ENZYMOLOGY

Topic: Enzyme Electrode

BY

DR. ANNIKA SINGH

DEPARTMENT OF LIFE SCIENCES AND BIOTECHNOLOGY

- The enzyme electrode is a miniature chemical transducer which functions by combining an electrochemical procedure with immobilized enzyme activity.

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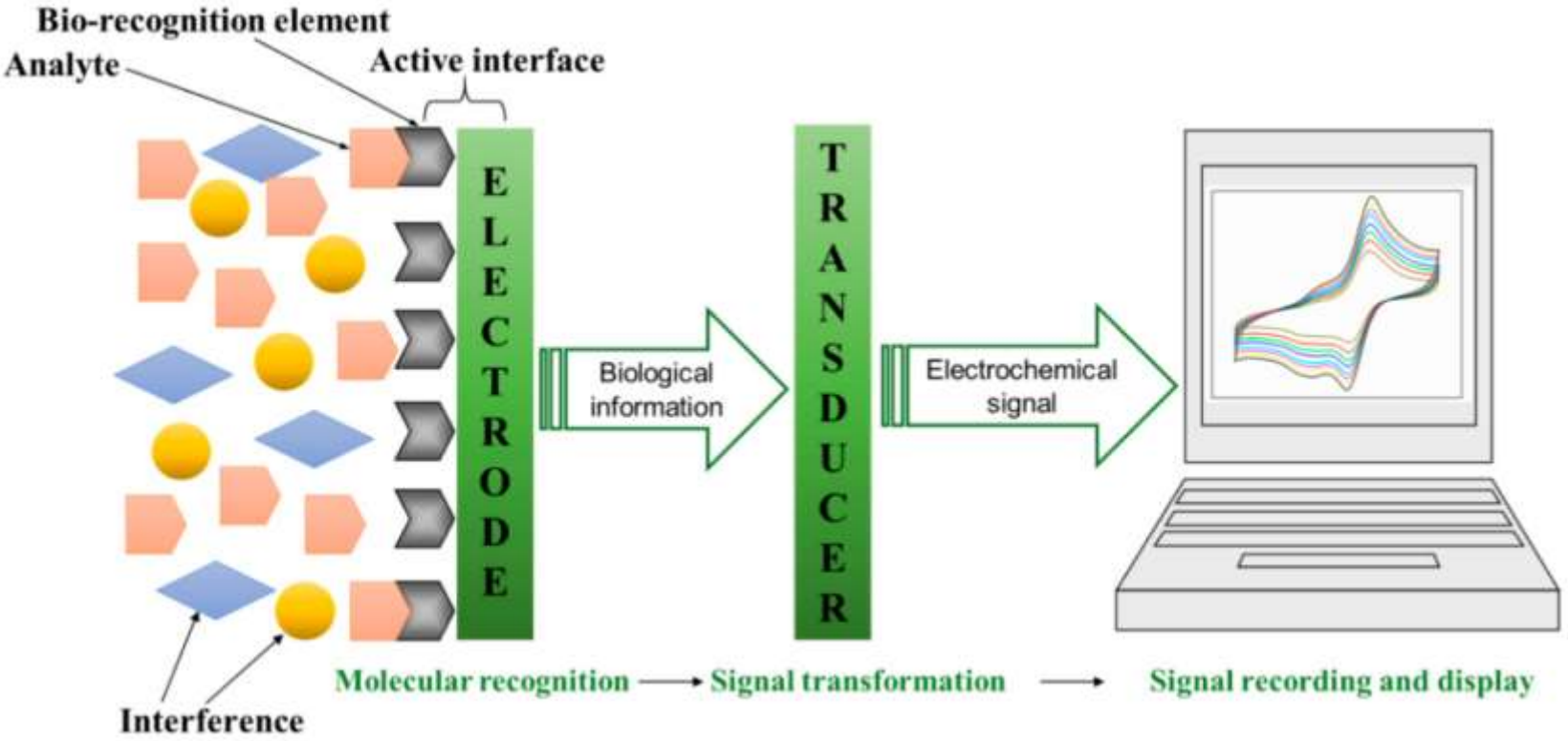
- A biosensor typically consists of a bio-receptor (enzyme/antibody/cell/nucleic acid/aptamer), transducer component (semi-conducting material/nanomaterial), and electronic system which includes a signal amplifier, processor & display.



Cox

A biosensor essentially comprise of the following two major parts

1- Biological component-

For sensing the presence as well as concentration of analyte.

In the presence of a certain molecule the biological system changes the environment.

The measuring device sensitive to this change sends a signal.

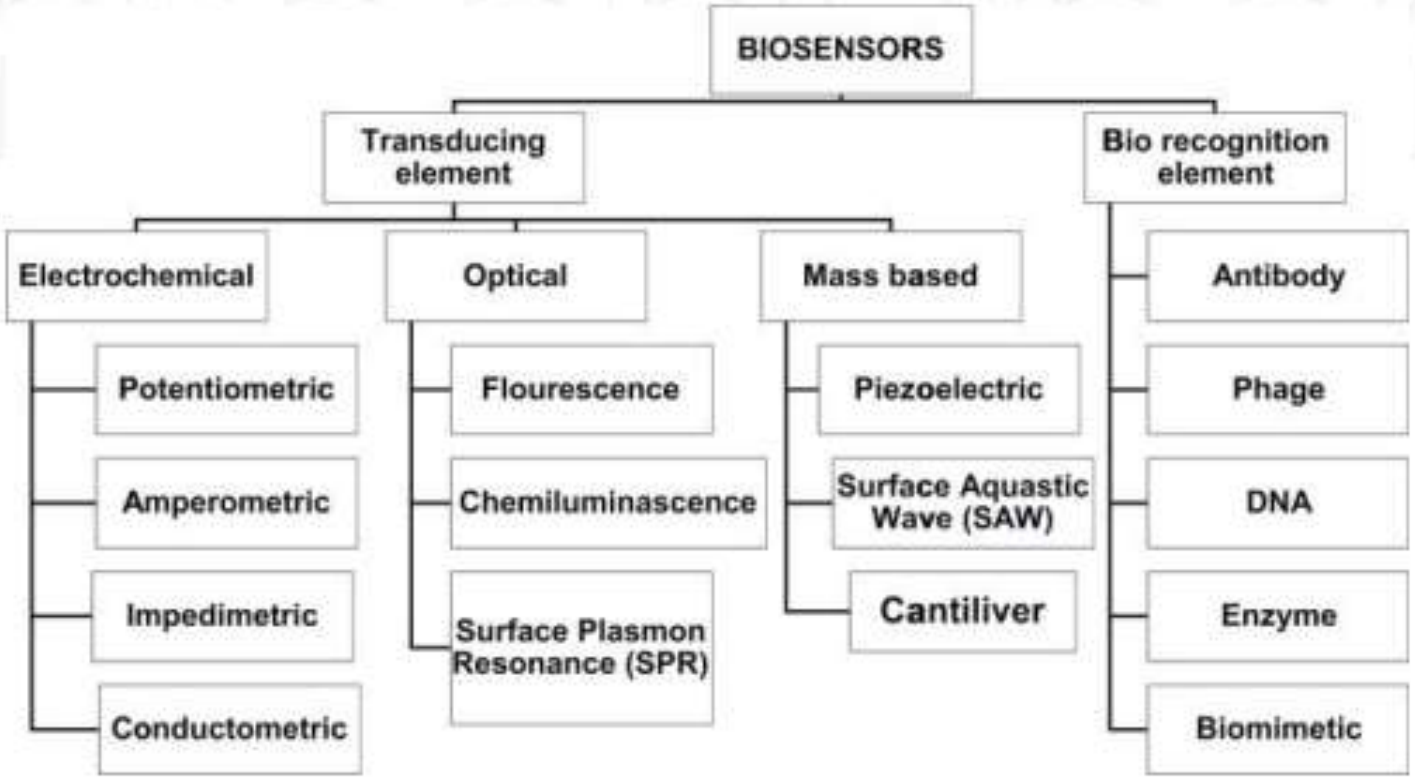
This signal can be converted into the measurement parameter.

2- Physical component:-

Transducer: A device that converts energy from one form into another e.g. A biosensor is a sensing device that consists of a biological component coupled to a transducer that converts biochemical activity into, most commonly, electrical energy.

- Updike and Hicks demonstrated the first biosensor in 1967
- Biosensors have mainly two parts and they are a molecular recognition element (MRE) and a transducer
- Animal or plant cells, receptors, organelles, anti-bodies, tissues, microbes and enzymes have been widely used as MREs
- Artificial materials like MIPs (Molecularly imprinted polymers) and PNAs (Peptide Nucleic Acids) have also been used as MREs.
- MREs can be grouped into two categories namely affinity and catalytic bases.
- Cata-lytic base MREs includes plant or animal cells, microbes, organelles and enzymes. Whereas affinity base MREs includes MIPs, nucleic acids receptors and antibodies
- MIPs are of two types namely covalent bonding type and non-covalent bonding type. MIPs can be used to develop biomedical sensors such as beta-estradiol sensor herbicide sensors and chloramphenicol sensors

References
P. MohankumarJ. AjayanT. MohanrajR.



References
1. Lehninger PRINCIPLES OF BIOCHEMISTRY Fourth Edition: David L. Nelson and Michael M. Cox
2. BIOCHEMISTRY; DONALD VOET and JUDITH G. VOET

Characteristics of Biosensors

（1） The biosensor uses the immobilized bioactive substance as the catalyst, and the expensive reagent can be reused many times, which overcomes the shortcomings of the high cost of enzyme analysis reagent and complicated chemical analysis in the past.

（2） Strong specificity only reacts to a specific substrate, and not affected by color and turbidity.

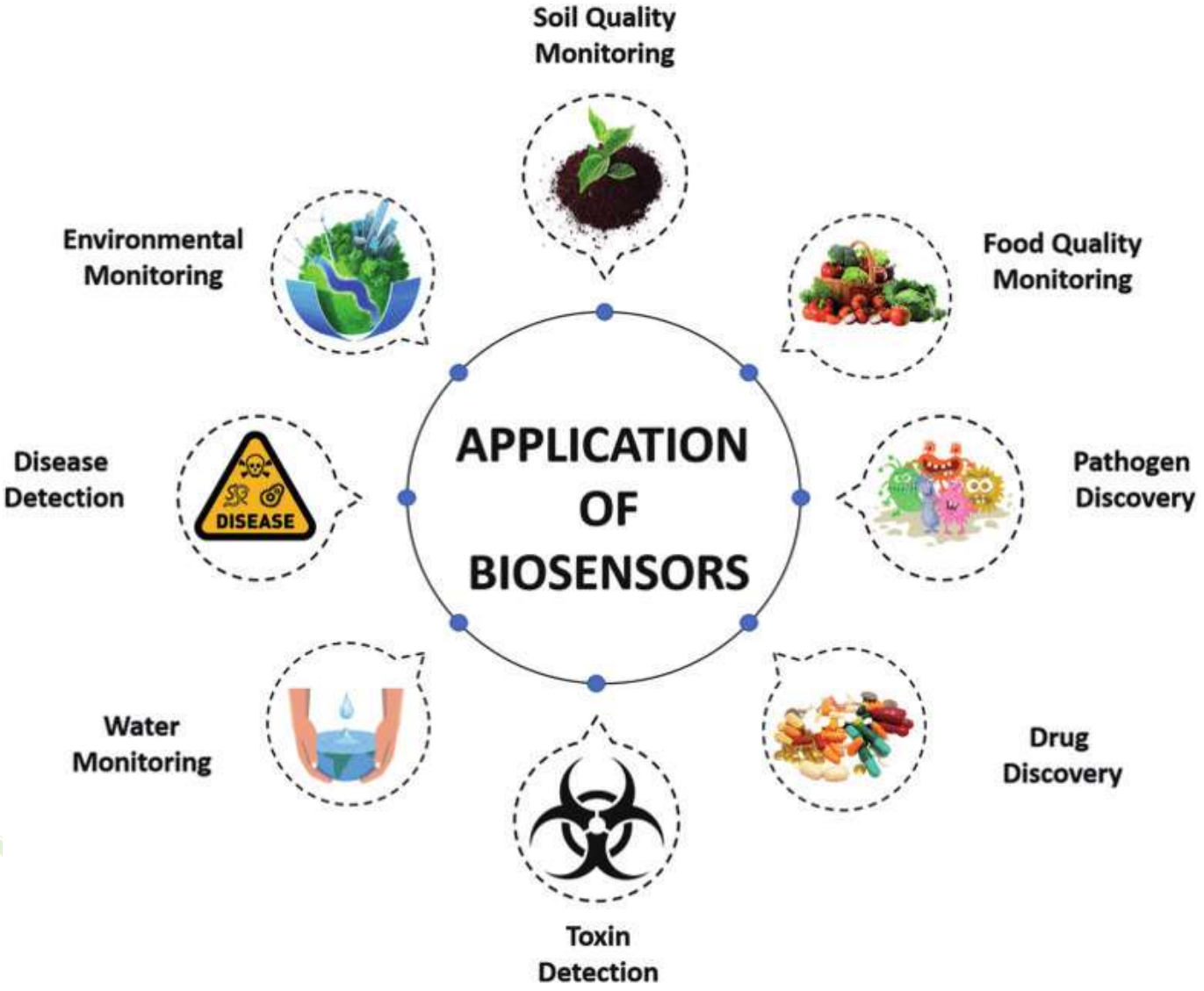
（3） The analysis speed is fast, and the results can be obtained in one minute.

（4） High accuracy; general relative error can reach 1%.

（5） The operating system is simple and easy to realize automatic analysis.

（6） Low cost; only a few cents per measurement in continuous use.

（7） Some biosensors can reliably indicate the oxygen supply and by-products in the microbial culture system. In the process of production control, much complex information can be obtained only by the comprehensive action of physical and chemical sensors. At the same time, they also pointed out the direction of increasing the yield of products.


Soil Quality Monitoring
Environmental Monitoring
Food Quality Monitoring
APPLICATION OF BIOSENSORS
Disease Detection
DISEASE
Pathogen Discovery
Water Monitoring
Drug Discovery
Toxin Detection

- Enzyme-based biosensors play an essential role in the health application by early detection of diseases
- pregnancy test kits where hCG protein is detected in the urine
- glucose-based biosensors
- Blood glucose level monitoring in diabetes patients using commercial biosensors has been established Accu-Chek, One Touch, Glucocard, Freestyle, etc.
- Advanced techniques such as enzyme-linked immune sorbent assay, fluorimetric, and immune-affinity column assay have been developed for cardiovascular disease detection.
- Biosensors are also being employed in detection of cancer biomarkers, DNA, peroxides, etc., different biosensors are available for the early diagnosis of cancer based on detection of tumor-associated antigen and its corresponding antibodies
- A biochip is available for quick and accurate detection of multiple cancer markers
- Real-time in vivo detection of dopamine with the immobilization of tyrosine onto the implantable microelectrode surface is by an enzyme-based carbon fiber micro biosensor

Site-directed mutagenesis

- Site-directed mutagenesis is an invaluable tool to modify genes and study the structural and functional properties of a protein, based on the structure, function, catalytic mechanism, and catalytic residues of enzymes.
- Site-directed mutagenesis includes single and combinational mutations.
- Site-directed mutagenesis is the basis for structure and function studies
- Single site-directed mutagenesis and multiple mutations have been used to expedite and simplify methods for mutagenesis
- The properties of enzymes can be improved markedly by the combination of site-directed mutagenesis with other methods.
- For example, commonly used laundry detergents may contain subtilisin, whose wild-type form has a methionine that can be oxidized by bleach, significantly reducing the activity the protein in the process
- This methionine may be replaced by alanine or other residues, making it resistant to oxidation thereby keeping the protein active in the presence of bleach
- Therminator DNA Polymerase is derived from the Family B *Thermococcus* contains mutations in the conserved exonuclease domain (separate from the polymerase active site domain) (D141A/E143A) and a mutation (A485L) in the conserved polymerase active site Region III
- The D141A/E143A mutations inactivate the 3’-5’ exonuclease activity so that any modified nucleotide that is incorporated is not subsequently removed by the 3’-5’ exonuclease proofreading activity.