Hand Book on

Emerging Applications of Biosensors

22ETC152

For First Year BE, VTU, Belgaum

Course Title:	**Emerging applications of biosensors**		
Course Code:	22ETC152	CIE Marks	50
Course Type (Theory/Practical /Integrated)	Theory	SEE Marks	50
		Total Marks	100
Teaching Hours/Week (L:T:P: S)	3:0:0:0	Exam Hours	3 hrs of Theory
Total Hours of Pedagogy	40 hours	Credits	03

Course objectives

To learn the Fundamentals of biosensors.

To acquaint the student with design and construction of biosensors.

To expose the students to recent advances in application of biosensors in health, environment, agriculture and food industry.

Module-1 (8)

INTRODUCTION TO BIOSENSORS

Introduction to biosensor, General components of biosensor, Biomolecules in biosensors such as enzyme, DNA, antigen antibody, protein, Classification of biosensors based on principle: amperometric, potentiometric biosensors, optical, acoustic, piezoelectric, and calorimetric biosensors, scope of biosensors and its limitations.

Module-2 (8)

BASIC DESIGN AND TRANSDUCER

Design Considerations: calibration, dynamic Range, signal to noise, sensitivity, selectivity, Interference recognition. Transduction membrane protein sensors: ion channels, Types of Transducer, Optical; Fiber Optic, ECL, Surface Plasmon Resonance, Electro chemical; FET, Impedance, Piezoelectric; Cantileaver,

Module-3(8)

APPLICATIONS OF BIOSENSORS IN HEALTH AND ENVIRONMENT

Biosensors and diabetes management, Microfabricated biosensors and point-of-care diagnostics systems, Noninvasive biosensors in clinical analysis; Surface plasmon resonance and evanescent wave biosensors, Biosensor in cancer and HIV early diagnosis.

Module-4(8)

APPLICATIONS OF BIOSENSORS IN FOOD AND AGRICULTURE INDUSTRY

Detection of product content, allergic components, pathogens, pesticide residues. Monitoring of raw material conversions. Detection of crop diseases, pathogens in plants, Detection of soil nutrients, pesticide and its residual detection.

Module-5 (8)

APPLICATIONS OF NANOMATERIALS IN BIOSENSORS

Nano Materials in biosensors; Carbon based Nano Material, Metal oxide and nano particle, Quantum dots, Role of nano material in Signal Amplifications, Detection and Transducer Fabrication

Course outcome (Course Skill Set)

At the end of the course the student will be able to:

CO1	Classify types of biosensors based on principle
CO2	Able to differentiate different types of transducers based on their physicochemical characteristics
CO3	Apply bio sensing techniques in health, environment, and agriculture and food industry.
CO4	Use biomaterial and nanomaterials in biosensors for signal amplification, Detection and Transducer Fabrication

Module 1

INTRODUCTION TO BIOSENSORS

1.1 Introduction to biosensor,

1.2 General components of biosensor,

1.3 Biomolecules in biosensors such as enzyme, DNA, antigen antibody, protein,

1.4 Classification of biosensors based on principle: amperometric, potentiometric biosensors, optical, acoustic, piezoelectric, and calorimetric biosensors,

1.5 Scope of biosensors and its limitations.

1.1 Introduction to Biosensor

- Sensor is a device which detects changes in a physical quantity like temperature, humidity, water flow, intensity of light etc. and converts it into a quantity that can be measured and/or analyzed.
- Biosensor is an analytical device that converts a biological response into a more useful electrical signal using biological responsive material or bio recognition element such as microorganisms, cells, enzyme, antibody or nucleic acid.
- Biosensor is a combination of a Biological sensing element and a transducer, which converts the data into electrical signals. Additionally, there will be an electronic circuit which consists of a Signal Conditioning Unit, a Processor or Microcontroller and a Display Unit.
- Biosensors are 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 and Reproducibility.
- Biorecognition elements should be highly specific for the analyte.
- Biorecognition elements should be stable under assay conditions over a very large number of assays
- The reaction should be independent of physical parameters such as stirring, pH and temperature
- Response should accurate, precise, reproducible, and linear over useful analytical range.
- Invasive biosensors should be tiny, biocompatible and sterelizable, if required.
- Complete biosensor should be cheap, small, portable and easy to operate.
- The desired biological material is usually in the form of an enzyme due to operate.

- One of the commonly used biorecognition element is enzyme due to its advantages such as specificity, rate enhancement, regulation, reuse, stability, immobilisation etc.
- General components of biosensors are bio recognition element, transducer, amplifier, signal processing unit and display unit.
- Biosensors have become very important in the fields of medicine, clinical analysis and in general health monitoring.
- The advantages of biosensors over lab based equipment are their small size, low cost, quick results, and very easy to use.
- Biosensors are used in Medicine, Clinical and Diagnostic Applications, Environmental Monitoring, Industrial Applications, Food Industry and Agriculture Industry
- Apart from the desired medicine and health based applications, Biosensors have also found critical applications in several other fields like industrial processing, agriculture, food processing, pollution control etc.
- Commercial Biosensor in the field of personal health care are becoming quite popular, especially, self-monitoring of blood glucose.

1.2 General components of biosensor

- Generally, biosensors are composed of three main components as depicted in Figure 1.
- These include a biological sensing element, transducer, and signal processing unit.
- The block diagram of the biosensor consists of three segments namely, biological sensing element, transducer, and signal processing unit**.**

1.2.1 Biological sensing element:

- This component is also known as a sensor or detector element and is responsible for sensing or detecting the presence and/or the concentration of the target analyte or substance.
- This is a biological component, which serves as a biochemical receptor that specifically recognizes the target analyte.
- When the biological receptor interacts with a target analyte, it generates a signal in the form of light, heat, pH, charge or mass change.
- This material should be highly specific, stable under storage conditions and must be immobilized.
- Furthermore, the biological receptor should be capable of selectively detecting the target compound or analyte in the test sample.
- Biological receptor determines the sensitivity of the entire device through the generation of the physicochemical signal that is monitored by the transducer.

- This component can be a tissue, microorganism, organelle, cell receptor, enzyme, antibody or nucleic acid etc. These can be grouped into two categories, namely catalytic and non-catalytic receptors.
- The catalytic group of biological receptors are used in devices intended for continuous monitoring of substances at millimolar or micromollar concentrations.
- These include enzymes, tissues and microorganisms.
- The non-catalytic group is used mainly in biosensor devices that measure analytes such as steroids, drugs, and toxins etc. which usually occur at very low concentrations (micro to picomollar range)
- These are non-reusable devices which can only be used once and discarded thereafter. Such receptors include antibodies, antigens, nucleic acids etc.

1.2.2 Transducer:

- The second segment of the biosensor is the transducer and it is a physical component.
- Transducer converts the biochemical signal into proportional electrical signals.
- Generally, a transducer is a material that is capable of converting one form of energy to another.
- In a biosensor, a transducer is responsible for converting the biochemical signal received from the biological receptor, which is a result of the interaction between the target analyte and the biological receptor, into a measurable and quantifiable signal which can be piezo-electrical, optical, electrochemical etc.
- The transducer detects and measures the change that occurs during biological receptor – analyte interaction.
- An example of a transducer is a pH sensor in a glucose biosensor.
- An enzyme, known as glucose oxidase, is used as a biological receptor which binds glucose and converts it to gluconic acid in the presence of oxygen.
- The pH sensor (transducer) then detects the change in pH (due to production of gluconic acid) and converts it into a voltage change.
- The following features are recommended when a transducer is designed; specificity to the target analyte, analyte concentration range, response time and suitability for practical applications.
- Ideally, a transducer should be highly specific to the analyte, give measurement at the lowest analyte concentration within the shortest time possible.

1.2.3 Signal processing Unit:

- The output of the transducer will be either current or voltage relying on the type of enzyme.
- If the output is voltage, then it is fine.

- But if the output is current, then this current needs to be converted into equivalent voltage (using an Op-Amp based current to voltage converter) before proceeding further.
- The output voltage signal is generally very low in amplitude and is superimposed on a high frequency noise signal.
- Thus, the signal is amplified (using an Op-Amp based Amplifier) and then it is passed through a Low Pass RC Filter.
- Signal Processing Unit or a Signal Conditioning Unit is accountable for performing the process of amplifying and filtering the signal. The output of the signal processing unit is termed as an analog signal.
- This output is equivalent to the biological quantity being measured.
- The analog signal can be exhibited directly on an LCD display but usually, this analog signal is passed to a Microcontroller, where the analog signal is converted into digital signal.
- This is done since it is easy to analyse, process or store a digital signal.
- Signal processing unit is the associated part which consists of Signal Conditioning Unit, a Processor or Micro-controller and a Display Unit.

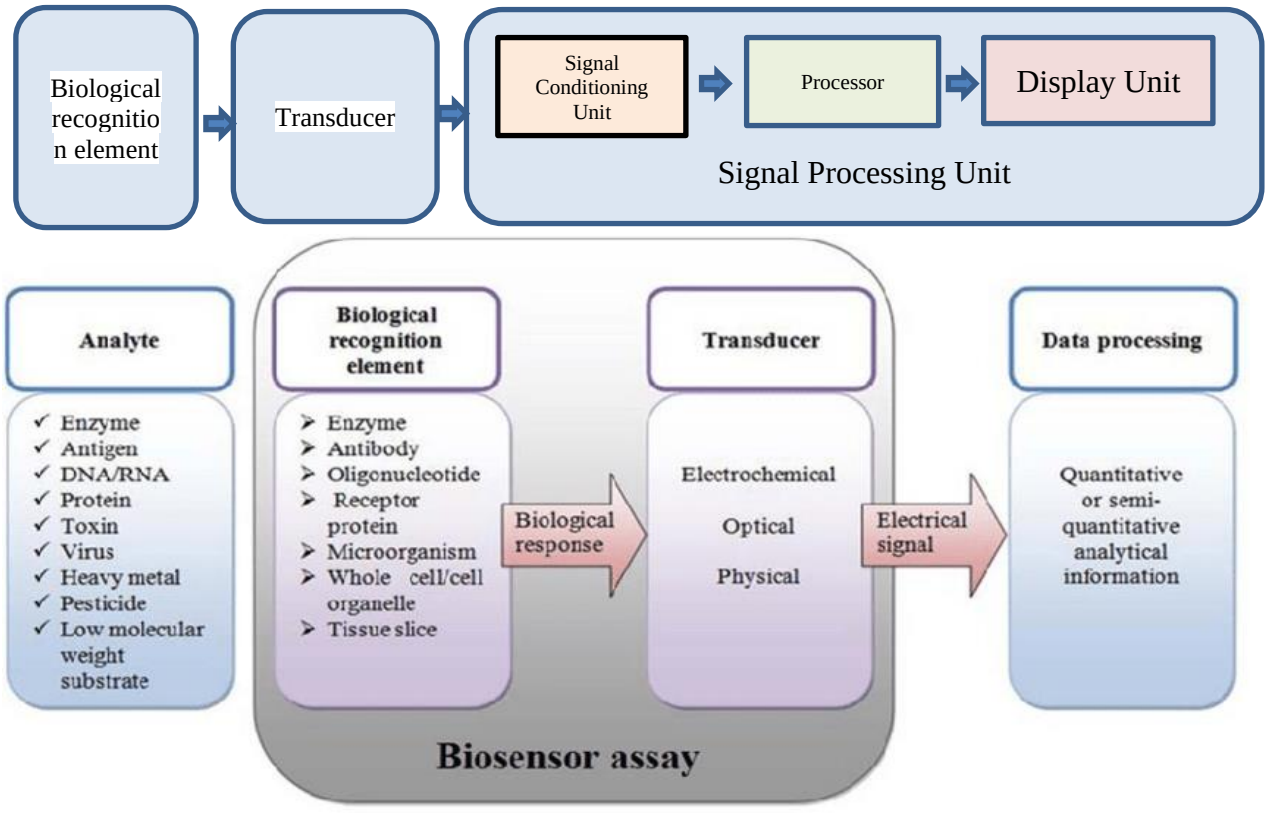


Fig 1.Generalised representation of Biosensors

1.3 Biomolecules in biosensors such as enzyme, DNA, Antigen-Antibody, Protein.

1.3.1 Enzyme based biosensors

- Enzyme based biosensors are most popular biosensors due to its specialist features such as specificity, rate enhancement, regulation and mild working condition.
- Enzyme biosensors are useful tools for monitoring rapid changes in metabolite levels in real-time, include pure enzyme preparations or biological processes.
- They have been derived on immobilization processes such as van der Waals forces, ionic or covalent bonding.
- The well-known enzymatic biosensors today are glucose and urea biosensors.
- However, glucose biosensors are most popular among researchers and are reportedly the mostly commercialized biosensors.
- The glucose biosensor, which was developed by Clark, is made up of glucose oxidase immobilized within a dialysis membrane which is integrated inside oxygen electrodes.
- Enzymatic biosensors are known for their prolonged use and reusability due to the fact that enzymes used as biological receptors cannot be consumed.
- Thus, the detection limit and the lifetime of enzyme based biosensors is greatly enhanced by the stability of the enzyme.

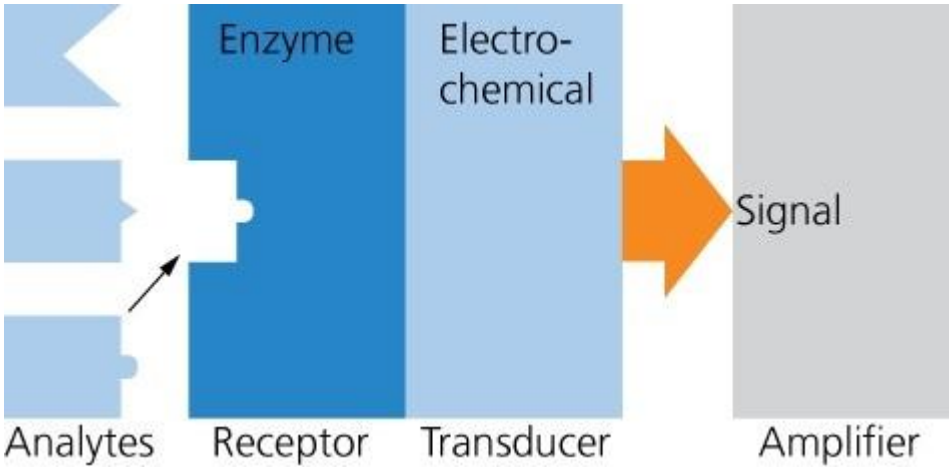


Fig 2 Enzyme based biosensors

1.3.2 DNA based biosensors

- Another group of biosensors based on a biological receptor is DNA biosensors.
- The most attractive feature of biosensors is the high selectivity of biosensors for their target analytes in a matrix of chemical or biological elements.
- DNA biosensors, which use nucleic acids as their biological receptors, detect proteins and non-macromolecular compounds that interact with certain DNA fragments known as DNA probes or DNA primers.

- The interaction observed stems from the formation of stable hydrogen bonds between the double helix nucleic acid strands.
- To develop DNA biosensors, immobilization of the probe becomes the most crucial step.
- The strong pairing of lined up nucleotide strands between bases in their complementary parts influences biosensors based on DNA, RNA, and peptide nucleotide acids to be the most sensitive tool.
- That probes, which are short oligonucleotides capable of hybridization with individual areas of the target nucleotide sequence, together with various chemical composition and conformational arrangements, were employed in the development of DNA biosensors.
- Extremely high sensibility and selectivity is needed to maximize the hybridization efficiency and minimize non-specific binding.

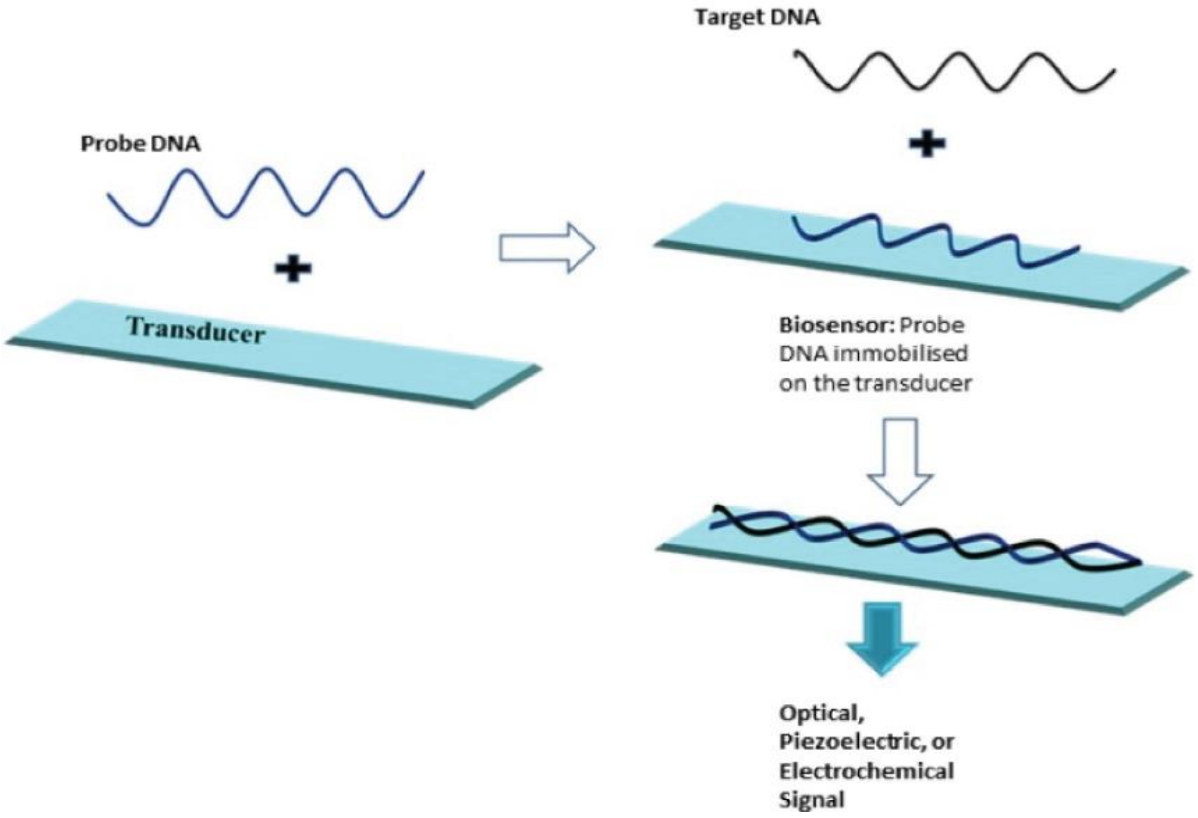


Fig 3 DNA based biosensors

1.3.3 Antibody based sensors

- Antibody-based biosensors or immunosensors have revolutionized diagnostics for the detection of a plethora of analytes such as disease markers, food and environmental contaminants, biological warfare agents and illicit drugs.
- Antibodies are ideal biorecognition elements that provide sensors with high specificity and sensitivity.

- Antibodies are large Y-shaped proteins produced by plasma cells that are utilized by the immune system to identify and target pathogens such as bacteria and viruses.
- Their small size, high stability and easy genetic manipulation make recombinant antibody fragments valuable and robust tools for the fabrication of immunosensors.
- Antibody-based biosensors have revolutionized diagnostics for the detection of a plethora of analytes such as food and environmental contaminants, biological warfare agents, illicit drugs and disease markers.
- Immobilization of antibodies on to a sensor surface without altering their specificity and immunological activity is one of the most crucial steps in the fabrication of a successful immunosensor.
- The immobilization step affects the detection limit, sensitivity and overall performance of the immunosensor.
- Orientation of antibodies on sensor surfaces can be controlled by the interaction between specific reactive groups on the surface and on the antibody.

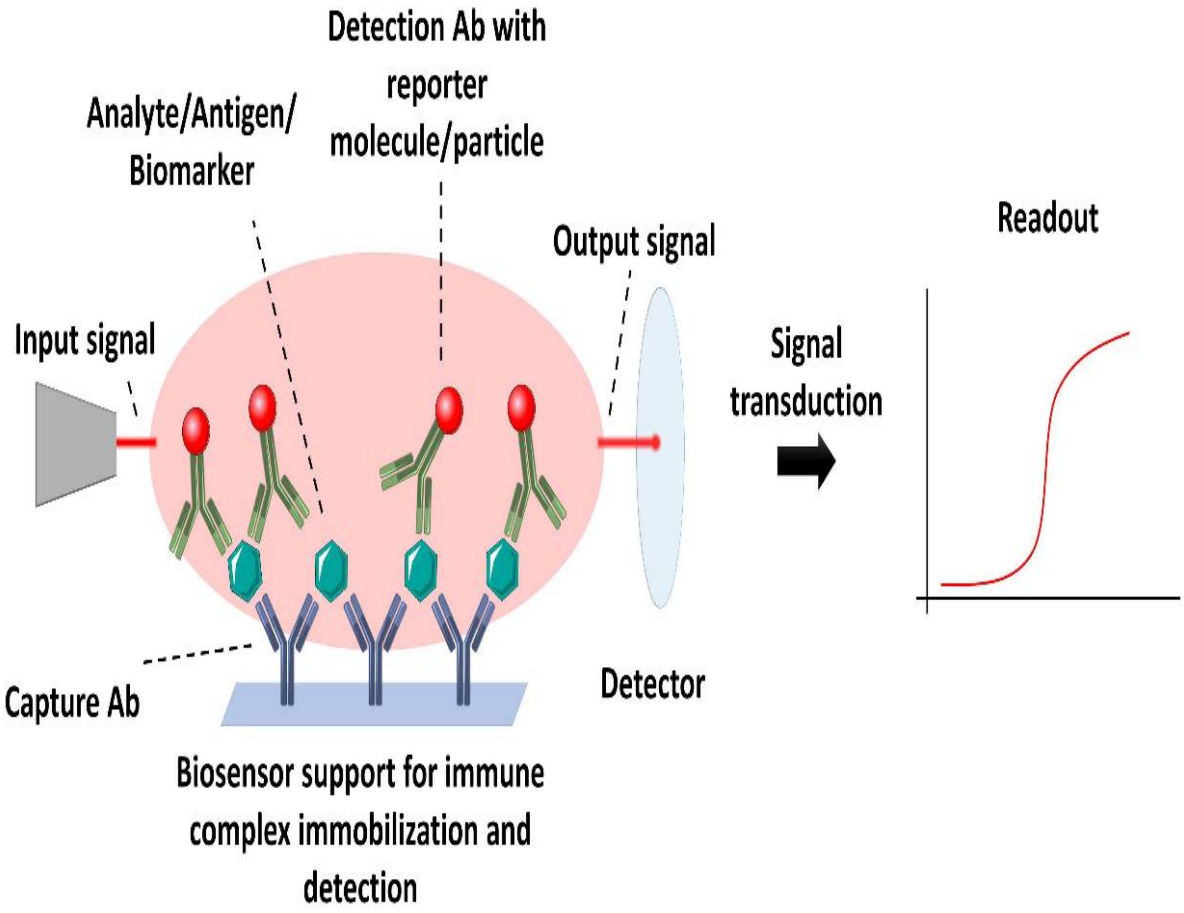


Fig 4 Antibody based biosensors

1.3.4 Protein based sensors

- Peptides/peptides have been used as components in biological analysis and fabrication of novel biosensors for a number of reasons, including mature synthesis protocols, diverse structures and as highly selective substrates for enzymes.
- Bio-conjugation strategies can provide an efficient way to convert interaction information between peptides and analytes into a measurable signal, which can be used for fabrication of novel peptide/protein-based biosensors.
- Many sensitive fluorophores can respond rapidly to environmental changes and stimuli manifest as a change in spectral characteristics, hence environmentally-sensitive fluorophores have been widely used as signal markers to conjugate to peptides to construct peptide-based molecular sensors.
- Additionally, nanoparticles, fluorescent polymers, graphene and near infrared dyes are also used as peptide-conjugated signal markers.
- On the other hand, peptides/proteins may play a generalist role in peptide-based biosensors.
- Peptides/proteins have been utilized as biorecognition elements to bind various analytes including proteins, nucleic acid, bacteria, metal ions, enzymes and antibodies in biosensors.
- The selectivity of peptides/proteins as an enzymatic substrate has thus been utilized to construct enzyme sensors or enzyme-activity sensors.
- In addition, progress on immobilization and microarray techniques of peptides has facilitated the progress and commercial application of chip-based peptide biosensors in clinical diagnosis.

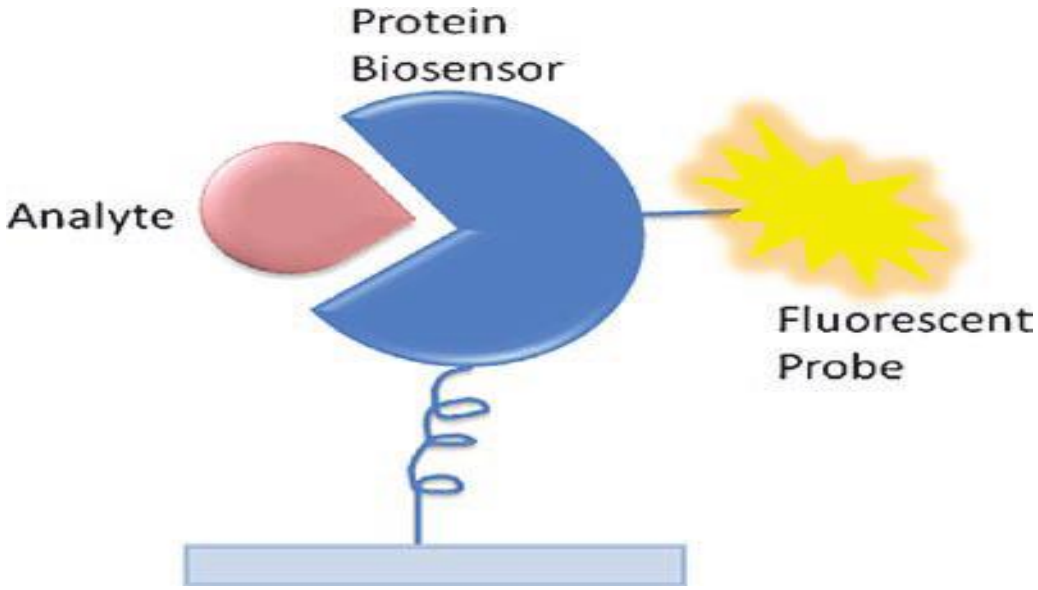


Fig 5 Antibody based biosensors

1.4 Classification of biosensors based on principle: Amperometric, Potentiometric, Optical, Acoustic, Piezoelectric, and Calorimetric Biosensors

- The most commonly applied classification of biosensors is based on the type of transduction element used in the sensor.
- These biosensors are grouped into three main categories, amperometric, potentiometric, optical, acoustic, piezoelectric, and calorimetric biosensors.
- The working principles of each of the three biosensors are different and can thus be implemented in a variety of applications.

Based on the working principles of Transducer of the biosensor, classification is done as follows

- **Amperometric Biosensor:** Based on the movement of electrons produced in the redox reaction.
- **Potentiometric Biosensor:** Based on the changes in the distribution of charge causing an electrical potential to be produced.
- **Optical Biosensor:** Light output during the reaction or light absorption difference between the reactants and products.
- **Acoustic Biosensor:** Utilizes acoustic or mechanical waves as a detection mechanism to obtain medical, biochemical, and biophysical information about the analyte of interest
- **Piezoelectric Biosensor:** Piezoelectric effect due to the mass of the reactants
- **Calorimetric Biosensors:** Heat output input by the reaction

1.4.1 Amperometric biosensors

- Amperometric biosensors function by the production of a current when a potential is applied between two electrodes.
- The simplest amperometric biosensors in common usage involve the Clark oxygen electrode.
- This consists of a platinum cathode at which oxygen is reduced and a silver/silver chloride reference electrode.
- When a potential of -0.6 V, relative to the Ag/AgCl electrode is applied to the platinum cathode, a current proportional to the oxygen concentration is produced.
- Normally both electrodes are bathed in a solution of saturated potassium chloride and separated from the bulk solution by an oxygen-permeable plastic membrane (e.g., Teflon, polytetrafluoroethylene).
- The following reactions occur:

Ag anode

$$\left\{\begin{array}{l} 4Ag \longrightarrow 4Ag^{+} + 4e^{-} \\ 4Ag^{+} + 4Cl^{-} \longrightarrow 4AgCl \end{array}\right\}$$

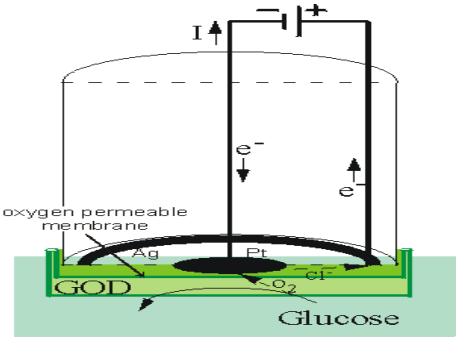
$$4Ag^{+} + 4Cl^{-} \longrightarrow 4AgCl + 4e^{-}$$

Pt cathode

$$\left\{\begin{array}{l} O_2 + 2H_2O + 2e^{-} \longrightarrow 2H_2O + 2OH^{-} \\ 2H_2O + 2e^{-} \longrightarrow 2OH^{-} \end{array}\right\}$$

$$O_2 + 4H^{+} + 4e^{-} \longrightarrow 2H_2O$$

- The efficient reduction of oxygen at the surface of the cathode causes the oxygen concentration there to be effectively zero.
- The rate of this electrochemical reduction therefore depends on the rate of diffusion of the oxygen from the bulk solution, which is dependent on the concentration gradient and hence the bulk oxygen concentration.
- It is clear that a small, but significant, proportion of the oxygen present in the bulk is consumed by this process; the oxygen electrode measuring the rate of a process which is far from equilibrium, whereas ion-selective electrodes are used close to equilibrium conditions.
- This causes the oxygen electrode to be much more sensitive to changes in the temperature than potentiometric sensors.
- A typical application for this simple type of biosensor is the determination of glucose concentrations by the use of an immobilised glucose oxidase membrane.
- The reaction results in a reduction of the oxygen concentration as it diffuses through the biocatalytic membrane to the cathode, this being detected by a reduction in the current between the electrodes.
- Other oxidases may be used in a similar manner for the analysis of their substrates (e.g., alcohol oxidase, D- and L-amino acid oxidases, cholesterol oxidase, galactose oxidase, and urate oxidase).



- Figure 6. Schematic diagram of a simple amperometric biosensor. A potential is applied between the central platinum cathode and the annular silver anode.

- This generates a current (I) which is carried between the electrodes by means of a saturated solution of KCl.
- This electrode compartment is separated from the biocatalyst (here shown glucose oxidase, GOD) by a thin plastic membrane, permeable only to oxygen. T
- he analyte solution is separated from the biocatalyst by another membrane, permeable to the substrate(s) and product(s).
- This biosensor is normally about 1 cm in diameter but has been scaled down to 0.25 mm diameter using a Pt wire cathode within a silver plated steel needle anode and utilising dip-coated membranes.

1.4.2 Potentiometric biosensors

- Potentiometric biosensors make use of ion-selective electrodes in order to transduce the biological reaction into an electrical signal.
- It consists of an immobilised enzyme membrane surrounding the probe from a pH-meter (, where the catalysed reaction generates or absorbs hydrogen ions.
- The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly from the pH-meter's display.
- Here electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction.

$Hg \leftrightarrow Hg^{+} + e-$
$HgCl_2 \leftrightarrow 2\,Hg^{+} + 2Cl^{-}$
$KCl \leftrightarrow K^{+} + Cl^{-}$

$Ag \leftrightarrow Ag^{+} + e-$
$AgCl \leftrightarrow Ag^{+} + Cl^{-}$
$HCl \leftrightarrow H^{+} + Cl^{-}$

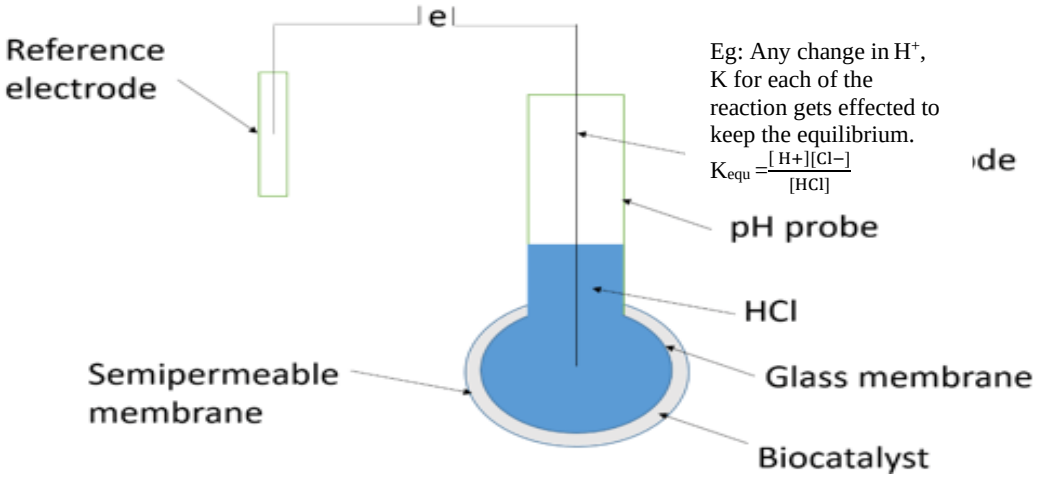


Fig 7. A simple potentiometric biosensor. A semi-permeable membrane surrounds the biocatalyst entrapped next to the active glass membrane of a pH probe.

The electrical potential is generated between the internal Ag/AgCl electrode bathed in dilute HCl and an external reference electrode. Semipermeable membrane surrounds the enzyme allows the analyte to move in. Potentials at reference electrode are unaffected by chages in H^+. Reaction generates or absorbs H^+ at glass electrodes. So, equilibrium of the reaction alters to keep the K constant for each of the reaction. Reaction. Changes in H^+ glass electrodes ultimately changes the electron concentration of the reaction. Measure of the current between two electrodes will be measure of $H^+$of the solution.

1.4.3 Optical biosensors

- An optical biosensor is a compact analytical device containing a biorecognition sensing element integrated with an optical transducer system (Figure 8).
- The basic objective of an optical biosensor is to produce a signal which is proportionate to the concentration of a measured substance (analyte).
- The optical biosensor can use various biological materials, including enzymes, antibodies, antigens, receptors, nucleic acids, whole cells and tissues as biorecognition elements.
- Surface plasmon resonance (SPR), evanescent wave fluorescence and optical waveguide interferometry utilize the evanescent field in close proximity to the biosensor surface to detect the interaction of the biorecognition element with the analyte.
- There are a huge number of variations in the construction of optical biosensors.

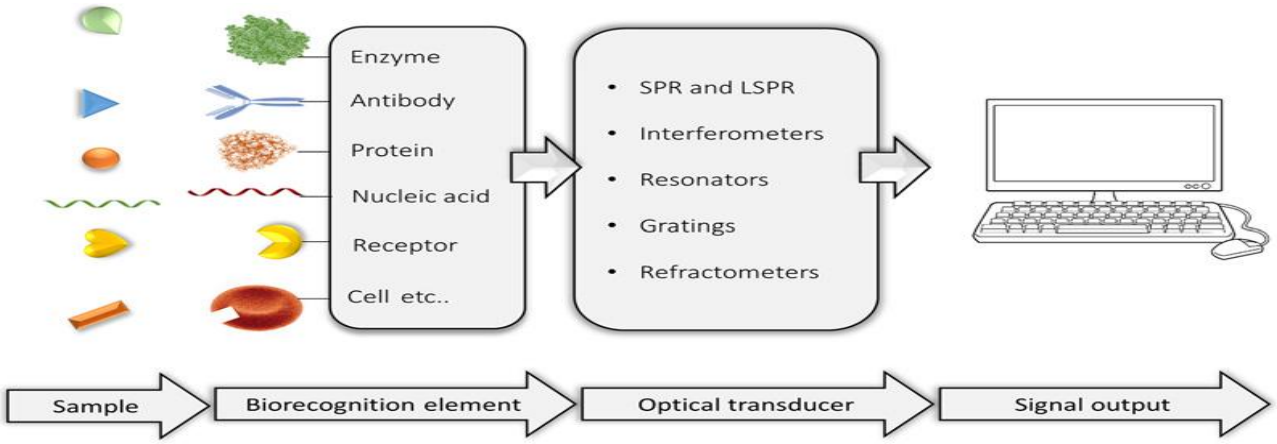


Figure 8 Generalised representation of optical biosensors.

Surface plasmon resonance biosensors (SPR)

- The SPR phenomenon occurs on the surface of metal (or other conducting materials) at the interface of two media (usually glass and liquid) when it is illuminated by polarized light at a specific angle.
- This generates surface plasmons and consequently a reduction of the intensity of reflected light at a specific angle known as the resonance angle.

- This effect is proportionate to the mass on the surface.
- A sensorgram can be obtained by measuring the shift of reflectivity, angle or wavelengths against time.
- In all configurations, the SPR phenomenon enables direct, label-free and real-time changes of refractive index at the sensor surface, which is proportionate to the biomolecule concentration.
- To measure a ligand–analyte interaction, one interacting molecule must be immobilized on the sensor surface.
- A practical SPR instrument combines an optical detector part, usually measuring intensity shift, a sensor chip with a gold surface and a layer enabling ligand immobilization, which is integrated with a fluidics system enabling a flow-through operation.
- The operating principle of a typical SPR instrument is presented in Figure 9

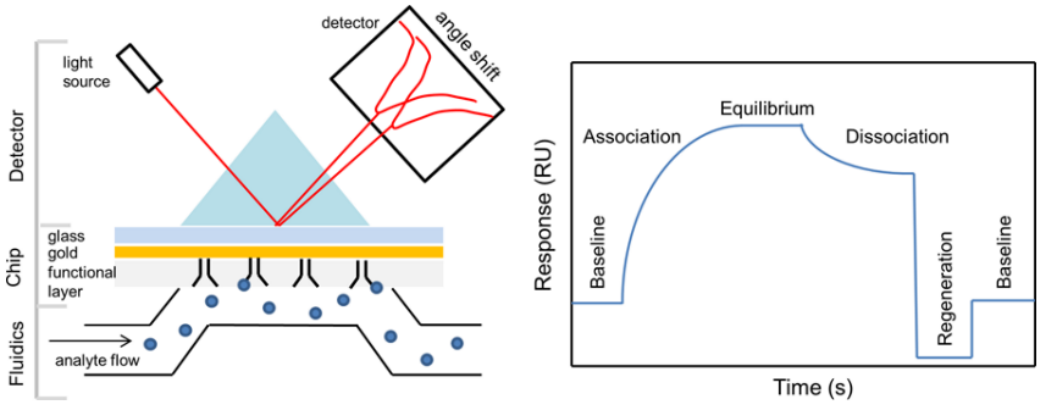


Figure 9: The operating principle of a typical SPR instrument

Surface plasmon resonance imaging (SPRi)

- SPR imaging (SPRi) takes the SPR analysis a step further by merging the sensitivity of SPR and spatial imaging in a microarray format allowing the simultaneous study of multiple different interactions.
- SPRi allows simultaneouslystudying multiple different interactions on an array of precisely patterned molecules (Figure 10).
- High throughput, sensitivity and obtaining the spatially resolved images of biointeractions open up a great future for SPRi to be applied in clinical chemistry and medicine for the screening of biomarkers and therapeutic targets.
- For example, a successful application of this method was the kinetic study of the binding between an immunosuppressive drug (FK506) and its target protein (FK506-binding protein 12 (FKBP12)) in a high-throughput SPRi format with a detection limit of 0.5 nM .

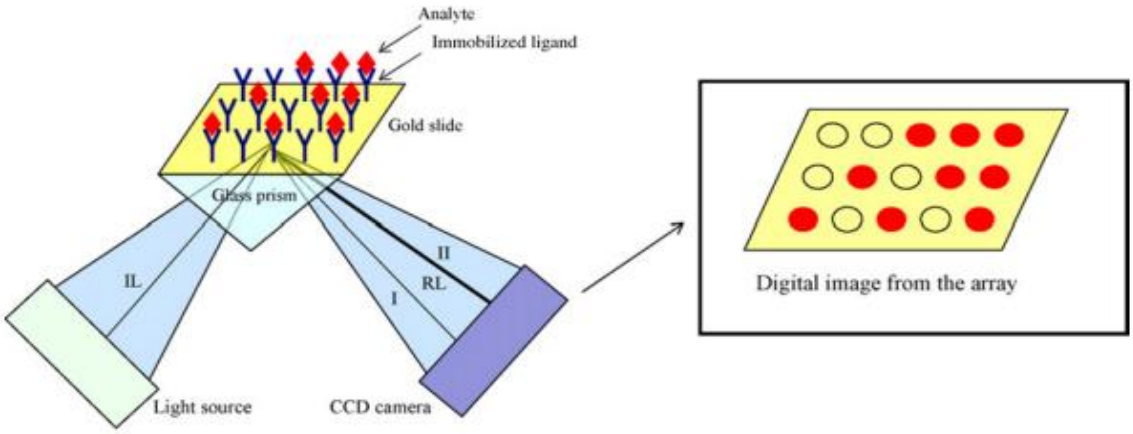


Figure 10: The operating principle of a typical SPRi

Localized surface plasmon resonance

- Localized SPR (LSPR) is based on metallic nanostructures (MNPs) (Au, Ag, etc.) having unique optical properties which are not seen in larger metal structures.
- A particularly striking example of such phenomenon is the red colour of aqueous dispersions of colloidal gold particles, which is a manifestation of LSPR.
- The optical phenomenon of LSPR occurs when incident light interacts with MNPs, the electromagnetic field of the light induces collective electron charge oscillations confined in MNPs and the subsequent absorbance of light within the ultraviolet–visible (UV-VIS) band (Figure 4).
- Thus, the major difference between SPR and LSPR is that induced p plasmons oscillate locally on thenanostructure rather than along the metal/dielectric interface (Figure 11).

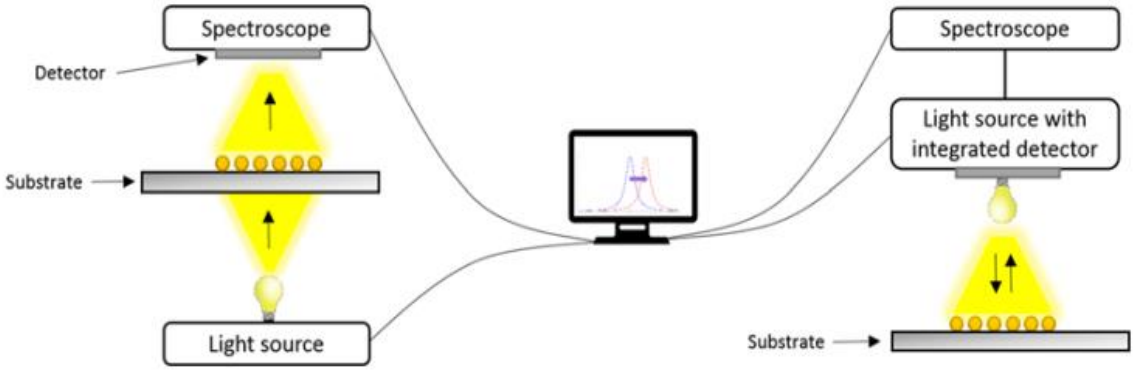


Fig 11 Localized surface plasmon resonance

Evanescent wave fluorescence biosensors

- In these biosensors, the biological recognition and the consequent binding event occur within the confines of an evanescent wave.
- The evanescent wave arises from the manner in which light behaves when confined in an optical waveguide or fibres.

- Guided light is totally internally reflected when it meets the interface of the waveguide/fibre and a surrounding medium with a lower index of refraction, as a result an electromagnetic field called an evanescent wave extends out from the interface into the lower index medium.
- The evanescent wave decays exponentially with distance from the surface, generally over the distance of 100 nm to approximately a wavelength.
- Since the evanescent wave is such a near-surface phenomena, detection employing evanescent wave excitation to generate the fluorescent signal is surface-sensitive, meaning that only fluorescent molecules near the surface are excited (Figure 12).

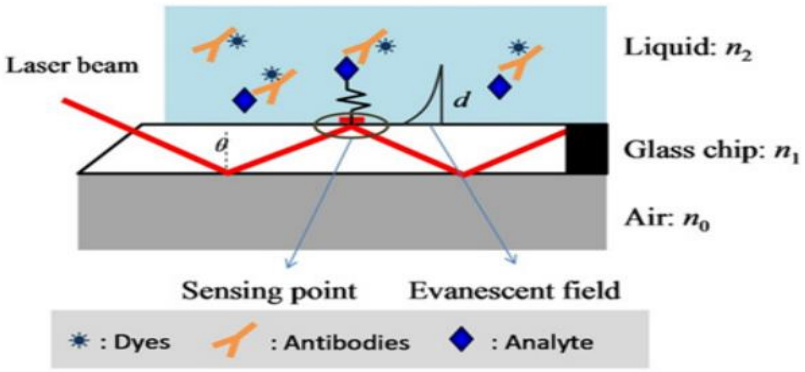


Fig 12 Evanescent wave fluorescence biosensors

- **Bioluminescent optical fibre biosensors:** This technique uses recombinant bioluminescent cells and the bioluminescent signal is transferred from the analyte by an optical fibre.
- **Optical waveguide interferometric biosensors**: An integrated planar optical waveguide interferometric biosensor is a combination of evanescent field sensing and optical phase difference measurement methods. By probing the near-surface region of a grating sensor area with the evanescent field, any change of the refractive index of the probed volume induces a phase shift of the guided mode compared with a reference field, typically of a mode propagating through the reference arm of the same waveguide structure. The interfering fields of these modes produce an interference signal detected at the sensor's output, whose alteration is proportional to the refractive index change and the signal is related to the concentration of the analyte.
- **Ellipsometric biosensors:** An ellipsometric biosensor measures changes in the polarization of light when it is reflected from a surface. This platform was applied in detecting the binding of influenza A virus strains with a panel of glycans of diverse structures.
- **Reflectometric interference spectroscopy biosensors:** Reflectometric interference spectroscopy (RIfS) is a label-free and time-resolved method where the simple optical setup is based on white light interference at thin layers. Changes in the phase and amplitude of polarized light provides information about the thickness and refractive index of the adsorbed protein layer.

- **Surface-enhanced Raman scattering biosensors:** Surface-enhanced Raman scattering (SERS) is a biosensing technique which enhances the intensity of the vibration spectra of a molecule by several orders of magnitude when it is in close proximity to nano-roughened metallic surfaces or nanoparticles made of gold or silver

1.4.4 Acoustic biosensors

- Bulk acoustic wave (BAW) biosensors employ either longitudinal or shear waves, although the latter is often preferred to reduce acoustic radiation in the medium of interest.
- They are the oldest and the simplest acoustic wave devices.
- BAW devices consist of a parallel electrode placed on both sides of the thin piece of crystal. BAW sensor can technically employ any piezoelectric element, and typically quartz is used, as it is an inexpensive material readily available in nature and easily synthesizable in abundant quantities.
- In addition, thin disks of quartz are more stable at high temperatures than other piezoelectric elements.
- When an alternating electric field is applied, it results in a potential difference between the two electrodes and the shear deformation of the crystal.
- As a result, there is mechanical oscillation of a standing wave across the bulk of the quartz.
- The frequency of the vibrations is dependent on quartz properties such as density, size, and phase in contact with the crystal surface.
- Currently, thickness shear mode (TSM) resonator and shear horizontal acoustic plate mode (SH-APM) sensors remain the most widespread BAW sensors (Fig 13).
- The other two common BAW sensors that lie beyond the scope of this entry are the thin rod acoustic wave sensors and the flexural plate wave devices.

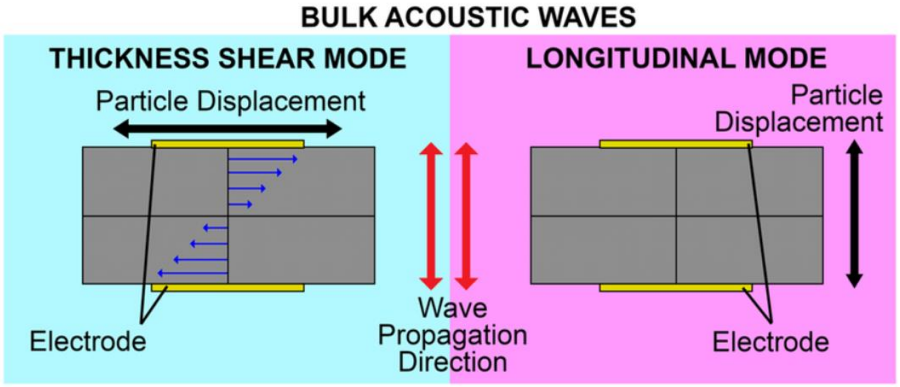


Fig 13 Modes of BAW. Thickness shear mode (TSM) and longitudinal mode

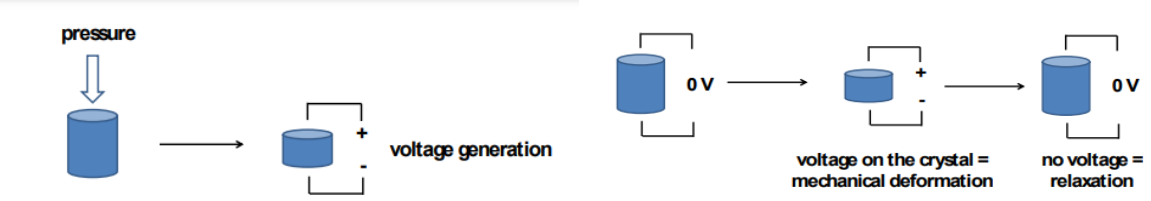
- **Thickness Shear Mode (TSM) Resonator:** TSM resonator, also known as quartz crystal microbalance (QCM), is the simplest and most widespread acoustic wave device today. TSM typically composes of a quartz plate sandwiched by electrodes on opposite faces. Electric field

crosses through this plate when voltage is applied to the electrodes, resulting in a shear mechanical strain or displacement in the quartz. By oscillating the voltage frequency, a mechanical resonance can be generated, where the maximum displacement of crystal occurs at the surfaces.

- **Shear Horizontal Acoustic Plate Mode (SH-APM) Sensor**:
  SH-APM sensors use a thin piezoelectric substrate, or a plate, to guide the acoustic wave and to confine its energy within the plate's top and bottom surfaces. Most of the production and analysis principles employed in SH-APM sensors are used in a TSM resonator. Their most striking difference is that SH-APM sensors employ inter digital transducers (IDT) rather than electrode plates. IDTs are deposited on opposite ends of a surface, where one IDT generates displacement waves through application of an oscillating voltage and the other receives it . The surface without IDT is immersed in the targeted liquid and acts as the sensor, so the device will not suffer from corrosion problems as electrode plates do in biological solutions.

1.4.5 Piezoelectric biosensors

- Anisotropic crystals i.e. crystals without center of symmetry can generate electric dipole when mechanically squeezed.
- The electric dipole is also called piezoelectricity.
- The described effect can work in oppose way when an anisotropic crystal become deformed due to voltage imposed on it.
- The mechanical deformation is, however, a simple situation and oscillation is rather chosen in the common applications like here described analytical devices.
- In the case of oscillation, an alternating voltage is imposed on the crystal and mechanical oscillation then occurs



a b

Figure 14. Piezoelectric effect when voltage is generated because of mechanical deformation (a), when mechanical deformation is initiated by an applied voltage (b)

- The oscillations can have many appearances depending upon material and other conditions like electrical contacts, shape of the crystals etc.

- The oscillations occur in adiabatic waves which are typically spread over the mass like the acoustic one.
- In the oscillating crystals, the both surface acoustic waves spreading on the material and bulk acoustic waves occurring in deep matter can take place.
- In standard analytical applications, frequencies of oscillations are measured and interaction with either crystal alone or electrode leading electricity impulse on the crystal surface can serve for the determination of analyte.
- Piezoelectric biosensors are a group of analytical devices working on a principle of affinity interaction recording.
- A piezoelectric platform or piezoelectric crystal is a sensor part working on the principle of oscillations change due to a mass bound on the piezoelectric crystal surface.
- Biosensors having their surface modified with an antibody or antigen, with a molecularly imprinted polymer, with genetic information like single stranded DNA, and biosensors with bound receptors of organic of biochemical origin, are presented and discussed.
- **Piezoelectric Immunosensors:** The piezoelectric immunosensors are biosensors which contains an antibody as a biorecognition element and specificity of the antibody significantly influences specificity of the whole immunosensor.

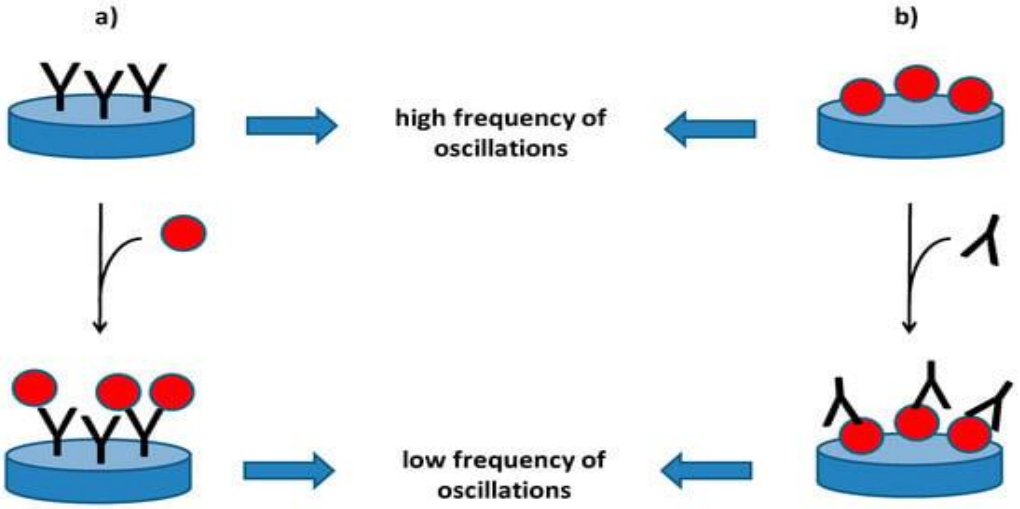


Figure 15. Piezoelectric immunosensors for the determination of an antigen (a) or an antibody(b)

- **Molecularly Imprinted Polymers on Piezoelectric Platform:** Molecularly Imprinted Polymers are specific artificial materials that can substitute antibodies or antigens as a biorecognition part in a biosensor.

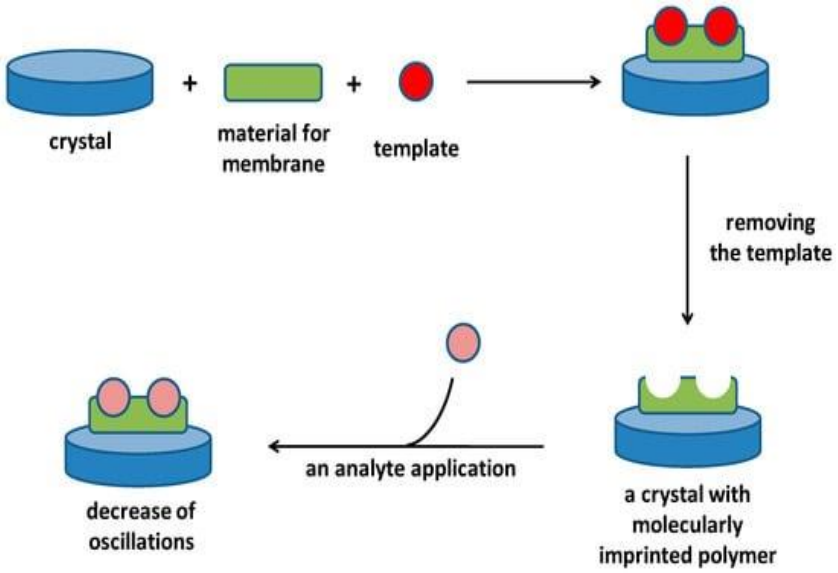


Figure16. Covering of crystal with a Molecularly Imprinted Polymer and following assay of an analyte chemically identical or close to the template.

- **Genetic Information Using Piezoelectric Biosensors:** Genetic information can be employed as a biorecognition part of various biosensors. Single-strand short strains of DNA or RNA can by written down as typical examples of genetic information forms that are suitable for biosensors construction.

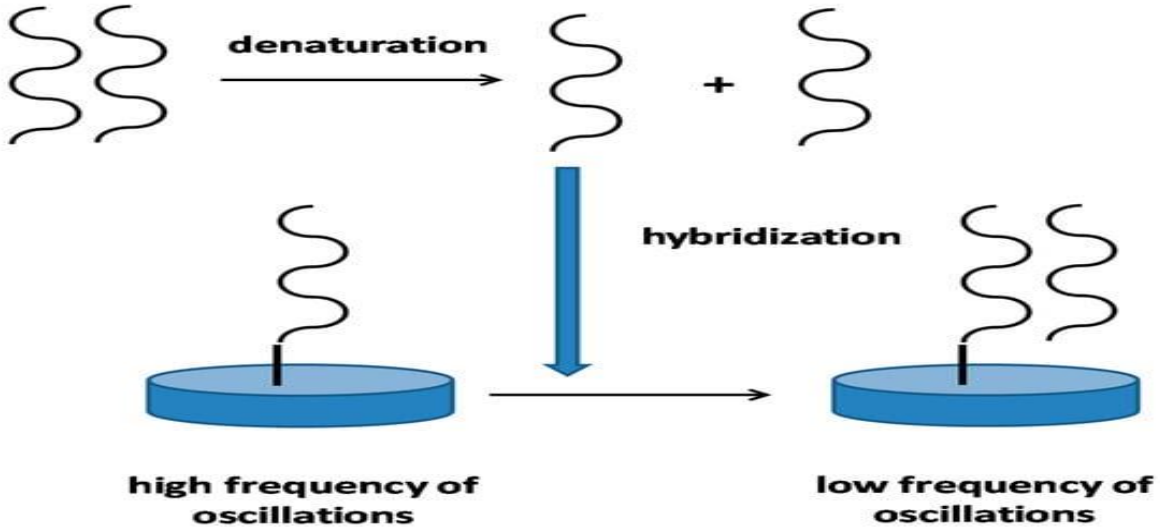


Figure 17. DNA piezoelectric biosensor.

1.4.6 Calorimetric biosensors

- Many enzyme catalysed reactions are exothermic, generating heat which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration.
- This represents the most generally applicable type of biosensor.

- The temperature changes are usually determined by means of thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment (Figure 18).
- Under such closely controlled conditions, up to 80% of the heat generated in the reaction may be registered as a temperature change in the sample stream.
- This may be simply calculated from the enthalpy change and the amount reacted.
- Calorimeter sensors are widely used in different areas such as biochemical, clinical, and pharmaceutical industries, and many more.
- In the calorimetry sensor, the energy released during a biochemical reaction is calculated as the measure of the interaction of the tested molecules.
- Two processes are used with the calorimetry sensor: (1) Adiabatic calorimetry, where there is no heat exchange between the external environment and the reaction vessel, and (2) Heat conduction calorimetry, which involves the heat transfer from a vessel to the surrounding heat sink.

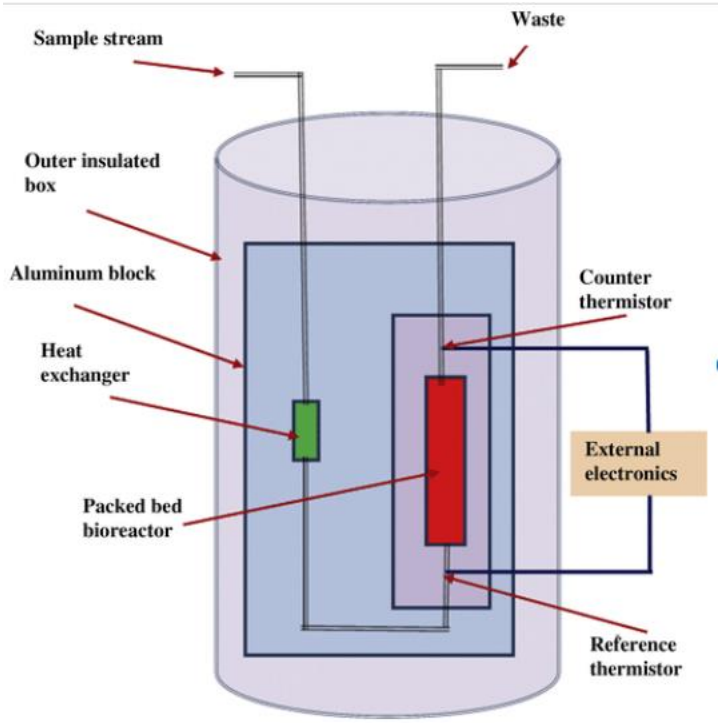


Figure 18. Calorimetric biosensors. Schematic diagram of a calorimetric biosensor. The sample stream passes through the outer insulated box to the heat exchanger within an aluminium block. From there, it flows past the reference thermistor and into the packed bed bioreactor, containing the biocatalyst, where the reaction occurs. The change in temperature is determined by the thermistor and the solution passed to waste. External electronics (l) determines the difference in the resistance, and hence temperature, between the thermistors.

1.5 Scope of biosensors and its limitations.

- There is a huge application of biosensor in the medical industry for testing purpose.
- The biosensor is used for pathogens detection purpose.
- Biosensors are also used for water treatment purpose.
- Biosensors are used for environmental monitoring purposes such as gas detection or tracing.
- They used for toxic metabolites detection.
- The biosensor is used to identify the contaminants in water such as heavy metal ions. This process is generally used when river water is treated for drinking.
- Biosensors are used in the security system and biodefense technology.
- Blood Glucose biosensor is used for glucose monitoring inside the human body.
- The biosensor also used for testing of cholesterol.
- Biosensors are used for the measurement of vitamins, biotin, folic acid, etc.
- Biosensors are used in Agriculture, Biotech industries to continuously monitor types of chemical, their properties in a specific system.

Biosensors	Transduction	Advantages	disadvantages	Applications
Amperometric and Potentiometric	Oxygen electrode	- simple - very specific (more selective)	- low sensitivity	glucose, sulfites, glutamate, ethanol, sucrose, lactate, cholestérol…
Amperometric	Hydrogen peroxide electrode	- simple - faster, more sensitive, more precise and more accurate than potentiometric biosensors	- little specific (less selective)	sulfites, glucose, glutamate, amino acids
Potentiometric	Hydrogen peroxide electrode	- simple - sensitive - very short response times	- not very specific (less selective)	sulfites, glucose, glutamate, amino acids
optical	Optical systems	- remote use - low costs - miniaturizable - no electrical interference	- need for high energy sources - narrow concentration range - Interference of incident light.	nitrate, glucose, glycerol, ethanol, galactose…
calorimetric	Calorimetric	no optical interference like color and turbidity	-expensive, - bulky - need for large amounts of enzyme	glucose, lactate, ascorbic acid, cholesterol, galactose, ethanol, lipids.

Table 1: Scope of biosensors and its limitations

Beneficial Features: A successful biosensor must possess the following features:

- The biocatalyst must be highly specific for the purpose of the analyses be stable under normal storage conditions except in the case of calorimetric enzyme strips and show good stability over a large number of assays (i.e. much greater than 100).
- The reaction should be as independent of physical parameters as like stirring, pH and temperature.
- The response should be accurate, precise, reproducible and linear over the useful analytical range without dilution or concentration. It should also be free from electrical noise.
- If the biosensor is to be used for invasive monitoring in clinical situations the probe must be tiny and biocompatible having no toxic or antigenic effects. If it is to be used in fermenters it should be sterilisable. This is preferably performed by autoclaving but no biosensor enzymes can presently withstand such drastic wet-heat treatment. In either case the biosensor should not be prone to fouling or proteolysis.
- The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.
- The biosensors have been considered to be superior and more sensitive, in comparison to physical instruments due to the following reasons:
  - In a biosensor the immobilized biological material is present in intimate contact of a suitable transducer so that the biochemical signal is quickly converted into an electrical signal.
  - The immobilization of biomolecules permits reuse of these molecules (which are expensive) and allows simplification of the entire apparatus.
  - The biological sensing element is present in a small area and is very sensitive, thus facilitating analysis of substances in small quantities.
  - Biosensors may be developed according to specific needs and can be highly specific or show broad spectrum**.**

Disadvantages:

- Heat sterilization is not possible as this would denature the biological part of the biosensor.
- The membrane that separates the reactor media from the immobilized cells of the sensor can become fouled by deposits.
- The cells in the biosensor can become intoxicated by other molecules that are capable of diffusing through the membrane.
- Changes in the reactor broth (i.e., pH) can put chemical and mechanical stress on the biosensor that might eventually impair it.

Module 2

BASIC DESIGN AND TRANSDUCER

2.1 Design Considerations: calibration, dynamic Range, signal to noise, sensitivity, selectivity, Interference recognition.

2.2 Transduction membrane protein sensors: ion channels,

2.3 Types of Transducer, Optical; Fiber Optic, ECL, Surface Plasmon Resonance, Electro chemical; FET, Impedance, Piezoelectric; Cantileaver,

2.1 Design Considerations: Calibration, Dynamic Range, Signal To Noise, Sensitivity, Selectivity, Interference Recognition.

- A successful biosensor is composed of two main components, mainly a biological receptor or sensor element and a transducer.
- The first step in developing a biosensing device involves investigating the target analyte and understanding how this analyte interacts with certain biological molecules.
- Once this has been established, the following tasks are critical: Selection of a biological receptor: the specificity and selectivity of a biosensor to the analyte of interest is dependent upon the biological receptor used.
- A suitable receptor with high affinity for the analyte is thus recommended.
- Having knowledge of the advantages and disadvantages of various biological receptors in different biosensor applications is very important in selecting a suitable receptor.
- **Selection of a suitable immobilization method**: for any biological molecule to operate reliably as a biological receptor, it requires attachment onto the surface of a transducer, the process is known as immobilization.
- Various methods have been used for this task and include adsorption, entrapment, covalent attachment, micro encapsulation and cross linking.
- **Selection of a transducer element**: the transducer element greatly influences the sensitivity of the biosensor device.
- Employing the right transducer will result in a device with increased sensitivity while the sensitivity is more likely to be compromised by the use of an ineffective transducer

2.1.1 Calibration

- Sensor Calibration in simple terms can be defined as the comparison between the desired output and the measured output.
- On-site monitoring requires enhanced sensitivity, selectivity, rapidity, and ease of operation of the analytical equipment, which should provide reliable continuous information in real-time and demonstrate sufficient stability of action.
- We use different systems and types of equipment for measuring various physical quantities.
- The accuracy of the measurement depends upon various factors.
- The equipment used for measurements can lose their precision when used at higher temperatures, high moisture or humidity conditions, subjected to degradation, subjected to external shocks, etc…This can be observed as the error in the measurement.
- To tackle this error and make necessary changes to the equipment calibration methods are used. Today sensors are being used for making various measurements.
- There are sensors to measure temperature, color, humidity, etc…Sensor Calibration plays a crucial role in removing the errors in sensor measurements.
- Sensors are electronic devices. They are sensitive to the changes in their working environment. Undesirable and sudden changes in the working environments of the sensors give undesired output values. Thus, the expected output differs from the measured output. This comparison between the Expected output and measured output is called Sensor Calibration.
- Sensor calibration plays a crucial role in increasing the performance of the sensor. It is used to measure the Structural errors caused by sensors. The difference between the expected value and the measured value of the sensor is known as the Structural Error.
- Sensor calibration helps in improving the performance and accuracy of the sensors.
- There are two well-known processes in which sensor calibration is done by industries.
- In the first method companies **add an In-house calibration process** to their manufacturing unit to perform individual calibration of the sensors. Here the company also adds necessary hardware to their design for sensor output correction.By this process, the sensor calibration can be changed to match the application-specific requirements. But this process increases the time to market.
- The alternative of this **In-house calibration process**, several manufacturing companies provides sensor packages with a high-quality automotive-grade MEMS sensor along with complete system-level calibration. In this process, the companies include an onboard digital circuitry and software to help designers to improve the functionality and performance of the sensors.
- To reduce the product design time and component count, digital circuitry such as voltage regulation and Analog signal filtering techniques are included.

- To improve the overall performance and functionality, the onboard processor is provided with sophisticated sensor fusion algorithms.
- Some of the sophisticated onboard signal processing algorithms also help in reducing the manufacturing time enabling the faster time to market.

Standard Reference Method

- Here the sensor output is compared with a standard physical reference to know the error in some sensors. Examples of sensor calibration are rulers and meter sticks, For temperature sensors- Boiling water at 100C, Triple point of water, For Accelerometers- "gravity is constant 1G on the surface of the earth".

Calibration Methods:

- There are three standard calibration methods used for sensors. They are-**One point calibration, Two-point calibration, and Multi-Point Curve Fitting**.
- Before knowing these methods we have to know the concept of Characteristic curve.
- Every sensor has a characteristic curve that shows the response of the senor to the given input value. In the calibration process, this characteristic curve of the sensor is compared with its ideal linear response.
- Some of the terms used with the characteristic curve are-
- **Offset** – This value tells us whether the sensor output is higher or lower than the ideal linear response.
- **Sensitivity or Slope** – This gives the rate of change of sensor output. A difference in slope shows that the sensor output changes at a different rate than the ideal response.
- **Linearity** – Not all sensors have a linear characteristic curve over the given measurement range.
- One point calibration is used to correct the sensor offset errors when accurate measurement of only a single level is required and the sensor is linear.
- Temperature sensors are usually one point calibrated.

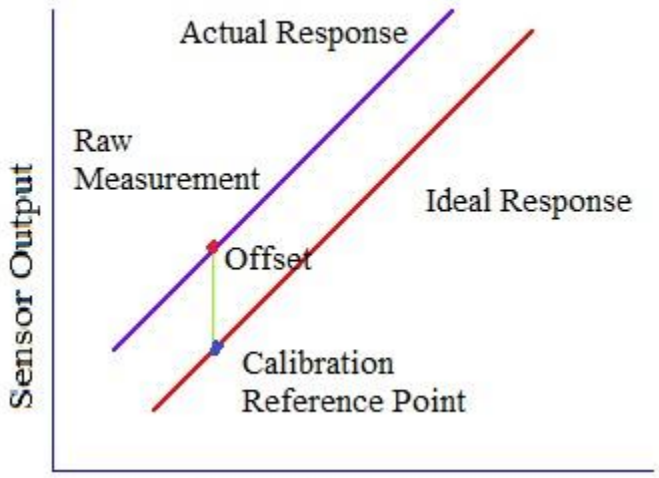


Fig 19: One-Point-Calibration

- Two-point calibration is used to correct both slope and off-set errors. This calibration is used in the cases when the sensor we know that the sensor output is reasonably linear over a measurement range. Here two reference values are needed- reference High, reference Low.

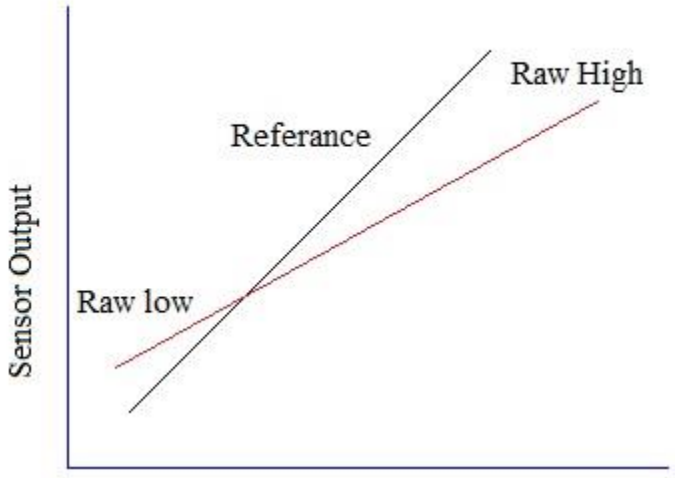


Fig 20 Two-Point-Calibration

- Multi-point Curve fitting is used for sensors that are not linear over the measurement range and require some curve-fitting to get the accurate measurements.
- Multi-point curve fitting is usually done for thermocouples when used in extremely hot or extremely cold conditions.
- For all the above calibration process, the characteristic curves of the sensors are drawn and compared with the linear response and error is known.
- The calibration process helps us to determine the following results-No error noted on the DUT,

- An error is noted and no adjustment is made.
- An adjustment is made to remove the error and the error is corrected to the desired level.
- For sensor calibration sensor models are used. Sensor calibration is applied in Control systems to monitor and adjust the control processes. Automatic systems also apply te sensor calibration to get error-free results.
- Use of Sensor Calibration: The calibration process is used to increase the performance and functionality of the system. It helps in reducing errors in the system. A calibrated sensor provides accurate results and can be used as a reference reading for comparison.

2.1.2 Dynamic range

- Dynamic range is one of the design parameter, when digital sensors are component of sensor device.
- Here, dynamic range of measurement will be also related to the number of binary digits (bits) used in a digital numeric representation in which the measured value is linearly related to the digital number.
- Often this dynamic range of measurement is limited at one end of the range by saturation of a sensing signal sensor or by physical limits that exist on the motion or other response capability of a mechanical indicator.
- The other end of the dynamic range of measurement is often limited by one or more sources of random noise or uncertainty in signal levels that may be described as defining the sensitivity of the sensor or metrology device.
- The range of concentrations over which the biosensor exhibits a change in output is the operational range of the biosensor.
- Operational range is determined by measuring the concentrations over which the biosensor shows a graded, concentration-dependent change in response (Figure 21).

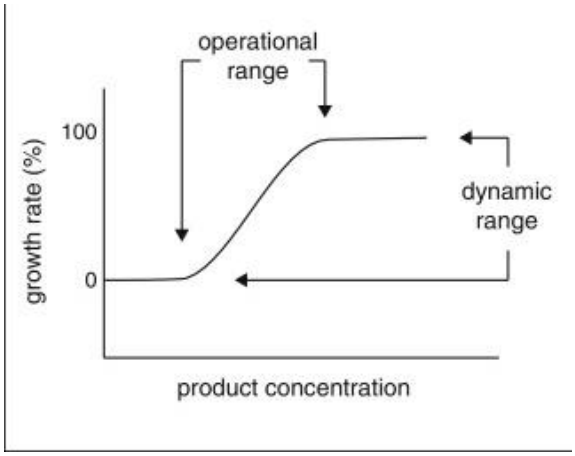


Fig 21 Biosensor transfer function

- Defining engineering parameters for biosensors is a prerequisite for biosensor-based measurements.
- The relationship between biosensor output and product concentration is the biosensor transfer function.
- The range of concentrations over which the biosensor functions is the operational range. The intensity of the biosensor response is the dynamic range.
- Sensor dynamic range quantifies the ability of a sensor to acquire both high and low signals.
- It is defined as the ratio of the largest nonsaturating input signal to the smallest detectable input signal.
- The false positive rate of a biosensor system determines the maximum number of designs that can be evaluated in a given experiment.
- The signal-to-noise of a biosensor, also referred to as the dynamic range of the system, can be quantified as the ratio of the highest measured output of the biosensor to the lowest measured output of the biosensor.
- Dynamic range is the ratio between the largest and smallest values that a certain quantity can assume.
- Various strategies are used to tune, extend, and narrow the dynamic range of Biosensors that use either optical or electrochemical readouts.
- These strategies enable one to tune the affinity of biosensors that detect nucleic acids, small molecules, heavy metal ions, pH, and temperature.
- In addition, environmental changes and material sizes are also used to adjust the dynamic range of Biosensors.
- However, biosensors with extended dynamic range display reduced precision, while highly precise sensors display narrowed dynamic range.
- Biosensor design strategy should be to develop dual-signaling biosensor architecture that simultaneously provides both a highly sensitive "signal-on" readout over a small fixed dynamic range as well as a "signal-off" readout, enabling quantification over a large, extended dynamic range.

2.1.3 Signal-to-noise ratio

- In quite many applications of sensors, noise adds to the useful signal, while in others, noise is proportional to signal.
- Whatever the case, the larger the noise fluctuations, the more difficult it is to detect or measure the useful signal.

- Figure 22 shows a typical detector output with respect to time shows that the performance of an electro-optical sensor does not depend on its signal alone but on its signal to noise ratio.

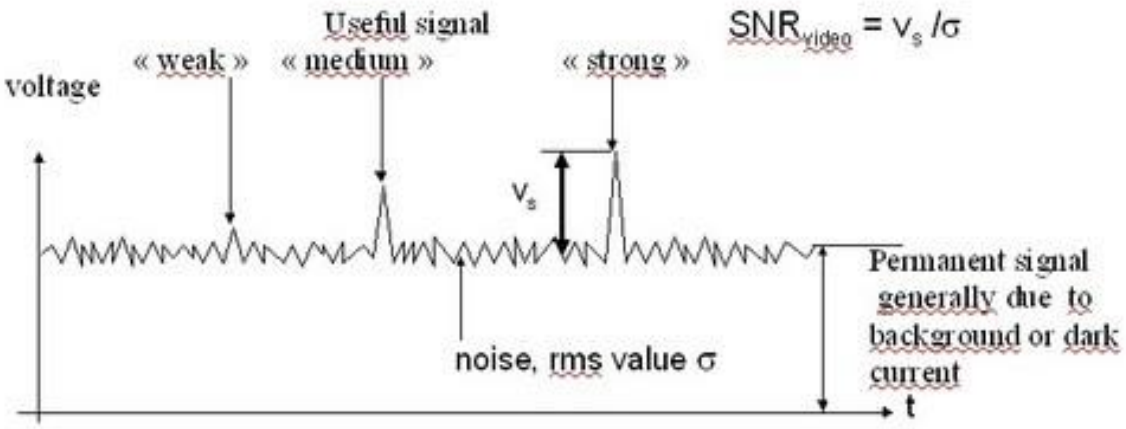


Figure 22: Signal to noise ratio

- Since the sensor output is noisy, it fluctuates above and below its average value by an instantaneous amount, for example ib (t) if one is concerned with the output current. The corresponding current or voltage variances, σ2i or σ2v, inside the electronic bandpass of the sensor generate the following electrical noise power Pn across the load resistor RL:
- If is is the instantaneous pertinent output from the detector, the corresponding electric power of the signal is :
- By definition, the power signal to noise ratio of the sensor at that corresponding instant is the ratio between the electrical pertinent signal power and that of the noise, both being evaluated inside the sensor bandpass:
- Spatial (geometric) and spectral filtering are aimed at minimizing shot noise, due for example to stray light, and at maximizing lens transmittance for the pertinent signal.
- Spatial filtering eliminate stray light from intense sources of light outside the field of view, by means of diaphragms, baffles, or protective screens.
- Spectral filtering separates useful from parasitic radiations.
- In Electronic filtering after detection, if the variation in time of the expected signal is known, and this signal is band limited and if the noise spectrum is white, signal processing techniques such as matched filtering are a good choice.

2.1.4 Sensitivity

- The sensitivity of the sensor is defined as the slope of the output characteristic curve (DY/DX in Figure 23) or, more generally, the minimum input of physical parameter that will create a detectable output change.
- In some sensors, the sensitivity is defined as the input parameter change required to produce a standardized output change.
- In others, it is defined as an output voltage change for a given change in input parameter. For example, a typical blood pressure transducer may have a sensitivity rating of 10 mV/V/mm Hg; that is, there will be a 10-mV output voltage for each volt of excitation potential and each mm Hg of applied pressure.
- The sensitivity is the derivative of the output with respect to the stimulus. For a transfer function, A, the sensitivity, b, for a particular input value B, so, is given by;
- $b = \left.\frac{dA}{dB}\right|_{s_o}$
- Therefore, in basic terms, the sensitivity is simply the smallest fractional change in a device that can be measured.
- Sensitivity is considered as the most important characteristic of a biosensor.
- The sensitivity of a biosensor is defined as the relationship between the change in analyte concentration and the intensity of the signal generated from the transducer.
- Ideally, a biosensor should generate a signal in response to small fluctuations in the concentration of the target analyte.
- Depending on the application, biosensors are required to detect analytes in the ng/ml or fg/ml concentration ranges.
- This is usually important for medical applications and environmental monitoring purposes.

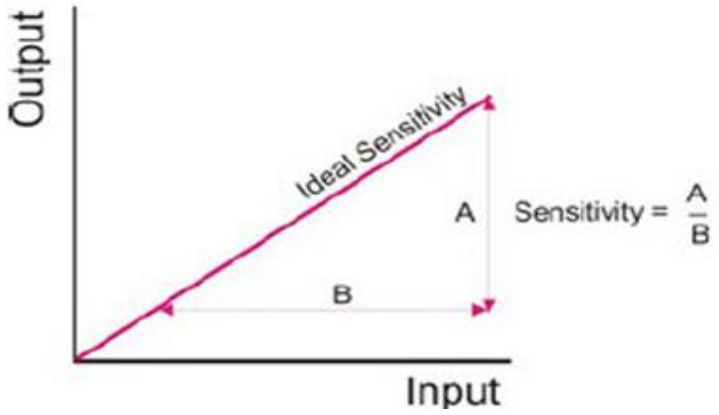


Figure 23: Illustration of sensitivity in biosensor

2.1.5 Selectivity

- This refers to the ability of the biosensor to selectively bind and respond only to the desired analyte, in the presence of other molecules or substances.
- When a signal or response is generated from interactions with an analyte that is different from the target analyte such is termed a false positive result.
- This is common in biosensors with poor selectivity, thus failing in clinical applications.
- Selectivity is a very important feature especially in medical applications where the test sample or sample matrix, usually blood or urine, contains numerous molecules that are quite similar to the target analyte and compete for binding to the biological receptor.
- The selectivity of a sensor is the ability to discriminate the target from the interference molecules and display a target-specific sensor response.
- It is a critical trait for chemical sensors that are used in real-time air pollution control, hazardous materials detection, food quality inspection and personal health monitoring.
- Attaining high target selectivity ensures that sensors will exhibit accurate information about the existence and concentration of a target gas, which is essential for reliable sensor response.
- To obtain target selectivity, it is critical to determine the optimum modification technique and receptor materials as well as to understand how each method works and how it could be designed for a specific target.

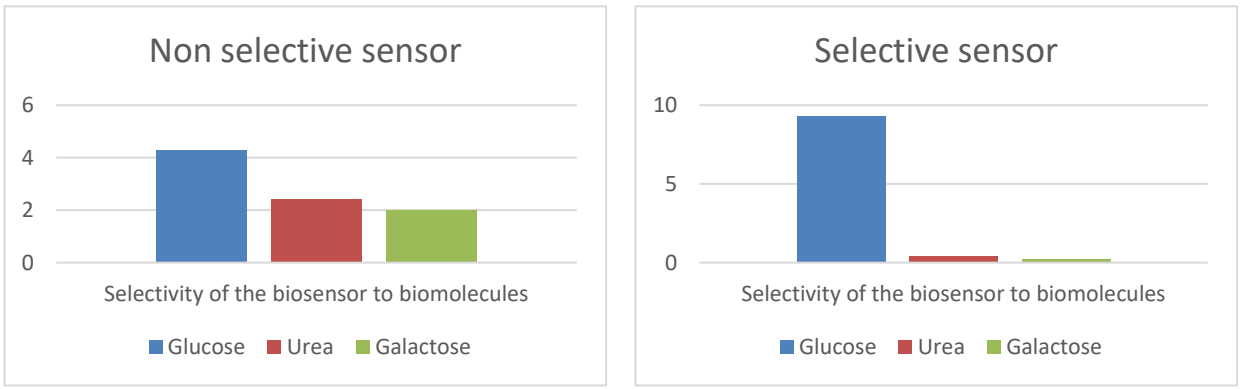


Figure 23: Selectivity test of sensor with respect to different biomolecules

2.1.6 Interference recognition

- Sensor systems are used every time a microcontroller needs to interact with the physical world.
- They are abundant in home automation, factory control systems, critical infrastructure, transport systems and many, many other things.
- In a sensor system, a sensor transforms a physical quantity into an analog signal which is sent to an ADC and a microcontroller for digitization and further processing.
- Once the measurement is in digital form, the microcontroller can execute tasks according to the measurement.
- Electromagnetic interference (EMI) can affect a measurement as it is transferred to the microcontroller.
- An attacker can manipulate the sensor output by intentionally inducing EMI in the wire between the sensor and the microcontroller.
- The nature of the analog channel between the sensor and the microcontroller means that the microcontroller cannot authenticate whether the measurement is from the sensor or the attacker.
- If the microcontroller includes incorrect measurements in its control decisions, it could have disastrous consequences
- The active sensors communicate by transmitting probe signals.
- The communication of probe signals may result in cross-interference which may vary in time.
- Cross-interference is detected, and can later be avoided, by determining a difference between signals received in a first part of a timeslot and signals received in a second part of the timeslot.
- In order to do so probe signals comprising two non-zero pulses are transmitted in respective parts of the timeslot.
- Applications are, for example, active presence sensors in lighting control applications in indoor as well as outdoor environments.
- It has been discovered that cross-interference across active sensors (such as sensors based on ultrasound, or radio frequency) is a problem in indoor as well as outdoor sensing applications.
- Cross-interference across active sensors generally depends on the dimensions of the monitored space and presence/absence of objects therein.
- For instance, when an object is moved (or added/removed) the cross-interference pattern across sensors tends to vary.
- This affects proper operation of the presence sensing systems.

2.2 Transduction membrane protein sensors: ion channels

- Biological membranes are one of the essential components of living organisms, forming physical boundaries in biological cells, such as the plasma membrane and the organelle membranes.
- The principal components of membranes are phospholipids and membrane proteins.
- Phospholipids are amphiphilic molecules consisting of a hydrophilic head group and hydrophobic tails.
- They form a bilayer-membrane configuration in aqueous environments, which is attributed to the hydrophobic interactions of their hydrocarbon chains.
- Lipid bilayer membranes function as hydrophobic barriers against soluble and ionic molecules and prevent the entry of such molecules into the cytoplasm and organelles.
- Membrane proteins are incorporated in the lipid bilayer and allow signal transduction and transport of ligand molecules across the membrane.
- Binding of an odorant molecule to the ionotropic receptor directly triggers the influx of cations into the cell through the membrane; this influx stimulates the neurons.
- Since these protein receptors act as ligand-sensing elements, numerous studies have attempted to use this sensing property of the highly specific membrane receptors for the development of biosensors.
- Moreover, sensory systems often include a mechanism of input amplification that enhances the output signal-to-noise ratio.
- Biosensors that embed sensory systems are based on two major platforms: the lipid bilayer-based platform and the cell-based platform (figure 24).

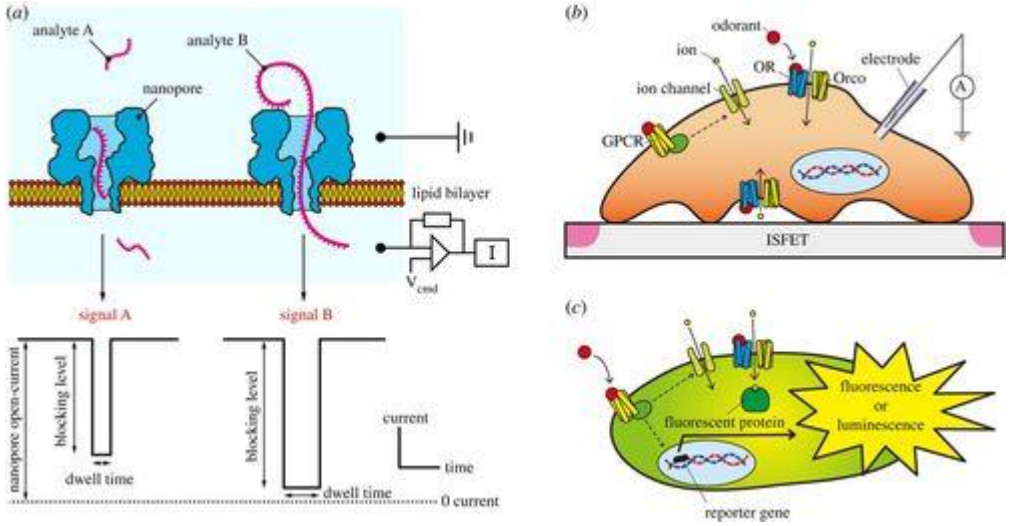


Figure 1. Membrane protein-based biosensors

- Figure 1. Membrane protein-based biosensors on (a) a lipid-bilayer platform and (b,c) cell-based platforms. On the lipid bilayer platform, the nanopore protein is incorporated in the lipid bilayer.

Single analyte molecules are detected based on the signatures of the current trace that translates the interaction between the analytes and the nanopore. On the cell-based platforms, such as those for odorant sensing, cell responses to odorants can be determined by (b) measuring the electrical alterations of the cell using electric signal measuring systems, including ion-sensitive field effect transistor (ISFET), (c) detection of fluorescence or luminescence changes initiated by olfactory stimuli. In this case, utilized cells express G protein-coupled receptor (GPCR) or olfactory receptor (OR) with OR co-receptor (Orco). (Online version in colour.)

Direct and indirect transduction

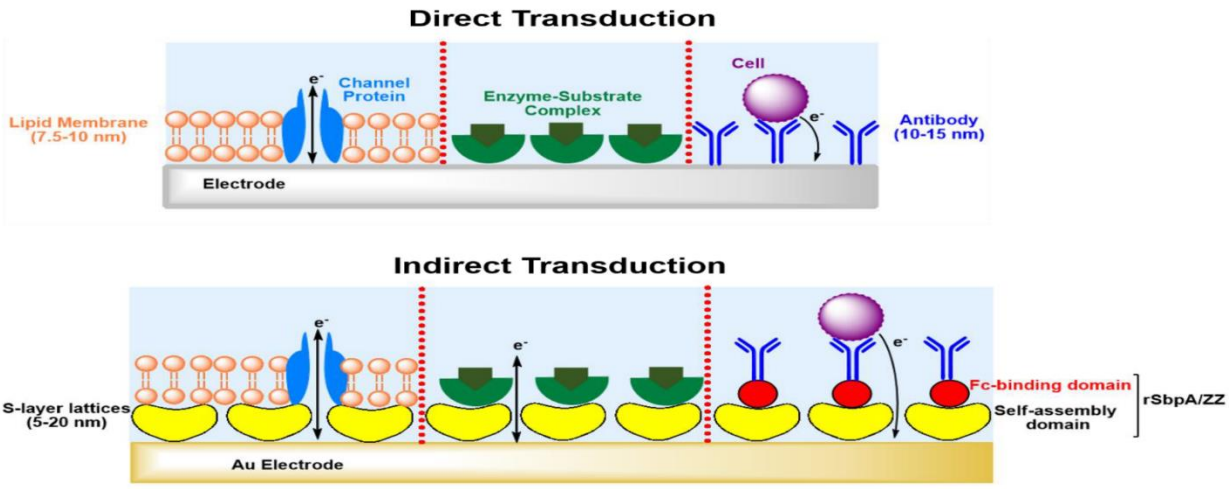


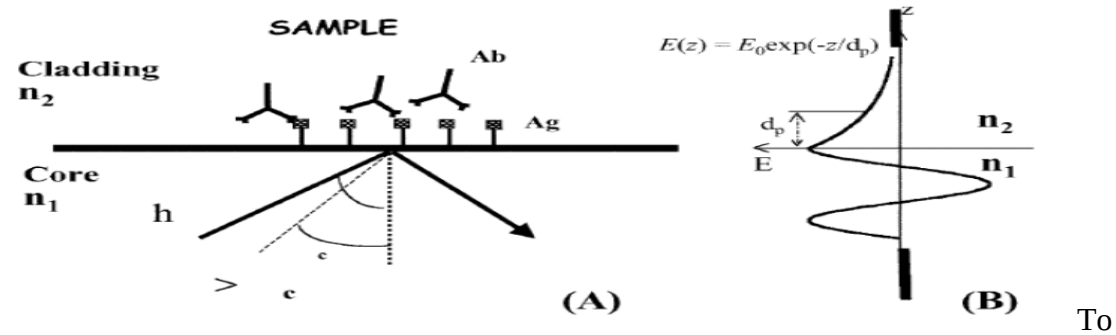
Fig: 24 Direct and indirect transduction

- In direct approach, the electron transfers are close to the surface, whereas in the indirect one, electron shuttles between the reaction site and the sensor surface.
- In indirect approach, The S-layer protein lattice constitutes an intermediate matrix. In the lipid-based biosensor (left), electrons transfer from the outer membrane to the inner membrane and vice versa via a channel protein.
- In the detection biosensor (middle and right), electrons transfer between the enzyme–substrate complex and cell/antibody and electrode surface, respectively. The S-layer lattice provides an immobilization matrix and ion reservoir.
- The pores of the S-layer lattice ensure no impact on the electron transfer. Fc: fragment crystallizable; rSbpA/ZZ: recombinant S-layer protein from Lysinibacillus sphaericus CCM 2177 with fused Fc-binding Z-domain (synthetic analog of immunoglobulin G (IgG-binding B—domain) of protein A of Staphylococcus aureus).

2.3 Types of Transducer, Optical; Fiber Optic, ECL, Surface Plasmon Resonance, Electro chemical; FET, Impedance, Piezoelectric; Cantilever,

2.3.1 Fibre optic Biosensors

- Optical fibers transmit light on the basis of the principle of total internal reflection (TIR).
- When this phenome-non occurs the light rays are guided through the core of the fiber with very little loss to the surroundings.
- The optical fiber is formed by a core with a refractive index n1and a cladding with a refractive index n2 (Fig.1).
- For light propagation by TIR the refractive index of the core (n1) must be larger than that of the cladding (n2), i.e. n1>n2.
- When a ray of light strikes the boundary interface between these transparent media of different refractive index and the angle of incidence is larger than the critical angle, defined by the Snell's law ($\theta c = \sin^{-1}[n2/n1]$), it will be totally internally reflected and propagated through the fiber.
- When the incident light is totally internally reflected, its intensity does not abruptly decay to zero at the inter-face.
- A small portion of light penetrates the reflecting medium by a fraction of wavelength, far enough for recognition of the different refractive index.
- This electromagnetic field, called the evanescent wave, has an intensity that decays exponentially with distance, starting at the interface and extending into the medium of lower refractive index.
- The penetration depth (dp), defined as the distance required for the electric field amplitude to fall to 1/e (0.37) of its value at the interface, increases with closer index matching and it is also a function of the wavelength of the light and the angle of incidence.
- The evanescent wave can interact with molecules within the penetration depth, thereby producing a net flow of energy across the reflecting surface in the surrounding medium (i.e. that with refractive index n2) to maintain the evanescent field.
- This transfer of energy will lead to attenuation in reflectance which can be used to develop absorption sensors based on evanescent waves (attenuated total reflection (ATR) sensors).
- When the evanescent lights electively excites a fluorophore, the fluorescence emitted can be directed back into the fiber and guided to the detector.



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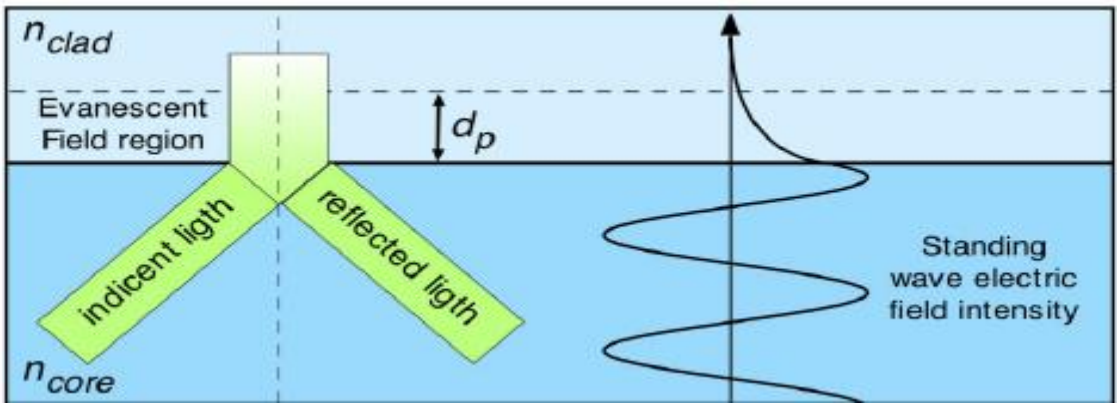


Fig: 25 Total Internal refraction [a] and electrical field amplified[b].

2.3.2 Electro chemiluminescence Biosensor (ECL)

- Electrochemiluminescence (ECL) is a chemiluminescence phenomenon resulting from the electrochemical excitation of a luminescence system (luminophore) that emits light when it returns to its fundamental state.
- The mechanisms associated with these phenomena are classified into two main types, annihilation pathway and Co-reactant pathway (Fig 26).

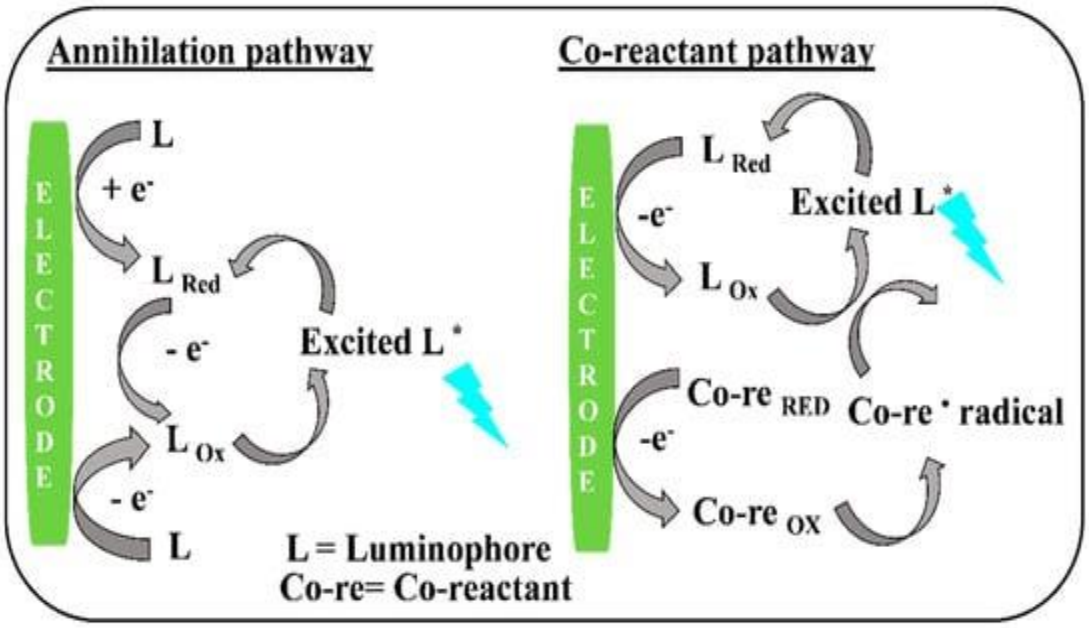


Fig 26: Mechanisms associated with Electrochemiluminescence

The annihilation pathway:

- A reduced specie and an oxidized specie (charged radical ions) are simultaneously generated at the electrode surface by applying alternating pulse potentials.
- These two species react between them generating an excited form, which in the relaxation process to the ground state emits a photon.

Co-reactant pathway:

- A co-reactant is a chemical specie that is reduced or oxidized at the electrode surface, generating a very reactive intermediates that react with the reduced or oxidized luminophore (specie capable of emit light) present in the solution to produce the excited state.
- Finally, the excited state returns to the ground state to cause chemiluminescence.
- Employing a co-reactant is especially useful when either radical charged ions are not stable enough for the ECL annihilation reaction, or radical ions cannot both be formed because of the solvent has a narrow potential window.
- With a co-reactant ECL can be generated by applying a potential in one direction.
- There are two reaction paths to produce the excited state of the ECL emitter, reductive-oxidation or oxidative-reduction ECL.
- For instance, oxalate ion ($C_2O_4^{2-}$) [4,5] and several amines [6,7,8,9] can be used for oxidative-reduction ECL where an oxidative step produces a strong reductant, whereas peroxidisulfate ion ($S_2O_8^{2-}$) is frequently used for reductive-oxidation ECL.
- As described above, ECL reactions require a luminophore.
- Few compounds and their derivatives primarily utilized for aqueous-based ECL bioanalytical detection methods, are luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and ruthenium (II) chelates $[RuL_3]^{2+}$.
- Nowadays, new luminophores such as semiconductor nanomaterials are being widely used with great results. This fact is one of the main reasons ECL sensor and biosensor are having a great and successful advance.

2.3.4 Surface Plasmon Resonance Biosensor

- Surface Plasmon Resonance is a phenomenon that occurs when polarized light hits a metal film at the interface of media with different refractive indices.
- SPR techniques excite and detect collective oscillations of free electrons (known as surface plasmons) via the Kretschmann configuration, in which light is focused onto a metal film through a glass prism and the subsequent reflection is detected (Fig. 1).

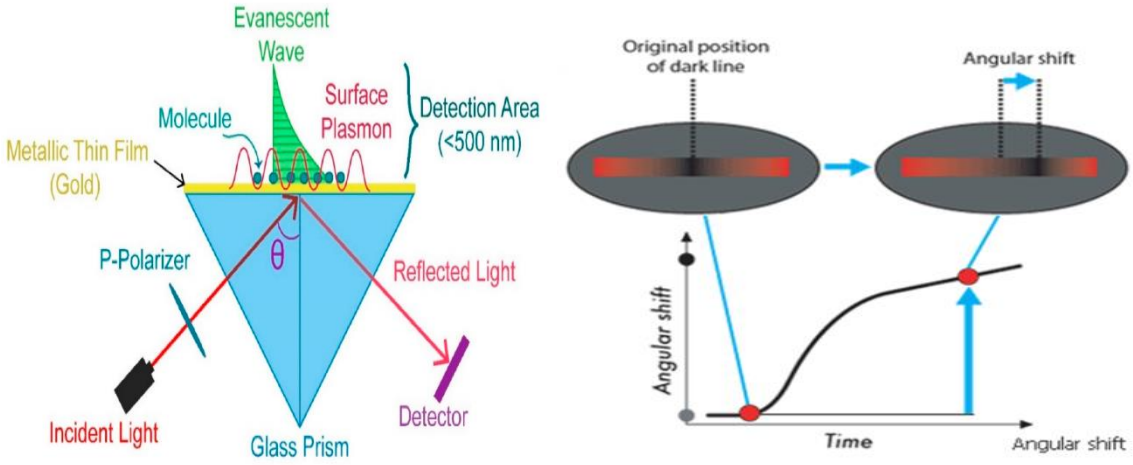


Fig 27: Basics of Surface Plasmon Resonance

- At a certain incident angle (or resonance angle), the plasmons are set to resonate with light, resulting in absorption of light at that angle. This creates a dark line in the reflected beam that contains a wealth of information.
- The resonance angle can be obtained by observing a dip in SPR reflection intensity.
- Comparing to the conventional diagnostic tools, SPR biosensors have multiple advantages such as easy preparation, no requirement of labeling, real-time detection capability, cost- effectiveness, and high specificity and sensitivity.
- However, for the label-free detection of low concentrations of analytes with small molecular weight its sensitivity is not enough.
- Therefore, considerable efforts have been invested to overcome these challenges and improve the sensitivity of the SPR biosensor such as ligands or functional nanomaterials with keeping all its advantages.
- The enhancement of the SPR biosensor needs modification of its surface with suitable ligands to capture the target compound (the analyte) and neglect other molecules available in the sample as shown in Figure 28.

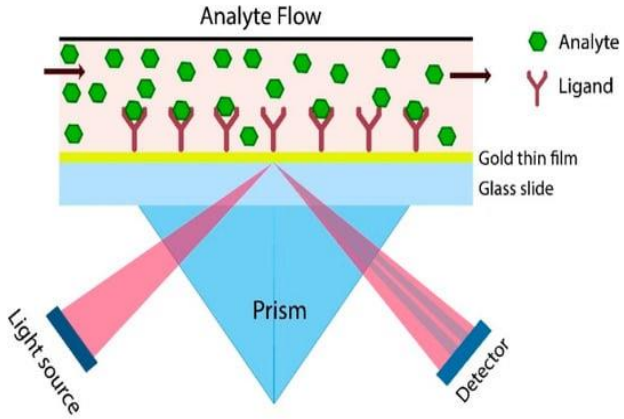


Figure 28. Direct label free detection SPR biosensor.

- These ligands can be permanently or temporarily immobilized on the sensor surface.
- The analyte accumulation results in a RI change in the evanescent field detected.
- When the ligand captures the analyte, the measurable signal rises and this is called direct label free detection.
- Following in time the resonance angle or wave length shift at which the dip is observed produces the sensogram (Figure 29), then the amount of adsorbed species after injection of the original baseline buffer can be determined, and a study of the kinetics of the biomolecular interaction can be done.

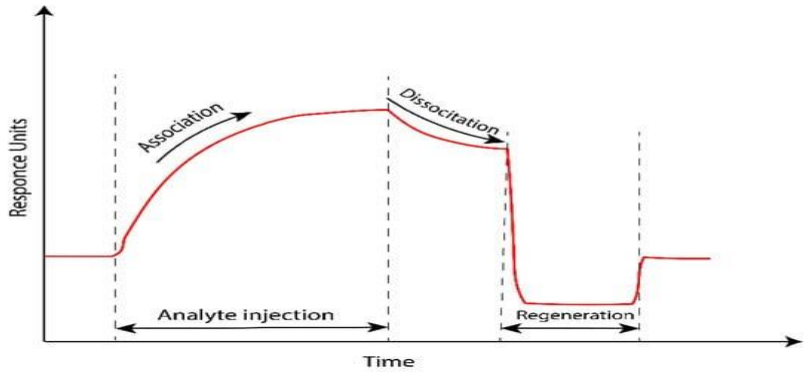


Figure 29. Surface plasmon resonance (SPR) sensogram.

2.3.5 Field-effect transistor (FET) biosensors

- Among various kinds of biosensors, field-effect transistor biosensors (Bio-FETs), an integrated between bio-receptors and ion-sensitive field-effect transistors (ISFET), emerged as the most developed candidates because of several advantages.
- In a typical FET system, the sensing elements are immobilized on the sensing channels (semiconductor path), which are connected to source (S) and drain (D) electrodes, to capture the targets (usually via high specificity and binding affinity).
- A bias potential is applied and modulated to a third electrode (gate).

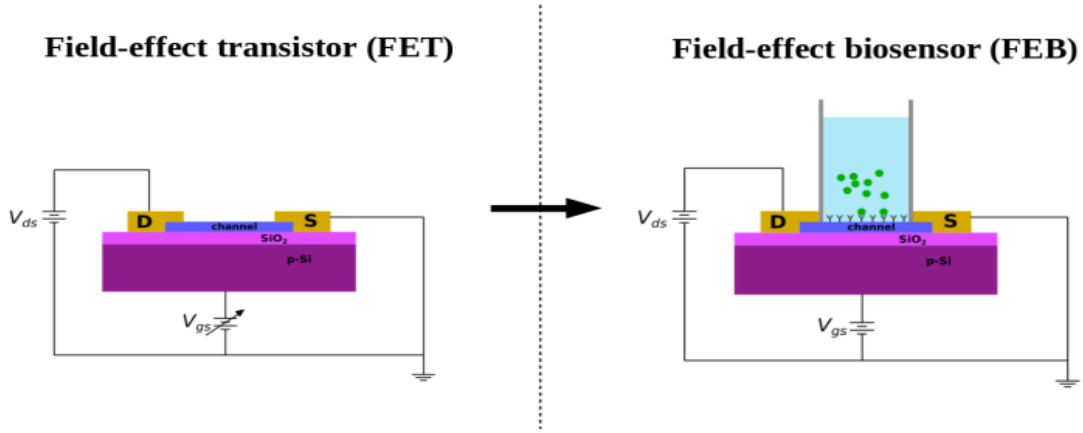


Figure 30. Field-effect transistor based biosensors.

- The channel conductance, which is varied by detection of the targets, is recorded and further processed by an electrical measurement system.
- There are two kinds of FETs: n-type with electrons as the main charge carriers and p-type with holes as the primary charge carriers.
- In an n-type FET system, if the probes detect positively charged molecules, the charge carriers (electrons) will accumulate on the sensing channels and increase the conductance.
- If negatively charged targets are recognized, the conductance will be decreased due to the depletion of the electrons.
- Conversely, for a p-type FET system, binding with positive charges results in conductance decline due to a reduction of the charge carriers (holes) and capturing negative charges raises the conductance because of hole accumulation.
- Outburst of nanotechnology triggers combination between biosensors and nanomaterials for sensing application with breakthrough designs in which biomolecules (antibodies, nucleotides and so on) as receptors are immobilized on the surface of nanotransducers (nanowires, nanotubes, nanoparticles, etc.).

2.3.6 Impedimetric biosensors

- Label-free electrochemical biosensors include impedimetric biosensors which measure the impedance, i.e., the opposition presented to a current in an alternating current (AC) circuit when a voltage is applied.
- The impedance is a complex quantity, and a common graphical representation is the Nyquist plot.
- This is a frequency response plot, where the values of the real part are plotted on the x-axis and those of the imaginary part on the y-axis.
- An ideal Nyquist plot shows a semicircle resulting from the dominating, kinetically limited charge transfer through the electric double layer at the electrode.
- Binding of analyte molecules to the electrode will influence the charge transfer and, hence, result in a shift of the Nyquist plot.
- As a consequence, the opportunity for charge transfer is an additional requirement for sensing layers of impedance biosensors.
- At low frequencies, Nyquist plots may show straight lines with a slope of 45°. This is characteristic for diffusion limited processes and described by the Warburg impedance.
- Electrodes of impedance biosensors are typically made of gold.
- A well-established procedure for the introduction of functional groups on this material is to use suitably substituted thiols forming self-assembled monolayers (SAMs).

- Thiols with aliphatic hydrocarbon spacers of sufficient chain length lead to well-defined and stable SAMs of high density.
- The brush-like structure of such layers makes it possible to effectively reduce nonspecific protein adsorption on the underlying gold surface.
- However, such SAMs may result in insulating layers, hindering the charge transfer required for the transduction principle of impedimetric biosensors.
- The use of aromatic hydrocarbons featuring delocalized π-electrons would be more beneficial for charge transfer processes, but nonspecific protein adsorption in the subsequent measurements may increase because of a reduced density of the layer.
- Conductive polymers would offer an alternative, but are often linked with coating procedures more complex than wet chemistry.
- Thiolated single-strand DNA (ssDNA) oligomers, on the other hand, can be packed densely on the gold surface by wet chemistry methods similar to those of thiolated hydrocarbons.
- Coimmobilization of thiolated ssDNA with thiolated hydrocarbons may be recommended to improve the integrity of the brush-like structure.
- The negatively charged backbone of the DNA oligomers—resulting from the composition of alternating sugar (deoxyribose) and phosphate groups—promises lower initial impedance values and, hence, the possibility of charge transfer events.
- With impedance sensors, immobilized ssDNA or immobilized oligonucleotides has been used directly as probe for DNA or protein detection, respectively.

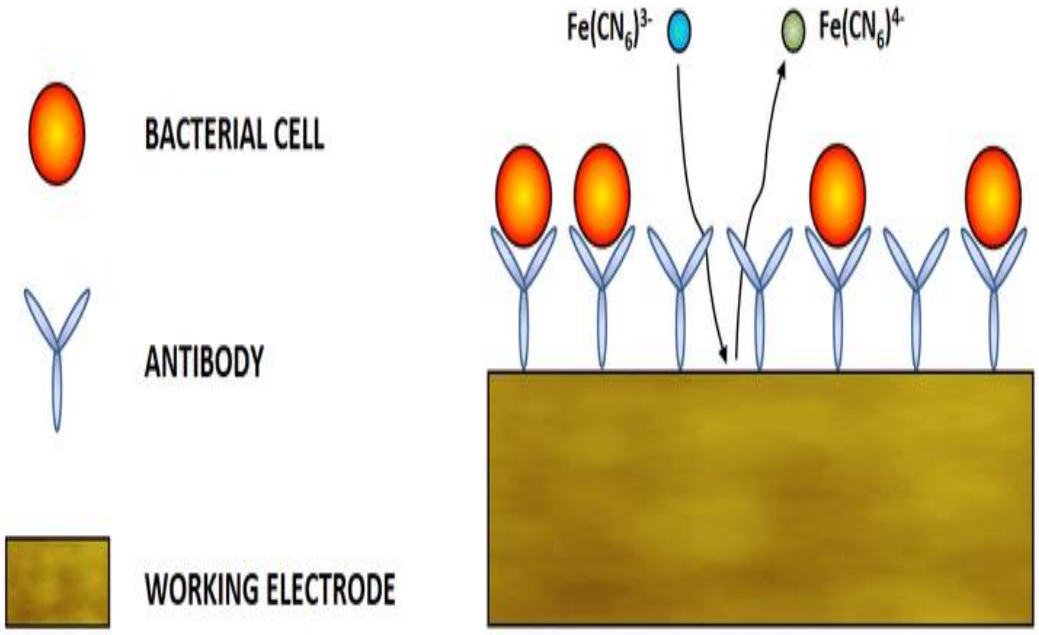


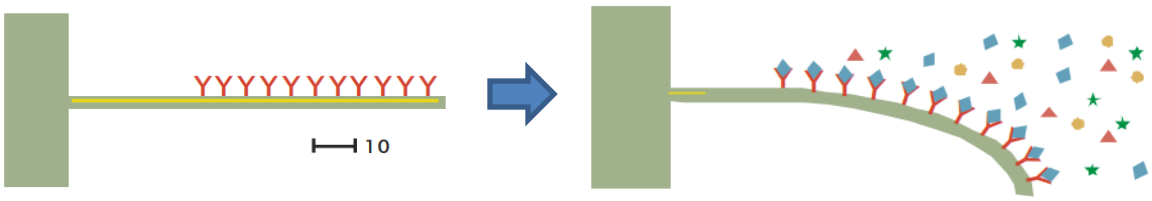
Fig 31 Impedimetric biosensors

2.3.7 Piezoelectric biosensor;

- Piezoelectric sensors utilize crystals which undergo an elastic deformation when an electrical potential is applied to them.
- The applied alternating potential produces a standing wave in the crystal at a characteristic frequency.
- This characteristic frequency is highly dependent on the elastic properties of the crystal.
- If a crystal is coated with a biological recognition element the binding of a (large) target analyte to a receptor will produce a change in the resonance frequency, giving a binding signal.
- In a mode that uses surface waves (SAW), the sensitivity is greatly increased.
- This is a special application of the Quartz crystal microbalance in biosensor.
- The piezoelectric generator is the method of transforming high frequency electric oscillations into mechanical oscillations.
- It is most frequently used at the present day for producing ultrasonic and it allows us to attain the highest frequencies now possible.
- Two classes of bio-recognition processes-bio-affinity recognition-and bio-metabolic recognition, offer different methods of detection.
- Both processes involve the binding of a chemical species with another, which has a complementary structure.
- This is referred to as shape-specific binding. In bio-affinity recognition, the binding is very strong, and the transducer detects the presence of the bound receptor-analyte pair.
- The most common types of processes are receptor-ligand and antibody-antigen binding.
- In bio-metabolic recognition, the analyte and other co-reactants are chemically altered to form the product molecules.
- The biomaterials that can be recognized by the bio-recognition elements are as varied as the different reactions that occur in biological systems.
- Almost all types of biological reactions, (chemical or affinity), can be exploited for biosensors. The concept of shape-specific recognition is commonly used to explain the high sensitivity and selectivity of biological molecules, especially antigen-antibody systems.
- The analyte molecule has a complementary structure to the antibody, and the bound pair is in a lower energy state than the two separate molecules. . The interaction of antibodies with their corresponding antigens is an attractive reason for attempting to develop antibody-based chemical biosensors, i.e. immunosensors

2.3.8 Cantilever Biosensor

- Cantilevers (springboard) are nanomechanical biosensors, microfabricated with the standard silicon technology.
- Due to their intrinsic flexibility, together with the availability of techniques designed to monitor bending, cantilevers have become versatile tools.
- This technology is a multifunctional and highly sensitive technique, and a real time method useful for a variety of applications, such as plastic explosive detection using gas biosensors, whole microorganism detection as part of liquid biosensors, or DNA and proteins studies.
- By incorporating a piezoresistor to each cantilever in a Wheatstone bridge type configuration, it is possible to read resistance changes as voltage changes.
- The Wheatstone bridge configuration uses a pair of cantilevers; one of them will be used as reference.
- The differential signal between both cantilevers will be the output of this configuration.
- The signal-noise relation is substantially improved with this configuration, and the noise originated by unspecific binding, thermal fluctuation, or vibrations is eliminated.
- Non-specific binding to the surface is a general problem that must be minimized in all analyses.
- Although the complete elimination of this parameter is not possible, its influence on detection could be controlled with the use of the reference cantilever.
- The immobilization of molecules on the cantilever surface is required for its use as a nanomechanical sensor (Figures 32).
- The immobilized molecules provide the cantilever with specificity for the analyte.
- The specific molecular interactions taking place at the flexible surface of cantilever increase surface tension, forcing the cantilever to bend.
- This type of surface tension induced by molecular interactions is not generally observed on the surface of common materials.
- The cantilever senses the tension and bends in response to the free energy changes taking place at its surface.



Figures 32 Nanomechanical cantilever sensor

Module 3

APPLICATIONS OF BIOSENSORS IN HEALTH AND ENVIRONMENT

3.1 Biosensors and diabetes management,

- Several factors have combined over the past few years to make glucose biosensor one of the most financially attractive areas in medical diagnosis.
- Generally, glucose measurements are based on interactions with one of three enzymes: hexokinase, glucose oxidase (GOx) or glucose-1-dehydrogenase (GDH).
- The hexokinase assay is the reference method for measuring glucose using spectrophotometry in many clinical laboratories.
- Successful glucose biosensor must also meet the diabetic's expectations.
- There are many theories of operations of biosensors such as Reflectance based method, Electrochemical methods, Enzymes and reagents, Hexokinase methods, Glucose oxidase based- Peroxidase optical method, Organic mediator optical methods, Perssian Blue method, Ferricyanide electrochemical method, Ferrocene Electrochemical method, reusable sensors etc.
- Electrochemical sensors may be subdivided into potentiometric, amperometric, or conductometric types.
- Enzymatic amperometric glucose biosensors are the most common devices commercially available, and have been widely studied over the last few decades.
- Amperometric sensors monitor currents generated when electrons are exchanged either directly or indirectly between a biological system and an electrode

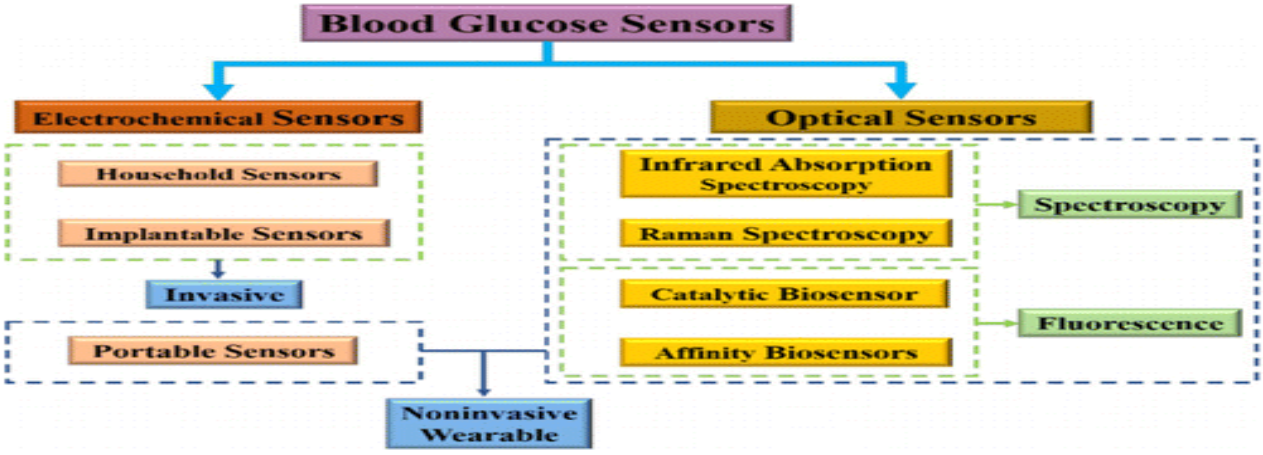
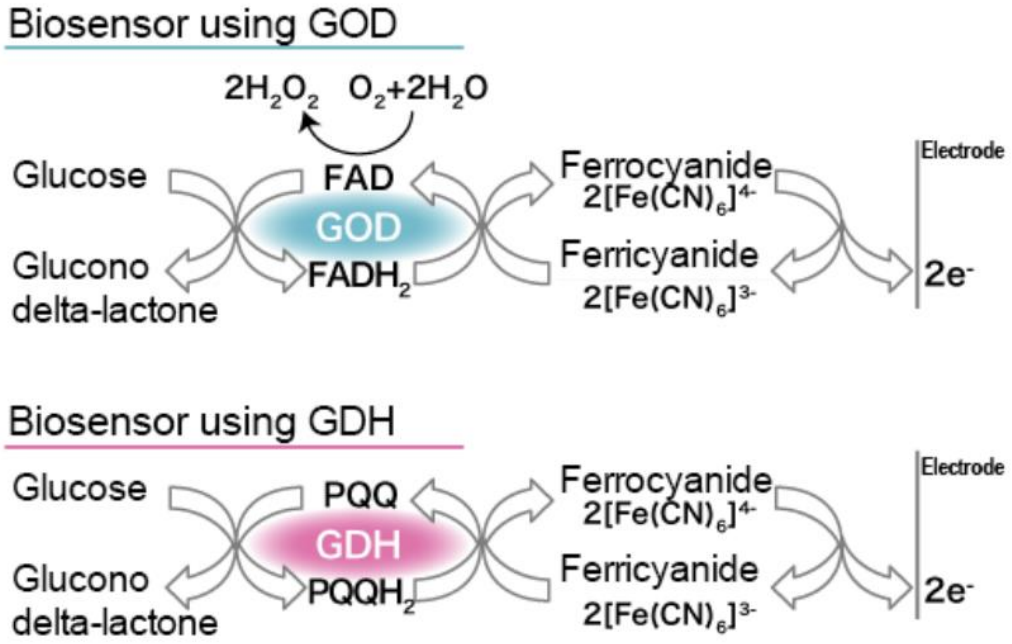


Fig. 33. Classification of blood glucose sensors in this review.

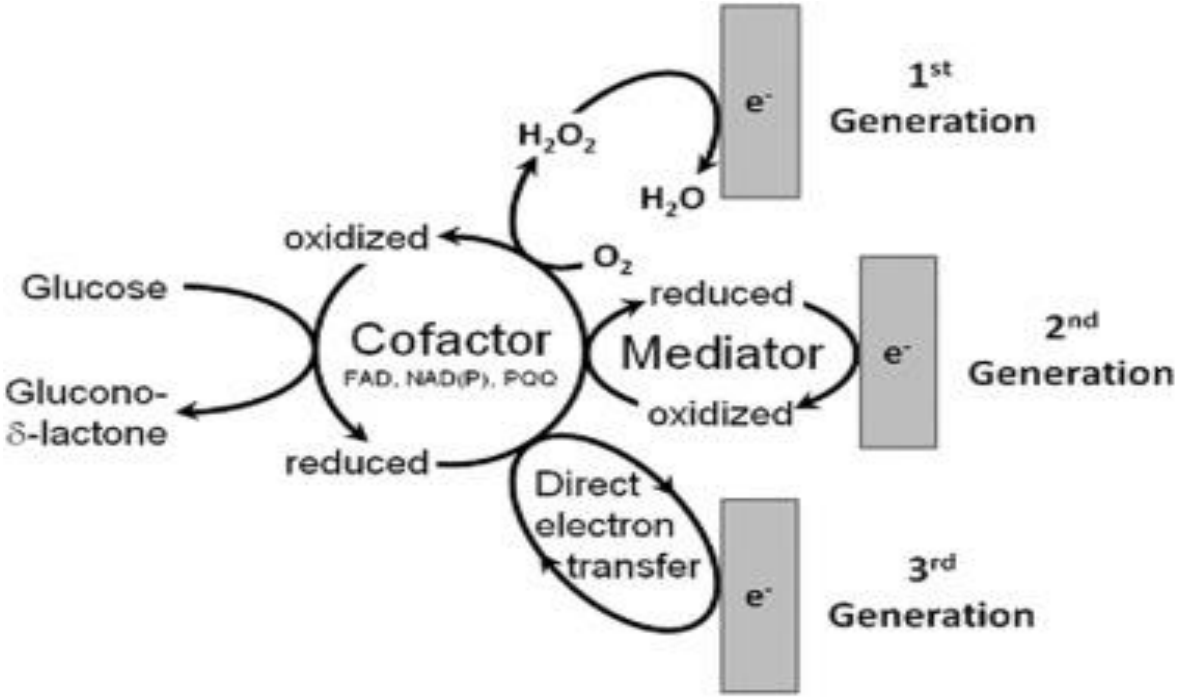
- Electrochemical methods are popular, and over the years, enzymatic amperometric glucose sensors were the first and widespread glucose sensors available
- Electrochemical glucose sensors can be divided into potentiometric (employed to detect variations of surface charge onto a counter electrode), amperometric (charge flow between the counter electrode and the bio-system), or conductometric (variations in ionic conductance between electrodes.
- They are generally fabricated by using two families of enzymes, the glucose oxidase and the glucose dehydrogenase (GDH). The reaction, catalyzed is as follows:



Figures 32 Glucose sensor reaction scheme

- GOx is the standard enzyme for biosensors; it has a relatively higher selectivity for glucose. GOx is easy to obtain, cheap, and can withstand greater extremes of pH, ionic strength, and temperature than many other enzymes, thus allowing less stringent conditions during the manufacturing process and relatively relaxed storage norms for use by lay biosensor users
- The concept behind a glucose biosensor is based on the fact that the immobilized GOx catalyzes the oxidation of D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide.
- To function as a catalyst, GOx requires a flavin adenine dinucleotide (FAD) redox cofactor. FAD functions as an initial electron acceptor and is reduced to FADH2.
- This enzymatic electrode evaluates the glucose level by the amperometric tracking of the released hydrogen peroxide.

- Glucose dehydrogenases are instead defined as oxidoreductases which are unable to use oxygen as an electron acceptor and therefore transfer electrons to other natural and artificial acceptors.
- GDHs also need a cofactor. These are mainly nicotine adenine dinucleotide (NAD+ or NADH depending on the oxidation state) or pyrroloquinoline quinone (PQQ).
- FAD, NAD+ and PQQ remove hydrogen, H+ and e-, from glucose.
- GDH-PQQ is a particularly efficient enzyme system, with a fast electron transfer rate, but it is relatively expensive.
- GDH with NAD+ as a cofactor produces NADH rather than H_2O_2.
- Nicotine adenine dinucleotide is an important electron acceptor in glucose oxidation, during which NAD's nicotinamide ring accepts one hydrogen ion and two electrons, equivalent to one hydride ion.
- In this reaction, the generated reduced form of this cofactor is NADH, which can be electrochemically oxidized.



Figures 33 Glucose sensor designs

- 1st Generation: Based on the sensor designed by Clark and Lyons
  Formation of hydrogen peroxide
  Oxygen as an electron acceptor
  Errors due to interference from other electroactive species
- 2nd Generation Replacement of oxygen as an electron acceptor
  Introduction of the non-physiological mediator

	Limitations in the transfer from the enzymatic active site to the electrode
3rd Generation	Absence of mediator
	Direct transfer between enzyme and electrode
	Low operating potential, higher selectivity, less interference

- An electrochemical biosensor is composed by working electrodes (on which the reaction of interest, responsible for the measurement, takes place), reference electrodes and auxiliary electrodes (to ensure that the current does not circulate through the electrode).
- Glucose concentration is mostly evaluated using the amperometric method that monitors the current flowing between the working electrode and the reference electrode.
- The basic concept of the glucose biosensor is based on the fact that the immobilized GOx catalyzes the oxidation of β-D-glucose by molecular oxygen producing gluconic acid and hydrogen peroxide. In order to work as a catalyst, GOx requires a redox cofactor—flavin adenine dinucleotide (FAD). FAD works as the initial electron acceptor and is reduced to FADH2.

$$\text{Glucose} + \text{GOx} - \text{FAD}^{+} \rightarrow \text{Glucolactone} + \text{GOx} - \text{FADH}_2$$

- The cofactor is regenerated by reacting with oxygen, leading to the formation of hydrogen peroxides.

$$\text{GOx} - \text{FADH}_2 + \mathbf{O}_2 \rightarrow \text{GOx} - \text{FAD} + \mathbf{H}_2\,\mathbf{O}_2$$

- Hydrogen peroxide is oxidized at a catalytic, classically platinum (Pt) anode. The electrode easily recognizes the number of electron transfers, and this electron flow is proportional to the number of glucose molecules present in blood [36].

$$H_2O_2 \rightarrow 2H^{+} + O_2 + 2e$$

- Three general strategies are used for the electrochemical sensing of glucose; by measuring oxygen consumption, by measuring the amount of hydrogen peroxide produced by the enzyme reaction or by using a diffusible or immobilized mediator to transfer the electrons from the GOx to the electrode.
- The number and types of GDH-based amperometric biosensors have been increasing recently. The GDH family includes GDH-pyrroquinolinequinone (PQQ) and GDH-nicotinamide-adenine dinucleotide (NAD). The enzymatic reaction of GDH is independent of the dissolved oxygen. The quinoprotein GDH recognition element uses PQQ as a cofactor.

$$\text{Glucose} + \text{PQQ(ox)} \rightarrow \text{gluconolactone} + \text{PQQ(red)}$$

- This mechanism requires neither oxygen nor NAD^{+}. GDH-PQQ is a particularly efficient enzyme system, with a rapid electron transfer rate, but it is relatively expensive [17].

- GDH with NAD as a cofactor produces NADH rather than H_2O_2. NAD is a major electron acceptor in the oxidation of glucose, during which the nicotinamide ring of NAD^+ accepts a hydrogen ion and two electrons, equivalent to a hydride ion. The reduced form of this carrier generated in this reaction is called NADH, which can be electrochemically oxidized.

$$\text{Glucose+NAD+}\rightarrow\text{gluconolactone+NADHNADH}\rightarrow\text{NAD++H++2e}$$

3.2 Microfabricated biosensors and point-of-care diagnostics systems

- Even with tremendous growth in hospital sector, providing centralized clinical testing facility is inadequate.
- Patient admitted to the hospital with number of possible life-threatening causes for symptoms during initial assessment may get unattended in initial and comprehensive qualitative and quantitative assessment to get information that is crucial in managing the disease by any doctor.
- Without clinical chemistry or microbiological parameters physician is often unable to make any reliable diagnosis.
- To do this clinical samples need to be draw from the patient, labelled, transported to laboratory, segregated, tested, analysed, certified and reported back that often located at central place that takes several hours due to remote locations of the laboratory and its expensive equipment.
- So, such delay is major concern as physician is unable to initiate timely effective treatment.
- Hence, approach to solve such issues can only be solved by addressing laboratory-based steps, and not through transportation logistic problem.
- However, approached in delaying the testing time by automation of the analysis lead to increase in the coast of testing due to expensive instruments.
- Hence, "Point-of-Care" clinical sample analysis system incorporates simple, safe, maintenance-free home-use monitoring system works with whole clinical samples rater than clinical samples that requires preclinical treatments, so precludes all the laboratory instruments such as centrifuges, filters etc. that requires money and time.
- Such "Point-of-Care" blood analysis system should requires design that (a) replace in-line calibration with factory calibration, (b) replace wash step with single-use analytical component, (c) eliminate need for metered samples, (d) simple reusable reading device to display results.
- "Point-of-Care system" that can be utilised along the patient's "bed-side" rather than the laboratory's "table-top" with the same analytical quality, along emergency room, intensive care unit, critical care unit etc.

- "Point-of-Care system" is crucial for estimating clinical parameters such as blood gases, electrolytes, metabolites that covers many conditions such as arrhythmia, dehydration, anemoia, respiratory problems, and diabetes.
- A successful hospital "Point-of-Care system" would, at minimum, need to perform all of these.
- The microfabrication technology focuses on the miniaturization of engineering systems and has evolved from the mature process technology in semiconductor device fabrication.
- The main techniques of microfabrication are thin films deposition, layers doping, patterning via photolithography, etching to obtain the required design, polishing and bonding.
- Microfabrication technology has been widely used for the development of complex electronic components, integrated micro-electromechanical systems (MEMS), and different types of sensors for a wide range of applications.
- The benefits of developing sensors with such technological processes include low costs, large scale fabrication of nominally identical structures, the possibility of integration with other devices and the compatibility with a numerous of technologies, such as complimentary metal-oxide semiconductor technology (CMOS) for the manufacturing of integrated circuits or silicon on insulator (SOI) technology for semiconductor engineering.
- The most common assembly of electrodes for electrochemical applications is the three-electrode system.
- A three-electrode structure consists in a working electrode (WE), a reference electrode (RE) and a counter electrode (CE).
- The working electrode serves as the transduction element in the (bio)chemical reaction, while the counter electrode establishes a connection to the electrolyte solution so that a current can be applied to the working electrode.
- This system is also beneficial because it averts the RE from pushing the current which could modify its potential.
- The potential is applied between the WE and the RE and the CE provides the mandatory current to sustain electrolysis at the WE.

Fundamental of POC biosensors

- Biosensors are analytical devices used for the detection of a biological substance. In general, biosensors consist of three components: a receptor (specific for a disease) that recognizes the analyte, a transducer that converts the bio-recognition event into a measurable signal, and a reader.
- This technology allows precise control and manipulation of fluids, which typically requires much less sample volume than that of conventional assays.

- The efficient liquid mixing in biosensors also enhances the interaction between assay reagents and target biomarkers, which shortens the assay duration and provides fast readout.
- Moreover, the portability of biosensors makes them ideal candidates for POC field settings. To date, researchers around the globe have developed various types of biosensors for wide applications in POC settings such as the diagnosis of infectious diseases, food safety analysis, and environmental monitoring.
- These biosensors include chip-based, paper-based, and other biosensors (textile-based or nanomaterial-based biosensors), which will be briefly discussed in the following sections.
- For POC testing of chronic and infectious diseases, there is always an increasing demand for low-cost, portable, and integrated biosensors, which can provide rapid results with low sample consumption.
- Chip-based biosensors are one of the POC biosensors used for POC diagnosis of many infectious diseases.
- They are prepared either by miniaturizing conventional biochemical assays on a microchip scale or by integrating novel detection principles with microfluidic chips, reffered as " "Lab-on-chip".
- These biosensors are mainly made of polymethyl methacrylate (PMMA), polytetrafluoroethylene (PTEE), or polydimethylsiloxane (PDMS).
- PDMS biosensors are commonly used due to their cost-effectiveness, high specificity, and minimal reagent consumption.
- Specifically, they consist of multiple channels which enable nucleic acid testing steps, including nucleic acid extraction, amplification, and amplicon detection, to be performed in an automated manner.
- In recent years, smartphone has also been integrated into chip-based biosensors for imaging and signal analysis.
- For instance, chip-based biosensors coupled with a smartphone have been used to rapidly detect amplicon signals within an hour for the diagnosis of H1N1 and Zika virus infections.
- Paper-based biosensors have been broadly used for rapid testing of infectious diseases, which show potential to substitute the conventional laboratory tests and chip-based biosensors.
- Paper is inexpensive, readily available, and biodegradable, showing a promising tool for onsite rapid diagnosis.

- It allows the diffusion of a biological sample through a capillary effect, eliminating the need for external power sources.
- Earlier studies have introduced lateral flow test strips and microfluidic paper-based analytical devices (μPAD) for POC testing.
- The assay usually involves hybridization of single-stranded DNA or RNA with a complementary probe to produce double-stranded nucleic acids or interaction between antigen and antibody to produce an Ag-Ab complex, generating signals such as colorimetric, fluorescence, or chemiluminescence signals.
- Their special characteristics such as simple, affordable, and ease of fabrication, modification, and functionalization have made them possible to achieve rapid, onsite POC testing.
- Other biosensors like film-based, textile-based, and nanomaterial-based biosensors have also been used for the diagnosis of infectious diseases.
- For instance, film-based biosensors which are made of transparent polyester substrate film have been used for the detection of pathogens.
- This material has the ability to withstand thermal cycling and amplification process.
- Textile-based biosensors are biosensors which are typically made of thread, fabric, or clothes which are inexpensive and readily available with low sample consumption. For example, textile-based biosensors are cheaper and require a smaller sample volume (optical
- Emerging point-of-care biosensors for rapid diagnosis of COVID-19 have exploited these biosensors for POC diagnoses, especially by integrating with mobile phones.
- The device packaged in a compact portable assembly shows a bright potential for performing fast and accurate cytokine assays for COVID-19 in clinics and POC settings for responsive disease management

3.4 Noninvasive biosensors in clinical analysis;

3.5 Surface plasmon resonance and evanescent wave biosensors,

3.5 Biosensor in cancer and HIV early diagnosis.