Biosensors for Environmental and Clinical Monitoring

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Abstract
Biosensors are analytical devices composed of a recognition element of biological origin and a physico-chemical transducer. Immobilization plays a major role in developing the biosensor by integrating both the above components. In this article, studies carried out at BARC on immobilization of biological elements and their association with transducers for the development of biosensors for environmental and clinical monitoring, are reviewed. Biosensors for methyl parathion pesticide, catechol, glucose, urea, cholesterol and dopamine are being studied.

Concept of Biosensor
A biosensor is a compact analytical device, incorporating a biological or biologically derived sensing element, either closely connected to, or integrated within a transducer system. The principle of detection is the specific binding of the analyte of interest to the complementary biorecognition element immobilized on a suitable support matrix (Fig. 1). The specific interaction results in a change in one or more physico-chemical properties (viz. pH change, electron transfer, mass changes, heat transfer, uptake or release of gases or specific ions) which can be detected and measured by the transducer. The usual aim is to produce an electronic signal, which is proportional to the concentration of a specific analyte or group of analytes, to which the biosensing element binds.

Fig.1: Principle of biosensors
Biosensors can be classified according to bio-recognition system. The biological elements used in biosensor technology are the enzymes, antibody/antigens and nucleic acids/complementary sequences. In addition, microorganisms, animal or plant whole cells and tissue slices, can also be incorporated in the biosensing system. Depending on the method of signal transduction, biosensors can also be divided into different groups: electrochemical (amperometric, potentiometric or conductometric), optical, thermometric and piezoelectric. Biosensors offer many advantages over conventional analytical techniques. The selectivity of the biological sensing element offers an opportunity for the development of highly specific devices for real-time analysis in complex mixtures, without the need for extensive sample pre-treatment or large sample volumes. The function of a biosensor will depend on the biochemical specificity of the biologically active material. Biosensors also promise highly sensitive, rapid, reproducible and simple-to-operate analytical tools. Biomolecules have poor stability in solutions hence it is necessary to stabilize them by immobilization. Thus immobilization plays a key role in developing stable biocomponent for integration with transducers.

This review summarizes some of our studies related to immobilization of biological molecules on different supports using suitable techniques for the development of biosensors for environmental and clinical monitoring.

**Biosensor for Environmental Monitoring**

Towards monitoring of different environmental pollutants, our laboratory has been working on the development of biosensors for pesticides and phenolic compounds. Methyl parathion pesticide is extensively used in the field of agriculture despite its high toxicity and contributes a major share in terms of restricted use in India. Among the various biosensors for methyl parathion detection, major systems are based on acetylcholinesterase (AChE) and organophosphorus hydrolase (OPH) enzymes. AChE biosensor is based on enzyme inhibition mechanism, hence it requires longer incubation time and also has poor specificity because of interference from carbamate pesticide and metals. OPH catalyzes hydrolysis of methyl parathion pesticide into detectable product p-nitrophenol (PNP) and generates two protons as a result of the cleavage of the P-O bond. Products that are chromophoric and/or electroactive can be detected by colorimetric and electrochemical methods, and are exploited to develop biosensors for detection of methyl parathion pesticide. The analyte can be determined, as the rate of product formation is directly proportional to the concentration of the analyte. As the OPH is a periplasmic enzyme, whole cells can be immobilized directly on the matrix and integrated with transducers for biosensor development. In our laboratory different types of microbial biocomponents (from disposable to reusable) were developed by immobilizing microbial cells on different supports and associated with different transducers (optical and electrochemical) for simultaneous analysis of single to multiple samples. An optical microbial biosensor was developed in which disposable biocomponent was prepared by immobilizing whole cells of *Flavobacterium sp.* on glass fibre filter paper disc and associated with an optical fibre transducer for the detection of methyl parathion. In the second study, *opd* gene, which codes for OPH enzyme, was cloned to make recombinant *E.coli* with high periplasmic expression of enzyme. Recombinant *E.coli* cells were immobilized on screen-printed carbon electrode (SPCE) for preparing the biocomponent and an electrochemical microbial biosensor was developed based on cyclic voltammetry for the detection of methyl parathion. In this study, biocomponent was reusable and biosensor required low volume of sample. In our third study, an optical biosensor was developed for detecting a large number of samples in a single platform in a short period of time. For this, a soil bacterium, *Sphingomonas sp.*
JK1 was isolated and identified which hydrolyzes methyl parathion. Microbial cells were then immobilized onto the surface of the wells of microplate (96 wells) and used as a reusable biocomponent, providing a convenient system for simultaneous analysis of multiple numbers of samples. In another experiment, *Sphingomonas* sp. JK1 was also immobilized on the inner epidermis of onion bulb scale as a natural support and directly placed in the wells of microplate and associated with the optical transducer for monitoring of methyl parathion pesticide.

Phenolic compounds include a large variety of analytes having significance in health care and environmental pollution monitoring. Phenolics constitute a large group of pollutants, which originate from a variety of industrial processes such as manufacture of plastic, paper, dyes, drugs and pesticides. Catechol is one such phenolic derivative which is readily absorbed by the gastrointestinal tract, causing vasoconstriction, renal tube degeneration, decrease in liver function, cancers, and neurodegenerative diseases. Tyrosinase is a polyphenol oxidase enzyme that catalyzes the oxidation of phenolic compounds via hydroxylation with molecular oxygen to catechols and subsequent dehydrogenation to o-quinones which can be reduced at low potentials. Hence this enzyme was employed for the development of a biosensor for low potential detection of phenols and catechol in foods, pharmaceuticals, and clinical and environmental samples. In one study, tyrosinase enzyme was entrapped in agarose–guar gum composite matrix and an electrochemical biosensor was developed for catechol. In another study, tyrosinase was also entrapped on oxidized porous silicon and a conductivity-based biosensor was developed for quantitative estimation of catechol.

**Biosensor for Clinical Monitoring**

Enzymes are well-known as biological sensing materials in the development of biosensors due to their specificity and play a key role in clinical diagnosis. Enzymes like glucose oxidase (GOD), urease, choline oxidase (ChOx) and tyrosinase etc., were immobilized on suitable supports for better stability and reusability and associated with transducers for biosensor monitoring of clinical metabolites such as glucose, urea, cholesterol and dopamine etc.

Detection of glucose has been the most studied analyte in diabetic patients. Most of the glucose biosensors are based on the glucose oxidation catalyzed by GOD. Immobilized GOD converts glucose into gluconolactone with the consumption of oxygen. Amperometric response was monitored, by measuring the depletion of oxygen from sample using oxygen-sensitive dissolved oxygen (DO) electrode. A method was optimized to prepare a synthetic polyvinyl alcohol (PVA) membrane using high and low degree of polymerization of PVA, acetone, benzoic acid and cross-linking by UV treatment and GOD was immobilized and integrated with the DO probe for biosensor application. GOD was also immobilized on inner epidermal membrane of the onion bulb scales as a natural support and applied for biosensor application for detection of glucose in association with DO probe.

Urea estimation is of utmost importance in monitoring kidney functions and associated disorders. Determination of blood urea nitrogen is an important routine test widely used in clinical laboratories. Urease is one of the key enzymes which can be utilized for developing urea biosensor. The catalytic action of urease over substrate urea liberates ammonium ion, which can be detected by ammonium ion selective electrode as transducer. In our studies, urease enzyme was entrapped in PVA and polyacrylamide (PAA) composite polymer membrane prepared on the cheesecloth support by gamma-irradiation induced free radical polymerization and urea biosensor was developed. The performance of the biosensor was monitored using a flow-through cell in conjugation with the...
ammonium selective electrode and ammonium produced as a result of enzymatic reaction was monitored potentiometrically\textsuperscript{11}. Whole cells of \textit{Brevibacterium ammoniagenes}, as a source of urease, were also immobilized in polystyrene sulphonate–polyaniline (PSS–PANI) conducting polymer on a Pt twin wire electrode, by potentiostatic electropolymerization and the change in resistivity of the sensor was used for calibration of the urea biosensor \textsuperscript{12}.

Cholesterol is an important lipid found in cells and membrane of all animal tissues. High cholesterol accumulation in the blood serum is strongly correlated with diseases such as coronary heart disease, arteriosclerosis, brain thrombosis, lipid metabolism dysfunction and cerebral infarction (stroke). Decrease in the level of cholesterol in blood causes hypothyroidism, anemia, malabsorption and wasting Syndrome. Cholesterol oxidase (ChOx) enzyme can be used for the development of a biosensor for the determination of cholesterol. Immobilization of ChOx onto the electrode surface is a critical step. A method was developed for immobilization of positively charged ChOx and the negatively charged multi walled carbon nanotubes (MWCNTs) on graphite electrode surface using layer-by-layer technique. The modified electrodes showed electrocatalytic activity towards reduction of oxygen which was associated with cyclic voltammetry and electrochemical impedance spectroscopy for the determination of cholesterol\textsuperscript{13}.

Dopamine plays an important role in the function of central nervous, renal, hormonal and cardiovascular systems. It is of great clinical importance to measure the dopamine level in extracellular fluid to monitor neurotransmission processes and diagnose Parkinson’s disease. We have used tyrosinase for the development of dopamine biosensor. Tyrosinase was extracted from a plant source \textit{Amorphophallus companulatus} and immobilized in a novel composite of two biopolymers: agarose and guar gum. The composite matrix-containing enzyme forms a self-adhering layer on the active surface of glassy carbon electrode, making it a selective and sensitive biosensor. Modified electrode was associated with electrochemical analyzer to determine dopamine by direct reduction of biocatalytically liberated quinone\textsuperscript{14}. In another study, tyrosinase was also immobilized on glutaraldehyde activated eggshell membrane for the development of an electrochemical biosensor for dopamine detection\textsuperscript{15}.

**Conclusion**

Microbial OPH and tyrosinase enzymes were used for the development of biosensors for the environmental monitoring of methyl parathion and catechol. Biosensors were also developed using immobilized GOD, urease, ChOx and tyrosinase for monitoring of clinical metabolites glucose, urea, cholesterol and dopamine respectively, for clinical analysis.

**References**


