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Enzymatic Electrochemical Biosensors-A Mini Review

Mahin Basha Syed[1*] and Peddisetty Shiva Kumar[2]

[1]*Chemical and Biochemical lab, Department of Chemistry, A1 Global Institute of Engineering and Technology, Markapur, Andhra Pradesh, India*

[2]*Dean and HOD, Department of Civil Engineering, A1 Global Institution of Engineering and Technology, Markapur, Andhra Pradesh, India*

**Corresponding Author e-mail id: sdmahinbasha@gmail.com*

Abstract

Electrochemistry can be broadly defined as the study of charge transfer phenomena. As such, the field of electrochemistry includes a wide range of different chemical and physical phenomena. Although wide ranging, electrochemistry has found many practical applications in analytical measurements. Enzymatic Biosensors is used in electrochemistry to measure different compounds. This review introduces the principles and working of Antibody biosensors, DNA based biosensors, Optical biosensors, Potentiometric biosensors, Amperometric biosensors, Conductometric biosensors. Biosensors have extremely broad applications and can be found in a variety of industries ranging from healthcare to agriculture to defense. The global market for biosensors is quite large and only expected to grow in coming years as interest in food quality, health care monitoring, disease diagnostics, and national security continue to develop. Commercial biosensors released into the market along with the current and modern instrumentation, are also presented.

Keywords: Antibody biosensors, DNA based biosensors, Optical biosensors, Potentiometric biosensors, Amperometric biosensors

Introduction

The term “biosensor” is short for “biological sensor.” analytical devices that are based on recognition of a biological element (Turner 2013). Enzymes have high specificity and reactivity of an enzyme towards its substrate are properties being utilized in biosensor technology (Liang *et al.,* 2000). An enzyme-based biosensor ideal for biomedical

applications. Biosensors possess advantages such as sensitivity, reliability, accuracy, ease of handling, and low-cost compared with conventional detection methods. A biosensor consists of two main components: a bioreceptor and a transducer (Economou, *et al.*, 2017).

The first one is a biological element (e.g., enzyme, an antibody or a nucleic acid) that recognizes selectivity of the desired analyte (e.g., glucose, urea, toxic metal ions etc.). The bioelement interacts with the analyte being tested and the biological response is converted into an electrical signal by the transducer. The biological recognition system translates information from the biochemical domain, usually an analyte concentration, into a chemical or physical output signal with a defined sensitivity. The main purpose of the recognition system is to provide the sensor with a high degree of selectivity for the analyte to be measured. The second component is a transducer that converts the recognition event into a readable signal. A typical biosensor construct also normally incorporates signal-processing elements (amplification, filtering, data processing, and storage) and a display of the final result.

Application of enzymes as bioreceptor molecules in biosensors is due to (Borgmann *et al.*, 2011)

a) The large number of enzymatic reactions that can be utilized for analytical purposes;

b) The wide array of detectable species – in addition to direct detection of the substrate and product of the enzymatic reaction, inhibitors (compounds that inhibit the enzymatic reaction) and mediators (compounds that enhance the catalytic activity) can be monitored indirectly;

c) The flexibility in detection – different types of transduction (electrochemical, optical, and thermal) can be used to detect the analyte of interest;

d) The low consumption of enzymes, since they are not consumed during the analysis;

e) The high selectivity of enzymatic reactions;

f) The commercial availability of enzymes at high purity.

The disadvantages of using enzymes in biosensors devices are the following:

a) Enzymes are bulky proteins and, often, the active site of the enzyme is not readily accessible to the substrate; therefore, the activity of the enzyme is reduced.

b) Enzymes have an inherently limited lifetime, and can be deactivated by components in the sample, or by extreme chemical and physical conditions prevailing in the sample. Therefore, biosensors exhibit only limited long-term stability.

c) The enzymatic activity is dependent on pH, ionic strength, chemical inhibitors and temperature.

d) The cost of some commercial enzymes is often high.

The development of an enzymatic biosensor involves:

a) Selection of a suitable enzyme;

b) Selection of a suitable immobilization method;

c) Selection of a suitable transducer;

d) Optimization of the biosensor in terms of dynamic range, linearity, and minimization of interferences;

e) Packaging of the biosensor;

f) Commercialization.

The features of an ideal biosensor are (Vaddiraju *et al.*, 2010; Preda *et al.*, 2011):

a) Wide applicability to many sample matrices;

b) High accuracy and precision;

c) Excellent sensitivity and specificity;

d) Wide dynamic range;

e) Rapid response time for real-time monitoring;

f) High operational and physical robustness (i.e. insensitivity to variations of pH, ionic strength, temperature, pressure etc);

g) Long-term stability, lifetime and reliability;

h) Amenability to testing and calibration;

i) Low service requirements, running and capital costs;

j) Product safety (biocompatibility if the biosensor is to be used for invasive monitoring in clinical situations, and in environmental applications the host system must not be contaminated by the sensor);

k) Small size, portability and low power requirements.

Types of Biosensor

Biosensors can be grouped according to their biological element or their transduction element. Biological elements include enzymes, antibodies, microorganisms, biological tissue, and organelles. Antibody-based biosensors are also called immune sensors. When the binding of the sensing element and the analyte is the detected event, the instrument is described as an

affinity sensor. When the interaction between the biological element and the analyte is accompanied or followed by a chemical change in which the concentration of one of the substrates or products is measured, the instrument is described as a metabolism sensor. Finally, when the signal is produced after binding the analyte without chemically changing it but by converting an auxiliary substrate, the biosensor is called a catalytic sensor. The method of transduction depends on the type of physicochemical change resulting from the sensing event. Often, an important ancillary part of a biosensor is a membrane that covers the biological sensing element and has the main functions of selective permeation and diffusion control of analyte, protection against mechanical stresses, and support for the biological element. The most commonly used sensing elements and transducers are described below.

Biosensor Based on Bio-Recognition Method

Here, the biosensors are classified according to the nature (molecules, whole cells, etc.) or function (affinity or catalysis) of the bio recognition element. Isolation and purification are mandatory for the best performance of the sensor. Several molecules or whole cells can function as bio recognition elements some of which are described below:

Enzymatic biosensors

Enzymes are proteins with high catalytic activity and selectivity towards substrates. Their commercial availability at high purity levels makes them very attractive for mass production of enzyme sensors. Their main limitations are pH, ionic strength, chemical inhibitors, and temperature, which affect their activity. Most enzymes lose their activity when exposed to temperatures above 60ºC. Most of the enzymes used in biosensor fabrication are oxidases that consume dissolved oxygen and produce hydrogen peroxide (Newman & Setford., 2006; Prodromidis & Karayannis 2002). Enzymes have been immobilized at the surface of the transducer by adsorption, 28 covalent attachment, and entrapment in a gel or an electrochemically generated polymer, in bilipid membranes or in solution behind a selective membrane. Enzymes are commonly coupled to electrochemical and fiber optic transducers.

Antibody biosensors

Antibodies are proteins that show outstanding selectivity. They are produced by B-lymphocytes in response to antigenic structures, that is, substances foreign to the organism. Molecules larger than about 1 kDa can stimulate an immune response. Smaller molecules like vitamins or steroids can be antigenic (also called haptens) but they do not cause an immune

response unless they are conjugated to larger ones like bovine serum albumin. Many antibodies are commercially available and commonly used in immunoassays (North *et al.*, 1985). Antibodies are usually immobilized on the surface of the transducer by covalent attachment by conjugation of amino, carboxyl, aldehyde, or sulfhydryl groups. The surface of the transducer must be previously functionalized with an amino, carboxyl, hydroxyl, or other group. Antibodies share similar limitations with enzymes. Furthermore, binding may not be reversible and regeneration of the surface may require drastic changes in conditions like low pH, high ionic strength, detergents, etc. Therefore, efforts are being made to produce low cost, single use sensors. Probably the main potential advantage of immune sensors over traditional immunoassays is that they could allow faster and infield measurements. Immuno sensors usually employ optical or acoustic transducers (Conroy *et al.*, 2009).

DNA Based Biosensors

DNA biosensors are commonly employed to detect specific sequences of DNA. They can reach high levels of selectivity and affinity based on the hybridization between a DNA target and its complementary probe, which is present either in solution or on a solid support. These systems can be based on optical or electrochemical detection (Conroy *et al.*, 2009).

Biosensor Based on Transduction Method

Based on the signal transduction method, biosensors are classified as optical, colorimetric, electrochemical, acoustic, piezoelectric, etc. Of these optical and electrochemical sensors are the most important and therefore they are briefly described below

Optical biosensors

Fiber optic probes on the tip of which enzymes and dyes (often fluorescent) have been co-immobilized are used. These probes consist of at least two fibers. One is connected to a light source of a given wavelength range that produces the excitation wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength. Surface plasmon resonance transducers, which measure minute changes in refractive index at and near the surface of the sensing element, have been proposed. Surface plasmon resonance (SPR) transducers have been proposed. SPR measurement is based on the detection of the attenuated total reflection of light in a prism with one side coated with a metal. When a p-polarized incident light passes through the prism and strikes the metal at an adequate angle, it induces a resonant charge wave at the metal/dielectric interface that

propagates a few microns. The total reflection is measured with a photo detector, as a function of the incident angle (Fan *et al.*, 2008).

Electrochemical biosensors

An electrochemical biosensor is a self-contained integrated device, capable of providing quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with an electrochemical transduction element (Thevenot *et al.*, 2001). The biochemical signals can be used to generate a current/charge or may change conductivity between the two electrodes. The corresponding transduction device can be potentiometric, amperometric, and conductometric/impedimetric.

Potentiometric biosensors

Potentiometry is commonly used to measure glucose concentrations greater than 10-5 M, which is in the physiological range in most cases. The potential difference between the reference electrode and the indicator electrode is measured at zero current flow. The ideally non polarizable reference electrode provides a constant potential, while the indicator electrode shows an erratic potential depending on the concentration of the analytes. The zero current potential applied between the two electrodes is recorded as a function of the concentrations of target analytes in a logarithmic manner (Wang *et al.*, 2008). Nernst equation describes potential of electrochemical cell as a function of concentrations of ions taking part in the reaction.

The capability for their continuous measurement is also an interesting possibility for environmental applications. The apparatus is inexpensive, portable, and is well suited for in situ measurements. The main disadvantage is the high limit of detection and the poor selectivity.

Amperometric sensors

Amperometry is based on the measurement of the current resulting from the electrochemical oxidation or reduction of an electroactive species. It is usually performed by maintaining a constant potential at platinum, gold or carbon-based working electrode or an array of electrodes with respect to a reference electrode, which may also serve as the auxiliary electrode, if currents are low (10-6 to 10-9 A). The resulting current is directly

correlated to the bulk concentration of the electroactive species or its production or consumption rate within the adjacent biocatalytic layer. As biocatalytic reaction rates are often chosen to be first-order dependent on the bulk analyte concentration, such steady-state currents are usually proportional to the bulk analyte concentration. This signaltransduction mechanism is frequently used for enzymatic and catalytic biosensors (Thevenot *et al.*, 1999). The main advantage of this class of transducer is the low cost and hence the electrodes are dispensed after use. The high degree of reproducibility with these electrodes eliminates the cumbersome requirement for repeated calibration. The type of instrument used for these measurements is also easy to obtain and can be inexpensive and compact, allowing for the possibility of in situ measurements. Limitations for this signal transduction mechanism include the potential interferences to the response if several electroactive compounds generate false current. These effects have been eliminated in clinical applications through the use of selective membranes, which carefully control the molecular weight or charge of the compounds that have access to the electrode (Habermuller *et al.*, 2000).

Conductometric / Impedimetric Biosensors

It is used to measure the ability of an analyte (e.g. electrolyte solutions) or a medium (e.g. nanowires) to conduct an electrical current between electrodes or reference nodes. The measured parameter is the electrical conductance/resistance of the solution. When electrochemical reactions produce ions or electrons, the overall conductivity or resistivity of the solution changes and the same is measured and calibrated to a proper scale. Conductance measurements have relatively low sensitivity. The electric field is generated using a sinusoidal voltage (AC), which helps in minimizing undesirable effects such as Faradaic processes, double layer charging and concentration polarization.

Conclusion

In summary, this work has demonstrated about working of antibody biosensors outstanding selectivity. They are produced by B-lymphocytes in response to antigenic structures. This project brings research focuses on advanced DNA based biosensors, DNA biosensors are commonly employed to detect specific sequences of DNA. They can reach high levels of selectivity and affinity based on the hybridization between a DNA target and its complementary probe. Fiber optic probes on the tip of which enzymes and dyes (often fluorescent) have been co-immobilized are used. These probes consist of at least two fibers. One is connected to a light source of a given wavelength range that produces the excitation

wave. The other, connected to a photodiode, detects the change in optical density at the appropriate wavelength, electrochemical sensors that show promise in revolutionizing the way monitoring, quantitative or semi-quantitative analytical information was using a biological recognition element. Potentiometry is commonly used to measure glucose concentrations greater than 10-5 M, which is in the physiological range in most cases. The potential difference between the reference electrode and the indicator electrode is measured at zero current flow. Amperometry is based on the measurement of the current resulting from the electrochemical oxidation or reduction of an electroactive species. Conductometric biosensor is used to measure the ability of an analyte or a medium to conduct an electrical current between electrodes or reference nodes. The measured parameter is the electrical conductance/resistance of the solution. This work was focused equally on the development and characterization of an enzymatic biosensor. It gives a thorough explanation for how and why each component of the sensor was developed; additional work can and should be done to further understand the performance of each sensor.

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