

## Biosensor Devices: A review on their biological applications

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### ABSTRACT

The natural biosensors are chemical sense organs specially designed on the basis of smell and taste likewise. Biosensor is a device that detects, transmits and records information regarding physiological or biochemical changes. Basically it is the probe that integrates a biological component with an electronic transducer thereby converting biochemical signals into electrochemical, optical, acoustic and electronic ones. The function of a biosensor depends on specificity of biological active material and the analyte to be detected such as chemical compound, antigen, microbes, hormones, nucleic acid or any subjective parameter like smell and taste. The biological sensing elements have been used as enzyme, antibody, DNA, receptor, organelles and micro-organism as well as animal and plant tissues. Types of biosensor includes immunosensors, microbial biosensors, whole cell based, electrochemical, optical and acoustic biosensors, which have vast applications in biomedical research, healthcare, pharmaceutical, environmental monitoring, homemade security and battlefields. In this review a summary of relevant aspects concerning biosensor integration in efficient analytical setups and the latest applications of biosensors in diagnostic applications focusing on detection of molecular biomarkers in real samples is included. An overview of the current state and future trends of biosensors in this field is given.

**KEY WORDS:** AMPEROMETRIC, BIOSENSORS, BIORECEPTORS, CONDUCTOMETRIC, POTENTIOMETRIC, TRANSDUCERS

### INTRODUCTION

In recent years, however intensive research has been undertaken to decentralize such tests as that they can be performed virtually anywhere and under field condition (Griffiths and Hall, 1993, Fan *et al.*, 2008). Hence development of portable, rapid and sensitive biosensor

technology with immediate on the spot interpretation of result are well suited for this purpose to records information regarding a physiological or biochemical changes (D'Souza, 2001). Areas for which biosensors show particular promise are clinical, diagnostics, food analysis, bioprocess and environmental monitoring, (Rogers and Gerlach 1999). The importance of biosensors results

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from their high specificity and sensitivity which allows the detection of broad spectrum of analysis in complex sample matrices (blood, serum, urine, food), with minimum sample pretreatment, (Rogers,1995, Rogers and Mulchandani,1998). The ability to detect pathogenic and physiologically relevant molecules in the body with high sensitivity and specificity offers a powerful opportunity in the early diagnosis and treatment of diseases, (Perumal and Hashim, 2014).

In past two decades the biological, medical and veterinary fields have seen great advances in development of biosensors, capable of characterizing and quantifying biomolecules, (Dinh and Cullum, 2000). Biosensor is defined as a device that uses specific biochemical reactions mediated by isolated enzymes, immune system, tissue, organelles or whole cell to detect chemical compounds usually by electrical, thermal or optical signals (D'Souza, 2001), or simply biosensor is a sensor incorporating biological element such as enzymes, antibodies, receptors, proteins, nucleic acids, cells or tissue sections as the recognition element coupled to a transducer. Leland C. Clark (Jr) 1918-2005 is the father of Biosensors, which have been classified as electrochemical, optical, acoustic and electronic (Ivnitski *et al.*,1999). Biosensors represent the end product of a rapidly growing field, which combines fundamental, biological, chemical and physical science with engineering and computer science to satisfy the need in a broad range of application area, (Patel *et al.*, 2010). An important consideration for the development of biosensors is the adsorption of the biorecognition element to the surface of a substrate, ( Bhakta *et al.*, 2015).

The current review focuses on the biosensors, their types and some recent developments in the related field. The field of biosensors has journeyed in leaps and bounds and now has become one of the essential states of the art technology in laboratory medicine, especially in point of care testing. The idea of biosensors has revolutionized the concept of self testing by the patients in

many clinical conditions especially diabetes mellitus. (Ramasamy *et al.*, 2014). The advent of nanotechnology provides a new perspective for the development of nanosensors and nanoprobe with nanometer dimensions and is appropriate for biological and biomolecular measurements. Because of the constraints of other approaches, such as ultralow detection, large detection range, high cost, and knowledge complexity, the implementation of effective approaches using carbon-based materials is vital. Carbon nanotubes (CNTs) with superior electrical performance are essential in designing modern biosensors CNT-based biosensors have an economical production process, rapid response, high sensitivity, and good selectivity and are easily available in the market. The different methodologies for the transduction of the produced biological events are considered and the applications to forensic toxicological analysis, classified by the nature of the target analytes, as well as those related with chemical and biological weapons critically commented, (Kiani *et al.*, 2013, Zheng *et al.*, 2013, Yanez-sedeno *et al.* 2014).

## BIOSENSOR SYSTEM: PRINCIPLE OF BIOSENSOR

A biosensor in general utilizes a biological recognition element that senses the presence of analyte (The species to be detected). A biosensor system can be defined as the combination of different entities such as sampling, a biosensor, a system for replenishing information and a data analysis system to implement a biological model which provide information to a human or automated controller (Dey and Goswami 2011). The choice of biological material will depend on a number of factors viz the specificity, storage, operational and environmental stability and creates a physical or chemical response that is converted by a transducer to a signal (Cooper,2004, Serra, 2010). The details are given in figure

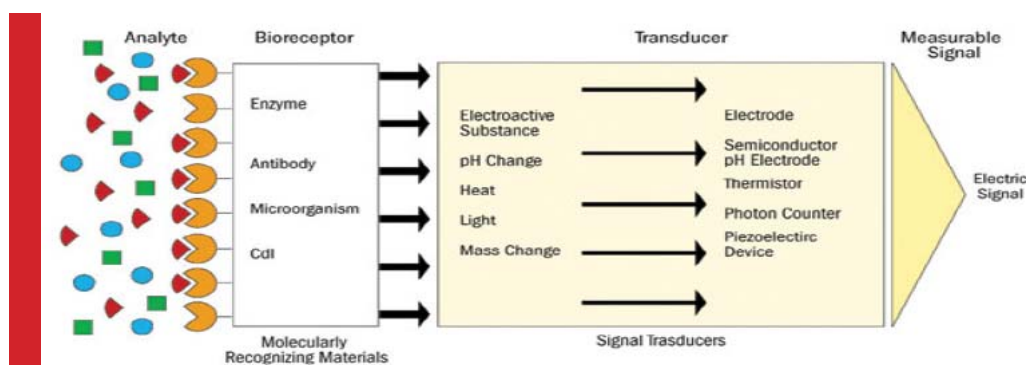


FIGURE 1: Ideal requirement for biosensor based detection assay Biosensor (Kivirand *et al.*, 2013)

**Accuracy:** False positive and false negative result must be low or preferably zero

**Assay time:** Biosensors should produce a real-time response especially when perishable foods are being tested.

**Sensitivity:** Failure to detect false negative result, lowers the sensitivity of assay, which cannot be tolerated. **Specificity:** The biosensor should easily discriminate between the target organism or toxin or other organism. **Reproducible:** Each assay should be highly reproducible and easy to calibrate. **Robust:** The biosensor must be able to resist changes in temperature, pH, ionic strength and be sterilizable. **User friendly:** The assay should be fully automated and requires minimal operator skills for routine detection.

**Validation:** The biosensor assay should be evaluated against current standard technique.

**Classification of Biosensors:** Biosensors are classified based on two parameters: one based on transduction mechanism and the other based on the bio recognition element.

**Microbial biosensor:** These are analytical device that couples microorganism with a transducer to enable rapid accurate and sensitive detection of target analyte in field as diverse as medicine, environmental monitoring, defense, food processing and safety. Microorganism offer advantages of ability to detect a wide range of chemical substances, amenability to genetic modification and broad operating pH and temperature range, making them ideal as biological sensing materials. Microorganisms have been integrated with a variety of transducers such as amperometric, potentiometric, calorimetric, conductimetric, colorimetric, luminescence and fluorescence to construct biosensor devices.

## WHOLE CELL SENSORS

Bioreceptors refer to intact, living microbial cells that have been genetically engineered to produce measurable signals in response to a specific chemical or physical agent in their environment. Bioreporters contain two essential genetic elements, a promoter gene and reporter gene. The promoter gene is turned on (Transcribed) when the target agent is present in the cells environment. The promoter gene in a normal bacterial cell is linked to other genes that are likewise transcribed and then translated into proteins that help the cell in either combating or adopting to the agent to which it has been exposed. In the case of bioreporter, these genes or portion thereof has been removed and replaces with a reporter gene. Activation of reporter gene leads to production of reporter proteins that ultimately generate some detectable signal. Therefore the presence of signal indicates that the bioreporter has sensed a particular target agent

in its environment originally developed for fundamental analysis of factors affecting gene expression. Bioreporters were early applied for the detection of environmental contaminant have since evolved into field as diverse as medical, diagnostics, precision agriculture, food safety assurance, process monitoring control and biomicro-electronic computing, (Ivnitski *et al.* 1999; Hofmann *et al.*, 2013, Wang *et al.*, 2013).

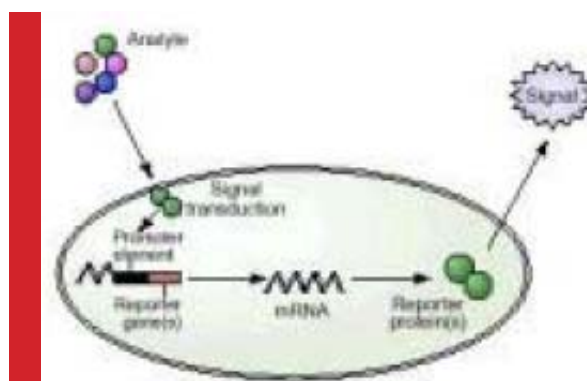


FIGURE 2: Whole cell sensor (Wang *et al.*, 2013)

## ELECTROCHEMICAL DNA BIOSENSORS

DNA biosensors are constructed by the immobilization of oligonucleotide sequence (Probe) in to a transducer that is able to convert the biological event into measurable signals (Dominguez and Acros, 2006). Sometimes the probe can be free in a solution but in all cases the principal of specific hybridization between single stranded (DNA) ssDNA known as the probe and target sequence which must be detected plays a key role in DNA based biosensors (Epstein *et al.*, 2002). The converted signal is a key role in DNA based biosensors. The converted signal is a response to hybridization of the probe and target sequence can be optical, electrochemical or piezoelectrical (Junhui *et al.*, 1997, Rivas and Pedano, 2006). The novel use of conducting polymers for sensors fabrication has allowed to develop DNA biosensors which have characteristic of low detection limits, allowing rapid detection of pathogens (Leonard *et al.*, 2003, Velusamy *et al.*, 2009). An important consideration for the development of biosensors is the adsorption of the biorecognition element to the surface of a substrate, (Bhakta *et al.*, 2015).

## DESIGNING THE BIORECOGNITION INTERFACE

It is necessary to have a biorecognition element for biosensors to detect which is selective sequence of DNA to be detected, it is also important that this biorecognition element is integrated with the signal transducer. Integration is most commonly achieved by immobilizing ss-DNA on the electrode surface. Conducting polymers

are more suitable material currently employed for the fabrication of biosensors. Common conducting polymers are polyacetylenes, polypyrroles, polythiophenes, polyterthiophenes, polytetrahydrofulvalenes, polynaphthalenes, poly(3,4-ethylenedioxythiophene), polyparaphenylenylene, polyazulene, polyaniline, polythiophene and polypyrrole are biocompatible (Geise *et al.*, 1991). However polyanilines and polypyrroles are the most extensively used biosensors for food born pathogen detection, polypyrrole is used mostly in biosensors and immunosensor because of its best biocompatibility and the ease of immobilization of various biologically active compound. To detect bio-analytes at a physiological pH, biosensing material must be electro active in neutral environment unlike polyaniline and polythiophene. To overcome this problem polypyrrole is attractive because it can be more easily deposited from neutral pH aqueous solution of pyrrole monomers. DNA can form a strong bond with polypyrrole based on the interchanging of a strong bond with molecules and hence it has attracted attention for application of DNA biosensors.

Recently, conducting polymer nanocomposition with the enhanced properties has been developed to overcome the inherent limitation of pure conducting polymers. Biosensors can play an important role as efficient tools in this field considering their well known advantages of sensitivity, selectivity, easy functioning, affordability and capability of miniaturization and automation. (Yanezsenedo *et al.* 2014). Electrochemical biosensors currently dominate the field, but are focussed mainly on metabolite monitoring, while bioaffinity monitoring is carried out principally using optical techniques (Turner, 2013).

There are three types of electrochemical biosensors: amperometric, potentiometric and conductometric. These operate at fixed potential with respect to reference electrode and involved the detection of the current generated by the oxidation or reduction of species at the surface of the electrode. Amperometric microbial biosensors have been widely developed for determination of biochemical oxygen demand (BOD) for measurement of biodegradable organic pollutant (Liu and Mattiasson, 2002) Besides BOD, amperometric microbial biosensors have been applied for measurement of several other chemicals. Because of its importance in fermentation industry and clinical toxicology (D'Souza *et al.*, 1997), microbial biosensor ethanol has generated the second most research attention after BOD. Different microorganism metabolizing ethanol such as *Acetobacter aceti*, *Candida vini*, *Gluconobactor suboxydans*, *Aspergillus niger* have been immobilized on oxygen electrode to fabricate the ethanol biosensor.

Conventional potentiometric microbial biosensors consist of an ion-selective electrode (ammonia, chloride)

or gas sensing electrode ( $p\text{CO}_2$  and  $\text{PNH}_3$ ) coated with an immobilized microbial layers. Microbial consuming analyte generates a change in potential resulting from ion accumulation or depletion. Potentiometric transducers measure the difference between a working electrode and a reference electrode and the signal is correlated to the concentration of analyte. Several microbial biosensors are based on modification of glass pH electrode with genetically engineered *E.coli* expressing organophosphorus hydrolase intracellularly and on the outer surface of cell and wild type organophosphorus bacteria *Flavobacterium species* have been reported (Simonian *et al.*, 1998). The principle of detection is based on the detection of the proton released by organophosphorus hydrolase catalysis of organophosphorus and correlating to the concentration of organophosphorus.

Similarly recombinant *E.coli* and penicillinase synthesis immobilized on pH electrode using gluten and acetylcellulase membrane entrapment respectively were developed for monitoring penicillin (Chao and Lee, 2000). An ammonium ion selective electrode was coupled with urease-yielding *Bacillus species* isolated from soil to develop a disposable microbial biosensor for monitoring the presence of urea in milk (Verma and Singh, 2003).

## SURFACE PLASMON RESONANCE BASED BIOSENSORS SPR

SPR is a phenomenon that occurs during optical illumination of a metal surface and it can be harnessed for bimolecular interaction analysis (BIA) (Liedberg *et al.*, 1995). It is best described as a charge density in oscillation at the interface between two media with oppositely charged dielectric constants. Plasmon represent the excited free electron which is provided by compatible light energy photon. The amplitude of the resulting Plasmon electromagnetic or evanescent wave is maximum at emergent (ambient) medium (Salmon *et al.*, 1999). The ambient medium is generally aqueous phase and thus less dense with correspondingly lower refractive indices and is penetrated by the evanescent wave to a depth of approximately one wave length typically guided waves propagate in a confining structure such as an optical fiber, whereas the surface Plasmon wave (SPW) is guided by the metal dielectric interface (Tubb *et al.*, 1997). The following figure provides a simplified overview of the detection principle.

## ACOUSTIC WAVE –BASED BIOSENSORS

These are based on detection of mechanical acoustic waves and incorporate a biological component. These are mass sensitive detectors, which are operated on the

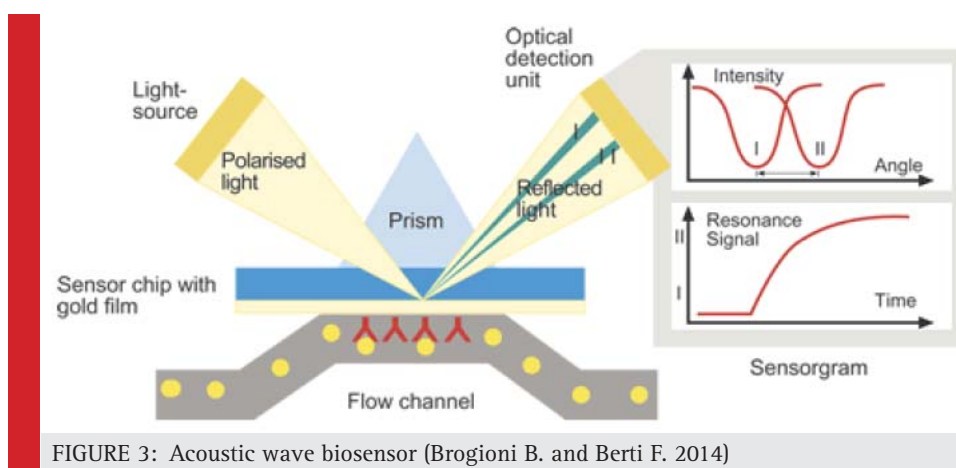


FIGURE 3: Acoustic wave biosensor (Brogioni B. and Berti F. 2014)

basis of an oscillating crystal that resonates at a fundamental frequency (Babacon *et al.*, 2000).

After the crystals has been coated with biological reagents (such as antibody) and exposed to a particular antigen a quantifiable change occurs in the resonant frequency of the crystal, which correlates to mass changes at the crystal surface (Griffiths and Hall 1993). The vast majority of acoustic wave biosensors utilizes piezoelectric materials as the signal transducers. Piezoelectric material are idea for use in this application due to their ability to generate and transmit acoustic waves in a frequency dependent manner. The physical dimensions and properties of the piezoelectric material influenced the optimal resonant frequency for the transmission of the acoustic wave. The most commonly used piezoelectric materials includes quartz ( $\text{SiO}_2$ ) and lithium niobate ( $\text{LiTaO}_3$ ) which influenced the optimal resonant frequency for the transmission of the acoustic wave .

Emerging science, driving new sensors to deliver the molecular information that underpins all this, includes the development of semisynthetic ligands that can deliver the exquisite sensitivity and specificity of biological systems without the inherent instability and redundancy associated with natural molecules. Currently aptamers, affibodies, peptide arrays and molecularly imprinted polymers are particularly promising research directions in this respect. Chances of success are enhanced by the potential utility of some of these materials for novel therapeutic, antimicrobial and drug release strategies, since these complimentary areas will drive investment in these approaches.

Although conventional method for the detection and identification can be very sensitive, inexpensive and present both quantitative and qualitative information they can require several days to yield result. Biosensor offer an exciting alternative to the more traditional method, allowing rapid real-time and development of portable, rapid and sensitive biosensor technology with

immediate on the spot multiple analysis that are essential for detection, clinical diagnostics, food analysis, bioprocess and environmental monitoring, improvement flow cell design and production. The determination of glucose levels using biosensors, particularly in the medical diagnostics and food industries, is gaining mass appeal. Glucose biosensors detect the glucose molecule by catalyzing glucose to gluconic acid and hydrogen peroxide in the presence of oxygen. This action provides high accuracy and a quick detection rate. Biomedical applications, such as blood glucose detection, demand a great deal of research activities.

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