BIOSENSORS: DEFINITION, DESIGN, DEVELOPMENT AND APPLICATIONS

Dr. Bhawana Pandey
Head of the Department of Biotechnology & Microbiology
Bhilai Mahila Mahavidyalaya
Chhattisgarh.

I. INTRODUCTION

Biosensors is an analytical devices that combine a biological component with a physicochemical detector to measure the presence of specific substance. These devices leverage the specificity of biological molecules, like enzymes, antibodies and nucleic acids, to detect specify analytes. The interaction between the biological component and the analyte generates a signal, typically electrical, optical, or thermal, then it is converted into a measurable output with the help of transducer.

II. DEFINITION OF BIOSENSORS

A biosensor can be defined as a compact analytical device incorporating with a biological/ biologically derived sensing element called bioreceptor, either integrated within or intimately associated with a physicochemical transducer. The primary function of a biosensor is to provide either quantitative or semi-quantitative analytical information using a biological recognition element.

Biosensors are

- Analytical devices that consists a combination of biological detecting elements like sensor system and a transducer is termed as biosensor.
- Biosensors can be defined as self-sufficient integrated devices that has capacity to provide specific qualitative or semi-quantitative analytical information using a biological recognition element which is in direct-spatial contact with a transductional element.
- In simple words, biosensors are analytical devices that detects changes in biological processes and transform the biological data into electrical signal.
- The main features of biosensors are:

- ❖ Stability
- ❖ Economical
- ❖ Sensitivity
- ❖ Reproducibility

What are Biosensors?

A Biosensor is an analytical device that detects changes in Biological processes and converts them into an electrical signal. The term Biological process means biological element or material like enzymes, tissues, microorganisms, cells and nucleic acids.

So, a Biosensor is a Biological sensing element and a transducer, which converts the data into electrical signals. Additionally, an electronic circuit which consists of a Signal Conditioning Unit, a Microcontroller or Processor and a Display Unit.

III. DESIGN OF A BIOSENSOR

1. **Bioreceptor:** The biological recognition element responsible for the selective interaction with the target analyte. Common bioreceptors include:

   - Enzymes
   - Antibodies
   - Nucleic acids
   - Microorganisms
   - Cells

2. **Transducer:** The component that converts the biological interaction into a measurable signal. Types of transducers include:

   - Electrochemical (potentiometric, amperometric, conductometric)
   - Optical (fluorescence, absorbance, surface plasmon resonance)
   - Piezoelectric (quartz crystal microbalance)
   - Thermal

3. **Signal Processor:** The electronic system that amplifies, processes, and converts the signal from the transducer into a readable output. This often involves analog-to-digital conversion and signal conditioning.

4. **Display:** The interface that presents the results to the user, which can range from simple digital readouts to complex graphical displays on computers or smartphones.

The following block diagram showing the components of a Biosensor.

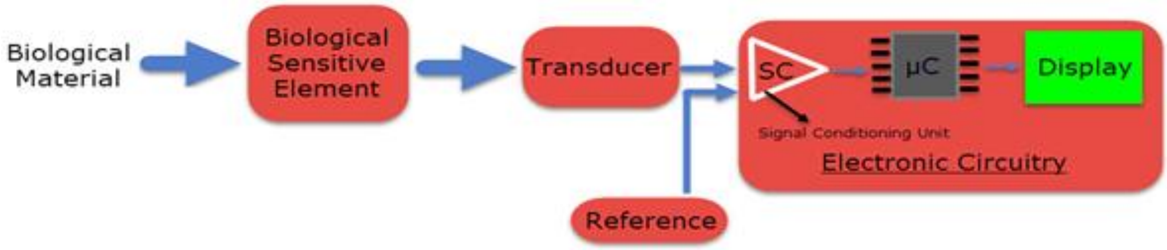


Figure 1: Components of Biosensor

IV. GENERAL FEATURES OF BIOSENSORS

A biosensor has two distinct components.

- **Biological Component:** Nucleic acid, enzyme, cell etc.
- **Physical Component:** Transducer and amplifier.

The biological component recognises and interacts with the analyte to produce a physical change that can be detected, by the transducer. The biological material is appropriately immobilized on to the transducer and the so prepared biosensors can be repeatedly used several times (may be around 10,000 times) for a long period.

Development of Biosensors

The development process of biosensors involves several stages:

1. **Selection of Bioreceptor:** Based on the target analyte and the required specificity, stability, and operational conditions.

2. **Transducer Integration:** Choosing an appropriate transducer that matches the type of signal generated by the bioreceptor interaction.

3. **Immobilization Techniques:** Developing methods to immobilize the bioreceptor onto the transducer surface without losing its activity. Common techniques include physical adsorption, covalent bonding, entrapment, and affinity interactions.

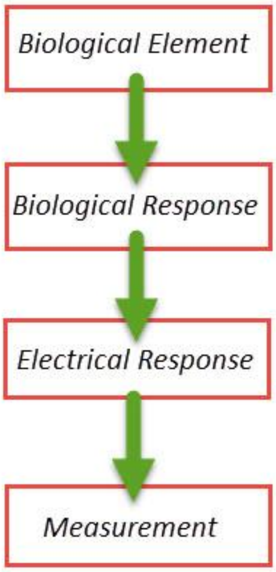
4. **Optimization:** Fine-tuning the biosensor components for optimal performance in terms of sensitivity, specificity, response time, and stability.

5. **Characterization and Calibration:** Testing the biosensor's performance with known standards to ensure accuracy and reliability.

6. **Prototyping and Validation:** Creating prototypes and validating the biosensor under real-world conditions.

Principle of a Biosensors

The desired biological material which is usually an enzyme. By a process Electroenzymatic approach (a chemical process) of converting the enzymes into specific electrical signals (usually current) with the help of a transducer.



- Biosensors works on the principle of signal transduction and biorecognition of any element.
- All the biological materials including-enzyme, antibody, nucleic acid, hormone, organelle or whole cell as sensor or detector in a device. But the bio-receptor is usually a specific deactivated enzyme which is placed in to the transducer.
- The tested analyte attached to the specific enzyme (bio-receptor) and inducing a change in biochemical property of enzyme. The change gives an electronic response through an electroenzymatic approach.
- Electroenzymatic process is the chemical process of converting the enzymes into specific electrical signals of transducer.

- Now, the outcome from transducer i.e. electrical signal is a representation of the biological material (analyte and enzyme) being measured.
- The electrical signal is usually converted into physical display for its proper analysis.

Working of Biosensors

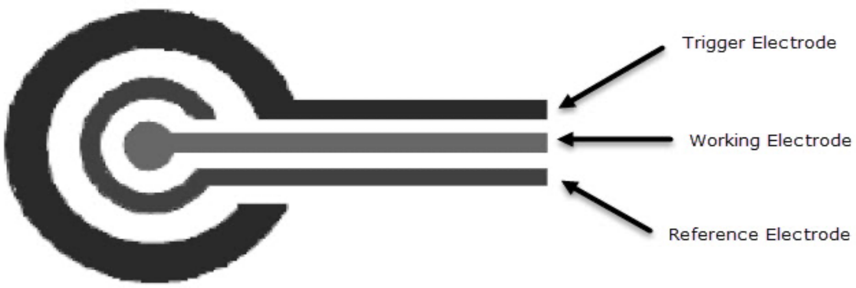
The combination of biological element and a transducer will convert the biological material into a corresponding electrical signal. Depending on the type of enzyme, the output will be either current or voltage.

The output voltage signal is usually very low in amplitude and it is superimposed on a high frequency noise signal. So, this signal is amplified and then passed through a Low Pass RC Filter. The output of the signal processing unit is an analog signal that is equivalent to the biological quantity being measured.

The analog signal is displayed directly on the LCD display but usually, this analog signal is passed to Microcontroller, where the analog signal is converted into digital signal, to analyze, process or store a digital signal.

Example of Biosensor

Here the example of a Biosensor: The Glucometer, which is one of the most common applications. Diabetes is characterized by the levels of glucose in the blood. Regularly checking the blood glucose levels is necessary for diabetes patients. Usually, it consists of a test strip, which collect a small sample of blood to analyze the patients glucose levels. This particular sensor implements the Electroenzymatic approach i.e. oxidation of glucose present in blood.



Glucometer Test Strip

In the test strip a trigger electrode and a reference electrode is present. When blood is placed on the test strip, after a chemical reaction an electrical current is generated, which is directly proportional to the concentration of glucose.

V. DIFFERENT TYPES OF BIOSENSORS

Biosensors are classified into two groups i.e. either based on the Biological Element used in the analysis. Some commonly used biological elements are DNA, enzymes, antibodies, microorganisms, tissues etc.

Another classification of Biosensors is based on the type of transduction used in the sensor i.e. type of physiochemical. The biosensors based on method of transduction are again divided into three types:

- Mass based Biosensors
- Optical based Biosensors
- Electrochemical Biosensors

There are few subclasses of each of these types of biosensors.

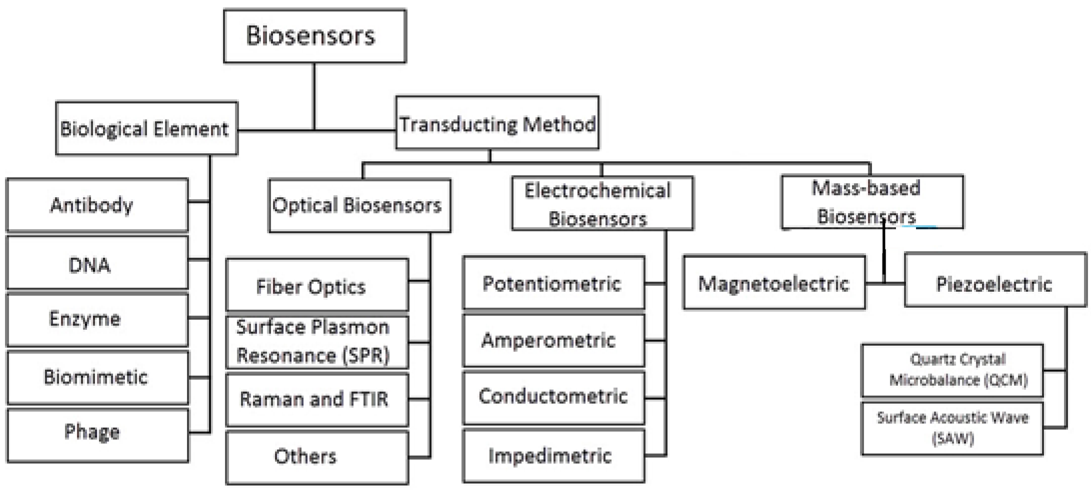


Figure 2: Types of Biosensors

In Modern classification the are several types of biosensors based on the sensor devices and the type of biological materials used.

1. **Electrochemical Biosensors:** These are simple devices based on the measurements of electric current, ionic or conductance changes carried out by bio electrodes.

2. **Amperometric Biosensors:** These biosensors are based on the movement of electrons as a result of enzyme-catalysed redox reactions. Normally, a constant voltage passes between these electrodes which can be easily determined. In an enzymatic reaction, the substrate or product can transfer an electron with the electrode surface to be oxidised or reduced.

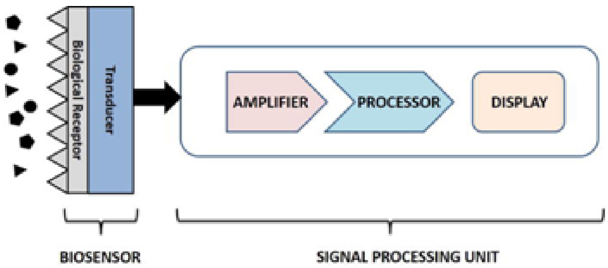


Figure 3: Amperometric Biosensors

This results in an altered current flow that can be measured. The magnitude of the current is proportional to the substrate concentration. Clark oxygen electrode in which determines reduction of O_2 occurs is the example of amperometric biosensor.

3. **Potentiometric Biosensors:** The changes in ionic concentrations are determined by use of ion- selective electrodes in this type of biosensors. pH electrode is the most commonly used ion-selective electrode, because many enzymatic reactions involve the release or absorption of hydrogen ions. The other electrodes are ammonia-selective and CO_2 selective electrodes.

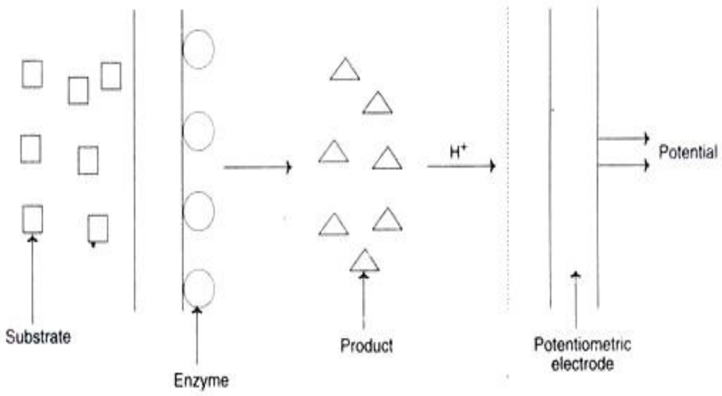


Figure 4: Potentiometric Biosensors

The potential difference obtained between the potentiometric electrode and the reference electrode can be measured which is proportional to the concentration of the substrate. The major limitation of the above biosensors is the sensitivity of enzymes to ionic concentrations such as H^+ and NH^+_4. Ion-selective field effect transistors (ISFET) are the low cost devices that can be used for miniaturisation of potentiometric biosensors. An example of ISFET biosensor used to monitor intra-myocardial pH at the time of open-heart surgery.

4. **Conduct Metric Biosensors:** In this type of biosensor there are several reactions in biological systems occur that bring about changes in the ionic species. These ionic species are responsible for alteration of electrical conductivity which can be measured. An example of conduct metric biosensor is the urea biosensor utilising immobilised urease. In this urease catalyses the following reaction.

The above reaction is reason for alteration in ionic concentration which can be used for monitoring urea concentration. These urea biosensors are successfully used during dialysis and renal surgery.

5. **Thermometric Biosensors:** Number of biological reactions are associated with the production of heat and it is the basis of thermometric biosensors. They are also known as thermal biosensors or calorimetric biosensors. It consists of a heat insulated box fitted with aluminium cylinder heat exchanger.

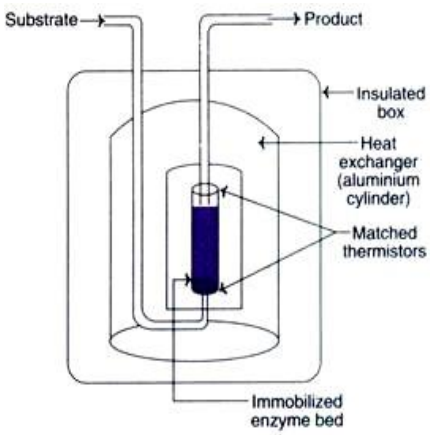


Figure 5: Thermometric Biosensors

The reaction takes place in a small enzyme packed bed reactor. When substrate enters in the reactor, it gets converted to a product and heat is generated. The temperature difference between the substrate and product is measured by thermistors. Even a small change in the temperature can be detected by thermal biosensors.

This biosensors is used for the estimation of serum cholesterol. When cholesterol gets oxidized by cholesterol oxidase enzyme, heat is generated which can be measured. Another example is estimations of glucose (enzyme-glucose oxidase), urea (enzyme-urease), uric acid (enzyme-uricase) and penicillin G (enzyme-P lactamase) can be done by these biosensors. Thermometric biosensors can be used as a part of enzyme-linked immunoassay and the new technique thermometric ELISA (TELISA).

6. **Optical Biosensors:** Optical biosensors are those devices which are utilises the principle of optical measurements (absorbance, fluorescence, chemiluminescence etc.). The fibre optics and optoelectronic transducers are used in this biosensor. The word optrode, is combination of two words optical and electrode. In Optical biosensors enzymes and antibodies are used as the transducing elements.

   Optical biosensors allow a safe non-electrical remote sensing of materials and they do not require reference sensors, as the comparative signal can be generated using the same source of light as the sampling sensor.

   - **Fibre Optic Lactate Biosensor**

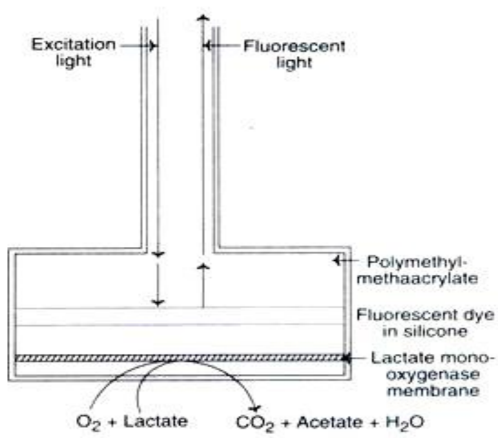
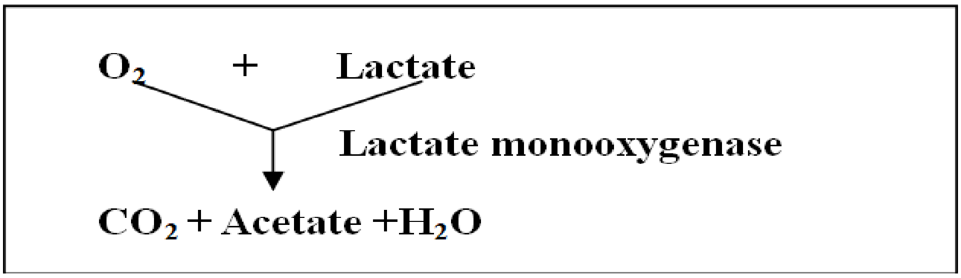


Figure 6: Fibre Optic Lactate Biosensor

Its working is based on the measurement of changes in molecular O_2 concentration by determining the quenching effect of O_2 on a fluorescent dye. The reaction is catalysed by the enzyme lactate mono-oxygenase.



The amount of fluorescence generated by the dyed film is dependent on the O_2 quenching (reducing). As the concentration of lactate in the reaction mixture increases, O_2 is utilized, and decrease in the quenching effect. That results an increase in the fluorescent output which can be measured.

- **Optical Biosensors for Blood Glucose:** This biosensor estimates blood glucose through 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 reactions is :

Glucose oxidase
Glucose --- -------------- ------Gluconic acid + H2O2

Peroxidase
Chromogen+ 2H2O2 -------------------- Colour dye + 2H2O+ O2

The colour of the dye can be measured by using a portable reflectance meter. Colorimetric test strips of cellulose coated with appropriate enzymes and reagents are used for the estimation of blood and urine parameters.

- **Luminescent Biosensors to Detect Urinary Infections:** This biosensor is used to detect microorganisms in the urine, causing urinary tract infections, For this purpose, the immobilized enzyme namely luciferase is used. The microorganisms, on lysis release ATP which can be detected by the following reaction. The quantity of light output can be measured by electronic devices in this biosensor.

Leuciferin + ATP + O_2

↓ Luciferase

Oxyluciferin + CO2 +AMP+ LIGHT

7. **Piezoelectric Biosensors:** Piezoelectric biosensors also called as acoustic biosensors are based on the principle of acoustics (sound vibrations). Piezoelectric crystals with positive and negative charges vibrate with characteristic frequencies are the basis of these biosensors.. Adsorption of certain molecules on the crystal surface alters the resonance frequencies which can be measured by electronic devices. Some enzymes with gaseous substrates can also be attached to these crystals. A biosensor for cocaine in gas phase has been created by attaching cocaine antibodies to the surface of piezoelectric crystal. This biosensor for organophosphorus insecticide has been developed by incorporating acetylcholine esterase. Likewise, a biosensor for formaldehyde has been developed by incorporating formaldehyde dehydrogenase

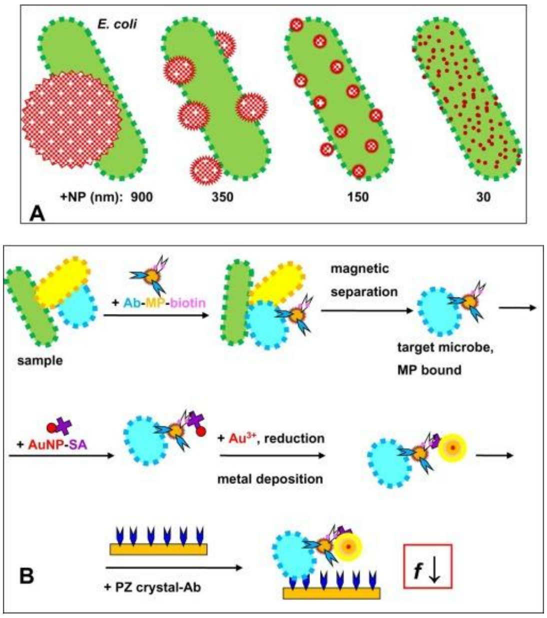


Figure 7: Piezoelectric Biosensors

8. **Limitations of Piezoelectric Biosensors:** Because the crystals may cease to oscillate completely in viscous liquids, So **it** is very difficult to use these biosensors to determine substances in solution.

Whole Cell Biosensors: These biosensors are useful for multi-step or cofactor requiring reactions. These biosensors may employ live or dead microbial cells.

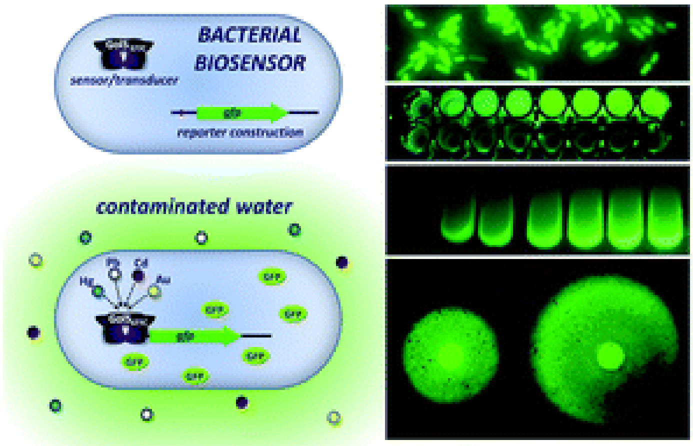


Figure 8: Whole Cell Biosensors

Advantages of Microbial Cell Biosensors: The microbial cells are cheaper with longer half-lives. Further, they are less sensitive to variations in pH and temperature compared to isolated enzymes.

9. **Immuno-Biosensors:** Immuno-biosensors work on the principle of immunological specificity, coupled with measurement based on amperometric or potentiometric biosensors.

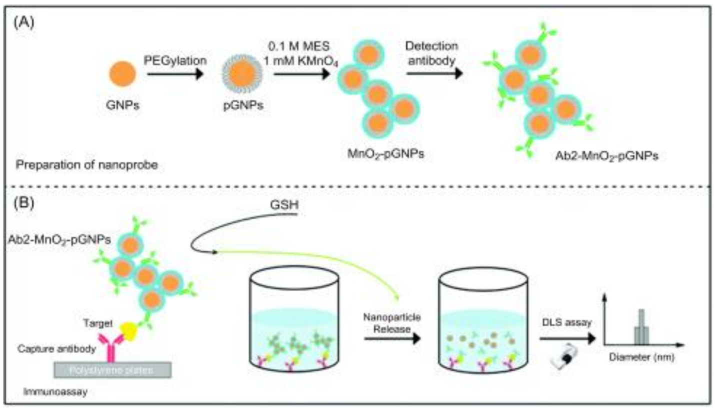


Figure 9: Immuno-Biosensors

An immobilized antibody to which antigen can directly bind.

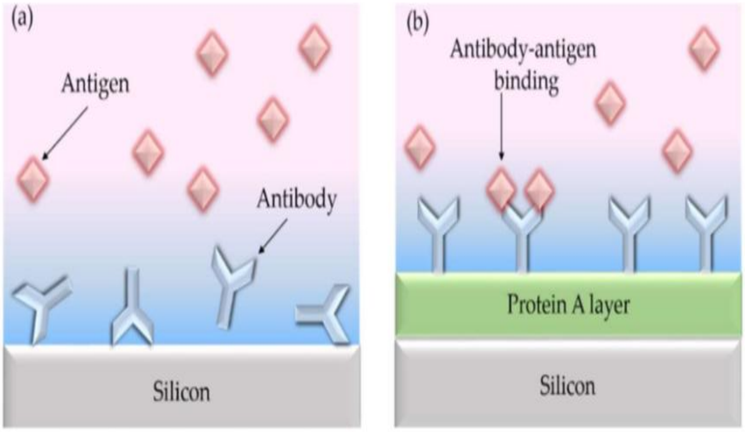


Figure 10: Direct Bind of Antigen and Antibody

An immobilized antigen that binds to antibody which in turn can bind to a free second antigen.

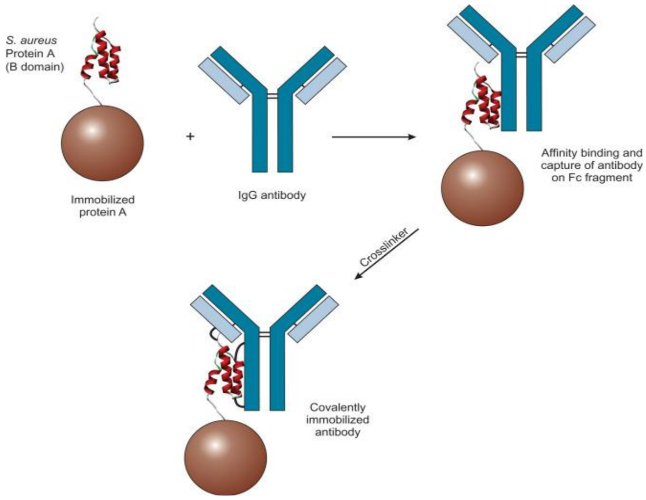


Figure 11: Binding of Antibody and Immobilized Antigen

VI. APPLICATIONS OF BIOSENSORS

Biosensors have become very important in the fields of medicine, and in general health monitoring. The advantages of biosensors are as follows:

- Small size
- Low cost
- Quick results
- Very easy to use

Biosensors have also found critical applications in several other fields like industrial processing, agriculture, food processing, pollution control etc.

1. **Medicine, Clinical and Diagnostic Applications:** The main application of Biosensor is in the Medicine, Clinical and Diagnostics. Electrochemical based Biosensors are used in biochemical labs and clinics to monitor and measure glucose and lactic acid. It is used to monitor glucose levels in diabetes patients, detecting pathogens, measuring blood gases, and identifying biomarkers for various diseases.

   Now these days Biosensor in the field of personal health care are becoming quite popular, especially, self-monitoring of blood glucose. The main advantage of this method is the blood samples cannot be contaminated and undiluted for more accurate results.

2. **Environmental Monitoring:** Another major application of Biosensor is in the field of Water Pollution Monitoring. There are number of pollutants contaminating ground water and due to which drinking water quality is getting worse. Biosensors with sensing elements for nitrates and phosphates are becoming common for battling water pollutants.

   Another important application is for the military to detect chemicals and hazardous biological specimens used as bio-weapons. Detection of pollutants, toxins and pathogens in water, air, and soil.

3. **Industrial Applications:** Fermentation is a processes used in dairy, alcohol and other similar products. Large scale Bacteria and cell culture must be maintained for this purpose. Biosensors are designed to monitor and measure the generation of a fermented product. It is used for Ensuring food safety and quality by detecting contaminants, pathogens, and ensuring proper fermentation processes

4. **Food Industry:** Some Biosensors that can measure carbohydrates, acids, alcohol, etc. are already available in the market. These biosensors are used in food industry for measurement of amino acids, carbohydrates, alcohols, gases, etc. in the common food industries like Wine, Beer, Yogurt, soft drinks etc.

5. **Agriculture Industry:** Biosensors in the field of agriculture are generally used for detection of pesticides and plant pathogens, managing crop conditions, monitoring soil health etc.

6. **Security and Defense:** Biosensors in the field of security and defense are used to detect biological and chemical warfare agents, explosives, and illegal drugs etc.

VII. FUTURE PERSPECTIVES OF BIOSENSOR

The future of biosensors looks promising with advancements in nanotechnology, materials science, and molecular biology include:

1. **Nanobiosensors:** Utilizing nanomaterials to enhance sensitivity and enable miniaturization.

2. **Wearable Biosensors:** Integrating biosensors into wearable devices for continuous health monitoring.

3. **Lab-on-a-Chip:** Developing compact devices that integrate multiple laboratory functions on a single chip for point-of-care diagnostics.

4. **Smart Biosensors:** Combining biosensors with wireless technology and data analytics for real-time monitoring and decision-making.

VIII. CONCLUSION

Biosensors represent a powerful tool for detecting and quantifying various substances with high specificity and sensitivity. Their design and development involve the integration of biological elements with advanced transducers, optimized through rigorous testing and validation. The diverse applications of biosensors span across multiple industries, demonstrating their versatility and critical role in modern technology. As research progresses, biosensors are expected to become even more integral to fields ranging from healthcare to environmental monitoring, driving innovation and improving quality of life.