

A Review on Immobilization Techniques of Biosensors

Tanu Bhardwaj

Department of Instrumentation
Shaheed Rajguru College of Applied Sciences for Women
University of Delhi
Delhi, India

Abstract -- In the progressing field of biosensors, most prominent footstep is to immobilize bioreceptors onto the transducer. Immobilization step in the development of biosensors is engaged with an extremely crucial role. The rewards provided by an effective immobilization method are extended utilization of the biosensor and predicted lengthened storage stability. Choice of right immobilization technique gives rise to an efficient, simple and cheap biosensor which can be commercialized certainly. This review paper focuses on several types of immobilization techniques and the battle between them due to their own significance individually.

I. INTRODUCTION TO THE FOUNDATION OF BIOSENSORS

Inauguration of the biosensor technology field started with the development of enzyme electrode by Ieland C. Clark in 1962. The term "Biosensor" was inserted into the dictionary of research by Cammann in 1977. A biosensor creates a bridge between entirely diversified fields of material science, biology, optics, electronics, physics and chemistry. Today, one of the matured domains is a biosensor in which new advancements get birth everyday. In the real world, biosensors are practically substitutes of conventional analytical techniques that are tedious, costly, complex and not appropriate for in situ supervising. A Biosensor is a chemical analytical device unifying a biological element with a transducer adumbratively. It consolidates a biological element within or in intimate contact with a transducer which yields an electronic signal proportional to a single analyte that is further conveyed to a detector [1-9] as shown in Figure 1. It embraces three fundamental components that are bioreceptor (biological element), transducer and electronic circuit [1,10]. Bioreceptor or biological element is a biomolecule that is embedded with the transducer like enzyme, DNA, protein, whole cell, antibodies etc [11].

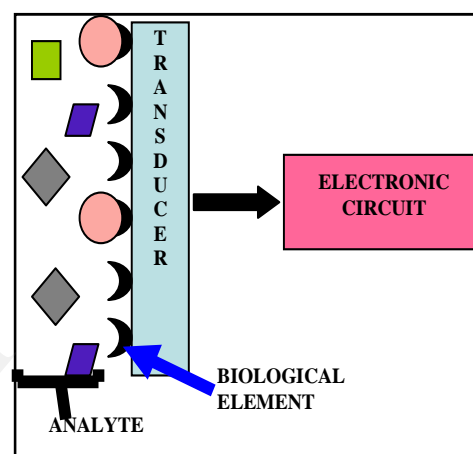


Figure 1 : Fundamental units of a biosensor

The transducer is a device that renovates one form of energy into another like chemical energy into an electrical energy. Electronic circuit comprises of a signal processing system that converts an electrical signal into a processable signal. As shown in Figure 1, the biological elements are immobilized in/on a permeable membrane. Only specific analyte communicates with the immobilized biological elements residing within/on the membrane which grant products like heat, capacitance, electric potential, current, impedance, pH etc. The transducer reform the product signals into an electrical signal that is promoted to the electronic unit of the biosensor. The pillar behind a biosensor is "A chemical reaction occurring on the surface of the transducer with the biological element to bring about an electrical signal". With relation to biosensors, the most superior and first footstep is the immobilization of the biological element onto the transducer surface. Multiple strategies exist for biological elements immobilization, for e.g. binding to resin, crosslinking and encapsulation. A high quality immobilization must assemble the subsequent necessities: decent and rapid, no percolation of immobilized element from the substrate and biological elements must retain their identity after immobilization [12-14]. Various transducers are attainable in consideration of biosensors : electrochemical, optical, thermal, piezoelectric, etc. Electrochemical biosensors generate an electrical signal when a

chemical reaction appears on the floor of the transducer [15]. Optical biosensors create alteration in the light rays properties when analyte communicates with biological element [16,17]. Figure 2 is shown below which presents different types of biosensor's components.

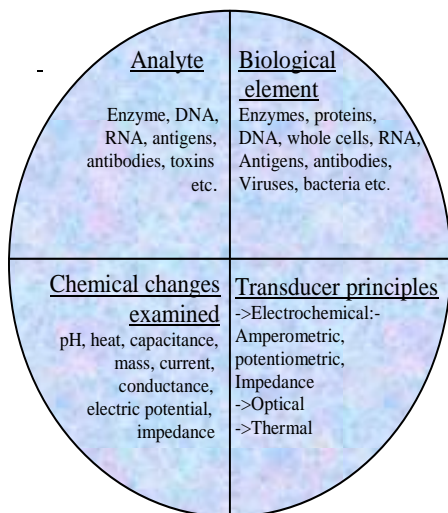


Figure 2: Examples of biosensors unit

II. MENU OF IMMOBILIZATION TECHNIQUES

As previously conversed, there are three techniques of immobilization:- adsorption, covalent bonding, crosslinking and encapsulation. Immobilization of biological elements onto a surface must be stable, permit diffusion of substrates and products, and allow excellent electron transfer [18]. Adsorption and encapsulation belong to physical methods, and crosslinking and covalent bonding are placed under chemical methods of immobilization [19]. Simplest manner of explaining adsorption is, the act of sticking of atoms, ions, biomolecules or molecules of gas, liquid or dissolved solids to a surface as shown in Figure 3. This is accomplished by non-covalent interactions like electrostatic, vander waals, hydrophobic etc between sensing molecule and surface. Protocol used for the adsorption method is the easiest.

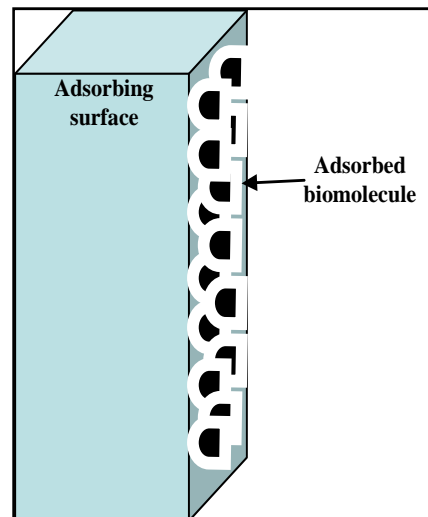


Figure 3: Biomolecules adsorbed on a surface

Ling et al., 2011 has worked on an alanine dehydrogenase enzyme based electrochemical biosensor immobilized into the polymer matrix of poly(2-hydroxyethyl methacrylate) (pHEMA) on a screen printed carbon paste electrode for the detection of ammonium ions. Interaction between NADH, pyruvate and ammonium ions is catalysed by alanine dehydrogenase and cyclic voltammograms of the biosensor show jump in oxidation peak of NADH with rise in ammonium ions concentration [18]. Deacetylation of chitin allows formation of chitosan which is often exploited for adsorbing enzyme. Magalhaes et al., 1998 has reported work performed on chitosan for the same. He executed four experiments with chitosan with different modifications in each, and then resulted out the effects of modification on enzyme activity encapsulated and biosensor which shows that the modification of chitosan adds some new qualities but decreases enzyme activity [19]. Soares et al., 2012 has reported urease immobilization in polypyrrole film that is polymerized electrochemically by cyclic voltammetry procedure which does not need ruck of information about chemical reactions and the monomer is of low-cost also [20]. Ferreira et al., 2004 has developed a glucose amperometric biosensor where the glucose oxidase enzyme is adsorbed in layer-by-layer films of poly(allylamine) hydrochloride (PAH) on Prussian blue (PB) layer modified indium-tin oxide substrate [21].

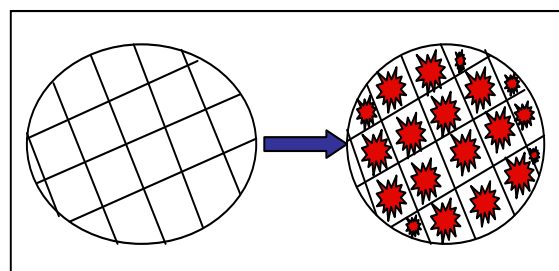


Figure 4: Encapsulation of biomolecules within the matrix

In encapsulation, the enzyme is caught in the trap of porous

polymer matrix as shown in Figure 4. The matrix can be a polymer or sol-gel etc [4,12,13]. Tsai et al., 2003 has reported a sol-gel based biosensor for detection of heavy metals in which FITC-dextran is immobilized together with urease to identify the change in pH. FITC-dextran is pH sensitive fluorescent indicator whose intensity of fluorescence increases along with increase of pH value [17]. Sol-gel is a kind of glass which has micro porous structure on its surface which can be utilized for biomolecule entrapment. It is one of the types of encapsulation methods. Sol-gel matrix offers wide range of benefits as if (1) it can entrap huge amount of enzymes in it (2) no covalent modification with the supplementation of enzyme (3) chemically inactive (4) optically clear (5) thermally stable (6) low temperature process [17, 22-27]. Ramanathan et al., 1997 has reported comparison between the two physical methods of assemblage: adsorption and sandwich technique of encapsulation using same sol-gel solution in each of the methods, but the protocol regarding application of enzyme was different. He observed that lactate dehydrogenase enzyme was more restricted in encapsulation rather than adsorption. 75% percolation was noticed in case of adsorption [27]. Again, an examination is implemented to show the differences among adsorption, sandwich entrapment and microencapsulation utilizing enzymes cholesterol oxidase and horseradish peroxidase by Kumar et al., 2000 which infers that the sandwich entrapment is best among physical assemblage methods [28].

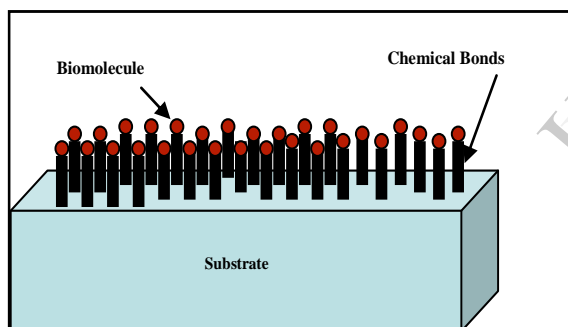


Figure 5: Biomolecules attached covalently with Substrate

Covalent bonding utilizes the phenomenon of chemical bond formation between the sensing molecule and the solid support as shown in Figure 5 [13]. Urease was covalently bonded to cellulose acetate by Gill et al., 1992 [29]. Rajesh et al., 2005 has reported a method of immobilization exploiting the principle of covalent bonding to reduce the desorption of enzyme after adsorption of enzyme on the surface and obtain a lifetime stable biosensor. He, first, prepared monomer N-(3-aminopropyl)-pyrrole which was intermingled with PTS (para-toluene sulfonate) which has free NH_2 groups to bind with enzyme covalently. After leaching examination of this film, no percolation of the enzyme was observed [30]. Koncki et al., 1995 has reported a carboxylated PVC layer whose carboxyl group binds covalently to an enzyme that provides a better kind of support compared with plain PVC film [31].

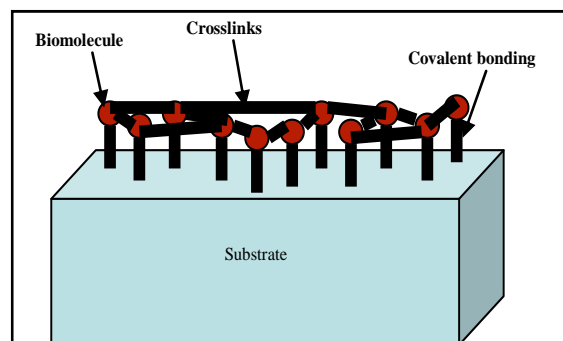


Figure 6: Crosslinking between Biomolecules

As shown in Figure 6, modification of solid support is performed by its treatment with glutaraldehyde, and then the procedure is known as cross-linking. Chitosan bead linked to urease via Glutaraldehyde is reported by Liang et al., 2005 [32]. Urease immobilization on quartz crystal utilizing modifications on the crystal surface by cysteamine and glutaraldehyde was reported by Erdamar et al., 2008 [33].

III. BATTLE OF IMMOBILIZATION TECHNIQUES

While selection of an immobilization technique for a biosensor, there is always a fight between these techniques due to the fact that each technique has its own advantage over other. Various researchers have worked on immobilization techniques and come to the discussed below conclusion. Prodromidis et al., 2002 has reported that the unique feature of encapsulation is that their arrangement and design can be easily adjusted [4]. Lin et al., 1997 has also discussed in his research paper that in adsorption, the biological elements percolate out from the floor of solid base due to which the biosensor response declines with time and hence, they suffer from short life span [12]. Singhal et al., 2002 has developed a biosensor by experimenting both types of physical immobilization methods. For encapsulation of enzyme, he has intermingled enzyme with poly(N-vinyl carbazole) and stearic acid which is further transformed into a film by Langmuir-Blodgett technique, and for adsorption of enzyme, he has applied enzyme onto the already made LB poly(N-vinyl carbazole) and stearic acid. He experimented, observed and concluded that adsorption techniques show more leaching as compared to encapsulation technique [34]. Lakard et al., 1998 has reported comparison among different immobilization techniques like adsorption and encapsulation of enzyme in polyethylenimine films, and furthermore, reticulation of polyethylenimine film with crosslinking agent glutaraldehyde solution to motivate covalent linkages in the film with enzyme. Completion of experimentation shows that in physical immobilization techniques, the necessity to go through the chemical structure of enzyme is not needed, but problems also arise along with the method that are short term stability and weak bonds between matrix and enzyme due to weak non-covalent forces, resulting in leaching problem. Chemical bonding wins the examination in context of reduction in enzyme loss and better binding between matrix and enzyme. Chemical

linkages has its own disadvantages, one of them is the study of complicated networks of chemical linkages in enzymes [35]. Hedayatollah et al., 2004 has observed in his work that chemical bonding gives rise to better immobilization as compared with encapsulation. He immobilized urease enzyme by chemical bonding protocol exploiting glutaraldehyde and electropolymerization of pyrrole monomer [36]. Chen et al., 2000 has reported blend of two immobilization techniques called entrapment and crosslinking. The method constitute of advantages of both the techniques. He prepared an ammonium ion selective membrane from poly(vinyl chloride) and polyurethane onto which urease containing gelatin layer was imposed to get entrapped urease on gelatin matrix. This film was modified by dipping it in into the glutaraldehyde solution to initiate crosslinking. After urea and urease reaction, ammonium ions go through the ammonium ion selective polymer layer and electrode senses the alteration. But the major problem in this kind of sensor is that other cations at the ammonium optical membrane interfere while sensing of ammonium ions [37]. Major advantage of the sol-gel encapsulation over covalent bonding is that the method does not need any attention towards functionalities of the enzyme. Another advantage is physical entrapment secures the integrity and prevents directional changes of the enzyme. The unparalleled feature of sol-gel is that it is not limited by the size of the pores because the pore size formed is in accordance with the size of the biomolecule [23].

VI. CONCLUSION

Adsorption process is simplest but the main drawback is percolation due to which this technique has short lifetime. Covalent bonding and cross-linking immobilization technique has longer life span in comparison to adsorption because of stronger bond formation between the sensing molecule and the solid support. Factors like pH, ionic strength etc. can not approach the covalent bonds. But the process is complicated and time consuming, and may involve hazardous chemicals. Covalent bonding has longest life span. Loss of activity of sensing molecule is also observed to get reduced in covalent bonding. Encapsulation process is the blend of the advantages and eliminates the anomalies of the above two methods: covalent bonding and adsorption. Encapsulation can provide sufficient life span and less percolation than covalent bonding. The main crux of selecting an immobilization technique is the compatibility of the bioreceptor with the immobilization technique and the sensing technique.

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