

# Biosensors in clinical chemistry: An overview

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## Abstract

Biosensors are small devices that employ biological/biochemical reactions for detecting target analytes. Basically, the device consists of a biocatalyst and a transducer. The biocatalyst may be a cell, tissue, enzyme or even an oligonucleotide. The transducers are mainly amperometric, potentiometric or optical. The classification of biosensors is based on (a) the nature of the recognition event or (b) the intimacy between the biocatalyst and the transducer. Bioaffinity and biocatalytic devices are examples for the former and the first, whereas second and third generation instruments are examples for the latter. Cell-based biosensors utilizing immobilized cells, tissues as also enzyme immunosensors and DNA biosensors find variegated uses in diagnostics. Enzyme nanoparticle-based biosensors make use of small particles in the nanometer scale and are currently making a mark in laboratory medicine. Nanotechnology can help in optimizing the diagnostic biochips, which would facilitate sensitive, rapid, accurate and precise bedside monitoring. Biosensors render themselves as capable diagnostic tools as they meet most of the above-mentioned criteria.

**Key Words:** Biocatalyst transducer, biosensor, DNA biosensor, nanotechnology

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## INTRODUCTION

The field of biosensors has crossed lots of leaps and bounce and now become as one of the essential state 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 patient in many clinical conditions especially diabetes mellitus. In this review we have discussed about biosensors, their classifications, mechanisms involved in their function and their diagnostic applications.

## Historical perspectives

Professor Clark father of biosensor, has published his monumental paper on oxygen electrode using the enzyme glucose oxidase (GOX) in transducer in measurement of glucose. Where he describes different methods of electro-chemical sensors made analytically viable,<sup>[1]</sup> Guilbault and Montalvo elaborated the potentiometric enzyme electrode based on the urease immobilization technique. This remains the trigger for the development of thermal enzyme probes and enzyme thermistors.<sup>[2,3]</sup> The idea of incorporating bacteria as the biological element in microbial electrodes revolutionized the biosensor industry.<sup>[4,5]</sup> In 1975, fiberoptic sensors (optodes) with immobilized indicators were also released for a commercial purpose, and are presently proving their worth as regards *in vivo* measurement of pH, pCO<sub>2</sub>, and pO<sub>2</sub> (blood gas parameters).<sup>[6]</sup> Subcutaneous implantation of the needle-type *in vivo* enzyme electrode of glucose biosensors was reported successfully, and its utility has

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immense value in diabetic management.<sup>[7]</sup> Liedberg *et al.* elaborated on the basis of fixing antibodies to a piezoelectric or potentiometric transducer in direct immunosensors.<sup>[8,9]</sup> Involving the nucleic acid biosensor as a microchip and enzyme nanoparticle-based biosensor has also been making rapid strides in clinical diagnostics and therapeutics as well.<sup>[10,11]</sup> These biosensors of recent years, e.g., horseradish peroxidase nanoparticles, afford reagent less electronic biosensors, which are simple, sensitive and accurate, and could be extended to clinical chemistry assays.<sup>[12]</sup>

## WHAT IS BIOSENSOR?

Biosensors are small devices that utilize biological reactions for detecting target analytes.<sup>[13]</sup> The analytical device essentially consists of a *biocatalyst* and a *transducer*.

The biocatalyst in biosensor can be an enzyme, cell, tissue or even an oligonucleotide, etc.

The transducer effectively converts the biological or biochemical signal produced by the catalyst into a quantifiable signal. The common transducing elements including optical and electrochemical generate light and current signals, respectively.<sup>[14]</sup>

Analyte → Bioreceptor ... Recognition → Biocatalyst → Transducer → Measurement.

## LOGISTICS

For a biosensor to be pragmatic, efficient and capable, it must inherently possess the following features and the biocatalyst must be highly specific for the analyte under investigation:

- It must not be labile when subjected to normal storage conditions
- It should exhibit good and uniform stability over a significant number of assays
- The reaction should be independent of various physical parameters
- The cofactors/coenzymes must be co-immobilized with the biocatalyst
- Accurate, precise and linear results must be obtained without the need to resort to dilution/concentration
- While using the biosensor for invasive monitoring, care must be taken to ensure that the probe is tiny and biocompatible and should eschew toxic or antigenic effects.

## TRANSDUCERS

The key component of a biosensor is the transducer, which utilizes a physical change accompanying the given

reaction. The change may be heat absorbed or released by the reaction: Calorimetric biosensors, or may refer to the distribution of charges leading to an electrical potential to be produced: Potentiometric biosensors. Alternatively, it may represent the movement of electrons produced in a redox reaction: Amperometric biosensors. In addition, transducers also signify light output during the course of the reaction or difference in light absorbance between the reactants and products (optical biosensors), and those denoting effects are attributable to the mass of the reactants or products (piezo – electric biosensors).<sup>[14]</sup>

Table 1 enumerates the various transducers and their modes of functioning.

Some of the well-documented applications are also depicted therein.

## CLASSIFICATION OF BIOSENSORS

Broad classification for biosensors includes the following criteria:

- Based on the nature of the recognition event
  - *Bioaffinity devices*: These rely on the selective binding of target analyte to a surface-restricted ligand partner (e.g., antibody, oligonucleotide, DNA, cell...)
  - *Biocatalytic devices*: An immobilized enzyme is used herein to help recognize target substrate. e.g., Sensor strips with immobilized GOX have been in vogue for personal/home monitoring of diabetes mellitus.<sup>[12]</sup>

Glucose biosensors account for 90% of the market worldwide. In this review, we briefly summarize the principles of biosensors, the current commercial devices available for glucose and glycol-hemoglobin measurements and the recent work in the area

**Table 1: Transducers in biosensors**

| Transducer                             | Mode           | Applications  |
|--|----------------|---|
| Ion selective electrode                | Potentiometric | Ions in biological media, enzyme electrodes                 |
| Gas sensing electrode                  | Potentiometric | Gases, enzymes, organelles, cell or tissue electrodes       |
| Field effect transistors               | Potentiometric | Ions, gases, substrates and immunological analytes          |
| Optoelectronic and fibre-optic devices | Optical        | Hydrogen ion concentration, enzymes, immunological analytes |
| Thermistors                            | Calorimetric   | Subcellular organelles, enzymes, vitamins, antibiotics      |
| Enzyme electrodes                      | Amperometric   | Enzymes, immunological systems                              |
| Conductimeter                          | Conductance    | Enzyme substrates   |
| Piezoelectric crystals                 | Acoustic mass  | Volatile gases and vapors, antibodies                       |

(Adopted from Malhotra *et al.* 2003)<sup>[15]</sup>

of artificial receptors, and the potential for the development of new devices for diabetes specifically connected with *in vitro* monitoring of glucose.<sup>[16]</sup>

- Based on different generation instruments
  - *First generation instruments*: Herein, the two components, viz. biocatalyst and transducer, may be resolved and both may in turn remain functional in the absence of the other
  - *Second generation instruments*: Enzyme membrane electrodes incorporating with mediators in detection are available, e.g., GOX association with peroxide detection are available as regards diluted samples.<sup>[17]</sup>

However, rapidly responding sensors are prepared by covering metal electrodes with a porous enzyme layer. The key feature as regards the second generation biosensors is that auxiliary enzymes and/or co-reactants are co-immobilized with the principal enzyme, thereby enhancing the analytical quality. The oxidizable interferences are limited by using a GOX/peroxidase complex that communicates with the electrode at a low working potential. Enzymatic recycling of the analyte and/or accumulation of intermediates increases the sensitivity several folds. For instance, the inclusion of Nicotinamide Adenine Dinucleotide NAD bound to polyethylene glycol in the glucose dehydrogenase layer allows a reagentless glucose measurement. Second generation biosensors use an artificial electron mediator that replaces O<sub>2</sub> as the electron shuttle. Ferrocene, quinones, quinoid-like dyes, etc., have been used as mediators<sup>[18,19]</sup> [Figure 1].

Diabetes is a chronic disorder resulting from insulin deficiency and hyperglycemia, and has a high-risk of development of complications for the eyes, kidneys, peripheral nerves, heart, and blood vessels. There is a large population in the world suffering from this disease, and the healthcare costs increase every year. Quick diagnosis and early prevention are critical for the control of the disease status. Traditional biosensors such as glucose meters and glycohemoglobin test kits are widely used *in vitro* for this purpose because they are the two major indicators directly involved in diabetes diagnosis and long-term management. The market size and huge demand for these tests makes it a model disease to develop new approaches to biosensors.

Most oxidase enzymes are not choosy with respect to oxidizing agent, and allow substitution of a variety of artificial oxidizing agents, as depicted in the following reaction with GOX.



In this category, the biocatalyst and transducer interact in a more intimate fashion, and the removal of either of the two components affects the functioning of the other.

- *Third generation instruments*: Herein, the biochemistry and electrochemistry are more closely associated without mediators. [Figure 1] When the electrochemistry occurs at a semi-conductor, the biochip may be applied to such instruments. Third generation sensors are reagentless,

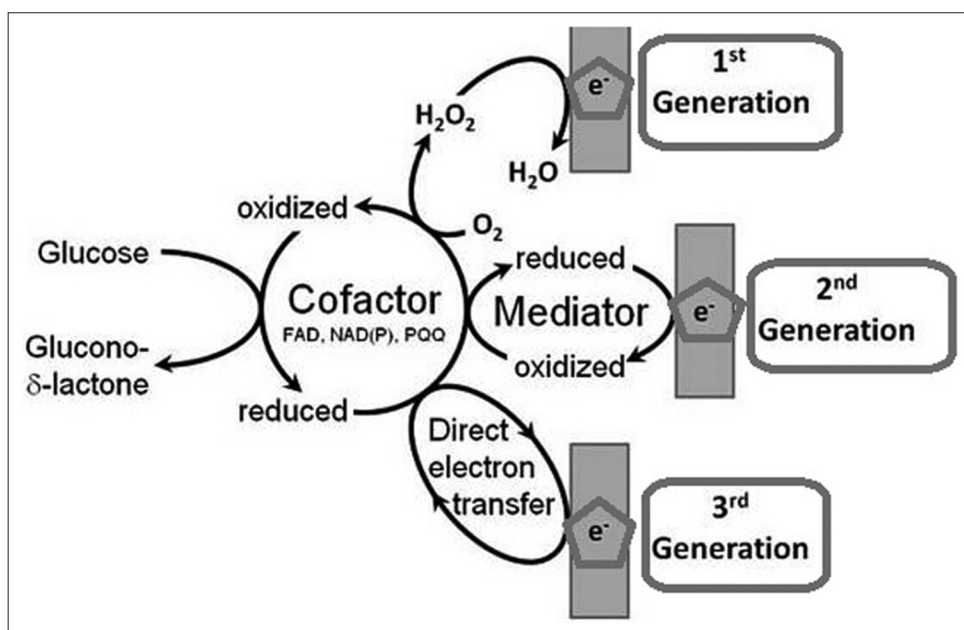


Figure 1: Schematic representation of three generations of glucose biosensor: Adapted from Ferri *et al.*<sup>[30]</sup>

characterized by the progression from use of a freely diffusing mediator (O<sub>2</sub> or artificial) to a system wherein the biocatalyst and mediator are coimmobilized at an electrode surface, thereby making the bio-recognition component an integral part of the electrode transducer. The process of coimmobilization of enzyme and mediator could be achieved by redox mediator labeling of the enzyme followed by enzyme immobilization, enzyme immobilization in a redox polymer or even enzyme and mediator immobilization on a suitably conducting polymeric surface.<sup>[16,20]</sup>

There has been an explosion of research into the physical and chemical properties of carbon-based nanomaterials since the discovery of carbon nanotubes by Iijima in 1991.<sup>[21,22]</sup> Carbon nanomaterials offer unique advantages in several areas, like high surface-volume ratio, high electrical conductivity, chemical stability and strong mechanical strength, and are thus frequently being incorporated into sensing elements. Carbon nanomaterial-based sensors generally have higher sensitivities and a lower detection limit than conventional ones.<sup>[21]</sup>

### Mechanism of first generation instruments

#### Step 1

The biocatalyst within a biosensor responds to the substrate in solution by catalyzing the specific reaction.

#### Step 2

The rate of the reaction is measured by various ways using different transducers.

e.g., Estimation of glucose using GOX



The rate of the reaction can be measured by the following below-mentioned steps:

- The rate of consumption of O<sub>2</sub> can be measured amperometrically by its reduction at a platinum cathode polarized at - 0.6 V as against the standard calomel electrode (Clark oxygen electrode)
- The rate of production of H<sub>2</sub>O<sub>2</sub> can be measured by its oxidation at a platinum anode polarized at + 0.7 V as against the standard calomel electrode
- The rate of production of gluconic acid can be measured using a pH electrode to measure the accompanying decrease in pH. Several reports are available pertaining to the role of hydrogen peroxide and peroxidase in biosensors.<sup>[7,23,24]</sup>

### Mechanism of second generation instruments

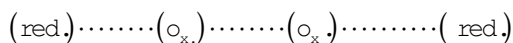
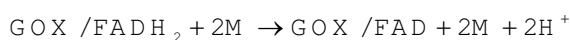
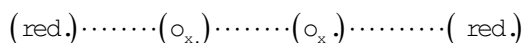
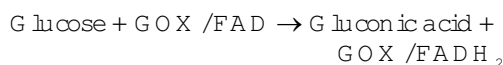
E.g., Glucometer (home monitoring of blood glucose).

In the above device, the intimacy between biocatalyst and transducer is increased by facilitating the design

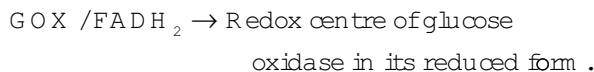
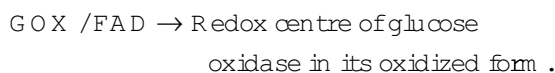
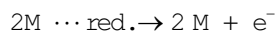
of an electrode surface that is competent of capturing electrons transferred in the redox reaction.

In this device, the rate of oxidation of glucose is measured not by the rate of disappearance of substrate or appearance of product but by the rate of electron flow from glucose to an electrode surface.

The reactions that occur in the device are sequentially described below:



At the electrode



M → Mediator (e. g., Ferrocane)

The electrons donated to the electrode produce a current that is proportional to the rate of oxidation.<sup>[25-27]</sup>

### Third generation instruments

Such instruments are essentially at the research level, primarily to eliminate complicated artificial mediators by adding metals, to overcome the difficulty to relate to electron transfer between electrode and enzyme. Presently, commercial viability is vague due to temperature, pH, toxic chemical and metals. Although most biosensors in articles perform well in research labs, these could not be used for his/her sample. Therefore, it may take quite some time to make commercially available.<sup>[28,29]</sup>

### CELL-BASED BIOSENSORS

These are based on the use of immobilized whole cells and tissues.<sup>[31]</sup>

#### Merits

- More economical to manufacture as compared with those depending on enzymes



- No requirement for complex biocatalyst isolation and laborious purification procedures.<sup>[32]</sup>

#### Demerits

- Cells contain many enzymes and extreme care has to be taken so as to ensure selectivity of the unique response
- Cells possess longer response time than do enzyme-based sensors
- The time taken for cell-based sensors to return to baseline potential after usage is lengthy.<sup>[33,34]</sup>

#### TYPES OF CELL-BASED BIOSENSORS

- Single cell type
- Multicell type
- Combination of cell and enzyme.

##### Single cell type

Estimation of cholesterol using microorganisms that act a source of cholesterol oxidase is immobilized to polyacrylamide or agar on an oxygen electrode.



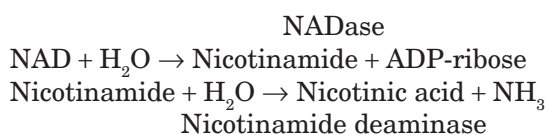
Cholesterol can be estimated by either using an oxygen electrode to measure the rate of O<sub>2</sub> uptake or amperometric measurement of H<sub>2</sub>O<sub>2</sub> production.<sup>[35]</sup>

##### Multicell type

It can be used to enhance the number of potential applications.

##### Combination of cell type and enzyme

Purified NAD are from *Neurospora crassa* in association with *Escherichia coli* (rich in nicotinamide deaminase activity), which would catalyze the following reactions.



The ammonia released can be detected by gas sensing electrode (transducer) to produce an NAD<sup>+</sup> sensitive biomarker.<sup>[36]</sup>

##### Cell types other than microorganisms

In addition to the above-mentioned procedures, immobilization of banana pulp in an oxygen electrode to design a dopamine-sensitive biosensor has also been attempted.

Banana pulp is rich in the enzyme polyphenol oxidase, and the enzyme found in banana pulp possesses a high selectivity for the neuroactive agent dopamine.<sup>[37]</sup>

Cell-based biosensors are variedly used in cancer research as well, for assessing the tumor cell sensitivity to pharmacological drugs, detection of toxins and chemical substances and clinical trial of new drugs.<sup>[38]</sup> A recent study by Ellis and Wolfgang has developed a cell-based biosensor to study fatty acid metabolism using malonyl CoA responsive element.<sup>[39]</sup>

##### Enzyme immunosensors

These biosensors combine the use of molecular recognition properties of antibodies with the high sensitivity of an enzyme-based analytical method.

A non-labeled immunosensor, whose selectivity depends on immunochemical affinity of an antigen for its corresponding antibody, has been developed as the basis for the potentiometric determination of an antigen with an antibody-bound membrane or electrode. These immunosensors are available for syphilis antibody, blood group typing, human chorionic gonadotropin (hCG) and human serum albumin. In contrast, the labeled immunosensors may be characterized by a pronounced increase of sensitivity. Of these labeled immunosensors, mention must be made of enzyme immunosensors that use the chemical amplification of a labeling enzyme for sensitivity. Enzyme immunosensors with an oxygen electrode have been fabricated to determine alpha-fetoprotein AFP, hCG, IgG and toxin. Bioaffinity sensors with a pre-formed metastable ligand-receptor complex that are homologous to the enzyme immunosensor have been used in the determination of thyroxine (T4), biotin and insulin.<sup>[40-42]</sup>

##### Mechanism

Competition between the enzyme-labeled antigen and unlabeled antigen for an antibody immobilized on an appropriate transducer forms the basis.

Following are the steps that need to be carried out while using an immunosensor for IgG using an amperometric oxygen electrode.<sup>[43]</sup>

###### Step 1

Oxygen electrode that contains a membrane onto which will be bound an anti-IgG antibody.

###### Step 2

Free IgG is labeled with the enzyme catalase.

###### Step 3

A known amount of this labeled IgG is mixed with a sample containing an unknown amount of unlabeled IgG.

###### Step 4

This mixture is then placed into the chamber of the oxygen electrode and the labeled and the unlabeled IgG compete for the antibody on the membrane.

#### Step 5

Rinse the sensor to remove any non-specifically associated IgG.

#### Step 6

The sensor is then filled with H<sub>2</sub>O<sub>2</sub> solution, which acts as a substrate for catalase. The more unlabeled IgG that is present, lower the amount of the labeled IgG and lower the rate of oxygen evolution.

### Regeneration

The biosensor is regenerated by rinsing with an appropriate acidic buffer such as glycine – HCl to dissociate the antigen from the antibody.

### Transducers in immunosensors

- Amperometric transducers
- Potentiometric transducers
- Optical transducers measuring fluorescence/chemiluminescence that could measure analytes in nanomol concentrations.<sup>[44]</sup> (Refer Table 1 depicts different transducers mode and their applications).

### Limitations

The time taken for each assay (antigen-antibody coupling) takes several hours. To overcome the limitations, binding and rinsing processes are carried out as separate stages and then the membrane is introduced to the transducer. This will allow one sensor to be used with several antibody-linked membranes and measure numerous samples over a short period of time.

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## DNA BIOSENSORS

DNA biosensors are based on nucleic acid recognition processes whose task is to envisage, rapid, simple and economical testing of genetic and infectious diseases and also for enabling the detection of DNA damage and interactions. A DNA biosensor microchip that is suited clinically has also been developed.<sup>[45]</sup>

### Advantages

The greatest merit is that nucleic acid recognition layers can be readily synthesized and regenerated for multiple uses. This is unlike enzymes or immunoglobulins.<sup>[44,46,47]</sup>

### Types of DNA biosensors

Sequence-specific hybridization biosensor:

- Optical biosensors
- Electrochemical biosensors
- Mass sensitive devices
- DNA microarrays.

## SEQUENCE-SPECIFIC HYBRIDIZATION BIOSENSORS

They depend on the immobilization of a single stranded ss DNA probe onto the transducer surface. The duplex formation can be aptly detected following the association of an appropriate hybridization indicator or through other changes resulting from the binding event. The immobilization step should lead to a well-defined probe orientation that is readily accessible to the target. Depending upon the nature of the physical transducer, various schemes can be used for attaching the DNA probe to the surface.<sup>[48]</sup>

The following possibilities exist:

- Use of thiolated DNA for self-assembly into gold transducers and covalent linkage to the gold surface through a functional alkanethiol-based monolayer are examples of schemes that could be utilized for attaching the DNA probes.<sup>[49]</sup>
- Use of biotinylated DNA for complex formation with a surface-confined avidin is another possible scheme. Covalent coupling to functional groups on carbon electrodes and, alternately, a simple adsorption onto the carbon surface are other instances of available schemes.<sup>[50]</sup>

Nucleic acid recognition is the basis on which the DNA sensors are constructed. The advent of peptide nucleic acid (PNA) has opened up new vistas in biosensors. In PNA, the sugar phosphate backbone is replaced with a pseudopeptide. The features of solution phase PNA could be extended onto the transducer surfaces while designing the uniquely selective DNA biosensors. Surface-confined PNA recognition layers attribute sequence specificity onto DNA biosensors that could detect even single base mismatch. The ramified superstructures possessing numerous single stranded arms that could hybridize to the complementary DNA sequence are the DNA dendrimers. Immobilizing these dendritic nucleic acids onto the physical transducers imparts greatly increased hybridization capacity and is synonymous with a substantially amplified response.<sup>[51]</sup>

### Optical DNA biosensors

Fiberoptics are essential devices that carry light form one place to another through a series of internal inflections, and DNA optical biosensors employ such

devices. The ss DNA probe is placed at one end of the fiber and fluorescent changes resulting from the association of a (fluorescent) indicator with the double stranded ds DNA hybrid is measured. The first developed DNA optical biosensor centered around the use of ethidium bromide as an indicator. Extremely low concentrations (femtomolar) could be detected in comparison with other conventional fluorescent indicators. In general, the hybridization of fluorescently labeled complementary oligonucleotides is monitored by observing the increase in fluorescence that accompanied the binding. Optical transduction based on evanescent wave devices provides real-time label-free optical detection of DNA hybridization. These rely on monitoring changes in surface optical properties.<sup>[52,53]</sup>

The coupling of chemi-luminescence with sandwich hybridization, magnetic bead capture and flow injection operation have been used in the rapid tests for detection of hepatitis B virus DNA.

In recent years, yet another innovative technique based on molecular beacons (Mbs) has been developed. Mbs are oligonucleotides (stem and loop structures) labeled with a fluorophore and a quencher on the two extremities of the stem that acquire fluorescence upon hybridization. This procedure monitors capability and Mb offers high sensitivity and specificity in mutational analysis.<sup>[54]</sup>

### Electrochemical biosensors

Electrochemical detection of DNA hybridization pertains to monitoring a current response under controlled potential conditions. The hybridization event is commonly detected through the increased current signal of a redox indicator (that recognize duplex DNA) or from other hybridization-induced changes in electrochemical parameters. The use of a threading intercalator, viz. ferrocenyl naphthalene diimide, in binding to the DNA tightly has also been launched. Use of enzyme labels for electrochemical detection of DNA hybridization is in vogue. A direct amperometric monitoring of the hybridization event could be achieved with regard to the use of horse radish peroxidase-labeled target. The hybridization event eventually resulted in the “Wiring” of the enzyme to the transducer, thereby letting in a continuous electroreduction current attributable to hydrogen peroxide.

Novel label-free electrochemical detection schemes affording rapid and simpler assays have come into existence. Changes occurring in the intrinsic electroactivity of DNA as a result of hybridization could be monitored.<sup>[55]</sup> One of the useful indicators of

free detection scheme is provided by mass sensitive devices wherein quartz crystal microbalance (QCM) transducers are employed. These facilitate dynamic monitoring of hybridization events. Basically, QCM hybridization biosensors consist of an oscillating crystal with the DNA probe immobilized.<sup>[56]</sup>

A highly sensitive device has been developed to help detect the genetic disorder, Tay-Sach’s disease. Complex DNA samples with reference to their sequence and information could be analyzed by the integration of multiple biosensors in association with DNA microarrays.<sup>[57]</sup>

### NANOTECHNOLOGY

Nanotechnology is a term covering a wide range of technologies concerned with the structures and processes on the nanometer scale. Enzyme nanoparticle-based biosensors are being put to use, which are simple and sensitive, and effective methods are available, wherein an enzyme electronic biosensor could be fabricated by immobilizing enzyme nanoparticles onto the gold electrode surface. Horseradish peroxidase nanoparticles have been successfully mobilized to develop reagentless electronic biosensors for H<sub>2</sub>O<sub>2</sub> detection without calling for promoters and mediators and hence offer a great potential to develop elegant enzyme-based and competent electronic biosensors.<sup>[58]</sup>

### Application in diagnostics

Nanotechnology can help in optimizing the

**Table 2: Overview applications of biosensor**

|  |   |
|--|---|
| Fatty acid binding protein: An earliest marker of myocardial infarction elevated within 3 h using disposable electrodes <sup>[60]</sup>                  | Schreiber <i>et al.</i> 1997 <sup>[60]</sup>      |
| Along with glucose and lactate are used in differential diagnosis of coma, metabolic disorders, shock also in sports medicine, etc., <sup>[61, 62]</sup> | Heinemann <i>et al.</i> 2009 <sup>[62]</sup>      |
| Nowadays continuous glucose monitoring as glucowatch is also possible <sup>[61]</sup>  | Jijun <i>et al.</i> 2005 <sup>[61]</sup>          |
| Adapted glucometer tried in urea, creatinine cholesterol and triglyceride useful renal, musculo-skeletal and cardiovascular diseases <sup>[63]</sup>     | Osaka <i>et al.</i> 1996 <sup>[64]</sup>          |
| Skin allergy test using cell based biosensor. <sup>[64]</sup>  | Hofmann <i>et al.</i> 2013 <sup>[64]</sup>        |
| Sialic acid recent marker in glycol biology altered in various hypertensive disorder, cancer, etc., <sup>[65,66]</sup>                                   | Kwak <i>et al.</i> 2012 <sup>[66]</sup>           |
| Organo phosphorus, carbamates and other pesticides affecting nervous system are the commonest agricultural hazards in countries like in India            | Pundir 2012 <sup>[67]</sup>                       |
| DNA biosensor extensively used in genotyping, mutational study, cancer diagnostics and infectious diseases   | Monosik <i>et al.</i> 2012 <sup>[68]</sup>        |
| Immunoglobulin levels altered in plasma cell disorders can be estimated pizelectrical crystal in biosensor <sup>[69]</sup>                               | Rosales-Rivera <i>et al.</i> 2012 <sup>[69]</sup> |



diagnostics biochips. These diagnostic chips can be used to analyze thousands of genes simultaneously. For this purpose, the gene fragments with known properties synthesized in the laboratory are bonded to the chip surface, and the diagnosis depends on which gene pieces of the DNA from a patient's blood gets deposited on. Viruses and cell types can be identified on the basis of surface properties, which make them adhere to certain nanostructures. Re-regenerative medicine widely uses nanotechnology recently.<sup>[59,60]</sup> Also refer Table 2 for other applications of biosensor.

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