Microbiology
US05EMIC26
Bioinstrumentation and Biotechniques

Unit- IV (a) Biosensors:

Principle, Method and Application

Reference Books:

1. **Instrumental Methods of Chemical Analysis by Chatwal and Anand**
2. **Practical Biochemistry and Molecular Biology – Wilson and Walker**
3. **Instrumental Methods of Analysis in Biotechnology**
   - D. K. Chantana and P.S. Mehra
4. **Microbiology- Prescott 8th edition.**
5. **Biophysical Chemistry- Principles and Techniques**
   - Upadhyay, Upadhyay and Nath

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Introduction

- Analyte is a substance whose presence and concentration needs to be determined & monitored.
- Routine estimation & monitoring the level of certain analyte can be done conventionally through physical instruments.
- Here, In biosensor biological molecules or living cells have been used to developed sensitive device that are used for such analysis. such sensitive device is known as Biosensor.

- Biosensor are superior & sensitive as compares to physical sensors because

(1) In biosensor, the biological material is immobilized & is in intimate contact with the transducer. so,that biochemical signal is quickly converted into an electric signal.

(2) Re use of immobilized biomolecule simplifies the entire apparatus.

(3) The biological sensor is present in a small area & is very sensitive & therefore allow the analysis of a substances even in small quantities.

(4) Biosensor can be developed depending upon the specific need & can be highly specific or broad spectrum.

(5) Simplicity & speed of measurement.

History

- The first biosensor was developed by L.L.Clark in Cincinate,USA in1950.
- This biosensor had an oxygen electrode (clark electrode after Its discover) which could measure the dissolved oxygen in blood.

- Later glucose oxidase enzyme in a gel was coated & immobilized on the oxygen electrode to measure blood sugar.
- Enzyme urease was used (in combination with an electrode specific for NH_4^{++} ions)for measuring urea in body fluids like blood & urine.
- These two biosensors developed in early years of history of Biosensors made use of two different transducer technologies.

(a) In first case ,estimates were made by measurment of electric current (amperometric)

(b) In second case the estimates were made by measurement of charge on the electrode(potentiometry)

Biosensor(Defination)

"An analytical device consisting of a Biocatalyst (enzyme or tissue) & a Transducer –which can convert a biological or a biochemical signal or response into a quantifiable electrical signal"

- It is highly specific ,very sensitive & can measure wide range of molecules.
- Its is categorized as Fisrt,Second & Third generation based on intimacy between biocatalyst & transducer.

First generation instruments:

- Two components easily separated –both remain functional in absence of other.
- Here ,the biocatalyst within a biosensor responds to the substrates in solution by catalyzing reaction.
- Very fast response time and require only 25 mm^3 sample.

- Example: YSI(yellow springs instruments) model 23

- Its glucose biosensor in which enzyme glucose oxidase linking to a probe to measure H_2O_2

Glucose+O_2-----------gluconic acid+ H_2O_2

- The rate of consumption of the substrate O_2 can be measured by its reduction at a platinum cathode
- The rate of production of the product H_2O_2 can be measured by its oxidation at a platinum anode
- The rate of production of the product gluconic acid can be measured using a pH electrode.

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Second generation instruments:

- **The two components interact in a more intimate fashion & removal of one of the two components affects the usual functioning of the other.**
- **Here, the rate of oxidation of glucose is measured by the rate of flow of e^- from glucose to an electrode surface.**
  Example: Exactech blood glucose meter
- **It is constructed by designing an electrode surface that is capable of capturing electrons which are usually transferred in the oxidation reduction reactions.**
- **Size of a pen model.**
- **Smaller devices designed to implanted under the skin.**

1. Glucose + GO/FAD $\longrightarrow$ Gluconic acid + GO/$FADH_2$
(red) (oxi) (oxi) (red)

2. GO $FADH_2$ + $2M^+$ $\longrightarrow$ GO/FAD + 2M + $2H^+$
(red) (oxi) (oxi) (red)

3. At electrode 2M $\longrightarrow$ $2M^+ + 2e^-$
(red) (oxi)

- e^- donated to the electrode and form current which is proportional to rate of oxidation of glucose, this proportional to glucose concentration in blood.

Third generation instruments:

- **The biochemistry & the electrochemistry occurs at a semiconductor ,the term biochip may be applied to describe such instruments.**
- **These instruments involve the most intimate interactions of the biocatalyst and transducer.**
- **A glucose biosensor operating on the principle of Exac Tech meter but in which the enzyme was directly reduced at the electrode surface (no need of a mediator) is an example of such an instrument**

Cell based biosensors:

- Immobilized whole cells or tissues are used to produce biosensors.
- More recent immobilization techniques have intended to use gentler physical methods such that cell viability is retained.
- The advantage of this is that such cells may be involved in converting substrate into product via a complex multi enzyme pathway without having to immobilize each of the enzymes & then provide them with expensive coenzymes.

- Eg., *Nocardia erythropolis* immobilized in poly acrylamide an oxygen electrode

$$\text{Cholesterol} + O_2 \xrightarrow[\textit{N.erythropolis}]{\text{Chol.oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

- The oxygen electrode measures the rate of oxygen uptake & this can be related to the cholesterol content of the biological sample

Advantages

- Cheaper
- No requirement for a complex biocatalyst
- They have longer response times than do enzyme based sensors

Disadvantages

- Cells contain many enzymes. Hence, care has to be taken to ensure selectivity of response
- Time taken for cell based biosensor to return to base line potential after use is more.

Enzyme immunosensors

- Several kinds of enzyme immunosensors have been developed
- They combine the molecular recognition properties of antibodies with the high sensitivity of enzyme based analytical methods
- The enzyme is used as a marker as it reacts with its substrate, giving changes that can be detected by a transducer.
- There is similarity between such methods & ELISA techniques.
- A similar assay can be carried out for HCG .
- Catalase is used to label HCG & oxygen evolution noted by oxygen electrode

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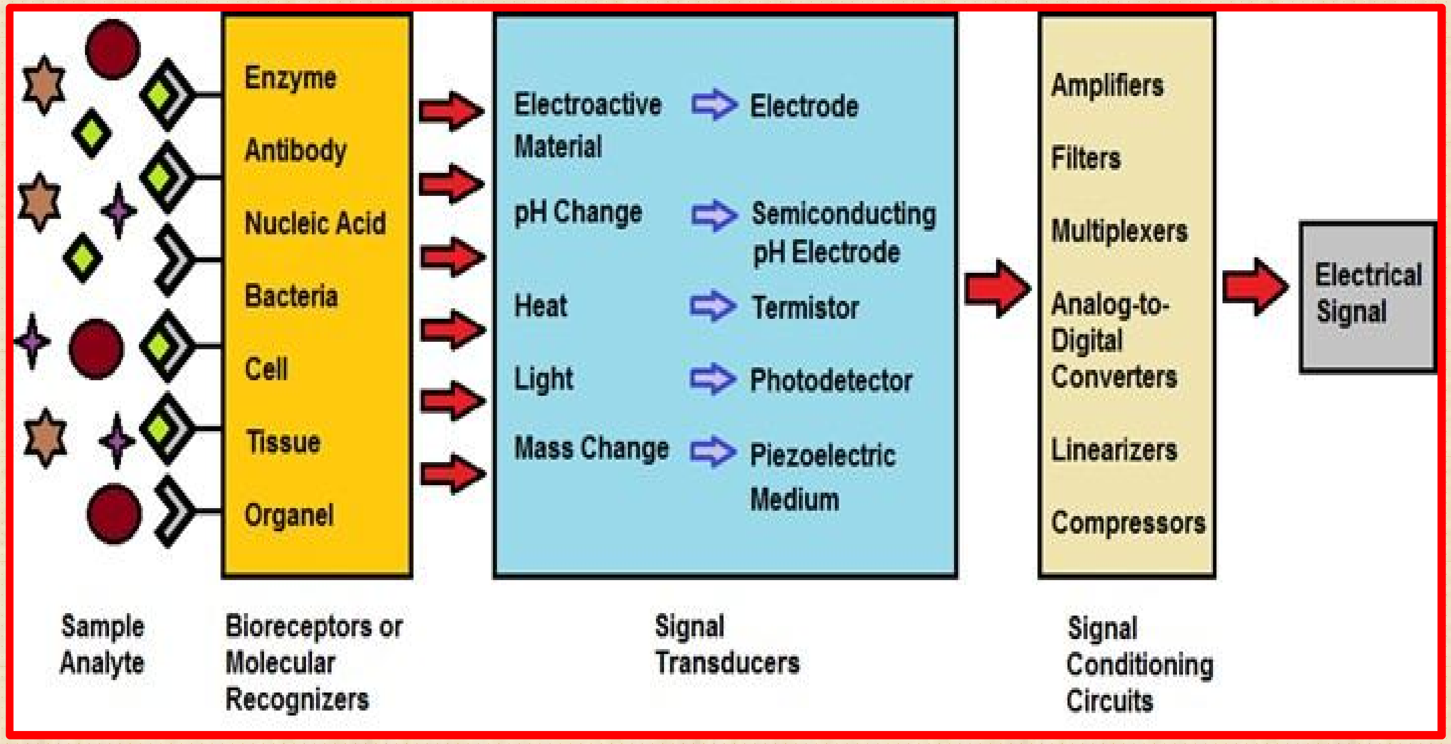
- In attempts to construct enzyme immunosensors, bioluminiscence,chemiluminiscence & fluorescence principles are exploited because of their great sensitivity
- A luminescent immunoassay with catalase has been used to detect human serum albumin at only 1 ng/cm3

- **Enzyme based biosensors** are used in different analyzers for quantification of glucose (PO_2 electrode),urea, creatinine etc. where the enzyme is immobilized on the sensor.
- **Affinity sensors** have immobilized molecules with specific high affinity binding properties like binding proteins, antibodies, aptamers (DNA SENSORS).

Principle

- Analyte diffuses from the solution to the surface of the biosensor.
- Analyte reacts specifically & efficiently with the Biological component of the Biosensor.
- This reaction changes the physicochemical properties of the Transducer surface.
- This leads to a change in the optical/electronic properties of the Transducer Surface.
- The change in the optical/electronic properties is measured/converted into electrical signal, which is detected.



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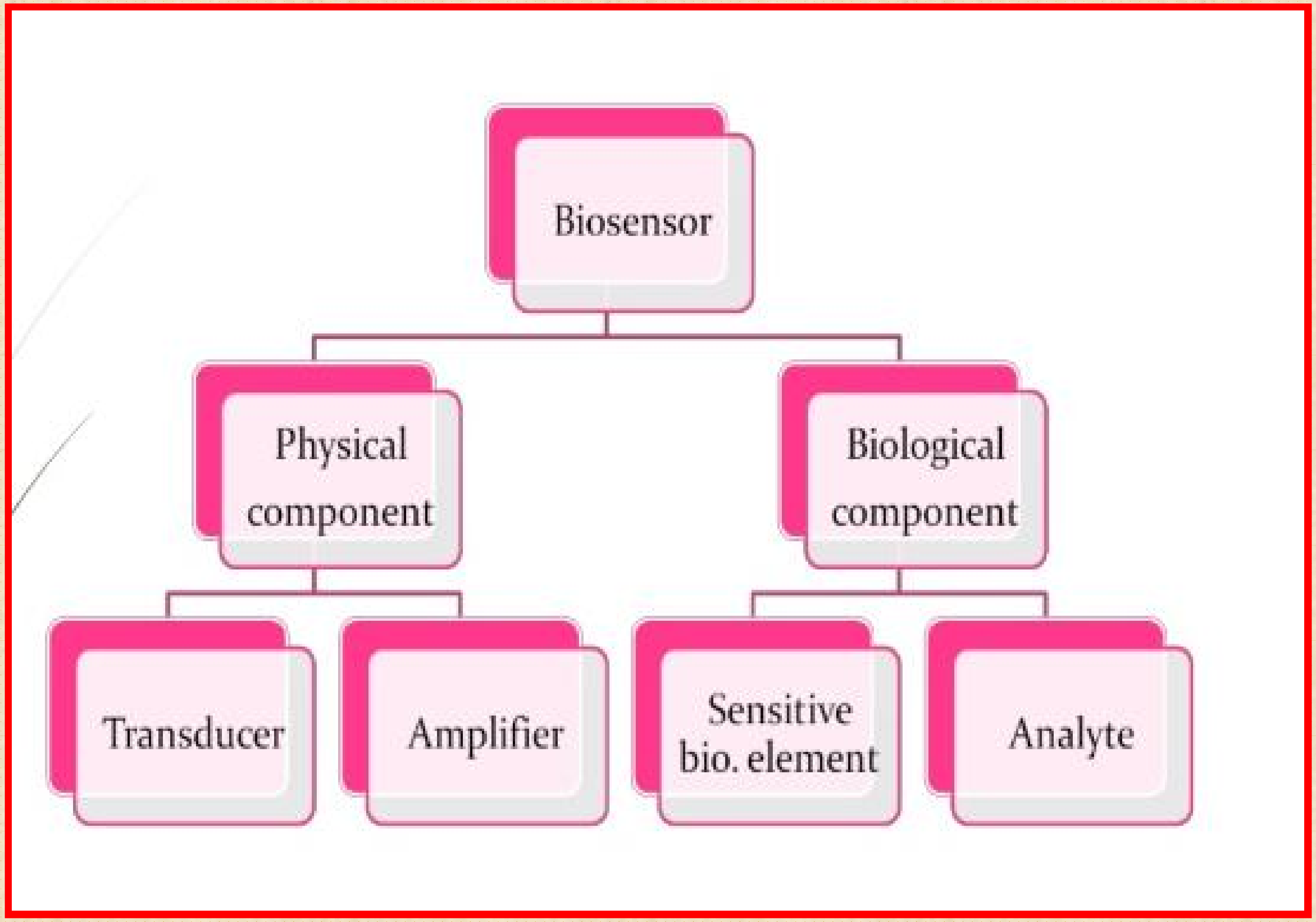

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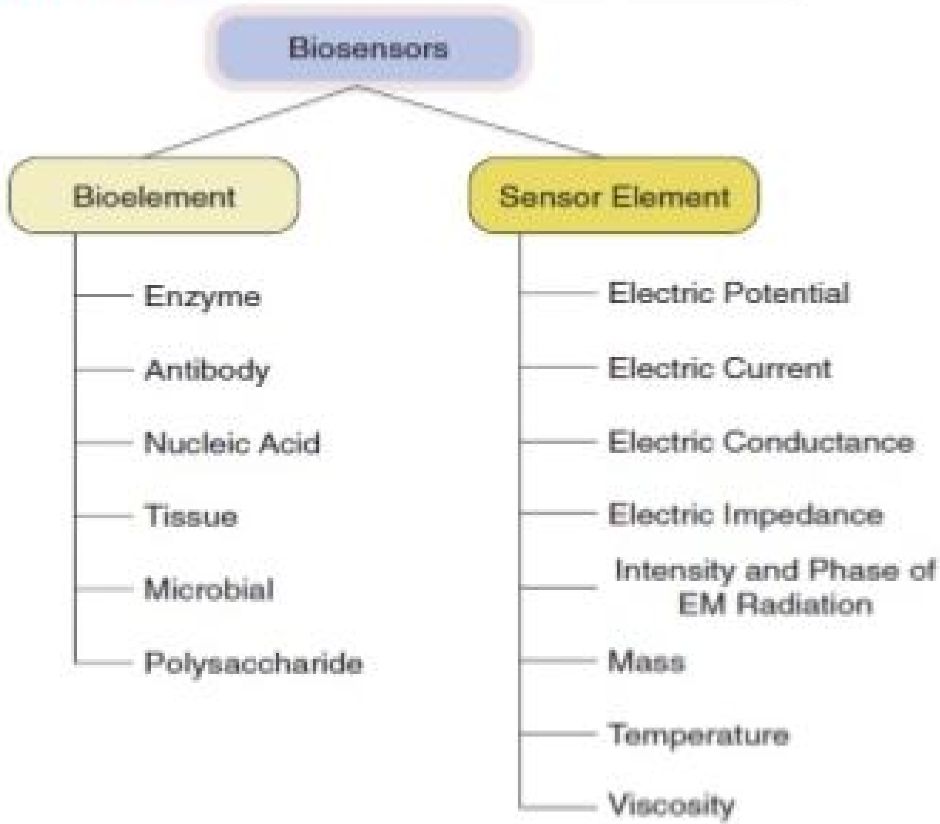
ELEMENTS OF BIOSENSORS



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Biological Element:

- Function to interact specifically with a target compound i.e. the compound to be detected.
- It must be capable of detecting the presence of a target compound in the test solution.
- The ability of a bio-element to interact specifically with target compound (specificity) is the basis for biosensor.

Physiochemical 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.

Electro active Substance → Electrode

pH Change → Semiconductor pH Electrode

Heat → Thermistor

Light → Photon Counter

Mass Change → Piezoelectric Device

Detector:

- 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.

Principle of Detection

TYPES

- Calorimetric/Thermal Detection Biosensors.
- Optical Biosensors.
- Resonant Biosensors.
- Piezoelectric Biosensors.
- Ion Sensitive Biosensors.
- Electrochemical Biosensors.
  - Conductimetric Sensors.
  - Amperometric Sensors.
  - Potentiometric Sensors.

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Calorimetric / Thermal Detection Biosensors

- **Uses Absorption / Production of Heat.**
- **Total heat produced/absorbed is α̃ Molar Enthalpy/Total number of molecules in the reaction.**
- **Temp. measured by Enzyme Thermistors.**

Advantages:

- **No need of Frequent recalibration.**
- **Insensitive to the Optical & Electrochemical Properties of the sample.**

Uses:

Detection of: (1) Pesticides . (2) Pathogenic Bacteria.

Optical Biosensors

- **Colorimetric for color - Measures change in Light adsorption.**
- **Photometric for Light Intensity - Detects the Photon ‘ output.**

Resonant Biosensors

- **An Acoustic Wave Transducer is coupled with bioelement.**
- **Measures the change in Resonant Frequency.**

Piezoelectric Biosensors

- **Uses Gold - To detect specific angle at which ê waves are emitted when the substance is exposed to laser light/crystals like quartz, which vibrates under the influence of an electric field.**
- **Change in Frequency α̃ Mass of Absorbed material.**

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Ion Sensitive Biosensors

- Are semiconductor FETs with ion-sensitive surface.
- Surface Electrical Potential changes when the ions & semiconductors interact.
- Measures the Change in Potential.

Uses: pH Detection.

Electrochemical Biosensors

- Underlying Principle – Many chemical reactions produce or consume ions or ês causing some change in the electrical properties of the solution that can be sensed out & used as a measuring parameter.

Uses: Detection of : Hybridized DNA
DNA- binding Drugs &
Glucose Concentration.

Conduct metric Sensors.

- Measures Electrical Conductance/Resistance of the solution. Conductance Measurements have relatively Low sensitivity.
- Electrical Field is generated using sinusoidal(ac) voltage, which helps in minimizing undesirable effects like:
  i. Faradaic processes.
  ii. Double layer charging &
  iii. Concentration polarization.

Amperometric Biosensors

- High Sensitivity Biosensor.
- Detects electro active species present in the biological test samples.
- Measured Parameter – Current.

Potentiometric Sensors

- **Working Principle – When ramp voltage is applied to an electrode in solution, a current flow occurs because of electrochemical reactions.**
- **Measured Parameter – Oxidation / reduction Potential of an Electrochemical reaction.**

APPLICATIONS OF BIOSENSORS

- **Food Analysis**
- **Study of biomolecules and their interaction**
- **Drug Development**
- **Crime detection**
- **Medical diagnosis (both clinical and laboratory use)**
- **Environmental field monitoring**
- **Quality control**
- **Industrial Process Control**
- **Detection systems for biological warfare agents**
- **Manufacturing of pharmaceuticals and replacement organs**

Food Analysis

- Food quality check using nanoparticles.
- Detection of various protein.
- Various proteins are sensed out in body fluids.

Advantage:

- Highly sensitive
- 96% accuracy

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Biological Applications

- DNA Sensors: Genetic monitoring, disease diagnosis
- Immunosensors: HIV, Hepatitis, other viral diseases drug testing, environmental monitoring…
- Cell-based Sensors: functional sensors, drug testing… Point-of-care sensors: blood, urine, electrolytes, gases, steroids, drugs, hormones, proteins, other…
- Bacteria Sensors: (E-coli, streptococcus, other): food industry, medicine, environmental, other.
- Enzyme sensors: diabetics, drug testing, other.

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