PREPARATION OF AMPEROMETRIC GLUCOSE BIOSENSOR BY MEANS OF ELECTROPOLYMERISATION OF POLYANILINE ONTO GRAPHITE

A DISSERTATION
SUBMITTED FOR THE PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE MASTER OF
SCIENCE DEGREE IN CHEMISTRY

BY

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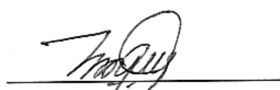
SEPTEMBER 2013

BOARD OF EXAMINER AND CERTIFICATE OF APPROVAL

This dissertation entitled **"Preparation of amperometric glucose biosensor by means of electropolymerisation of polyaniline onto graphite"**, submitted by "*Mr. Suresh Prasad Bhusal* ", under the supervision of Associate Professor *Dr. Amar Prasad Yadav*, Central Department of Chemistry, Tribhuvan University, Nepal is here submitted for the partial fulfillment of the Master of Science (M.Sc.) Degree in Chemistry. This dissertation has not been submitted in any other university or institution previously for the award of a degree.



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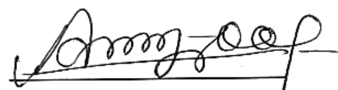

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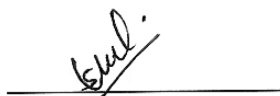

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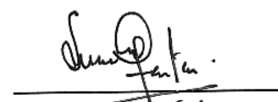

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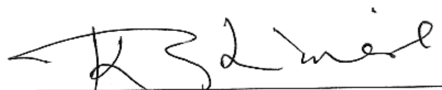

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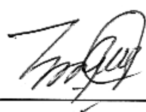
RECOMMENDATION LETTER

This is to certify that the dissertation work entitled **"Preparation of amperometric glucose biosensor by means of electropolymerisation of polyaniline onto graphite** ", has been carried out by **Suresh Prasad Bhusal** as a partial fulfillment for the requirement of M.Sc. Degree in Chemistry under my supervision. To the best of my knowledge, this work has not been submitted to any other degree in this institute.



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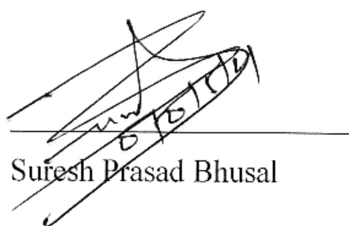
DECLARATION

I, "*Suresh Prasad Bhusal*" hereby declare that the work presented herein is genuine work done originally by me and has not been published or submitted elsewhere for the requirement of a degree program. Any literature, data or work done by others and cited in dissertation has been given due acknowledgement and listed in the reference section.



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DEDICATION

Dedicated to my parents

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September, 2013 ***Suresh Prasad Bhusal***

ABBREVIATIONS

M	molar
mV	millivolt
sec	second
pH	negative logarithm of hydrogen ion
Aq	aqueous
s	solid
mL	milliliter
L	litre
mm	millimeter
cm	centimeter
m	meter
g	gram
LR	lab reagent
GOx	glucose oxidase
PANi	polyaniline
FAD	flavin adenine dinucleotide
YSI	yellow spring instruments
KD	kilodaltons

ABSTRACT

In this study, an amperometric glucose biosensor with immobilization of glucose oxidase enzyme on electrochemically polymerized polyaniline film onto graphite surface has been prepared. The immobilization of the enzyme glucose oxidase has been accomplished via the physical entrapment technique. Electropolymerization of aniline onto the suface of the graphite was carried out by cyclic voltammetry (potential range of -0.4V to 1.2V, scan rate 100mV/sec, 10 cycles) by using standard calomel electrode (SCE) as reference and platinum wire as counter. Characterization of the PANi/Graphite was carried out with the aid of cyclic voltammetry in 1M HCl, obtaining three couple of peaks from which the two pair were distinct, having proved the electro-activity of the PANi film. The enzyme was potentiostatically immobilized onto the surface from 0.1M phosphate buffer of pH 6.0 with 200U/mL glucose oxidase (GOx) enzyme. The amperometric response was studied by using hydrodynamic amperometric mode. Determination of glucose was carried out by the measurement of amperometric response obtained during the reduction of enzymatically produced hydrogen peroxide at - 0.4 V *vs* calomel electrode and platinum wire as counter in phosphate buffer of pH 6.0. Biosensor was found to be responsive within the concentration range of $0.01ML^{-1}$-$0.1ML^{-1}$. All experiments were carried out at room temperature.

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CHAPTER 1

1. INTRODUCTION

1.1 General Introduction

A biosensor is an analytical device that converts the concentration of the analyte into a signal (*e.g.* electrical signal) by integrating biological sensing element into a transducer [1]. Biosensors are portable, simple-to-use, and high specific analytical tools, which are compatible with data-processing technologies. Therefore, biosensors will have promising applications in various fields, such as pharmacy, health care, pollution monitoring, food and agricultural product processing *etc.*

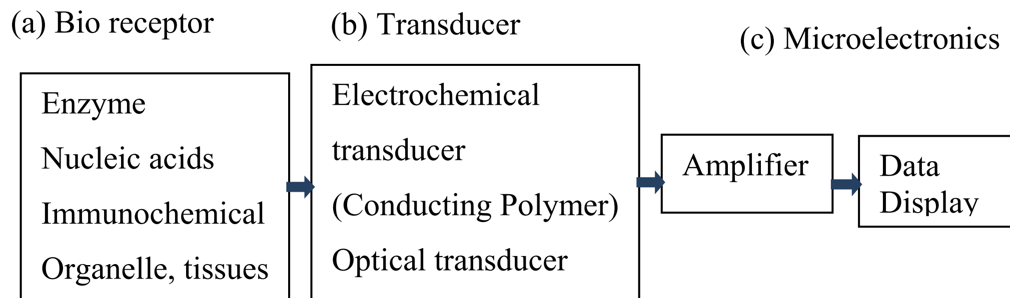


Fig: 1.1 Schematic Diagram of Biosensor

Fig. 1.1 shows the main components of a biosensor. Enzymes, nucleic acids, antibodies, tissue slice, binding protein, whole cells or other bio-receptors have been used as sensing probes in biosensor fabrication [2]. They have an intimate contact to the transducer. The transducer can be an electrochemical, optical or piezoelectric device. Bio receptor (a) and transducer (b) together are sometimes referred to as biosensor membrane. A very important advancement in producing biosensor membranes is the use of electrically conducting membranes that contain the enzyme, cofactor, and mediator. There are many different kinds of conducting polymers that can be chosen. The conducting polymer can act as the electrochemical transducer to convert the biological information into an electrical signal. It has been used in analytical applications already for a while [3].

Biosensors are used in physics, chemistry and biology. Biosensors are used to analyze various physical, chemical and biological processes. Heat, light and electricity are the results of various reactions. Those properties are exploited to analyze reactions.

1.2 Types of Biosensors

There are different types of biosensors depending on the type of property used to analyze a reaction. Followings are the different types of biosensors.

1.2.1 Thermometric biosensors

Thermometry is a technology which is used to measure the temperature. There are various ways to measure the temperature such as optical, mechanical and electrical technique. Thermometric biosensors are constructed by combining enzymes with temperature sensors. When the analyte is exposed to the enzyme, the heat of reaction of the enzyme is measured and is calibrated against the analyte concentration.

1.2.2 Piezoelectric biosensors

The charge which accumulates on a solid material due to mechanical strain is defined as piezoelectricity. The word piezoelectricity means electricity resulting from pressure. A device which can measure the piezoelectricity is known as piezoelectric sensors. This technology is used in physiology, physical chemistry, biology, etc. When it comes to biology; the piezoelectric sensor is renamed as piezoelectric biosensor. The development of a piezoelectric biosensor based on nucleic acids interaction is presented focusing on the methodology for probe immobilization. This is a key step in any DNA biosensor development. Often, the detection limits and, in general, the analytical performances of the biosensor can be improved by optimizing the immobilization of the receptor on the transducer surface.

1.2.3 Optical biosensors

Optical biosensors are analytical devices comprising optical element and biological recognizing molecule. Optical biosensors can be used to detect the light produced by chemical reactions.

Optical biosensors provide the most comprehensive analysis of optical activities of different technologies. When light is reflected at an optical interface where there is a change of refractive index, there is a decay of energy from the point of reflection into the surrounding medium. This energy field which extends into the medium depends upon the medium in which the wave guide is dipped. The resultant changes of luminescence, absorption or fluorescence can hence be determined. When the glass surface of the biosensor is coated with a thin layer of metal (silver, gold), the intensity of the resonance angle changes depending on the concentration of the medium in which electrode is immersed. This phenomenon is called the surface plasma resonance (SPR).

1.2.4 Amperometric biosensors

Amperometric biosensors are type of biosensors which exploit potential difference between two electrodes to analyze the process. These biosensors are also called potentiometric biosensors.

1.2.5 Acoustic wave biosensors

Acoustic biosensor is sound based biosensor

1.2.6 Hybrid biosensors

Hybrid biosensors are the biosensors made by combination of various types of biosensors.

The enzyme electrode is a combination of any electrochemical probe (amperometric, potentiometric or conductometric) with a thin layer (10 -200 mm) of immobilized enzyme. Enzyme immobilization is defined as the restriction of enzyme mobility in a fixed space. In order to make a viable biosensor, the biological component (enzyme) has to be properly attached to

the transducer. Design, preparation procedure and immobilization procedures is the key to construct a successful biosensor so that enzymes should be stabilized and easy to be contacted by substrates. Choice of immobilization technique to immobilize enzyme is extremely important in terms of biosensor operational stability and long-term use. Methods selected must be compatible with the enzyme and substrates. Poor technique will result in significant loss of enzyme activity and thus low sensor response. A critical step in fabrication of these devices is effective enzyme immobilization while maintaining free diffusion of the substrates to the enzyme layer.

1.3 Methods of Enzyme Immobilization

There are many methods to immobilize the enzymes [4]. They are adsorption, covalent binding, cross linking, physical entrapment etc.

1.3.1 Adsorption

In adsorption, enzyme is attached on the surfaces of support/carrier particles by weak but sufficiently large forces to allow reasonable binding forces such as Vander Waals, ionic and hydrogen bonding interaction and possibly hydrophobic forces as illustrated in Fig.1.2 This method is only suitable for exploratory work over short periods of time. This method may be stabilized by cross-linking of glutaraldehyde but this might denature some of the proteins. Many substances adsorb enzymes on their surfaces but the surfaces of the support materials may need to be pretreated either chemically or physically for effective immobilization. The advantages of choosing adsorption as the immobilization technique are that usually no reagents are required and involve minimal preparation and cleanup work. Nearly full activity of the enzyme is retained since the active site of the adsorbed enzyme is unaffected. Despite its simplicity, adsorbed enzymes are susceptible to ambient conditions such as pH, temperature, ionic strength, polarity etc. which will cause leakage of enzymes from supports. Another disadvantage is that there is no specific binding by substrate or contaminants to the carrier which may result in diffusion limitations and mass transfer problems.

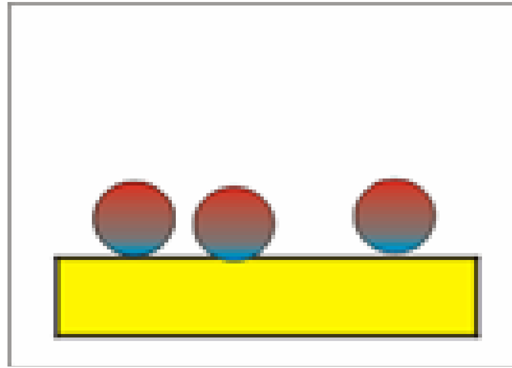


Fig: 1.2 Adsorption of the enzyme to the surface.

1.3.2 Covalent binding

Covalent binding is the retention of enzymes on support surfaces through the formation of a covalent bond between functional group on the carrier and the enzyme as illustrated in Fig. 1.3. Those on enzymes are usually amino acid residues such as amino group from Lysine or Arginine, carboxyl group from Aspartic acid and Glutamic acid, hydroxyl group from Serine and Threonine and sulfhydryl groups from Cysteine. Formation of covalent bonds must not inactivate the enzyme. This is ensured by blocking the active site by flooding the enzyme solution with a competitive inhibitor prior to covalent binding. This mode of attachment often involves three steps including the activation of the support, the modification of the activated electrode surface and the enzyme coupling. Reactions have to be carried out at mild temperature and low pH. This method is widely applicable since it provides a more stable immobilized biomolecule layer and modification with more than one layer is possible. Besides, the enzyme is permanently attached to the support.

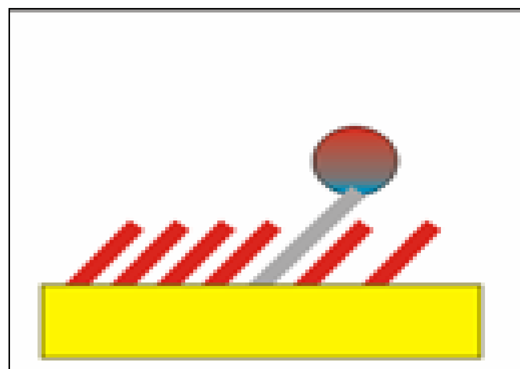
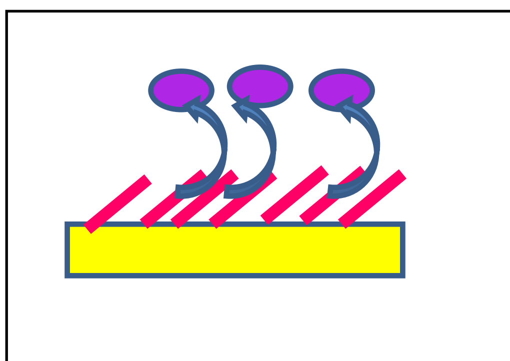


Fig: 1.3 Covalent linkage to the electrode or a self-assembled monolayer.

1.3.3 Cross-linking

This method joins the enzyme to each other or to another supporting material to form a large 3D structure. This can be done by using bi-functional agents such as gluteraldehyde, bis-diazobenzidine and 2, 2-disulfonic acid. In cross-linking, enzymes can be cross-linked with glutaraldehyde to form an insoluble aggregate, adsorbed enzymes may be cross-linked or cross-linking may take place following the impregnation of porous support material with enzyme solution as shown in Fig.1.4. Cross-linking is a useful method to stabilize adsorbed enzyme. It may cause significant changes in the active site of enzymes and thus, tests must be done to ensure that the active site remains free and available for catalytic activity. It may result in severe diffusion limitations and poor mechanical strength or rigidity



. Fig: 1.4 Cross Linking of the enzyme to the substrate

1.3.4 Entrapment

Entrapment is a physical method to immobilize or physical enclosure of enzymes in a small space. Enzyme remains free in the solution, but restricted in movement by the lattice structure of a gel. Pore size of the gel lattice is controlled so that the structure is tight enough to prevent enzyme leakage while allowing free movement of substrates and products. This method is applicable to many enzymes, may provide no or little perturbation of the native structure and function of the enzyme, very selective to the enzyme sensed and is a good method to trap microorganisms. Some of the problems associated with entrapment are due to the loss of enzymatic activity

and large response time. Matrix entrapment and membrane entrapment; including microencapsulation are the two major methods of entrapment. The matrix can be a particle, a membrane or a fiber. Figure 1.5 shows the entrapment of enzyme into a gel or polymer network.

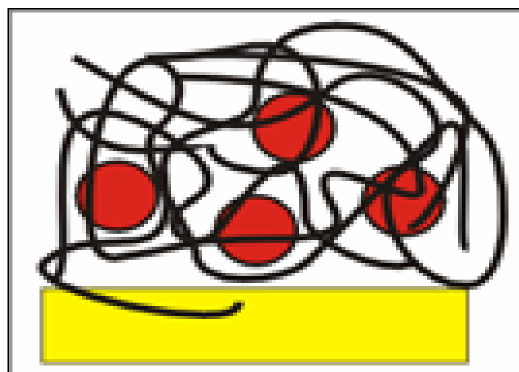


Fig: 1.5 Entrapment into a gel or polymer network.

A special form of membrane entrapment is microencapsulation. The main types of membrane used are cellulose acetate, polycarbonate, collagen, polytetrafluoroethylene (TEFLON), nafion and polyurethane. This is the method used in the early biosensors such as Clark type sensors. Microscopic hollow spheres are formed and it contains the enzyme solution while being enclosed within a porous membrane. There is a close attachment between the enzyme and the transducer and it gives a good stability to change in temperature, pH, ionic strength and substrate concentration.

1.4 Glucose Biosensor

Determination of glucose is essential due to its clinical and industrial importance. Rapid determination of blood sugar is very important for treatment and control of diabetes. Thus numerous efforts have been devoted to the development of glucose biosensors with fast and accurate response. Most electrochemical glucose biosensors are based on the glucose oxidase (GOx) enzyme, which catalyzes the oxidation of glucose to gluconolactone which is hydrolyzed to gluconic acid and hydrogen peroxide. GOx is a dimeric protein as shown in Fig. 1.6 with a molecular weight of 160, 000 KD. It contains one tightly bound flavin adenine dinucleotide (FAD) per monomer as cofactor which means that each enzyme will have two FAD-sites. FAD can be released

from the protein following partial unfolding of the protein since it is not covalently bound.

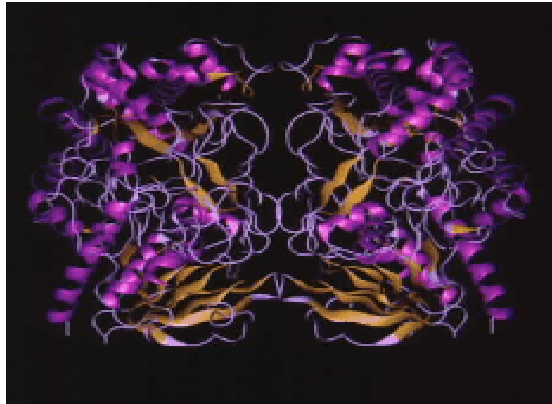
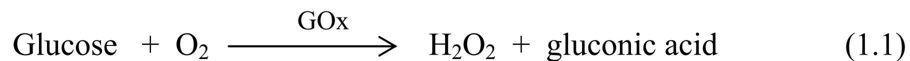


Fig: 1.6 Overall topology of Glucose oxidase.

GOx is made up of two identical subunits with a molecular weight of 80,000 KD each. These two monomers are connected non-covalently via a long but narrow contact area. There are either salt linkages or hydrogen bonds between the dimers.

The quantification of glucose can be achieved via electrochemical detection of the enzymatic release of H_2O_2 [5-6].



$$\text{Glucose} + O_2 \xrightarrow{\text{GOx}} H_2O_2 + \text{gluconic acid} \quad (1.1)$$

Glucose concentration can be measured by electrochemically either by potentiometric or Amperometric technique

Potentiometric Glucose Biosensor

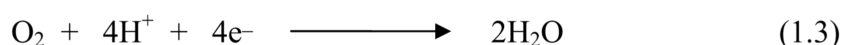
The potentiometric detection of enzyme activity is based on the measurement of the change in pH in the enzymatic layer on the sensor surface. The pH change (*e.g.* release of acid) is caused by oxidation of H_2O_2 produced by the enzymatic reaction as indicated in equation (1.2). The enzyme catalyzes the conversion of the target analyte in reactions consuming or releasing protons. The sensitivity limit of a potentiometric enzyme electrode is the sensitivity of the pH transducer. Conventional pH transducers, *e.g.* glass electrode or pH sensitive field effect transistors, can be used for this purpose.

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \qquad (1.2)$$

Amperometric Glucose Biosensor

There are two types of amperometric glucose biosensors. Oxygen based or hydrogen peroxide based**.**

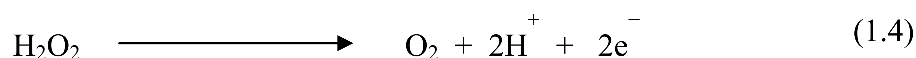
For the oxygen-based enzyme glucose since oxygen is consumed during the enzymatic reaction, oxygen concentration in the glucose oxidase membrane will be a linear function of glucose concentration [7-8]. The oxygen concentration can be detected by coupling the membrane containing immobilized glucose oxidase to an electrochemical oxygen sensor. Since oxygen is also present in the sample, a similar reference oxygen sensor without the enzyme needs to be incorporated in the system. The signal current is then subtracted from the reference electrode and these results in a glucose-dependent difference current. The advantages of this type of sensor is that it has low electrochemical interference due to the use of a nonporous hydrophobic membrane that will only allow gaseous molecules to reach the electrode and it can also provide information on oxygen variations in the system. Furthermore, the immobilization of the enzyme catalase along with glucose oxidase will help prolong the active lifetime of glucose oxidase as catalase promotes the degradation of hydrogen peroxide to oxygen and water. However, the differential setup makes it a more complicated device and renders it hard to miniaturize. A negative potential is applied to the Pt working electrode for a reductive detection of the oxygen consumption as



$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O \qquad (1.3)$$

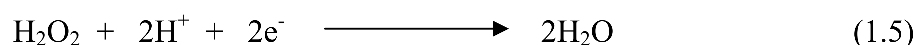
In the hydrogen peroxide-based enzyme glucose sensor the amount of hydrogen peroxide is usually detected by measuring the current during the oxidation or reduction of hydrogen peroxide [9-10]. The anodic or cathodic current will be the linear function of glucose concentration. The hydrogen peroxide-based enzyme glucose sensor has found wide application in the development of a glucose biosensor, especially an implantable version, due to its simple sensor configuration that facilitates ease of miniaturization. Unlike oxygen, hydrogen peroxide is not present in the sample to be analyzed, so no

differential set-up is needed. The oxidation of the hydrogen peroxide is given as



$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (1.4)$$

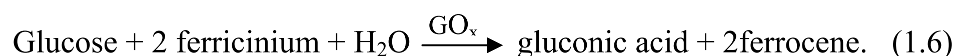
The direct reduction of H_2O_2 in a slightly acidic medium [11]



$$H_2O_2 + 2H^+ + 2e^- \longrightarrow 2H_2O \quad (1.5)$$

The oxygen and hydrogen peroxide based glucose sensors are called first generation amperometric glucose sensors.

Second generation glucose sensors make use of mediators to shuttle electrons from the enzyme to the electrode [12-15]. This type of system is supposed to eliminate the dependency of the enzymatic reaction to oxygen. If the system is oxygen deficient, the glucose sensor will become insensitive to glucose and will only respond to changes in oxygen concentration. However, as oxygen remains in the system, the mediator must be able to compete effectively for the electrons. Ferrocene and its derivatives are the best-known artificial electron carriers. If the enzyme membrane contains ferrocene and glucose oxidase, the following reaction will occur.



$$\text{Glucose} + 2\ \text{ferricinium} + H_2O \xrightarrow{GO_x} \text{gluconic acid} + 2\text{ferrocene}. \quad (1.6)$$

At a positively biased electrode, this reaction follows:

$$2\ \text{ferrocene} \longrightarrow 2\ \text{ferricinium}^+ + 2e^- \quad (1.7)$$

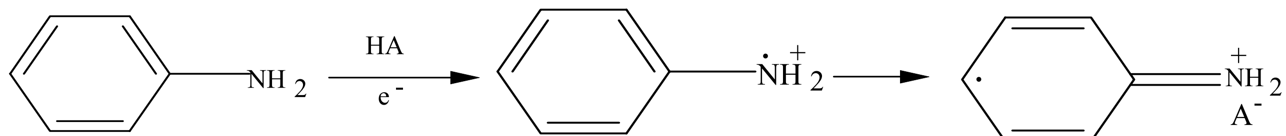
Glucose sensors based on this principle can display a linear range up to 30mM even when oxygen concentration in the system is very low. The advantage of this system is that the oxidation of the mediator can be carried out at a significantly lower potential than that of hydrogen peroxide resulting in lower electrochemical interference from endogenous species. However, the mediators can be toxic, thus eliminating the possibility of *in vivo* use. Heller and his group [16] have designed a recent version of the mediator sensor, which is not diffusible. The mediator is bound to a cross-linked polymer.

Glucose oxidase is tethered to the electrode with a hydrogel formed of a redox polymer with a bound complexed osmium redox center.

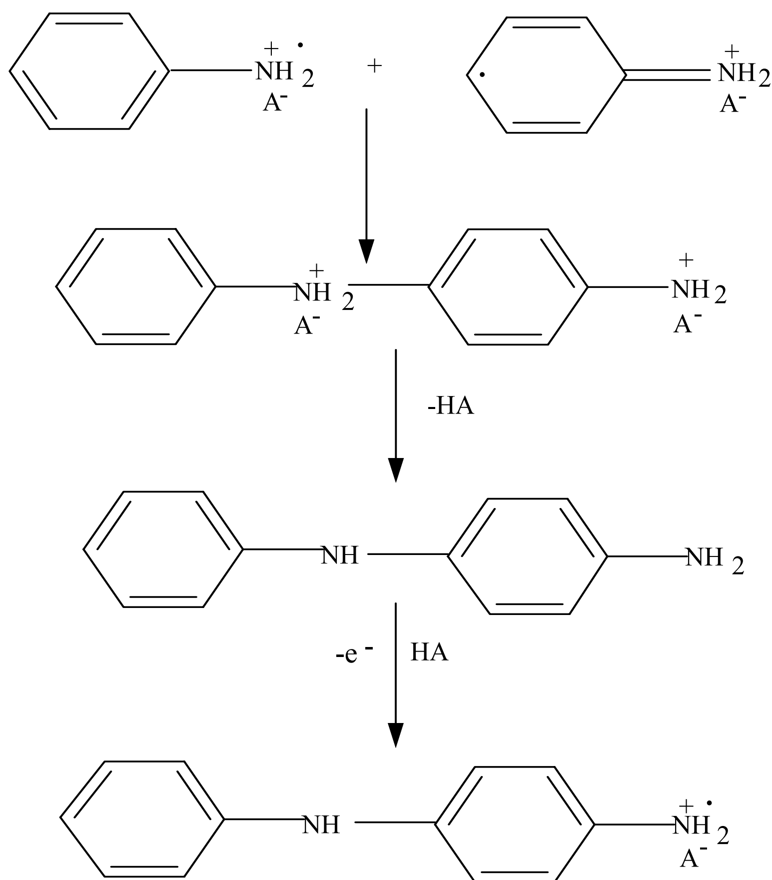
Third generation amperometric glucose sensors are based on the use of conducting organic salts or polymers. The films are grown electrochemically and glucose oxidase is entrapped in the membranes. Polypyrrole [17] and polyaniline [18] have been investigated as potential membrane materials for the entrapment of the enzyme. The electrode responds to glucose concentration via peroxide oxidation. The advantage of this system is that manipulation of the electropolymerization can give a film that extends the linear range for glucose detection and reduces oxygen dependence.

Polyaniline is probably the eldest known electroconducting polymer, since it was used for textile coloring one century ago [19]. The great interest in research of polyaniline is connected to discovery of its conductivity in the form of emeraldine salt and existence of different oxidation forms [20].

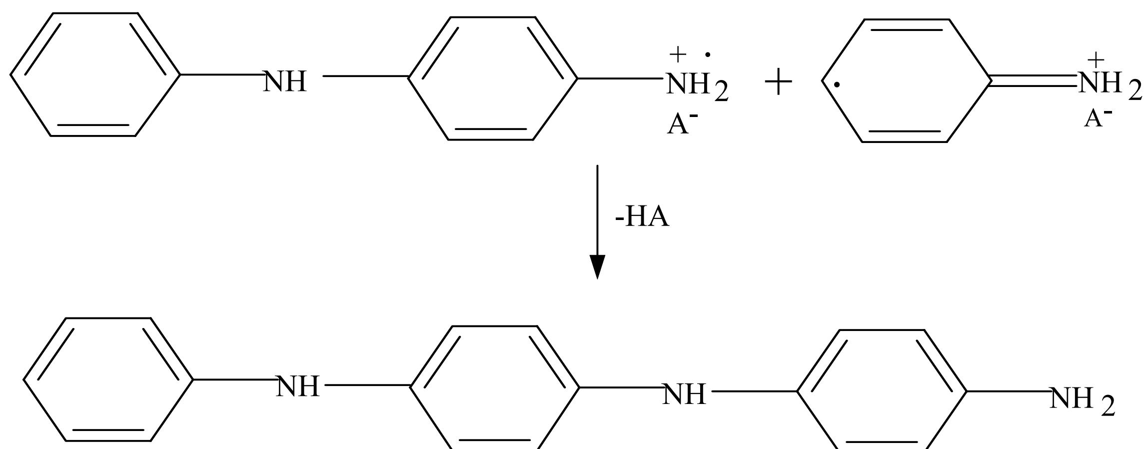
Generally, electroconducting polymers are obtained by either chemical or electrochemical oxidative polymerization, although reductive polymerization was also reported. Chemical polymerization is used when large quantity of polymer is requested. Electrochemical polymerization is favorable, since in the most cases the polymer is directly deposited on the electrode facilitating analysis. On the other hand, electrochemical polymerization is especially useful if polymer film electrode is needed. By proper design of electrochemical polymerization of aniline, polymer thickness and conductivity can be easily controlled. It is believed that electrochemical polymerization is consisted of three different steps, in first, oxidation of the monomer at anode lead to formation of soluble oligomers in the diffusion layer, in the second, deposition of oligomers occurs through nucleation and growth process, and finally, the third step is responsible for chain propagation by solid state polymerization Unfortunately, a general mechanism of electrochemical polymerization could not be established, since it was evidenced that various factors had influence. However, it was observed that first step of the electrochemical polymerization



Coupling of radicals



Polyaniline propagation



Dimer of aniline

Fig: 1.7 Schematic diagram of the mechanism of electrochemical polymerization of Anlline

was formation of reactive cation radicals. The next step, strongly dependent on the experimental conditions, is believed to be essential for the polymer growth. The knowledge on the kinetics of the nucleation and growth process during electrochemical synthesis of electro conducting polymers is also of great interest, since it would be useful in control of the morphology, density, crystallinity etc. of the desired polymer.

Both the mechanism and the kinetics of the electrochemical polymerization of aniline were extensively investigated. Electrochemical, similarly to chemical, polymerization of aniline is carried out only in acidic electrolyte, since higher pH leads to formation of short conjugation oligomeric material, with different nature. The mechanism of electrochemical polymerization of aniline is shown in Fig.1.7. As stated before, it is generally accepted that the first step of the polymerization process of aniline involves formation of aniline cation radicals, by anodic oxidation on the electrode surface, which is considered to be the rate-determining step The existence of aniline radical cation was experimentally confirmed, by introducing molecules, (resorcinol, hydroquinone, benzoquinine etc.), capable of retarding or even stopping the reaction, which evidenced a radical mechanism. The oxidation of the aniline monomer is an irreversible process, occurring at higher positive potentials than redox potential of the polyaniline.

Unlike other known electro conducting polymers, polyaniline can exist, depending on degree of oxidation, in different forms, known as: leuoemeraldine, emeraldine and perningraniline. leucoemeraldine e.g. leucoemeraldine base, refers to fully reduced form; emeraldine, e.g. emeraldine base, is half-oxidized, while perningraniline, e.g. perningraniline base, is completely oxidized form of polyaniline. The only conducting form of polyaniline is emeraldin salt, obtained by doping or protonation of emeraldine base [21]. The unique feature of mentioned polyaniline forms is ease of its mutual conversions by both chemical and electrochemical reactions as it can be seen in Fig. 1.8 [22].

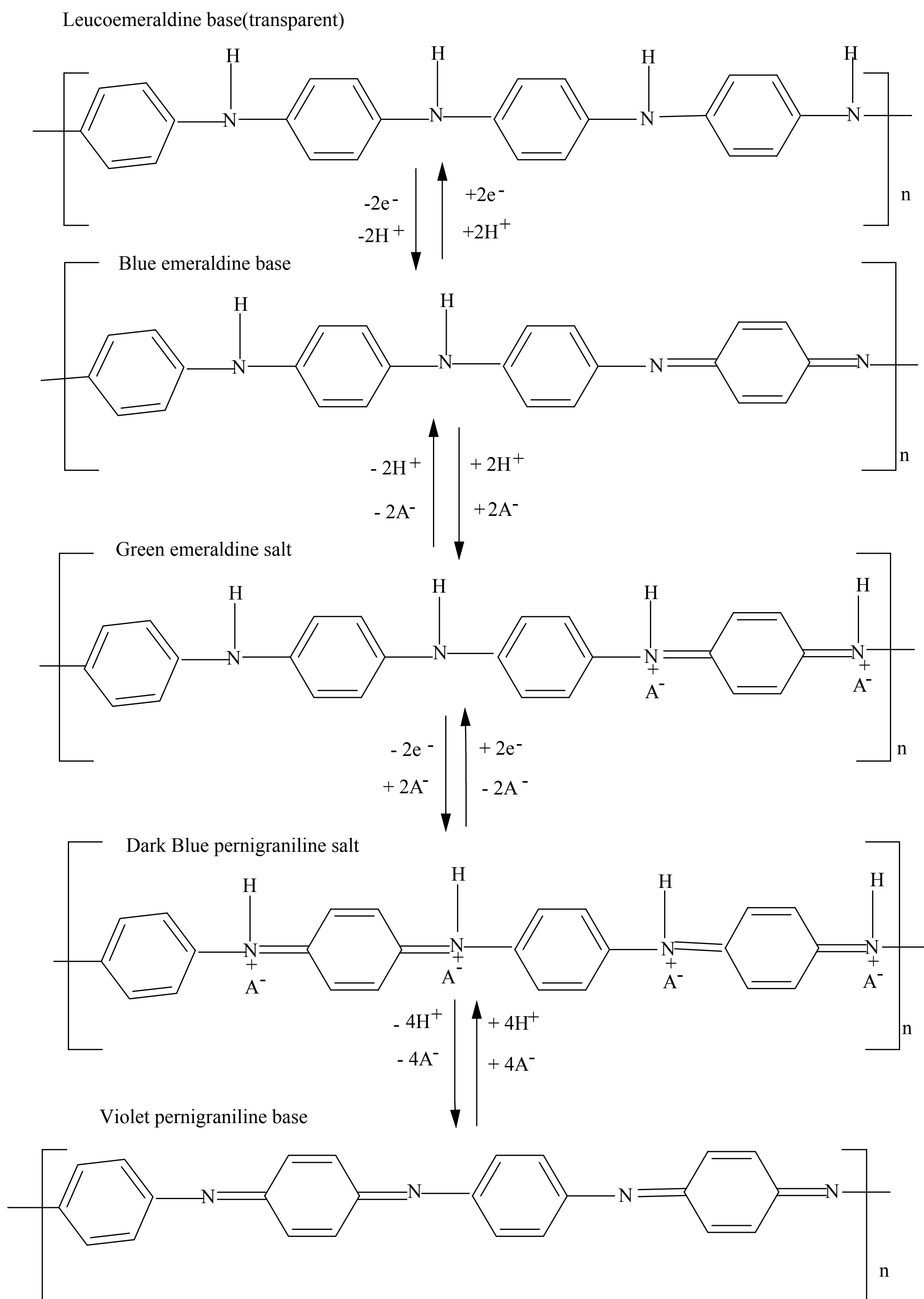


Fig: 1.8 Different forms of polyaniline

Apart from the changes in oxidation levels, all the transitions among polyaniline forms are manifested by color and conductivity changes [23]. The conducting protonated emeraldine in the form of green emeraldine salt, obtained as a product of electrochemical polymerization of aniline in acidic electrolytes, can be easily transformed by further oxidation to fully oxidized dark blue perningraniline salt, which can be treated by alkali to form violet perningraniline. Emeraldine salt can also be reduced to transparent leucoemeraldine, or can be transformed by alkali to blue non conducting emeraldine. The two blue forms of polyaniline, perningraniline salt and emeraldine have different shades of blue [23]. Both reductions of emeraldine salt to leucoemeraldine and oxidation to perningraniline states are followed by decrease in conductivity [24].

The mechanism of polyaniline conductivity differs from other electroconducting polymers, owing to the fact that nitrogen atom are involved in the formation of radical cation, unlike most of the electroconducting polymers whose radical cation is formed at carbon. On the other hand, nitrogen is also involved in the conjugated double bonds system. Therefore, electrical conductivity of polyaniline is dependent both on the oxidation and protonation degrees [21]. As mentioned before, polyaniline is characterized by existence of various oxidation forms. Polyaniline in the form of emeraldine base can be doped (protonated) to conducting form of emeraldine salt. Emeraldine base, half oxidized form, is consisted of equal amount of amine (-NH-) and imine (=NH-) sites. Imine sites are subjected to protonation to form bipolarone or dication (emeraldine salt form). Bipolarone is further dissociated by injection of two electrons both from electron pairs of two imine nitrogen, into quinodiimine ring, and the third double bond of benzenoid ring is formed [24].

Unpaired electrons at nitrogen atoms are cation radicals, but essentially they represent polarons. The polaron lattice, responsible for high conductivity of polyaniline in the form of emeraldine salt is formed by redistribution of polarones along polymer chain, according to schematic representation given in Fig. 1.9 [25].

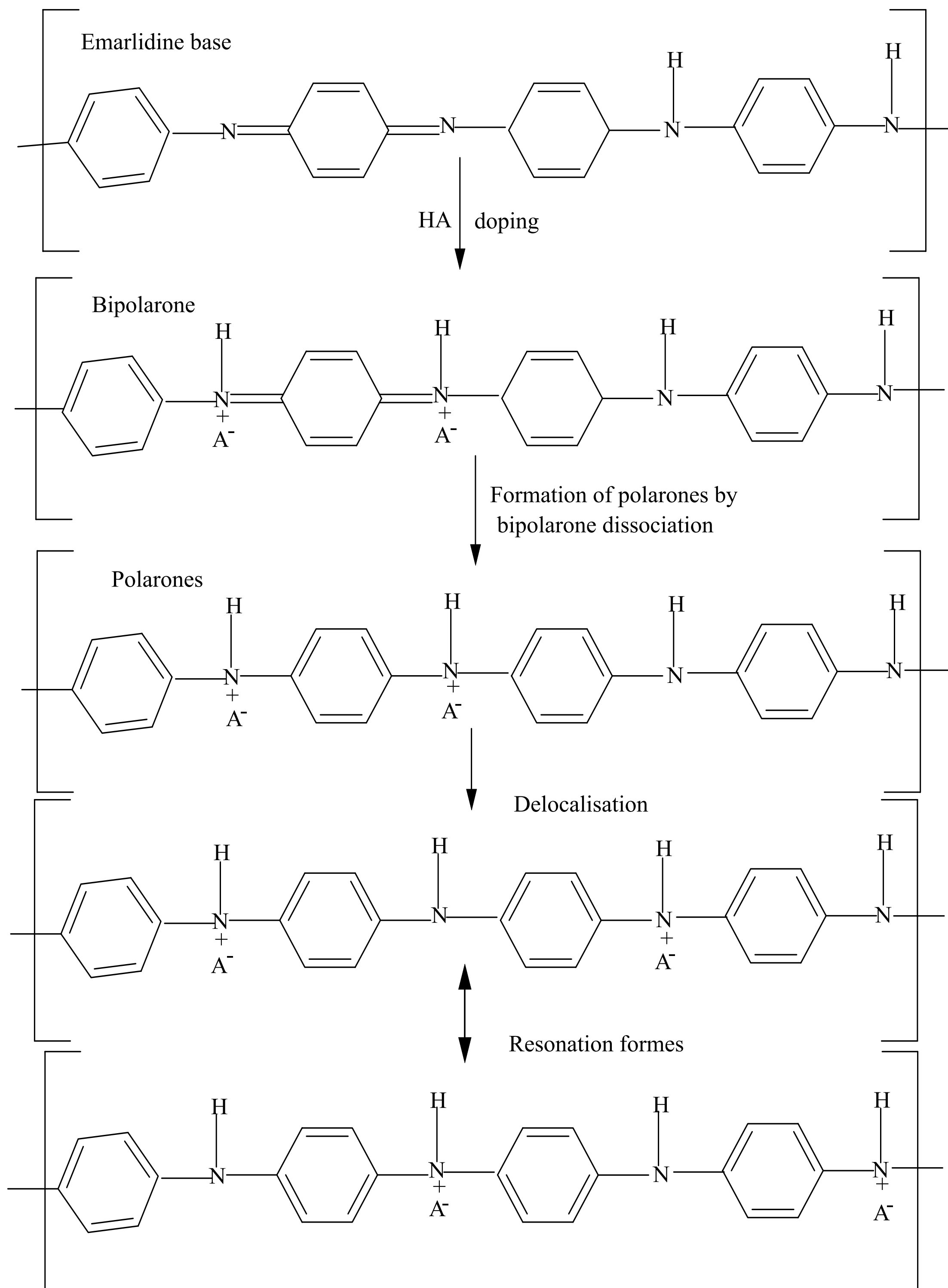


Fig: 1.9 Schematic diagram for showing the sequence of polyaniline conductivity

Although both bipolarone and polarone theoretical models of emeraldine salt conductivity were proposed [25] it was lately confirmed that, beside from the fact that few of spineless bipolarones exist in polyaniline, formation of polarones as charge carriers explained high conductivity of polyaniline [26] as mentioned, unique property of polyaniline is conductivity dependence on the doping (proton) level [27].

The maximal conductivity of polyaniline is achieved at doping degree of 50%, which corresponds to polyaniline in the form of emeraldine salt [28]. For higher doping degrees some of the amine sites are protonated, while lower doping degrees means that some of the imine sites were left unprotonated, [25] explaining why, in the light of the polaron conductivity model, reduction of emeraldine salt to leucoemeraldine and oxidation to perningraniline states decrease the conductivity. The order of magnitude for conductivity varies from 10^{-2} S cm^{-1}, for undoped emeraldine, up to 10^{3} S cm^{-1} for doped emeraldine salt [20].

Beside the fact that doping degree has the pronounced effect on the conductivity, various other factors such as: moisture amount [29], morphology [30], temperature [31] etc. were also found to have influence on the polyaniline conductivity.

In this study the glucose biosensor was prepared by using physical entrapment technique in polyaniline membrane on graphite surface. Polyaniline surface was prepared by electropolymerisation.

1.5 Literature Review

The idea of a glucose enzyme electrode was proposed in 1962 by Clark and Lyons from the Children Hospital in Cincinnati [32]. Their first device relied on a thin layer of GOx entrapped over an oxygen electrode (via a semipermeable dialysis membrane), and monitoring the oxygen consumed by the enzyme-catalyzed reaction:

$$\text{glucose} + \text{oxygen} \xrightarrow{GO_x} \text{gluconic acid} + \text{hydrogen peroxide} \quad (1.8)$$

Clark's original patent [33] covers the use of one or more enzymes for converting electro inactive substrates to electro active products. The effect of interferences was corrected by using two electrodes (one covered with GOx) and measuring the differential current. Clark's technology was subsequently transferred to Yellow Spring Instrument Company that launched in 1975 the first dedicated glucose analyzer (the Model 23 YSI analyzer) for the direct measurement of glucose in 25 mL samples of whole blood. Updike and Hicks [34] developed further this principle by using two oxygen working electrodes (one covered with the enzyme) and measuring the differential current for correcting for the oxygen background variation in samples. Guilbault and Lubrano [35] described in 1973 an enzyme electrode for the determination of blood glucose based on amperometric (anodic) monitoring of the liberated hydrogen peroxide:

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (1.9)$$

Good precision and accuracy were obtained in connection to 100 mL blood samples. A wide range of amperometric enzyme electrodes, differing in the electrode design or material, membrane composition, or immobilization approach have since been described.

During the 1980s biosensors became a 'hot' topic, reflecting the growing emphasis on biotechnology. Intense efforts during this decade focused on the development of mediator-based 'second-generation' glucose biosensors [36-37], the introduction of commercial strips for self-monitoring of blood glucose [38-39], and the use of modified electrodes for enhancing the

sensor performance [40]. In the 1990s the intense activity towards the establishment of electrical communication between the GOx redox center and the electrode surface [41-42], and the development of minimally-invasive subcutaneously implantable devices [43– 45] was studied.

Table 1 summarizes major historical landmarks in the development of electrochemical glucose biosensors.

Table – 1

Date	Event	References
1962	First glucose enzyme electrode	[60]
1973	Glucose enzyme electrode based on peroxide detection	[36]
1975	Launch of the first commercial glucose sensor system	YSI Inc.
1982	Demonstration of in vivo glucose monitoring	[60]
1984	Development of ferrocene mediators	[37]
1987	Launch of the first personal glucose meter	Medisense Inc.
1987	Electrical wiring of enzymes	[42]
1999	Launch of a commercial in vivo glucose sensor	Minimed Inc.
2000	Introduction of a wearable noninvasive glucose	Cygnus Inc.

Based on the technological improvement of electrochemical glucose biosensors. Three generation of glucose biosensor have been reported [37].

The first generation glucose biosensors estimated glucose concentration in the sample based on hydrogen peroxide production by glucose oxidase (GO_x) utilizing dissolved oxygen. First-generation devices have relied on the use of the natural oxygen cosubstrate, and the production and detection of hydrogen peroxide. Such measurements of hydrogen peroxide formation have the advantage of being simpler, especially when miniaturized sensors are concerned. A very common configuration is the YSI probe, involving the entrapment of GOx between an outer diffusion-limiting/biocompatible polycarbonate membrane and an inner anti-interference cellulose acetate one.

The amperometric measurement of hydrogen peroxide requires application of a potential at which coexisting species, such as ascorbic and uric acids or acetaminophen, are also electroactive. The anodic contributions of these and other oxidizable constituents of biological fluids can compromise the selectivity and hence the overall accuracy. Extensive efforts during the 1980s were devoted for minimizing the error of electro active interferences in glucose electrodes. One useful strategy is to employ a perm selective coating that minimizes access of such constituent to the transducer surface. Different polymers, multilayers and mixed layers, with transport properties based on size, charge, or polarity, have thus been used for discriminating against coexisting electro active compounds [46]. Such films also exclude surface-active macromolecules, hence imparting higher stability. Electropolymerized films, particularly poly (phenylenediamine) and over oxidized poly-pyrrole, have been shown particularly useful for imparting high selectivity (based on size exclusion) while confining the GOx onto the surface [46-47]. Other widely used coatings include the negatively charged (sulfonated) Nafion or Kodak AQ ionomers, size-exclusion cellulose acetate films, and hydrophobic alkanethiol or lipid layers.

Combining the properties of different films, offers additional advantages. For example, alternate deposition of cellulose acetate and Nafion was used for eliminating the interference of the neutral acetaminophen and

negatively charged ascorbic and uric acids, respectively [48].

Efforts during the 1990s focused on the preferential electro-catalytic detection of the liberated hydrogen peroxide. This has allowed tuning of the detection potential to the optimal region (0.0 to 0.20 V vs. Ag/AgCl) where most unwanted background reactions are negligible. The remarkably high selectivity thus obtained was coupled to a fast and sensitive response. Metalized (Rh, Ru)-carbon [49- 50] and metalhexacyanoferrate [51-53] based transducers have been particularly useful for enhancing the selectivity towards the target glucose substrate. Additional improvements can be achieved by combining this preferential catalytic activity with a discriminative layer, e.g., by dispersing rhodium particles within a nafion film [54].

Since oxidase-based devices rely on the use of oxygen as the physiological electron acceptor, they are subject to errors accrued from fluctuations in the oxygen tension and the stoichiometric limitation of oxygen. Such limitation (known as the ‘‘oxygen deficit’’) reflects the fact that normal oxygen concentrations are about an order of magnitude lower than the physiological level of glucose.

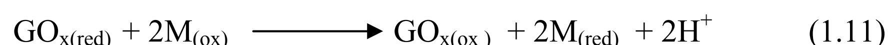
Several routes have been proposed for addressing this oxygen limitation. One strategy relies on the use of mass-transport limiting films (such as polyurethane or polycarbonate) for tailoring the flux of glucose and oxygen, i.e., increasing the oxygen/glucose permeability ratio [55]. A two-dimensional electrode, designed by Gough’s group [55], has been particularly attractive for addressing the oxygen deficit by allowing oxygen to diffuse into the enzyme region of the sensor from both directions and glucose diffusion only from one direction. It has recently addressed the oxygen limitation of glucose biosensors by designing an oxygen-rich carbon paste enzyme electrode [56]. The new biosensor is based on a fluorocarbon (Kel-F oil) pasting liquid, which has very high oxygen solubility, allowing it to act as an internal source of oxygen. The internal flux of oxygen can thus support the enzymatic reaction even in oxygen-free glucose solutions. It is possible also to circumvent the oxygen demand issue by replacing the GOx with glucose dehydrogenase (GDH) that does not require an oxygen cofactor [57].

Further improvements (and attention to the above errors) can be achieved by replacing the oxygen with a nonphysiological (synthetic) electron acceptor, which is able to shuttle electrons from the redox center of the enzyme to the surface of the electrode. Glucose oxidase does not directly transfer electrons to conventional electrodes because a thick protein layer surrounds its flavin redox center. Such thick protein shell introduces a spatial separation of the electron donor-acceptor pair, and hence an intrinsic barrier to direct electron transfer, in accordance to the distance dependence of the electron transfer rate [58].

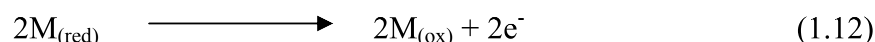
The minimization of the electron-transfer distance (between the immobilized GOx and the electrode surface) is thus crucial for ensuring optimal performance. Accordingly, various innovative strategies have been suggested for establishing and tailoring the electrical contact between the redox center of GOx and electrode surfaces.

Particularly useful has been the use of artificial mediators that shuttle electrons the FAD center and the surface by the following scheme.

$$\text{Glucose} + GO_{x(ox)} \longrightarrow \text{Gluconic acid} + GO_{x(red)} \quad (1.10)$$



$$GO_{x(red)} + 2M_{(ox)} \longrightarrow GO_{x(ox)} + 2M_{(red)} + 2H^{+} \quad (1.11)$$



$$2M_{(red)} \longrightarrow 2M_{(ox)} + 2e^{-} \quad (1.12)$$

Where M(ox) and M(red) are the oxidized and reduced forms of the mediator. Such mediation cycle produces a current dependent on the glucose concentration. Diffusional electron mediators, such as ferrocene derivatives, ferricyanide, conducting organic salts (particularly tetrathiafulvalene-tetracyanoquinodi methane (TTFTCNQ), phenothiazine and phenoxazine compounds, or quinone compounds have thus been widely used to electrically contact GOx (Fig. 1.10) [37 - 38]. As a result of using these electron-carrying mediators, measurements become largely independent of oxygen partial pressure and can be carried out at lower potentials that do not provoke interfering reactions from coexisting electro active species.

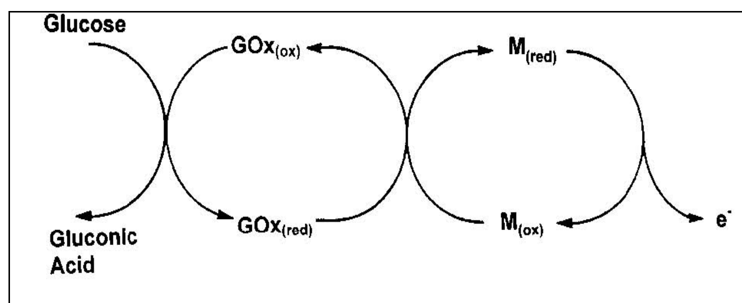


Fig: 1.10 Sequence of events that occur in 'second-generation' (mediator based) glucose biosensors mediated system.

In order to function effectively, the mediator should react rapidly with the reduced enzyme (to minimize competition with oxygen), possess good electrochemical properties (such as a low redox potential), have low solubility in aqueous medium, and must be nontoxic and chemically stable (in both reduced and oxidized forms). Commercial blood glucose self-testing meters, described in the following section, commonly rely on the use of ferrocene or ferricyanide mediators. Most in vivo devices, however, are mediator less due to potential leaching and toxicity of the mediator.

In order to avoid complication offered by synthetic or natural mediators in second generation biosensors a lot of work is being done for finding new strategies for direct electron between the electrode and active center of enzyme. This led to development of highly selective and sensitive third –generation biosensors. Heller's group [41] developed an elegant nondiffusional route for establishing a communication link between GOx and electrodes based on 'wiring' the enzyme to the surface with a long flexible poly-pyridine polymer having a dense array of osmium-complex electron relays. The resulting three-dimensional redox-polymer/enzyme networks offer high current outputs and stabilize the mediator to electrode surfaces.

Chemical modification of GOx with electron-relay groups represents another novel avenue for facilitating the electron transfer between its redox center and the electrode surface. Willner and co-workers reported on an elegant approach for modifying GOx with electron relays (Fig. 1.11) [42]. For this purpose, the FAD active center of the enzyme was removed to allow positioning of an electron-mediating ferrocene unit prior to the reconstitution

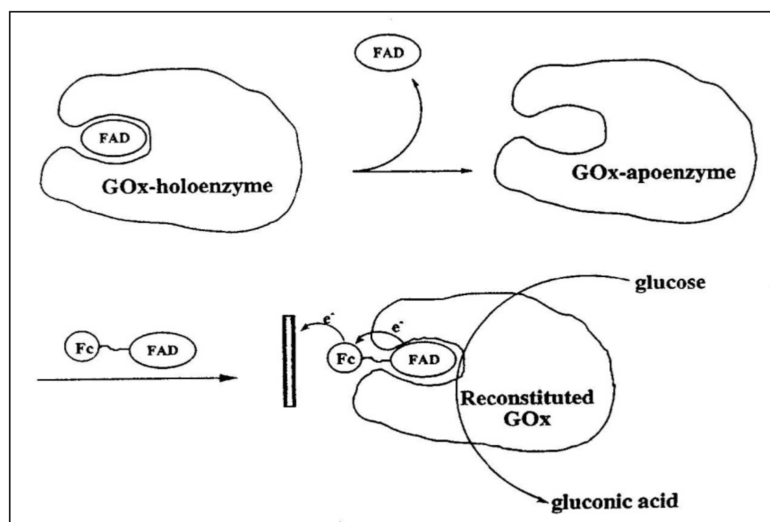


Fig:1.11. Electrical contacting of a flavoenzyme by its reconstitution with a relay-FAD semisynthetic cofactor.

of the enzyme. The attachment of electron-transfer relays at the enzyme periphery has also been considered for yielding short electron-transfer distances [59]. More sophisticated bioelectronic systems for enhancing the electrical response, based on patterned monolayer or multilayer assemblies and organized enzyme networks on solid electrodes, have been developed for contacting GOx with the electrode support. Functionalized alkanethiol modified gold surfaces have been particularly attractive for such layer-by-layer creation of GOx/mediator networks.

Electrochemical biosensors are well suited for satisfying the needs of home (personal) glucose testing. The majority of personal blood glucose meters are based on disposable (screen printed) enzyme electrode test strips. Such single-use disposable electrode strips are mass produced by the thick-film (screen printing) micro fabrication technology. Each strip contains the printed working and reference electrodes, with the working one coated with the necessary reagents (i.e., enzyme, mediator, stabilizer, linking agent). Such reagents are commonly being dispensed by an ink-jet printing technology. A counter and an additional ('baseline') working electrode may also be included. Such single-use devices obviate problems of carry over, contamination, or drift.

The control meter is typically light and small (pocket-size), battery operated, and relies on a potential-step (chronoamperometric) operation. Such devices offer considerable promise for obtaining the desired clinical information in a faster, simpler (‘‘user-friendly’’), and cheaper manner compared to traditional assays. The first product was a pen-style device (the Exactech), launched by Medisense Inc. in 1987, that relied on the use of a ferrocene-derivative mediator. Various commercial strips and pocket-sized test meters, for self-monitoring of blood glucose Based on the use of ferricyanide or ferrocene mediators have since been introduced [59].

In most cases, the diabetic patient pricks the finger, places the small blood droplet on the sensor strip, and obtains the blood glucose concentration (on a LC display) within 15–30s. Recent efforts have led to new strips, requiring sub-micrometer blood volumes and enabling ‘‘lesspainful’’ sampling from the arm. In addition to small size, fast response, and minimal sample requirements, such modern personal glucose meter has features such as extended memory capacity and computer downloading capabilities.

Although self-testing is considered a major advance in glucose monitoring it is limited by the number of tests per 24 h period. Such testing neglect night time variations and may result in poor approximation of blood glucose variations. Tighter glycemic control, through more frequent measurements or continuous monitoring, is desired for triggering proper alarm in cases of hypo-and hyperglycemia, and for making valid therapeutic decisions [44]. A wide range of possible *in vivo* glucose biosensors has thus been studied for maintaining glucose levels close to normal. The first application of such devices for *in vivo* glucose monitoring was demonstrated first by Shichiri et al. in 1982 [60]. Continuous *ex vivo* monitoring of blood glucose was proposed already in 1974 [61].

The major requirements of clinically accurate *in vivo* glucose sensors have been discussed in various review articles [44]. These include proper attention to the issues of biocompatibility/biofouling, miniaturization, long-term stability of the enzyme and transducer, oxygen deficit, baseline drift, short stabilization times, in vivo calibration, safety, and convenience. The

sensor must be of a size and shape that can be easily implanted and cause minimal discomfort. Under biocompatibility one must consider the effect of the sensor upon the in vivo environment as well as the environment effect upon the sensor performance. Problems with biocompatibility have proved to be the major barriers to the development of reliable implantable devices. Most glucose biosensors lack the biocompatibility necessary for a prolonged and reliable operation in whole blood. Alternative sensing sites, particularly the subcutaneous tissue, have thus received growing attention. While the above issues represent a major challenge, significant progress has been made towards the continuous monitoring of glucose.

Most of the recent attention has been given to the development of subcutaneously implantable needle-type electrodes [43-45]. Such devices are designed to operate for a few days and be replaced by the patient. Success in this direction has reached the level of short-term human implantation continuously functioning devices, possessing adequate (1 week) stability, are expected in the near future. Such devices would enable a swift and appropriate corrective action (through a closed-loop insulin delivery system, i.e., an artificial pancreas). Algorithms correcting for the transient difference (time lag) between blood and tissue glucose concentrations have been developed [45]. The recently introduced CGMS unit of Minimed. Inc. (Sylmar, CA) offers a 72 h of such subcutaneous monitoring, with measurement of tissue glucose every 5 min. and data storage in the monitor's memory. After 72 h, the sensor is removed, and the information is transferred to a computer for identifying patterns of glucose variations. In addition to easily removable short-term implants, efforts are continuing towards chronically implanted devices (aimed at functioning reliably 6–12 months).

Noninvasive approaches for continuous glucose monitoring represent a promising route for obviating the challenges of implantable devices. In particular, Cygnus Inc. has developed an attractive wearable glucose monitor, based on the coupling of reverse iontophoretic collection of glucose and biosensor functions [62]. The new GlucoWatch biographer provides up to three glucose readings per hour for up to 12 h (i.e., 36 readings within a 12 h period). The system has been shown to be capable of measuring the

electroosmotically extracted glucose with a clinically acceptable level of accuracy. An alarm capability is included to alert the individual of very low or high glucose levels. Other routes for ‘‘collecting’’ the glucose through the skin and for noninvasive glucose testing are currently being examined by various groups and companies.

Over the past fifty years scientists have witnessed an intense activity and tremendous progress towards the development of electrochemical glucose biosensors. Major advances have been made for enhancing the capabilities and improving the reliability of glucose measuring devices. Such intensive activity has been attributed to tremendous economic prospects and fascinating research opportunities. The success of glucose blood monitors has stimulated considerable interest *in vitro* and *in vivo* devices for monitoring other physiologically important compounds. Despite the impressive advances in glucose biosensors, there are still many challenges related to the achievement of tight, stable and reliable glycemic monitoring. The development of new and improved glucose biosensors thus remains the prime focus of many researchers.

As this field enters the fifth decade of intense research we expect significant efforts coupling fundamental sciences with technological advances. Such stretching of the ingenuity of researchers will result in greatly improved electrical contact between the redox center of GOx and electrode surfaces, enhanced ‘‘genetically engineered’’ GOx, new ‘‘painless’’ *in vitro* testing, advanced biocompatible membrane materials, the coupling of minimally invasive monitoring with compact insulin delivery system, new innovative approaches for noninvasive monitoring, and miniaturized long-term implants. These, and similar developments, will greatly improve the control and management of diabetes.

1.6 Objective of the Study

Electrochemical biosensors (enzyme electrodes) have been under extensive investigation during the last two decades because they combine the selectivity of substrate-specific enzymes with the versatility and simplicity of electrochemical electrode system. Development of glucose biosensor for continuous monitoring is still of paramount importance due to the significance of glucose determination in analytical and clinical laboratories, monitoring of glucose levels in fermentation reactors, glucose estimation in the food industry, and the pharmaceuticals processes. Various attempts have been made to improve the quality of glucose biosensor for the accurate determination of glucose level since long time. The high accuracy, low detection limit and longtime durability of the biosensor are being searched in various laboratories. This study is aimed to,

1. Electropolymerisation of aniline onto graphite surface
2. Study of the H_2O_2 response of PANi /Graphite surface.
3. Immobilization of the glucose oxidase enzyme on PANi /Graphite surface.
4. Determine the response of the immobilized glucose oxidase membrane towards Glucose.

CHAPTER 2

2. EXPERIMENTAL

2.1 Materials

The electrochemical studies were carried out using Hokuto Denko HA 151 Potentiostat with three electrode cell. The working electrode was graphite electrode (surface area of 0.5cm^2). The auxillary and reference electrodes were Pt wire and calomel (with saturated KCl) electrode. pH value of the buffer solution were measured with an Denver instrument model 270 pH meter. Analytical grade aniline, glucose oxidase, sodium monohydrogen-orthophosphate ($Na_2HPO_4 \cdot H_2O$), sodium dihydrogen orthophosphate ($NaH_2PO_4 \cdot 2H_2O$), and D (+) glucose were purchased from Merck. Hydrogen peroxide (100 volume) and graphite rod of commercial cell was used. Water used throughout the experimental work was doubly distilled.

2.2 Preparation of Solutions

2.2.1 Preparation of HCl solution

250mL of 1M and 500mL of 2M HCl solution were prepared by diluting the 11.6M HCl.

2.2.2 Preparation of 0.2 M aniline solution

The aniline was doubly distilled by using common laboratory distillation apparatus and 4.56mL of aniline was added to the 250mL volumetric flask and 2M HCl was added to the mark.

2.2.3 Preparation of 0.1M potassium nitrate (KNO_3) solution

250mL of potassium nitrate solution was prepared by dissolving 2.55 gm of potassium nitrate in distilled water.

2.2.4 Preparation of phosphate buffer solution of different pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5)

Phosphate Buffer solution of different pH were prepared by using

Henderson's equation by mixing the solution of sodium monohydrogen orthophosphate ($Na_2HPO_4 \cdot H_2O$) (0.1M), and sodium dihydrogen ortho-phosphate ($NaH_2PO_4 \cdot 2H_2O$) (0.1M),

$$pH = pKa + \log \frac{[salt]}{[acid]} \qquad (2.1)$$

Where pK_a is the dissociation constant of sodium dihydrogen orthophosphoric acid. The salt NaH_2PO_4 is completely dissociated:

$$NaH_2PO_4 \rightleftharpoons Na^+ + H_2PO_4^- \qquad (2.2)$$

(The ion $H_2PO_4^-$ acts as monoprotonic acid)

$$H_2PO_4^- \rightleftharpoons H^+ + HPO_4^- \qquad (2.3)$$

For which the dissociation constant K (K_2 for phosphoric acid) is 6.2×10^{-8} mol^{-1}. Na_2HPO_4 can be used as salt.

2.3 Electrochemical Synthesis of PANi onto Graphite Electrode

The surface of graphite was polished with silicon carbide1200/2000 grinding paper and sonocated. Then the surface was washed with distilled water and dried. Electtropolymerisation of Polyaniline on mild steel surface has been previously reported [63]. Polyaniline film deposition of aniline in strongly acid medium was performed at room temperature using graphite rod as working electrode *vs* calomel electrode as reference and Pt wire as counter. A schematic diagram of PANi synthesis is shown in Fig. 2.1 and photograph is shown in Fig. 2.2.

Then galvanostastic, potentiostatic, and cyclic voltammetric methods were used for electopolymerisation. Galvanostatic method was used by using current density of $2mAcm^{-2}$, $3mAcm^{-2}$, $4mAcm^{-2}$ and $5mAcm^{-2}$. Potentiostatic method was used by the application of 0.75V, 0.90V and 1V. The polymerization by cyclic voltammetry was carried out by applying the potential range between -0.4V to 1.2V with the scan rate of 100 mV/sec and 10 cycles.

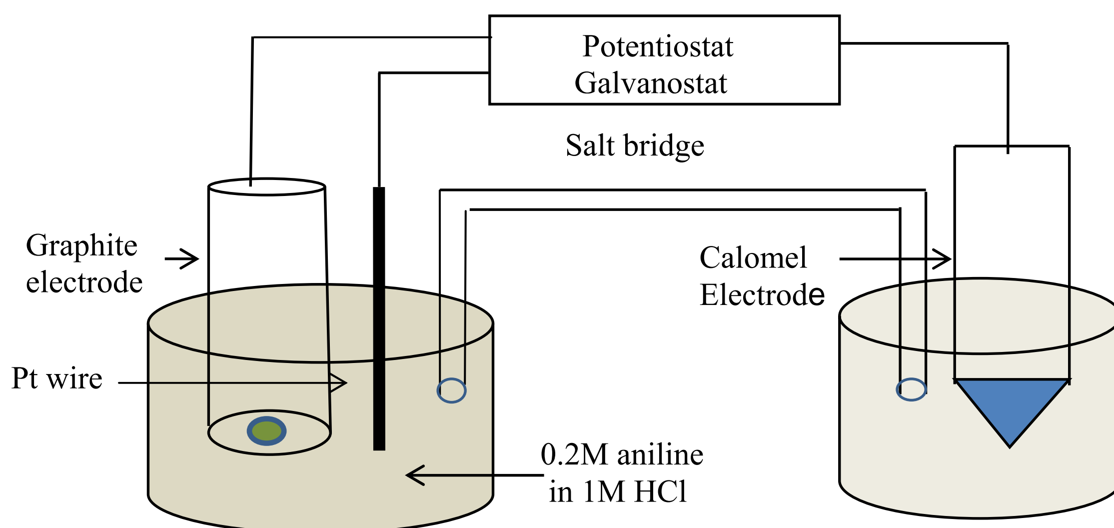


Fig: 2.1 A Schematic diagram of the electropolymerisation of PANi onto the graphite surface.

Fig: 2.2 Photograph of the electropolymerisation of PANi onto the graphite surface.

Out of three methods the best coating was obtained by cyclic voltammetric method. Thus the cyclic voltammetric method was selected. After the coating the electrode was washed with buffer solution after the coating process.

2.4 Study of the Response of PANi/Graphite Electrode to Hydrogen Peroxide

The PANi/Graphite electrode was immersed into phosphate buffer (0.1M) at different pH containing 0.1M potassium nitrate as supporting electrolyte. In an amperometry experiment, current following through the PANi/Graphite electrode at -0.40V *vs* calomel as reference and platinum wire as counter was recorded and a steady background current was obtained. After then hydrogen peroxide solution was added with the help of micropipette and current was measured as a function of concentration hydrogen peroxide. The electrolyte was stirred uniformly by a PTFE covered magnetic bar during the experiment. A concentration range from 1mM to 25mM was studied. Subsequent to each addition of H_2O_2 the electrodes showed rapid response in increasing the current. Then the cathodic current values (Δi = ib - ia) were plotted against the hydrogen peroxide concentration.

Where Δ_i= difference of current
i_b = total Current
ia = steady background current

2.5 Immobilization of Glucose Oxidase Enzyme on the PANi/Graphite Surface.

The PANi/Graphite electrode was placed into 0.1 M phosphate buffer (pH 6.0) containing 200U/mL glucose oxidase and 0.1 M potassium nitrate as supporting electrolyte. Afterwards, the film was oxidized at the potential of 0.75V against calomel as reference and platinum wire as counter for 40 minutes and GOx was potentiostatically attached. Again, the freshly prepared GOx/PANi/Graphite electrode was carefully rinsed with distilled water to remove non-attached enzyme.

2.6 Amperometric Biosensor Response Measurements Towards Glucose

Amperometric response studies were carried out in phosphate buffer (0.1M, pH = 6.0) and KNO_3 (0.1M) as supporting electrolytes. Biosensor response was studied via the application of -0.4V *vs* calomel electrode and Pt-wire as counter. After the background current reached a stable value, glucose solution was added to the cell by using micropipette. The reduction current was measured by using hydrodynamic amperometric mode.

CHAPTER 3

3. RESULTS AND DISCUSSION

3.1 Cyclic Voltammetry of PANi/Graphite Surface

Typical cyclic voltammogram of PANi/Graphite surface is shown in Fig: 3.1. Three subsequent responses with the parent peaks can be observed in the potential range studied, indicating the existence of the respective redox couples. According to the previously reported mechanism behind this process, the first peak appears at a potential of +220 mV, representing the oxidation of leucoemeraldine into emeraldine. This corresponds to the oxidation from the fully reduced form into the partly oxidized polyaniline. An anodic peak occurring at about +800 mV then corresponds to the transformation of emeraldine into perningraniline -the fully oxidized form of polyaniline. Finally, a couple of small waves between the two evident peaks can be attributed either to p-benzoquinone or to some other degradation products.

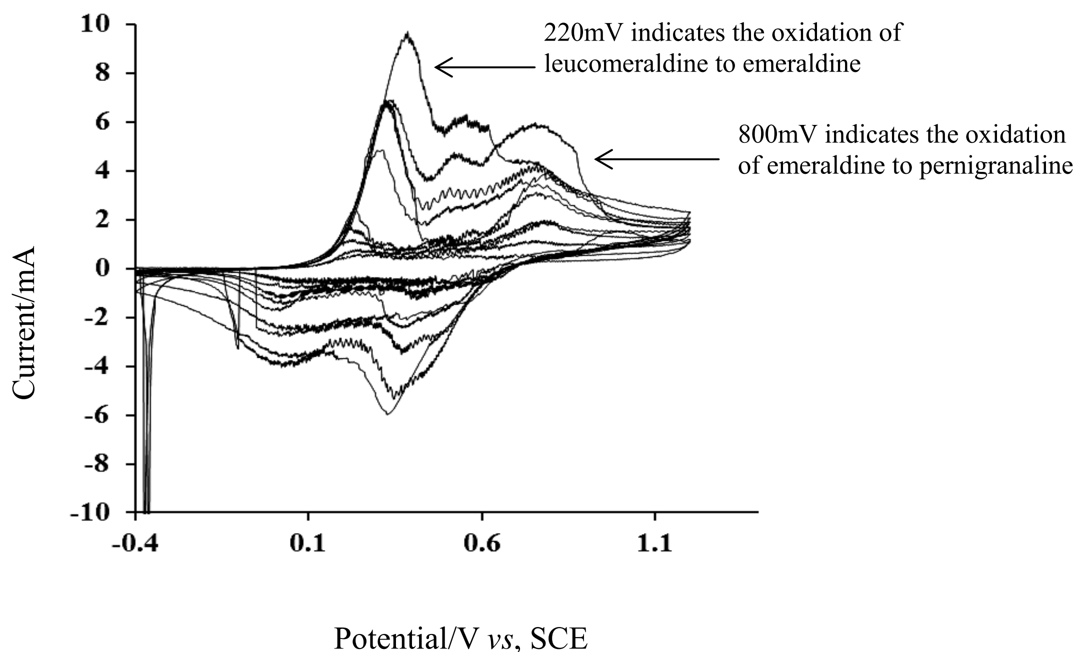


Fig: 3.1 Cyclic voltammogram of PANi /Graphite surface. Experimental conditions: 0.2M aniline monomer in 1M HCl. potential limits: -0.4V-1.2V, scan rate 100 mV S^{-1}, number of cycles, n = 10.

Fig: 3.2 Photograph of PANi /Graphite surface

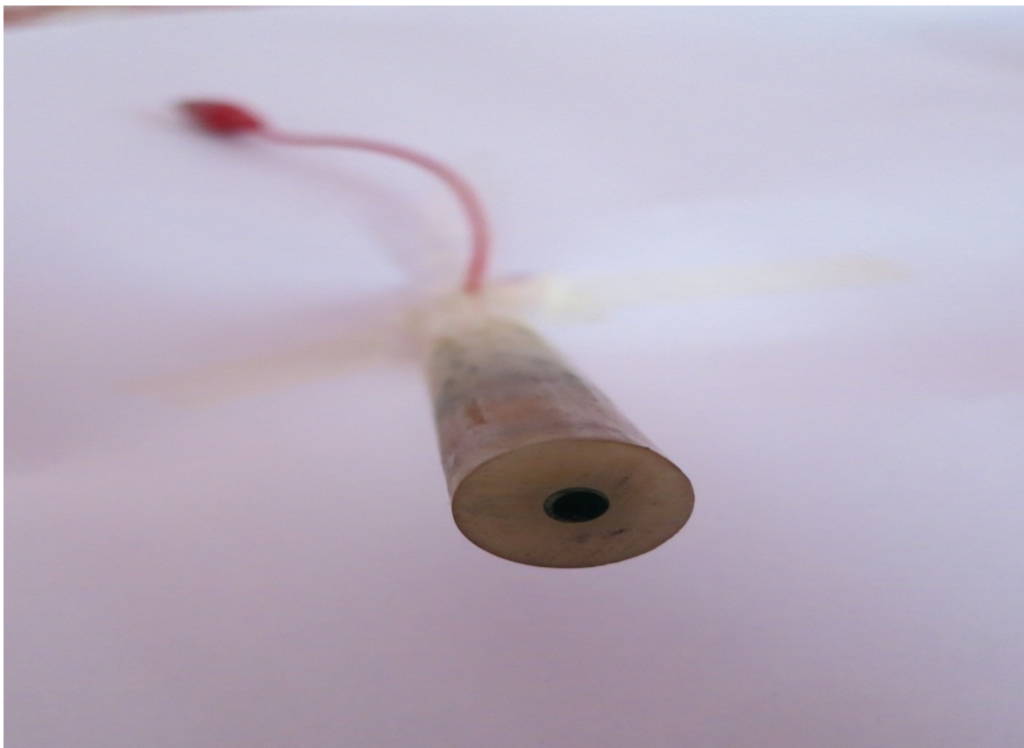


Fig: 3.3 Photograph of GOx/ PANi /Graphite surface.

The photograph of the PANi/Graphite and GOx/PANi/Graphite surface is shown in Fig. 3.2 and Fig. 3.3 respectively.

3.2 Response of PANi/Graphite Surface to Hydrogen Peroxide at Buffer Solution of Different pH

A graphical representation of the response current of the PANi/ Graphite electrode against H_2O_2 concentration at different pH is shown in Fig. 3.4. When the graph is examined it can be seen that good linear response has been obtained in the pH range of 5.0 -7.0. The optimum pH stability range for glucose oxidase enzyme is pH 5.5 to pH 7.0. The hydrogen peroxide can be reduced in slightly acidic medium. The pH 6.0 was selected for biosensor response measurement due to the better conductivity and stability of polyaniline for long time in acidic medium. In this pH enzyme glucose oxidase is stable and hydrogen peroxide can be reduced.

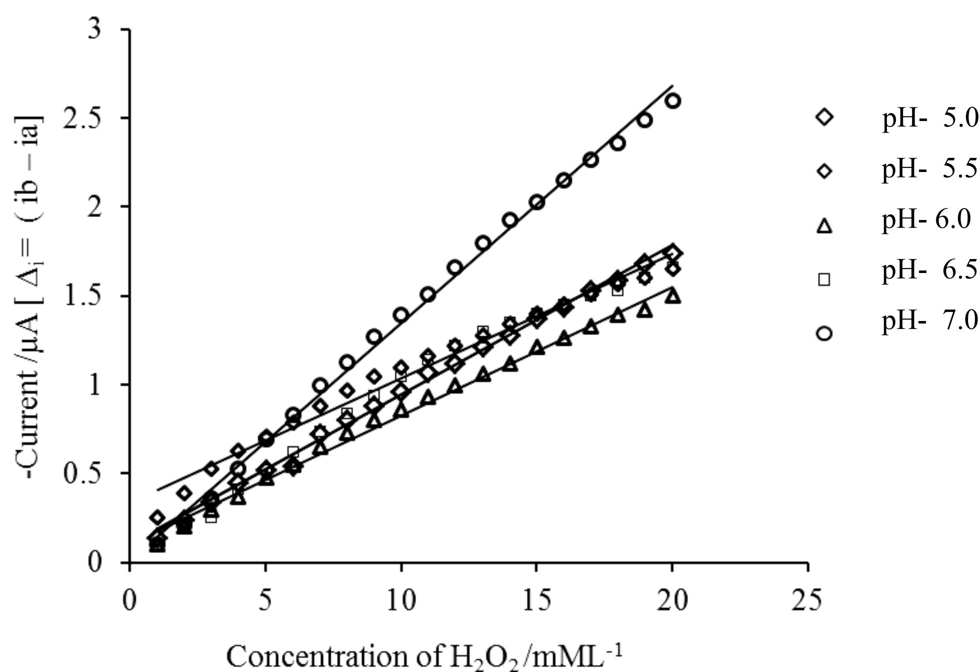


Fig: 3.4 Plot of reduction current *vs.* concentration of H_2O_2 at different pH

3.3 Response of PANi/Graphite Surface to Hydrogen Peroxide at Different Reduction Potential

The response of PANi/Graphite electrode with hydrogen peroxide was studied at -0.3V, -0.4V, -0.5V reduction potential. A graphical representation

of the response current of the PANi/Graphite electrode against H_2O_2 concentration at different reduction potential has shown in Fig. 3.5. From the graph good linearity of response current has been seen as the reduction potential increases. But the biosensor response was measured at -0.4V to reduce the interferences and better stability of enzyme. At higher reduction potential various interfering species may reduce and biosensor becomes less sensitive.

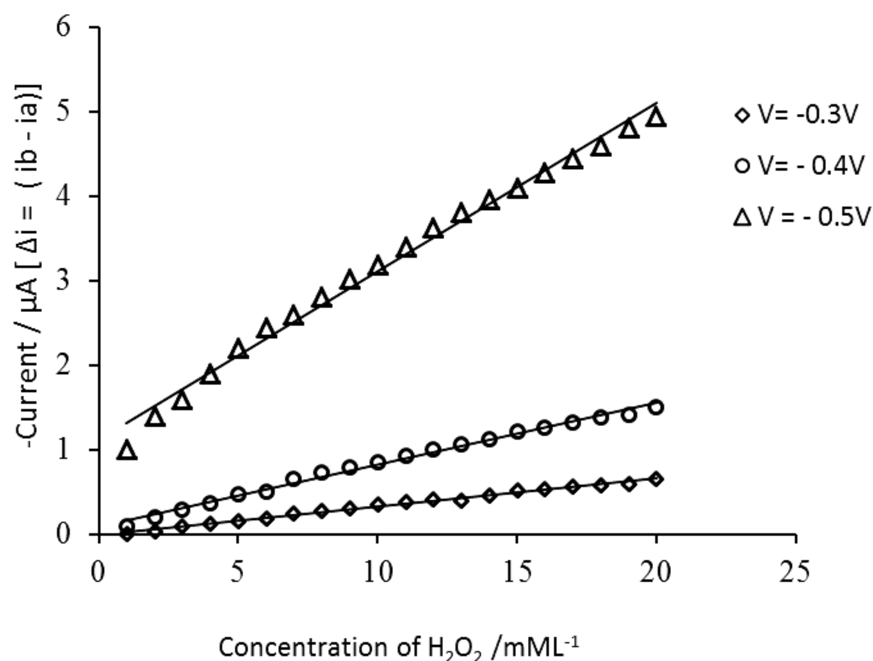


Fig: 3.5 Plot of reduction current *vs.* concentration of H_2O_2 at different reduction potential

5.5 Study of the Response of Biosensor

The glucose biosensor was responsive towards glucose solution. First the response of biosensor towards glucose solution with time was studied. It was found that initially current response increased gradually and a steady current was obtained after 4 minutes as shown in Fig: 3.6.The graphical representation of response of glucose biosensor against glucose concentration has been shown in the Fig: 3.7. A linear response was obtained within the concentration range 0.01M L^- to 0.1 M L^-. After the range of 0.1M the current response was started to deviate from linearity remaining constant due to saturation of electrode surface.

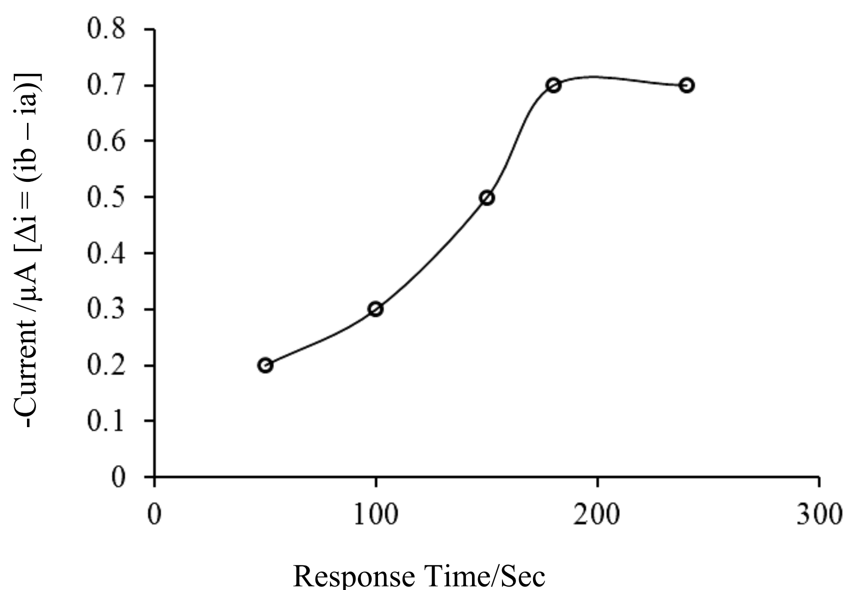


Fig: 3.6 Plot of the response of biosensor with time

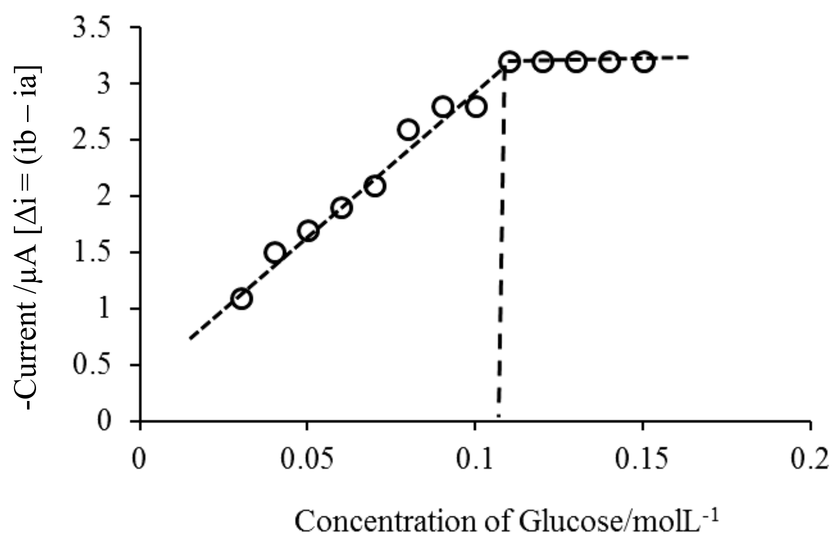


Fig: 3.7 Plot of reduction current *vs* concentration of glucose

When the glucose solution was added it was oxidized by glucose oxidase enzyme (GOx) of GOx/PANi/Graphite electrode into the gluconic acid and hydrogen peroxide. This hydrogen peroxide detected and due to which cathodic response was obtained. Thus by correlating with this response current, concentration of glucose can be analyzed.

4. CONCLUSIONS

This study is aimed at preparing amperometric glucose biosensor. The polyaniline was electropolymerised by cyclic voltammetry onto the graphite surface in acidic medium. The glucose oxidase enzyme (GOx) was successfully immobilized onto PANi/Graphite film by applying the potential of 0.75V *vs.* calomel electrode as reference and Pt wire as counter. Apparently one of the most interesting features of the graphite-biosensor developed is the dual function of the PANi film, providing suitable conditions for the effective, one-step enzyme immobilization (i.e., without use of a cross-linking agent) and, at the same, acting as a mediator. The PANi/Graphite film gave the linear response to hydrogen peroxide between the studied concentration range of 1mM to 25 mM in the pH range of 5.5- 7.0 at -0.4V reduction potential. Film gave the good linear response at different reduction potential between -0.30V to -0.50V in the phosphate buffer of pH 6.0. But the best result was obtained at reduction potential of -0.4V and in the buffer solution of pH 6.0. Thus the reduction potential of -0.4V and phosphate buffer of pH 6.0 were selected for the glucose biosensor response measurements. The actual configuration of the GOx/PANi/Graphite, tested herein in the hydrodynamic amperometric mode, has been subjected to the basic characterization and optimization of key experimental parameters in an effort to define typical operational conditions. Biosensor gave linear response to D- Glucose solution within the concentration range of $0.01ML^{-1}$-$0.1L^{-1}$ and a calibration curve was drawn. Thus by correlating with this curve, concentration of glucose can be analyzed within the specified range.

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