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Conducting polymers in the fabrication of efficient biosensors

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Fabrication of efficient biosensors for industrial and medical applications is a challenging problem. Several polymers have been used for interfacing a biological component to the electrode surface in order to attain higher performance efficiency and faster electron transfer processes between the enzyme and the electrode. In this minireview, some of the successful matrices used in biosensor fabrication have been discussed.

1 Introduction

Biosensor is a synergic combination of analytical biochemistry and microelectronics. These devices offer the prospects of simplified, virtually non-destructive analysis of turbid biological fluids. In general, a biosensor consists of a biological component (B) in intimate contact with a suitable transducer (T) coupled through immobilisation (Fig. 1). The biological component gives rise to a signal as a result of the biochemical reaction of the analyte (A), which is detected by transducer to give an electrical signal (OS). This can be used with or without amplification for the estimation of the concentration of the analyte in a given sample[1]. Although, the recognition biomolecule incorporated within a biosensor possesses an exquisite level of selectivity, it remains as a structurally weak component of the system and is vulnerable to extreme conditions, such as, *p*H, temperature and the ionic strength[2]. The immobilisation of the biological component, though decreases its activity, imparts stability to the biological component against the environmental conditions[3,4].

Immobilisation of biocatalysts in a suitable matrix is an important practice in biomedical, industrial and basic enzymology for repetitive and continuous processes and helps in economic utilisation of the biocatalysts[5,6]. The activity of the immobilised biomolecules depends on surface area, porosity, hydrophilic character of the immobilising matrix, reaction conditions and the methodology chosen for immobilisation.

Immobilisation of the biological component can be done in a number of ways, depending on the type of the component. The immobilisation can be achieved through chemical bonding or physical retention. A schematic diagram representing classification of different types of immobilisation procedures is given in Fig. 2. Binding of biocatalyst to solid supports by chemical method can be achieved directly or thorugh crosslinking. The bonding can be through ionic or covalent interactions. The crosslinking can be either through linking to itself or through co-crosslinking with a structural protein such as bovine serum albumin. Bifunctional reagents such as glutaraldehyde are often used as the crosslinking agents. Physical retention consists of entrapment in a matrix in the form of beads, fibers, or enclosing in the matrix by encapsulation or incorporating in membrane reactors.

Many biosensor fabrications are based on electrochemical transduction of the biological signal. This is because of the fact that about 30% of the biological reactions involve consumption/liberation of protons, electrons or ions, which are electrochemically active. Moreover, electrochemical methods are more economical compared to other transducer devices such as piezoelectric crystals, field-effect-transistors, etc. Efficient electrochemical transducers requi-

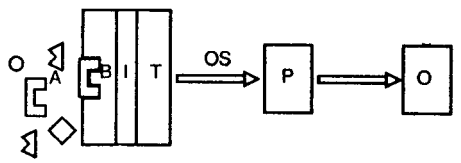

Fig. 1—Schematic representation of a biosensor [B—biological component is interfaced (I) to the transducer (T). The interaction between B and the analyte (A) gives rise to the output signal (OS), which can be recorded or displayed (D) with or without further processing (P)].

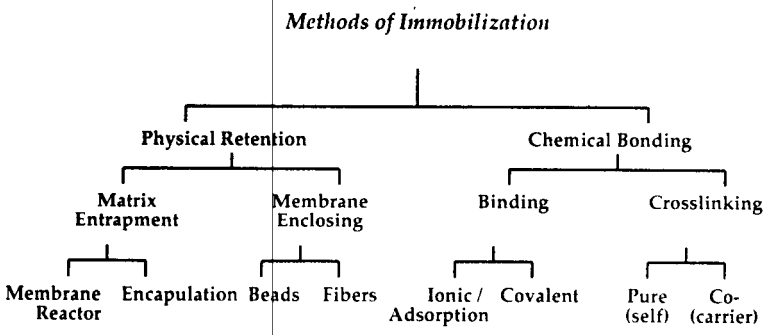

Fig. 2—Classification of immobilisation techniques of biological components onto a solid support on the basis of methodology involved in the process.

re a conducting immobilising matrix so as to probe the redox reaction at the enzyme active site directly, instead of using the electroactive reactant or product as the measuring probe. The electron transfer reactions of biological molecules are frequently slow at ordinary electrodes. To overcome this problem, and thus to facilitate the direct coupling of biological redox reactions to electrodes, various types of modified electrodes have been used[7-17]. These include electrodes modified by deposition of polymer species and electrodes based on conducting polymers or conducting salts.

The conducting immobilising matrix facilitates relay of the biological signal to the detecting device (T). There are a number of polymers which have been used for fabrication of efficient biosensor in terms of sensitivity and stability. Table 1 gives a list of some of the polymer matrices used for immobilisation. Some of the successful immobilisation matrices have been discussed in brief below.

2 Conducting salts

Conduting salts are formed by a combination of donor (D) and acceptor (A) systems. These species are generally planar, having delocalised π-electron density both above and below the molecular plane. Tetrathiafulvalene (TTF) and tetracyanoquinodimethane (TCNQ) are typical examples of donor and acceptor systems. Conducting salts formed out of TCNQ with different donors have been used as electrode materials for the fabrication of biosensors[18,19]. The fabrication of a conducting salt based enzyme electrode is a multi-step process[18]. It consists of preparation of the conducting salt from the acceptor and the donor, preparation of the conducting salt electrode and finally immobilisation of enzyme on this electorde. A brief description of the steps involved in each process is given below.

Table 1—Different immobilising matrices used in immobilisation of biological components for various applications such as fabrication of enzyme electrodes

Matrix	Biological component	References
TTF.TCNQ	Ascorbic acid oxidase	[19]
	Methanol dehydrogenase	[32]
	Glucose oxidase	[18]
Polypyrrole	Glucose oxidase	[8, 20, 23, 24]
	Ps. Putida cells	[21]
Poly(ethylene-vinyl)alcohol	Alcohol dehydrogenase	[28]
Polyphenol	Glucose oxidase	[16, 17]
	D-Amino oxidase	[16]
Poly(*o*-phenylenediamine)	Glucose oxidase	[10]
Polyurethance	Glucose oxidase	[27]
Polyethylene-g-acrylic acid	Glucose oxidase	[13]
Viologen-acrylamide copolymer	Nitrate reductase	[33]
Nafion	Cytochrome c_{551}	[30]

The donor-acceptor salts can be prepared[18] as explained here for TTF-TCNQ system. This is prepared by direct reaction of the uncharged donor and acceptor in acetonitrile by mixing equal amounts of TTF and TCNQ solutions in acetonitrile to give a black precipitate. The reaction mixture is cooled overnight with stirring, filtered with suction and dried under vacuum. The precipitate is washed with acetonitrile and then with ether till the washings are colourless.

The conducting salts can be used in working electrodes in a number of ways. One such method is packed

cavity electrode, which has been used for constructing a conducting organic salt electrode[18]. It is constructed by press fitting a platinum disk attached to brass rod into a Teflon sheath.

0.015 g of PVC dissolved in 2 ml THF to make a polymerised solution was evaporated to 0.5 ml and then mixed with 0.09 g conducting salt crystallites. The mixture was smeared into cavity and packed well to ensure good electrical contact and then dried. The enzyme can be used either in membrane electrodes or simply as adsorbed enzyme. Construction of the membrane electrode is a simple process. Enzyme solution is placed at the electorde surface and covered with dialysis bag. It is stored in buffer potentiostated at +0.05 V with respect to saturated calomel electrode.

Conducting salt electrodes have been used for flavo and other redox proteins (Table 1).

3 Polypyrrole

Polypyrrole, a polymer of pyrrole, has been used for the immobilisation by many groups for glucose sensors[12,14,20-23]. The procedure of immobilisation of enzymes in polypyrrole matrix is rather simple and reproducible. When applied potential at the working electrode is cycled between 0 and +0.8 V at 5 mV/s in 10 m*M* sodium phosphate buffer (*p*H 5.6) against Ag/AgCl electrode containing 0.1 *M* sodium perchlorate, 1 m*M* pyrrole and glucose oxidase (≈200 U/mL), a thin film of polypyrrole gets deposited on the electrode surface. The resulting electrode can be washed with fresh electrolyte and stored in buffer.

Simultaneous immobilisation of ferrocene, an electron relay used in the fabrication of glucose sensor in polypyrrole matrix is an involved process[8]. Synthesis of monomeric [(ferrocenyl)amidopentyl] amidopropyl)pyrrole (FAPAPP) from ferrocenoyl chloride is a multi-step process. Copolymers of pyrrole and FAPAPP are formed by electropolymerisation of mixtures of monomers in aqueous electrolyte, at a redox potential intermediate between those for the two monomers.

Simultaneous immobilisation of glucose oxidase and hydroquinone sulphonate in the polypyrrole films has been reported by Kajiya *et al.*[24]. Pyrrole is polymerised in the presence of glucose oxidase and sodium hydroquinone-sulphonate to give a glucose oxidase electrode with coimmobilised hydroquinone sulphonate in polypyrrole matrix.

4 Polyurethane

Polyurethane is a polymer formed by water induced polymerisation of its prepolymers[25,26]. Polyurethane prepolymer is a water miscible polyether diol of poly(ethylene glycol) and poly(propylene glycol) with isocyanate groups at the two ends. Prepolymers get polymerised in the presence of water with the liberation of carbon dioxide. The relative composition of poly(ethylene glycol) and poly(propylene glycol) in the prepolymer determines the hydrophilicity of the polymer. Immobilisation of biological components such as enzymes and whole cells in polyurethane polymer matrix by physical entrapment involves simple steps[25-27].

The electrode surface is cleaned to remove the oily contaminations. Aqueous soltion of the biological component is mixed with the prepolymer at the electrode surface. The prepolymer is allowed to polymerise (≈30 min) and then washed with aqueous buffer and stored in buffer. Doping of polymer matrix with redox relays such as ferrocene can be done during the polymerisation, which will entrap the relay in the matrix.

5 Polyphenol

Adsorption of glucose oxidase at a platinum electrode followed by immobilisation in an electrochemically polymerised phenol film has been found to be a reproducible method of electrode fabrication with a shelf life of the order of 40 days[16,17]. The immobilisation steps involve electrochemical polymersation of phenol in the presence of the enzyme.

The electrode is immersed in the growth medium containing glucose oxidase, tetraethylammonium tetrafluoroborate (0.1 *M*) and 0.05 *M* phenol in 0.15 *M* disodium hydrogen orthophospahte buffer at *p*H 7.0 for 20 s at 0.0 V. The potential at the electrode is stepped to +0.9 V and held for 8 min. It is then stepped back to 0 volt, kept at this value for 20 s and then electrode was washed with buffer. The electrode is stored in glucose free buffer solution at 4°C between measurements.

6 Poly(ethylene-vinyl alcohol)

Poly(ethylene-vinyl alcohol) is yet another conducting polymer used for the imprisonment of enzymes[28]. The immobilisation process involves the following steps.

The poly(ethylene-vinyl alcohol) (EVAL) is aminoacetylised using 2-dimethylaminoacetoaldehyde dimethylacetal and 3-(N, N-dimethylamino-*n*-propane-diamine)propionaldehyde dimethylacetal in a mixture of 45 mL of water, 25 g of concentrated sulphuric acid, 25 g of anhydrous sodium sulphate and 5 g of aminoacetal. The aminoacetalised membrane is washed with water and dried. This membrane is stirred in 10 mL of aqueous solution of enzyme at room temperature, followed by washing with water.

7 Redox polymer films

Oxidoreductases can be electrically wired to electrodes by chemical or electrostatic binding of redox polymers. The electron flow is from the enzyme to the electrodes through the polymer. One such polymer, a cross-linkable poly(vinylpyridine) complex of $[Os(bpy)2Cl]^{+/2+}$ has been reported[29]. Uncomplexed pyridines are quaternised with two types of groups, one promoting the hydrophilicity (bromoethanol) and the other with an active ester (N-hydroxysuccinamate). The ester forms amide bonds with the free lysines of the protein and the added polyamine cross-linking agent (triethylenetetramine, trien). The cross-linking forms a rugged electroactive film on the electrode surface.

8 Entrapment in Nafion matrix

The polyelectrolyte nafion entraps heme proteins such as cytochrome c_{551} with the prosthetic group exposed to the electrode[30]. The immobilisation method is rather simple.

Cytochrome c_{551} and 5% solution of the polyelectrolyte Nafion were mixed in 1:3 ratio in sodium phosphate buffer (0. 1 *M*, *p*H 7.2). The mixture was spread on the cleaned surface of graphite electrode. A second layer was added containing only the polymer fo stability.

The method is useful for anionic proteins or redox enzymes. Cationic proteins such as cytochrome c bind too tightly to the polymer and, hence, they do not react with the electrode surface.

9 Future prospects

Prediction of the overall behaviour of a biosensor on the basis of existing models or the activity of the bioreagent in a bulk solution is difficult[2]. This unpredictability explains, to some extent, the diversity of approaches used towards the assay of any given analyte. The problem lies in the selection of appropriate matrix to have maximum biological activity and better sensitivity of the immobilised biological component. Doping the matrix with properly chosen mixed valence materials of organic (such as electron acceptors) or inorganic (such as metallo-complexes) origin[31], acting as mediator can improve the performance for improved electron transfer from the enzyme-active site to the electrode surface.

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