APPLICATION OF POLY (2-ETHYL ANILINE) IN UREA BIOSENSOR

A Major Project Submitted To The Faculty Of Technology,

University Of Delhi, Delhi

In

The Partial Fulfilment Of

The Requirements For The Award

Of The Degree Of

MASTER OF ENGINEERING

In

POLYMER TECHNOLOGY

By

VIKAS SINGH SHISHODIA

DEPARTMENT OF APPLIED CHEMISTRY

AND POLYMER TECHNOLOGY

DELHI COLLEGE OF ENGINEERING

DELHI-110042

CERTIFICATE

This is to certify that **VIKAS SINGH SHISHODIA (Roll No. 08/poly/03)**, a student of **Master of Engineering (Polymer (Technology)** in the Department of Applied Chemistry and Polymer Technology, Delhi College of Engineering Delhi, has individually completed a project entitled **"Application** of Poly (2-Ethyl Aniline) In Urea Biosensor**"** under the joint supervision of **Dr. D. Kumar,** Assistant Professor, Department of Applied Chemistry and Polymer Technology, Delhi College of Engineering, Delhi and **Dr. B. D. Malhotra,** Scientist-F, Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, Delhi, Dr. K.S. Krishnan Marg, New Delhi.

To the best of our knowledge and belief, this work has not been submitted to any other university or institution for the award of any degree or diploma.

The project work has been carried out from 13-10-04 to 30-06-05.

Dr. B.D. Malhotra
Scientist-in-Charge,
BECPRG,
National Physical Laboratory,
Dr. K.S. Krishnan Marg,
New Delhi-110012

Dr. D. Kumar
Assistant Professor
Department of Applied Chemistry,
Delhi College of Engineering,
Delhi-110042

Prof.G.L.Verma
Head,
Department of Applied Chemistry,
Delhi College of Engineering,
Delhi-110042

ACKNOWLEDGEMENT

I am grateful to my individuals to many who made their time and knowledge available to help to work on **"**Application of Poly (2-Ethyl Aniline) In Urea Biosensor**".** The matter in this project has not been submitted anywhere else for the award of any other degree/diploma, to the best of my knowledge. Firstly my most sincere regards to my guide, **Dr. D. Kumar**, Assistant Professor, Department of Applied Chemistry and Polymer Technology, Delhi College of Engineering, University of Delhi, Delhi, for his kind discussion, support, co-operation and guidance. I, sincerely express deep sense of gratitude to my co-guide **Dr. B.D. Malhotra** "Scientist-in-charge" Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, New Delhi, for accepting me to work under his guidance and supervision. I am highly indebted to Dr. B.D. Malhotra for his useful discussion and constant encouragement during the course of my project work. I am also thankful to **Dr. G.L.Verma**, Head, Department of Applied Chemistry and Polymer Technology, Delhi College of Engineering, Delhi for giving me permission to do my project work at NPL, New Delhi. I am also thankful to **Dr. Vikram Kumar**, Director, NPL, New Delhi for extending the laboratory facilities during my project tenure.I am also thankful to Dr. S.P. Singh, Dr.Pratima, Dr. R.P.Singh, Mr. M.K.Pandey, Mr. Sunil, and rest of the member of the lab for their kind help, moral support and inspiration. I am also grateful to **Dr. S.K.Chakladhar**, Head HRD Group, NPL, who got me permission from the Director, NPL, to carry out my project work.

I express my gratitude to my parents and friends for their kind help and continuous encouragement during my project work.

VIKAS SINGH SHISHODIA

Table of Contents

Chapter 1

Conducting polymer

1.1 General Introduction

Polymers are long chain molecules that are composed of repeating units of monomers e.g., plastics, elastomers, and fibers. Polymers are considered to be insulators, recently electrically conducting polymers have been discovered. The requirement of a polymer to be electrically conductive is that it should consist of alternating single and double bonds, i.e., conjugated double bonds.

Conducting polymers are increasingly replacing natural and inorganic materials in application requiring excellent mechanical properties and light weight. The mechanical properties of polymers can be tailored to provide strong materials with high toughness and low resistance.

In early 1970s, a key discovery in the evolution of conductive polymers was the high conductivity of polysulfur nitride $(SN)_X$ (1), an inorganic polymer which becomes superconducting at about 0.3 K. This was the first polymeric material that was shown to have metallic properties. In the late 1970s many researchers directed their interests to organic conducting polymers because they believed that these materials probably could be processed using conventional plastic technology.

Polymers are insulators because the atoms in the polymer chain are covalently bonded. In the covalent bonded molecules of saturated carbon compounds, there is no scope of delocalization of valence electrons and consequently, either charge carriers nor path of their movements are available. Since in the conjugated molecule of carbon compounds, delocalization of electron may occur through the interaction of π- bonded electrons, such molecules may be conducting. Thus it was thought that a long chain conjugated molecule, such as polymer of acetylene, may prove to be conducting. In fact, it was proposed, purely

from theoretical consideration that properly substituted polyacetylene [PA] molecule would exhibit even superconducting behavior at room temperature (2).

In 1977, the high electrical conductivity of an organic polymer, doped polyacetylene was reported by H.Shirakawa in Japan, Alan G. MacDiarmid of University of Pennsylvania and A.G.Heeger, University of California, Santa Barbara (3). They discovered that partial oxidation with iodine or other reagents made polyacetylene films material with a metallic conductivity. They proved that plastics could indeed, under certain circumstances, be made to behave very much like a metal, for which H. Shirakawa, Alan G. MacDiarmid and A.G.Heeger were awarded the **Noble Prize** in chemistry for the year 2000.

The conductivity of doped polyacetylene as high as 10^3 S/cm was higher than that of any known polymer. Polyacetylene, because of its processing difficulty and rapid fall in conductivity when exposed to air, has limited commercial application. Iodine doped polyacetylene is not the only conducting polymer, several others have also been found to exhibit conductivity on doping. Ever since the discovery of highly conducting polyacetylene, a few new polymers have been added to the list of conducting polymers such as polypyrrole (4), polythiophene (5), polyparaphenylene (6), polyphenylene vinylene (7), polyaniline (8) etc.

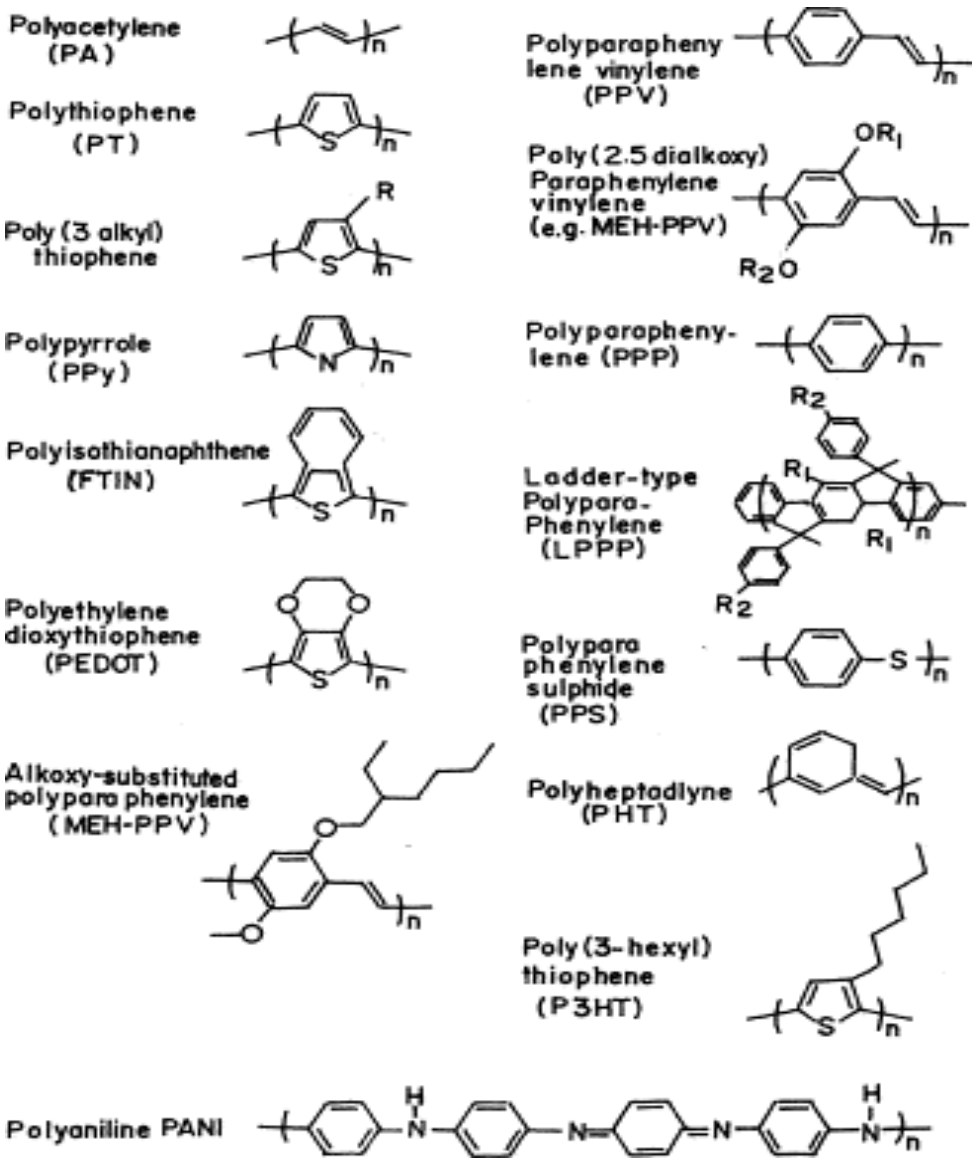


Fig. 1.1 Chemical structure of some conducting polymers

Figure 1.1 shows the chemical structure of some of the polymers that become conducting upon "doping" with an oxidizing or reducing agent.

The most interesting aspect is that the conductivity can be made to vary over a very wide range, starting from insulating to semiconductor and towards metallic, by varying the concentration of the dopant. Fig. 1.1 gives the comparison of the electrical conductivities of conducting polymers along with a few inorganic materials. Some of problems associated

with trans polyacetylene [PA] include its extreme sensitivity to oxygen and its solubility in common organic solvents. Tourillion and Garnier synthesized (5) poythiophene electrochemically. It can be doped with both p and n type dopants and the doping level up to 50% can be achieved. Such a high value of dopant has been associated with the partial crystalline nature of this conducting polymer. Conducting polyparaphenylene [PPP] can be synthesized electrochemically and its electrical conductivity can be increased by several orders of magnitude on suitable doping. This polymer has also been found to unstable in air and hence cannot be used for industrial application (6).

Polypyrrole [PPy] with high conductivity as high as 8 S/cm can be prepared as a dense conducting film by electro-chemical polymerization. A disadvantage is that it is not possible to produce conducting PPy with electron donating species. It is however, a good electrode material and a number of applications for PPy have been proposed (4). Such a conducting polymer though stable in air but not soluble in common organic solvent. A significant advantage over other doped polymers is the excellent stability of doped polyphenylene vinylene [PPV] under normal atmospheric conditions and at temperatures in excess of 673K.

Amongst the various conducting polymers polyaniline [PANI] has rapidly become the subject of considerable interest for physicists, chemists and material scientists. Polyaniline exists in several oxidation states with electrical conductivity varying from 1-10 S/cm to more than 10 S/cm. However, only one form, called the emeralidine salt, is electrically conducting. It can be easily synthesized by electrochemically or chemical oxidation of aniline in aqueous acidic media, using common oxidant, such as ammonium peroxydisulphate. Mechanically flexible, dark blue films of conductive polyaniline also has been achieved by protonic doping of emeralidine films cast from N-methylpyrrolidone [NMP] solutions. Protonic doping accomplished either by dipping of emeralidine films in acid or passing a gas, protonates the imine nitrogen atoms in the backbone of the polymer. The conductive emeralidine salt becomes the insulating emeralidine base when treated with aqueous alkali.

In the early 1980s, excitement ran high when several prototype devices based on conducting polymers, such as rechargeable batteries and current rectifying p-n junction diodes, were

announced. A number of predictions were made about the bright future of these new materials. One was that by the late 1980s, conductive polymers would find dozens of uses in electrical wires and polymer batteries powering devices from watches to automobiles. Perhaps this prediction would have been realized, had the early conducting polymers being process-able and their electronic structures better understood. But far from behaving as plastics, the first conducting polymers were insoluble, infusible and brittle, some polymers were even unstable in air.

It is essential to understand the conduction mechanism and charge carriers in these exciting "Conducting Plastics" and also their application in molecular/electronic devices. Though, these polymers are used in several applications, their process-ability and stability are the major problems which most of the polymer scientists are trying to overcome.

1.2 Conduction Mechanism &Electrical Behavior in Conducting Polymers

The conducting polymers are a class of organic semiconductors having a negative temperature coefficient of conductivity and hence the theory of conventional semiconductors was used to discuss the conduction mechanism. A key requirement for a polymer to become intrinsically conducting is that there should be overlap of molecular orbital to allow the formation of delocalized molecular wave functions. Besides this, molecular orbital must be partially filled, so that there is a free movement of electrons throughout the lattice (9). In band theory, the atomic orbital of each atom overlap with the same orbital of neighboring atoms in all directions to produce molecular orbital similar to those in small molecules. When these orbitals are spaced together in a given range of energies, they form what looks like continuous energy bands.

The electrical properties of conventional inorganic semi-conducting materials depend on the band structure. When the bands are partially filled or empty, no conduction occurs. If the band gap is narrow, at room temperature, thermal excitation of electrons from the valance bands to conduction gives rise to conductivity. This is what happens in classical

semiconductors. When the band gap is too wide, thermal excitation is insufficient to excite electrons across the gap and solid is an insulator. The high conductivity of metals is due to partially occupied bands, a partially filled conduction bands, a partially empty valence band or a zero band gap. In order to understand the behavior of conducting polymers, it is essential to know about the type of charge carriers and the band structure.

A molecular doped polymer represents a genuine molecular system wherein; the charge transport is carried out by the hopping mechanism between the dopant molecules, which acts like hopping site (10). This mechanism can be visualized using the two centers model (11) where the carriers hop from one centers to other. Different factors such as temperature (T), separation between centers (R), distribution of hopping energy and electric field (F) would govern this type of charge transport by increasing the hopping probability. By considering these factors one can obtain the corresponding conduction parameters.

1.3 Charge Carriers in Conducting Polymers

Conducting polymers are peculiar in that they conduct current without having a partially filled or empty band. There electrical conductivity cannot be explained well by simple band theory. For example, simple band theory cannot be used to explain why the charge carriers, usually electrons or holes, in polyacetylene and polypyrrole are spin less.
The electronic phenomena in these electronic polymers cannot be completely explained by using the theory of conventional inorganic semiconductors.

The mechanism of conduction and behavior of charge carriers in the conducting polymers have been explained using the concept of solitons, polarons, and bipolarons. A radical cation that is partially delocalized over some polymer segment is called a **polaron.** It stabilizes itself by polarizing the medium around it. It is really a radical cation and has a spin of ½.
When an electron is removed from the top of the valency band of a conjugated polymer, a vacancy (hole or radical cation) is created that does not delocalize completely, as would be expected from classical band theory. Only partially delocalization occurs, extending over several monomeric units and causing them to deform structurally. The energy level

associated with this radical cation represents a destabilized bonding orbital and thus has a higher energy than the energy in the valency band. This rise in the energy is similar to rise in energy that takes place after an electron is removed from a filled bonding molecular orbital.

If another electron now is removed from the already oxidized polymer containing the polaron, two things can happen. This electron could come from either a different segment of the polymer chain, thus creating another independent polaron level, or from the first polaron level (remove the unpaired electron) to create a special dication, which is called a **bipolaron.** Low doping levels give rise to polarons, where higher doping levels produce bipolarons. Compared to polaron, bipolaron is doubly charged but spinless.

The bipolaron also has structural deformation associated with it. The two positive charge of the bipolaron are not independent, but acts as a pair, much like the copper pair in the Bardeen-Copper-Schrieffer (BCS) theory of superconductivity. Both polarons and bipolarons are mobile and can move along the polymer chain by the rearrangement of double and single bonds in the conjugated system that occurs in an electric field. If many bipolarons are formed, say as a result of high doping, their energies can start overlapping at the edges, which creates narrow bipolaron bands in the band gap.

In polyacetylene, which has a degenerate ground state (two equivalent resonance forms), the bipolarons dissociate into independent cations, which are spin less and are called solitons. Solitons do not form in polymers with non-degenerate ground state, such as polypyrrole, polythiophene and polyphenylene. These polymers are called nondegenerate because their resonance forms are not identical if they are superimposed. Doping with a suitable dopant can increase the concentration of charge solitons.

Table 1: Properties of solitons, polarons and bipolarons

Defect	Spin	Charge
Soliton	½ 0	0 +e or -e
Polaron	½	+e or -e
Bipolaron	0	+2e or -2e

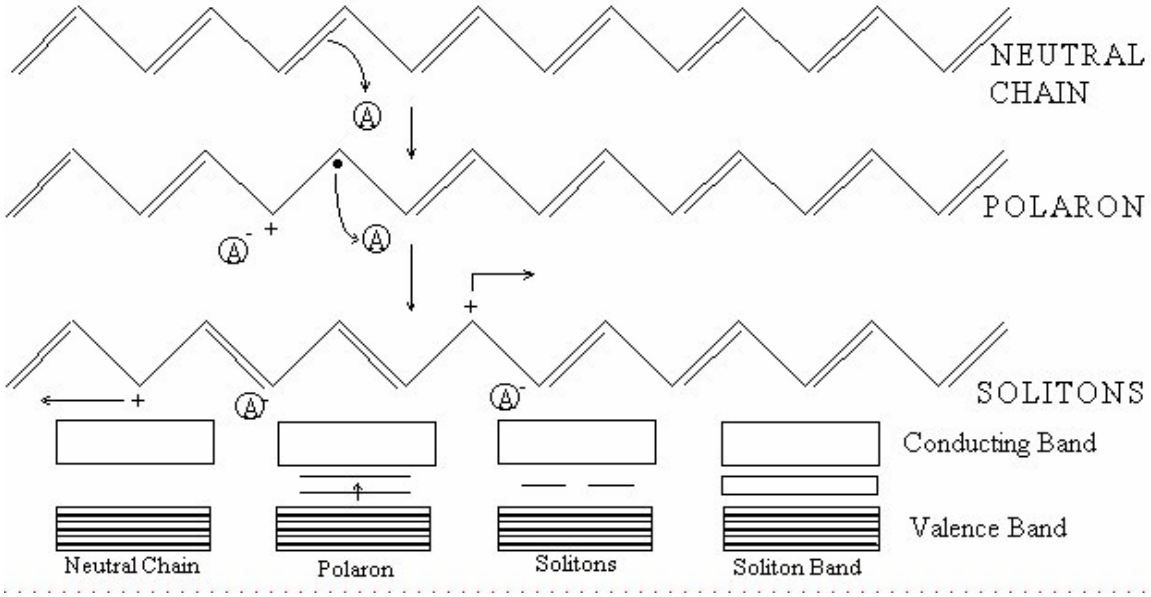


Fig. 1.2 Band structure of polaron, bipolaron and soliton in polyacetylene

1.4 Band gap in conducting polymers

The energy spacing in between the highest occupied and lowest unoccupied bands is called the band gap. The highest occupied band is called the valence band, and the lowest unoccupied band is called the conduction band. Conducting polymers, scientist envisioned, might be a class of these polymers that would have a zero energy band gap or a very low band gap.

The optical band gap controls the electronic and optical properties of conducting polymers. Attempts have, therefore, been made to reduce band gap in conducting polymers by various technique.

The band gaps of many of well-suited conducting polymers are greater than 2.0 eV. Thus, for example, that of poly (p-phenylene) is 2.7 eV (12-13), that of poly (p-phenylene vinylene) is 2.4 eV. (14-15), that of polythiophene is 2.0-2.1 eV (16-17), and that of polypyrrole is 3.2 eV (18). Polyacetylene has the lowest band gap of about 1.5 eV (19) to 1.7 eV (20).

1.5 Synthesis of conducting polymers

There are several methods known for the synthesis of conducting polymers, however, the most widely used technique is the oxidative coupling involving the oxidation of monomers to form a cation radical followed by coupling to di-cations and the repetition leads to the polymer. Electro-chemical synthesis is also rapidly becoming the preferred general method for preparing electrically conducting polymers because of its simplicity and reproducibility. The advantage of electro-chemical polymerization is that the reaction can be carried out at room temperature. By varying either the potential or current with time, the thickness of film can be controlled. For conducting polymers intractability is still a problem. The following are the preliminary details relating the method used for synthesis of conducting polymers.

1.5.1 Electrochemical polymerization

Polymers such as polypyrrole, polyaniline, polythiophene, are prepared by electro-chemical polymerization. In this method appropriate potential is applied to working electrode soaked in aqueous electrolytic solution containing the monomer. This method enables exact control of thickness by passing suitable electrical charge. Electro-chemical polymerization also helps in doping to enhance usefulness and also produces freestanding films. It is cost effective because film can be deposited over small area of electrode.

Electro-chemical polymerization of conducting polymers is generally employed by: (1) constant current or galvanstatic; (2) constant potential or potentiostatic; (3) potential scanning/cycling or sweeping methods. Standard electro-chemical technique which employs a divided cell containing a working electrode, a counter electrode and a reference electrode generally produces the best films. The commonly used anodes are chromium, gold, nickel, palladium, titanium, platinum and indium-tin oxide coated glass plates. Semi-conducting materials such as n-doped silicon (21) gallium arsenide (22) cadmium sulphide and semi-metal graphite (23) are also used for the growth of polymer films.

1.5.2 Chemical polymerization

Chemical polymerization is the most useful method for preparing large amounts of conducting polymers, since it is performed without electrodes. Chemical polymerization (oxidative coupling) is followed by the oxidation of the monomers to cation radical and their coupling to form dications and the repetition of this process generates a polymer. All the classes of conjugated polymers may be synthesized by this technique.

1.5.3 Chain Growth polymerization

Natta was the first to prepare polymer by using chain polymerization method. He carried out extensive investigation into the direct polymerization of acetylene, and reported that bubbling of acetylene gas through a solution of a typical Ziegler catalyst in hydrocarbon solvent resulted in the preparation of trans-polyacetylene as semi-conductor red powder. Poly-acetylene is prepared by the passage of pure, dry acetylene gas over $TiCl_4$/ $Al(C_2H_5)_3$ catalyst solution in toluene at 195 K. The polymer film is obtained on the surface of the catalyst solution. The film obtained was 98% cis-polyacetylene.

Conducting polymers can be synthesized by usual chemical polymerization method like chain growth method or step growth methods. For chain growth methods catalysts like Ziegler – Natta are frequently used. Synthesis of conducting polymers by anionic chain growth process the carbon disulfide has been used. Earlier these polymers were synthesized

by heating the monomer at moderately high temperatures. Some example of the conducting polymers, which have been synthesized by chain polymerization methods using iron, copper chloride, as catalysts include polypyrrole, and polythiophene etc. These conducting polymers are obtained in the form of powder.

1.5.4 Step polymerization

Step polymerization involves condensation between two reactive groups of different molecules. For instance polyphenylene is prepared by step polymerization method.
Polyphenylene sulfide is prepared by polycondensation of para-dichlorobenzene with sodium sulfide. Similarly polyvinylene sulfide and polythiophene sulfide have been prepared by polycondensation between appropriate dichloro compounds and anhydrous sodium sulfide. Photochemical and other methods are also used for film development, however these methods are not frequently used.

The step growth polymerization proceeds usually through the condensation reaction bifunctional monomers to give a linear polymer. This polymerization is used to synthesize a polymer containing heteroatom in the backbone. Polymerization reaction is carried out either at 413K or at 19 atm.

1.6 Doping Techniques

Doping of polymers may be carried out by the following methods.
(a) Gaseous doping
(b) Solution doping
(c) Electrochemical doping
(d) Self doping, and
(e) Radiation induced doping

Of these, first three methods are widely used because of convenience and low cost. In the gaseous doping, polymers are exposed to the vapours of dopants under vacuum (24). The

level of dopant concentration in the polymers may be easily controlled by the temperature, vacuum and the time of exposure. Solution doping involves the use of solvent (Toluene, Acetonitrile, Tetrahydrofuran, Nitro-methane etc.) in which all the products of doping are soluble.

In electrochemical doping only ionic type dopants are used as the electrolyte in polar solvents such as nitro-methane, acetonitrile, dichloromethane, tetrahydrofuran etc. Self-doping does not require any external doping agent. In the polymer chain the ioniozable group, for example, sulphonate group of poly [3(2-ethane sulphonate) thiophene] acts as a dopants for the polymer (25).

In radiation induced doping, high-energy radiation such as gamma rays, electron beam and neutron radiation are used for the polymers. For example gamma rays irradiation in the presence of SF_6 gas (26) and neutron diffraction in the presence of I_2 (27) has been used for doping in polythiophene:

1.7 Stability of conducting Polymers

1.7.1 Thermal stability

Thermal stability of most of the conducting polymers is poor, except the heterocyclic polymers. The rate of thermal degradation is very much dependent on the environment. Polyacetylene, for example, degrades at room temperature in air or oxygen atmosphere, but in helium, its degradation starts at 320ºC (28). Similarly, polythiophene and its derivatives are stable up to 200º to 250ºC in air, but do not decompose up to the 700ºC in inert atmosphere (29). Nature of dopants also affects the thermal stability of polymers. Polypyridine, for example, doped with arylsulphonate is stable up to 80ºC in humid environment, but when doped with BF_4 it retains its stability upto 150ºC (30). Incorporation of heteroatoms having non bonding electron pairs in the conjugated chain structure increase the thermal stability of the polymers.

A few papers report the thermal stability of polypyrrole and polythiophene (31-34). Very few studies have been found in the literature on the aging of conducting polymers. In particular, Mohammad et al (35) have been made some interesting observations on the decay of conductivity in ambient and dry conditions for polyacetyle, polypyrrole and polythiophene at 353 K and 448 K.

1.7.2 Environmental stability

Conducting polymers, on doping become unstable in environmental conditions. The rate of reduction in conductivity of iodine-doped polyacetylene under room temperature is higher than that of its virgin state (36). However, in dry oxygen iodine doped polyacetylene loss its conductivity more slowly than its undoped state. Polyphenylene sulphide (PPS), which is stable in virgin state, is unstable in the presence of moisture in doped state. But in dry air or oxygen, doped PPS is stable. Polyparaphenylene is stable in air in the presence of moisture but when doped with oxidizing dopants, it is unstable in moisture. But polythiophene and poly (3-methyl thiophene) are stable both in doped and undoped state in the presence of moisture or air and oxygen. The stability of these polymers is attributed to the delocalization of electrons in the chain through participation of the non-bonding pair of electrons of the sulphur atom. This principle of stabilization of conducting polymers has also received support from polyethynyl sulphide and poly (phenylphosphoethynediyl). These two polymers do not show any reduction in conductivity even after a year of storage in normal environment.

1.8 Factors affecting the conductivity of conducting polymers

Various factors affecting the conductivity of polymers are.

1.8.1 Conjugation length

One of the structural features common to all the conducting polymers is the presence of conjugation. It has been found that it is the conjugation length of the polymer chain and not its chain length which is important for its electrical conductivity.The conjugation length of a polymer chain is the average distance between two defects, which interrupt the conjugation. Evidently, chain ends are such interruption but there are also other such as O═ C─CH_2.The effect of conjugation breaking defects on the conductivity is very drastic. Experimentally, it has been found that the conductivity decreases rapidly with decreasing conjugation length. If for example, the conjugation length is decreased from its pristine value (100A°) to about 10A° in the case of Trans –PA chains, the conductivity decreases by eight orders of magnitude.

1.8.2. Temperature

In order to understand the effect of temperature on the conductivity polymers, it is reasonable, first of all, to define two conductivity regimes:The high conductivity with conductivity 1000 S $cm^{-1}$and the modest and low conductivity regime with conductivity 100 Scm^{-1}. Majority of conducting polymers belong to the moderate and low conductivity regime. The temperature difference is different in the two conductivity is different in the two conductivity regimes. In both the conductivity regimes, the conductivity decreases when the temperature is lowered implying hereby that the temperature coefficient for a synthetic metal is different from that of metal in which the conductivity increases on cooling the metal. In moderate and low conductivity system, the conductivity vanishes as the temperature approaches to zero whereas in high conductivity systems, the conductivity remains finite.

The conductivity dependence of the conductivity also varies with the level of doping. For low-doped PA samples, for example, the temperature dependence of the conductivity is very drastic. As the doping level increases, the dependence of conductivity on doping become less and less and for most highly doped samples, the conductivity is found to be nearly temperature dependent.

1.8.3 Doping level

The conductivity of the conjugated polymers is, in general found to increase with increase in the doping level of the polymers. The doping level of the polymer is determined by the dopant concentration expressed in mol%.

The enhancement of electrical conductivity primarily depends on the chemical reactivity of the dopant with the polymer. The same dopant cannot be effective for the different polymers. Iodine for example, enhances the conductivity of polyacetylene by 10-12 orders of magnitude but it fails to dope polyphenylene sulfide or polyparaphenylene because of its weak oxidizing ability

1.9 Conducting polymers to biosensors

Conducting polymers have much attracted interest as a suitable matrix of enzymes (37-38). Conducting polymers are used to enhance speed, sensitivity, and versatility of biosensors in diagnostics to measure vital analytes. Conducting polymers are thus finding ever increasing used in diagnostic medical reagents (39). The techniques of incorporating enzymes into electro-depositable conducting polymeric films permits the localization of biologically active molecules on electrodes of any size or geometry and is particularly appropriate for the fabrication of multi-analyte micro-amperometric biosensors (40). Electrically conducting polymers have considerable flexibility in the available chemical structure, which can be modified as required. By chemical modeling and synthesis, it is possible to modulate the

required electronic and mechanical properties. Moreover, the polymer itself can be modified to bind protein molecules (41-43). Another advantage offered by conducting polymers is that the electrochemical synthesis allows direct deposition of the polymer on the electrode surface while simultaneously trapping the protein molecules (44-45). It is thus possible to control the spatial distribution of the immobilized enzymes, the film thickness and modulate the enzyme activity by changing the state of the polymer. The development of any kind of technology in this field heavily depends on the understanding of the interaction at the molecular level, between the biologically active protein, either as a simple composite or through chemical grafting. For the proper relay of electrons from the surface of the electrode to the enzyme active site, the concept of 'electric wiring' has been reported (46-47). Conducting polymers are likely to provide a 3-dimensionsal electrically conducting structure for this purpose.

Conducting polymers are also known to be compatible with biological molecules in neutral aqueous solutions. They can be reversibly doped and undoped electrochemically accompanied by significant changes in conductivity and spectroscopic properties of the films that can be used as a signal for biochemical reaction (48-49). The electronic conductivities of conducting polymer changes over several orders of magnitude in response to change in pH and redox potential or their environment (50).

Conducting polymers have the ability to efficiently transfer electric charge produced by the biochemical reaction to electronic circuit (51). Moreover conducting polymers can be deposited over defined areas of electrode. This unique property of conducting polymers along with the possibility to entrap enzymes during electrochemical polymerization has been exploited for the fabrication of amperometric biosensors (52-57). Besides this conducting polymers exhibit exchange and size exclusion properties due to which they are highly sensitive and specific towards substrate (58-61). Numerous papers published reveal that electro-deposition of conducting polymers serve as good matrices for the immobilization of enzymes. They provide good detect ability and fast response as the redox reaction of the substrate, catalyzed by an appropriate enzyme, takes place in the bulk of the polymer layer.

1.10 Advantages of Conjugated molecules

1. Easy deposition, e.g., with spin coating or ink jet printing like process
2. Handling under ambient conditions, possible-nitrogen atmosphere is preferable though
3. Relatively cheap large scale production
4. Electronic tenability
5. High absorption coefficients in comparison to inorganic semiconductors
6. Polymers can be mixed easily when they dissolve in the same solvent, or can be separated in phases equally simple if they use incompatible solvents.
7. Many mechanical and chemical characteristics (e.g., solubility and strain, cross linking properties) can be fine tuned by adding and removing side groups.

Chapter 2
Biosensors

2.1 Introduction

A biosensor is an analytical device, which converts a biological response into an electrical signal (Figure 2.1). The term 'biosensor' is often used to cover sensor devices used in order to determine the concentration of substances and other parameters of biological interest even where they do not utilize a biological system directly. This very broad definition is used by some scientific journals (e.g., Biosensors, Elsevier Applied Science) but will not be applied to the coverage here. The enzymes as the biologically responsive material, but it should be recognized that other biological systems may be utilized by biosensors, for example, whole cell metabolism, ligand binding and the antibody-antigen reaction. Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate, the major impetus coming from the health-care industry (e.g., 6% of the western world are diabetic and would benefit from the availability of a rapid, accurate and simple biosensor for glucose) but with some pressure from other areas, such as food quality appraisal and environmental monitoring. The estimated world analytical market is about £12,000,000,000 $year^{-1}$ of which 30% is in the health care area. There is clearly a vast market expansion potential as less than 0.1% of this market is currently using biosensors. Research and development in this field is wide and multidisciplinary, spanning biochemistry, bioreactor science, physical chemistry, electrochemistry, electronics and software engineering. Most of this current endeavour concerns potentiometric and amperometric biosensors and colorimetric paper enzyme strips. However, all the main transducer types are likely to be thoroughly examined, for use in biosensors, over the next few years.

A successful biosensor must possess at least some of the following beneficial features.

1.The biocatalyst must be highly specific for the purpose of the analyses, be stable under normal storage conditions and, except in the case of colorimetric enzyme strips and dipsticks, show good stability over a large number of assays (i.e., much greater than 100).

2. The reaction should be as independent of such physical parameters as stirring, pH and temperature as is manageable. This would allow the analysis of samples with minimal pre-treatment. If the reaction involves cofactors or coenzymes these should, preferably, also be co-immobilized with the enzyme.

3. The response should be accurate, precise, reproducible and linear over the useful analytical range, without dilution or concentration. It should also be free from electrical noise.

4. If the biosensor is to be used for invasive monitoring in clinical situations, the probe must be tiny and biocompatible, having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case, the biosensor should not be prone to fouling or proteolysis.

5. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.

6. There should be a market for the biosensor. There is clearly little purpose developing a biosensor if other factors (e.g., government subsidies, the continued employment of skilled analysts, or poor customer perception) encourage the use of traditional methods and discourage the decentralisation of laboratory testing.

The biological response of the biosensor is determined by the biocatalyst membrane, which accomplishes the conversion of reactant to product. Immobilized enzymes possess a number of advantageous features, which makes them particularly applicable for use in such systems. They may be re-used, which ensures that the same catalytic activity is present for a series of analyses. This is an important factor in securing reproducible results and avoids the pitfalls

associated with the replicate pipetting of free enzyme otherwise necessary in analytical protocols. Many enzymes are intrinsically stabilized by the immobilization process but even where this does not occur there is usually considerable apparent stabilization. It is normal to use an excess of the enzyme within the immobilized sensor system. This gives a catalytic redundancy, which is sufficient to ensure an increase in the apparent stabilization of the immobilized enzyme. Even where there is some inactivation of the immobilized enzyme over a period of time, this inactivation is usually steady and predictable. Any activity decay is easily incorporated into an analytical scheme by regularly interpolating standards between the analyses of unknown samples. For these reasons, many such immobilized enzyme systems are re-usable up to 10,000 times over a period of several months. Clearly, this results in a considerable saving in terms of the enzymes' cost relative to the analytical usage of free soluble enzymes.

When the reaction, occurring at the immobilized enzyme membrane of a biosensor, is limited by the rate of external diffusion, the reaction process will possess a number of valuable analytical assets. It follows that the biocatalyst gives a proportional change in reaction rate in response to the reactant (substrate) concentration over a substantial linear range, several times the intrinsic K_m. This is very useful as analyte concentrations are often approximately equal to the K_ms of their appropriate enzymes which is roughly 10 times more concentrated than can be normally determined, without dilution, by use of the free enzyme in solution. The independence of the reaction rate with respect to pH, ionic strength, temperature and inhibitors. This simply avoids the tricky problems often encountered due to the variability of real analytical samples (e.g., fermentation broth, blood and urine) and external conditions. Control of biosensor response by the external diffusion of the analyte can be encouraged by the use of permeable membranes between the enzyme and the bulk solution. The thickness of these can be varied with associated effects on the proportionality constant between the substrate concentration and the rate of reaction (i.e. increasing membrane thickness increases the unstirred layer which, in turn, decreases the proportionality constant, k_L, in equation. Even if total dependence on the external diffusion rate is not achieved (or achievable), any increase in the dependence of the reaction rate on external or internal diffusion will cause a

reduction in the dependence on the pH, ionic strength, temperature and inhibitor concentrations.

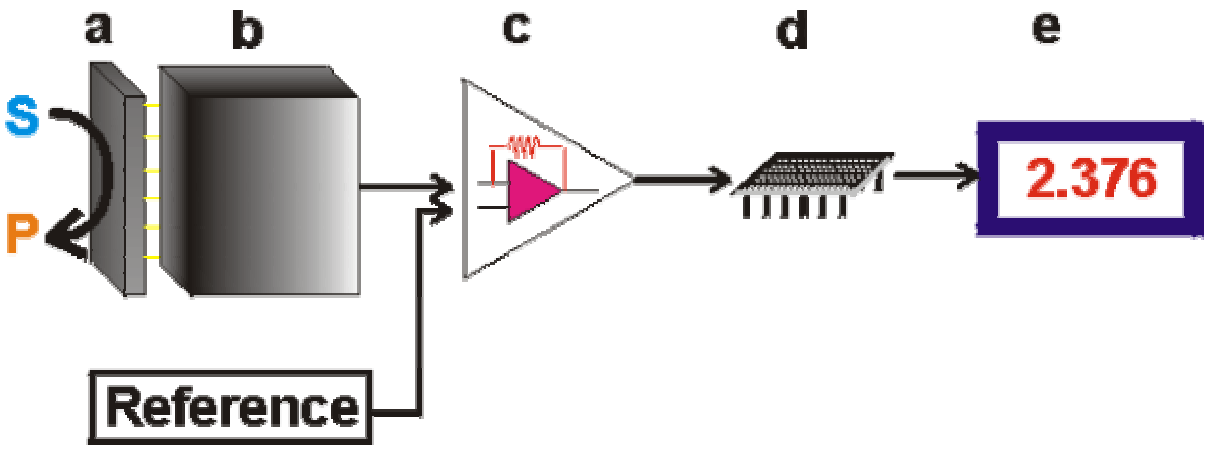


Fig. 2.1. Schematic diagram showing the main components of a biosensor. The biocatalyst (a) converts the substrate to product. This reaction is determined by the transducer (b) which converts it to an electrical signal. The output from the transducer is amplified (c), processed (d) and displayed (e).

The key part of a biosensor is the transducer (shown as the 'black box' in Figure 2.1) which makes use of a physical change accompanying the reaction. This may be

1. The heat output (or absorbed) by the reaction (calorimetric biosensors),

2. Changes in the distribution of charges causing an electrical potential to be produced (potentiometric biosensors),

3. Movement of electrons produced in a redox reaction (amperometric biosensors),

4. Light output during the reaction or a light absorbance difference between the reactants and products (optical biosensors), or

5. Effects due to the mass of the reactants or products (piezo-electric biosensors).

There are three so-called 'generations' of biosensors; First generation biosensors where the normal product of the reaction diffuses to the transducer and causes the electrical response, second generation biosensors which involve specific 'mediators' between the reaction and the transducer in order to generate improved response, and third generation biosensors where the reaction itself causes the response and no product or mediator diffusion is directly involved.

The electrical signal from the transducer is often low and superimposed upon a relatively high and noisy (i.e., containing a high frequency signal component of an apparently random nature, due to electrical interference or generated within the electronic components of the transducer) baseline. The signal processing normally involves subtracting a 'reference' baseline signal, derived from a similar transducer without any biocatalyst membrane, from the sample signal, amplifying the resultant signal difference and electronically filtering (smoothing) out the unwanted signal noise. The relatively slow nature of the biosensor response considerably eases the problem of electrical noise filtration. The analogue signal produced at this stage may be output directly but is usually converted to a digital signal and passed to a microprocessor stage where the data is processed, converted to concentration units and output to a display device or data store (62).

2.2 Immobilization of enzymes

Stable immobilization of macromolecular biomolecules on conducting micro surfaces with complete retention of their biological retention properties is a crucial problem for the commercial development of new generation biosensors. The commonly used techniques of immobilization are;

1. Adsorption on inert carriers
2. Cross linking by bifunctional reagents into the macroscopic particles
3. Physical entrapment in gel lattices
4. Covalent binding of water insoluble matrices
5. Micro encapsulation within the wall spheres
6. Electrochemical entrapments

All the methods can be broadly divided into two parts: attachment and entrapments. The entrapped enzymes are isolated from large molecules, which cannot diffuse into the matrix. The attached enzyme can be exposed to the molecules of all sizes. Thus different forms of kinetics are observed during the reaction. The different methods of enzyme immobilization are discussed in the following section.

2.2.1 Adsorption

This is the simplest method of enzyme immobilization and requires no reagent. This results less disruption to the enzyme as against the chemical methods. However it suffers from greater susceptibility to change in pH, temperature, ionic strength and substrate and requires considerable optimization. This method is not widely applicable because the bio-component may leach out from the electrode surface, but cross-linking can minimize this. Lifetime of the bio-component is very short compared to other methods of immobilization (63).
Physical adsorption of an enzyme onto an electrode is usually done by evaporation of a buffered solution containing the enzyme of interest. Temperature is maintained around $4^{o}C$, so the enzyme does not thermally degrade. Cross – linking can then follow the adsorption.

2.2.2 Covalent immobilization

This method provides a more stable immobilized bio-component than covalent attachment and is more widely applicable. The bio-component immobilized in a matrix by covalent bonding will not reverse by change in pH or ionic strength. The attachment most often involves three steps viz. activation of the support, enzyme coupling, and removal of loosely bound enzyme (64). Experimental conditions must be maintained with the help of the bio-component, nature of the matrix and coupling agent. Activation of matrix is usually accomplished with chemicals such as silane or cyanuric chloride (65).

The enzyme is then attached to the activated surface directly or is linked to a bifunctional agent, which has been attached to the surface. Conducting polymers bearing adequate

functional groups bind directly to bio-component of interest. The entrapment occurs in sequence, namely electro-polymerization and covalent bonding. The advantage of sequential steps are that polymerization of monomer can take place even when the condition which are deteriorating for enzymes but necessary for polymerization i.e., high potential values, organic solvents, and generate free radicals during reaction. Also covalent binding can be carried out in aqueous buffer solution containing additives and stabilizers that preserve the catalytic activity and/or the recognition properties of the biomolecule. Hernandez et al. (66) have developed a potentiometric biosensor of urea prepared by covalent attachment of urease to polypyrrole film electro generated groups.

2.2.3 Cross-linking Immobilization

Physical adsorption and electro-chemical entrapments are often supported by cross-linking to prevent leaching of bio-component. Cross-linking alone can also be used for immobilization on an electrode surface. Best use of cross-linking is for creation of enzyme membrane by employing bi-functional agents, which bind to the bio-component, and either the gel/polymer or the electrode. The parameters controlled during polymerization are pH, ionic strength, temperature and reaction time. Some bi-functional agents are glutraldehyde and bovine serum albumin. In them, cross-linking results in formation of a bigger molecule on the polymer surface (67).

2.2.4 Electrochemical Immobilization

Amongst the available immobilization methods, electro-chemical entrapment is mostly used. Electro-chemical oxidation of given monomer, containing aqueous solution of enzyme, is the simplest method of immobilization at the working electrode surface, and results in the formation of a conducting polymer layer containing entrapped enzyme molecules. This is a single step entrapment without affecting the activity of the enzymes. A small volume of bio-component is distributed uniformly with defined thickness of film over the defined areas on the electrodes. Conducting polymers provide closest proximity between active site of an enzyme and conductive surface of the electrode. A number of enzymes can be immobilized

simultaneously into single conducting polymer film. Electro-polymerization is most often done in aqueous solution of pH, close to neutral values using a potentiostatic or galvanstatic conditions (69-72). Thickness depends upon the amount of electric charge passed and the amount of immobilized enzyme.

Use of buffer solution is advisable because it prevents the denaturation of an enzyme due to the local acidification of the solution during the electro-polymerization (73). Mediators can be immobilized simultaneously with the enzyme during electro-polymerization. This well controlled procedure of enzyme immobilization by electro-chemical method is of great significance in fabrication of micro sensors, in preparation of multi-layered devices and multi-enzymes.

Electro-polymerized films of conducting polymers are convenient matrix for the immobilization of enzyme. Redox reactions of substrate, catalyzed by an appropriate enzyme, take place in bulk of the polymer layer, providing good detect ability and fast response.

A number of conducting polymers such as polypyrrole, polyaniline, poly (N-methyl pyrrole), poly (o-phenylenediamine) etc., have been used for entrapment of enzyme. Substitituted conducting polymers can also be used but unsubstituted polypyrrole is the most favored as the polymer matrix, although it was modified with mediators.

2.3 Types of biosensors

Biosensors can be classified on the basis of the transducer used, i.e., how the transduction of signal occurs. The most commonly used methods are the electrochemical transduction involving amperometric and potentiometric determinations. The broad classification of the transduction methods including those on amperometric and potentiometric methods are listed in following section.

1. Movement of electrons produced in a redox reaction (as in amperometric biosensors)
2. The changes in the distribution of charge causing an electrical potential to be produced (as

in potentiometric biosensors)

3. The light output during the reaction or a light absorbance difference between the reactants and products (as in optical biosensors)
4. The heat output (or absorbed) by the reaction (as in calorimetric biosensors)
5. Effects produced due the mass of the reactants or products (as in piezo-electric biosensors)

2.3.1 Amperometric biosensors

Amperometric biosensors function by the production of a current when a potential is applied between two electrodes. They generally have response times, dynamic ranges and sensitivities similar to the potentiometric biosensors. The simplest amperometric biosensors in common usage involve the Clark oxygen electrode (Figure 2.2). This consists of a platinum cathode at which oxygen is reduced and a silver/silver chloride reference electrode. When a potential of -0.6 V, relative to the Ag/AgCl electrode is applied to the platinum cathode, a current proportional to the oxygen concentration is produced. Normally both electrodes are bathed in a solution of saturated potassium chloride and separated from the bulk solution by an oxygen-permeable plastic membrane (e.g. Teflon, polytetrafluoroethylene). The following reactions occur:

Ag anode $4Ag^0 + 4Cl^- \longrightarrow 4AgCl + 4e^-$

Pt cathode $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$

The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be effectively zero. The rate of this electrochemical reduction therefore depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration. It is clear that a small, but significant, proportion of the oxygen present in the bulk is consumed by this process; the oxygen electrode measuring the rate of a process which is far from equilibrium, whereas ion-selective electrodes are used close to equilibrium conditions. This causes the oxygen electrode to be much more sensitive to changes in the temperature than potentiometric sensors. A typical application for this simple type of biosensor is the determination of

glucose concentrations by the use of an immobilized glucose oxidase membrane. The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes. Other oxidases may be used in a similar manner for the analysis of their substrates (e.g., alcohol oxidase, D- and L-amino acid oxidases, cholesterol oxidase, galactose oxidase, and urate oxidase)

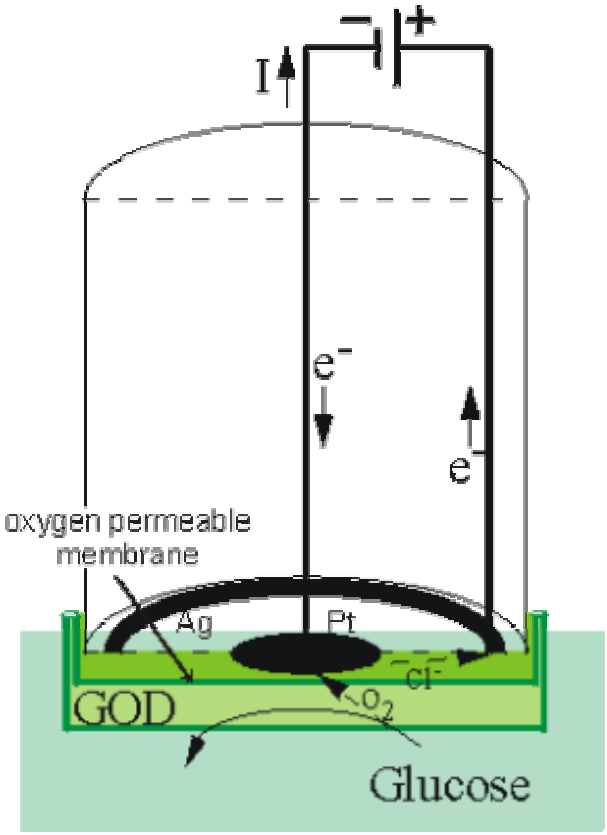


Fig. 2.2. Schematic diagram of a simple amperometric biosensor.

A potential is applied between the central platinum cathode and the annular silver anode. This generates a current (I), which is carried between the electrodes by means of a saturated solution of KCl. This electrode compartment is separated from the biocatalyst (here shown glucose oxidase, GOD) by a thin plastic membrane, permeable only to oxygen. The analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate(s)

and product(s). This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilizing dip-coated membranes.

The major problem with these biosensors is their dependence on the dissolved oxygen concentration. This may be overcome by the use of 'mediators' that transfer the electrons directly to the electrode bypassing the reduction of the oxygen co-substrate. In order to be generally applicable these mediators must possess a number of useful properties.

1. They must react rapidly with the reduced form of the enzyme.

2. They must be sufficiently soluble, in both the oxidized and reduced forms, to be able to rapidly diffuse between the active site of the enzyme and the electrode surface. This solubility should, however, not be so great as to cause significant loss of the mediator from the biosensor's microenvironment to the bulk of the solution. However soluble, the mediator should generally be non-toxic.

3. The over potential for the regeneration of the oxidized mediator, at the electrode, should be low and independent of pH.

4. The reduced form of the mediator should not readily react with oxygen (74).

2.3.2 Potentiometric biosensors

Potentiometric biosensors make use of ion-selective electrodes in order to transducer the biological reaction into an electrical signal. In the simplest terms this consists of an immobilized enzyme membrane surrounding the probe from a pH-meter (Figure 2.3), where the catalyzed reaction generates or absorbs hydrogen ions The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly from the pH-meter's display. Typical of the use of such electrodes is that the electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction.

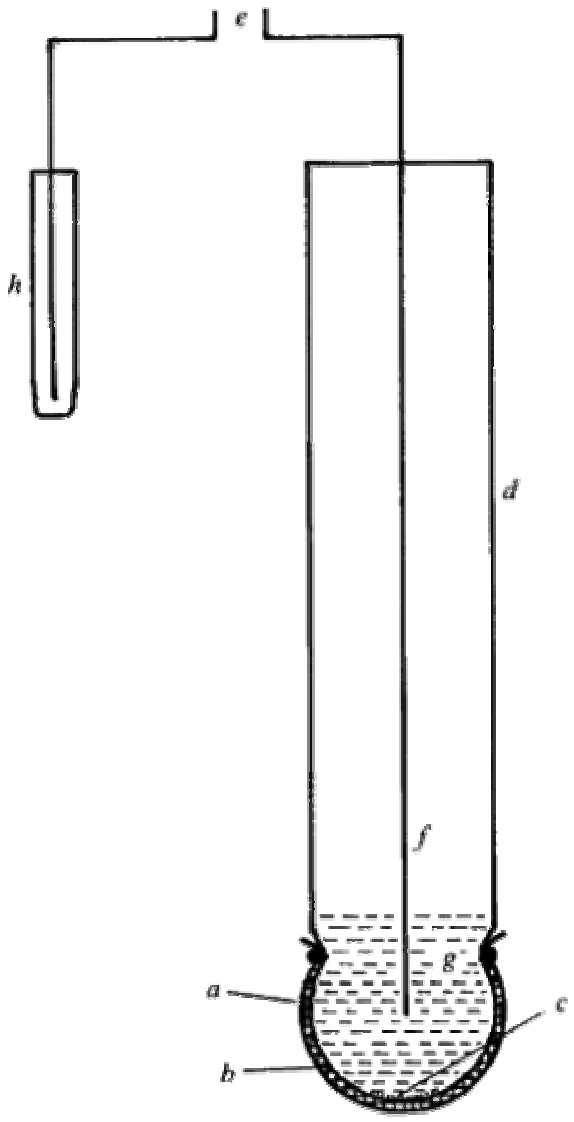


Fig. 2.3. A simple potentiometric biosensor

A semi-permeable membrane (a) surrounds the biocatalyst (b) entrapped next to the active glass membrane (c) of a pH probe (d). The electrical potential (e) is generated between the internal Ag/AgCl electrode (f) bathed in dilute HCl (g) and an external reference electrode (h).

There are three types of ion-selective electrodes, which are of use in biosensors

1. Glass electrodes for cations (e.g., normal pH electrodes) in which the sensing element is a very thin hydrated glass membrane, which generates a transverse electrical potential due to the concentration-dependent competition between the cations for specific binding sites. The selectivity of this membrane is determined by the composition of the glass. The sensitivity to H^+ is greater than that achievable for NH_4^+.

2. Glass pH electrodes coated with a gas-permeable membrane selective for CO_2, NH_3 or H_2S. The diffusion of the gas through this membrane causes a change in pH of a sensing solution between the membrane and the electrode that is then determined.

3. Solid-state electrodes where the glass membrane is replaced by a thin membrane of a specific ion conductor made from a mixture of silver sulphide and a silver halide. The iodide electrode is useful for the determination of I^- in the peroxides reaction (75).

2.3.3 Conductometric biosensors

Conductometric biosensors measure the change in the conductance of the biological component arising between a pair of metal electrodes. Contractor et al. (76) have constructed biosensors for the estimation of glucose, urea neutral lipid/lipase and hemoglobin/pepsin by monitoring change in the electronic conductivity arising as a change in redox potential and/or pH of the microenvironment in the polymer matrix. Ramanathan et al. (77) have studied the application of polyaniline LB films as glucose biosensor. They have also investigated the dielectric spectroscopic measurements of PPY/glucose biosensor (78). Conductivity biosensors based on conducting polymers were developed for pencillin (79) and also for glucose, urea, lipids and hemoglobin [Contractor et al. 1994]. Microelectronic devices have been fabricated with sensitivity to urea concentration in millimolar range in measurement of conductance, capacitance and admittance using urease immobilized in a PPY layer on gold thin film electrodes. A flow injection system was developed with covalently immobilized penicillinase on polypyrrole films and change in conductivity due to pH change was measured.

2.3.4 Optical biosensors

Optical biosensors are based on the measurement of light absorbed or emitted as consequence of a biochemical reaction. In such type of biosensors, light waves are guided by means of optical fibers to suitable detectors. These types of biosensors have been used for the detection of pH, O_2 and CO_2 etc. Gerard et al. (80) have measured the pyruvate concentration optically by immobilizing LDH onto polyaniline electrode. These electrodes were stable for 15 days and had a response time of 90 seconds. Chaubey et al., (81) have immobilized LDH on PPY-PVS electrodes on ITO plates and detected the lactate concentration by optical measurements. Singhal et al., (82) immobilized glucose oxidase on polyhexyl thiophene by LB technique and fabricated optical glucose sensor using UV-visible spectroscopy.

2.3.5 Calorimetric biosensor

The basic principle of such biosensors is that all biochemical reactions involve a change in enthalpy. Such a change in enthalpy is detected by calorimetric biosensors. Mossbach and Danielsson (83) developed an enzyme thermister where the temperature change in an enzyme reaction was measured by thermister device. A number of substrates, enzymes and antigens have been estimated using thermister biosensors. Xie et al., (84) investigated a ferrocene mediated thermal biosensor that combines electrochemistry and calorimetry. For detection,of thermal signal generated by the redox reaction was measured as opposed to measuring the electrochemical signal.

2.3.6 Piezoelectric biosensor

These biosensors operate on the principle of generation of electric dipoles on subjecting an anisotropic natural crystal to mechanical stress. Due to the adsorption of an analyte, the mass of the crystal is increased resulting in altered frequency of oscillations. Such types of biosensors have been utilized for the measurement of ammonia, hydrogen, methane, carbon monoxide, nitrous oxide and other organophophorous compounds.

The diagnosis and monitoring of chronic human diseases requires intensive examination of blood samples and other vital visceral components. These routine examinations are based upon the determination of behavior patterns of certain "analytes" using typical analytical techniques and require appropriate manpower and time for collecting samples and performing clinical tests. The behavior patterns of such "analytes" are known to be specific for a given disease and hence can be helpful in monitoring its progress. The healthcare industry has developed various biochemical tests based on the above principle to diagnose and monitor the progress of human diseases. The clinical utility of a biochemical test is determined by its sensitivity, ability to detect the disease with no false negatives and its specificity, i.e., ability to avoid false positives in non-diseased persons. In addition to this the test should be less time consuming (fast) and should use inexpensive devices and components.

2.4 Biosensors for health care

There has been great demand for rapid and reliable methods, which can be used in biochemical laboratories for determination of substances in biological fluids such as blood, serum and urine etc. There is also a demand to move clinical analysis form centralized laboratories to a doctor clinic and patients self-testing at home. Most of the available methods in market for rapid detection are based on enzyme electrodes. They provide for a negligible enzyme consumption of < 1 micro gram per sample. Although biosensors have found immense applications in various fields, their use in health care monitoring is of immense importance. Recently, measurement of metabolites in media other than blood has a great demand. Such types of measurement are important. Where there is a need of continuous monitoring of analytes, such as glucose, urea etc. therefore invasive biosensing sometimes proves to be very painful for the patients undergoing self-testing. Therefore, the concept of non-invasive testing in sweat, saliva or skin has become popular.

2.4.1 Glucose biosensor

Detection of glucose has been the most studied analyte in diabetic patients. The level of the glucose can be monitored either in-vivo or in-vitro. The first approach for in-vitro study was pioneered by Shichir et al. (85) A number of glucose biosensors have been reported which are based on conducting polymers. Ramanathan et al., (86) covalently attached glucose oxidase on poly (o-amino benzoic acid) and fabricated the screen-printed electrodes made of this material. These electrodes have been shown to be useful for glucose estimation from 1 to 40 mM and stability of about 6 days. The i-STAT portable clinical analyzer that is a significant commercially available biosensor can measure a range of parameters, like sodium chloride, potassium, glucose, blood urea nitrogen (BUN) and haematcrit. The NPL glucosense developed at the National Physical Laboratory, India is based on the screen-printed graphite electrode have a mediator incorporated in the working electrode. The product is available with the Indian markets for the consumers. The sensors are fabricated using thin film micro fabrication technology on a disposable cartridge (87). Recently, Singhal et al, (88) reported that poly (3-dodecylthiophene)/stearic acid/ glucose oxidase (P3DT/SA/GOX) Langmuir Blodgett films based glucose biosensors can be used for at least 35 measurements and was found to be stable upto 40 days. The amperometric response of the P3DT/SA/GOX LB electrodes shows almost same response for about 10 days after which there is gradual decrease in response for about 40 days. The half-life of these electrodes has been determined as about 25 days.

2.4.2 Lactate Biosensor

Lactate measurement is helpful in respiratory insufficiencies, shocks, heart failure, metabolic disorder and monitoring the physical conditions of athletes. Many biosensors have been reported to date (89-94). Two different technologies have been approached for the development of the miniaturized systems. Thin film electrodes have been developed which can be either used as either implantable catheter type devices of for in-vivo monitoring in combination with micro-dialysis system, [Dempsey 1997, (95) Secondly, disposable type

sensors were developed for the purpose of online analysis (96-97). National Physical Laboratory has developed a screen-printed electrode based lactate biosensor.

Li and coworkers (98) have reported the sol-gel encapsulation of lactate dehydrogenase for optical sensing of L-Lactate. This disposable lactate sensor has a linear dynamic range from 0.2 to 1 mM of lactate and stability of about 3 weeks. The sensor was found to have a diminished enzyme activity (about 10%) and leaching of the enzyme from the matrix.

2.4.3 Cholesterol biosensor

Determination of cholesterol is clinically very important because abnormal concentrations of cholesterol are related with hypertension, hyperthyroidism, anemia, and coronary artery diseases. Determination based on the inherent specificity of an enzymatic reaction provides the most accurate means for obtaining true blood cholesterol concentration. Reports on the development of cholesterol biosensors are available (99-106). Vengatajalabathy and Miztuani (107) demonstrated an amperometric biosensor for cholesterol determination by a layer-by-layer self-assembly using ChOx and poly (stryenesulfonate) on a monolayer of microperoxidase covalently immobilized on Au-alkanethiolate electrodes. The sensor was found to be responsive even in the presence of potential electrical interferents, L-ascorbic acid, pyruvic acid and uric acid. Kumar et al., (Insert Ref 99) presented a cholesterol biosensor by co-immobilization of cholesterol oxidase and peroxidase on sol-gel films and utilized these films for estimation of cholesterol.

2.4.4 DNA biosensor

DNA biosensors have an enormous application in clinical diagnostic for inherited diseases, rapid detection of pathogenic infections, and screening of cDNA colonies required in molecular biology. Conventional methods for the analysis of specific gene sequences are based on either direct sequencing or DNA hybridization (108) use of its simplicity, most of the traditional techniques in molecular biology are based on hybridization. Several immobilization techniques such as adsorption (109) covalent attachment (110) utilization

involving avidin-biotin complexation (111) were adopted for DNA probe to the surface of an electrochemical transducer. The transducer was made from carbon (112) gold (113-115) or conducting polymer (116-117). In the case of a common sandwich assay the signal generating species is an enzyme, such as horseradish peroxidase (118). Lund et al., (119) linked the tagged DNA to the surface of the micro sphere using a suitable reagent. Another effort is the use of micro-fabrication system and micro mechanical technology to the preparation of DNA samples and their analysis, e.g., DNA chip. Gambhir et al., (Insert ref. 115) have attempted to immobilize DNA on conducting polypyrrole/ polyvinyl sulphonate films and demonstrated the adsorption characteristics. The researcher are of the opinion that anion doped polypyrrole undergoes ion exchange with PO_4^{-3} of DNA facilitating the adsorption. Presently, DNA probes and biosensors have widely attracted attention for diagnosis of various disorders (120-121).

2.4.5 Urea and creatinine biosensors

Urea estimation is of utmost importance in monitoring kidney functions and disorders associated with it. Most of the Urea biosensors available in literature are based on detection of NH_4^+ or HCO_3^- sensitive electrodes (122-128). Osaka et al. (129) constructed a highly sensitive and rapid flow injection system for urea analysis with a composite film of electropolymerised inactive polypyrrole and a polyion complex. Gambhir et al. [Use ref. 127] have co-immobilized urease and glutamate dehydrogenase on electrochemically prepared polypyrrole/ polyvinyl sulphonate for the fabrication of urea biosensor. Singhal et al., [Use ref. 122] have recently immobilized urease on poly (N- vinyl carbazole/ stearic acid) (PNVK/SA) Langmuir – Blodgett films and observed a potentiometric response. Two linear ranges were obtained viz., 0.5 – 10 and 10 – 68 mM. It has been shown that such a urease electrode can be used for about 10 times.

Creatinine is an analyte used for the determination of renal and muscular dysfunction. The attempts to fabricate a potentiometric device began in 1976 with Meyerhoff and Rechnitz (130). Later systems with improved operational and storage capacity were developed (131-

132). Recently, an impedimetric device has been reported to assay urea and creatinine in serum using poly (methyl vinyl ether)/maleic anhydride modified screen-printed electrodes (133).

2.4.6 Urea biosensors

Urea is a bio-molecule that is known to play a variety of roles for the welfare of mankind. The well-known role of urea is as a fertilizer, which satisfies the nitrogen requirement of the plant. However, in the human body it is a waste product. The urea sensor becomes indispensable in diabetics monitoring, in order to predict the nature and cause of diabetes and also as a direct indication for the onset of kidney failure or malfunctioning of the liver With this sole motivation of monitoring urea levels in blood for an early diagnosis of organ dysfunction, an attempt been made for the fabrication of urea sensor based on the membrane immobilization technology.

2.4.6.1 Ptoduct and their application

As the product has been designed with the sole aim of monitoring urea levels in body fluid, it will be of direct applied value, in paramedical sciences, to medical practitioners, common man and chronic patients. It can also be used as an analytical tool for routine laboratory estimations of urea concentration in different solutions. An important application is in the area of agriculture and hydroponics where the precise estimation of urea can be performed in soil samples. Statistical evidence can thus be obtained on the role of urea based fertilizers on crop yield. The sensor will enable rapid experimentation and thereby provide an indispensable tool for crop management.

2.4.6.2 Demand potential

The need for a fast sensing urea probe is an absolute requirement of the present-day society with the increasing problem of diabetes and malfunctioning of kidney and liver due to air pollution and in take of toxic compounds. The sensor will also serve as a bedside tool for

rapid monitoring of urea in suspected patients and will help medical practioners for providing preventive medicine. As the quantity of sample required is only one drop of blood, it is practically a user-friendly technology and will be friend even a layman. Secondly, it would serve an important requirement in the agricultural sector where a rapid analytical tool for measurement of urea content in soils and fertilizers is an absolute necessity. The interesting features of this device, its low cost, ease of operation and quick results. An additional feature would be the display of messages advising the patient on his blood urea status.

2.4.6.3 Manufacturing process

The manufacturing process consists of two stages (1) the synthesis and characterization of membrane containing immobilized urease (the enzyme for specific detection of urea) and (2) the caliber. Presently, the method of detection is based on the principle of potentiometry. This can however be made amperomtric by suitably modifying the measurement techniques using conducting polymers. In the potentiometric mode of detection the change in the potential at the working action of the P? Probe for monitoring P? Changes related to urea concentration. Electrode the contains the immobilized enzyme and which is the site of the reaction is monitored with respect to a standard reference electrode. The change in potential is measured as a function of time and the value of potential at a given time is used for developing a calibration curve. However, for the amperometric mode of detection the integration of the sensing electrode with the reaction products is systematically investigated. Results obtained so far indicate that a plausible model for amperometric mode of urea detection can be used for real-time monitoring of urea. The important aspect is the standardization of the pH Probe to be used for the detection of the pH changes due to the breakdown of molecule in the presence of urease (134).

2.5 Urease

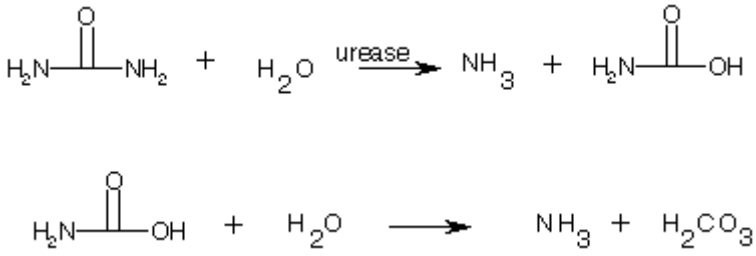
Urea + H_2O ---------- Ammonium ions + Bicarbonate ions.

The urea can be monitored either in the buffer solution, in blood serum or in whole blood. The pH probe is calibrated using standard buffer, to an accuracy of± 0.01 units. It may be mentioned that in the reaction of urease with urea it generates ammonia and carbon dioxide

as the end products. The ammonia in the aqueous medium gets converted to ammonium ions whereas the carbon dioxide dissolves in the aqueous medium and gets converted to bicarbonate ions. However bicarbonate being a weakly dissociating acid, contributes very little to pH changes, but the ammonium ions are strongly alkaline and hence the net pH shifts towards the alkaline side.

2.5.1 Urease Introduction (Urease kra1)

Urease, a nickel-dependent metalloenzyme catalyzes the hydrolysis of urea to ammonia and carbamate. The carbamate then spontaneously hydrolyzes to form carbonic acid and a second molecule of ammonia. At physiological pH, the carbonic acid proton dissociates and the ammonia molecules equilibrate with water becoming protonated, resulting in a net increase in pH.



The rate of catalyzed hydrolysis is 10-14 times the rate of an uncatalyzed reaction. Nickel-dependant ureases are found in a wide array of different organisms, many have been isolated from various bacteria, fungi, and higher plants. Urease has a wide variety of functions. The primary environmental role is to allow organisms to use external and internally generated urea as a nitrogen source. In plants urease may participate in the systemic nitrogen transport pathways and possibly act as a toxic defense protein. Medically, bacterial ureases are important virulence factors. Various bacterial ureases have been implicated in the pathogenesis of many clinical conditions such as: pyelonephritis, ammonia encephalopathy, hepatic coma, urinary catheter encrustation, peptic ulceration, and are directly associated with the formation of infection-induced urinary stones. Various urease inhibitors such as acetohydroxamic acid maybe an answer for treatment of the listed conditions, however, early studies show adverse side effects which include, depressed bone marrow biosynthesis,

inhibition of DNA synthesis, and high doses have been shown to be teratogenic. Ureases and urea catabolism may also have more practical uses. In an agricultural setting rapid hydrolysis of fertilizer urea by soil bacterial ureases results in unproductive volatilization of nitrogen and may cause ammonia toxicity or alkaline-induced plant damage. Studies have shown that urease inhibitors can be combined with fertilizers to increase the overall efficiency of nitrogen utilization. (Jabri E, et al. 1995)

2.5.2 Structure

2.5.2.1 Structural feature

Klebsiella structure aerogenes urease consists of a trimer of (alpha, beta, and gamma) units in a triangular arrangement with three active sites per enzyme (one active site per unit). The interactions between the individual subunits are fairly intricate, all three of subunits make extensive contacts to build the trimer. Each alpha subunit packs between the two related alpha subunits, contacting two beta and two gamma subunits to form the sides of the triangle arrangement. Each beta subunit packs between two of the adjacent alpha subunits at the corners of the triangle. Each of the remaining gamma subunits interact with two alpha subunits and tightly with the other two gamma subunits at the crystallographic threefold axis. Because of the high degree of interaction, it is not obvious which alpha, beta, and gamma chains make up the primary unit. High conservation of sequences and the extensive interaction of the trimer suggest a similar trimer structure in all ureases. (Jabri E, et al. 1995)

To the side is a model of one of the (alpha, beta, gamma) units that forms urease. (1kau is used as representative coordinate file as it contains the fully coordinated nickel metallocenter) Each subunit can be further broken down. The gamma subunit consists of an alpha-beta domain with four helices and two antiparallel strands. Two helices (b and c) and the two strands pack together like the helices of a four-strand bundle with right-handed up-down-up-down topology. One of the remaining helices (a) is lined up peripherially and interacts at the three-fold axis with the other two gamma subunits, tightly packing against itself against the two other (a) helices in an orthogonal manner. The last helix (d) is only a turn.

The first fifteen residues of the beta subunit form two antiparallel beta sheets with the amino-terminal residues of the alpha subunit. The core of the beta subunit forms an imperfect six-stranded antiparallel beta jellyroll. The jellyroll is left-handed and may be the first of its kind observed. This subunit stabilizes the trimer by interacting with domain two of its own alpha subunit and domain one of a symmetry related alpha subunit.

The alpha subunit consists of two domains, an (alpha, beta) 8 barrel domain and a primarily beta domain. The (alpha, beta) 8 barrel is the only domain that contributes to the active site. It's barrel is rather elliptical with the long axis connecting strands 1 and 7. Strands 9 and 10 extend the lower portion of strands 1 and 3. The active site is located at the carboxyl termini of the strands. A flap is formed that covers the active site by a helical excursion between strand 7 and helix 7. More information about the active site and its mechanism will be explained further down the page.

Mixed four-stranded and eight-stranded beta sheets form the wall of a U-shaped canyon, which makes up the second beta domain. The walls are connected by the long strands 5 and 6 together with strands, 10 and 11 which run go down one side and up the other. (Jabri E, et al. 1995)

2.5.2.2 Active site structure

The active site in Klesiella urease is located solely in the (alpha, beta) 8 barrel domain of the alpha subunit. Each of the three active sites includes a metallocenter consisting of two-nickel ions approximately 3.5 angstroms apart.

2.5.2.3 Mechanism

Urea binds into a pocket adjacent nickel to the near Wat-502. The H2-H4 mobile flap may open to allow access into the active site. Once in the active site the Urea binds to Ni-1 via Oxygen coordination. The Urea is then placed in a manner that allows coordination of its Oxygen with the N epsilon of His 219 forming a hydrogen bond. The Urea also accepts a hydrogen bond from interactions of its amide nitrogen with the side chain of His 320. These interactions orient the urea in manner that the carbonyl of urea is polarized and subject to

attack by hydroxide (Wat-502) that is coordinated to Ni-2, this forms a tetrahedral intermediate. As a proton is transferred from His 320 to the Nitrogen leaving group the intermediate collapses into ammonia and carbamate. The carbamate further hydrolyzes to form carbonic acid and a second molecule of ammonia. (Pearson M, et al. 1997)

2.5.3 Urease

Urease catalyzes the hydrolysis of urea:

$$(NH_2)_2CO + 3H_2O \longrightarrow CO_2 + 2NH_4OH$$

Jespersen (1975) reports that ammonium carbamate is produced in citrate and Tris buffer.

Urease occurs in many bacteria, several species of yeast and a number of higher plants. Varner (1960) has reviewed it. Two of the best sources are: Jack beans (*Canavlia ensiformis*) from which it has been crystallized and thoroughly studied, and *Bacillus pasteurii*.

The enzyme is important in assaying for urea. See Guilbault and Montalvo (1970). Its immobilization has been reported, *et al*. (1974), James and Pring (1975), Messing (1974), Nakamoto *et al*. (1975), Sundaram (1973) and Tran-Minh and Broun (1975).

2.5.3.1 Characteristics of urease from the jack beans

Molecular weight: 480,000 (Fishbein *et al*. 1970)

Composition: Monomeric (a) urease can polymerize to form six unit polymers of about three million daltons. (Fishbein *et al*. 1970; Fishbein and Nagarajan 1972a). Andrews and Reithel (1970) report on the sulfhydryl groups. Contaxis and Reithel (1971) indicate the molecule can be split in half with no loss of activity. See also Contaxis and Reithel (1972), Fishbein and Nagarajan (1972b), Lynn (1970), and Bailey and Boulter (1969).

Optimum pH: 7.4 (Cesareo and Langton, 1992)

Km: 1.3mM in Tris HCl (Cesareo and Langton, 1992)

Inhibitors: Heavy metals, NH^{4+} ions formed. See also Fishbein and Carbone (1965). Sodium and potassium ions are inhibitors (Cesareo and Langton, 1992).

Specificity: Urease is specific for urea and hydroxyurea (Fishbein and Carbone 1965). See also Sundaram and Laidler (1970).

Stabilizers: EDTA in concentrations of 1 X 10^{-3} M. 50% glycerol solutions protect urease crystalline suspension for several months at 4°C (135).

2.7 Biosensor for environment monitoring

Biosensors show a potential to complement both laboratory based and field analytical methods for environmental monitoring (136). Although wide range of biosensors has been reported foe environmental application, a few have commercialized.

The conducting polymer based gas sensors for environmental monitoring based on the fact that upon exposure to vapors, the polymer shows rapid conductivity changes, which are reversible at room temperature. The change in the conductivity results from a reversible reduction of the polymer on exposure to the organic vapors as well as change in the moisture content in the film (137-138).

A thin film of conducting polymer based on microelectronic ammonia gas sensor device has been developed which is the first application of conducting polymer in gas sensors device for commercial application. This device is resistor element which shows large variation when ammonia is presents in the environmental atmosphere in very low concentration and enable the necessary action or alarm activation (www.ett.bwe.hu).

Use of conducting polymers in impedence type sensors provide a possibility to develop low cost, highly sensitive and selective room temperature gas sensor (139-140). They describe a number of sensors for organic vapours based PPY, N-Methyl pyrrole, poly (5-Carboxyindole) and polyaniline. These materials are however advantageous due to lack of specificity. They show response to wide range of different gases and vapours but their

selectivity is found to be better than the inorganic materials based gas sensitive is found to be better than the inorganic materials based gas sensitive resistors.

AAI-Abtech a biotechnology company in Pennsylvania, have developed a novel sensor based on conducting PPY on an array of microelectrodes. It has been shown that a simple system using Mo (V)-catalysed conversion of iodide to iodine, H_2O_2 converts the iodide to iodine, which oxidizes the PPY, resulting in the measurable change in conductivity.

Thomas fare and coworker have developed an immunosensor based on conducting polymer for screening of pesticide atrazine. New approach for the flow amperometric biosensing of formate with single, double and triple layered arrangements of the Ferro-cyanide mediated PPY based biosensors was described (141). The detection of formate concentration has been found to be important in atmosphere, natural waves and sediments; this layered structure comprised of formate dehydrogenase, NAD and mediator has been demonstrated for the flow amperometric biosensing of formate in aqueous media, with the detection limit of 2-5 mM.

A cost effective polyaniline base ammonia gas sensor has been described (142). Hyey deposited sensing film of polyaniline, electrochemically doped by cyclic voltametry on thin and thick film electrodes. They investigated the sensitivity, selectivity, temperature dependency and long-term behaviors.

2.8 Biosensor for food industries

Food industry needs rapid and affordable methods to determine compounds in the products for quality control in food processing. In this respect, application of biosensor technique may be an alternative to the expensive and inefficient method. Extensive work on development of analytical devices for estimation of glucose, sucrose, lactate, citric acid, ascorbic acid etc in the food product have been persuaded during the last few years (143-144). A few reports on conducting polymers based biosensors for application to food industries are available (145-146).

Ghos et al have developed (147) biosensors for the estimation of fish freshness based on amperometric method using three ferrocene carboxylic acid mediator incorporated conducting polypyrrole enzyme electrode with immobilized xanthine oxidase, nucleoside phosphoxylase and nucleotidase enzyme for quantitative measurement of hypoxanthine, inosine, and inosine monophosphate metabolites in fish tissues.

Chapter 3
Substituted derivatives

3.1 Substituted Derivative introduction:

Conducting organic polymers have shown great potential for many applications, including rechargeable batteries, corrosion protection, light emitting diodes, molecular sensors, electro chromic devices and gas separation membrane (148-153) For these applications, the polymers must be highly process-able and chemically stable for long period of time. Polyaniline (PANI) has been one of the most widely studied conducting polymers because of its chemical and oxidative stability. However, as it is common with other conjugated polymers, polyaniline is limited by poor thermal process ability and solvent solubility (154). Improved solubility can be achieved by introducing bulky alkyl substituents into the polyaniline backbone, but limitations are than imposed on the conductivity of the polymer produced. The conductivity of polyaniline and the solubility of substituted polyaniline can be achieved by co-polymerization. A deep knowledge of electrochemical properties and the study and characterization of homopolymers is very important path to find the route to synthesize copolymers with tailored properties.

Polymeric films present many complicated mechanism aspects due to electronic transfer through the films specially to the sites, which are far from the polymer/substrate interface. The movement of polymeric chains to allow the accommodation of the different species like ions and solvent molecules inside the films is very important. The form of the voltammograms strongly depends upon counter ion, solvent thickness, etc; so a wide variety of E/I profiles have been found (155).

Kaufman et al., (156) have proposed a model for charge transportation through polymeric films in the sense that propagation of charge takes place by auto exchange electronic reactions among oxidized and reduced species positioned at neighboring sites in the films. It has been shown that large substituents like ethyl and the chemical nature of counter ion are

very important parameters in determining the energy of the electronic bands of PANI films. The presence of substituent significantly perturbs the geometry of the chains, leading to a hypsochromic shift of the polaronic absorption band. It has been shown, that depending upon the size of counter ion, the presence of alkyl group in the ortho position influences the participation of this anion in the ion exchange process due to steric hindrance. The electronic donating effect of ethyl substituent is completely disregarded in comparison to the steric effect that produces an increase of the torsion angle between phenyl rings. The electronic effect is important in the second oxidation process and it is evidenced by the diminution of the redox potential of the emeraldine/pernigraniniline couple.

One of the major problems associated with the conducting polymers is their poor process ability that is attributed to the fact that these polymers have a poor solubility in conventional solvents. Various methods have been adopted by chemist to increase the solubility and therefore, process ability of these polymers. Polyaniline is observed to be no exception and has been found to be soluble in some solvents like as N-methyl-pyrolidone. Attempts have been made to improve upon the solubility of polyaniline. One such approach reported in the literature related to the synthesis of the substituted polyaniline.

The electrical conductivity of N- substituted polyaniline is much less as compared to that of unsubstituted polyaniline due to following reasons:

1. Increase in torsion angle

2. Hindrance in delocalization

3. Lack of imine site

4. Stearic hindrance

N-substituted polyaniline can be prepared by the two methods:

(a) By taking substituted monomers followed by polymerization

(a) Substituting a parent polyaniline backbone by the dehydrogenation reaction

3.2 Need of Alkyl substituted Derivatives

Several workers are carrying out substitution of polymer either in their monomer form or in polymer form. The necessity of substitution, arises due to poor processibility of several useful conducting polymers like polypyrrole, polyaniline, polythiophene etc. which have been find use in field of molecular and microelectronics. Polymerization of substituted monomer is one of the most successful approaches for the synthesis of solution process-able conducting polymers. Conducting polymers such as polyphenylacetylene (157), poly (3-hexylthiophene) (158) and poly (3-octylthiophene) (159) are some example of such an approach. These conducting polymers have been found to be soluble in the organic solvents in their undoped states. Various general approach like block copolymerization (160) and polymerization of substituted monomers (161) have been made to overcome the problem of intractability of conducting polymer. The process-ability can be achieved either by copolymerization or preparation of composites (162-163) or by incorporation of certain functional groups in the chain structure. Electrically conducting polymers were also obtained by either loading the insulating polymer with a conducting powder, either a metal or graphite, or pyrolysing an insulating polymer. The attraction of polyaniline amongst various organic conducting polymers is its ease of synthesis, yield, novel protonic acid doping and good thermo-environmental stability. Polyaniline degrades before melting and has been shown to have little solubility in solvents like N-methyl pyrolidone (NMP), acetone, tetrahydrofuran (THF), DMSO,and DMFetc. Despite this technical problem, polyaniline is presently commercially available and it is now used as one of the electrodes in rechargeable lightweight batteries (164). The problem of process-ability of polyaniline, has been overcome by resorting to various approaches like, addition of side groups to the polymers backbone grafting of polyaniline to a non-conducting polymer, direct polymerization of intractable polyanline into the final desired shape and making a composite or blend of conducting polyaniline and a non-conductive but easily process-able polymer etc. These techniques have been a great success and process-able polyaniline have been successfully synthesized (165).

Substitution of polyaniline by alkyl, aryl, halogen groups etc., has been investigated by several workers (166-172). Poly (o-toludine) has been chemically synthesized by Green et al

(173) and poly (o-anisidine) has been synthesized by Macinnes et al (174). Both these polyaniline derivatives are soluble in common organic solvents. At higher pH a severe loss of electrical conductivity has been observed in polyaniline along with its electro-activity (175). This problem has however been overcome by synthesis of sulphonated polyaniline, which is, water soluble in conducting state (176). Most polymerization of substituted polyaniline involves alkyl group functionalization to improve the solubility in organic solvents (177). Structure of some of the substituted polyaniline are shown in Figure 3.1

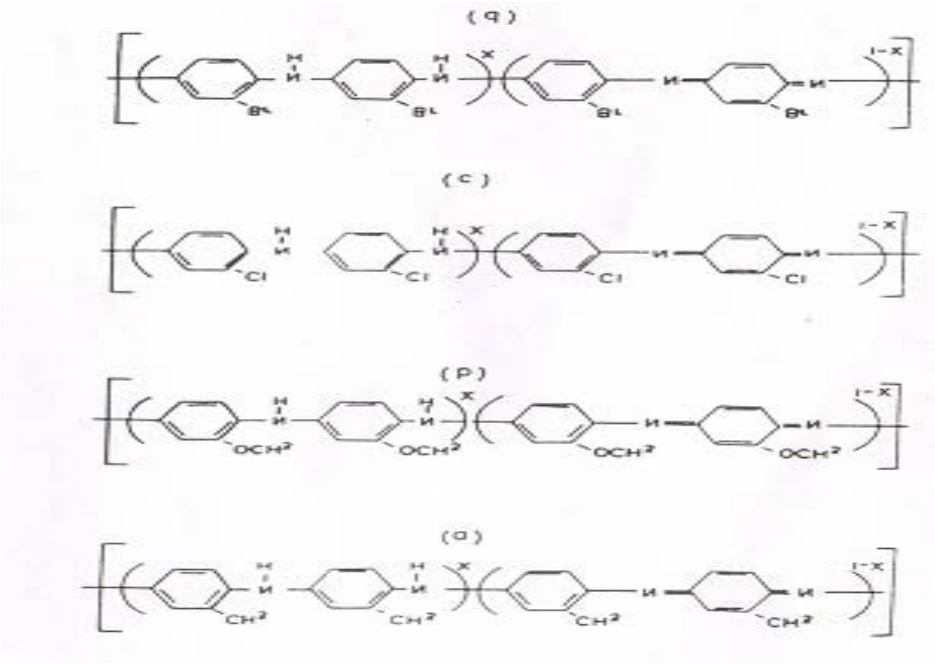


Fig. 3.1 Chemical structures of some substituted polymers

However, very little research has been focused on the effect of functionalizing aniline with a hydrophobic, electron-withdrawing group. Effect of withdrawing groups namely fluorine, chlorine, bromine etc., on polyaniline has been investigated (169-171). Koros et al (178) have used fluoremethyl functionalized polyamides to improve the separation of gases through polyamides membranes and postulated that the fluoro-substitution might enhance the solubility of oxygen in these membranes. Poly (2-fluoroaniline) has been synthesized previously, from chromic acid oxidation or electrochemically, however, polymer was not fully characterized and freestanding films were not formed from the polymer powder (162-164). Kang et al (170) have prepared poly (2-chloroaniline) and poly (2-fluoroaniline) by using chromic acid in aqueous 1M HCl solutions. Misra et al (171) have recently prepared

poly (2-bromo-aniline) and poly (2-chloroaniline) by chemical and electrochemical method. The results of Raman and infrared studies on poly (2-fluoro aniline) indicate that localized polaronic moieties are responsible for the poor electrical conductivity (168).

The various applications of conducting polymers in electronics may be due to fact that the polymers can easily be prepared by chemical and electrochemical technique and their conductivity can be controlled over a wide range extending to more than 10 orders of magnitude (179-180). In these devices, conducting polymers serves as metal (181) or p-type semiconductors (182-183) or both (184). It has been reported earlier (185-189) that the Schottky devices can be fabricated by the thermal evaporation of metals in vacuum. Compos et al (170) reported I-V and C-V characteristics of the Schottky diode using polyparaphenylene with metal over wide temperature range. Pandey (173) et al reported the work function, ideality factor, barrier height, and Richardson constant through I-V measurements on metal/polyaniline Schottky diode. In this chapter, the results on the synthesis and characterization of alkyl-substituted polyaniline are reported. The polymer was synthesized by both chemically and electrochemically methods. The solubility of chemically synthesized powder has also been experimentally examined. Suitable solvent was chosen for solution casting and spin casting. The powder was also used for preparing vacuum coated films.

The powders and films were characterized by a number of technique like Fourier transform infra-red (FT-IR) spectrometry, UV-vis spectrophotometer, Differential scanning calorimetry (DSC), Thermo gravimetric analysis (TGA), Gel permeation chromatography (GPC), Scanning electron micrography (SEM), X-ray diffraction (XRD) electrical conductivity of the polymer, in form of powder and thin film, were also measured by four points probe set up. The thickness of the film was measured through Telystep instruments based on stylus movement. Redox behavior of the electrochemically synthesized polymer film was also determined by an electrochemical interface.

Chapter 4
Characterization technique

4.1 Cyclic voltammetry

Electrochemistry provides methods for preparing conducting polymers in the conventional form of thin films on electrode surfaces. The polarization studies, cyclic voltammetry, chronocoulometry and chronoamperometry techniques have been extensively used for electro-analytical characterization, such as, redox behavior, electrochemical stability, electrochemical degradation and mechanism of polymerization etc of conducting polymers. Cyclic voltammetry (CV) is the most versatile electro analytical technique for the mechanistic study of redox behavior, number of electrons involved in the redox reaction, electrochemical studies, degradation studies, diffusion coefficient estimation and study of reversibility of redox couples in conducting polymer systems. By varying the potential between working electrode and counter electrode in an electrochemical cell, change of color (according to their oxidation level) from one state to another can be investigated. Redox couples can be characterized from the potentials of the peaks on the cyclic voltammetry and from the changes caused by the variation of the scan rate. The block diagram of the apparatus used for CV is shown below:

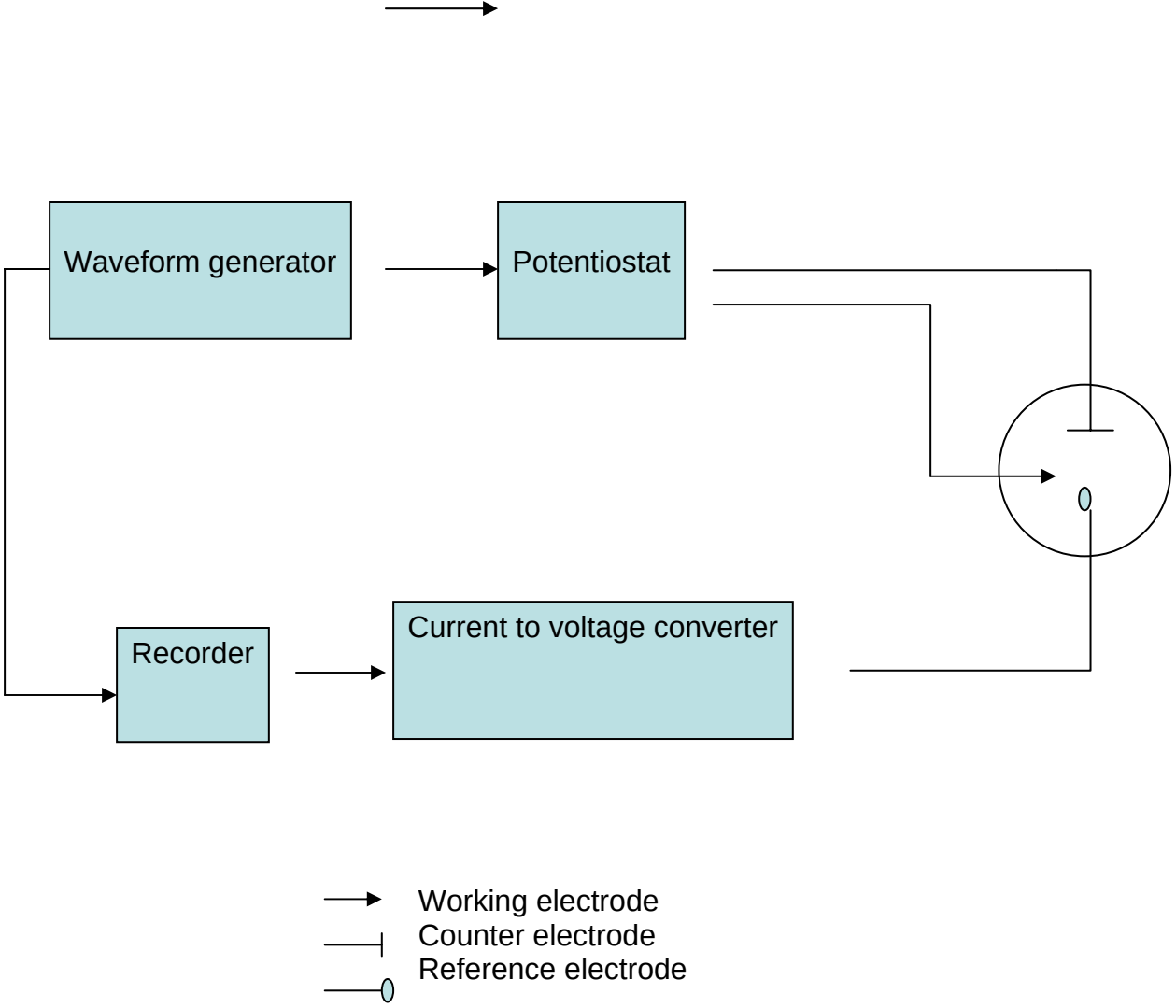


Figure 4.1 Block diagram for cyclic voltammetry (CV)

Figure 4.1 shows one compartment cell comprising of three electrodes. An ITO coated glass plate, platinum foil and silver/silver chloride have respectively been used as working, counter and reference electrodes. A Schlumberger (Model SI 1286) Electrochemical Interface, coupled with an HP plotter has been used to record the cyclic voltammogram of the conducting polymers under study. When potential is slowly increased between electrodes in the cell containing monomer along with 1M HCl, after a certain threshold voltage, there a steep rise in the value of the current, which is termed as polymerization voltage. This value has been used for synthesis of the conducting polymers in potentiostatic mode and as an outer potential limit for recording the desired cyclic voltammogram.

The important parameters of the CV are the magnitude of the peak current, Ipa (anodic current) and Ipc (cathodic current) and the peak potential, Epa and Epc (anodic and cathodic

peak potential). For electrochemically and chemically reversible systems the following equation is applied:

$$\Delta Ep = Epa-Epc = 0.059/n$$

Where, n is the number of electrons transferred and Epa and Epc are the anodic and the cathodic peak potentials (volts), respectively. ΔEp is independent of scan rate for the reversible couple. The value of i_{pa} and i_{pc} are similar in magnitude for a reversible couple with the kinetic complications.[23]. Peak current (anodic/cathodic) depends upon square root of the scan rates in any redox couple. In ideal reversible systems anodic peak current should be equal to cathodic peak current or $i_{pa}/i_{pc}=1$

In this work poly (2-ethyl aniline) were synthesized electrochemically on an indium-tin-oxide coated glass plates and platinum wire. These conducting polymer thin films were characterized by the cyclic voltammetry. Potassium ferrocyanide/ferricyanide was used as a standard system before using the polymeric samples. CV of polymeric films has been recorded in phosphate buffer pH (7.0).

4.2 Spectroscopic analysis

The spectroscopic characterization of the polymeric samples is usually carried out by either preparing a thin film or making a solution of the polymer transparent to the incident radiation. In case of powders, a few mg of sample is mixed with potassium bromide (KBr) for making the pellets to the IR studies. UV-visible spectra give the energy band gap and defect states, while IR spectroscopy identifies and confirms the structure and presence of the various linkages in the polymer. This is very important characterization technique, specially, when substitution, cross-linking and co-polymerization involved.

4.2.1 Fourier Transform Infrared (FT-IR) Spectroscopy

A molecule absorbs radiation only when the natural frequency of vibration of some part of the molecules (i.e., atoms or groups of atoms comprising it) is the same as frequency of the

incident radiation. After absorbing the correct wavelength of the radiation, the molecules vibrate at increased amplitude. This occurs at the expense of the energy of the IR radiation, which has been absorbed.

Infrared spectroscopy is one of the most powerful analytical techniques, which offers the possibility of chemical identification. One of the most important advantages of the infrared spectroscopy over the other usual methods of structural analysis (X-ray diffraction, electron spin resonance etc.) is that it provides useful information about the structures of the molecules and bonding quickly, without tire-some evaluation methods. Moreover, FT-IR provides a very faster and easier method of identifying the chemical structures especially those of organic ones.

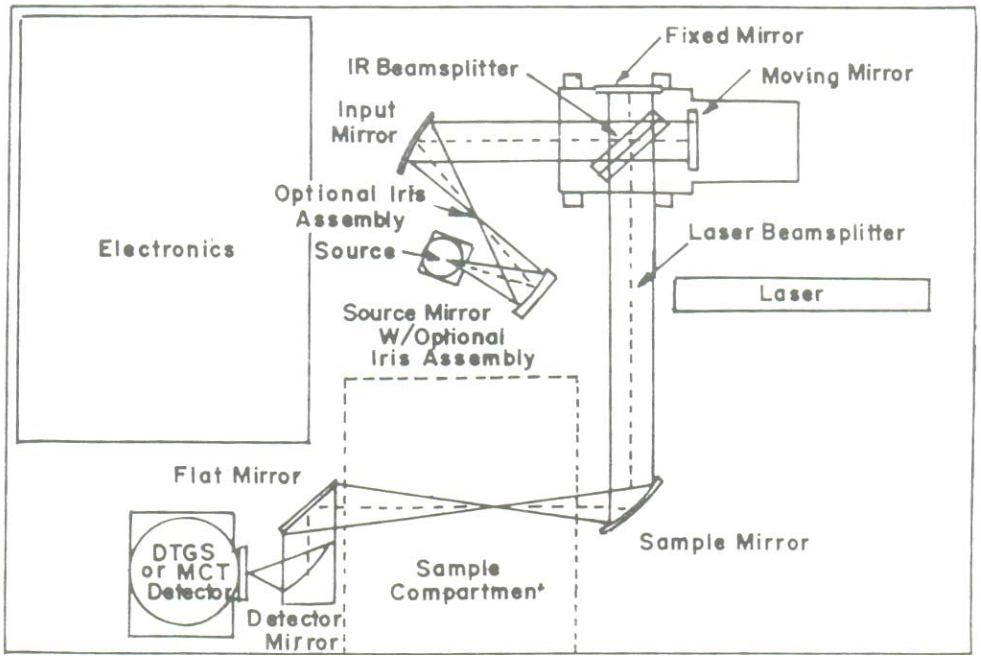


Figure 4.2: FT-IR Set up

FT-IR spectroscopy employs an interferometer in place of monochromator. This device generates the Fourier transforms of the infrared spectrum, which is converted to the spectrum itself by the computer. This approach have the advantageous of providing much higher source radiation throughout, increase signal to noise (S/N) ratio, and higher wave number accuracy than is possible with a conventional light dispersive spectrometer.

This technique is based upon the simple fact that a chemical substance shows marked selective absorption in infrared region giving rise to the closed packed absorption bands, called an absorption spectrum, which may be extended over wide wavelength range. Various bands presents in an IR spectrum corresponding to the characteristics functional groups and bonds presents in the chemical substances. IR spectrum of the chemical substances is thus a finger- print for its identification. Band position in an infrared spectrum may be expressed conventionally by the wave number ν.

The relation between velocity of light c, wavelength λ and frequency ν , is as follows:

$$\nu = c/\lambda$$

Band intensities in IR spectrum may be expressed by either as a transmittance (T) or absorbance (A). Transmittance is defined as the ratio of the radiant power transmitted by a sample to the radiant power incident on the sample. In the most of the spectrum transmittance versus wave number (cm^{-1}) has been plotted. In the present work, FT-IR spectra of the sample has been recorded in the range 4000 to 400 cm^{-1} at 4 cm^{-1} resolution using FT-IR spectrophotometer of Nicolet, USA, Model 510P.

4.2.2 UV-Visible spectroscopy

The absorption of UV radiation by organic compounds in the visible and ultraviolet region involves promotion of electrons in σ, π and n-orbitals from the ground state to higher energy state. These higher energy states are described by molecular orbitals that are vacant in the ground states and are commonly called antibonding orbitals. The antibonding orbitals associated with the σ bond is called the σ* orbital and that associated with the π bond is called the π* orbital. As the n electrons do not form bonds, there antibonding orbitals are not associated with them. The electronic transitions that are involved in the ultraviolet and visible regions are of various types viz; $\sigma \rightarrow \sigma^*$, $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$. Transitions to antibonding π* orbitals are associated only with unsaturated centers in the molecule.

Compounds containing isolated double bonds absorbs in the range 162 to 192 nm, whilst conjugated molecules (those containing single and double bonds) absorbs above 210 nm. Extension of the conjugated systems intensifies the absorption peaks and shifts it further to higher wavelengths, towards the visible spectrum.

This technique not only provides information about the different bonding but also is an excellent tool for determining the band energy, which is an important parameter used in investigating the conduction mechanism in the organic conductors. A block diagram of the UV-vis spectrophotometer used is shown in the Fig.4.3

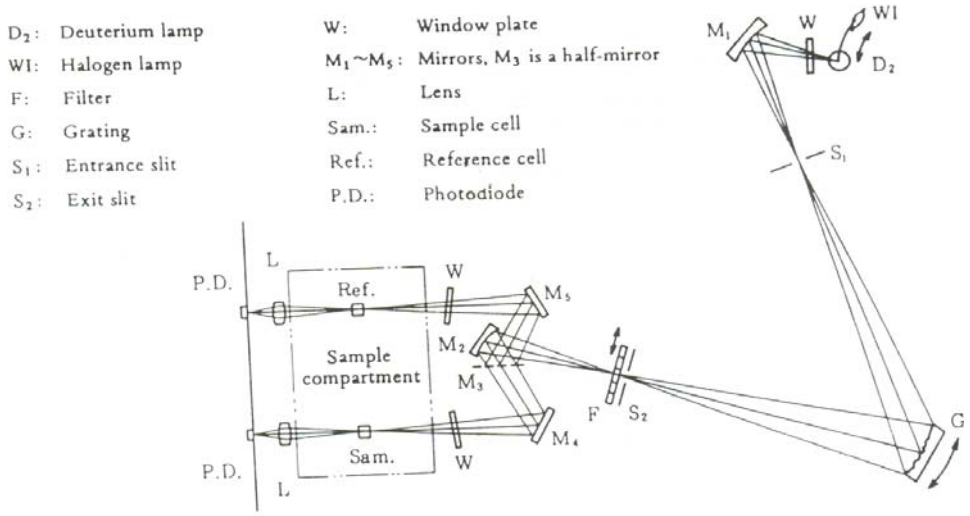


Figure 4.3 Block diagram of the optical detection systems

Figure 4.3 gives the block diagram of optical detection systems used in the UV- visible spectrometer. Deuterium and halogen lamps are used to produce ultra-violet (200 nm) and visible (400-1100 nm) spectrum respectively. The lamp is positioned automatically so as to provide the appropriate wavelength. UV- visible absorption spectra of the all polymeric samples has been recorded in the ultra-violet and visible range (200 to 1100 nm) using UV-visible spectrophotometer (Model Shimadzu model 160A) dual beam systems. The relationship between the energy absorbed in an electronic transition and the frequency (υ) or wavelength (λ) of the radiation producing the transition is :

$$\Delta E = h\upsilon = hc/\lambda$$

Where, h is Planck's constant and c is the velocity of light, ΔE is the energy absorbed in an electronic transition in the molecule.

The intensity of the absorption may be expressed as transmittance (T) =I/Io, where Io is the intensity of the radiant energy striking the sample, and I is the intensity of the radiation emerging from the sample. Alternatively one can express the absorption intensity in the following way:

$$Log_{10}I/Io= k.c.b= A$$

Where, k = a constant characteristics of the solute, c = concentration of solute (moles/liter), b = path length through the sample, A = absorbance

The UV-visible data is also used for determination of the band gap (i.e. difference between conduction band energy and valency band) in case of various conducting polymers by using the following relationship (28a):

$$(\alpha.d) =(h\upsilon-\varepsilon_g)^{1/2}$$

$$\alpha.h\upsilon = \alpha\ (h\upsilon-\varepsilon_g)^{n}$$

Where α is the absorption coefficient and d is the thickness of the sample, ε_g is the energy band gap, n (1/2, 1, 2) is a constant and depend upon the degree of the transition. Hυ is the incident photon energy.

4.3 Electrical conductivity measurement

Electrical conductivity of conducting polymers depends upon numerous factors. Significant among these are: (a) nature of chemical reactivity of the dopants (b) process of doping (c) doping level (d) method and condition of polymer synthesis (e) processing of the polymer and (f) degree of crystallinity.

The mechanism of conduction in conducting polymers is an interesting aspect to be investigated. Several mechanism of conduction in conducting polymers has been reported and yet many are to be revealed. The charge transport is well understood by investigating the electrical properties using the conductivity/resistivity measurements. The conductivity is given as below:

$$\sigma (T) = J/E = nq\mu$$

Where, J is the current density, n is the total number of charge carriers and q is the charge of the electron and μ is the mobility of the charge carrier. The conductivity measurement as a function of temperature reveals lot of information about the type of conduction. The temperature variation of electrical conductivity is defined as:

$$\sigma (T) = \sigma_o \exp (- E_A/kT)$$

Where, Ea is the activation energy, k is the Boltzmann constant and T is the absolute temperature. The determination of activation energy provides the information about the type of conduction viz; hopping, ionic conductivity etc. To measure the electrical conductivity, four-point probe technique has been used.

4.3.1 Four-point-probe technique

The method of measurement of conductivity depends upon the range of conductivity to be measured. It is well known fact that conducting polymers in their undoped forms behave like insulator with increasing doping levels, these electronic organic materials show a wide variation in the conductivity, varying from semiconductor to metal. In the present studies, four-point-probe method, which is normally used in conducting polymers is used. For measurements of low resistivity, the contact resistance between the measurements electrodes and the specimen is a matter of concern. Contact resistance may be reduced by painting electrodes directly on the surface of specimen instead of relying on pressure contact with

metal plates or foils. However this problem is completely eliminated by the four-point-probe method. The diagrammatic representation of four-point-probe set-up is given in the Fig. 4.4.

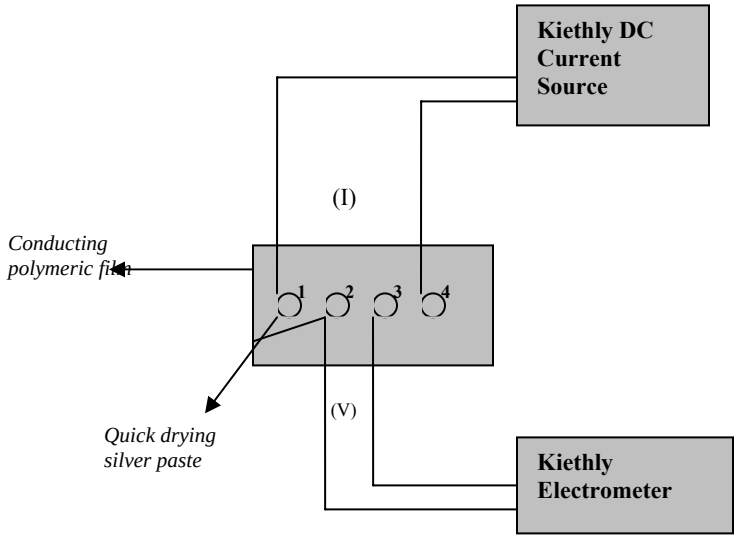


Fig. 4.4 Diagrammatic representation of four-point-probe set-up

By carrying out measurements through four-point-probe method, effect of contact resistance is avoided, provided that the contact resistance is much smaller than the input resistance of the voltameter. In certain cases, where the sample resistance is very high, the current, I, which flows is very small and it can only be determined by measuring the voltage drop across a standard high resistor R with an electrometer may be kept relatively low, so as to maintain the virtual earth condition at the input. Four-point-probe becomes very different by the way that in this a known current I, is, applied by the electrode 1 and collected at 4, whilst the potential difference ΔV between electrodes 2 and 3 is measured.

A constant current source (Keithly Model 224) is used to pass a steady current through the two outermost probe and the voltage drops across inner two are measured. A d.c. voltage is applied across the electrodes and the current passing through the materials is measured by the Keithley electrometer (Keithley Model 617) in vacuum.

The temperature variation was measured by placing the sample holder into the home made furnace. The furnace used is a muffle of 4 inch diameter and 10 inch in length, on which canthol wires were wound. The voltage was controlled using the variac. The temperature was measured using the copper-constantan thermocouple. The temperature could be maintained up to ±2°C. Teflon coated wires were used for contacts. Samples in the form of the pellets, thin films, prepared by electrochemical, spin coating, solution cast methods were used.

In this work, this technique is used for the determination of conductivity of electrochemically prepared poly (2-ethyl aniline) and urease immobilized on electrochemically prepared poly (2-ethyl aniline) samples.

Chapter 5

Experimental

5.1 Introduction

Polymer coated electrodes have attractive features as to the immobilization of an electro catalyst at the surface in high concentration. The preparation of the polymer-coated electrodes by the anodic polymerization of the corresponding monomer has become very popular because the electrochemical technique can prepare polymer films of controlled thickness. There are a number of enzymes immobilized electrochemically, namely, urease, lactate dehydrogenase, cholesterol oxidase, DNA etc. Various methods were employed for the immobilization of urease and their response measurements.

5.2 Materials

Urease E.C.3.5.1.5 (Urea amidohydrolase) from the Jack beans, (Canvlia ensiformis) procured from Sigma Chemical Company with specific activity of 50 units/mg. Nesslers's reagent (king's) for determination of urea in blood purchased from the Qualigens fine chemicals a division of Glaxo India Limited, acetone from Merck company and $HClO_4$ from the Merck (India limited, Mumbai). All materials are of analytical grades. Water purification systems- Model Milli Q 1075, UV-visible from Shimadzu- Model 160 A, Four point probe conductivity measurement apparatus from Keithly electrometer model (EC 617) have been used during my project work.

5.3 Instruments have been used during my studies

Electrochemical measurements were carried out at room temperature using three-electrode system, consisting of an indium-tin-oxide (ITO) coated glass plate as working electrode (1 cm^2), platinum as counter electrode, and silver as reference electrode. Poly (2-ethyl aniline) was prepared by applying a constant potential of 0.95 V using programmable

electrochemical analyzer model 273A. A constant potential is applied to this electrochemical cell and the current is recorded as a function of this potential. Deionised water was obtained from Millipore water purification system (Model milli Q 1075).

5.4 Preparation of the 2-ethyl aniline solution

2-ethyl aniline was distilled prior to its use. 2-ethyl aniline (0.1M) solution was prepared in 1M $HClO_4$ solution. Perchloric acid ($HClO_4$) was used as a dopant.

5.5 Immobilization of urease

10 μL of 50 units of urease was immobilized onto 1 cm^2 surface of electrochemically prepared poly (2-ethyl aniline) deposited on ITO coated glass plates. After urease adsorption, these ITO glass plate were allowed to dry overnight. The urease immobilized on poly (2-ethyl aniline) films were washed with buffer solution to remove any unadsorbed urease prior to its use and stored at 4°C when not in used.

5.6 Buffer preparation

A phosphate buffer of pH= 7 was prepared at room temperature by 0.2 M monobasic Sodium dihydrogen phosphate (5.44 gm.) and 0.2 M dibasic Sodium hydrogen phosphate (7.095 gm.).

5.7 Electrochemical synthesis of poly (2-ethyl aniline)

The poly (2-ethyl aniline) films were prepared electrochemically by oxidation of 2-ethyl aniline monomer with perchloric acid ($HClO_4$) as dopant. The commercially available 2-ethyl aniline monomer was distilled prior to use. Electrochemical synthesis was carried out in three-electrode system. An indium-tin-oxide (ITO) coated glass plate was used as working

electrode with platinum as counter and silver/silver chloride as reference electrode. The polymerization was carried out at 0.95 V at room temperature for a period of 600 s.

5.8 Chemical synthesis of alkyl substituted polyaniline

2-Ethyl aniline (2.4 ml) was dissolved in 500 ml of 1M HCl, and the solution was cooled to 5ºC in an ice cooled water bath. A few drops of the $FeSO_4$ were added as a catalyst. A precooled solution of 9.2 gm (0.020 mol) of $(NH_4)_2S_2O_8$ in 200 ml of 1M HCl was then added drop wise with vigorous stirring during a period of 30 minute. After 30 minutes reactants mixed, the solution started to a blue green tint and became intense green as precipitate formed. After 2 hrs precipitate was filtered and then collected in a Buchner funnel. The blue green powder was then transferred into the beaker containing 200 ml of 1M HCl to ensure complete protonation. After stirring at room temperature for 10 hr, the mixture was filtered and the precipitate cake was washed with 500 ml of 1M HCl until the filtrate become colorless. Upon drying under dynamic vacuum at room temperature for 24-48 hrs poly (2-ethylaniline) formed. For conversion of poly (2-ethyl aniline) into base form the doped powder was suspended into aqueous NH_4OH (approximately 100 ml of 0.1 NH_4OH was used per gm of hydrochloride) with stirring for 16 hrs at room temperature. The pH of solution was periodically adjusted to 10 by addition of small amount of 1M NH_4OH . The suspension was then filtered and then precipitate was finally washed with 400 ml of 0.1 NH_4OH followed by 5-50 ml portions of 1:1 mixture of methanol and 0.2 M NH_4OH. The polymer was than dried under dynamic vacuum at room temperature for about 48 hrs. The resulting polymer obtained from the above synthesis contains some unreacted monomer, oligomers and additional amounts of oxidants. In such cases, higher conductivity was not achieved even after complete protonation. So simple washing was not sufficient to remove such undesired materials. Extraction using suitable solvents has been reported to be the best technique for removal of monomer, oligomers etc. Hence, the powder was further washed with methanol to extract varying amounts of lower molecular weight oligomeric impurities. The powder synthesized by the above method gets partially doped due the reaction with acidic medium, viz, HCl and further processing was required to obtain the polymer into the undoped form.

In order to obtain the undoped poly (2-ethylaniline) the polymer collected from the above method was treated with aqueous ammonium hydroxide solution (pH=12.0) and then continuously stirred for about 48 hrs. This powder turned into the bluish color and collected in a Buchner funnel and washed with distilled water several times. The resulting powder was dried at room temperature for about 5 hrs in a vacuum oven. Chemically synthesized undoped poly (2-ethylaniline) powder that was obtained from the vacuum oven was mixed in 500 ml of 1M HCl for about 10 hrs and was continuously stirred using a magnetic stirrer. The precipitate was then vacuum filtered using a vacuum filtration flask and a vacuum pump and then washed repeatedly with deionized water. The powder was transferred to a Petri dish and dried in a vacuum oven for about 5 hrs.

5.9 Conductivity Measurement by Four-points-probe Technique

The conductivity of Poly (2-ethyl aniline) with and without urease films deposited on ITO was measured by the four-point probe technique. On these films, we attached four Cu electrodes with the help of silver paste and after drying the paste a known current I supplied by the electrode 1 and collected at the electrode 4, whilst the potential difference ΔV between electrodes 2 and 3 was measured.

A constant current source (Keithly Model 224) is used to pass a steady current through the two outermost probes and the voltage drop across inner two is measured. A dc voltage is applied across the electrodes and the current passing through the material is measured by the Keithly electrometer (Keithly Model 617) in vacuum.

5.10 Amperometric response

Amperometric studies were carried out using an electrochemical analyzer (model 273A) at a scan rate of 30 mV/s. The electrochemical solutions consist of phosphate buffer pH 7. Three-electrode cell configuration was used when urease immobilized onto poly (2-ethyl aniline) film as a working electrode, Ag/AgCl as a reference and platinum foil as counter electrode. Cyclic Voltagram was run for poly (2-ethyl aniline) and urea biosensor separately.

Chapter 6
Results and discussion

Electrochemical characterization of poly (2-ethyl aniline)

6.1 Cyclic votammetric studies

The knowledge of the oxidation-reduction potential for the polymers is important for device application especially when they are used as biosensors. This type of study., redox behaviour of npolymers has been carried out by the cyclic voltammetry.

6.1.1 Cyclic voltammetery of poly (2-ethyl aniline)

The cyclic voltammetry of poly (2-ethyl aniline) was done in the phosphate buffer (pH 7) at a scan rate of 30 mV/s. The cyclic voltammetric studies reveal that the poly (2-ethyl aniline) films coated on ITO glass plate give an oxidation peaks around 0.2V and 0.6 V as shown in fig. 6.1. These oxidation peaks are in agreement with the values as reported in the literature. It indicates that these poly (2-ethyl aniline) films are electro-active and can be used for immobilization of urease.

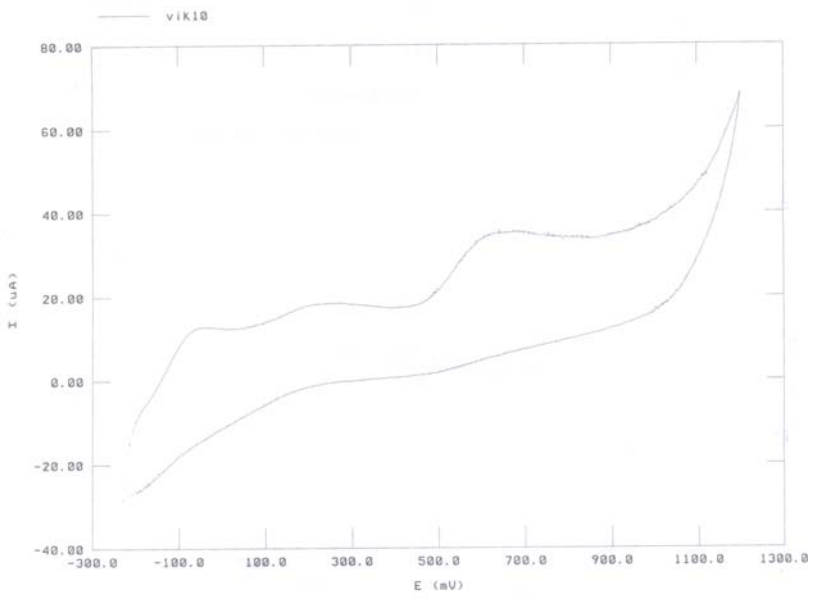


Fig. 6.1 Cyclic voltammogram of poly (2-ethyl aniline)

6.1.2 Cyclic voltammetry of poly (2-ethyl aniline)/urease

Cyclic voltammetric studies of poly (2-ethyl aniline)/urease were carried out at room temperature in phosphate buffer (pH7). Urease about 10 μL was put on ITO glass plate for immobilization purpose. These poly (2-ethyl aniline)/urease films were used as a working electrode, platinum foil as a counter electrode and Ag/AgCl as a reference electrode. A cyclic voltammogram of poly (2-ethyl aniline)/urease shown in the fig. 6.2. It shows an oxidation peak around 0.6 V. The oxidation peak appears for poly (2-ethylaniline)/urease slightly shifts downwards due to the suppression of original peak which indicates the immobilization of urease enzyme in polymer matrix.

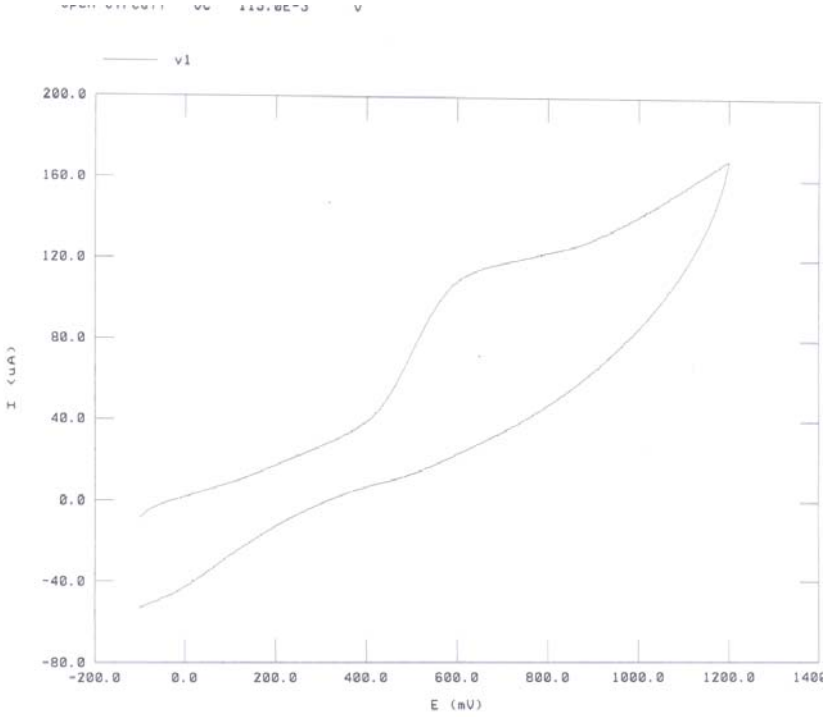


Fig 6.2 Cyclic voltammogram of poly (2-ethyl aniline)/urease

6.2 FT-IR Studies

6.2.1 FT-IR spectra of electrochemically prepared poly (2-ethyl aniline)

The FT-IR spectra of electrochemically synthesized poly (2-ethyl aniline) on ITO glass plate using 1M $HClO_4$ as electrolyte solution are shown in the figure 6.3. The characteristics peaks appear at 3030, 2800, 1564, 1460, 1244, 1027, and 720 cm^{-1}. Peak observed at 3030 cm^{-1} is due to the aromatic –CH stretch vibration. The peaks at 1564 and 1460 are due to -NH bending vibration and due to the asymmetric vibration of CH_3 overlaps with CH_2 bending respectively. The peaks appear at 1244 and 720 cm^{-1} are due to the –CN stretch and due to ortho substituted derivative. The peak appears at 2800 cm^{-1} is due to the presence of ethyl moiety in the polymer. A dopant peak of chloride ion due to perchlorate doping is seen at 1564 cm^{-1}

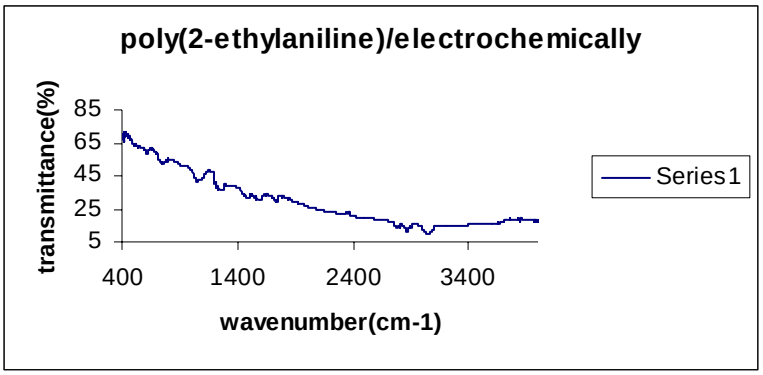


Fig 6.3 FT-IR spectra of the electrochemically prepared poly (2-ethyl aniline)

6.2.2 FT-IR spectra of electrochemically prepared poly (2-ethyl aniline)/urease

The FT-IR spectra of electrochemically synthesized poly (2-ethyl aniline)/urease on ITO glass plate using 1M $HClO_4$ as electrolyte solution are shown in figure 6.4. The peaks appear at 3256, 3030, 1564, 2800, 1460, 1244, 1027, and 720 cm^{-1}. Peak observed at 3030 cm^{-1} is due to the aromatic –CH stretch vibration. The peaks at 1564 and 1464 cm^{-1} are due to -NH bending vibration and asymmetric vibration of CH_3 overlaps with CH_2 bending respectively. The peaks appear at 1244 and 720 cm^{-1} are due to the –CN stretch and due to ortho-substituted derivative. The peak appears at 2800 cm^{-1} is due to the presence of ethyl moiety in the polymer. A dopant peak of chloride ion seen at 1564 cm^{-1}.

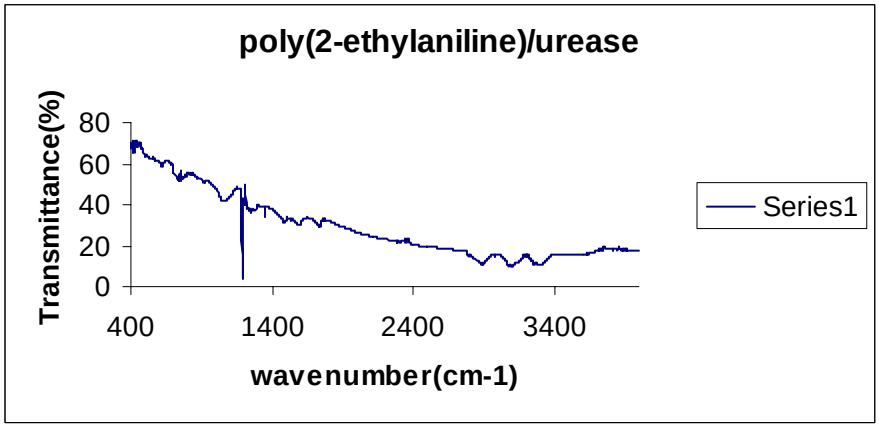


Fig 6.4 FT-IR spectra of the electrochemically prepared poly (2-ethyl aniline)/urease

It has been observed from above two spectra that characteristics peaks appear almost at the same region in both cases except few peaks of the urease enzyme. The peptide group of urease (protein) backbone causes five vibrations in the -CONH plane and five vibrations out of plane –CONH vibrations. The characteristics peaks appear at about 3256 $cm^{-1}$and 3030 cm^{-1}have been assigned to amide A and amide B respectively. Amide A band is caused by the –NH stretching vibration whereas amide B band arises due to the first overtone of amide B vibration that become intensify by the Fermi-resonance with the amide A vibration.

6.3 UV-visible spectra

6.3.1 UV-visible spectra of electrochemically prepared poly (2-ethyl aniline)

UV-visible spectra of electrochemically prepared poly (2-ethyl aniline) on ITO glass plate using 1M $HClO_4$ as electrolyte shown in fig.6.5. The absorption peak obtained for the electrochemically prepared poly (2-ethyl aniline) is found about at 410 nm.

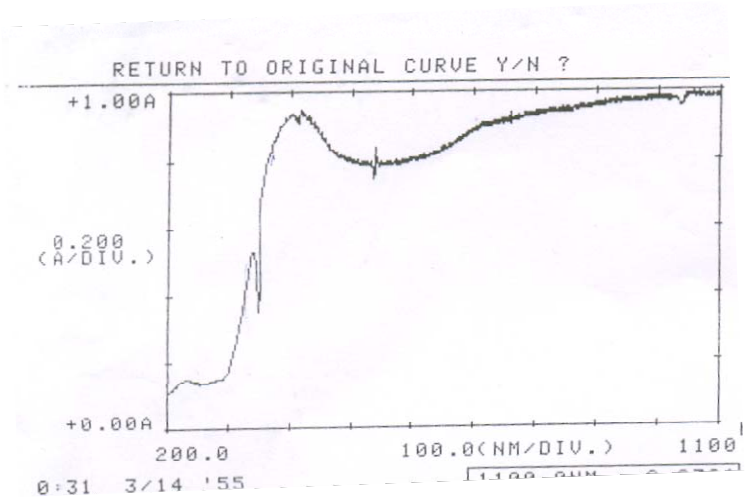
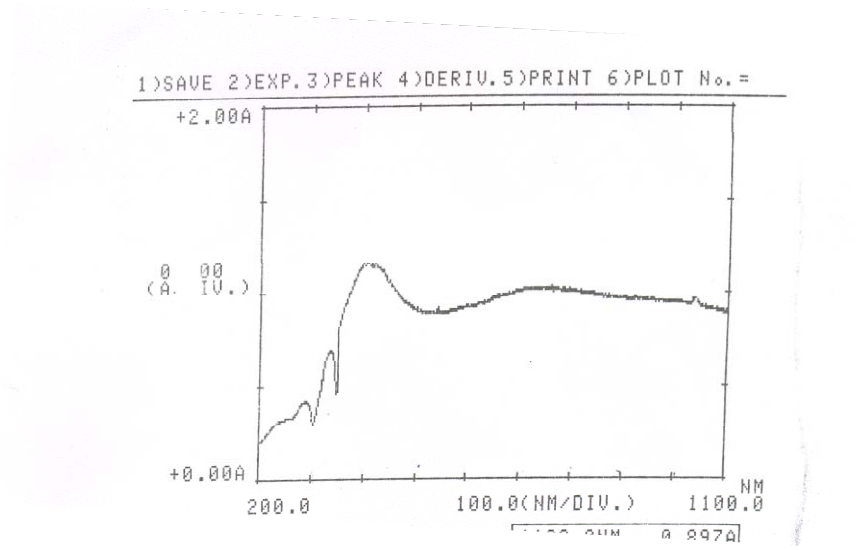


Fig. 6.5 UV-visible spectra of electrochemically prepared poly (2-ethyl aniline)

6.3.2 UV-visible spectra of electrochemically prepared poly (2-ethyl aniline)/urease

UV-visible spectra of electrochemically prepared poly (2-ethyl aniline)/urease on ITO glass plate using 1M $HClO_4$ as electrolyte are shown in the fig. 6.6. The absorption peak for the electrochemically prepared poly (2-ethyl aniline) is about at 410 nm and peak appears for the urease, which immobilized physically on ITO glass plate is found about at 280 nm.The absorption peak of urease suggested that urease immobilized on ITO glass plate is active.



.**Fig. 6.6 UV-visible spectra of electrochemically prepared poly (2-ethyl aniline)/urease**

6.4 Conductivity of electrochemically prepared poly (2-ethyl aniline) by using Four Point Probe Technique

6.4.1 The electrical conductivity of poly (2-ethyl aniline)

The electrical conductivity of poly (2-ethyl aniline) with and without urease was measured by the Four-point probe technique. The electrical conductivity of this polymer is reported in table 6.1 and 6.2 given below:

Table 6.1

Electrical conductivity measurements of electrochemically synthesized Poly(2-ethyl aniline)

Current I = 10 mA					Current I = 15 mA		
S.No.	Time interval in seconds	Potential (mV)	Resistance (Ω)	Conductivity (Ω^{-1})	Potential (mV)	Resistance (Ω)	Conductivity (Ω^{-1})
1.	0	565	56.5	0.017696	840	56.00	0.0178571
2.	20	568	56.8	0.017605	845	56.33	0.0177514
3.	40	560	56.0	0.017857	840	56.00	0.0178571
4.	60	555	55.5	0.018001	835	55.16	0.0179640
5.	80	550	55.0	0.018181	842	56.13	0.0178147

6.4.2 Electrical conductivity measurements of electrochemically synthesized Poly(2-ethyl aniline)/urease

Table 6.2

Current I = 10 mA					Current I = 15 mA		
S.No.	Time interval in seconds	Potential (mV)	Resistance (Ω)	Conductivity (Ω^{-1})	Potential (mV)	Resistance (Ω)	Conductivity (Ω^{-1})
1.	0	750	75.0	0.0133333	1085	72.33	0.0138248
2.	20	755	75.5	0.0132450	1080	72.00	0.0138888
3.	40	768	76.8	0.0130208	1075	71.66	0.0139534
4.	60	765	76.5	0.0130718	1090	72.66	0.0137614
5.	80	760	76.0	0.0131578	1085	72.33	0.0138888

Summary

(1) Poly(2-ethyl aniline) was synthesized was synthesized by both electrochemically and Chemical polymerization method. The characterization of polymer sample was carried out by the different techniques

e.g., Cyclic voltammetry (CV), UV-visible spectroscopy, Fourier Transform Infra Red spectroscopy(FT-IR) and Four Point Probe conductivity measurement techniques.

(2) The results obtained from UV-visible and FT-IR techniques also supporting the utility of Poly(2-ethyl aniline in biosensor device.

(3) The cyclic voltammograms of Poly(2ethyl aniline and Poly(2-ethyl aniline)/urease suggest that the suitability of this polymer for its utility in biosensor devices.

(4) The conductivity of this polymerwith and without enzymes is good and this implies that the it can be used to biosensor.

REFERENCES

1. V.V. Walatka, M.M. Labes and J.H.Perlstein., Phys. Rev. Lett. 31 (1973) 1139.
2. W.A.Little., Phys. Rev., 134A (1964) 1416; Sci. Amer. 212 (1965) 21.
3. H. Shirarkawa, E.J. Louis, A.G. MacDiarmid, C.K. Chiang and A.G. Heeger., J.Chem. Soc. Chem. Commun. (1977) 578.
4. A.F.Diaz, K.K.Kanazawa.and G.P.Gardini., J.Chem. Soc. Chem.Commun. (1979) 635.
5. G. Tourillon and F. Garnier., J.Electroanal. Chem, 135 (1982) 173.
6. D.M. Ivory, G.G. Miller, J.M. Sowa, L.W. Shacklette, Chance abd R.H. Baughman. J. Chem. Phys., 71 (1979) 1506.
7. J.D. Capistran, D.R.Gagnon, S. Antoun, R.W. Lenz and F.E.Karasz, Polym. Prepr. (Am.Chem Soc Div Polym Chem), 25 (1984) 282.
8. A.F. Diaz and J.A. Logan., J. Electroanal. Chem. 111 (1980) 111.
9. D. Bloor and B. Movagher., IEEE Procedings. 130 (5) (1983) 225.
10. J. Mort, G.Pfister and S. Grammatica., Solid State Commun. 18 (1976) 693.
11. S. Santos, M.V. Tartsev., Physica B 205 (1995) 4.
12. G. Grem, G. Leditzky, B. Ullrich and G. Leising., Synth. Met. 51 (1992) 383.
13. G. Grem, G. Leditzky, B. Ullrich and G. Leising., Adv. Matar. 4 (1992) 36.
14. K. Yoshino, T. Takiguchi, S. Hayashi, D.H. Park and R.I. Sugimoto., Jpn. J. Appl. Phys. 25 (1986) 881.
15. J. Obrzut and F.E. Karasz, J. Chem. Phys. 87 (1987) 2349.
16. M.A. Sato, S. Tanaka and K. Kaeriyama, Synth. Met 14 (1986) 279.
17. T.C. Chung, J.H. Kaufman, A.J. Heeger and F. Wudl., Phys. Rev. B 30 (1984) 702.
18. J.L.Bredas in “Handbook of Conducting Polymers” (T.A. Skotheim, ed.) Marcel Dekker, New York, 2 (1986) 859.
19. A.J. Heeger in“Handbook of Conducting Polymers” (T.A. Skotheim, ed.), Marcel Dekker, New York, 2 (1986) 729.
20. J.H. Burroughes and R.H. Friend in Conjugated Polymers: The Novel Science and Technology of High Conducting and Nonlinear Optically Active Materials (J.L. Breadas and R. Silvey, eds.), Kluwer Academic, Dordrecht, The Netherlands, 1991, p. 555.

21. R. Noufi, A.J. Frank, A.J. Nozik, *J. Am. Chem. Soc.* 103(1981)1849-1850
22. R. Noufi, D. Tench, L.F. Warren, *J. Electrochem. Soc.* 127(1980)2310-2311
23. R.A. Bull, F.R. Fan, A.J. Bard, *J. Electrochem. Soc.* 130(1983)
24. J.C.W. Chien, Polyacetylene: Chemistry, Physics, and Materials Science (Academic press, New York) 1984.
25. A.O. Patil, Y. Ikenoue, N. Basescu, N. Colaneri, J. Chen, F. Wudl and A.J. Heeger., Synth. Met. 20 (1987) 151.
26. K. Yoshino, S. Hayashi, K. Kaneto, J. Okube, T. Moriya, T. Matsuyama H. Yamaoka., Mol. Cryst. Liq. Cryst. 121 (19850 255.
27. S. Hayashi, S. Takeda, K. Yoshino and T. Matsuyama., Synth. Met. 18 (1987) 591.
28. J.C.W. Chien, P.C. Uden and J.L. Fan., J. Polym. Sci. Polym. Chem. Edn. 20 (1982) 2159.
29. G. Tourillon and F.Garnier., J.Electrochem. Soc. 130 (1983) 2042.
30. H. Munstedt, H. Naaarmann and G. Kohler., Mol. Cryst. Liq. Cryst. 118 (1985) 129
31. M. Satoh, K. Imanishi, Y. Yasuda, R. Tsushima, H. Yamasaki, S. Akoi and K. Yoshino., Synth. Met. 30 (1989) 133.
32. K. Sasto, M. Yamaura, T. Hagiwara, K. Murata and M. Tokumoto., Synth. Met. 40 (1991) 35.
33. F.Mohammad, P.D.Calvert and N.C.Billingham., Synth., Met. 66 (1994) 33.
34. F.Mohammad, P.D.Calvert and N.C.Billingham., Bull Mater. Sci. 18 (1995) 255.
35. F.Mohammad, P.D.Calvert and N.C.Billingham., Bull. Electrochem. 10 (1994) 508.
36. H. Keiss, G. Meyer, D. Baeriswyl and G. Harbeke., J.Electron Mater 9 (1990) 763.
37. S.B. Adeloju, *Analyst*, 121(1996)699-703
38. P.N. Bartlett, P.R. Birkin, *Synth. Met.*, 61(1993)15-21
39. W.J. Sung, Y.H. Bae, *Anal. Chem.* , 72(2000), 2177-2181
40. A. Heller, *Acc. Chem. Res.*, 23(1990)128-134
41. P.R. Unwin, A.J. Bard, *Anal. Chem*, 64(1992)113-119
42. W. Lu, H. Zhao, G.G. Wallace, *Anal. Chim. Acta.*, 315(1995)27-32
43. A. Mulchandani, C.L. Wang, *Electroanalysis*, 8(1996)414-419
44. M. Situmorang, D.B. Hibbert, J.J. Gooding, *Electroanalysis*, 12(2000)111-119
45. P.N. Bartlett, R.G. Whitaker, *Biosensors*, 3(1988)359-379

46. A. Gambhir, M. Gerard, S.K. Jain, B.D. Malhotra, *Appl. Biochem. Biotechnol.* (2001) in press
47. B.A. Gregg, A. Heller, Anal. Chem. 62(1990)258-263
48. A. Heller, *Acc. Chem. Res.*, 23(1990)128-134
49. M.S. Wrighton, *Science*, 231(1986)32-37
50. G.P. Kittlesen, H.S. White, M.S. Wrighton, *J. Am. Chem. Soc.* , 106(1984)7389-7396
51. E.W. Paul, A.J. Ricco, M.S. Wrighton, *J. Phys. Chem.* , 89(1985)1441-1447
52. P. De Taxis du Poet, S. Miyamoto, T. Murakami, J. Kimura, I. Karube, *Anal. Chin Acta.*, 235(1990)255-264
53. M. Umana, J. Waller, *Anal. Chem.* , 64(1986)2979-2983
54. N.C. Foulds, C.R. Lowe, *J. Chem. Soc. Faraday Trans. I*, 82(1986)1259-1264
55. C. Iwakura, Y. Kajiya, H. Yoneyama, *J. Chem. Soc. Chem. Commun.*, (1988)1019-1020
56. P.N. Bartlett, R.G. Whitaker, *Biosensors*, 3(1988)359-379
57. P.C. Pandey, *J. Chem. Soc. Faraday Trans. I*, 84(1988)2259-2265
58. W.E. Price, G.G. Wallace, H. Zhao, *J. Membr. Sci*,, 87(1994)47-56
59. A. Mirmohseni, W.E. Price, G.G. Wallace, *Polymer Gels and Networks 1*, (1993) 61-77
60. P.R. Teasdale, G.G. Wallace, *Analyst*, 118(1993)329-334
61. M Trojanowicz, T.K. vel Krawcyzk, *Chem. Anal.*, 42(1995)199-213
62. www.isbu.ac.uk/biology/enztech/biosensors
63. P.W. Stoecker, A.M. Yacyncych, *Selective Electrode Rev.* 12 (1990) 137
64. A.P.F. Turner, I. Karube, G.S. Wilson, *Biosensors : Fundamentals and applications*, Oxford University Press, Oxford, England (1987) 86
65. P.W. Stoecker, A.M. Yacyncych, *Selective Electrode Rev.* 12(1990)137
66. E.C. Hernandez, A. Witkowski, S. Daunert, L.G. Bachas, *Mikrochim Acta.* 121(1995)63
67. A. Chaubey, M. Gerard, R. Singhal, V.S. Singh, B.D. Malhotra, *Electrochimica Acta.* 46(2000)723
68. Y. Xu, G.G. Guilbault, S.S. Kuan, *Enzym. & Microbl. Technol.* 12 (1990) 104
69. P. De Taxis du Poet, S. Miyamoto, T. Murakami, J. Kimura, I. Karube, *Anal. Chim.*

Acta., 235(1990)255-264

70. M. Umana, J. Waller, *Anal. Chem.*, 58(1986)2979
71. D. Belanger, J. Nadreau, G. Fortier, *J. Electroanal. Chem.*, 274(1989)143
72. D. Belanger, E. Brassard, G. Fortier, *Anal. Chim. Acta.*, 228(1990)311
73. P.N. Bartlett, R.G. Whitaker, *J. Electroanal. Chem.*, 224(1987)3762.
74. www.isbu.ac.uk/biology/enztech/amperometric
75. www.isbu.ac.uk/biology/enztech/potentiometric
76. A.Q. Contractor, T.N. Sureshkumar, R. Narayanan, S. Sukeerth, R. Lal, R.S. Srinivasa, *Electrochim Acta* , 39(1994)1321-1324
77. K. Ramanathan, M.K. Ram, B.D. Malhotra, A.S.N. Murthy., *Mater. Sci. Engg. C*, 3(1995)159-163
78. K. Ramanathan, M.K. Ram, M.M. Verghese, B.D. Malhotra., *J. Appl. Polym. Sci.*, 60(1996)2309-2316
79. M. Nishizawa, T. Matsue, I. Uchida, *Anal. Chem*., 64(1992)2642-2644
80. M. Gerard, K. Ramanathan, A. Chaubey, B.D. Malhotra., *Electroanalysis*,12(1999)450-452
81. A. Chaubey, R. Singhal, M. Gerard, B.D. Malhotra, Pro*ceeding of the 6th Asian Conference of Solid State Ionics: Science and Technology,* World Scientific Publishing Company Ltd., Singapore (1998)479-483
82. R. Singhal, A. Chaubey, K.K. Pandey, B.D. Malhotra., *4th National Conference on Solid State Ionics,* 3-5 March 2000 at Indian Institute of Technology, Mumbai (2000)
83. K. Mosbach, B. Danielsson, *Anal. Chem.*, 53(1981)83A-94A
84. Xie et al. [Insert from Gerard Paper at NPL Book.]
85. M.Shichiri, N. Asakawa, Y. Yamasaki, R. Kawamori, H. Abe, *Diabetes Care* 9(1986)298
86. K. Ramanathan, S.S. Pandey, R. Kumar, A. Gulati, A.S.N. Murthy, B.D. Malhotra, *J. Appl. Polym. Sci.*, 78(2000)662-667
87. K.A. Erickson, P. Wilding, *Clin. Chem.*, 39(1993)287
88. R. Singhal, W. Takashima, K. Kaneto, S.B. Samanta, S. Annapoorni, B.D. Malhotra, *Sens. Actuat. B*, 86(2002)42-48
89. A. Chaubey, K.K. Pandey, M.K. Pandey, V.S. Singh, Appl. Biochem. Biotechnol.

96(2001), 239-248

90. G. Palleschi, M. Mascini, L. Bernardi, P. Zeppli, Med. Biol. Eng. Comput., 28(1990)B25-28
91. E. Dempsey, D. Diamond, M.R. Smyth, G. Urban, G. Jobst, I. Moser, E.M.J. Verpoorte, A. Manz, H.M. Widmer, K. Rabenstein, Anal. Chim. Acta, 346(1997)341-349
92. T. Tatsuma, T. Watanabe, Anal. Chim. Acta 242(1991)85-89
93. F. Palmisano, D. Centonze, P.G. Zambonin, Biosense. Bioelectron. 9(1994)471-479
94. T.J. Ojhara, R. Rajagopalan, A. Heller, Anal. Chem. 66(1994)2451-2457
95. D. Pfeiffer, B. Moller, N. Klimes, J. Szeponik, S. Fischer, *Biosens. Bioelectron.* 12(1997)539-550
96. A.L. Hart, A.P.F. Turner, *Biosens. Bioelectron.* , 11(1996)263-270
97. N.G. Patel, A. Erlenkotter, K. Camman, G.C. Chemnitius, *Sens. Actuators B*, 67(2000)134-141
98. C.I. Li, Y.H. Lin, C.L. Shih, J.P. Tsaur, L.K. Chau, *Biosens. Bioelectron.* 17(2002)323-330
99. A. Kumar, Rajesh, S.K. Grover, B.D. Malhotra, *Anal. Chim. Acta*., 414(2000)43-50
100. A. Kumar, Rajesh, A. Chaubey, S.K. Grover, B.D. Malhotra, *J. Appl. Polym. Sci*., 82(2001)3486-3491
101. B.F.Y. Yon Hin, C.R. Lowe, *Sens. Actuators B*, 7(1992)339-342
102. J. Motonaka, L.R. Faulkner, *Anal. Chem.* 65(1993)3258-3261
103. M.A.T. Gilmartin, J.P. Hart, *Analyst*, 119(1994)2331-2336
104. H. Kumar, Biotechnol. *Appl. Biochem.* 30(1999)231-233
105. J.C. Vidal, E. Garcia, J.R. Castillo, *Anal. Sci.* 18(2002)537-541
106. G.K. Vengatajalabathy, F. Mizutani, *Sens. Actuators B*, 80(2001)272-277
107. G. Mararazza, I. Chianella, M. Mascini, *Biosens. Bioelectron.* 14(1999)43-51
108. J. Wang, X. Cai, G. Rivas, H. Shriaishi, N. Dortha, *Biosens. Bioelectron.* 12(1997)587-599
109. Cosnier, B. Galland, A. Le Pellec. *Electroanalysis* 10(1998)808-813
110. Millan, S.R. Mikkelsen, *Anal. Chem.*, 65(1993)2317-2323
111. Hashimoto, K. Ito, Y. Ishimori, *Sens. Actuat. B* 46(1998)220-225

112. Steel, T.M. Herne, *Anal. Chem.* 70(1998)4670-4677
113. Maruyama, J. Motonaka, Y. Mishima, Y. Matsuzaki, I. Nakabayashi, Y. Nakabayashi, *Sens. Actuator B,* 76(2001)215-219
114. Livache, A. Roget, E. Dejean, C.Barthlet, G. Bidan, R. Teoule, *Synth. Metals* 71(1994)2143-2146
115. A. Gambhir, M. Gerard, S.K. Jain, B.D. Malhotra, *Appl. Biochem. Biotechnol.*, 96(2001)303-309
116. T.T. Nikiforov, Y.H. Rogers, *Anal. Biochem.* 227(1995) 201-209
117. Lund, R. Schmid, D. Rickwood, E. Hornes, *Nucleic Acids Res.* 22(1988)10861-10880
118. Patolsky, Y. Weizmann, I. Willner, *J. Am. Chem. Soc.* 124(2002)770-772
119. Ferguson, F.J. Steemers, D.R. Walt, *Anal. Chem.* 72(2000)5618-5624
120. A.G. Frutos, S. Pal, M. Quesada, J. Lahiri, *J. Am. Chem. Soc.* 124(2002)2396-2397
121. Patolsky, A. Lichtenstein, J. *Am. Chem. Soc.* 123(2001)5194-5205
122. R . Singhal, A. Gambhir, M.K. Pandey, S. Annapoorni, B.D. Malhotra, *Biosens. Bioelectron. ,* 17(2002)697-703
123. S. Hirose, M. Hayashi, N. Tamura, *Anal. Chim. Acta.* 151(1983)377-382
124. P.C. Pandey, A.P. Mishra *Analyst* 113(1988)329-331
125. W.J. Cho, H.J. Hunag, *Anal. Chem.* 70(1998)3946-3951
126. R. Koncki, A. Radomska, S. Glab, *Talanta,* 52(2000)13-17
127. A. Gambhir, M. Gerard, A.K. Mulchandani, B.D. Malhotra, *Appl. Biochem. Biotechnol.* 96(2001)249-257
128. A. Gambhir, A. Kumar, B.D. Malhotra, B. Miksa, S. Slomkowski, e-polymers,(2002), http://www.e-polymers.org
129. T. Osaka, S. Komaba, Y. Fujino, T. Matsuda, I. Satoh, *J. Electro-chem. Soc.*, 146 (1999)615-619
130. M. Myerhoff, G.A. Rechnitz *Anal. Chim. Acta* 85(1976)277-285
131. G.G. Guilbault, P.R. Coulet, *Anal. Chim. Acta* 152(1983)223-228
132. A.J. Killard, M.R. Smyth, *Tibtech* 18(2000)433-437
133. W.O. Ho, S. Krause, C.J. McNeil, J.A. Pritchart, R.D. Armstrong, D. Athey, K. Rawson, *Anal. Chem.* 71(1999) 1940-1946

134. www.nrdcindia.com

135. www.chem.uwec.edu/chem 406/web pages 97

136. Marco M.P. and Barcelo D. (1996) Enviornment application analytical biosensors measurement Sci Technol, 7, 1547-1572.

137. Bartlett P. N. and Ling-chung S.K. (1989a) conducting polymer gas sensors part response of polypyrrole to methanol vapours, Sens. And Actuat B, 19, 141-150.

138. Bartlett P. N. and Ling-chung S.K. (1989b) conducting polymer gas sensors part II result for 4 different polymer and 5 different vapours Sens. And Actuat B, 20, 287-292.

139. Bartlett P. N., Archer P.B. M, Ling chung S.K. (1986c). conducting polymers gas sensors part I: fabrication and characterization, Sens and actuat. 19, 125-140.

140. Charlesworth J. M., Partridge A.C., Garrad N. (1993) Mechanistic studies in the interaction b/w polypyrrole and organic vapour, J. Phys. Chem. 97, 5418-5423.

141. Adeloju S.B. and yuan Y.J. (1999) polypyrrole based flow amperometric biosensor for formate , electrochem. News, online (http://www.Uws.edu.au)

142. Lepsenyi I., Reichardt A., Inzett G., Harsanyi G. (1999) highly sensitive and selective polymer based gas sensors, prog. of twelth european microelectronics and packaging conf., Harrogate, UK, 301-305.

143. Stephens S.K., Gullen D.C, Warner P. J. (1998) Biosensors for novel assay methods in food industry, New food, 1, 47-51.

144. Stredansky M, Pizzariello A, Stredansky S., Miertus S. (1999) Detrmination of D fructose in food stuff by an improved amperometric biosensor based on a solid binding matrix, Anal. Commun., 36, 57-61.

145. Umana M. and Waller J. (1986) protein modified electrodes, the glucose oxidase/ polypyrrole system. Anal. Chem., 58, 2979-2983.

146. Chaubey A., Pandey K.K., Singh V.S. and Malhotra B.D. (2000a) immobilization of lactate oxidase and lactate dehydrogenase on conducting polyaniline films Acta 407.

147. Ghosh (Hazra)S., Sarker D., Mishar T.N. (1998) Development of an amperometric enzyme electrode biosensor for fish freshness detection, Sens and Actuat B 53, 58-62.

148. Skothein, T. J. In *Handbook of Conducting Polymers*; Dekker, M., Ed.; New York 1986; Vols. 1 and 2. 149. Anderson, M. R.; Mattes, B. R.; Reiss, H.; Kaner, R. B. *Science* **1991**, *252*, 1412.

150. Liang, W. B.; Martin, C. R. *Chem. Mater.* **1991**, *3*, 390.

151. Gustafsson, G.; Cao, Y.; Traecy, G.M.; Klavetter, F.; Colaneri, N.; Heeger, J. *Nature* **1992**, *357*, 477.

152. Grem, G.; Leditzky, G.; Ullrich, B.; Leising, G. *Adv. Mater.* **1992**, *4*, 36. 153. Barlett, P. N.; Birkin, P. R. *Synth. Met.* **1993**, *61*, 15.

154. Conklin, J. A.; Huang, S. C.; Huang, S. M.; Wen, T. W.; Kaner, R. B. *Macromolecules*, **1995**, *28*, 6522.

155. Skotheim, T. A. In *Electroresponsive Molecular and Polymeric Systems*; Dekker, M., Ed.; New York, 1991, Vol 1, p.106.

156. Kaufman, F. B.; Schoeder, A. H.; Engler, E. M.; Kramer, S. R.; Chambers, J. Q. *J. Am. Chem. Soc.* **1980**, *102*, 483.

157. J.C.W. Chen. E. Wnek, F.E. Karasz and J.A. Hirsch, Macromolecules, 14 (1981) 479.

158. S.D.D.V. Roghooputh, S. Hotto, A.J. Heeger and F. Wudl J. Polym. Sci.Polym. Phys.25 (1987) 1071.

159. K. Yoshino .S. Nakajimma and R. Sugimoto, Jpn. J. of Appl. Phys. 26 L- (1987) 1038.

160. W.S. Huang, and J.M. Park, J.Chem Soc. Chem. Commun., (1987) 856.

161. M.Aldissi Polym. Plast. Technol. Engg. 26 (1997) 45.

162. S.S.Pandey,S. Annaporni and B.D. Malhotra Macromolecules, 26 (1993) 190.

163. C.H.Su, Synth. Met. 41 (1991) 671

164. B. Johnstone, Eastern Economic Review, 140 (1988) 78.

165. A. Andreatta, A.J. Heeger and P.Smith, Polym, Commun., 31 (1990) 275.

166. L.H.D ao, M. Leclerc, J.W.Chenalier, Synth. Met. 29 (1989) E377.

167.E.T. Kang, K.G. Neoh, K.L. Tan, M.H. Kuok and B.T.G. Tan, Liq. Cryst. Mol. Cryst., 178 (1990) 219.

168. A. Gruger, A. Novak, A. Regis and ph. Colomban, J. Mol. Structure, 328 (1994) 153.

169. P. Snauwaert, R. Lazzaroni, J. Riga, and J.J Verbist, Synth. Met. 18(1887) 335-340.

170. Dong-pil Kang and Mun-Soo Yun, Synth. Met. 29(1989) E 343-E3498.

171. B.M. Prasad, D. Singh. and R.A.Mishra, J. Polym. Mater, 13 (1996) 305.

172. S. Cattarain, L. Doubova, G. Mangoli and G. Zooti; Electrochemica Acta, 33 (1988) 1077-1084.

173. A.G.Green and A.E. Woodhead., J. Chem. Soc. Trans. 97 (1990) 2388.

174. D. Macinnes and B.L. Funt., Synth. Met. 25 (1988) 235.

175. J. Yue and A.J. Epstein., J. Am. Chem. Soc. 112 (1990) 2800.

176. J . Yue, A.J. Epstein and A.G. MacDiarmid.,m Mol. Crys. Liq. Crys. 189 (1990) 255.

177. M.R. Bryce, A. Chissel, P. Kathiramanathan. D. Parker and N.R.M. Smith., J. Chem. Soc. Chem. Commun. (1987) 466.

178. W.J. Koros, M.R. Coleman and D.R.B. Walker, Annu. Rev. Mater. Sci., 22 (1992)

179. G. Tourillon, D. Gourier, P. Garnier and D.Vivien., J. Phys. Chem. 88 (1984) 1049. 47.

180. G. Horowitz and F. Garnier., Sol. Energy. Meter 13 (1986) 47.

181. T. Skotheim, I. Lundstrom, A.E. Delahoy, F.J. Kampas and P.E. Vanier ., Apply. Phys. Lett. 40 (1982) 281.

182. Y. Ohmori, Y. Manda, H. Takahashi, T. Kawai and K. Yoshino ., Jpn. J.Appl. Phys. 29 (1990) L837.

183. P.M. Grant, T. Tani, W.D. Gill. M. Krounbi and T.C. Clarke., J. Appl. Phys. 52 (1981) 869.

184. K. Kaneto, S. Takeda and K. Yoshino., Jpn. J. Appl. Phys. 24 (1985) L553.

185. M. Campos, B. Bello; Jr., J. Phys., 26 (1993) 1274.

186. D.J. Elliot; Integrated Circuit Febrication Technology (Mcgrow Hill, New Yark 1982)

187. S.S. Pandey, M.K. Ram, V.K. Srivastava and B.D. Malhotra., J. of Appl. Poly. Sci. 65 (1997) 2745.
188. S.S.Pandey, S.C.K. Mishra, B.D. Malhotra and Subhash Chandra., J. of Appl. Poly. Sci. 44 (1992) 1911.
189. H.K. Caudhary and D.S. Kelker., J. Aoly. Sci. 61 (1996) 561