Damkohler Number..	20
• 3.3 Dependence of current intensity on Damkohler Number.....	21
• 3.4 Dependence of product concentration on Damkohler Number.....	22
➤ <b>CONCLUSION.....</b>	<b>24</b>
➤ <b>REFERNCES.....</b>	<b>25</b>

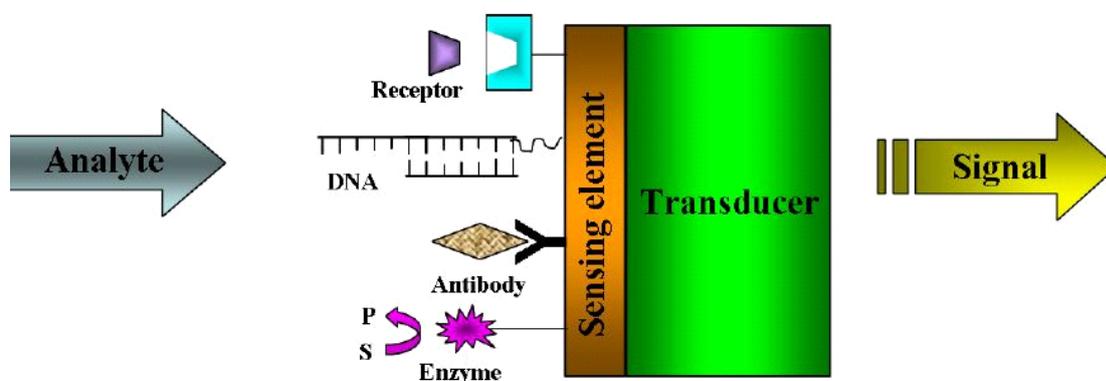
## LIST OF FIGURES

	<b>PAGE</b>
• Schematic representation of a Biosensor setup.....	2
• A schematic representation of biosensor.....	4
• Potential applications of biosensors .....	5
• Different Methods of coupling of a Biomaterial with the Sensor .....	6
• Principle of electrochemical biosensor.....	7
• Specificity of biosensor.....	9
• Transducers mechanism.....	9
• Schematic diagram of a Biosensor In 1-dimension.....	13
• Validation done against the substrate and product concentration.....	19
• Substrate concentraion Versus Enzyme layer.....	20
• Non-dimensional current density vs. the time.....	21
• Non-dimensional Product concentration versus Length of the Enzyme layer...	22

**CHAPTER-1**  
**INTRODUCTION**

## 1.1 Historical Review:

A biosensor is an analytical device that determines the presence of the species of interest and converts it into an appropriate electrical signal. In general, a biosensor is made of three major elements, the target analyte, the biosensing element and the transducers; which can recognize the target analyte and a transducer which is in close proximity with the biosensing element. The foremost biosensor was a glucose sensor, developed by Clark (Clark and Lyonse, 1962). Since then there has been a colossal progress in the field of biosensors. From Figure 1, we can have an overview of a biosensor setup which explains the working principle of a biosensor.



**Fig1. Schematic representation of a Biosensor setup**

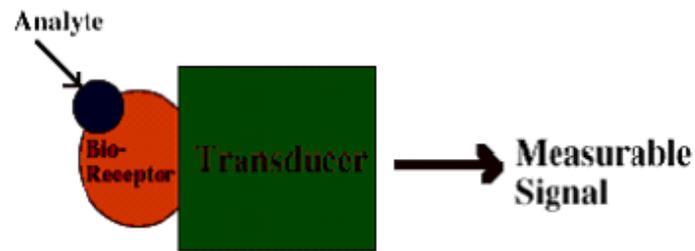
Owing to their exceptional performance, which include high specificity and sensitivity, fast response, low cost, compact size and user-friendly operations, biosensors have become an indispensable tool for detection of chemical and biological components in the areas of clinical, food and environmental monitoring. When electrochemical transducers combine

with an enzyme which acts as the biochemical component, it produces a signal, these biosensing systems that specifically depend on inhibition can be divided into three categories:

- Biosensors based on the immobilization of cells that act as the biochemical component (Schulmeister et al., 1987): The use of this type of biosensor can increase the sensor stability and render easy regeneration of the enzyme. However, such biosensors may suffer from side reactions due to the coexistence of numerous enzymes.
- Sensor devices tied with reactors that contain an immobilized enzyme matrix:  
The inhibitor passes through the reactor and then inhibits the enzyme (Lee et al., 2002). The residual activity of the enzyme is evaluated by measuring the enzymatic product before and after the inhibition of enzyme.
- Biosensors based on direct enzyme immobilization on a transducer device:  
The enzyme and transducer elements are in close contact with each other and incorporated in a single unit (Brain.r.eggins, 1996).

## **1.2 Biosensor:**

Biosensor is an analytical device with a biological element, which transforms chemical parameters within a system into an optical or electrical signal. Thus, a biosensor consists of a bioreceptor and a transducer. Bioreceptor is a biomolecule, which can identify the analyte target that has to be found out, but transducer is a component, which transforms the identified value into a particular signal that can be measured. These two components are integrated into a system to form a biosensor (Baronas et al. 2003).The schematic illustration of a biosensor is given in the Figure 2.



**Fig2. A schematic representation of biosensor**

The application fields of biosensors are wide:

- Clinical diagnosis and biomedicine
- In agriculture field and veterinary applications
- Fermentation control and analysis of the food and drink production
- Microbiology, bacterial and viral analysis
- In pharmaceutical field and medicine analysis
- Control of the industrial waste
- Control and monitoring of environmental pollutants
- Military applications

### **1.3 Types of Biosensors:**

Biosensors can be classified on the basis of different categories as follows:

a) Based on operating principle:

- Calorimetric Biosensors.
- Potentiometric Biosensors.
- Amperometric Biosensors.
- Optical Biosensors.
- Immunosensors.

b) Based on analytes used:

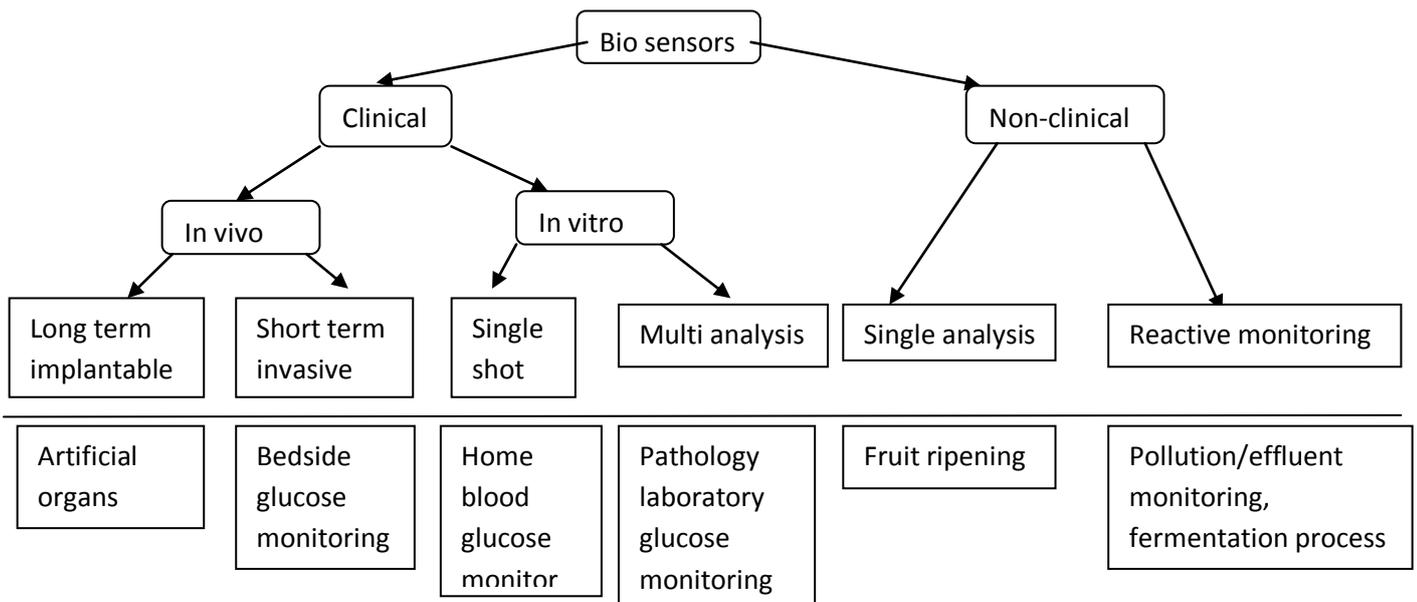
- Enzymes & Proteins: Enzyme electrode

- Antibodies: Immunosensors
- DNA: DNA Sensor
- Organelles & Microbial Cells: Microbial Sensor

c) Based on detection mode:

- Electrochemical Transducers: Potentiometric, amperometric and voltametric.
- Electrical Transducers: Surface and electrolyte Conductivity
- Optical Transducers: Fluorescence, Adsorption and Reflection
- Thermal Transducers: Heat of rejection and adsorption

The potential application of the biosensor is depicted in the flow chart as shown in the Figure 3.



**Fig 3. Potential applications of biosensors**

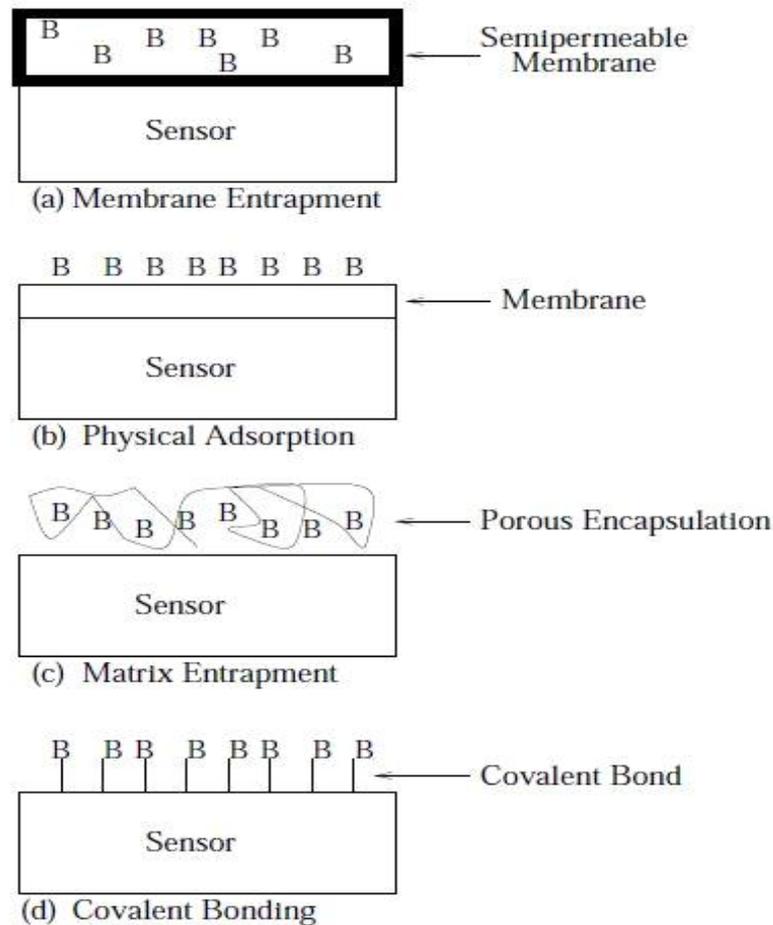
### 1.4 Concepts of Biosensor:

The “bio” and the “sensor” elements can be coupled together in one of the four possible ways listed below:

- Membrane Entrapment
- Physical Adsorption

- Matrix Entrapment
- Covalent Bonding

The approach to couple the bio and the sensor part is described below in Figure 4. These are also known as method of immobilization of enzyme on the transducers.



**Fig 4. Different Methods of coupling of a Biomaterial with the Sensor**

### 1.5 Key features of a biosensor:

1. The biocatalyst must be enormously specific for analysis purpose, and should be stable under normal storage conditions and shows a low deviation between assays.
2. The reaction should be independent on physical parameters such as stirring, pH and temperature. This allows analysis of samples with minimal pre-treatment.

If the reaction involves cofactors or coenzymes these should, preferably, also be immobilized with the enzyme.

3. The response should be accurate, precise, reproducible and linear over the concentration range of interest, without dilution or concentration. It should also be free from electrical or other transducer induced noise so as to have required accuracy (Kulyš et al 2006).

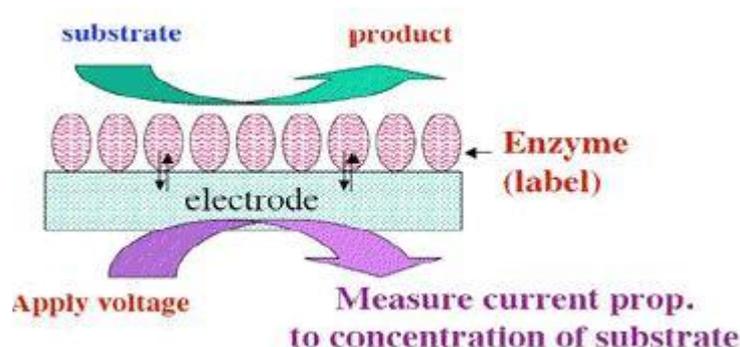
4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, and should not have toxic or antigenic effects. Furthermore, the biosensor should not be prone to inactivation or proteolysis.

5. For rapid measurements of analytes from human samples it is desirable that the biosensor can provide real-time analysis.

6. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators (Chaubey et al 2003).

## 1.6 Electrochemical Biosensor:

An electrochemical biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information by using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with an electrochemical transduction element. It is mainly used for the detection of hybridized DNA, DNA-binding drugs, glucose concentration; etc (Baronas et al 2003). The working principle of an electrochemical biosensor is given in Figure 5.



### Fig5. Principle of electrochemical biosensor

The electrochemical biosensor can be classified based on measuring electrical parameters as:

- Conductimetric
- Amperometric
- Potentiometric

Details about these 3 types are given below in the table 1.

**Table1. Different Electrochemical Sensing**

<b>Characteristics Measured Parameter</b>	<b>Conductimetric (Conductance/ Resistance)</b>	<b>Amperometric Current</b>	<b>Potentiometric (Potential/ Voltage)</b>
Applied Voltage	Sinusoidal(AC)	Constant Potential(DC)	Ramp Voltage
Sensitivity	Low	High	-----
Governing Equation	Incremental Resistance	Cottrell Equation	Nesrt Equation
Fabrication	FET+ Enzyme	FET+ Enzyme 2electrode	FET+ Enzyme Oxide electrode

### 1.7 Amperometric Biosensor:

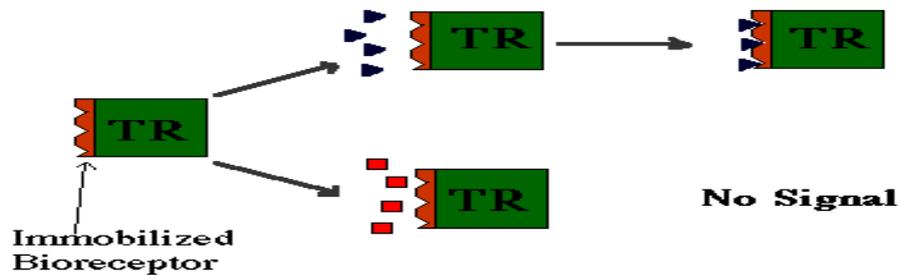
The amperometric biosensors is the one which measure the changes of the current of a working indicator electrode by direct electrochemical oxidation or reduction of the products of the biochemical reaction. In amperometric biosensors the potential at the electrode is made constant while the current is being measured. It produces a current proportional to the concentration of the substance to be detected. It is of three types

1. Single use amperometric biosensor
2. Intermittent use amperometric biosensor

### 3. Continuous use amperometric biosensor

#### 1.8 Transducers in Biosensors:

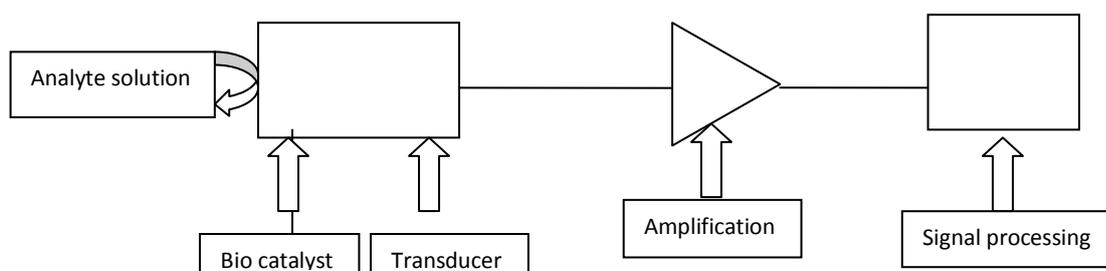
An enzyme is a bioreceptor as it is capable of recognizing a specific target molecule. Other biorecognizing molecules include antibodies, nucleic acids, and receptors. One major requirement for a biosensor is that the bioreceptor molecule has to be immobilized in the vicinity of the transducer. The working mechanism of the transducer is shown in Figure 6.



**Fig6. Specificity of biosensor (TR: transducer).**

The transducer converts the biochemical interactions into measurable signals. Electrochemical, electro-optical, acoustical, and mechanical transducers are few among the various types used (Malhotra 2002, Turner et al.,1997).

“Transduction of the biosensor signal is a process that is concurrent, and within the special environment of the biosensing element”. Transducers is also known as a device which can convert one form of energy to another form, i.e. a signal coming in form of mechanical or chemical energy to desired biological, optical or any form of signal required accordingly. The transducer mechanism is given in the Figure 7.



**Fig 7. Transducers mechanism**

## **1.9 Biomolecule model and Enzyme substrate interaction:**

A biomolecular interaction is a central element in understanding disease mechanisms and it is essential for devising safe and effective drugs. Amperometric biosensors usually involves biomolecular interaction, they are very often used for affinity relation test. The catalytic event that converts substrate to product involves the formation of a transition state. The complex, when substrate  $S$  and enzyme  $E$  combine, is called the enzyme substrate complex  $C$ , etc. Normally, we have two ways to set up experiments for biosensors: free enzyme model and immobilized enzyme model. The mathematical and computational models for these two models are very similar (Baronaset.al2004).

## **1.10 Literature Review:**

Enzymes are used to accelerate the rate of chemical reactions (both forward and backward) without being consumed in the process and tend to be very selective, with a particular enzyme accelerating only a particular reaction. Enzymes are important for regulation of biological processes, for example, as activators or inhibitors in a reaction. For understanding the role of enzyme kinetics it is necessary to study the rate of reaction the temporal behavior of various reactants and the conditions that influence the enzyme kinetics (Rubinow, 1975). Biosensors are analytical devices made up of a combination of a specific biological element, mainly an enzyme which recognizes a specific analyte (substrate) and the transducer which translates this biorecognition signal into an appropriate electrical signal (Tuner et al., 1987; Scheller et al., 1992). Amperometric biosensor is a type of biosensor which measure the current that arises on a working electrode by direct electrochemical oxidation or reduction of the biochemical reaction product. These biosensors are widely used in clinical diagnostics, environment monitoring, food analysis, and drug detection because they are reliable, highly sensitive and comparatively cheap. The proposed one-dimensional-in-space (1-D)

mathematical model does not consider the geometry of the enzymatic membrane and it also includes efficient diffusion coefficients. The quantitative value of diffusion coefficients is limited for one dimensional model (Schulmeister et al., 1993). Recently, a two-dimensional-in-space (2 D) mathematical model has been proposed considering the perforation geometry (Baronas et al., 2006; Baronas, 2007). However, a simulation of the biosensor based on the 2-D model is much more time-consuming than a simulation based on the corresponding 1-D model. This is more important when we investigate numerical peculiarities of the biosensor response in extensive ranges of catalytic and geometrical parameters. The multifold numerical simulation of the biosensor response based on the 1-D model is much more efficient than the simulation based on the corresponding 2-D model. The detection limit of the enzyme electrodes depends on the sensitivity of the amperometric system (Bladel and Boguslaski, 1978; Fuhrman and Spohn, 1998). The sensitivity of the enzyme electrode can be increased by the cyclic conversion of the substrate (Kulys, 1981; Schubert et al., 1985). The electrode cyclic conversion of the substrate is carried out by conjugation of the enzymatic reaction with the electrochemical process. The goal of this work is to make a model by which we can measure the biosensor response utilizing the amplification done by conjugated electrochemical and enzymatic substrate conversion. The developed model is based on non stationary diffusion equation (Crank, 1975) containing a non linear term related to the enzymatic reaction. Here we modeled an amperometric biosensor to detect the dependence of current density, substrate concentration and product concentration on Damkohler number.

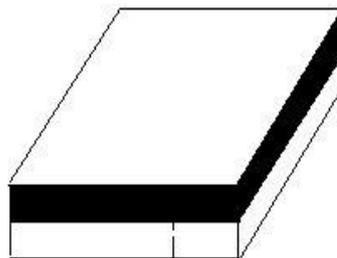
**Chapter-2**  
**Mathematical Modeling**

## 2.1 Mathematical model:

The amperometric biosensor is considered as an electrode and a relatively thin layer of an enzyme which is applied onto the electrode surface. The model involves enzyme layer where the enzymatic reaction as well as the mass transport by diffusion takes place where the analyte concentration is maintained constant (Petrukas et al., 2009).

## 2.2 Model Description:

The 1-D Model of the biosensor contains an enzyme layer and an electrode. The electrode acts as transducer of the biosensor and is covered by an enzyme immobilized layer. In the 1-D model the enzymatic layer is modeled by a homogeneous layer with an appropriate diffusion coefficient along with the reaction rate. In this model the enzyme layer is just above the electrode surface which is going to be modeled (Barronas et al., 2006). The diffusion coefficient and the enzymatic rate plays an important role in the response of a biosensor. The model of the one-dimensional biosensor is given below in the Figure 8.

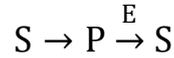


**Fig 8. Schematic diagram of a biosensor in 1-D**

## 2.3 Governing Equations:

Assuming the symmetrical geometry of the electrode and a uniform distribution of the immobilized enzyme in the enzymatic membrane, the mathematical model of the biosensor action can be defined in a one-dimensional in- space domain (Schulmeister et al.,1990).

The biosensor might be considered as an enzyme electrode, which contains a membrane with immobilized enzyme applied on the surface of the electrochemical transducer. Consider the scheme of substrate (S) electrochemical conversion to a product (P) following catalyzed with enzyme (E) product conversion to substrate:



We have assumed the symmetrical geometry of the electrode and homogeneous distribution of immobilized enzyme in the enzyme membrane. Coupling of the enzyme-catalyzed reaction in the enzyme layer with the one-dimensional-in-space diffusion, described by Fick's law, leads to the following equations:

$$\frac{\partial S}{\partial x} = D_S \frac{\partial^2 S}{\partial x^2} + \frac{V_{max} P}{K_M + P}, 0 < x < d, 0 < t \leq T$$

$$\frac{\partial P}{\partial x} = D_P \frac{\partial^2 P}{\partial x^2} - \frac{V_{max} P}{K_M + P}, 0 < x < d, 0 < t \leq T$$

where  $x$  and  $t$  stand for space and time, respectively,  $S(x, t)$  and  $P(x, t)$  denote the concentration functions of the substrate and reaction product, respectively,  $V_{max}$  is the maximal enzymatic rate attainable with that amount of enzyme, when the enzyme is fully saturated with substrate,  $K_M$  the Michaelis constant,  $d$  the thickness of the enzyme layer,  $D_S$  and  $D_P$  are the diffusion coefficients of the substrate and product, respectively,  $T$  is the full time of biosensor operation to be analyzed. Electrode surface is represented by  $x = 0$  plane while  $x = d$  represents the bulk solution/membrane interface. The operation of the biosensor starts when some substrate appears on the surface of the enzyme layer (Kulys et al., 2004). This is used in the initial conditions ( $t = 0$ ):

$$S(x, 0) = 0, S(d, 0) = S_0, 0 \leq x < d,$$

$$P(x, 0) = 0, 0 \leq x \leq d,$$

where  $S_0$  is the concentration of substrate in the bulk solution. During electrochemical conversion, the product is produced at the electrode. The rate of the product formation at the electrode is proportional to the rate of conversion of the substrate. When the substrate is well-stirred outside the membrane, then the thickness of the diffusion layer remains constant ( $0 < x < d$ ). Consequently, the concentration of substrate as well as the product over the enzyme surface (bulk solution/membrane interface) remains constant while the biosensor interacts with the solution of substrate. This is used in the boundary conditions ( $0 < t \leq T$ ) given by:

$$S(0, t) = 0,$$

$$S(d, t) = S_0,$$

$$D_p \frac{\partial P}{\partial x_{x=0}} = -D_s \frac{\partial S}{\partial x_{x=0}}$$

$$P(d, t) = 0.$$

## 2.4 Governing Equations in Non-Dimensional Form:

The governing differential equations are non-dimensionalized using the appropriate normalizing parameters. The followings are the non-dimensionalized parameters:

$$s^* = \frac{S}{K_M}$$

$$p^* = \frac{P}{K_M}$$

$$x^* = \frac{x}{d}$$

$$t^* = \frac{t}{\frac{d^2}{D_s}}$$

Using the above normalizing parameters, the equation for depletion rate of substrate can be written as:

$$\frac{\partial s^*}{\partial t^*} = \frac{\partial^2 s^*}{\partial x^{*2}} + \sigma^2 \left( \frac{P^*}{1 + P^*} \right)$$

where,  $\sigma^2 = \frac{V_{\max} d^2}{D_s K_m}$ , is the Damkohler number (Da).

Damkohler number is also termed as diffusion modulus which is used to compare the rate of enzyme reaction ( $V_{\max}/K_m$ ) with the rate of diffusion through the enzymatic layer ( $D_s/d^2$ ). In this whole procedure, if Damkohler number is less than 1 then the enzyme kinetics controls the biosensor response. And, if the Damkohler number is greater than 1 then the diffusion rate controls the biosensor response.

The two governing equations given below are the non-dimensional form of mathematical modeling. Equation 1 is for the rate of change of substrate concentration in non-dimensional form whereas equation 2 is for the rate of change of product formation in the non-dimensional form.

$$\frac{\partial s^*}{\partial t^*} = \frac{\partial^2 s^*}{\partial x^{*2}} + \sigma^2 \left( \frac{P^*}{1 + P^*} \right) \quad (1)$$

$$\frac{\partial P^*}{\partial t^*} = \frac{\partial^2 P^*}{\partial x^{*2}} - \sigma^2 \left( \frac{P^*}{1 + P^*} \right) \quad (2)$$

### **Initial Conditions:**

The initial conditions in nondimensional form are listed below,

$$S^*(x^*, 0) = 0$$

$$S^*(1, 0) = S^*_0$$

$$P^*(x^*, 0) = 0$$

### Boundary Conditions:

The boundary conditions in nondimensional form for the studied cases are:

$$S^*(0, t^*) = 0$$

$$S^*(1, t^*) = S^*_0$$

$$Dp \frac{\partial P}{\partial x_{x=0}} = -D_s \frac{\partial S}{\partial x_{x=0}}$$

$$P^*(1, t^*) = 0$$

### Current Density (I):

The current is measured as a response of a biosensor which depends upon the flux of the substrate S at the electrode surface when x equals zero. Simultaneously the current density I(T) can be obtained explicitly from Faraday's law and Fick's law using the flux of the concentration S at the surface of the electrode. The current density I(T) is expressed as,

$$I(T) = n_e F D_s \frac{\partial s}{\partial x_{x=0}}$$

which is normalized using  $I_0 = F V_{max} d$ .

The current density in non-dimensional form is obtained as follows,

$$I^*(T^*) = \frac{I(T)}{I_0} = \frac{n_e F D_s \frac{\partial s}{\partial x_{x=0}}}{F V_{max} d} = \frac{n_e D_s K_m}{V_{max} d^2} \cdot \frac{\partial s^*}{\partial x^*_{x^*=0}}$$

## **Chapter-3**

### **Results and Discussion**

### 3.1 Validation:

The present numerical model is validated against the published result of Baronas et al., 2004. They considered a case of an amperometric biosensor with initial substrate concentration of  $20\text{nmol/cm}^3$  and the thickness of enzyme layer was  $0.02\text{cm}$ . They considered the case of cyclic conversion of substrate. The results are obtained for variation of substrate and product concentrations with time. From Figure 9, it can be noticed that our numerical prediction matches quite well with the result obtained by Baronas et al., 2004.

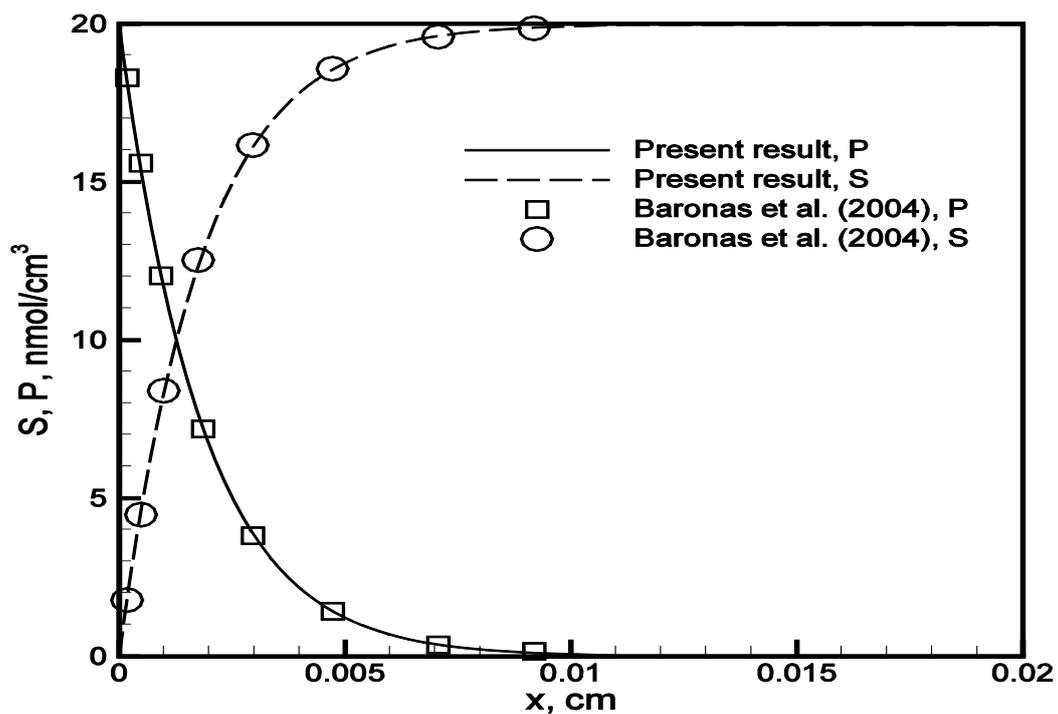


Fig 9. Validation of the present numerical model

Using numerical simulation, peculiarities of the biosensor action has been investigated for different values of the model parameters. The biosensor current density as well as substrate concentration along with product formation is dependent on its enzyme interface. A mathematical model was used to study the effect of Damkohler number on the response of the biosensor to know whether it is a diffusion rate driven or enzyme kinetic rate driven. Using computer simulation we have investigated the dependence of non-dimensional current density, product concentration, substrate concentration on Damkohler number.

### 3.2 Dependence of Substrate concentration on Damkohler Number:

The Figure 12 shows the variation of substrate concentration( $S^*$ ) with the enzyme layer thickness ( $X^*$ ). It can be seen that as the Damkohler number decreases the substrate

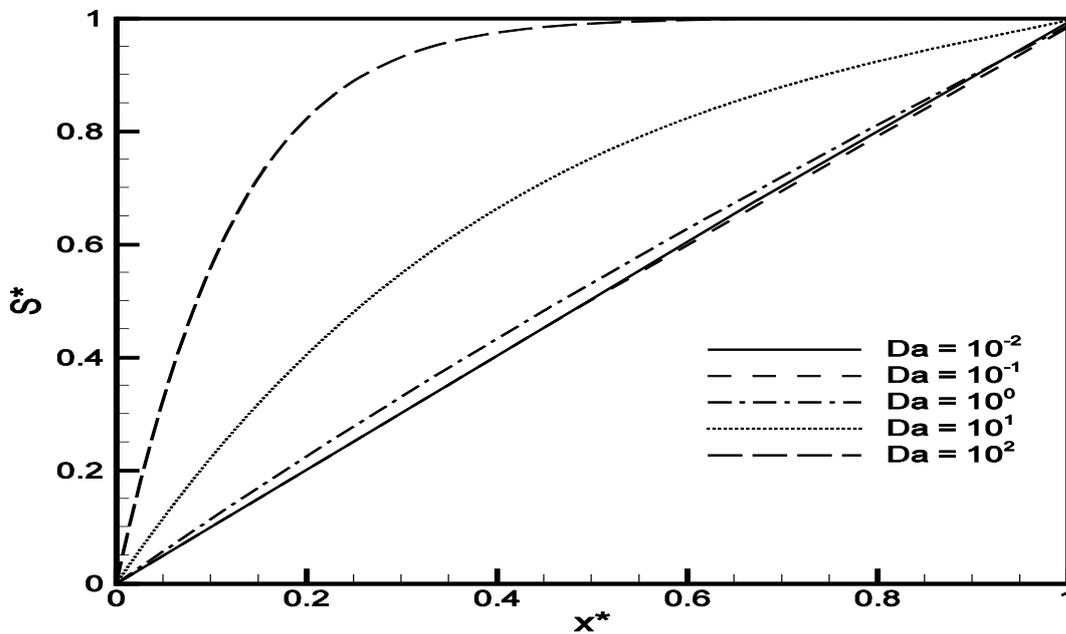


Fig 10. Substrate concentraion Versus Enzyme layer

concentration increases with the increase in enzyme layer thickness and when the Damkohler number is less than 1, the enzyme diffusion rate dominates which is reflected by almost a straight line passing through the origin. The variation loses its linearity for lower values of Damkohler number, which shows the dominance of enzyme kinetic rate over the enzyme diffusion rate. It is also interesting to observe that an instant of maximum substrate concentration corresponds to an instant of minimum product formation throughout the enzyme layer, which is the result of cyclic conversion of substrate.

### 3.3 Dependence of Current Density on Damkohler Number:

In the numerical simulation the Damkohler number is changed from  $10^{-2}$  to  $10^2$  by a factor of

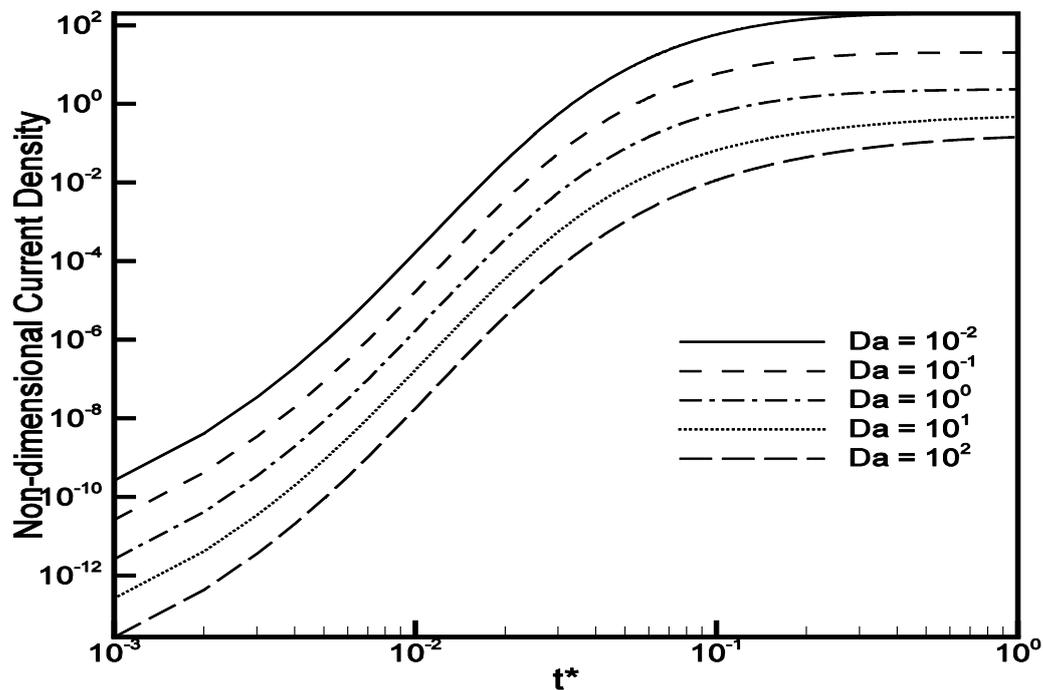


Fig 11. Non-dimensional current density vs. the time

10 and the change in the nature of the non-dimensional current density is measured. It can be

easily found from the Figure 10. that with decrease in the Damkohler number there is an increase in the non-dimensional current density. So it can be concluded from here that with the decrease in Damkohler number, the process is governed by diffusion controlled reaction which in turn results in an increase in the current density. In this we get an increase in the non-dimensional current density whereas whenever the process is enzymatic controlled rate driven, the current density in non-dimensional form decreases in comparison to diffusion controlled rate driven process.

### 3.4 Dependence of Product Concentration on Damkohler Number:

Now the product concentration that is formed depends on the enzymatic layer and the

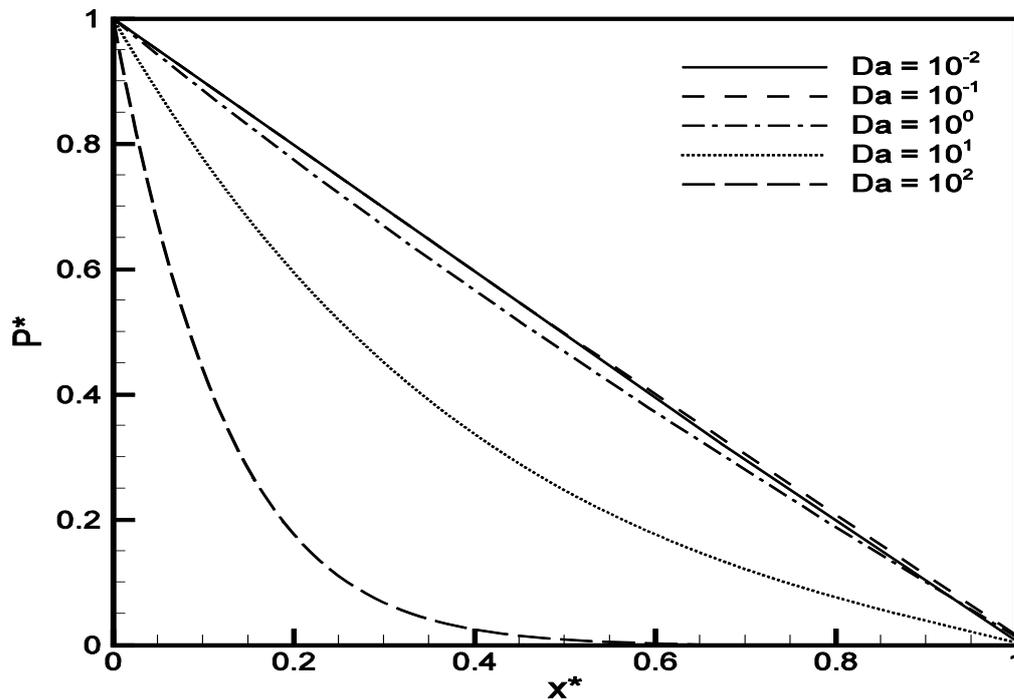


Fig 12. Non-dimensional Product concentration versus Length of the Enzyme layer

diffusion rate of the enzyme. From Figure 11, it is concluded that when the Damkohler number is less than 1, the enzyme diffusion dominates and it penetrates deeper into the

substrate, and the product concentration varies linearly. The enzyme catalytic reaction rate dominates the product formation when the Damkohler number is greater than 1. The nature of variation of product is quite different than the case when the reaction is governed by enzymatic diffusion. Now, the product concentration first decreases rapidly upto a certain point of enzyme layer thickness and then it decreases gradually with increase in the enzyme layer thickness.

## **CONCLUSION**

The non-dimensional mathematical model of an amperometric biosensor can be successfully used to investigate the response of biosensors with cyclic substrate conversion and it is also been used to determine the dependence of non-dimensional current intensity, substrate concentration, and the product concentration upon the Damkohler number. The Damkohler number states that whether the reaction rate is diffusion rate driven or enzyme kinematic rate driven. It has been found that the current density increases with the decrease in Damkohler number and vice-versa. Also, an instant of maximum substrate concentration corresponds to an instant of minimum product formation throughout the enzyme layer.

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