# APPLICATION OF BIOSENSORS IN ENVIRONMENTAL POLLUTION AND MEDICINE

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## ABSTRACT

In recent times, environmental wellness and health have won the attention of science and technology. As the world goes through an evolutionary transformation, environmental security is taken care with utmost observation and attention. Health is influenced by the ecology. Discharge of toxic pollutants such as chemicals toxins and pathogens necessitate a vigilant monitoring and a thorough check. On this account, detection and diagnosis of environmental pollutants and health hazards need scientific and technological tools.

Biosensors proved to be the most attractive analytical tools for better monitoring and to implement medical and environmental legislations in these areas. Biosensor technologies offer the potential to fulfill the parameters of diagnosis. The construction and working of biosensors base an interplay of nanotechnology and medicinal chemistry. This review encompasses the nuts and bolts of biosensors.

**KEYWORDS**: Biosensor, Environmental monitoring, Diagnosis of disease.

## 1.I NTRODUCTION

Traditional analytical method has constituted an important impediment for its application on a regular basis. The need for rapid, efficient and reliable instruments to monitor the environment and medical diagnosis has motivated the instrumentation industry to develop state of the art expertise with better methodologies which may have the capability to investigate the conventional and emerging analytes rapidly, reliably and economically. Biosensors proved to be the most attractive analytical tools for better monitoring and to implement medical and environmental legislations in these areas. (Scognamiglio, V.2010) Biosensor technologies offer the potential to fulfill these criteria through an interdisciplinary combination of approaches from nanotechnology, chemistry and medical science.(Krishnamurthy V, et al. 2010) At the moment biosensors have expansive application in medicine, agriculture, environmental monitoring and the bio-processing areas., (Anthony P. F. Turner 2013).

Biosensor is an improved analytical device or technique that can convert biological data into electric signals. In recognition to IUPAC definition "A biosensor is a self-contained integrated device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) which is in direct spatial contact with a transducer element. A biosensor should be clearly distinguished from a bio-analytical system, which requires additional processing steps, such as reagent addition. Furthermore, a biosensor should be distinguished from a bio-probe which is either disposable after one measurement, i.e. single use, or unable to continuously monitor the analyte concentration." (Thevenot DR et al.1999)A biosensor is a device composed of two elements:

1. A bioreceptor that is an immobilized sensitive biological element (e.g. enzyme, DNA probe, antibody) recognizing the analyte (e.g. enzyme substrate, complementary DNA, antigen). Although antibodies and oligonucleotides are widely employed, enzymes are by far the most commonly used biosensing elements in biosensors.

2. A transducer is used to convert (bio)-chemical signal resulting from the interaction of the analyte with the bioreceptor into an electronic one. The intensity of generated signal is directly or inversely proportional to the analyte concentration. Figure 1 explains the basic principle of a biosensor.

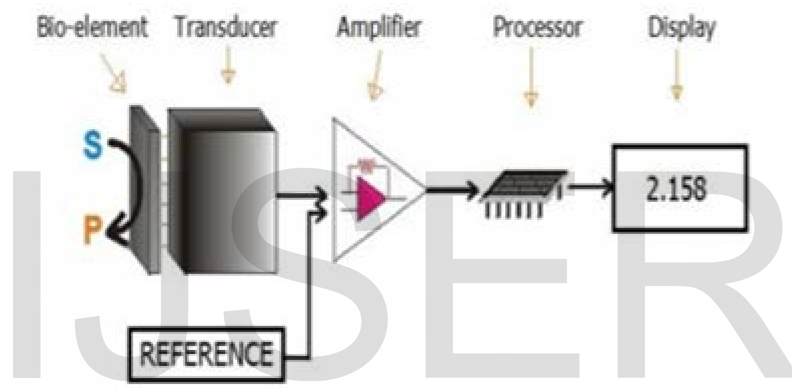


Fig.1: Schematic diagram of a Biosensor

Based on various scientific literature surveys, biosensors are categorized according to the basic principles of signal transduction and bio recognition elements. According to the transducing elements, biosensors can be classified as electrochemical, optical, and piezoelectric biosensors.

## 1.1. ELECTROCHEMICAL BIOSENSOR

Electrochemical Biosensors is a simple device. The investigated bio-electrochemical reaction would generate a measurable current (amperometric detection), a measurable potential or charge accumulation (potentiometric detection) or measurable conductivity change of a medium (conductometric detection) between electrodes. When the current is measured at a constant potential this is referred to as amperometry. Potentiometric, amperometric and conductometric measurement techniques form the types of electrochemical biosensors. Potentiometric sensors have an organic membrane or surface that is sensitive to an analyte. The reaction between them generates a potential (emf) proportional to the logarithm of the electrochemically active material concentration. This potential is compared with the reference electrode potential. (D. Grieshaber et. al 2008) Generally biological compounds (glucose, urea, cholesterol, etc.) are not electroactive, so the combination of reactions to produce an electroactive element is needed. This electroactive element leads a change of current intensity. This change is proportional to the concentration of analyte. Conductometric biosensors can measure the change of the electrical conductivity of cell solution. Most reaction involve a change in the composition of solution. Thus conductometric biosensors can detect any reactive change occuring in a solution. The advantage of electrochemical sensor is that they can sense materials without damaging the system.(Arora, 2012)

In an electrochemical system with three electrodes, the working electrode can be referred as either cathodic or anodic depending on the reaction on the working electrode is a reduction or an oxidation. There are many kind of working electrodes such as glassy carbon electrode, screen printed electrode, Pt electrode, gold electrode, silver electrode, Indium tin Oxide coated glass electrode, carbon paste electrode, carbon nanotube paste electrode etc. Screen printed electrodes are prepared with depositing inks on the electrode substrate (glass, plastic or ceramic) in the form of thin films. Different inks can be used to get different dimensions and shapes of biosensors. Screen-printed electrochemical cells are widely used for developing amperometric biosensors because these biosensors are cheap and can be produced at large scales. This could be potentially used as disposable sensor that decreases the chances of contamination and prevents loss of sensitivity. Performance factors of an electrochemical biosensor are selectivity, response time, sensitivity range, accuracy, recovery time, solution conditions and the life time of the sensor.

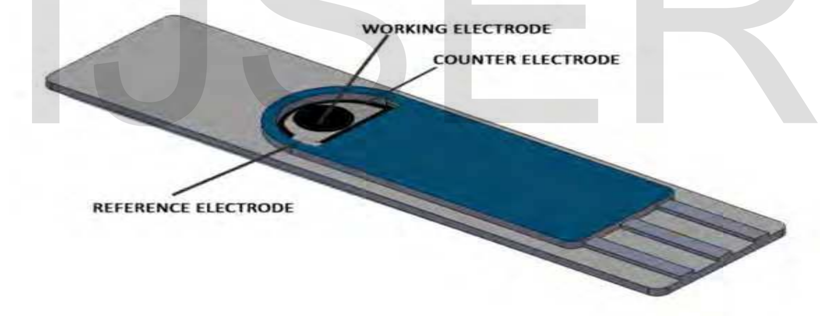


Fig.2: Electrochemical Biosensors

Figure 2 explains the working principle of an electrochemical biosensor, comprising of three electrode system. Sensing is accomplished with the aid of the working electrode.

**1.2.OPTICAL BIOSENSOR**

Optical biosensors are the devices that utilize the principle of optical measurements (absorbance, fluorescence, chemiluminescence etc.). They employ the use of fibre optics and optroelectronic transducers. The word optrode, representing a condensation of the words optical and electrode is commonly used. Optical biosensors primarily involve enzymes and antibodies as the transducing elements.Optical biosensors allow a safe non-electrical remote sensing of materials.(Long, F. et al. 2013) Another advantage is that these biosensors usually do not require reference sensors, as the comparative signal can be generated using the same source of light as the sampling sensor. Some of the important optical biosensors are the following. 1. Fibre optic lactate biosensor 2. Optical Biosensors for Blood Glucose 3. Luminescent biosensors to detecturinary infections.

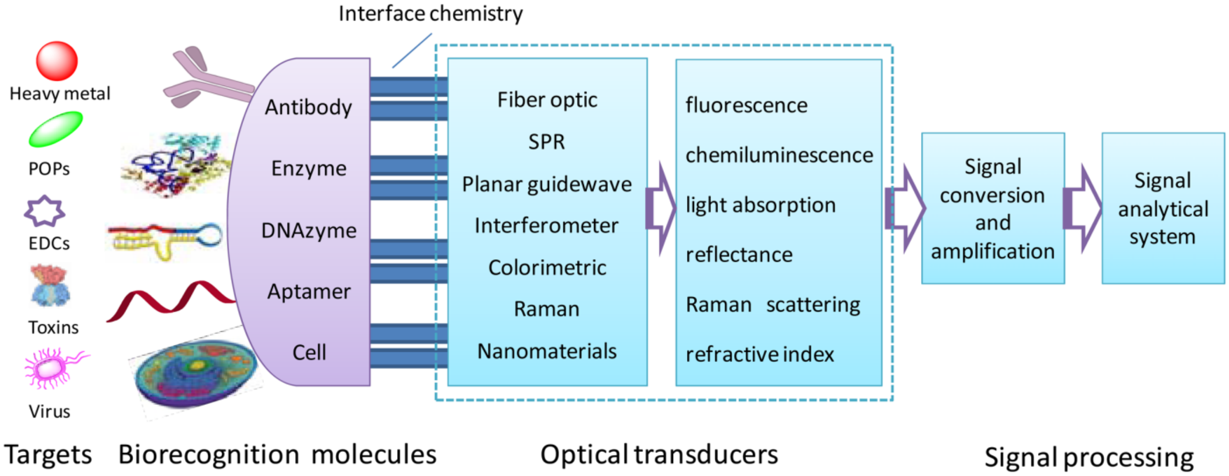


Fig.3: Optical Biosensors

Figure 3 explains the working principle of a optical biosensor. Reaction of biorecognition molecules with different targets is sensed by optical transducers with the aid of optical measuring technique such as absorbance, flouresecence etc, to produce biological signals that can be processed in electric signals.



## 1.3.PIEZOELECTRIC BIOSENSORS

Piezoelectric biosensors are based on the principle of acoustics (sound vibrations), hence they are also called as acoustic biosensors. Piezoelectric crystals form the basis of these biosensors. The crystals with positive and negative charges vibrate with characteristic frequencies. Adsorption of certain molecules on the crystal surface alters the resonance frequencies which can be measured by electronic devices. Enzymes with gaseous substrates or inhibitors can also be attached to these crystals.

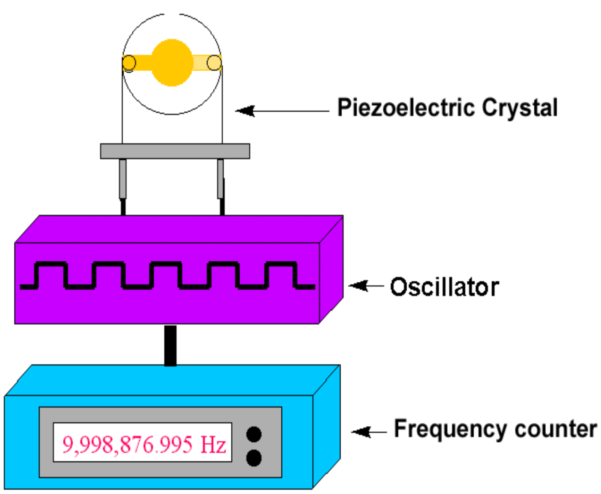


Fig.4: Piezoelectric Biosensors

Figure 4 explains the working principle of a piezoelectric biosensor based on resonance frequencies adsorbed in the crystal surface to produce electric signals. The sensitivity of this technique is determined by crystal oscillation.

A piezoelectric biosensor for organophosphorus insecticide has been developed incorporating acetylcholine esterase. Likewise, a biosensor for formaldehyde has been developed by incorporating formaldehyde dehydrogenase. A biosensor for cocaine in gas phase has been created by attaching cocaine antibodies to the surface of piezoelectric crystal. It is very difficult to use these biosensors to determine substances in solution. This is because the crystals may cease to oscillate completely in viscous liquids.

## 1.4.IMMUNOSENSORS

Immuno-biosensors or immunochemical biosensors work on the principle of immunological specificity, coupled with measurement (mostly) based on amperometric or potentiometric biosensors. There are several possible configurations for immuno-biosensors.( N. BojorgeRamírez et al. 2009)



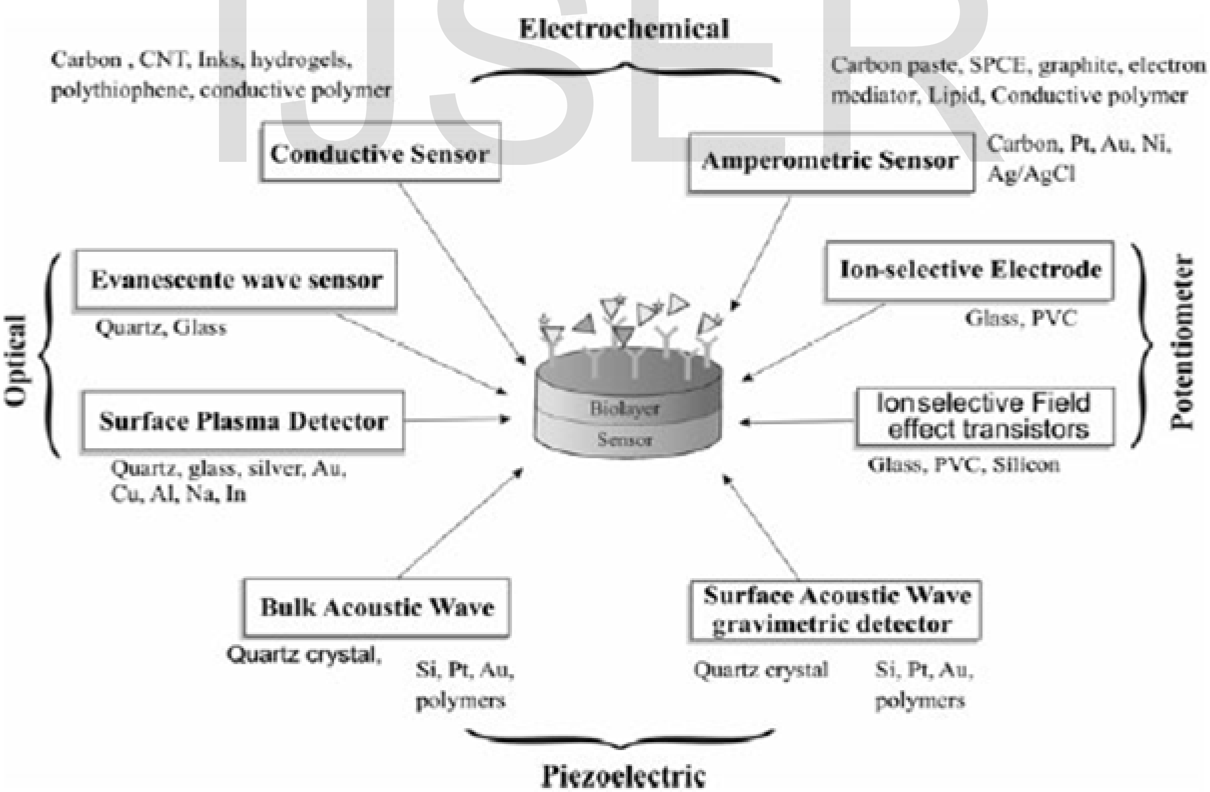


Fig.5: Immuno Biosensors

Figure 5 explains the working principle of an immuno-sensor which make use of electronic, optical, and piezoelectric as transducers to sense various compounds and polymers. This technique is used to analyse solid analyte, liquid analyte, gases and large molecular compounds.

Presently, there is an overall increase in research and investigation on biosensors, which reflects the considerable interest in the device. Though the level of technological and scientific development in high use of these devices in the real environmental and medical analysis is limited. (Mottram et al, 2003)

The application of bio-sensing technique is numerous, they include areas of clinical, diagnostic, medical applications, process control, bioreactors, quality control, agriculture and veterinary medicine, bacterial and viral diagnostic, drag production, control of industrial waste water, mining, military defense industry, etc. The focus of this paper is the application of biosensor for vironmental monitoring and medical uses.

## 2. BIOSENSORS FOR ENVIRONMENTAL POLLUTION

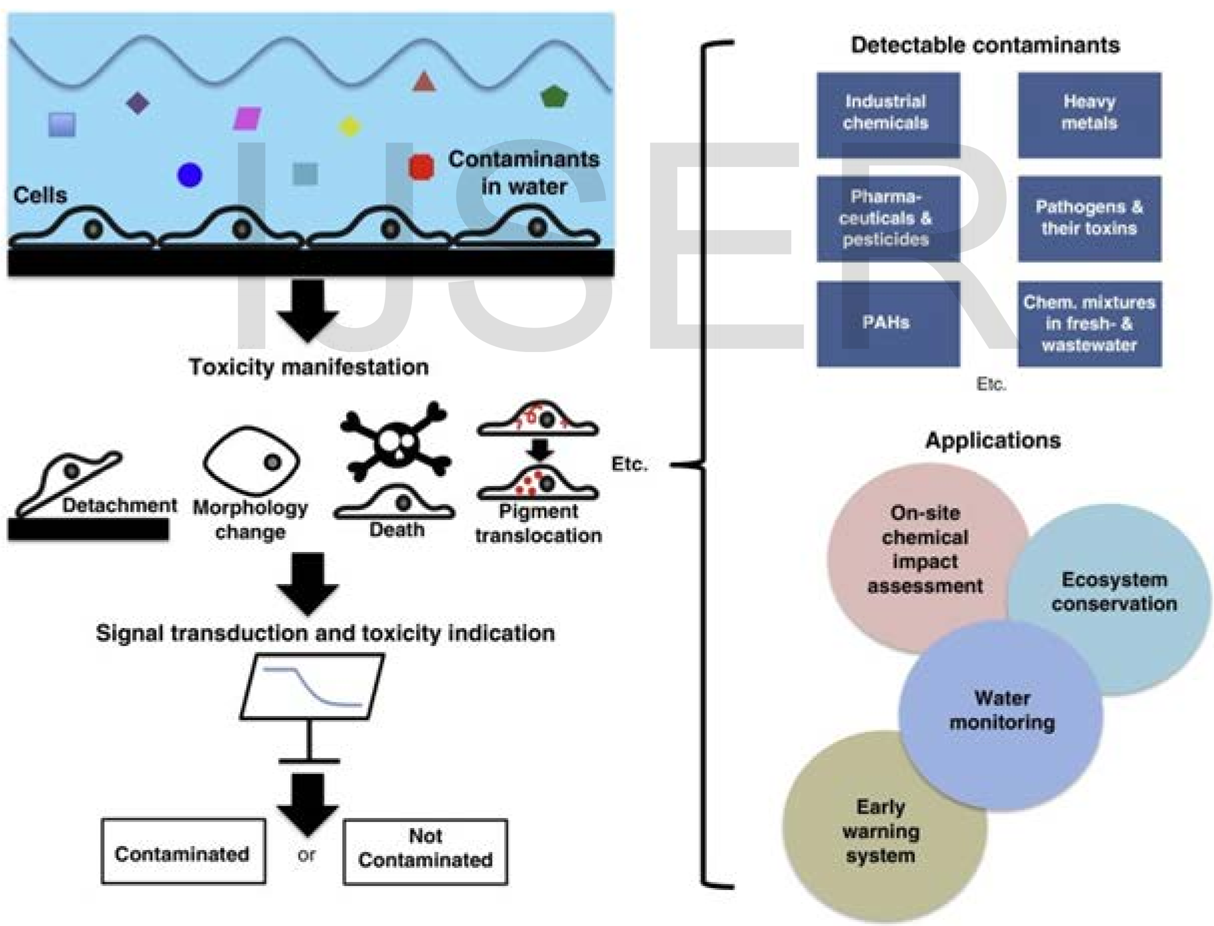


Fig.6: Biosensors in Environmental Monitoring

Figure 6 explains the use of biosensing technique in environmental monitoring of different detectable contaminants. Biosensor is applicable in areas of water monitoring, eco-system conservation, and on-site chemical impact assessment.

Biosensors are used for environmental qualitative monitoring of both inorganic and organic priority pollutants through physical, chemical, and biological assessments.(Lu tan, 2017) Pollutants are classified into various groups depending on the chemical structure, the mode of action and their effects. A wide variety of compounds of environmental concern are considered.

## 2.1. HEAVY METALS

Heavy metals are the most dangerous environmental contaminants, which present a threat to human health, even in trace quantity (Silva, et al. 2011) because they are non-biodegradable. The metal contaminants largely observed in the environment are: Lead, Chromium, Zinc, Mercury, Cadmium and Copper. (Brian, R.et al. 2000) Heavy metals are released in the ecological system in form of waste water, commercial fertilizers and pesticides. These are known for their bioaccumulation and toxicity in the food chain. Existing techniques for analysis of heavy metals such as spectroscopic, volumetric and chromatographic methods are precise but have the limitations such as high cost and lack of qualified technicians. Bacteria biosensors are currently used for the determination of heavy metals in different environmental samples. They make use of enzyme and DNA as bio-receptors, optical and electrochemical transduction systems. (Dhewa, 2015)



## 2.2. BIOCHEMICAL OXYGEN DEMAND (BOD)

Biochemical oxygen demand (BOD or $BOD_5$) is an important parameter mostly used in the estimation of the amount of biodegradable organic pollutant in water. This process is time consuming and considerably not suitable for online process monitoring. Based on this fact, BOD biosensor methods are used to achieve rapid determination of waste-water samples. An optical biosensor for parallel multi-sample determination of BOD in effluent samples is developed. The biosensor monitors the BOD concentration of effluent sample by oxygen sensing film immobilized at the end of glass sample vials. The rate of oxygen consumption is determined. Recently, BOD biosensor has been developed using yeast with oxygen probe which can detect organic contaminants within 15 minutes. (Dhewa, 2015)

## 2.3. NITROGEN COMPOUNDS

Nitrogen compounds (Nitrites) are commonly used as food preservatives(increase shelf-life) and soil fertilizers (increase soil fertility). These chemical compounds in continuous consumption can cause serious effects on human health. They contaminate ground and surface water destroying the aquatic environment. Their harmful effect is due to irreversible reaction with heamoglobin leading to severe health issues. Various biosensor devices are used for the determination of Nitrogen compounds in water samples. Another highly sensitive enzymatic conductometric biosensor, validated and used for natural water samples was established by Khandro et al.(2008)

## 2.4. POLYCHLORINATED BIPHENYLS (PCBs)

Polychlorinated biphenyls (PCBs) are extremely toxic organic compounds. They exist everywhere even when their production has been banned in several countries across the globe. Large portion of PCB accumulates in the food chain due to their highly lypophilic nature. More than 209 polychlorinated biphenyl congeners persist worldwide in the environment and food-chain. Gas chromatography and mass spectroscopy (GC-MS) were used for determination of PCBs(Centi, B. et al. 2008). Overtime, immunosensor , a class of biosensor resulted to be a better approach. It has an advantage of direct extraction without any additional purification steps. Immunosensor has a successful application for constructing low cost sensors for environmental monitoring by the use of sol-gel silica entrapment of viable *Pseudomonas species* (Gavlasova et al.2008)

## 2.5. PHENOLIC COMPOUNDS

Phenolic compounds are organic pollutants with high toxicity distributed commonly in the environment as industrial effluent. Phenolics are used in the production of drugs, antioxidants, polymers, pesticides, detergents, dyes, etc. Substituted phenols have toxic effects because they can easily penetrate the skin and cell membrane which affects the rate of biocatalyst reactions and the processes of respiration and photosynthesis.(Marco et al.2004) Electrochemical DNA sensors have been identified for environmental screening of toxic aromatic compounds and for molecular interaction existing among pollutants and DNA. Amperometric biosensor with tyrosinase have been developed for the determination of the phenol index in environmental samples. (Parellada et al.1998)

## 2.6. ORGANOPHOSPHORUS COMPOUND

Organophosphorus(OP) compounds are organic compounds majorly used as Insecticides, Herbicides, and Pesticides in agriculture for the control of pests, weeds, and disease- transmitting vectors.Pesticides are mixture of substances that prevent, destroy and lessen the damage caused by pest. Pesticides are present in water, soil and food. The toxic effect is a major source of concern globally. Enzymatic sensors are extensively used for the detection of these compounds. (Sara et al. 2006).Amperometric and optical transducers are employed for detection of herbicides. Dioxins are by-products of the chemical reaction involving chlorine. They are extremely toxic and carcinogenic. Therefore adequate research is needed to develop a biosensing technique in this regard.

## 2.7. CONTAMINATING MICROORGANISMS

The presence of pathogens (Bacteria, Viruses, etc) in waste, untreated and treated water is a huge environmental problem which contributes to public health issues. New technologies such as biosensors are developed for rapid identification of pollutants by microorganisms.(Koubova et al 2001) designed a surface plasmon resonance based sensor for the real time monitoring of pathogens in liquid samples. There is a need to explore the possibility of devising biosensors at the specific locations for constant and continuous monitoring.

## 3. BIOSENSORS IN MEDICINE

In the discipline of medical science, there is significantly rapid growth in the application of biosensors. The main focus of a biosensor in medicine is the diagnosis of infectious diseases. Among the various diseases of human, three of them namely, diabetes, cardiovascular disease and cancer can be monitored using biosensors.

### 3.1. BIOSENSOR FOR DIABETES APPLICATION

As the risk of cardiovascular disease is much higher for a diabetic, it is crucial that blood pressure and cholesterol levels are monitored regularly. There are several reported methods for glucose analysis. Glucose biosensor was the first recorded biosensor. (Clark and Lyons, 1962). Further technological advances, proved that glucose monitoring can be invasive and non-invasive. (Cibele Gouvea, 2011) Invasion glucose uses oxidase- based glucose electrochemical methods where the sensors are inserted into the fluid space. Non-invasive glucose monitors are to be developed. Optical or transdermal approaches are the most common non-invasive glucose sensing methods. Inspite of its ease and usage, this technique is not accepted by its low sensitivity, poor selectivity and difficulties in miniaturization. Considerable efforts have been made in the development of non - invasive glucose biosensor. A simple technique involving paper strips impregnated with reagents is used for this purpose. The strips contain glucose oxidase, horse radish peroxidase and a chromogen (e.g. toluidine). The following reactions occur.

$$\text{Glucose} \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$\text{Chromogen} + 2H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Colour dye} + 2H_2O$$

The intensity of the colour of the dye can be measured by using a portable reflectance meter. Glucose strip production is a very big industry worldwide. Colorimetric test strips of cellulose coated with appropriate enzymes and reagents are also in use for the estimation of several blood and urine parameters.

### 3.2. CANCER

The use of biosensor for the detection of cancer is an effective and accurate method. Biosensor technology has the potential to provide fast and accurate detection, reliable imaging of cancer cells, and monitoring of angiogenesis and cancer metastasis, and the ability to determine the effectiveness of anticancer chemotherapy agents. The site of tumor differs, that is identified using Biomarkers. They include breast, colon, esophageal, liver, lung, ovarian and prostate. Clinical cancer diagnosis is now focusing on developing analytical techniques, which are clearly capable of sensitive and parallel detection of biomarkers rendering useful point-of-care testing. For detecting cancer monoclonal antibodies, aptamers and antigens are used to bind micro Ribonucleic acids (miRNAs) corresponding to single stranded Deoxyribonucleic acid (ssDNA). The recognition signal is converted to electrical signal by a device called transducer. The transducer may be optical (luminescence, fluorescence, interferometry and colorimetric), calorimetric (thermistors), electrochemical (by, Amperometry, potentiometry and conductometry/impedimetry), or based upon mass changes (acoustic waves/ piezoelectric), and are needed because they give high noise signals, high performance, great resolution, cheap instrumentation and consistent results. (Zhang Y, 2013) Ion selective electrodes which detect electrical response whenever a specific element's molecular recognition occurs is used by potentiometric sensor. These biosensors have high potential in the diagnosis of cancer. Electrochemical biosensors help in detection of damaged DNA along with carcinogens that cause the damage. Laser-induced fluorescence is an optical biosensor for the monitoring and diagnosis of throat cancer. The patient swallows the biosensor, LASER beam is directed by the device and on the surface of the esophagus a specific wavelength of light is emitted. The walls of the esophagus reflect light of very specific wavelengths, and the variation in the visualization of various wavelengths is influenced by the presence or absence of cancer cells or normal cells. Over 200 patients have been tested by this sensor and have been found to be highly useful as compared to conventional methods. Surgical biopsies and the pain associated recovery are prevented by these biosensors. (Bohunicky B, 2010) Micro cantilever and immune-biosensors are based upon piezoelectric technologies for detecting tumor biomarkers. Over expression of human protein 53 gene's point mutations in several types of tumors has been reported with piezoelectric biosensors coupled with polymerase chain reaction amplification. (Tothill IE 2009)



### 3.3. CARDIO- VASCULAR DISEASE

The increasing incidence of cardiovascular disease is majorly caused by the increased cholesterol concentration in the blood.( Franco et al, 2012) Estimation of cholesterol is very important. This is carried out by the use of enzymes such as cholesterol oxidase and cholesterol esterase. Electrochemical and optical transducer and commonly used for this diagnosis. (Arya et al. 2008).The detection and quantification of biomarker proteins are critical for diagnosing and treating cardiovascular diseases. One such biomarker is C-reactive protein, which is produced in the liver and triggers inflammation in the body when it enters the bloodstream. The blood concentrations of this protein rapidly increase before the onset of cardiac diseases, and thus measuring the levels of this protein can be helpful for predicting the risks of cardiac diseases.The sensor was made by using a special type of nano-fibre, vertically aligned carbon nanofibres on silicon wafers. They then attached the probe molecule anti-C-reactive protein to the biosensor surface. This probe protein was used to capture C-reactive protein. Electrochemical studies were done to determine the efficacy of the biosensor in measuring C-reactive protein levels in solutions containing different concentrations of C-reactive protein. There was a decrease in current when C-reactive protein bound to the surface of the biosensor, indicating the specific concentration of this biomarker protein. The current decreased at faster rates for lower biomarker protein concentrations and lower rates for higher protein concentrations.The biosensor could detect extremely low concentrations of C-reactive protein, having a detection limit of 11 nanogram/millilitre. In addition, this biosensor can provide portable and multiple protein detection platforms, allowing rapid diagnosis and treatment of cardiac diseases. (Gupta, R. K. et al. 2014)

Other application of biosensors include, immunosensor array of clinical immunophenotyping of acute leukemia, lab- on a chip for quick and accurate detection of multiple cancerous biomarkers and luminescent biosensors to detect urinary infections.

## CONCLUSION

In biosensor development studies, suitable bio-receptor molecule, suitable immobilization method and transducer should be selected firstly. Knowledge in biology, biochemistry, chemistry, electrochemistry, physics, kinetics and mass transfer is required for this study. This means that developing a biosensor is an interdisciplinary process. The electrochemical biosensor, fiber-optic biosensor, carbon nano-tube, protein engineering for biosensors and wireless biosensors networks are other related topics that concerns biosensors. The environmental and medical application of biosensing technique involves detection and monitoring. The use of biosensor as a bio-analytical tool remains important, therefore more consideration should be put to the factors such as selectivity, molecular dynamics and environmental interactions, automation of parallel sensing and competitive cost. Further development of this technique leads to the production of sensors that common people can access them for environmental monitoring and diagnosis of diseases. To achieve these, better concept of alternative materials and process is needed. The outlook is promising for industrial use of multichannel application in different scientific and technological fields.

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