Polymers in Biosensors-A review

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ABSTRACT
Biosensors are small, special sensor devices for the concentration determination of compounds with biological relevance on a molecular basis. Three type of biosensors using polymer matarials are reviewed thoroughly.

Keywords*: Biosensers , Polymers*

Introduction-

Fundamental and application examples of polymeric materials in different type of biosensors are presented and discussed in view of their molecular st., biosensor design & construction. The role of series of polymer with respect to their typical application and their specific properties, like sensitivity and stability is highlighted.

Biosensors are small, special sensor devices for the concentration determination of compounds with biological relevance on a molecular basis. Polymer materials are essential components of biosensors because most of them cannot be constructed at all without them.

Generally, biosensors are composed of three units: the receptor as the bio-specific component, the transducer, which converts the measured physical effect, and the electronic component which combine the different parts.

The basis of biosensors is reaction between the immobilized species and the molecules to be sensed which set up a physical signal. During the function of biosensor, the following processes can be discerned: Specific recognition of the analytic transformation of the physiochemical parameter which is a result of the interaction with the receptor, into a signal and signal intensification and processing.
L.C. Clark[1] reported the first biosensor in 1956. His concept becomes commercial reality in 1975 with the successful launch of glucose analyser. According to the functional principles, biosensors are subdivided into several classes: Bioaffinity sensors (alternation of electron density), metabolism sensors (substrate consumption), coupled & hybrid systems and biomimetic sensors. A considerable number of different polymer materials have been investigated in view of their application in biosensors.

The four principal transducer classes are potentiometric, amperometric, optical and other physiochemical. Potentiometric devices measurer the accumulation of charge density the surface of an electrode. Amperometric sensors monitor currents generated when electrons are exchanged between a biological system and on electrode, an example being a blood glucose sensor. Optical biosensors correlate changes in concentration, mass or member to direct changes in the characteristic of light. Other physicochemical sensors monitor biological interactions though changes in enthalpyionic conduce and mass. Guitbault and montalvo[2] were the first to details a potentiometric enzyme electrode. They developed a urea sensor based on Urease immobilized at an ammonium-selective liquid membrane electrode.

Numerous research work have been published on a variety of biosensors with diverse application exploiting enzymes, antibodies, nucleic acids, cell receptors, microbs in electrochemical, optical and piezo electronic and thermometric transducers.

The first biosensors are often called enzyme electrodes and were first described by Clark and Lyons[3] for determination of glucose. Glucose biosensors are based on the fact that the enzymes glucose oridase (GOD) catalyzes the oxidation of glucose to gluconic acid and itself being reduced to GODH. In the early biosensors oxygen was used as the oxidizing agent which oxidizes GODH back to GOD. The consumption of oxygen was followed ampersometrically by electrochemical reduction at a platinum electrode. The glucose oxidation reaction catalyzed by glucose oxidase is
Glucose $+0_2 + H_20 \rightarrow$ gluconic acid $+ H_20_2$. At the electrode: $0_2 + 2\,\bar{e} + 2\,H^+ == H_20_2$

Polymer Films

Polymer films have been used in biosensors to protect the electrode surface from fouling, to block interferences to immobilize the biocomponent to entrap or incorporate a mediator to extend the linear range of the biosensor. "It is interesting to note that all commercial applications have employed polymer films to make the biosensor practical". There are three types of polymer films which are (i) conducting polymers are widely used, often to enhance electron transfer characteristics, (ii) Non conducting films are frequently used for their perm selective characteristics and (iii) composite films offer the strength of blending the favorable characteristics of more than one film.

Conducting Polymer Films

Conducting films are incorporated in biosensors to facilitate electron transfer foam bio component to the electrode surface. The important conducting polymers which are capable of conducting electronic charge are polypyrrole, polythiophene, and polyaniline[4] . Conducting polymers are divided into two categories, electronically conducting polymers for example polyacetylene[5] and redox conducting polymers. For analytical applications polyacetylene, polypyrrole, polythiophene and polyaniline are the most important polymers. The polymers can be produced chemically or through electrochemical polymerization. The biomolecular interaction capabilities of polymers are easily modified by attaching functional groups to the polymer backbone.

In some cases, polymeric precoatings can facilitate electrode position of the conducting polymer. When the electrode substrate was precoated with nafion, electrode position of the conducting polymer was facilitated. The sulfonate charge on the nafion backbone acts as a counter ion for the initial layers of polypyrrole, knitting the conducting polymer to the electrode surface. Conducting polymer coating undergo oxidation/reduction processes at moderate potentials.

A biosensor with an electropolymerized conducting polymer film was also constructed. Entrapment of glucose oxidase in a polyaniline film grown on a platinum electrode was carried out via electro polymerization pH 1.1. The low pH conditions yielded improved conductivity of polyaniline. The conductive polymer in this case facilitated electron transfer from a biocomponent to the electrode surface. Glucose oxidase was used because it tolerated the low pH conditions.

Reagentless enzyme electrodes have been developed by the covalent bonding of enzymes using redox mediators and coenzymes on the sensor surface to prevent contamination of the sample by sensor components. Mass production of enzyme electrodes require techniques for biosensor assembling avoiding manual deposition procedures. The electrochemical deposition of conducting polymer layers occurs at the surface of an electrode independent of its size and form. The morphology of the conducting polymer films can be adopted to exclude cooxidizable compounds firm approaching the electrode surface, thus improve the selectivity of the amperometric enzyme electrodes. Most of the amperometric biosensors described until now are constructed either by cross linking a suitable redox enzyme within a polymeric gel on the electrode surface or by assembling a preformed enzyme-containing membrane on top of the electrode.

Polymer films with defined thickness and morphology can be made by controlling the charge transferred during the electrochemical polymerization process, and parameters like temperature, monomer concentration, polymerization potential or current, as well as the concentration and nature of the supporting electrolyte. Hence, conducting polymer films show interesting properties concerning the decrease of the influence of interfering compounds due to their size-exclusion and ion-exchange characteristics[6].

Functionalized conducting polymers for the covalent binding of enzymes are polypymole, polyaniline and polythiophene. The use of functionalized conducting polymers is inevitable for the integration of bio-selective compounds and redox mediator in the conducting polymer. Functionalized monomers may be synthesized and subsequently polymerized along with the non-functionalized monomer.

Entrapment of enzymes within electrochemically grown conducting polymer films is unique method for entrapment of the enzymes within the growing polymeric network during the electrochemical polymerization process. The mass transfer characteristics will depend on the diffusion of the substrate through the conducting polymer films leading to a chamatic dependence of the signal on the film thickness, the morphology and porosity of the conducting polymer film[8].

Non Conducting Polymer films

Non conducting polymer films are incorporated into biosensors to prevent interferences, to prevent fouling of the electrode surface by proteins and other substances, to immobilize the bicomponent and to entrap mediator so that it does not leach away. Non-conducting films formed by electro polymerization techniques show characteristics of molecular self assembly. The typical film thickness for non-conducting polymer films formed by electro polymerization is about 10 nm. Sasso et al[9] developed a biosensor using a Platonized Reticulated Vitreous Carbon (RVC) electrode with immobilized glucose oxidase and an electro polymerized poly (1, 2 diaminobenzene) (DAB) film that drastically reduced interference from L-ascorbate, wate and L-cysteine and prevented fouling of the biosensor by proteins in blood serem. Additionally, thermal studies performed on electrodes with immobilized glucose oxidase that used carbodimide for covalent bonding or guitaraidehyde for cross linking demonstrated that increased thermal stability was obtained. Enzyme activity was maintained for one and one half months with daily use. The poly (1,2 – DAB) film was less stable than the enzyme (by about one week) and required repolymerization.

Sarkar [10] et al used polyethylerimine in the working electrode paste for aminoacid or protein biosensor and this increased device stability and reproducibility. Malitesta et al [11] immobilized glucose on platinum electrodes by electropolymerizing 1,2 – DAB (O-phenylenediamine) The film exhibited permselectivity in that as corbate., a common interference in physiological systems, was blocked. Barlette et al [12] constructed a glucose biosensor using on electropolymerized phend film to immobilize glucose oxidase subsequent to allowing adsorption into the surface of a platinum electrode.

Composite polymer films

In many cases composite membranes formed from two or more different types of polymers give better properties than the individual polymers. Koopal et al [13] reported the use of glassy carbon disk electrodes into which platinum was sputtered and a mixture of agarose solution and latex suspension was applied to create a porous membrane. Subsequently, electropolymerization of the monomers was performed galvanostatically in aqueous solution to construct a composite film on the electrode. Immobilization of Glucose oxidase was carried out by agitating composite membranes in a solution of glucose oxidase.

Polymer film fabrication

Polymer films on electrode surfaces can be produce by the following:

Solvent Casting-

This method has limited use since it is difficult to obtain complete and uniform coverage especially when one is forming a very thin film. In this approach a polymer film is formed by evaporation of a polymer solution placed on the electrode surface.

Spin coating-

To prepare film-covered electrodes by, this method, the polymer is dissolved in an appropriate solvent and micro liter quantities of the polymer solution are dropped onto the electrode which is mechanically fixed to aspinner that rotates the electrode at a high speed such as 400 rpm. The film is air-dried as it continues to spin for several minutes. Spin – coating is used extensively in the electronics industry.

Electropolymerization-

A thin film of poly pyrrole was deposited along with the glucose oxydase on the working electrode by electro-polymerization of the monomer from an electrolyte of the monomer and glucose oxydase. The unique feature of this work is that the electro polymerization was made on a screen printed electrode using platinum as reference electrode. Glucose oxydase was immobilized in a poly- cationic poly vinyl pyridine polymer that contained fercocene as mediator.

Electropolymerization offers many advantages such as the ability to coat very small as irregularly shaped electrode surfaces with a polymeric film, the ability to control film thickness based upon the amount of charges passed in the case of conducting films or by self regulation in the case of non-conducting films, the ability to influence the polymerization rate and the nature of the film via the applied potential used during electro-polymerization. In addition, electro-polymerization films have been successfully employed in the blockage of interferences that would otherwise undergo oxidation at the electrode surface and foul electrode surfaces.

Adsorption

Adsorption is to be carefully distinguished from absorption. The phenomenon of higher concentration of any molecular species at the surface than in the bulk of a solid is known as adsorption. The forces involved are, evidently inter molecular forces. It can roughly be divided into two classes: physisorption is usually weak and occurs via the formation of van der waals bonds, occasionally with hydrogen bonds or charged transfer forces. Chemisorption is much stronger and involves the formation of co valent bonds. Physical adsorption of a polymer film onto an electrode is a simpe technique for film formation. It is difficult to control film thickness with this method. Often an adsorption step preceeds electro-polymerization that is responsible for the film formation. Immobilization of antibody by cross linking with glutaraldehyde on the sensor surface was done to make the sensor very sensitive to penicillin in milk. The work of Cosneir and Innocent[14]utilized adsorption followed by electro-polymerization for polymer film formation. The cationic surfactant part of amio tyrosinase was substitutaed with pyrrole to form a modified monomer. This was adsorbed onto an electrode surface anol was then electro-polymerized.

Fouling and biocompatibility

A major problem that occurs in the analysis of biological samples is electrode fouling. It is defined as electrode surface passivation due to the adsorption of nonelectroactive species. High molecular weight substances , such as proteins, represent a major source of fouling, which gives decreased biosensor response with time.

A number of techniques to immobilize a biocomponent onto an electrode surface are available including adsorption, covalent attachment, microencapsulation, polymer entrapment, and crosslinking. All of these methods have been used in conjuction with polymer films. In cases where spatial resolution of different bio-components entrapped in a polymeric film is required, such as for different microarray electrodes, the immobilization process is facilitated by the use of electroploymerization techniques. Griffith et al 15 demonstrated that greater amount of enzymes could be entrapped by non conducting electropolymerized poly film. A variety of different bio components have been immobilized in polymeric films and used in construction of bio-sensors. These include glucose oxidase, choline oxidase, cholesterol oxidase, catalase, lactate oxidase, uricase, tyro-sinase, pyruvate oxidase, antibody, virus and chemoreceptors.

Conclusion

The most important part of a biosensor in terms of the material component is based on organic polymers. Their use in biosensors devices serves to enhance the stability, biocompatibility and selectivity. The application of such materials includes the use as supports for enzymes , antibodies, microorganisms, organelles, dyes and as component for the construction of membranes in the transducer of field effect transistors, thermistors, optical signal transformers and piezo electrical crystals.

Polymer films are incorporated in biosensors to facilitate electron transfer from bio-component to the electrode surface for the incorporation of mediators and in the immobilization of enzymes. Knowledge of possible electron transfer pathways plays an important role in the development of anperometric biosensors with improved operation characteristics.

Future work will be directed towards production of reagentless amperometric enzyme electrodes with low working potential, decreased influence of the oxygen partial pressure and interfering compounds.

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