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A concise assessment of electrochemically synthesized polypyrrole amperometric biosensors- A Review

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ABSTRACT

Amperometric biosensors have gained an immense popularity in the clinical diagnostic area. Conducting polymers in amperometric biosensors are used to inhibit the reduction of other electroactive species besides the substrate of interest. Polypyrrole is used as a matrix for the enzyme immobilization on its surface electrochemically, owing to its good electrical conducting properties. Hydrogels, polyacrylamide are used to prevent the leaching of this enzyme from the polymer film. With the development of nanotechnology some enzyme less biosensors have also been reported in which nanoparticles act as a recognition site for the analyte. The synergistic combination of biotechnology, nanotechnology and polymer chemistry yields a biological recognition element which when coupled with a compatible transducer device leads to the formation of electrobio composites of polypyrrole. This review describes the use of amperometric biosensors for the detection of glucose and cholesterol.

Keywords: Amperometric, Polypyrrole, Transducer, Biosensors, Enzymes.

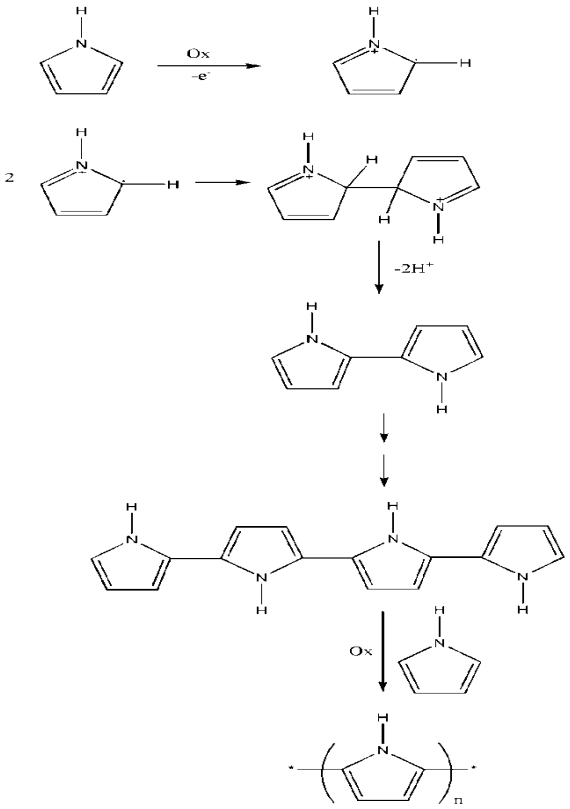
INTRODUCTION

Conducting polymers are the organic metals. Due to their unique properties like electronic conducting properties, optical properties, chemical and biochemical properties they have been used in wide range of applications including battery technology, photovoltaic devices, light emitting diodes, electrochromic displays, energy storage devices (solar cells) and more recently in biological application. With the discovery of biosensors in 1956, afore mentioned properties of conducting polymers make them potential candidates for sensing applications [1-3]. Among many conducting polymers like polythiophene and its derivative, polyaniline, poly*(p-phenylene vinylene)* etc. polypyrrole (PPy) is the choice of researchers as a biosensor due to its good conductivity and its ease of formation via electrochemical synthesis and also its stability towards air oxidation [4]. For sensing applications, biosensors convert chemical information or bio interactions into electrical or optical signals, which can easily be detected by modern techniques and the electronic properties of polypyrrole causes a fast, direct electron charge transfer between the polymer matrix and the analyte immobilized with it, hence yielding sensitive and selective signal [5]. In the formulation of biosensors, PPy acts as a matrix which provides covalent binding sites to enzyme therefore making an immobilized enzyme matrix substrate.

The compounds which possess relatively lower anodic oxidation potential and are susceptible to electrophilic substitution reaction can produce conducting polymers by electrochemical technique [6]. PPy can be easily prepared by either an oxidative chemical or electrochemical method. However synthetically conductive PPy is insoluble and infusible, which restricts its processing and applications in other fields [7]. Electrochemical polymerization of long-chain alkyl substituted polypyrrole yields a less conducting film, which is soluble in common organic solvents in its conducting state [8].The properties of PPy like tight adherence of PPy to electrode materials, the possibility of introducing functional groups, increase in electrode surface area due to polymer network etc. can be changed by changing the conditions during electrochemical polymerization. Biosensors basically are the diagnostic devices in which the biological component in conjunction with a transducer device converts a biochemical signal into an amplified electrical signal [9]. An efficient biosensor should have accurate, precise, reproducible and linear response over the useful analytical range, without dilution or concentration. It should also be free from electrical noise. The reaction should be as independent of physical parameters such 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 immobilized with the enzyme [10]. Depending on the nature of transducer used in biosensors, they can be categorized as calorimetric biosensors, potentiometric biosensors, amperometric biosensors, optical biosensors and piezo-electric biosensors. In this review our main focus is on amperometric biosensors based on polypyrrole matrix.

POLYPYRROLE

The electrical properties of polypyrrole make it a suitable candidate for biosensors. Polypyrrole (PPy) can be formed chemically or electrochemically through oxidative polymerization of pyrrole monomer. By controlling oxidation potential of the PPy, its conductivity can be varied over several orders of magnitude, covering a range that is suitable for using them as sensors (Scheme 1).



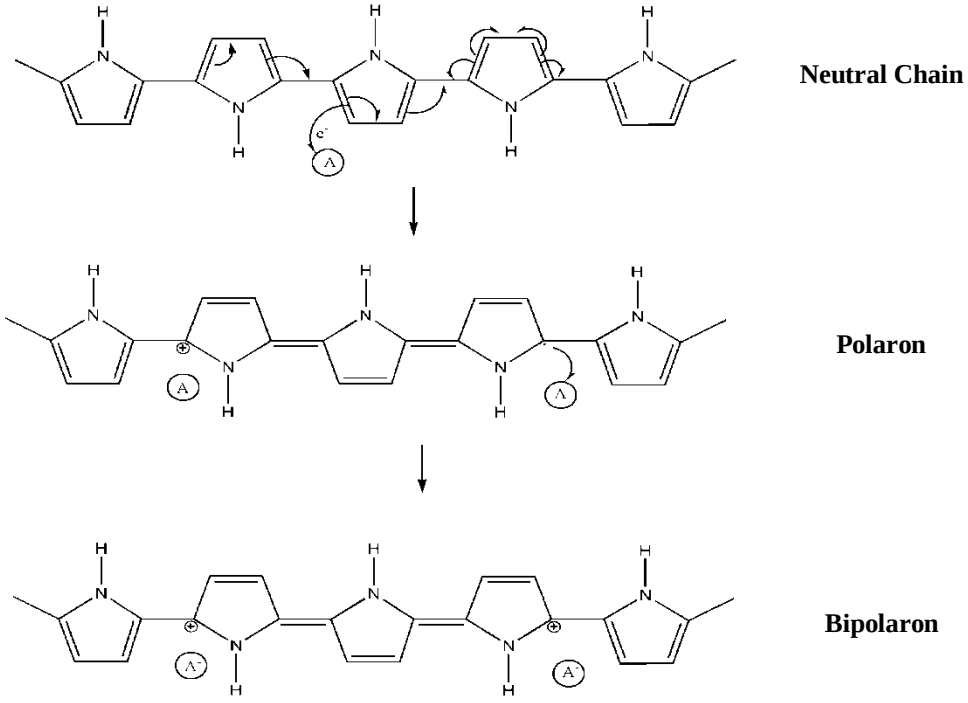
Scheme 1. Mechanism of polymerization of pyrrole

Electropolymerization of polypyrrole: Electropolymerisation cell assembly consists of three electrode system namely working electrode, reference electrode and counter electrode (auxiliary electrode) in presence of suitable electrolyte such as NaCl, KCl etc which furnish supporting ions in order to provide smooth drift velocity to the pyrrole molecules [11].

The **working electrode** is the electrode at which the reaction of interest is occurring. Common working electrodes can be made of inert materials such as Au, Ag, Pt, glassy carbon (GC) and Hg drop and film electrodes etc. The **reference electrode** must have a known and stable value of electrode potential and it is used as a point of reference in the electrochemical cell for the potential control and measurement. The high stability of the reference electrode potential is usually reached by employing a redox system with constant or buffered concentrations of each participant in the reaction. Counter electrode controls the amount of current supply to the reference electrode. Its role is to adjust the current flow through the reference electrode near zero (ideally zero) [12].

The oxidative electropolymerization of pyrrole to polypyrrole proceeds as shown in scheme 1. Formation of monomer radical cation by electrochemical oxidation takes place from +0.77 V to 0.8 V using Ag/AgCl reference electrode. The success of electropolymerization of pyrrole is due to the stability of the radical through charge delocalization and the ease of electro-oxidation. However polypyrrole formed by this method has a processability problem as it is very less soluble in solvents. To improve the processability, development of soluble and swollen PPy has been reported on adding suitable doping agent [7].

Conduction mechanism of polypyrrole: Polaron and bi polaron are the charge carriers responsible for the conduction in oxidized form of polypyrrole as shown in scheme 2 [13].



Scheme 2. Conduction mechanism of polypyrrole

AMPEROMETRIC BIOSENSORS

A biosensor is an analytical tool through which quantitative and qualitative information such as detection and concentration of the desired analyte from the system under study is obtained, such as measuring

glucose, lactate, alcohol, sucrose, galactose, uric acid levels in humans. It is a layered structure comprising of the biological recognition element complementary to the analyte attached to a matrix which is further joined to a transducer or directly attached to the transducer. Basically it performs four functions, firstly the biological recognition element interacts with the analyte, secondly this interaction then produces a chemical signal, thirdly this chemical signal is converted by the transducer into a readable output which is amplified by an amplifier and lastly the information is displayed on the computer. A representation of the working of biosensor is depicted in figure 1. Thus, the classification of biosensors is based on the type of biological recognition element used, on the type of transducer used, on the method by which the biological recognition element is attached to the matrix or transducer and method of interaction between analyte and biological recognition element. Table 1, representing the characterization is shown below.

Table 1. Classification of Biosensors

Type of biological recognition element	Biosensor
Proteins, enzymes Nucleic acids (DNA, RNA, etc.) Antibodies Microbes	Enzyme biosensor Nucleic acid biosensor Immunosensor Microbial biosensor
Type of transducer and Biosensor	Measured property
Electrochemical Optical Calorimetric Piezoelectric	Amperometric Potentiometric Impedence Absorbance Fluorescence Chemiluminiscence Heat changes Mass changes
Type of attachment between analyte and matrix/ transducer	
Covalent, crosslinking Adsorption, entrapment, encapsulation	Chemical biosensors Physical biosensors

Also the type of interaction taking place between the analyte and biological recognition element that is responsible for producing the signal, classifies biosensors into affinity, metabolism and catalytic biosensors. If the signal is produced by the binding of the two species it is called affinity biosensor, if the interaction causes a chemical reaction and the concentration of either the reactant or the resulting component is sensed then it is called as metabolism biosensor and if the signal producing event is due to the catalytic effect of the biological recognition element where it converts the analyte into a measurable species then it is known as catalytic biosensors [14-16].

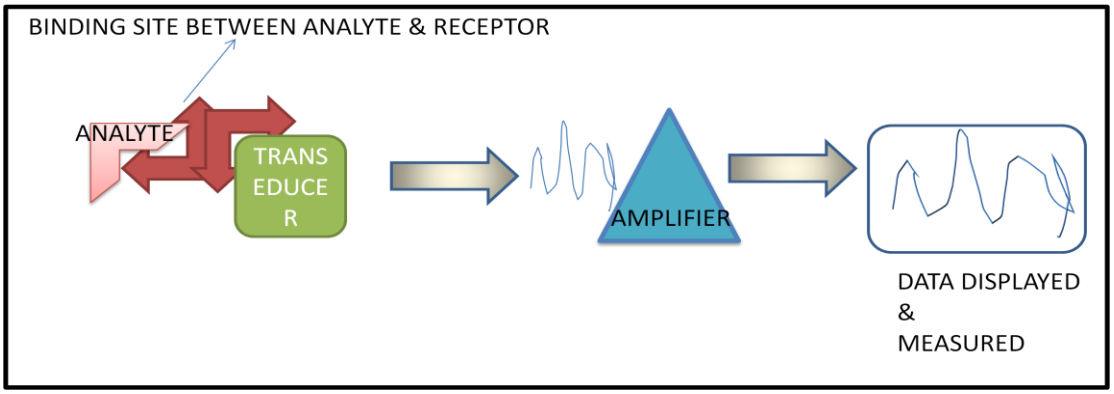


Figure 1. A representation showing working of biosensor

Amperometric biosensors are the ones in which the transducer used is elecetrochemical and the measured signal is the current which is proportional to the concentration of the analyte being sensed.

Amperometric biosensors are the oldest sensors to be developed starting the concept of biosensors. It started with the development of oxygen electrode in 1956 by Professor Leland C Clark Jnr. who is known as the Father of Biosensors and the first description of amperometric biosensor was made in 1962 by Clark and Lyon in the form of amperometric enzyme electrode for glucose. The technology of Clark was taken by the Yellow Springs Company which produced the first commercial glucose biosensor in 1975. In 1984 the first mediated amperometric glucose biosensor was made in which the mediator was ferrocene. All this while many other developments were taking place simultaneously in this field with the production of potentiometric and surface plasmon resonance based biosensors. The achievement of this field can be judged by looking at the number of publications increasing each year [17].

Principle of amperometric biosensors: In very simple words it can be stated that the interaction between the analyte and the bioreceptor is converted into the current signal by the amperometric transducer which is measured and displayed by the electronic processors which is then correlated with the analyte's concentration.

Amperometric measurements are done in three electrode cell system which comprises of the working electrode, reference electrode and the counter electrode. The working electrode is composed of the bioreceptor attached to the transducer, the reference electrode commonly used are Ag/AgCl electrode or the calomel electrode and the counter electrode generally used is the platinum electrode responsible for keeping the electrode potential of the reference electrode constant.

Amperometry is based on redox reactions in which the current is measured at a static potential value in an electrochemical cell. The value/ amount of the current produced depends on the substance present in the electrolytic solution capable of showing redox chemistry transferring electrons to the working electrode [18].

Three types of amperometric biosensors are known, first in which the reaction product which is electroactive causes the signal by its reduction or oxidation. This type of biosensor usually involves oxidase enzymes which work at higher electrode potentials thus causing other species in the environment to get oxidised resulting in miscalculation of the result. Moreover since it is based on the content of the dissolved oxygen, systems with scarce percentage of dissolved oxygen suffer. Second type are those in which helping agents are used which exist between the reaction and the transducer facilitating fast transfer of electrons between the redox centre and the working electrode and also eliminating the chances of extraneous species to be oxidised as these work on low electrode potentials thus preventing the result to be confounded thereby removing the disadvantage of the first type. Furthermore these helping agents reduce the dependence on dissolved oxygen content. Though quite successful in removing the short comings of the earlier one but couldn't completely eliminate them. Also its stability is an issue. The third type of amperometric biosensor produces the signal through the movement of electrons between the bioreceptor and the electrode [17-19].

In the first type the enzyme oxidises the analyte in the presence of dissolved oxygen and the product produced is H_2O_2 that is measured by applying a potential to oxidise it and the electrons involved are recognized by the working electrode or the decrease in concentration of dissolved oxygen can also be measured which is detected by applying a negative voltage on the working electrode to reduce it whereby the electrons involved are sensed by the electrode and current is produced, glucose biosensor being one of the examples. In the second type the enzyme oxidizes the analyte and the reduced enzyme comes back to its oxidized form not by using oxygen but by the helping agent which gets reduced. Then a potential is applied corresponding to the helping agent which is low, by which it gets oxidised and the number of

electrons are detected by the electrode, for example glucose, fructose, lactate biosensors in which the mediator is ferrocene, ferricyanide and ferrocene respectively. In the third type, the enzyme is capable of accepting electron from or giving the electron to the electrode directly without any middle agent. The redox enzyme gets reduced at a particular potential with respect to the reference electrode and then in the presence of analyte again gets oxidised while reducing the analyte, for example biosensors based on cytochrome c, horse-radish peroxidase etc. [18,20,21].

Applications of amperometric biosensors: Amperometric biosensors have proved useful in a number of areas namely pharmaceutical, medical, food and environment. These devices are used for the monitoring of alcohols, wines; for detecting harmful pesticides, chemicals and pathogens in food samples and also to check the freshness of food thus providing a control measure in order to provide safe and secure food products. In the medical field many biosensors are already in use namely glucose, urea, penicillin and L-alanine and pyruvate in biological fluids. These are also used in disease identification. Evaluation of pharmaceutical products is also carried out by amperometric biosensors. These have uses in environmental examination like sensing of phenolic compounds, inorganic phosphate, surfactants, pesticides, Escherichia Coli, and even in determination of biological oxygen demand [22-31].

Glucose amperometric biosensors: These biosensors are used to detect the glucose level in blood serum of human beings. Clark who is regarded as the father of biosensor, along with Lyon first discovered the glucose biosnsor in 1962. Enzyme electrode used in these biosensors, detect glucose by measuring, the oxygen consumption by the enzyme glucose oxidase by applying negative reduction potential.

$$C_6H_{12}O_6 + O_2 \xrightarrow{\text{Glucose Oxidase}} C_6H_{12}O_7 + H_2O_2$$

A negative potential was applied to the platinum cathode for a reductive detection of the oxygen consumption

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

Later on with more research, glucose detection was made on the basis of consumption of hydrogen peroxide amperometrically, measured by applying the more negative reduction potential to working electrode. This method was more precise and accurate, but, in blood along with glucose there are other electroactive species like acids (uric acid and ascorbic acid) which are also susceptible to undergo reduction, therefore causes interference with the detection of glucose.

In order to prevent the interference from electroactive species, enzymes are immobilized on the surface of conducting polymers and these bio composites of conducting polymers now prevent the movement of elctroactive species towards the surface of anode (working electrode). These biocomposites are selective in nature having good transport properties with suitable charge [32].

In glucose sensors, glucose oxidase enzyme is immobilized electrochemically on the surface of polypyrrole matrix and in order to prevent the leaching of this enzyme from the matrix, use of appropriate amount of buffers or stabilizing agent like polyacrylamide has been reported [33].

With the development of nanotechnology, incorporation of nanoparticles has also been reported with the objective to improve the binding sites between the analyte (glucose) and glucose oxidase immobilized on the surface of polypyrrole. This in turn increases the efficiency and selectivity thus making the detection of glucose less time consuming. Some nonenzymatic biosensors also have been reported in which nanoparticles acts as an active site for the oxidation of glucose [34]. Efficiency of some of the biosensors has been given in table 2.

Table 2. Various types of glucose biosensors and their efficiency

Type of biosensor	Amount of blood sample needed (for Glucose estimation)
Enzyme electrode system (oxygen based)	25 µl
Enzyme electrode system (hydrogen peroxide based)	100 µl
Enzyme immobilized on polypyrrole	5.6 mmol L^{-1}
Enzyme with carbon nanotubes and platinum nanoparticles	0.5 µM in 3 s
Enzymeless gold nanoparticles dispersed on the surface of polypyrrole	0.2–13 mM

Cholesterol amperometric biosensors: The determination of total cholesterol is a prime requirement for the clinical diagnosis of aberration in lipid metabolism and so hypertension causes life threatening heart stroke. These biosensors consist of an enzyme, cholesterol oxidase (ChOx) immobilized on the surface of polypyrrole electrochemically in presence of hydrogels. Hydrogles/ polypyrrole composites makes a stable, biocompatible biosensor and also prevent the leaching of this enzyme from the polymer matrix. Hydrogels also enhance the rate of electron transfer.

Cholesterol oxidase alone cannot detect cholesterol in the blood serum, therefore, cholesterol amperometric biosensors are based on the combination of two enzymes namely cholesterol esterase and cholesterol oxidase (ChOx). These two enzymes have been reported for total cholesterol determination. First cholesterol esterase produce free cholesterol from the esters of cholesterol present in blood, then cholesterol oxidase (ChOx) bind with free cholesterol and oxidize it and the transducer detects the signal. Efficiency of some of the amperometric cholesterol biosensors [35-36] has been tabulated in table 3.

Table 3. Efficiency of cholesterol biosensor

Type of biosensor	Quantity of cholesterol detected in blood
Cholesterol oxidase on polypyrrole with hydrogels composites	120 µM in 30 s
Cholesterol oxidase on polypyrrole with Prussian-Blue and nafion composites	8 µM in 60 s

From the table values, it is clear that hydrogels based cholesterol biosensors are more efficient and sensitive.

CONCLUSIONS

Biosensors have major clinical responsibility on them. We have seen the major change in their mode of action parallel with the advancement and development of the other fields. With passage of time sensitivity and reliability of these biosensors has been improved since their discovery. Although there are a lot of challenges to combat, along with their improved precession continuous evaluation of the substrate and optimization of the minimum amount of dose are the prime concerns related to these biosensors.

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