

ADVANCEMENT IN BIOSENSOR WITH NANOTECHNOLOGY

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Abstract: Biosensors are devices that combine the selectivity and specificity of biological sensing elements such as an enzyme, nucleic acid or an antibody with a suitable transducer. The development and performance of biosensors depend upon the materials employed for their construction, the physicochemical characteristics of the materials employed for the construction of the transducer, the matrices used for the enzyme immobilization, the stabilizers and the mediators. Based on transduction process biosensors are classified into different categories such as electrochemical, optical, piezoelectric, thermal and colorimetric. The emergence of nanotechnology offers great opportunities to improve the sensitivity, stability and anti-interference ability of the biosensing systems. With the development of nanotechnology, a number of novel nanomaterial have been fabricated and their novel properties are being gradually discovered and their applications have also greatly advanced biosensors performance. Nanobiosensors have generated a great deal of excitement due to their ability to detect a wide range of materials at incredibly small concentrations.

Keywords: Nanomaterials, Nanoparticles, Transducer.

I. INTRODUCTION

Biosensors in modern context can be defined as a portable sensor incorporating a biological element. Technically it is a miniaturized analytical tool comprising of highly specific biological sensing element like enzymes, antibodies, microbial cell, mitochondria, nucleic acids, tissue, cells and receptors etc. This biological sensing element is either integrated within or associated with transducer, which convert physicochemical interaction into discrete or continuous digital electronic signals, which are proportional to single or related groups of analytes. Biosensor is a synergic combination of bio-electrochemistry and microelectronics, which enable the signals produced by specific biochemical reactions to be registered, quantified and recorded. Leland C. Clark Jr. is considered as the father of biosensor concept. In 1956, Clark published his definitive paper on the oxygen electrode. Clark's idea became commercial reality in 1975 with the successful launch of glucose analyzer based on amperometric detection of hydrogen peroxide. This was the first of many biosensor-based laboratory analyzers to be built by companies around the world [1]. Since then research communities from various fields such as Physics, Chemistry, VLSI and Material science have come together to develop more sophisticated, reliable and mature biosensing devices for application in the fields of medicine, agriculture, biotechnology,

as well as military for bioterrorism detection and prevention etc. Biosensors have a few limitations: electrochemically active interferences in the sample, weak long term stability and troublesome electron-transfer pathways. Today a multitude of instruments referred to as biosensors could be find in labs around the world and there is a growing number of biosensors being used as diagnostic tools in point-of-care testing.

II. PRINCIPLE OF BIOSENSOR

In general, a biosensor consists of a biological component in intimate contact with a suitable transducer coupled through immobilization. The biological component gives rise to signal as the biochemical reaction of analyte, which is detected by transducer to give electrical signal. This reaction between the bioactive substance and the species (substrates) produces a product in the form of a biological or chemical substance electrochemical, heat, light or sound, then a transducer such as an electrode electrochemical, semiconductor, thermistor, counter or sound detector changes the product of the reaction into usable data (Fig.1) [2].

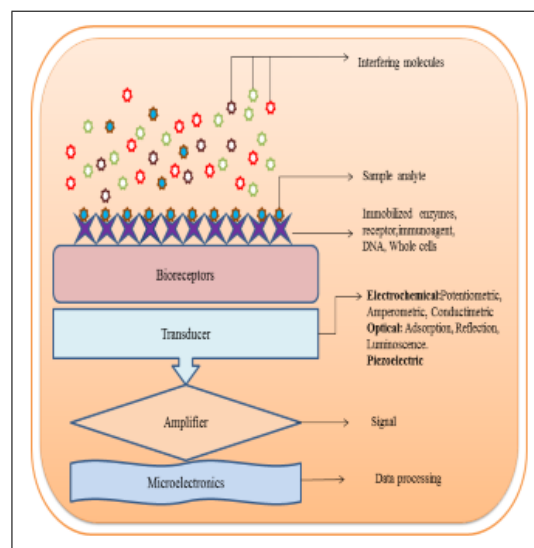


Figure 1: Operating principle of a biosensor

A. Biosensor Components

Biosensor consists of three parts :

- a) The sensitive biological element (e.g. Tissues, Microorganisms, Organelles, Cell receptors, Enzymes, Antibodies, Nucleic acids, Synthetic receptors, sensing organs etc).
- b) The transducer (Acts as an interface, measuring the physical change that occurs with the reaction at the bioreceptor then transforming that energy into measurable electrical output.) Physical transducers: Optical, Electrochemical, Opto-electronic, Piezoelectric, Magnetic Thermal, Mass.
- c) The detector element (Signals from the transducer are passed to a microprocessor where they are amplified and analyzed, The data is then converted to concentration units and transferred to a display or/and data storage device).

The transducer converts the biochemical interactions into a measurable electronic signal. Electrochemical, electro-optical, acoustical and mechanical transducers are among the many types found in biosensors. The transducer works either directly or indirectly.

B. Generation of Biosensors

Based on the level of integration, biosensors can be divided into three generations :

a) First generation biosensor

In the first generation biosensors the normal product of the reaction diffuses to the transducer and causes the electrical response. It was proposed by Clark and Lyons [3] and implemented by Updike and Hicks [4] who coined term enzyme electrode.

b) Second generation biosensor

In second generation biosensors use specific "mediators" between the reaction and the transducer in order to generate improved response. Ideally mediator is otherwise inactive, that is highly specific only for the desired electron transfer process between the recognition element and transducer. It involves the adsorption or covalent fixation of the biologically active component to the transducer surface and permits the elimination of semi-permeable membrane. In the second generation biosensors auxiliary enzymes and /or co-reactants were co-immobilized with the analyte, in order to improve the analytical quality and to simplify the performance [5].

c) Third generation biosensor

In third generation biosensors, reaction itself causes the response and no product or mediator diffusion is directly involved. So no normal product or mediator diffusion is directly involved in this. Conducting polymer-based biosensors come under this category. The direct binding of the biocatalyst to an electronic device that transduces and amplifies the signals is the basis for a further miniaturization of biosensors. Third generation biosensors have the mediator integrated with the enzyme and the electrode to ensure direct electron transfer. Direct electron transfer has been realized with the use of carbon nanotubes [6, 7].

III. CLASSIFICATION OF BIOSENSOR

A biosensor is an integrated receptor-transducer device, which is capable of providing selective quantitative or semi-quantitative analytical information using a biological recognition element. Biosensors may be classified according to the biological specificity-conferring mechanism, or nano based [8, 9] or the mode of signal transduction (Fig. 2).

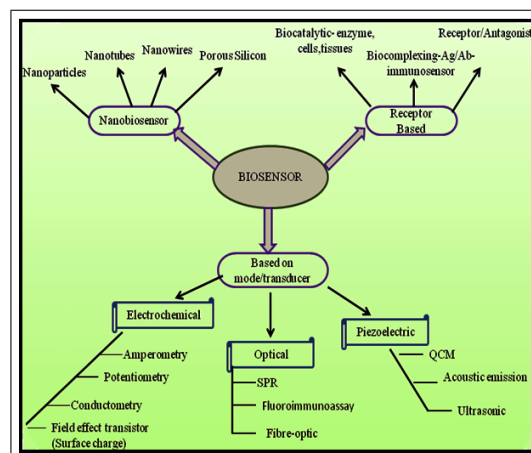


Figure 2: Classification of biosensors on the basis of different processes

a) Transducer based

- a) Electrochemical : Amperometric, Potentiometric, Voltametric, Conductometric.
- b) Piezoelectric : QCM, Acoustic emission, Ultrasonic.

b) Receptor based

- a) Biocatalytic - enzyme, cells, tissue.
- b) Receptor/Antagonist.
- c) Biocomplexing - Ag/Abimmunosenors.

c) Nanobiosensor Nanoparticles, Nanotubes, Nanowires, Porous silicon.

IV. BIOSENSOR BASED ON DETECTION

a) Direct Detection

Direct recognition sensors, in which the biological interaction is directly measured typically, use non-catalytic ligands such as cell receptors or antibodies.

b) Indirect Detection

Indirect detection sensors, relies on secondary labelled elements that are often fluorescently tagged antibodies or catalytic elements such as enzymes. Biosensors are also characterized by their specificity, or their ability to recognize a single compound among other substances in the same sample. The selectivity of biosensors is determined by both the bioreceptor and the method of transduction.

There are many potential applications of biosensors. The main requirements for a biosensor approach to be valuable in terms of research and commercial applications such as: Cancer diagnostics, Detection of pathogens, Drug discovery and evaluation of biological activity of new compounds, Glucose monitoring in diabetic patients etc. Determination of drug residues in food.

V. NANOBIOSENSORS

One of the most recent technological advances in this area has been the development of nanosensors, which are sensors with dimensions on the nanometer scale. There are currently several hypothesized ways to produce nanosensors :

- a) Top-Down Method
- b) Bottom-up Method
- c) Self-assembly

A. Biosensor/Nanobiosensor

Nanomaterials are exquisitely sensitive chemical and biological sensors. Each sensor should be sensitive for one chemical or biological component of a substance. Thus, by having sensor arrays it is possible to tell the composition of an unknown substance.

A biosensor generally consists of a biosensitive layer that can either contain biological recognition elements or be made of biological recognition elements covalently attached to the transducer. The interaction between the target analyte and the bioreceptor is designed to produce a physicochemical perturbation that can be converted into a measurable effect such as an electrical signal. Bioreceptor are important elements providing specificity for biosensor technologies, because they allow for binding of the specific analyte of interest to the sensor for the measurement with minimum interference from other components in complex sampling mixtures. Biological sensing elements can be either a biological molecular species (eg. an antibody, an enzyme, a protein, or a nucleic acid) or a living biological system (eg. cells, tissue, or whole organisms) that uses a biochemical mechanism for recognition.

Presently, there are several ways proposed to make nanosensors, including top-down lithography, bottom-up assembly, and molecular self-assembly.

Importance of Nanosensors in Cell Analysis :

- Cells in a population respond asynchronously to external stimuli e.g., apoptosis, cells differ in their ability to activate caspases.
- To study and understand molecular mechanisms that underlie such differences it is necessary to measure caspase activity in individual cells.

Nanomaterials are exquisitely sensitive chemical and biological sensors. Nanosensors with immobilized bioreceptor probes that are selective for target analyte molecules are called nanobiosensors. Nanosensors offer significant improvements in the field of cancer research. There are many developed biosensors and nanobiosensors, few of them are given below :

1) Optical Biosensor

The use of light instead of electrical potential or current to measure a biochemical reaction has been accelerated since the development of cheap light-emitting and photo-sensitive diodes together with fiber optic systems acting as transducers. Optical sensing techniques are especially attractive in high throughput screening since they enable biosensors to monitor multiple analytes simultaneously [10]. Standard optical biosensors modify fundamental properties such as light absorption, frequency or light emission. There are two main areas of development in optical biosensors. These involve determining changes in light absorption between the reactants and products of a reaction, or measuring the light output by a luminescent process. The most common use of this technology is for whole-blood monitoring in diabetes control. One of the initial optical biosensor consisted simply of membrane-dye combination to which a suitable enzyme was co-immobilized. Addition of the substrate to this complex generated a pH shift resulting in change of color of the dye. The color change was detected using a light-emitting diode of appropriate wavelength on one side of the membrane and a photodiode on the other [11].

2) Electrical Nanosensors

Nanosensors constructed at the molecular scale are promising and have proved to be extremely sensitive, selective and responsive. Nano biosensors are small enough to hold and measure individual proteins or even small molecules by using carbon nanotube. Electrical nanosensors incorporating nanostructures as sensing probes are highly promising devices for disease diagnostics in medicine; chemical or biomolecular binding events are directly converted into electrical signals, which allows for label-free readout in the case of DNA detection.

3) Electrochemical Biosensor

Nanomaterials are acquiring a big impact on progress of electrochemical biosensors. Nanotechnology brings new possibilities for biosensors construction and for developing novel electrochemical bioassays. The use of nanoscale materials for electrochemical biosensing has seen explosive growth in the past 5 years. Nanoscale materials have been used to achieve direct wiring of enzymes to electrode surface, to promote electrochemical reaction, to impose nanobarcode for biomaterials and to amplify signal of biorecognition event. Electrochemical sensors are the largest group of chemical sensors, representing approximately 58% of the total. An electrochemical biosensor is a biosensor with an electrochemical transducer. It is considered to be a chemically modified electrode (CME) as electronic conducting, semiconducting or ionic conducting material is coated with a biochemical film [12]. Electrochemical sensors are devices that extract information about sample from measurement of some electrical parameter. It is easy to categorize them according to the measured electrical parameter, because the three are linked together by Ohm's Law. So, if we measure difference of two potentials (in volts) we talk about "potentio-

BIOSENSORS	PRINCIPLE	APPLICATIONS
Optical biosensor	Colorimetric for color, Photometric for light intensity	Detection of cancer
Surface Plasmon resonance	Evanescence wave phenomenon	Biomolecular interactions
Electrochemical biosensor	Immobilization of biomolecules	Measure blood glucose concentration
Nanotube based biosensor	uses CNT as both an electrode and an immobilization	Application in immunoassays, nucleic acid probe assays, assays for clinical chemistry analytes
Piezoelectric biosensor	nucleic acids interaction	point mutation detection in PCR samples

Table 1: Different biosensors, principal and applications.

metric sensors", if the parameter of interest is current (in amperes) we talk about "amperometric sensors", and if we measure resistance (in ohms) or conductance we talk about "chemiresistors" or "conductometric" sensors.

4) Nanowire Biosensor

Nanowire based biosensor arrays have significant impact for detection of biological threats, early diagnosis of cancer, drug discovery, and medical treatment. The nanowire based biosensor arrays enables simultaneous detection of multiple analytes such as cancer biomarkers in a single chip, as well as fundamental kinetic studies for biomolecular reactions.

5) Viral Nanosensor

Virus particles are essentially biological nanoparticles. Herpes simplex virus (HSV) and adenovirus have been used to trigger the assembly of magnetic nanobeads as a nanosensor for clinically relevant viruses. This system is more sensitive than ELISA-based methods and is an improvement over PCR-based detection because it is cheaper, faster and has fewer artifacts.

6) Nanoshell Biosensors

Gold nanoshells have been used in a rapid immunoassay capable of detecting analyte within complex biological media without any sample preparation. Aggregation of antibody/nanoshell conjugates with extinction spectra in the near infrared is monitored spectroscopically in the presence of analyte. Nanoshells are already being developed for applications including cancer diagnosis, cancer therapy, diagnosis and testing for proteins associated with Alzheimer's disease.

7) Nanotube Based Biosensors

This is a carbon nanotube (CNT) based biological sensor for the detection of bio molecules. Carbon nanotubes are one of the most critical nanomaterials and applied in various fields due to their special physical characteristics, excellent electronic performance and stable chemical activity. They create special interest due to their novel electronic, metallic, and structural characteristics [13]. Carbon nanotubes are center of attraction due to their superior characteristics in electron transfer reaction and natively good mechanical properties. Carbon is the basic element of life. It exhibits a richness of allotropes with different

carbon-carbon bonds and different physical and chemical properties.

In the history of carbon, discovery of CNT is an important milestone [14]. Sumio Iijima in 1991 discovered CNT which is the allotrope of carbon with cylindrical nanostructure. CNT resemble graphite rolled up to a tube. Rolling of a single layer of graphite into a seamless cylinder composed single walled carbon nanotube (SWCNT) with diameter 1-2 nm [15]. Assembly of cylinder of SWCNT one within another formed MWCNT. Due to their unique structural, physical, chemical and electronic properties many researchers are involved in CNT study. These properties of carbon nanotube provide a wide range of applications such as DNA biosensor, field emission devices, scanning probe microscopy tips, gas sensors, chemical sensors, potential hydrogen storage material, batteries, nanoelectronic devices etc [16].

In recent past CNTs have been used for production of electrodes to improve electron transfer kinetics. So CNTs have acquired broad consideration as an electrode material. In comparison to SWCNT electrode, MWCNT electrode is easy to develop which shows promising electrochemical properties. CNT facilitate electron-transfer between electro-active species and electrode when it is used as electrode material [17]. Functionalization of CNTs improves their solubility in physiological solutions and selective binding to biotarget [18]. The walls of CNTs are hexagonal carbon rings and are generally formed in large bundles. The ends of CNTs are domed structures of six membered rings capped by five membered rings [19]. CNTs are functionalized by different acids; cap and sidewall break in different sites, producing defects on the CNT walls by introducing functional groups. Two types of acid treatments used for CNT are reflux with solution of nitric acid and exposing sample to mixture of HNO₃/H₂SO₄ (1:3) under ultrasonication for 6 h [20].

Examples of nanotube biosensor :

Kyotani et al., [21] used nitric acid for oxidation treatment of inner wall of multiwalled carbon nanotubes and claimed that during their experimental conditions no damage to the MWCNTs occurred. Main disadvantage of CNT is their crucial solubilization. To overcome this problem organic solvent like DMF or DMSO and aqueous solu-

tion of nafion are being used. Even highly carboxylated CNTs(c-CNTs) are dissolve in aqueous solutions without using surfactants. Carboxylic groups of c-CNT admit covalent bonding with biomolecules or solid surfaces [22]. Huang et al., [23], used disulfide to chemically modify MWCNT to interact with different biologic systems for biomedical applications. Disulfide is sensitive to the reductive intracellular environment, and such stimulus-responsive covalent bonds were used to modify carbon nanotubes. The weight percentage of the immobilized disulfide was estimated by thermogravimetric analysis (TGA). The biocompatibility of MWNTs was improved compared to that of MWNTs without functional groups. Wang et al., [24] prepared carbon-nanotube (CNT)-derived screen-printed (SP) electrochemical sensors fabricated, and evaluated based on a CNT ink. Carbon-based inks were commonly used to their low cost, wide potential window, and low background currents. Carbon-based inks composed of graphite particles, a polymeric binder and other additives. Electrodes fabricated from CNT-derived inks were mechanically strong (with good resistance to mechanical abrasion) and possessed an excellent adhesion to the ceramic substrate. Their well-defined appearance was similar to that of conventional screen-printed electrodes. The printed CNT surface remained rigid and stable after several washings and electrochemical measurements. The fabricated CNT strips combine the attractive advantages of CNT materials and disposable screenprinted electrodes. Such thick-film CNT sensors had a well defined appearance,mechanically stable, and exhibit high electrochemical reactivity.

Rubaines et al., 2003 prepared carbon nanotubes paste electrodes (CNTPE) by dispersion of multi-wall carbon nanotubes (MWNT) within mineral oil. The resulting electrode showed an excellent electrocatalytic activity toward ascorbic acid,uric acid, dopamine, 3,4-dihydroxyphenylacetic acid (dopac) and hydrogen peroxide. These properties permitted an important decreasein the overvoltage for the oxidation of ascorbic acid, uric acid and hydrogen peroxide as wellas a dramatic improvement in the reversibility of the redox behavior of dopamine and dopac, in comparison with the classicalcarbon (graphite) paste electrodes (CPE).

Goyaneset al., [25] Functionalized multiwalled carbon nanotubes with nitric acid and with mixture of nitric acid and sulfuric acid (1:3) by volume. The effect of acid treatment on multiwalled carbon nanotube (MWCNTs) was investigated through analysis of their morphologies followed by atomic force microscopy (AFM). The chemical changes were monitored by Fourier transform infrared (FTIR) and ultraviolet (UV/Vis) spectrophotometers. Both acid treatments applied during a short ultrasonication time do not show any relevant effect. However, an additional peak can be observed at 1200 cm⁻¹ in the FTIR spectrum of the MWCNTs treated with the acid mixture after 2 h but their UV/Vis spectrum did not change. These results could indicate that new C-O groups appear in the open

ends of the nanotubes without modifying the structure of their sidewalls. When the treatmentwith the acid mixture was prolonged, the sidewall of the nanotubes began to be destroyed up to their nearly complete destruction as observed by both UV/Vis and AFM. The results reported in this work showed that three simple and quick techniques help to control the carboxylation process often carried out before the functionalization of MWCNTs.

Laschi et al., [26] developed disposable electrochemical sensor for the detection of hydrogen peroxide, using screen-printed carbonbased electrodes (SPCEs) modified with multi-wall carbon nanotubes (MWCNTs) dispersed in a polyethylenimine (PEI) mixture. The modified sensors showed an excellent electrocatalytic activity towards the analyte, respect to the high overvoltage characterizing unmodified screen-printed sensors. The composition of the PEI/MWCNT dispersion was optimised in order to improve the sensitivity and reproducibility. The optimized sensor showed good reproducibility. Preliminary experiments carried out using glucose oxidase (GOD) as biorecognition element gave rise to promising results indicating that these new devices may represent interesting components for biosensor construction.

Raoofet al., 2009 used functionalized carbon nanotube electrode for fabrication of 1, 2-naphthoquinone-4-sulfonic acid sodium (Nq) on single-wall carbon nanotube (SWNT) modified glassy carbon electrode(GCE) by electrodeposition. This electrode was characterized by scanning electron microscopy (SEM) and the results showed that Nq can rapidly and effectively be deposited on the surface of SWNT film with high stability.The electrochemical properties of functionalized SWNT/GCE with Nq (SWNT-Nq/GCE) were studied using cyclic voltammetry, double step potential chronoamperometry and differential pulse voltammetrymethods. The results indicated that SWNT could improve the electrochemical behavior of Nq and greatly enhanced its redox peak currents.

Titus et al., [27] functionalized carbon nanotubes (CNT). Thefunctionalization of chemical vapor-deposited CNT was carried out by treating tubes with polyvinylalcohol through ultrasonication in water with the aid of a surfactant. The surfactant is expected to promotethe unbundling of aggregated CNT. The characterization of functionalized samples usedthermo-gravimetricanalysis, Fourier transform infrared spectroscopy, and Raman spectroscopy revealed that the CNTwere functionalized by the interaction of carboxylic acid and hydroxyl groups

Nguyen et al., [28] developed biosensor by using vertically aligned carbon nanotube (CNT) as a nanoelectrode platform. Prior to chemicalfunctionalization, metal catalyst particles at the ends of CNT are removed and the closed ends opened. We found that the oxidative treatmentfor generating the chemical functional groups at the opened ends of the CNT compromise the mechanical stability of the nanotubes, oftenleading to total collapse of the aligned CNTs. To solve this problem, we had developed a new approach for filling the gaps between CNTswith a spin-on

glass (SOG). Results from the coupling of nucleic acids to the CNT arrays suggest that the SOG enhanced the reactivity by providing structural support to the CNTs. The SOG also covered the length of the sidewalls of CNTs, leading to a less hydrophobic interface and thus may aid in improving the chemical reactivity.

A glucose sensor has been obtained by encapsulating GOx and Pt nanoparticles in a Nafion matrix in appropriate amounts [29]. Zhang et al., [30] reported glucose sensor prepared by encapsulating GOx and carbon nanotubes in chitosan matrix. Rubianes et al., [31] used multiwalled carbon nanotube paste electrode for Polyphenol oxidase (PPOx) in dopamine determination. Guo et al., [32] used layer by layer technique in ChOx /MWCNT/Gold sensor. Similarly in cholesterol sensor Singh et al., [33] used ChEt/ChOx/PANI composite film on ITO substrate and Li et al., [34] used cholesterol oxidase with MWCNT on carbon paste electrode. Arvinte et al., [35] prepared malate biosensor based on Nafion/MDH/SWCNT/GCE.

VI. CONCLUSION

The emergence of nanotechnology in biosensor is open new horizons for the development of nanosensors and nanoprobe with submicron-sized dimensions that are suitable for intracellular measurements.

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