Module No. 61. Light Sources for optical sensor

Introduction

1. Optical sensors used light for sensing techniques.
2. The light source are extremely important for the performance of optical sensors.
3. A focused light source is required with well known properties.
4. There is a set of properties for the light sources, that are used in optical sensors.

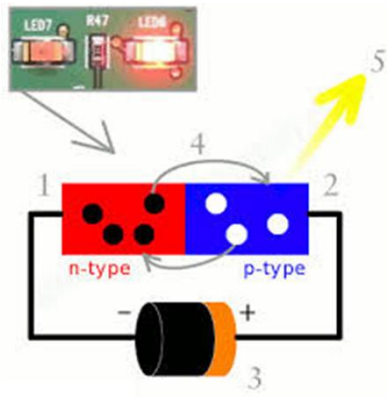
Properties and Types

In sensor Monochromatic light source is required. Monochromatic light consists of extremely narrow wave length. The two most commonly used optical sources are

Light-Emitting Diodes (LED) Example Traffic lights

Light Emitting Diode

LED consume much less power as compared to other and can produce single colour light. Light Emitting Diodes (Transistors). Electrons (n-type) in the semiconductor recombine with electron holes (p-type), releasing energy in the form of photons.Color of the emitted light is dependent on the material used for doping.



Types of Light Emitting Diodes

Diodes can emit different types of colours and they are classified on the basis pf production of colors.

1. Gallium Arsenide infra-red
2. Aluminium Gallium Phosphide – green
3. Gallium Indium Nitride (GaInN) – near ultraviolet, bluish-green and blue
4. Aluminium Gallium Nitride (AlGaN) – ultraviolet

To white LED light blue LED is quoted with phosphorous.In LEDs current can only flow in one direction. LEDs never burn out unless their current limit is passed.There is another type of LED that generates white light mimicking the natural sunlight referred as white LED.

LASER diodes that is light Amplification by Stimulated Emission of Radiation. Example LASER pointer.

A laser diode is a special type of LED that generate laser which is a coherent beam of light that is extremely mono chromatic. Laser diode are used in CD, DVD, laser printer and barcode scanner. They are also become very important in biosensor application specially

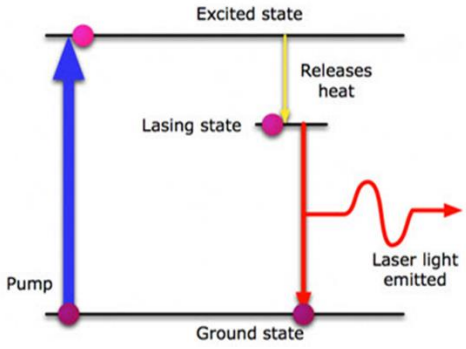
when light needs to be irradiated to the small area of interest. Such as micro capillary channels.

LASER gain Medium is a source from which LASER light is produced. There are different types of medium that can be used for LASER production.

1. Atom such as He-Ne , He-Cd.
2. Molecule such as carbon dioxide, Nitrogen Gas.
3. Liquids involving organic dyes dissolved in various soln.
4. Dielecric solids involving Neodymium.
5. Semiconductor materials such as Gallium crystals and ruby crystals.

Creation of a LASER Beam

1. n 1917, Einstein laid the foundation for the laser when he introduced the concept of stimulated emission; where a photon interacts with an excited molecule or atom and causes the emission of a second photon having the same frequency, phase, polarization and direction. The acronym LASER stands for "Light Amplification by Stimulated Emission of Radiation".
2. The First Laser A LASER is created, when the electrons in the atoms of LASER gain medium, become excited by absorbing energy and move to higher energy state.



3. Before returning to ground state, heat is released and electron reached at Lasing state.
4. Between the Lasing and ground state Laser light is emitted.

Theodore Maiman developed the first working laser at Hughes Research Lab in 1960, and his paper describing the operation of the first laser was published in Nature three months later. Since then, more than 55,000 patents involving the laser have been granted in the United States. Today's laser and all of its applications are the result of not one individual's efforts,

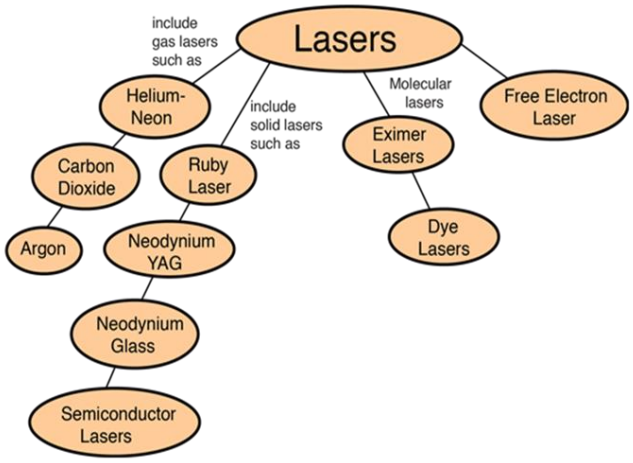
but the work of a number of prestigious scientists and engineers who were leaders in optics and photonics over the course of history.

Types of LASER

LASER are commonly typed by the LASING material.

1. Gas LASER
2. Solid LASER
3. Molecular LASER
4. Free Electron LASER

There are many other types of LASER classification based on their medical, industrial and commercial applications, wavelength and safety.



Types of LASER based on the name of lasing material. Some of them are discussed here.

Ruby LASER

A ruby laser is a solid-state laser that uses the synthetic ruby crystal as its laser medium. Ruby laser is the first successful laser developed by Maiman in 1960. Ruby laser is one of the few solid-state lasers that produce visible light. The ruby laser uses a solid medium of a crystal aluminum oxide (i.e., sapphire) containing chromium ions. It emits visible red radiation at a wavelength of deep red light of wavelength 694.3 nm.

Nd:YAG laser definition.

Neodymium-doped Yttrium Aluminum Garnet (Nd: YAG) laser is a solid state laser in which Nd: YAG is used as a laser medium. ... These lasers operate in both pulsed and continuous mode. Nd: YAG laser generates laser light commonly in the near-infrared region of the spectrum at 1064 nanometers (nm).

Eximer LASER

An excimer laser typically uses a combination of a noble gas (argon, krypton, or xenon) and a reactive gas (fluorine or chlorine). Under the appropriate conditions of electrical stimulation and high pressure, a pseudo-molecule called an excimer (or in the case of noble gas halides, exciplex) is created, which can only exist in an energized state and can give rise to laser light in the ultraviolet range. It is a form of ultraviolet laser which is commonly used in the production of microelectronic devices, semiconductor based integrated circuits or "chips", eye surgery, and micromachining.

Dye LASER

A dye laser is a laser that uses an organic dye as the lasing medium, usually as a liquid solution. Compared to gases and most solid state lasing media, a dye can usually be used for a much wider range of wavelengths, often spanning 50 to 100 nanometers or more.The dye rhodamine 6G, for example, can be tuned from 635 nm (orangish-red) to 560 nm (greenish-yellow), and produce pulses as short as 16 femtoseconds.

Main points.

There are two types of artificial light sources for optical sensor with unique properties LED and LASER diodes
LED are based on transistors and the color of light depends upon the dopants.
LASER waves are produced after exciting the LASING medium. Beam color is dependent of gain medium used.

Module No. 62. Types of optical Sensors
Photoconductive device output is resistance.Solar cell output is voltage

1. Photodiode output is current. Heat sensitive missiles.

Power generation by solar cells Optical sensors are extremely useful and have a number of applications.

Classification
Optical sensors could be classified in different ways. At least three different classes of Optical sensors are accepted.

1. Classification on the basis of location (extrinsic intrinsic)
2. Classification on the basis of output (resistance Current, Voltage)
3. Classification on the basis of applications

Types

Photovoltaic cell (solar cell) converts an amount of incident light into an output voltage.

Photodiodes convert an amount of incident light into an output current.

Photoconductive devices measure the resistance by converting a change of incident light into a change of resistance.

<u>Photo-voltic cell</u>

A photovoltaic cell (PV cell) is a specialized semiconductor diode that converts visible light into direct current (DC). Some PV cells can also convert infrared (IR) or ultraviolet (UV) radiation into DC electricity.A photovoltic cell is composed of positive and negative junctions of Boron doping (p-type) and phosphorus doping (n-type). Simply put, a solar panel works by allowing photons, or particles of light, to knock electrons free from atoms, generating a flow of electricity. Solar panels actually comprise many, smaller units called photovoltaic cells. (Photovoltaic simply means they convert sunlight into electricity.)

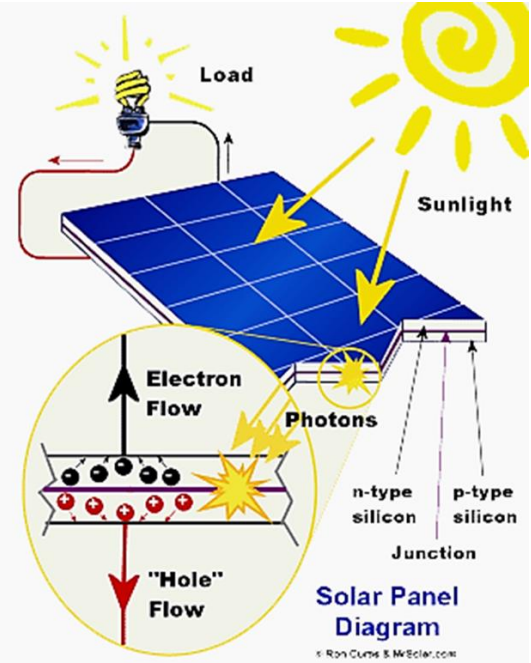


Fig shows a solar cell.

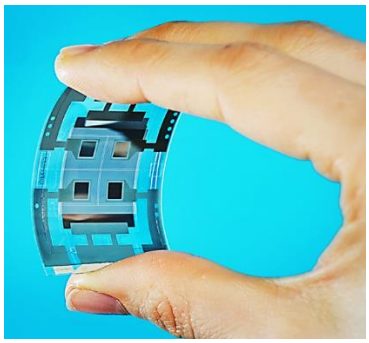
The common single junction silicon solar cell can produce a maximum open-circuit voltage of approximately 0.5 to 0.6 volts. To work, photovoltaic cells need to establish an electric field. Much like a magnetic field, which occurs due to opposite poles, an electric field occurs when opposite charges are separated. To get this field, manufacturers "dope" silicon with other materials, giving each slice of the sandwich a positive or negative electrical charge.

Specifically, they seed phosphorous into the top layer of silicon, which adds extra electrons, with a negative charge, to that layer. Meanwhile, the bottom layer gets a dose of boron, which results in fewer electrons, or a positive charge. This all adds up to an electric field at the junction between the silicon layers. Then, when a photon of sunlight knocks an electron free, the electric field will push that electron out of the silicon junction. Metal conductive plates on the sides of the cell collect the electrons and transfer them to wires. At that point, the electrons can flow like any other source of electricity.

Photodiodes Sensors

It is a form of light-weight sensor that converts light energy into electrical voltage or current. Photodiode is a type of semi conducting device with PN junction. ... The photo diode accepts light energy as input to generate electric current. It is also called as Photodetector, photo sensor or light detectorA photodiode is a semiconductor device that converts light into an electrical current. The current is generated when photons are absorbed in the photodiode. Photodiodes may contain optical filters, built-in lenses, and may have large or small surface areas.

A photodiode is designed to operate in reverse bias.t is a Photodiodes may contain optical filters, built-in lenses, Material used in the synthesis includes Silicon, Germanium, Indium Gallium, Mercury, etc. Silicon based photodiodes are very popular due to low noise.

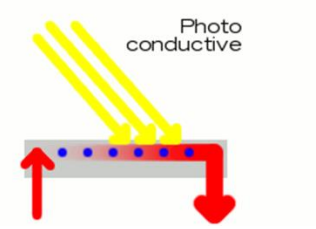


Organic photodiodes are extremely lightweight and also inexpensive to produce

A photodiode is a type of light detector that converts light into voltage or current, based on the mode of operation of the device.

Photoconductive Sensors

Photoconductivity is an optical and electrical phenomenon.

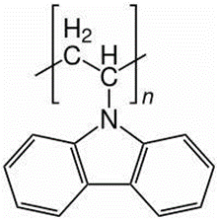


Applications for Photoconductive cells are in Light meter, in camera Daylight sensor, Elevator, safety stop ,Garage door.

The material becomes more electrically conductive due to the absorption of electromagnetic radiation. Visible light, UV, IR,, or gamma radiation. Photoconductive materials increases the flow of electrons and reduces the resistance.

Materials for Photo-conductives Sensors

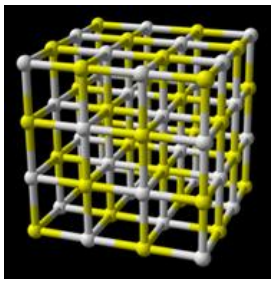
Polyvinylcarbazole is a temperature-resistant thermoplastic conductive polymer used in in copiers and laser printers.



Polyvinylcarbazole (PVK) is a temperature-resistant thermoplastic polymer produced by radical polymerization from the monomer N-vinylcarbazole. It is photoconductive and thus the basis for photorefractive polymers and organic light-emitting diodes.

Lead sulfide, Lead Sulfide is a crystalline solid used as a semiconductor and in photo optic applications, used in infrared detection applications, such as heat-seeking missiles. Lead sulfide finds applications in the following: Infrared detectors. ad sulfide is one of main materials used as detectors of infrared radiation in semiconductor optoelectronics. The infrared sensors and

transducers used in night vision instruments and thermal systems for missile targeting are created on its basis. For developing technologies for creating lead sulfide films with specific optical and photoelectric properties and crystal structures is of great interest. The pyrolysis of aerosols of thiourea coordination compounds (TCC) is one of the simplest and cheapest ways of accomplishing this, and it allows us to obtain high quality samples with specific propertie



Lead sulfide based heat sensitive messiles. Structure of Lead sulfide is shown Sulfur is shown in yellow dots while lead is shown in gray.

Photo optic applications. Slip property modifier used in friction industry for enhancing heat conduction and regulating friction coefficient

Selenium, employed in early television and xerography. Lead sulfide refers to two compounds containing lead and sulfur. Lead(II) sulfide, PbS, containing lead in the +2 oxidation state, naturally occurring as the mineral galena. Lead(IV) sulfide, PbS2, containing lead in the +4 oxidation state

Organic material based are also in market.

Module No. 63. Applications of Optical Sensors

Introduction

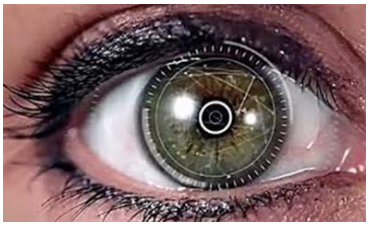
Optical sensors have many applications including health, diagnostics, defense, research, surveillance, environment and process control. Optical sensors address approximately all aspects of detection technology. The optical sensors ranges from micro-probes to large devices.

Biometric Verification

Biometric verification is any means by which a person can be uniquely identified by evaluating one or more distinguishing biological traits.

Retinal Scan

A biometric identifier known as a retinal scan is used to map the unique patterns of a person's retina. This beam traces a path on the retina.

A retinal scan is performed by casting an unperceived beam of low-energy infrared light into a person's eye as they look through the scanner's eyepiece. Because retinal blood vessels absorb light more readily than the surrounding tissue, the amount of reflection varies during the scan. Retinal imaging allows eye doctors to see signs of eye diseases.

Ambient Light Sensor

Ambient light is that light which already present in a scene, lights. Mostly we have seen this sensor on our mobile handsets.

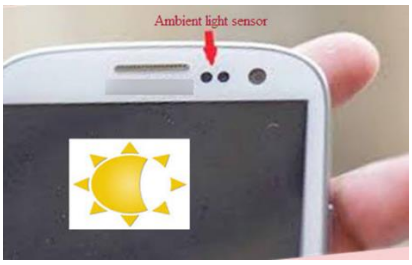
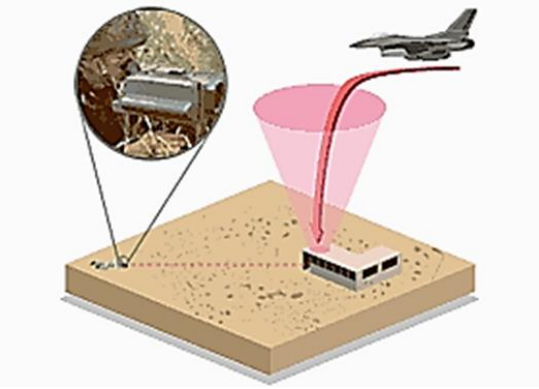


Fig shows an ambient light sensor in a smart phone.

There are three common types of ambient light sensor: phototransistors, photodiodes, and photonic integrated circuits, which integrate a photodetector and an amplifier in one device. It is a photodetector that is used to sense the amount of ambient light present. It appropriately dim the device's screen to match it. The standard international unit for the illuminance of ambient light is the lux. The typical performance of an ambient light sensor is from less than 50 lux in dim light to over 10,000 lux at noon.

LASER Guidence

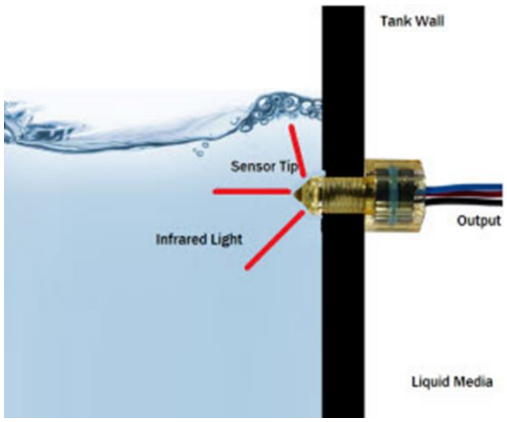
Laser guidance directs a robotics system to a target position by means of a laser be Laser guided technology is used by military LASER guided precision targeting. The key idea is to show goal positions to the robot by laser light projection instead of communicating them numerically.



Laser-guided bombs such as the Paveway II and III can home in on a coded pulse laser light which is indicated onto a target. Laser guided electronics are available in market including , video games, toys, communication and smart home technologies. The laser guidance of a robot is accomplished by projecting a laser light, image processing and communication to improve the accuracy of guidance.

Optical Sensor Based Liquid Level Indicator

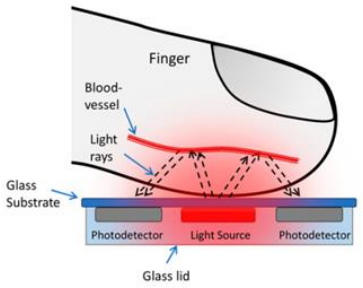
Optical Sensor Based Liquid Level Indicator consist of three components.(1). infrared Light Emitting Diode coupled with (2). a Light transistor, and a (3). Transparent prism tip.



1. Sensor is dipped in liquid
2. Light disperses throughout
3. Less is returned to the transistor, depending on the depth/level of liquid.

Pulse Oximeter

Pulse oximetry is a noninvasive method for monitoring a person's oxygen saturation. Though its reading of peripheral oxygen saturation (SpO2) is not always identical to the more desirable reading of arterial oxygen saturation (SaO2) from arterial blood gas analysis, the two are correlated well enough that the safe, convenient, noninvasive, inexpensive pulse oximetry method is valuable for measuring oxygen saturation in clinical use.



The oxygenated hemoglobin and deoxy hemoglobin have different rates of absorption of red and infrared light. Oxy-hemoglobin absorbs more infrared light (990 nm) and deoxy-hemoglobin absorbs more red light (660 nm).
Difference is detected and oxygen saturation

64.Temperature Sensors working and Applications

Temperature sensor or Probe. Temperature probes are another term for thermocouples, which are temperature measuring and monitoring devices that sense the electrical conductivity produced by heat in gas or liquids to maintain an appropriate temperature in a wide variety of different systems that use heat energy.

Temperature Measurements

Common scales of temperature measured in degrees:

1. Celsius (°C)
2. Kelvin (K)
3. Fahrenheit (°F)
4. Rankine (°R or °Ra),

which uses the Fahrenheit scale, adjusted so that 0 degrees Rankine is equal to absolute zero.

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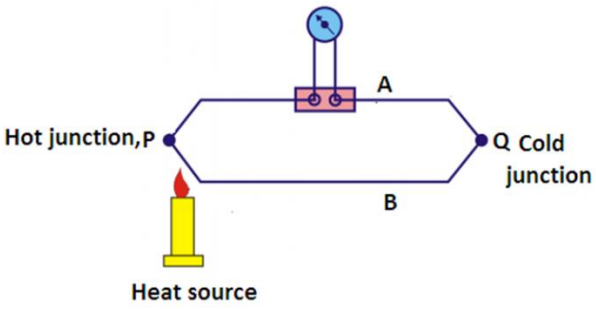
Working.

Classification

(i) Contact Temperature Sensor. They required to be in physical contact with the object being sensed. They use conduction to monitor changes in temperature.

(ii) Non-contact Temperature Sensor. They use convection and radiation to monitor changes in temperature. They can be used to detect liquids and gases that emit radiant energy

A temperature sensor basically senses temperature, but it does it in a number of ways. A contact temperature sensor will read the temperature of an object that it is attached to physically. Contact sensors work in many ways.



Thermocouples work using the Seebeck effect, which has to do with the temperature change in electrical circuits. Thermocouple temperature probes are devices that generate a temperature-

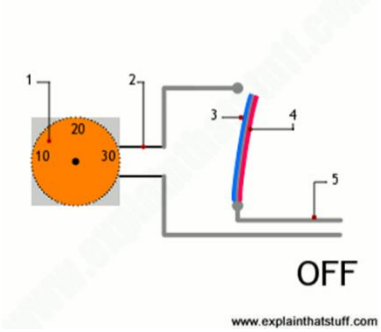
dependent voltage. The wires are joined at one end to form a measuring junction within a casing. To measure temperature, the probe is inserted into the fluid or medium and a voltage is generated.

Thermostat

It is a contact type electro-mechanical temperature sensor or switch,

It consists of two different metals such as nickel, copper, etc, that are bonded together to form a Bi-metallic strip.

The different linear expansion rates of the two dissimilar metals produces a mechanical bending movement when the strip is subjected to heat.



Temperature Sensor Types, An infrared thermometer is a sensor that consists of a lens to focus the infrared (IR) energy on to a detector, which converts the energy to an electrical signal that can be displayed in units of temperature after being compensated for ambient temperature variation.

Liquid crystal Infra red thermometer

Liquid crystals are chemical compounds and mixtures change color with changes in temperature.They are known as thermochromic liquid crystals (TLCs). Scientifically, they are identified as Cholesteric Liquid Crystals

Other types

Thermistors, detect thetemperature variability according to the changes of the electrical resistance.

Resistance temperature detectors that indicate the values according to the changes of the electrical resistance of metals.

Silicone-based sensors, show the spreading resistance analysis of semiconductors

Applications in Biosensors

There are Temperature Sensor applications

1. Motors
2. Home appliance
3. Computers
4. Industrial equipment
5. Exhaust
6. Food Production;
7. 3D printed
8. chocolates –

Summary

A temperature sensor is a device, typically, a thermocouple.

that provides for temperature measurement through an electrical signal.

A thermocouple (T/C) is made from two dissimilar metals that generate electrical voltage in direct proportion to changes in temperature.

Pressure Senor

A pressure sensor is a device for pressure measurement of gases or liquids. Pressure is an expression of the force required to stop a fluid from expanding, and is usually stated in terms of force per unit area. A pressure sensor usually acts as a transducer; it generates a signal as a function of the pressure imposed. For the purposes of this article, such a signal is electrical.

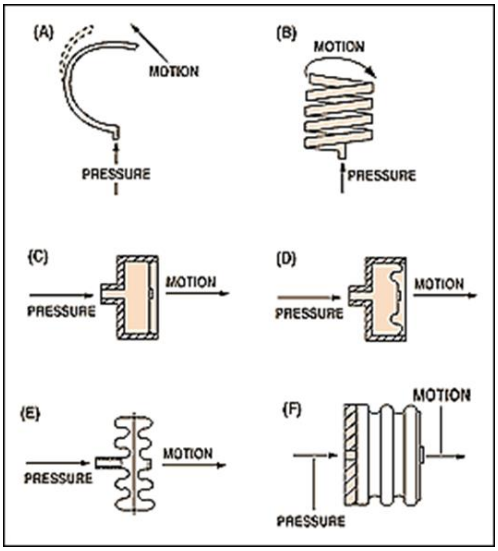
or

A pressure sensor is a device that detects a force exerted on a surface (pressure) and converts it to an electronic signal whose strength is relative to the strength of the force. Pressure sensors can also be used to measure the force exerted. Amperometry is one of an

Pressure sensing Elements and Basic Mechanism Pressure is sensed by mechanical elements such

1. plates,
2. shells,
3. tube and
4. diaphrams .

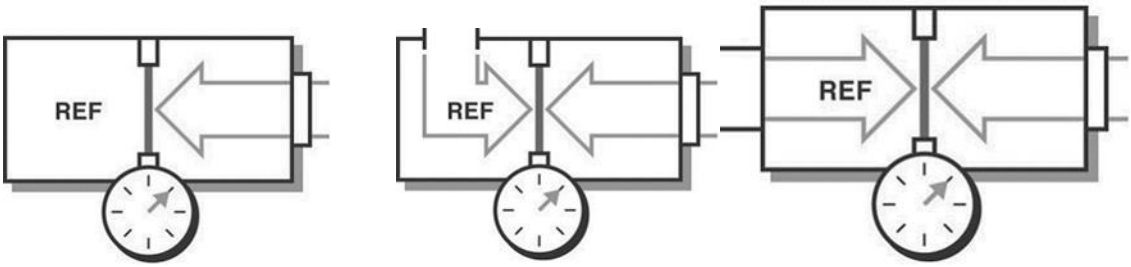
These elements are designed and constructed to deflect when pressure is applied. The basic mechanism in the pressure sensors is to convert the pressure to physical movement.



The basic pressure sensing element can be configured as a C-shaped Bourdon tube (A); a helical Bourdon tube (B); flat diaphragm (C); a convoluted diaphragm (D); a capsule (E); or a set of bellows (F).

Pressure sensors Types

There are three types of pressure measurements:



Absolute pressure measurement Guage Pressure measurement Differential p mreasuement

1. Absolute Pressure measurement is measured relative to a vacuum.
2. Gauge Pressure is measured relative to ambient atmospheric pressure.
3. Differential Pressure measurements are taken with respect to a specific reference pressure

Examples are discussed in detail in the lecture including of sensors in diagnostics include A cuff less wearable sensor blood pressure. Sensor for IOC in eye Measurement of Glucose.

Blood Pressure Apparatus

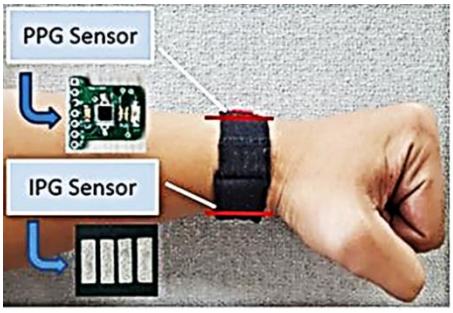
Blood pressure in is a good indicator of health. It is measured by a by sphygmomanometer. The measurement of blood pressure (BP) is critical to the treatment and management of many medical conditions. High blood pressure is associated with many chronic disease conditions, and is a major source of mortality and morbidity around the world. For outpatient care as well as general health monitoring, there is great interest in being able to accurately and frequently measure BP outside of a clinical setting, using mobile or wearable devices. In a sphygmomanometer blood pressure is measured by oscillations of pressure in by **deflation method. sphygmomanometer consists of**

1. An inflatable cuff
2. Mercury manometer
3. An operated bulb
4. A valve

5. A pump .

   methods utilize an occlusive cuff as an external pressure source, wrapping around a subject's upper arm to disclose the systolic and diastolic pressures within 30–60 s. Since the blood pressure monitor using the auscultatory or oscillometric method must include a cuff and some mechanical devices such as a pumping motor and a deflating valve

A wearable wrist Biosensor is available in market for monitoring blood pressure. The apparatus uses IPG and PPG techniques, as described below.



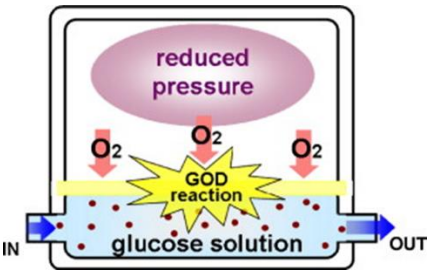
The impedance plethysmography (IPG) technique is a physical measure of the ionic conduction of a specific body segment in contrast with electrical conduction characteristics. Photoplethysmography, known most commonly as PPG, utilizes an infrared light to measure the volumetric variations of blood circulation. This measurement provides valuable information about the cardiovascular system. PPG is a non-invasive optical method for measuring blood volume changes per pulse. In other words, the PPG waveform represents the mechanical activity of the heart.

. Pressure Biosensor for Glucose measurement

A pressure biosensor is designed to measure differential pressure measurement of glucose level, using O levels.

Enzyme is immobilized on one side of the membrane

The pressure in the gas cell decreased continuously with consumption of O induced by (GOD) enzyme reaction.



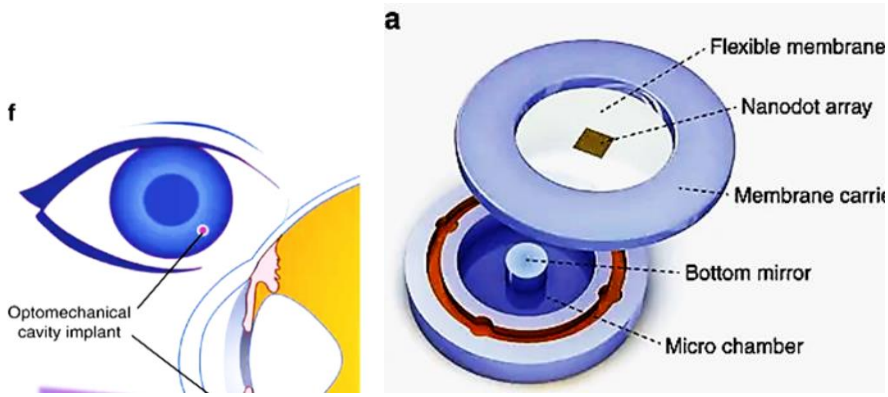
An enzyme based pressure biosensor for estimation ofglucose.

Sensor for Ocular Pressure

An implantable sensor is designed to provide convenient, accurate, on-demand Intra Ocular Pressure monitoring in the home environment.

The sensor is excited by near-infrared (NIR) light from a tungsten bulb.

The optical cavity of sensor reflects a pressure-dependent signal that can be converted to IOP.



Module No. 66 Physiological sensors

Physiological sensing deals with the sensors and analyses of different biological signals. Developed applications have mainly been for clinical and medical purposes in hospitals, healthcare centers, and clinics. Physiological sensors are also commonly known as physiological

monitors.They monitor the vital signs associated with the health of a person.The monitors are based on different sensing techniques, depending upon the output of the physiological signal. Smart sensors and wearable sensors are also inclided in Physiological sensors; .

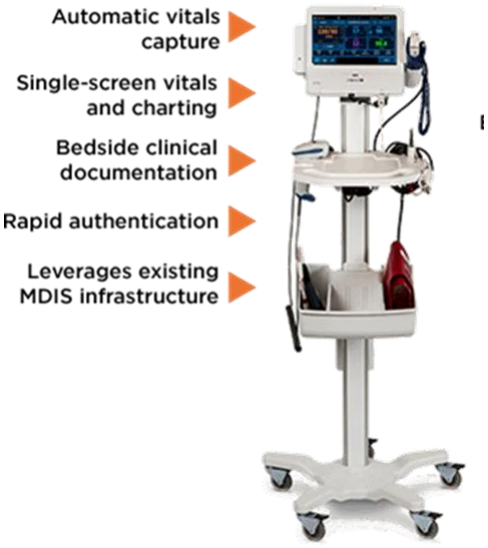
Types of Biosensors are discussed in the lecture. Biosensors are classified on the basis if Bioreceptor or sensing techniques. List of different parameters with range and Instrument.

Parameter	***Range***	***Technology/Instrument***
Rate of the heart	***0.5 - 4.0 mV***	***Electrodes***
Body temperature	***32.0 - 45.0 ℃***	***Temperatyure sensors***
Blood pressure	***10.0 - 400.0 mm Hg***	***Pressure sensors***
Respiration rate	***2.0 - 50.0 breaths/min***	***Pressure sensors*** ***Strain gauge***
Glucose in blood	***0.5 - 1.0 mM*** ***(millimoles per liter)***	***Electrochemical***
Pulse oxygenation	***80% -100%***	***Optical sensors***

Sensors/ Monitors

Physiologic monitoring systems monitor vital physiologic parameters so that clinicians can be informed of changes in a patient's condition. They typically consist of

1. A Central station
2. Bedside monitors
3. Telemetry transmitters
4. Receivers.

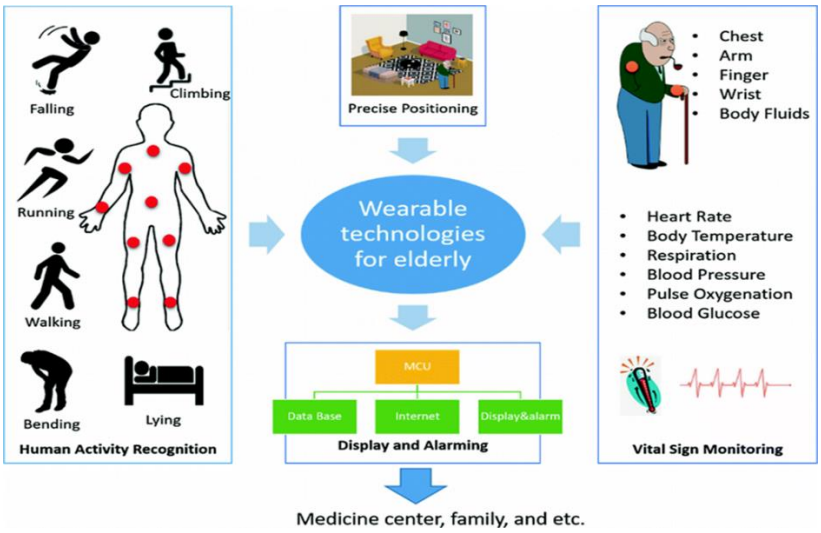


Types of Physiological sensors

Physiological sensors could be classified in different ways.

Classification on the basis of Function pressure sensor for determining BP.

1. Classification on the basis of sensor type used, for example optical sensor for Oxygen saturation.
2. Smart Sensors
3. Wearable sensors.



Module No. 67 Types of Biosensors

Introduction

Biosensors are classified on the basis of defend criteria.

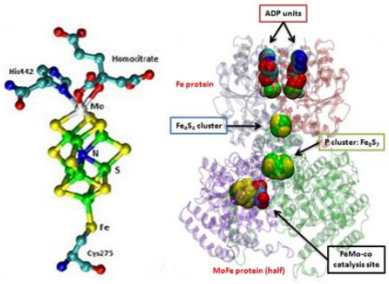
1. Generations of Biosensors
2. Biorecognition Element
3. Type of Sensor Element
4. Type of Application
5. Commercial Biosensors

The most convenient classification is based on either the use of BRE or the transducer.

Bio-memics strategy for detection

The approach is to synthesize Biomemic enzymes, that contains the key amino acid residues of the active site.These residues are involved in the catalytic and binding activity of the enzyme with the target molecule.

Nitrogenase enzyme is shown with Mo and Fe ions, involved in catalysis.



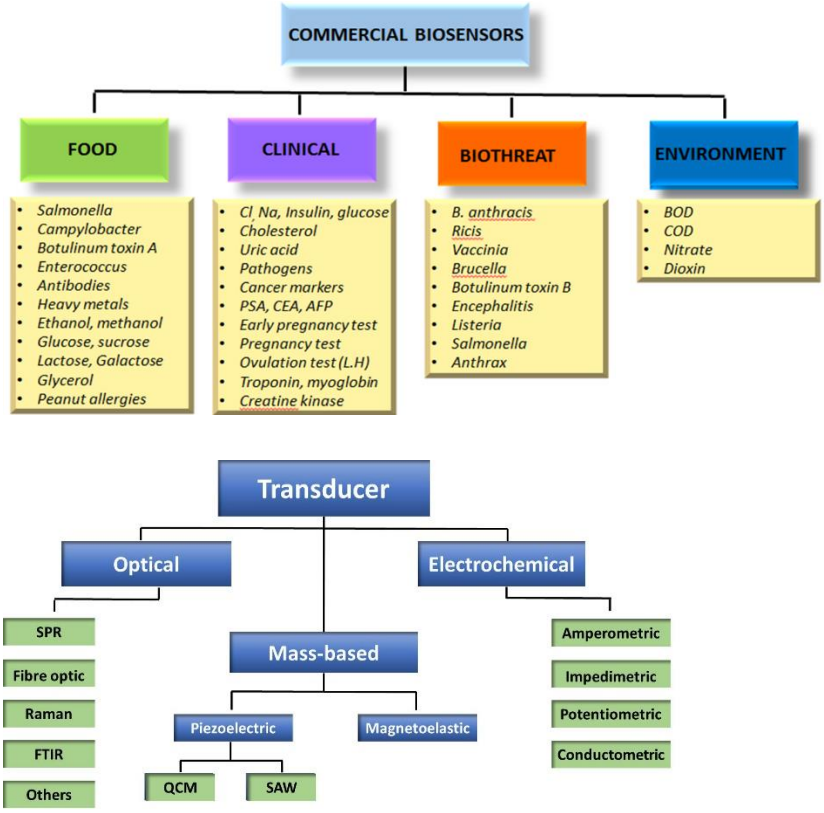
A) Molybdenum containing nitrogenase enzyme. B) Indicates the FeMo complex of the enzyme interacting with the key amino acid residues of the enzyme.

Commercial Biosensors

The commercial biosensors have small size and simple construction and they are ideal for point-of-care biosensing.

There are four main categories in commercial Biosensors.

1. Clinical Biosensors
2. Food Biosensors
3. Bio threat
4. Environment



68. Latest Research and current Trends

A sensor is an input device that can detect a change after receiving a signal or stimulus. weight, pressure etc.

Sensors are becoming the biggest growing markets because they have applications in almost every field of life.

I. Smart sensors
II. Internet of things
III. Robotics

Smart Sensor

A smart sensor is a small multi tasking device.

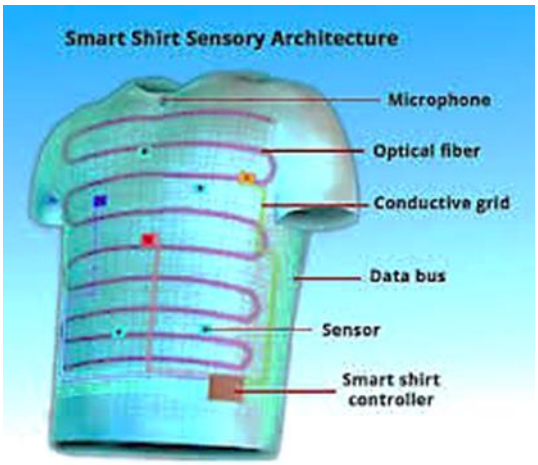
It can detect signals from physical environment. It saves time and energy.

I. A smart sensor contain an embedded Digital Motion Processor (DMP).
II. A microprocessor
III. Communication device

Examples of Smart Sensor

Wearable T smart shirt, it uses optical fibers to detect wounds. It can monitor the vital signs like heart rate, respiratory rate and temperature.

Oximeter is a smart sensor. It can measure Finger-based heart rate and Blood oxygen saturation level.



Internet of Things (IOT)

The Internet of Things (IoT) is a system of inter related things including Computing Devices, Mechanical Machines, Digital Machines, Objects, People and Animals.

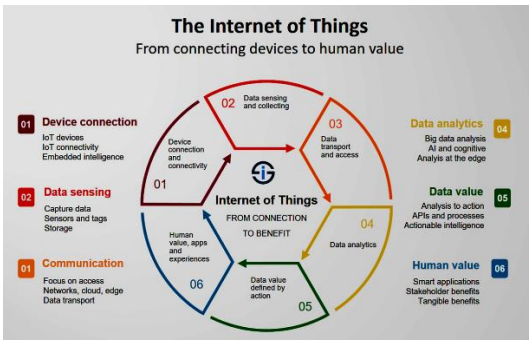
They are provided with a unique identity (UID) to access a facility, data, ability to transfer data without requiring human –human interaction, .

Robotic Sensors

A robot is a programmable machine, to carrying out a complex series of actions automatically.

Robotic sensors are used to estimate a robot's condition and environment, for further actions.

Sensors in robots are based on the functions of human sensory organs.



Biosenors Chapter 07

Enzymatic Biosensors and Immune Biosensors

Modules 69-90

Module No.	**Title**
69.	An introduction to Enzyme Biosensors
70.	Enzyme Stabilization and immobilization Method
71.	**Elimination of enzyme Leakage**
72	. Enzyme Stabilization by Pressure
73.	Nano particles in Enzyme Biosensors
74.	Hexokinase based Biosensor
75.	Cholesterol Oxidase Biosensor
76.	Glucose oxidase biosensors
77.	Lactate Oxidase biosensors
78.	Alcohol oxidase biosensors
79.	Xanthene oxidase biosensors
80.	Cardiac Troponin Biosensors
81.	An introduction to immuno Biosensors
82.	Enzyme Link Immunoabsorbent Assay (ELISA)
83.	Monocolonal Antibody
84.	Antibody Fragments
85.	Lateral Flow Assay (LFA)
86.	Optical Immunosensors
87.	Surface Plasmon Resolence Immunosensor
88.	Electrochemical Immuno Biosensors
89.	Impedance Immuno sensor
90.	Piezo Electric Immuno Sensor

Learning Outcome.

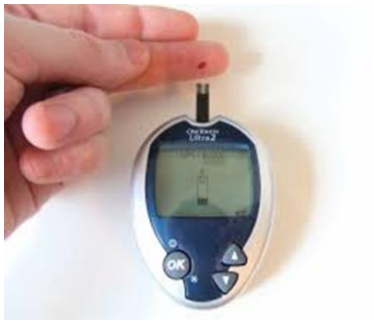
1. Students will ba able to understand the rationale of Enzyme Biosensors. Students will be able to learn the basic concepts of Enzyme Stabilization, immobilization and stabilization methods.
2. Students will be able to explain the use of nanoparticles in Biosensors.
3. Students will learn the fabrication of a variety of Enzyme based Biosensors.
4. Students will be able to explain the fabrication and designing of Cardiac Troponin Biosensors
5. Students will learn the basic concept of immuno Biosensors
6. Subjects related to Immunosensors like ELISA, AMonoclonal antibody and their fragments in biosensor fabrication will also be studied.Enzyme Link Immunoabsorbent Assay (ELISA)
7. Students will learn the basic principle, designing and fabrication of Lateral Flow Assay (LFA).
8. Students will learn a series of Immunobiosensor based on different techniques. Including

Module No. 69. An introduction to Enzyme Biosensors

An enzyme biosensor is an analytical device that combines an enzyme with a transducer to produce a signal proportional to target analyte concentration. The most successful product among Biosensor is an Enzyme Biosensor that is Glucometer. Chemical method for Glucose Estimation

Chemical method using DNS for glucose estimation requires

1. Large volume of sample
2. Pretreatment of sample
3. Drastic conditions
4. High temperature
5. Proper lab setup
6. Trained staff



Advantages of Enzyme based Biosensor

I. Glucometer work by analyzing a small amount of blood.
II. Glucometer is a small hand held device.
III. Glucometer works at ambient conditions .
IV. No pretreatment of blood sample is required.
V. Commercially available

Detection methods in Enzyme Biosensors

There is a huge number of enzymes that can act on their unique substrates and produce respective products and bi-products. The signal generated by catalysis can result from a change in proton concentration, release or uptake of gases, such as ammonia or oxygen, light emission, absorption or reflectance, heat emission, and so forth, brought about by the reaction catalyzed by the enzyme. The transducer converts this signal into a measurable response, such as current, potential, temperature change, or absorption of light through electrochemical, thermal, or optical means. This signal can be further amplified, processed, or stored for later analysis.

Enzyme Biosensors could work either by detecting the end product, bi-product or any step during catalysis (flow of electrons) by using different types of sensors.

Sensors in Enzyme biosensors

The selection of sensor in a Biosensor is dependent on the type of signal being detected detection of the signal

1. Optical senor could be used if there is a change in the optical properties (change in colour).
2. Amperometric Sensors a could be used to detect the flow of current.
3. Pressure sensor could be used for a change in pressure.

Dependencies of Enzyme Biosensors

1. The catalytic activity of enzymes is strongly dependent on pH, temperature and pressure.
2. Membrane biodegradtion by the enzymes could lead to Biocompatibility-based failure membrane biodegradation.
3. Example cellulase enzymes is immobilized on a cellulose membrane.

Summary

Enzyme based Biosensor are the most popular type of Biosensors (Glucometer).
A huge variety of signals could be detected by enzyme biosensors.
Glucometer has a big market for the product. unique features and economical availability.
Easy to use and cater the need of a common man.

70. Enzyme Stabilization and immobilization Method

ENZYME IMMOBILIZATION

Immobilization of Bioreceptor on the support layer is the first step in the fabrication of biosebsor. Stabilization of Bioreceptor molecule to retain its functional activity is of pivitol importance. The stabilization and immobilization of enzymes, biosensors involve, wiring *that is some sort of attachment)‖of an enzyme to the electrode.It involves the co-immobilizing the enzyme and mediator directly onto the electrode surface.

1. Carbon nanotubes
2. Gold nanoparticles
3. Polyelectrolyte
4. Self assembled monolayer

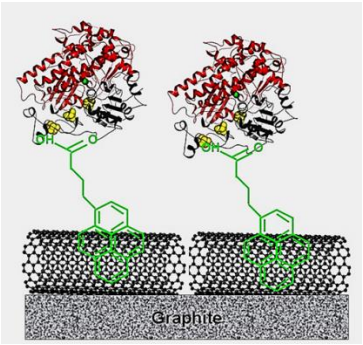
The effects of immobilization on activity, stability, and even selectivity of some enzymes are reported.

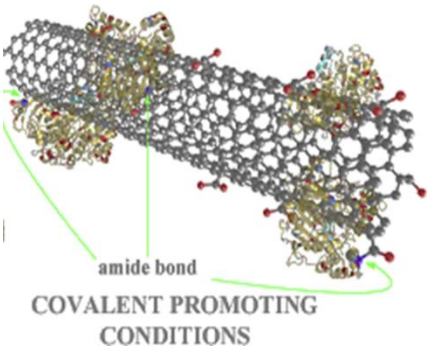
Immobilization of Enzyme onto CNT

Carbon nanotubes are made up of Graphene. They have aromatic structure and are hydrophobic. CNTs can act as a mediator for enzyme immobilization on electrode after treatment.

CNTs are used for

I. Physical adsorption
II. Chemical cross-linking,





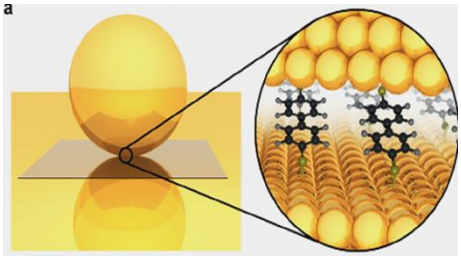
A. CNTs are functionalized by reducing agent such as citrate to the positively charged amino acid residues of the enzyme.
B. Chemical cross-linking involves covalent attachment of the enzyme onto CNT via a linker molecule using Glutaraldehyde' BSA or silca gels, sol gels.

Physical Adsorption on Gold Nano Particles

Physical adsorption is a simple and quick method.
It involves reducing the gold nanoparticles with a negatively charged ligand such as citrate.
Sulfur containing amino acid Cysteine is also used as a mediator or linker
The chemistry of sulfur absorption on gold surface is not clear yet.

A Self-Assembled Monolayer (SAM) is a one molecule thick layer of material that bonds to a surface.Self-assembled monolayers (SAM) of organic molecules are molecular assemblies formed spontaneously on surfaces by adsorption and are organized into more or less large ordered domains. SAMs are created by the chemisorption of "head groups" onto a substrate from either the vapor or liquid phase followed by a slow organization of "tail groups". The "head groups" assemble

together on the substrate, while the tail groups assemble far from the substrate. Areas of close-packed molecules nucleate and grow until the surface of the substrate is covered in a single monolayer. Adsorbate molecules such as Bioreceptor molecule in case of a biosensor, adsorb readily because they lower the surface free-energy of the substrate and are stable due to the strong chemisorption of the "head groups." These bonds create monolayers that are stable.



Self assembled monolayer and binding of receptor molecule is shoen in case of gold. A Self-Assembled Monolayer (SAM) is a one molecule thick layer of material that bonds to a surface. Thiol SAMs on gold are the most popular molecular films because the resulting oxide-free, clean, flat surfaces can be easily modified both in the gas phase and in liquid media under ambient conditions.

The most common methods of enzyme immobilization are

1. physical adsorption,
2. covalent tethering,
3. cross-linking, and
4. entrapment .

Physical adsorption results from hydrophobic, hydrophilic, or ionic interactions between the protein and a solid surface. There is evidence that physical adsorption of enzymes may stabilize their activity . However, this type of immobilization often leads to enzyme leakage, especially with changes in pH and temperature.

Adsorption is frequently used to build layer-by-layer architectures in combination with other immobilization techniques such as **cross-linking and/or entrapment** behind a membrane. **Sol-gels** have been used to immobilize enzymes . These techniques provide an environment similar to that of the enzyme in solution. However, the volume and pore size, change as condensation reactions continue.

Once the sol is dry and stable, the pore size is so small that the enzyme reaction is limited by diffusion . To prevent cracking and swelling of the hydrogel, organic-inorganic hybrid materials are being investigated). Several studies on enzyme entrapment in sol-gels for the development of biosensors focus on increasing sensitivity, measuring range, and decreasing response time .

Entrapment can also be achieved by chemical, electrochemical, or photo-polymerization in a monomer-enzyme solution. Amperometric biosensors entrapping GOX in electrochemically generated polymers have been developed.

Covalent attachment of enzymes to solid supports has been done by activation of enzyme carboxylic side chains with a carbodiimide followed by condensation with support surface amino groups.

Multipoint covalent attachment has been reported using soluble polysaccharides or solid agarose gel supports . Multiple covalent bonds result in reinforced quaternary and tertiary structures providing stabilization of up to 30,000-fold for a thermophilic esterase.

Summary

Enzymes are used BRE in biosensors. Immobilization and stabilization is a crucial step in the fabrication of biosensor. Use of different mediator is a successful approach to increase the stability and performance.Polyelectrolytes, Self-Assembled Monolayer, CNTs and GNP. Thiol SAMs on gold are the most popular molecular films because the resulting oxide-free, clean, flat surfaces can be easily modified both in the gas phase and in liquid media under ambient conditions. Different Physical and chemical methods are also discussed.

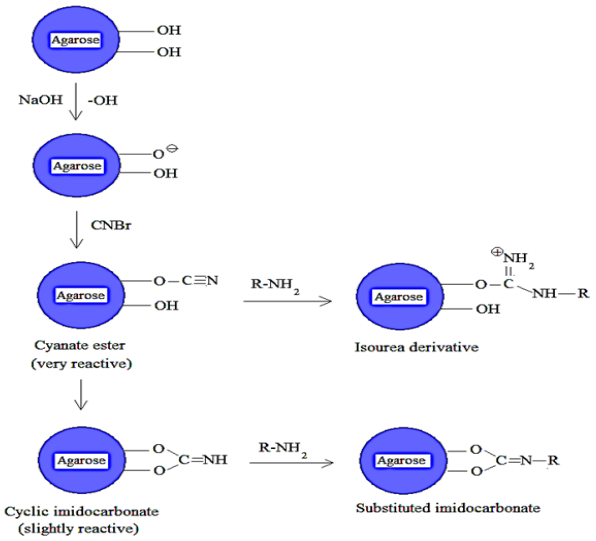
71. **Elimination of enzyme Leakage**

For artificial immobilization several methods are used. Use of chemical methods for immobilization is expected to be flaw less.It has been noted that the immobilized enzyme is eliminated, because the method relied on the completion of chemical reactoion and specific chemical bonding.

There are several different activated agarose gels that can be used to attach ligands activated Agarose gels are used for immobikization, Agarose is a polysaachride it is linear poly mer of D- Galactose and 3,6 anhydro-L-galactopyranose.

1. CNBr Agarose
2. Aminohexanoic acid Agarose
3. Carbonyldiimoazole Agarose
4. Epoxy activated Agarose

Cyanogen bromide is often used to immobilize proteins by coupling them to reagents such as agarose for affinity chromatography.Because of its simplicity and mild pH conditions, cyanogen bromide activation is the most common method for preparing affinity gels. CNBr-agarose is easy to use for the attachment of amines. The method is simple, works at pH and temperature and well documented. It is a rapid method and biological molecule retains the biological activity. Method offers multiple points for the attachment of the ligand molecules, It is rich is cyanate esters and imido carbonates, as shown in the figure.



Cyanogen bromide activation method

Limitations. It does not have long arm therefore the protein binding may be hindered. The disadvantages of this approach include the toxicity of cyanogen bromide and its sensitivity to oxidation. Also, cyanogen bromide activation involves the attachment of a ligand to agarose by an isourea bond, which is positively charged at neutral pH and thus unstable. Consequently, isourea derivatives may act as weak anion exchangers

Aminohexanoic acid-agarose and diaminohexane-agarose have relatively long methylene chains that keep the ligand a significant distance from the gel beads.

An alternative reagent for attaching amines is carbonyldiimadazole-agarose, and epoxy-activated agarose is used for alcohols. The type and number of covalent bonds between the matrix and the enzyme are crucial for both the functional properties of the immobilized catalyst and its leakage from the support.

Leakage population

There ateat least two populations of the covalently attached enzymes.

1. Liable to Leakage
2. Resistant to cleavage

The proportion of the two population varies with the extent of cross linking during CNBr activation. The process is not completely controllable for multiple binding sites.

Enzyme leakage

The isourea bond formed between the cyanate ester and amine group of protein is unstable.The weak bond constant leakage of the ligand. Low molecular weight ligands are slowly released from the polymeric support in the aqueous media above pH 5.0.

Although a number of leakage studies with CNBr-coupled ligands have been published data on the number of anchoring bonds are not as available. Leucine aminopeptidase, a hexameric enzyme with a molecular weight of 326000, is particularly rich in lysine residues.

There are average 22 residues are involved in enzyme-matrix linkages and there are 11 anchoring bonds in immobilized trypsin in case of trypsin enzyme.

It eliminates enzyme leakage and covalently immobilized enzymes sometimes show greater resistance to pH and temperature variations. Intermolecular cross-linking has been widely used in multipoint covalent immobilization. Intermolecular cross-linking can result in activity loss. Because of the random mechanism and fast reaction rate of the cross-linking reaction, it is difficult to obtain reproducible sensors.

The release of the enzyme from multipoint-attached preparations has not tallied well with theoretical predictions. This discrepancy lends considerable support to the idea that in multipoint-attached proteins there is a more or less broad distribution of the number of anchoring bonds and a heterogeneity of the microenvironment of these linkages.

The incomplete reaction of functional groups in polymers, even in the case of quite simple chemical transformations, is a phenomenon quite common to the polymer chemist. The reaction of the thiol-The single-point-attached enzyme that: (a) a certain amount of enzyme is released with a time course which follows quite closely a monoexponential process, as evidenced by the high correlation coefficient of the fit ; (b) the remainder is stable in the time range studied; (c) the absolute value of the detached and the remaining portion of enzyme varies unpredictably from preparation to preparation and is not correlated with the velocity of the cleavage reaction.

There are (at least) two populations of the covalently attached enzyme, one liable to solvolysis, the other resistant to cleavage. The proportions of the two populations vary with the extent of cross-linking during CNBr activation, which is not completely controllable.

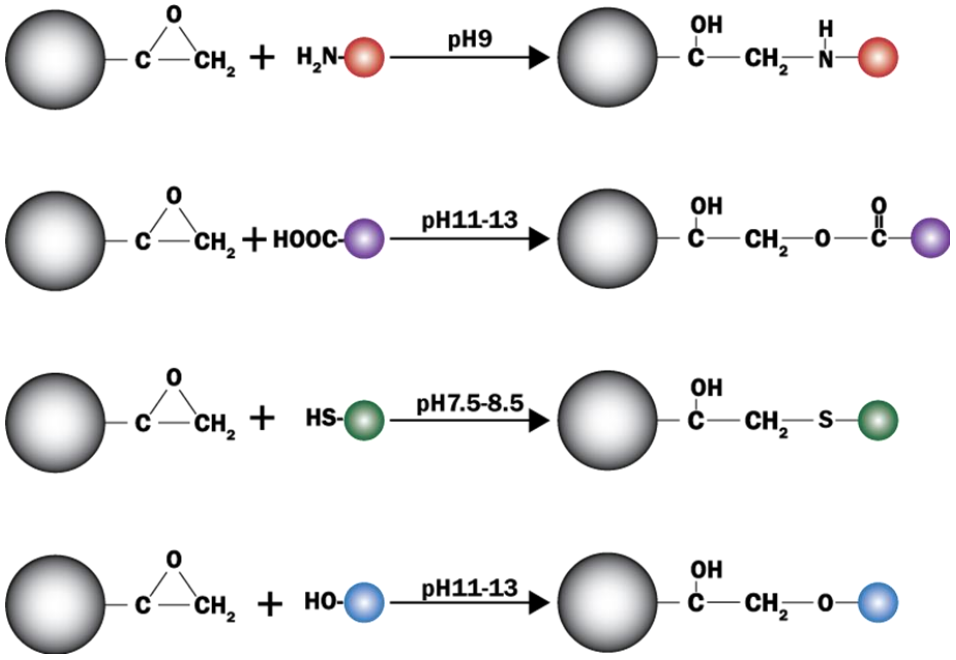
With monovalent ligands multivalent mediators between matrix and ligand a considerable increase in stability can be achieved. High-capacity and charge free polyacrylic-hydrazide-Sepharose derivatives proved to be particularly suitable.In CNBr method a large number of bonds, a considerable solvolytic release still takes place at room temperature above pH 7.

Affinity chromatography is the most popular and widely used method for purification of biomolecules including proteins, antibodies, lectins, carbohydrates, nucleic acids and glycoproteins. Affinity purification is based upon specific surface interactions among biological molecules such as antigen-antibody, enzyme-ligand etc. These specific surface interactions enable the purification of molecules from 1000 to 10,000 fold in a single step. In affinity chromatography, the ligand, antigen or one of the interacting molecules is covalently bound to the matrix and is used as a bait to fish out the target protein from any complex pool of proteins including cell or tissue lysate.

The matrix, such as agarose, needs to be activated for covalent binding of ligand for affinity purification. There are several pre-activated resins available commercially with different coupling chemistries. These pre-activated resins give wide range of options for scientists to choose from for binding ligand of their choice to purify the target

protein. Among them the epoxide chemistry is the most versatile as it can immobilize ligands via amine, thiol and hydroxyl groups.

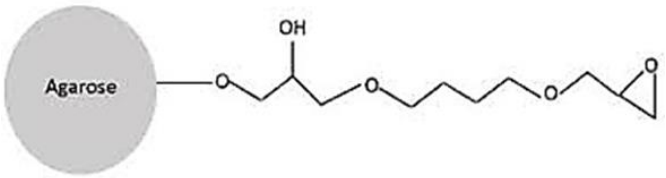
Epoxide-activated agarose is prepared by immobilization of oxiranes such as 1,4-butanediol diglycidyl ether onto matrix. For Amino group attachment pH 9 is required. For COOH group attachment pH 11-13 is required. For Thiol group attachement pH 7.5-8.5 is needed and OH group pH 11-13 is required.



This pre-activated resin provide hydrophilic 12 atom spacer arm. Epoxide-activated agarose offers several advantages: Epoxide-activated agarose can bind wide range of macromolecules

Epoxy Activated Resin Interactions

Epoxide-activates agarose has long hydrophilic spacer arm that favors binding of small ligands to the matrix including but not limiting to monosaccharide, peptides, and disaccharides.



Epoxy activated agarose

For example oxiranes such as 1, 4-butanediol diglycidyl ether provides 12 atom hydrophilic spacer to the matrix.

In summary Use of CNBr for immobization is widely is used, reported to be not a perfect method, as enzyme leakage is observed. Use of Epoxy activate Agarose is another alternative.

72. Enzyme Stabilization by Pressure

Pressure is force acting per unit area. An increase in pressure can improve the stability of the enzyme. Treatment of enzyme at high pressure is a latest approach to increase their stability and activation.It has different effects on different enzymes depending on the type and conditions. Glucose oxidase and polyphenol oxidase have a positive impact, with some changes in their 3D structure of the molecules.. Particularly notable is a significant number of thermophilic proteins that show improved stability under pressure. Effect of pressure on the biological activity of the enzymes is studied for long.

Pressure Effect

The effect of high pressure of enzyme is heterogeneous due to change in weak forces. It is dependent on the

1. Type of enzyme
2. Level of pressure
3. Effect of pH and temperature.

In some cases it can improve the biological activity and in some cases it is damaging for enzyme.

Effect of pressure on Hydrogen Bonds

Pressure is a physical force and it mainly effects on the physical forces of the enzyme molecule that are holding the enzyme structure. For example hydrogen bonds.

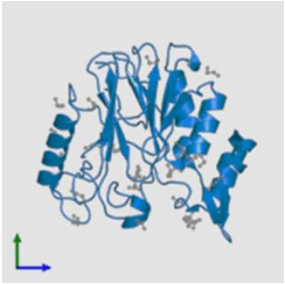
1. The amide groups form hydrogen bonds either with carbonyls or with water.
2. These hydrogen bonds are shortened by pressure.
3. The estimated shortened distances varied from site to site (0−0.11 Å)
4. Larger reduction in the turn but smaller in the interiors of secondary structures (alpha helix, beta sheets).

Stabilization and Activation of enzyme activity by pressure.

There are two examples for the Stabilization and activation of enzyme activity, due to high pressure.

1. Glucose Oxidase
2. Polyphenol oxidase

The high pressure promoted changes in the activity, stability, kinetic parameters and structure.



3D structure of Polyphenoloxidase

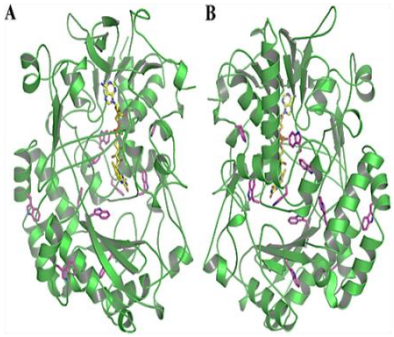
The high pressure homogenization process promoted changes in the glucose oxidase activity, stability, kinetic parameters and structure. The treatment improved the activity of the enzyme at non-optimum pH values (4.5 and 6.5) or at low temperatures (15–45°C) after enzyme treatment at 150 MPa. For the majority of the tested conditions, the HPH-treated glucose oxidase presented similar responses, independent of the pressure used in homogenization. In addition, the biophysical characterization showed no differences between the HPH samples homogenized at 75 and 150 MPa (Megapascal SI unit), indicating that the structural rearrangements induced at 75 MPa led to a stable and new conformation of the molecule. Taking together these findings, we conclude that HPH treatment at 75 and 150 MPa changed the three-dimensional conformation of GO, affecting directly on the functional characteristics of the enzyme.

Structural analysis of the GO structure from Aspergillus niger revealed several tryptophan residues located near to the surface, which could be easily exposed to the solvent by slight perturbations in the tertiary structure supporting our spectroscopic results. The same effect on fluorescence emission was observed for other HPH-treated enzymes , evidencing that HPH treatment effectively induces conformational changes in proteins. Based on that, we analyzed the fluorescence emission maximum around 333 nm that is quite sensitive to local environment of tryptophane residues along with minor contribution from tyrosine. The fluorescence emission spectrum of the native enzyme was distinct from that of the HPH-treated enzymes, highlighting conformational changes in GO upon HPH treatment.

Conformational changes

Activation and conformational changes of mushroom PPO after high pressure.

1. Loss of α-helix content
2. Change in β-sheet, β-turn and random coil
3. Trp and Tyr residues exposed to the less hydrophobic environment.
4. SH groups content on the surface increased.



Schematic representation of the GO structure from A. niger in two orientations (180°) indicating the positions of the tryptophan residues (carbon atoms in magenta).

Activation and conformational changes of mushroom PPO after high pressure microfluidization treatment were observed. After treated under pressures of 90 MPa, 110 MPa, 130 MPa and 150 MPa one pass, and 150 MPa 1, 2 and 3 passes, respectively, mushroom PPO exhibited an increase in activity. The highest relative activity of 110.74% was exhibited after one pass treatment at the pressure of 110 MPa while mushroom PPO showed a pass-related increase in activity after treated 1, 2 and 3 passes at 150 MPa. Values of two negative peaks of CD spectra at 208 and 222 nm

decreased, indicating a loss of α-helix content. Other secondary structures such as β-sheet, β-turn and random coil also changed to a certain extent. Tertiary and quarternary structure analysis of fluorescence emission spectra and UV absorption spectra indicated that Trp and Tyr residues in hydrophobic environments were partially unfolded to be exposed to the less hydrophobic environment. The sulphydryl groups content detection demonstrated that SH groups content on the surface of mushroom PPO was increased after high pressure microfluidization, indicating conformational changes of mushroom PPO. In addition, interestingly, we found that there were some indices that the increase of mushroom PPO relative activity was accompanied by the decline in the α-helix content.

73. **Nano particles in Enzyme Biosensors**

The unique properties of nanoparticles are extremely suitable for designing biosensors. For specific purposes surface medication is required.One step modification by using long alkyl tail and hydrophilic ligand head for multiple functionality of NP.

There are two types of nano particles which are used in Enzyme biosensors.

1. Organic nano particles
2. Inorganic nanoparticles.

For increasing the applications of nanoparticles it is required to modify the surface of nano particles. The surface could be modified by using physical methods and chemical methods. AuNPs may improve biosensor stability because of their excellent biocompatibility and large specific-surface area.

The important functions provided by nanoparticles include the

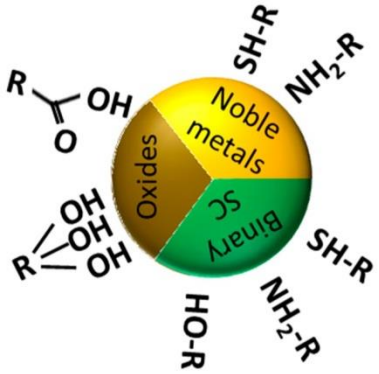
1. Immobilization of biomolecules
2. The catalysis of electrochemical reactions,
3. The enhancement of electron transfer between electrode surfaces and proteins
4. Labeling of biomolecules

Surface Modifications

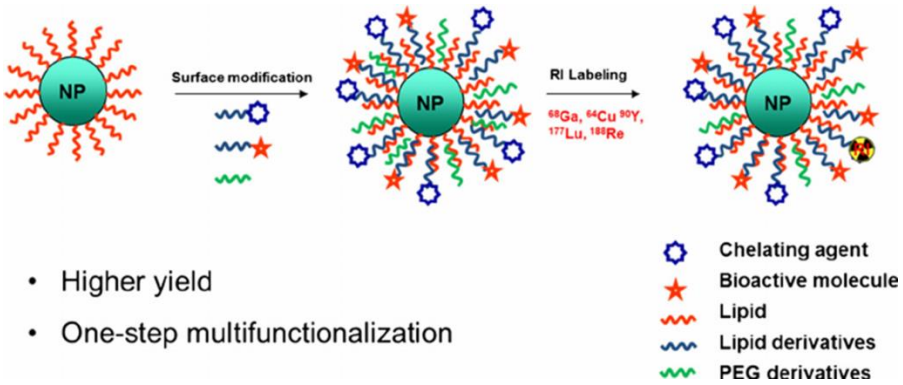
For biological application polar surface modifications are required for the attachment of biological molecules (Enzyme proteins), to increase the solubility and prevent them from aggregation. As per the requirements, NP could be functionalized by

1. Electrostatic adsorption
2. Hydrophobic Ligand
3. Hydrophilic Ligand

Depending of the NPs surface nature, different approaches have been developed based on the surface affinity towards different chemical groups. In general, we can recognize three main classes: (i) noble metals like Au and Ag (plasmonic materials) are normally functionalized with thiols or, to a lesser extent, amines and cyanides; (ii) oxides, typically used as components of magnetic NPs (e.g., iron oxides), can be easily coated via oxygen bonding with acidic and hydroxyl groups; (iii) binary compounds, f fluorescent semiconductor (SC) NPs (e.g., quantum dots), display high affinity towards thiols and hydroxyl groups, but also amino groups are often used. Figure shows the most commonly used functional groups to coat NPs of different materials.



. Schematic representation of the most commonly used functional groups in surface ligands and their preferred affinity towards binding different NP materials.



Surface modification of nanostructures with long alkyl tail and hydrophilic ligand head, which enable multi-functionality on nanoparticles in one step

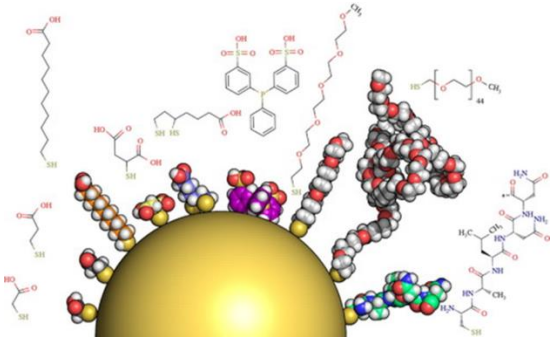
Electrostatic Adsorption

Electrostatic Adsorption is a widely used linkage approach, commonly used for carbons: amorphous activated carbon and carbon black.

Strong electrostatic adsorption is a method, in which metal (Pt) are strongly adsorbed over support surfaces by controlling the pH of the solution (NP size 1-2 nm).

Hydrophilic Ligand

For hydrophilic surface modifications a number of hydrophilic compounds are used such as



Binding of different groups on surface of gold nano particle.

1. Mercaptoacetic acid
2. Mercaptopropionic acid
3. Mercaptosuccinic acid
4. Bis-sulphonated triphenylphosphine
5. Polyethylene glycol SH
6. short peptide CALNN

Covalent immobilization of enzymes on gold nanoparticles (AuNPs) was done for the development of amperometric enzyme biosensors for detection of glucose.

, GOX has been entrapped in nanofilms electrochemically deposited on porous platinum, producing large increases in rates of catalysis and resulting in highly sensitive enzyme biosensors . The nanostructure served mainly to increase sensitivity.

Increased stability of glucose was reported for GOX covalently attached to citrate-stabilized AuNPs by derivatizing the nanoparticle (NP) with carboxyl groups and coupling the enzyme-amino side groups using the carbodiimide bioconjugation method. Attachment to NPs has also been done with glutaraldehyde (GA). Enzymes have been covalently tethered to, e.g., polysaccharides, vinyl polymers, polyamides (nylon), polyalkylenes, and polyester

ZnO has been employed in other ways as well for the fabrication of amperometric biosensors.

Carbon nanotubes are commonly employed in the fabrication of biosensors. A 3D nitrogen-doped carbon nanotube supported by a carbon-foam hybrid was fabricated as GOX's carrier matrix. The uniform pore distribution and high surface area of the nanostructured N-doped carbon clarified foam provide a large quantity of active sites for GOX immobilization, which improves sensitivity. After three weeks of storage at 4°C, the electrode retained 91.2% of its original sensitivity. A biosensor based on a Fe3O4 NP–polyvinyl alcohol composite. Operational stability was measured intermittently for a month, and the electrode was stored at 4°C in a phosphate buffer (pH 7) when not in use.

74. **Hexokinase based Biosensor**

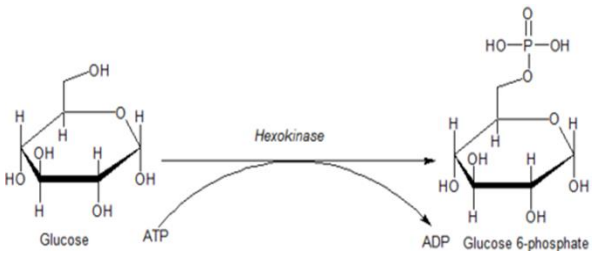
Hexokinase is a metabolic enzyme.The enzyme converts ATP into ADP. Biosensors are designed to detect the ATP in samples. GO is used with HK to detect the CK enzyme activity in samples.A fluorescent based HK biosensor for direct estimation of ADP

Hexokinase is an enzyme that phosphorylates hexoses (six-carbon sugars), forming hexose phosphate. In most organisms, glucose is the most important substrate of hexokinases, and glucose-6-phosphate is the most important product.

It is a Metabolic Enzyme. It acts on all hexoses. There are four mammalian types of this enzyme MW 50 KD-100 KD. It requires ATP And produces ADP.HK D is Glucokinase. Glucokinase has high Km value and has low affinity for glucose.

Hexokinase mechanism of Action

Hexokinase catalyzes the phosphorylation of hexoses and produced hexose phosphate. Glucose kinase is produced by liver, pancreas, hypothalamus, small intestine. Its deficiency is a genetic autosomal recessive disease that causes Chronic Haemolytic Anaemia.



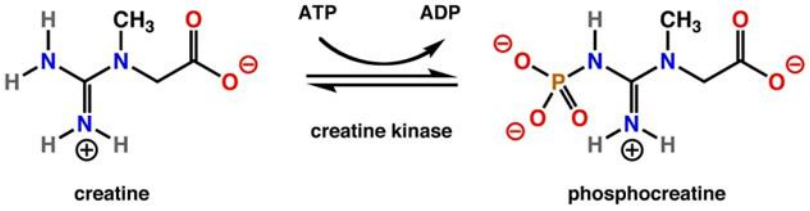
Catalysis f glucose by Hexokinase.

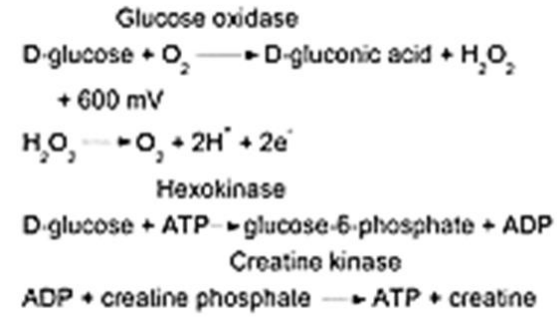
Hexokinase Based Biosensors.

Different biosensor for determination of adenosine triphosphate are created. They are based on enzyme hexokinase immobilized on inter digitated planar electrodes. Quick response, simplicity, and low cost are advantages of the biosensor. The biosensor can be used in for determination of ATP in water samples, as discussed in the lecture. Hexokinase enzyme is immobilized on the electrodes or the conducto metric support membrane. HK biosensors are quick to response, simple, and low cost. Mainly biosensor are used for the determination of ATP, in the samples.

Creatine Kinase.

Creatine kinase is an important enzyme of muscle cells. A biosensor for rapid and convenient determination of CK activity by measuring the rate of ATP production by this enzyme. GO was also used in the biosensor to determine the glucose concentration in the sample

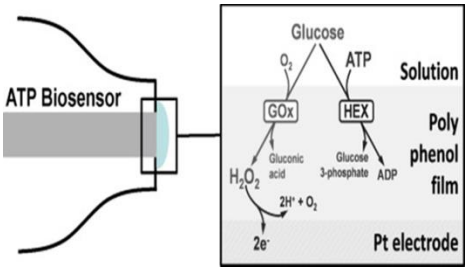




Fif shows the scheme for enzyme action in hexokinase based creatine biosensor..

ATP determination in tissues/ Cells.

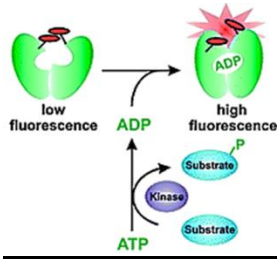
ATP is consumed during metabolic processes, by different enzymes. The level of enzymatic reactions could be determined indirectly. ATP biosensor was designed for the detection in ileum, colon. An ATP biosensor is designed to determine tyrosine phosphatase in osteoblastic cells.



Scheme for the hexokinase based Biosensor fabrication for ATP detection.

Hexokinase Based Fluorescent Biosensor

Fluorescent method for the determination of ADP.A fluorophore compound diethylamino-coumarin and genetic engineering techniques were used for the development of a biosensor for ADP.Biosensor shows high fluorescent binding with ADP.



Scheme for the hexokinase based fluorescent Biosensor .

<u>75. Cholesterol Oxidase Biosensor</u>

Cholesterol is an important lipid molecule in cell membranes and lipoproteins. Cholesterol is also a precursors of steroid hormones, bile acids, and vitamin D. Abnormal levels of cholesterol or its precursors have been observed in various human diseases, such as heart diseases, stroke, type II diabetes, brain diseases and many others. Therefore, accurate quantification of cholesterol is important for individuals who are at increased risk for these diseases.

Cholesterol is a lipid and synthesized by the animal involving 37 steps. Serum cholesterol level is required for the diagnosis and treatment of hypothyroidism, diabetes and liver diseases.

The Liebermann–Burchard or acetic anhydride test is used for the detection of cholesterol, in the presence of sulfuric acid.The formation of a green or green-blue colour after a few minutes is positive. Lieberman–Burchard is a reagent used in a colourimetric test to detect cholesterol, which gives a deep green colour.

Cholesterol catalyzing Enzymes

I. Cholesterol esterase (ChoE) or bile-salt activated lipase or sterol esterase catalyzes the hydrolytic cleavage of cholesterol.
II. Cholesterol oxidase (CHOx), a FAD-dependent enzyme of the oxido-reductase family catalyzes the oxidation of cholesterol to cholestenone.

cholesterol oxidase (EC 1.1.3.6) is an enzyme that catalyzes the chemical reaction for degradation of cholesterol. It has two reactants Cholesterol and Oxygen. Thus, the two substrates of this enzyme are cholesterol and O2, whereas its two products are cholest-4-en-3-one and hydrogen per oxide.

Cholesterol oxidase enzyme belongs to the family of oxidoreductases, specifically those acting on the CH-OH group of donor with oxygen as acceptor. The systematic name of this enzyme class is

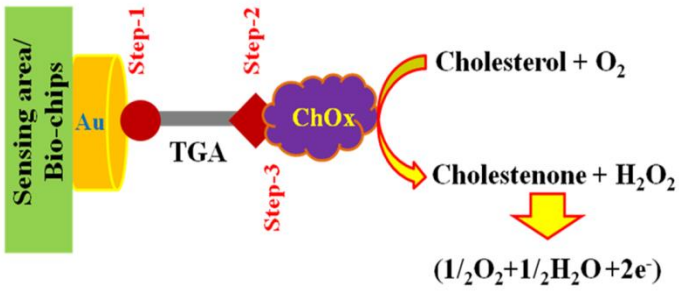
I. cholesterol:oxygen oxidoreductase. Other names in common use include
II. cholesterol- O2 oxidoreductase,
III. 3beta-hydroxy steroid oxidoreductase,
IV. 3beta-hydroxysteroid:oxygen oxidoreductase.

This enzyme participates in bile acid biosynthesis.

Cholesterol Oxidase

Cholesterol oxidase (CHOx), a FAD-dependent enzyme of the oxido-reductase family. It catalyzes the oxidation of cholesterol to cholestenone. It is produced by a large number of organisms including microbes.It has a molecular weight of 50Kd, optimum pH 8.

The substrate-binding domain found in some bacterial cholesterol oxidases is composed of an eight-stranded mixed beta-pleated sheet and six alpha-helices. This domain of the FAD cofactor bound by the FAD-binding domain and forms the roof of the active site cavity, allowing for catalysis of oxidation and isomerisation of cholesterol to cholest-4-en-3-one.



Fabrication of an amperometric cholesterol biosensor onto smart micro-chips based on cholesterol oxidase (ChOx) co-immobilized thioglycolic acid self-assembled monolayer (TGA-SAM).

Biosensor based on Cholesterol Oxidase and Cholesterol Estrase TMB and GQDs Pt Nano dots. Blue color shows the presence of cholesterol. Glucose oxidase is used to detect hydrogen peroxide produced by cholesterol oxidase in the presence of coloured substrate TMB.

76. **Glucose oxidase biosensors**

First ever Glucose Biosensor was composed of an

I. A platinum electrode,
II. An Oxygen permeable membrane

III. A thin layer of Gox enzyme
IV. Outer dialysis membrane.

Principle

It was based on the principle of measurement of Oxygen levels during the enzymatic reaction. 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).

First Generation Biosensors

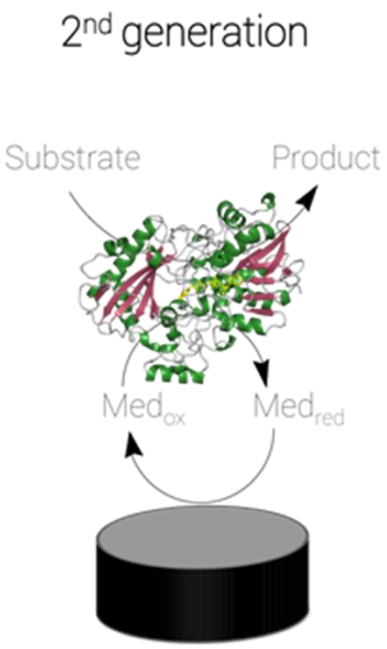
I. In first-generation biosensors work on the following principle.
II. The electrons generated by enzymatic REDOX reaction, are transferred to molecular oxygen.
III. The electrode was dipped into the biological sample.
IV. The dissolved Oxygen was utilized for the production of hydrogen peroxide.

Problems were associated with the first generation glucose biosensors.

1. Interference was observed by ascorbic acid, uric acid and certain drugs.
2. For example Acorbic Acid gets oxidize at the electrode and gives false high readings.
3. Oxygen deficit. The method is based on the availability dissolved of oxygen in the biological fluids.
4. It could be effected by certain factors lead to fluctuations in the Oxygen tension.

Second Generation

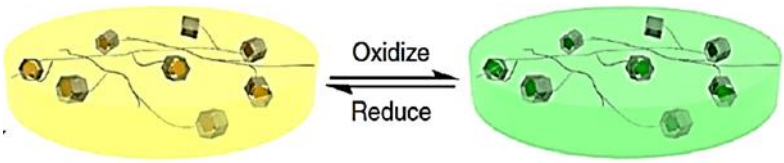
The second generation Biosensors are called mediated glucose Biosensors. Introduced as pen size Biosensor in 1987.A reduced mediator is formed by the action of enzyme. Reoxidation at electrode. Signals are detected for a change in the chemistry of mediator.



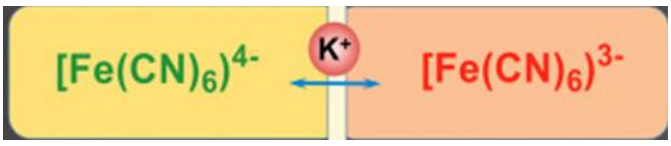
Mediators used in second generation glucose biosensors.

A variety of mediators were used, to improve the sensors performance.

1. Ferrocene
2. Ferricyanide
3. Are the iron based complexes.
4. Methylene blue is an organic dye and used as REDOX indicator.



Pxidised and reduced form of. Ferrocene.



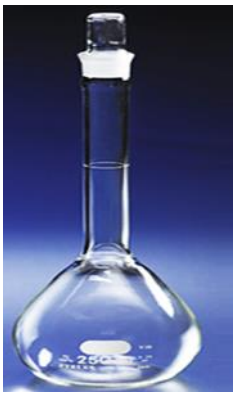
Oxidized and reduced form of Ferricyanide in the presence of K ions.

Qualities of Ferrocenes

A Ferrocenes fits all the criteria for good mediator.

1. Not reacting with Oxygen
2. Independent of pH
3. Stable in oxidized and reduced form
4. Shows reversible electron transfer Kinetics
5. Rapid reaction with GOx

Ferrocenes are used for e shuttling between Gox and GDH-PQQ and the electrodes.

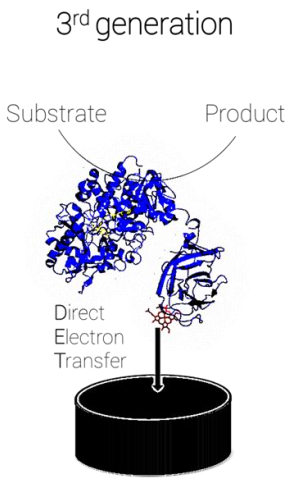


Methylene blue a pH indicator at different pH values

Third generation

In third-generation biosensors, the electrons are transferred directly from the enzyme to the electrode without any intermediate stages or use of nanoparticles.

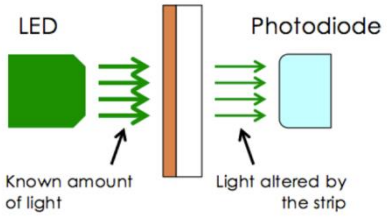
1. Reagent less
2. Mediator less
3. Direct transfer between enzyme and the electrode.



Latest Devices

Two types of latest devices are introduced.

1. Continuous Monitoring glucose system (CGMS)
2. Needle type enzyme electrode for Subcutaneous for subcutaneous implantation are used for CGMS.



An optical Non-Invasive Glucose Monitoring system is shown in Fig using a LED. Optical or transdermal approaches are used for non invasive GMS.

77. **<u>Lactate Oxidase biosensors</u>**

The enzyme lactate oxidase catalyzes the oxidation of L-lactate to pyruvate in the presence of dissolved oxygen. It forms hydrogen peroxide. Hydrogen per oxide is electrochemically active and can be either reduced or oxidized to give a current proportional to the L-lactate concentration.

Lactate detection and quantification plays a vital role in healthcare, especially in conditions such as hemorrhage, sepsis, tissue hypoxia, respiratory failure and kidney

failure. It also has a key role in sports medicine where it is essential in monitoring athletes. Lactate Oxidase is an enzyme which is predominantly used in the detection of Lactate. This has led to development of various biochemical sensors and devices utilizing Lactate Oxidase.

Lactate oxidase is an FMN (Flavin mononucleotide)-dependent alpha hydroxyl acid oxidizing enzyme. It employs Flavin mononucleotide (FMN) as a cofactor.

The enzyme belongs to the family of oxidoreductases, specifically those acting on single donors with O2 as oxidant and incorporation of two atoms of oxygen into the substrate (oxygenases).

Lactate Oxidase is used in biosensors and in vitro tests in order to detect lactate e.g. in blood. Lactate Oxidase participates in pyruvate metabolism. It employs one co-factor, FMN.The enzyme catalyzes the oxidation of L-lactate to pyruvate in the presence of dissolved oxygen and forms hydrogen peroxide:

Lactate Oxidase Sources

LOD is a globular flavoprotein can be obtained from a variety of bacterial sources, such as

1. *Pediococcus*
2. *Aerococcus*
3. *Mycobacterium*

3 D structure of lactate oxidase.

Lactate detection and quantification plays a vital role in healthcare, especially in conditions Hemorrhage , Sepsis, Tissue hypoxia including Respiratory failure.

Significance

Lactate level is widely used in clinical diagnostics for

1. Continuous surveillance in surgery
2. Sports medicine

3. Shock
4. Trauma
5. Food industry.

The baseline lactate level in blood ranges from 0.5 to 1.5 mmol/l at rest.

Lab method for the estimation

In clinical practice blood plasma levels are estimated by chemical method.Lactate oxidase catalyzes the oxidation of lactic acid to pyruvate and hydrogen peroxide. It catalyzes the reaction with a hydrogen donor, in the presence of 4-aminophenazone, to form a coloured complex.

In clinical medicine it is used for the quantitative determination of lactate in human plasma. Lactate oxidase catalyzes the oxidation of lactic acid to pyruvate and hydrogen peroxide. Peroxidase then catalyzes the reaction of hydrogen peroxide with a hydrogen donor, in the presence of 4-aminophenazone, to form a dye.

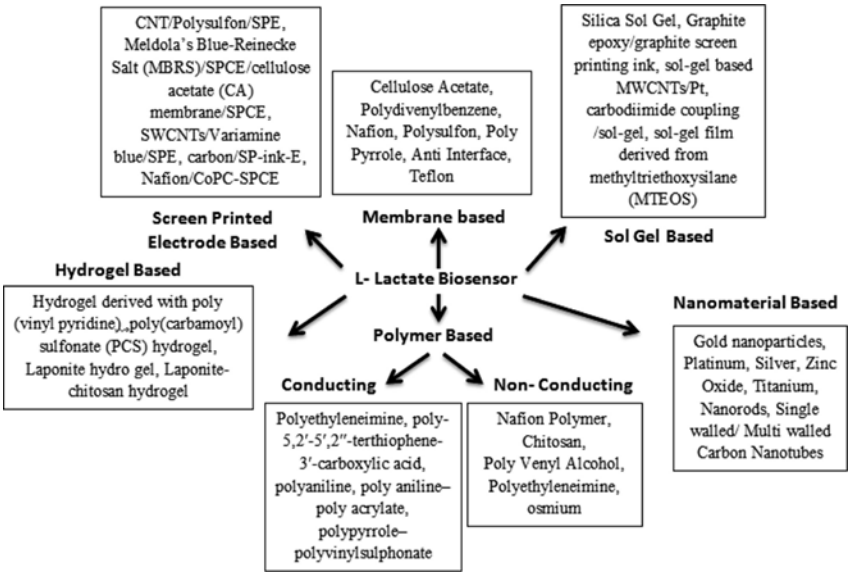
Lactate concentration has been widely used in clinical diagnostics for assessing patient health conditions and study of diseases and for continuous surveillance in surgery, sports medicine, and shock/trauma and food industry. The baseline lactate level in blood ranges from 0.5 to 1.5 mmol/l at rest, but can rise up to 25 mmol/L during the intense exertion. Lactate is a key metabolite of the anaerobic metabolic pathway.

1. Lactate Oxidase is used for the quantitative determination of lactate in blood plasma.
2. It is used as a reagent and enzymes in assays.
3. Due to its ability to catalyze the oxidation of L-lactate to pyruvate, Lactate Oxidase is the principle enzyme used in L-lactate biosensors.

Lactate Oxidase Biosensor

It utilize lactate oxidase and have applications in sports medicine.
It is used to monitor lactate production during exercise. Results in just 10 seconds.
Pocket-sized.It is simple, accurate , reliable lactate analyzer for sports performance monitoring.

Lactate Biosensors are device assemblies that has a biological element (example enzyme-Lactate Oxidase) and a transducer element that converts variations in physical quantity into current (electrical signal). This current is proportional to the quantity of Lactic acid concentration. The molecular weight of Lactate Oxidase is 80 Kda.



Summary

Lactate Oxidase is an enzyme which is predominantly used in the detection of Lactate. Estimation of lactate is important in health and diagnostics.Applications of Lactate Biosensors are found in Health and sports medicine. Recently a LO biosensor is introduced commercially for sportsmen.

78. <u>Alcohol oxidase based biosensor</u>

Alcohol oxidase is responsible for the oxidation of low molecular weight alcohols to the corresponding aldehyde or ketone. It uses molecular oxygen as the electron acceptor. It is a FAD dependent enzyme.

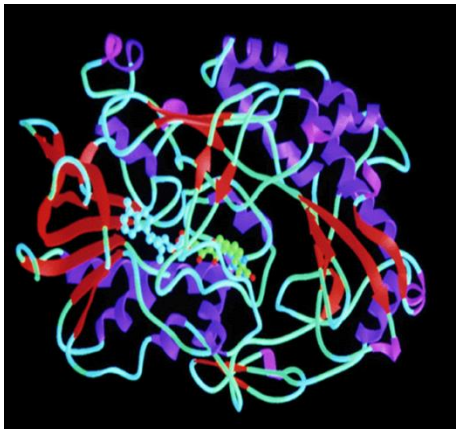
Types

Based on substrate specificity, alcohol oxidases may be categorized broadly into four different groups namely,

1. Short chain alcohol oxidase (SCAO)
2. Long chain alcohol oxidase (LCAO)
3. Aromatic alcohol oxidase (AAO) and
4. Secondary alcohol oxidase (SAO).

Alcohol Oxidase

Alcohol oxidase (AO) is the key enzyme of methanol metabolism. AO is a molecule of high molecular mass (600 kDa). It consists of eight identical subunits. Each subunit carry one non-covalently bound flavin adenine nucleotide (FAD) molecule as the prosthetic group.

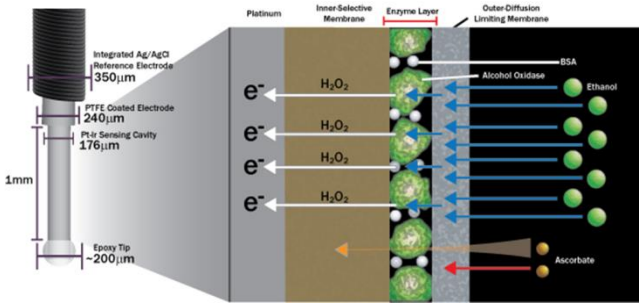


Ribbon representation of the 3D model of H. polymorpha AO monomer . α-helices are presented in purple, β-strands- in red. The ball-and stick model represents FAD.

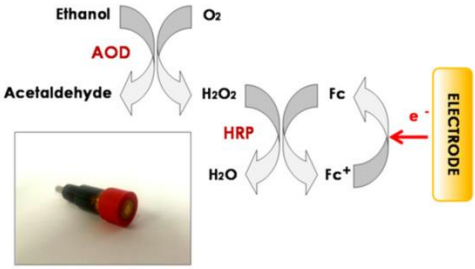
Assay methods rely on consumption of Oxygen or production of Hydrogen per oxide. Alcohol oxidase (AOX; Alcohol:O2 oxidoreductase, EC 1.1.3.13) is an oligomeric enzyme consisting of eight identical sub-units arranged in a quasi-cubic arrangement, each containing a strongly bound cofactor, flavin adenine dinucleotide (FAD) molecule. AOX is the first enzyme involved in the methanol oxidation, however, it is also able to oxidise other short-chain alcohols, such as ethanol, propanol and butanol. AOX is responsible for the oxidation of low molecular weight alcohols to the corresponding aldehyde, using molecular oxygen (O2) as the electron acceptor.

Ethanol Biosensor

Alcohol is produced during metabolic pathways. Alcohol is an important industrial product. Health and diagnostics, Food industry, Medical science, Agriculture and Environment. Ethanol biosensors based on alcohol oxidase have been reported to be stable and practical.



Alcohol Oxidase Biosensor for invivo applications



Automated Bioanalyzer Based on Amperometric Enzymatic Biosensors for the Determination of Ethanol.

The AOX biosensors that monitor the consumption of O2 are commonly used electrochemical methods, although optical methods can also be used. The most common method of monitoring O2 is based on a Clark-type O2 electrode. The first AOX electrode consisted of a platinum electrode on the surface of which the immobilised . AOX was mounted and secured with a nylon cloth and an O-ring. In fact, most ethanol sensors based on O2 probes consist of an electrode covered by a membrane onto which AOX was immobilised. The signal output of the electrode is the difference between the base oxygen level (100% saturation) and the level attained as a result of oxygendepletion by the enzymatic reaction.

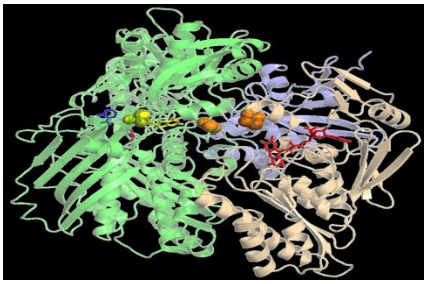
In the optical method, fluorescence-based biosensors for alcohols have been reported in literature. For instance, an optical bio-sniffer for ethanol vapours was constructed by immobilising AOX onto a tip of a fibre optic oxygen sensor coated with an oxygen sensitive ruthenium complex. The bio-sensing principle is based on quenching of the ruthenium complex, in the presence of O2 molecules (both liquid and gas-phases).

Summary

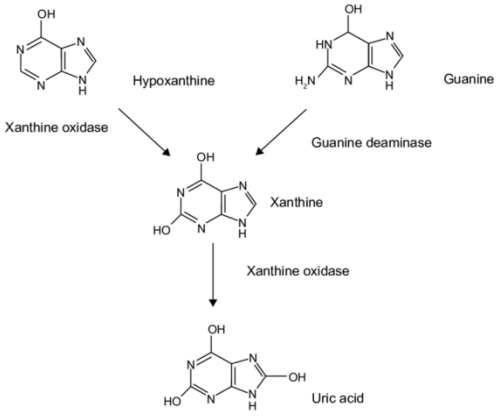
Alcohol oxidase is an Fad dependant important enzymes. Alcohol oxidase biosensors are used for the detection of alcohol in diagnostics and industries, to control the process technology. Biosensors are designed to invivo applications, such as to monitor brain activity.

79. XANTHINE OXIDASE–BASED BIOSENSORS

Xanthine and Hypoxanthine are the substrates for Xanthine oxidase. XO requires two Flavin adenine di nucnleotide molecules for its acrivity. It has two sites for binding with Molybdenum ions. XO can bind with eight FeS clusters.



Crystallographic structure (monomer) of bovine xanthine oxidase,. The bounded FAD (red), FeS-cluster (orange), with molydenium (yellow) are indicated.



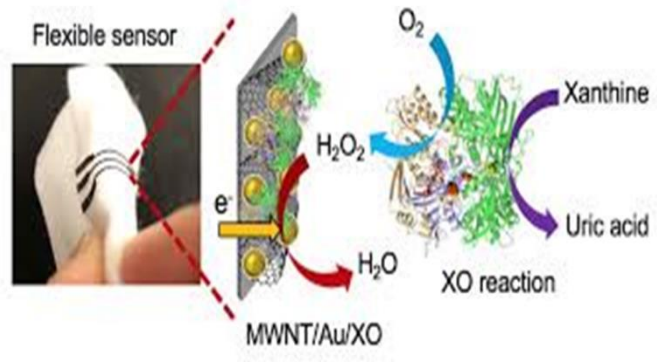
These metallic ions and co factor and take part in the reaction.

Clinical Significance

1. Xanthine oxidase is a superoxide -producing enzyme.
2. It is found normally in serum and the lungs, and its activity is increased during influenza A infection.
3. During severe liver damage, xanthine oxidase is released into the blood.
4. Blood assay for XO is a way to determine if liver damage has happened.

Xanthine Oxidase Biosensor

XO is a source of reactive oxygen species which can amplify and potentiate inflammation and delay wound healing.



For tracking the healing progress, a Xanthine oxidase biosensor is fabricated. Inhibition of XO holds a potential for improving wound healings.

The main application for xanthine biosensors in the food industry is for monitoring fish freshness. Upon the death of a fish, adenosine triphosphate (ATP) degrades to eventually produce xanthine, which can then be detected by an XOX biosensor. Loss of enzymatic activity is one of the factors.

Summary

Xanthine oxidase is a REDOX enzyme. Biosensors based on the enzyme are of great importance. The enzyme biosensor can monitor the wound healing. Xanthine oxidase Biosensors are also designed to monitor the fish and meat freshness.

80. Cardiac Troponin Biosensors

Troponins are a group of proteins found in skeletal and heart (cardiac) muscle fibers that regulate muscular contraction. Troponin tests measure the level of cardiac-specific troponin in the blood to help detect heart injury. After a heart attack, cardiac biomarkers are released from the damaged myocytes into the bloodstream

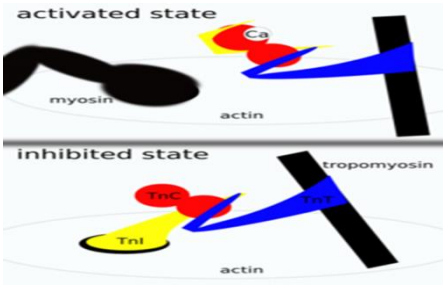
Troponin, or the troponin complex, is a complex of three regulatory proteins

1. Troponin C
2. Troponin I
3. Troponin T

It is integral to muscle contraction in muscle.

cTI and cTt are used as diagnostic and prognostic indicators in the management of AMI.

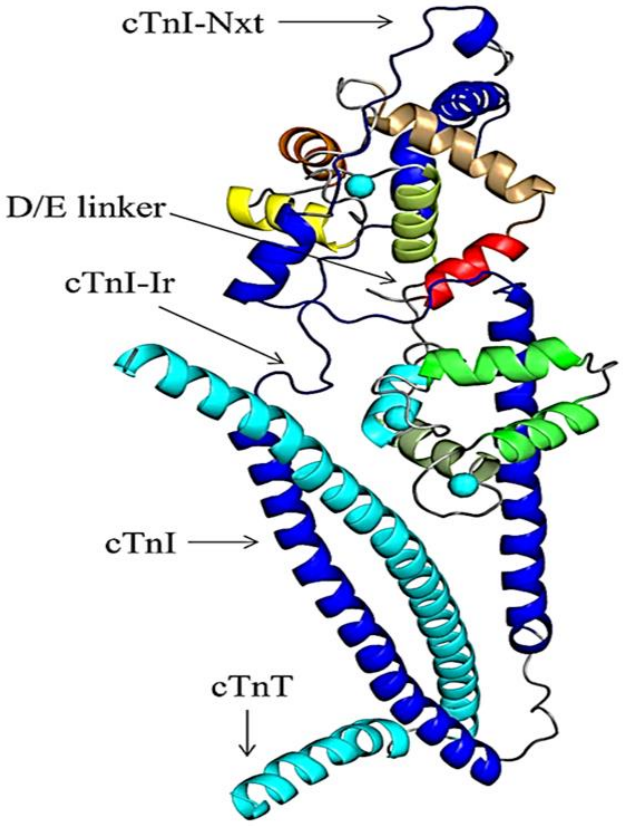
Troponin Complex Function



1. Troponin C binds to calcium ions to produce a conformational change in TnI.
2. Troponin T binds to tropomyosin, interlocking them to form a troponin-tropomyosin complex
3. Troponin I binds to actin in thin myofilaments to hold the troponin-tropomyosin complex in place.

Troponin Complex structure.

Troponin (Tn) is the sarcomeric Ca2+ regulator for striated (skeletal and cardiac) muscle contraction. On binding Ca2+ Tn transmits information via structural changes throughout the actin-tropomyosin filaments, activating myosin ATPase activity and muscle contraction.



Structure of troponin complex is shown in Fig. cT nT and cTnI are colored cyan(blue green) and blue respectively. The cTnC helices N, A, B, C, D in the N-domain are colored, red, mustard, orange, yellow and lime green. The bound Ca2+ ions at sites 2, 3, and 4 are rendered as spheres and colored cyan.

Troponin I

Cardiac Troponin I (cTnI) is a subtype of the troponin family, a marker for myocardial damage. Cardiac troponin I is a very sensitive and specific indicator of myocardium damage. Serum levels can be used to help differentiate between unstable angina and myocardial infarction.

Significance of cTI

The isoform of cTnI in myocardial tissue differs from the sequence of those in skeletal muscles. The cTnI conc. can go up to 50 ng/mL within 3–6 h, and finally to a level around 550 ng/mL. The cTnI level will go back to its standard status in 6–8 days, such that the diagnostic window is long enough for detection.

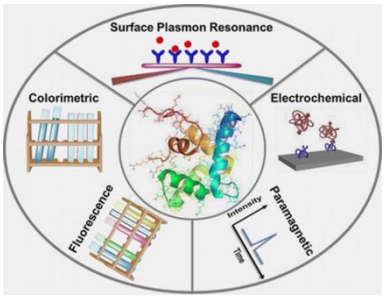
Results are given in nanograms per milliliter (ng/mL). The normal range for troponin is between 0 and 0.4 ng/mL. Other types of heart injury may cause a rise in troponin levels. It is noted that distinct rise and fall. typically rise 4-8 hours post onset of symptoms in MI. peaks at 18-24 hours. levels stay elevated for 10 days (allows late diagnosis of MI, may detect re-infarction with serial testing) The most important cardiac biomarkers related to MI which are measured through blood analysis are e different types of **troponin protein** .

Earliest Cardiac Markers

The earliest cardiac biochemical marker employed includes

1. Lactate dehydrogenase isoenzymes
2. Aspartate aminotransferase
3. Lactate dehydrogenase.
4. ECG (only 57% diagnosis)

However, none of enzyme display high specificity to AMI



Different types of Biosensord for the detection of tropin lavels are illustrated in Fig.

The electronic part is used to amplify and digitalize the physicochemical output signal from the transducer devices such as electrochemical (potentiometric, conductometric, capacitative, impedance, amperometric), optical (fluorescence, luminescence, refractive index), and microgravimetric devices.

Except troponin, the non-specificity against MI is an important diagnostic challenge in using some of these cardiac biomarkers. Nowadays, Myocardial infraction biomarkers have been detected by various methods, including immunosensing such as those based on the surface plasmon resonance, electrochemiluminescence , immunohistochemistry , enzyme-linked immunosorbent assay (ELISA) , fluorescence

resonance energy transfer and field-effect transistors, aptasensing, liquid chromatography , and chemiluminescence.

A few examples of troponin based Biosebsor are discussed in lecture.

81. An introduction to immuno Biosensors

The biochemical detection systems that use antibodies or antigens as the recognition element, known as immunosensors .The basis of such detection is the complex formation between antibody and antigen.Sometimes it is not required to use the whole antigen molecule, therefore AB fragments are used.

Structure of an immunobiosensor

A typical immune biosensor is composed of two main components.

1. Biological Component
2. Physical Component

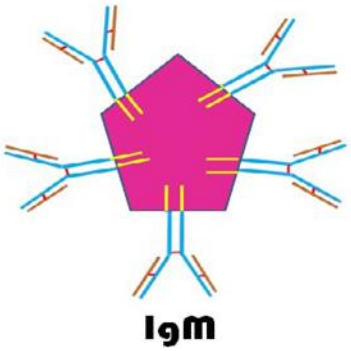
The biological component is composed of an immunogenic molecule, that is responsible for the detection of target molecule or analyte by binding with it.

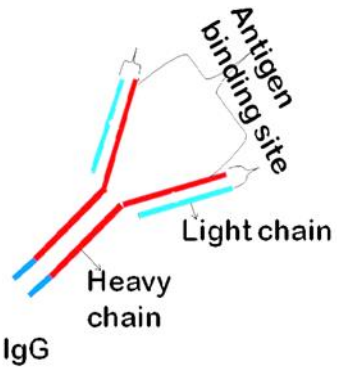
The physical component detect the biorecognition signals.Immuno-biosensor is a type of biosensor to detect the formation of an immunocomplex using an antibody or antigen as a bioreceptor. Immunosensors are affinity ligand-based biosensing devices that involve the coupling of immunochemical reactions to appropriate transducers.

Types of Antibody

There, there are 2 kind of antibodies .

1. IgM is the first antibody to fight against, and attack. IgM is a pentamer unit with ten antigen binding sites, but only five are functional.
2. IgG reacts later but help in the permanent eradication of antigens. IgG It has two high affinity binding sites.





Types of Antibody.

Antibody Affinity

Antibody affinity is the strength with which the epitope binds to an individual paratope (antigen-binding site) on the antibody.

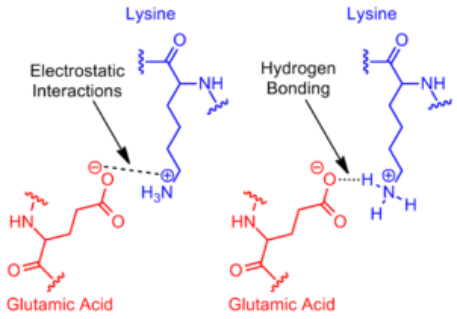
1. High affinity antibodies has following properties.
2. They bind quickly to the antigen.
3. They permit greater sensitivity in assays
4. They maintains this bond more readily under difficult conditions.

Forces involved in Binding

The formation of Ab-Ag complex is dependent on certain forces. All these forces are weak physical forces.

1. Hydrophobic forces
2. Hydrogen bonding
3. Salt bridge
4. Van der Wall forces
5. Electrostatic interactions
6. These forces could be altered by change in pH, salt conc.

In biochemistry, a salt bridge is a combination of two non-covalent interactions: hydrogen bonding and ionic bonding (Figure 1). Ion pairing is one of the most important noncovalent forces in Biochemistry, in biological systems, in different materials and in many applications such as ion pair chromatography. It is a most commonly observed contribution to the stability to the entropically unfavorable folded conformation of proteins.



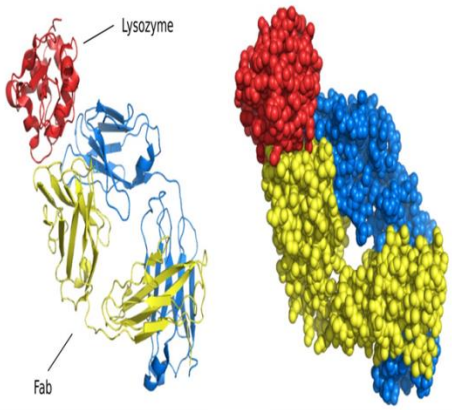
Example of salt bridge between amino acids glutamic acid and lysine demonstrating electrostatic interaction and hydrogen bonding.

Strength of Antibody and antigen complex

The strength of antigen-antibody complex is based on weak physical forces, that could be effected by pH, ionic strength and temperatureThe strength of the binding of the antigen-antibody complex could be characterized by its affinity constant that is K. The value of K in the order between 5x104 – 1x10 12 per mole liter , is expected to be for high affinity antigen-antibody complex.

Here, the highly specific reaction between the variable regions of an antibody and the epitopes of an antigen involves different types of bonding, basically hydrophobic and electrostatic interactions, van der Waals force, and hydrogen bonding.

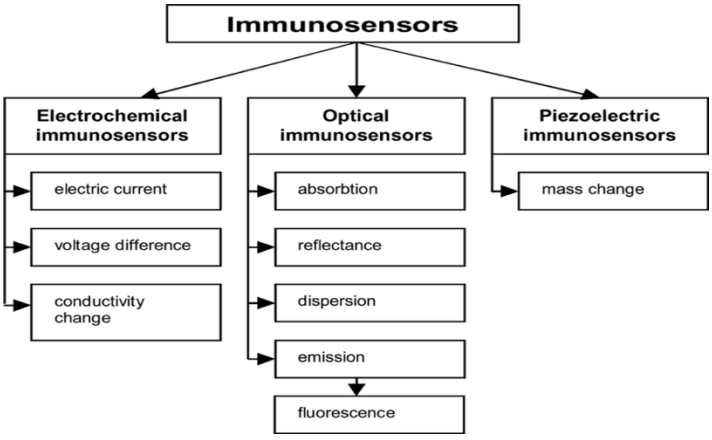
The antigen–antibody reaction is reversible and, owing to the relative weakness of the forces holding the antibody and antigen together, the complex formed would dissociate in dependence upon the reaction environment (e.g. pH and ion strength). The strength of the binding of an antibody to an antigen could be characterized by its affinity constant (K), which is of the order between 5 × 104 and 1 × 1012 L mol−1. The high affinity and specificity of this antigen–antibody binding reaction defines the unique immunosensor characteristics.



Binding of lysozyme and Fab fragment from antibody.

Immuno sensors.

The general immunosensor design consists of three individual parts in close contact: a biological recognition element, a physicochemical transducer, and an electronic part.



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Antibodies or antibody derivatives (antigens or haptens) usually serve as the biological recognition elements, which are either integrated within or intimately associated with a physicochemical transducer. This recognition reaction defines the high selectivity and sensitivity of the transducer device.

82 . Enzyme Link Immunoabsorbent Assay (ELISA)

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying soluble substances such as peptides, proteins, antibodies, and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same technology. In an ELISA, the antigen (target macromolecule) is immobilized on a solid surface (microplate) and then complexed with an antibody that is linked to a reporter enzyme. Detection is accomplished by measuring the activity

of the reporter enzyme via incubation with the appropriate substrate to produce a measurable product. The most crucial element of an ELISA is a highly specific antibody-antigen interaction.

The enzyme linked immunosorbent assay (ELISA) is a powerful method for detecting and quantifying a specific protein in a complex mixture. Originally described by Engvall and Perlmann (1971), the method enables analysis of protein samples immobilized in microplate wells using specific antibodies. ELISAs are typically performed in 96-well or 384-well polystyrene plates, which passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to use high-affinity antibodies and wash away non-specific bound materials makes ELISA a powerful tool for measuring specific analytes within a crude preparation.

The most commonly used enzyme labels are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Other enzymes have been used as well; these include β-galactosidase, acetylcholinesterase, and catalase. A large selection of substrates is available commercially for performing ELISA with an HRP or AP conjugate. The choice of substrate depends upon the required assay sensitivity and the instrumentation available for signal-detection (spectrophotometer, fluorometer, or luminometer).

ELISA formats–direct, indirect, and sandwich ELISA

There are several formats used for ELISAs. These fall into either direct, indirect, or sandwich capture and detection methods. The key step is immobilization of the antigen of interest, accomplished by either direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (such as labeled secondary antibody). The most widely used ELISA assay format is the sandwich ELISA assay, which indirectly immobilizes and indirectly detects the presence of the target antigen. This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies, each detecting a different epitope of the antigen–the capture antibody and the detection antibody. The sandwich ELISA format is highly used because of its sensitivity and specificity.

ELISA plates

When developing a new ELISA for a specific antigen, the first step is to optimize the pl ELISA

- E Enzyme
- L Linked
- I Immuno
- S Sorbent
- A Assay

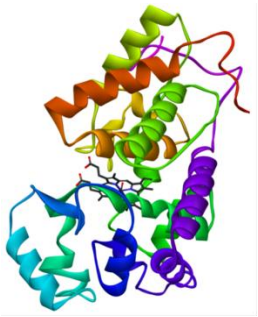
It is also known as enzyme immunoassay (EIA).

Two types of Biological molecules are involved.

1. Antibody
2. Enzyme

Enzyme Horse Reddish peroxidase

The enzyme horseradish peroxidase (HRP), found in the roots of horseradish, is used extensively in biochemistry applications. It is a metalloenzyme with many isoforms, of which the most studied type is C. It catalyzes the oxidation of various organic substrates by hydrogen peroxide. HRP belongs to the ferroprotoporphyrin group of peroxidases. The optimal pH is 6.0-6.5 with stability. stable in the pH range of 5.0-9. HRP is a single chain polypeptide containing four disulfide bridges with Mol Wt of about 44Kd with six Lysine residues suitable for conjugation with a protein. One commonly used enzyme conjugate in ELISA is horseradish peroxidase. Horseradish peroxidase (HRP) catalyzes the reduction of hydrogen peroxide (H2O2) to water (H2O).



3-D structure of Hore reddish per oxidase enzyme.

Enzyme conjugation

HRP can be conjugated to antibodies glutaraldehyde, periodate oxidation, through disulfide bonds, and amino and thiol directed cross-linkers. HRP is smaller and more

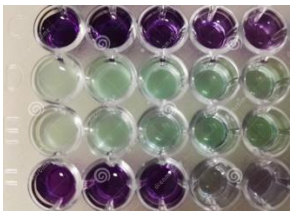
stable than the enzyme labels β-galactosidase and alkaline phosphatase. Its glycosylation leads to lower non-specific binding.

In market ELISA Plates are available with a variety of surfaces to optimize coating with the macromolecule of your choice. These plates are designed to deliver optimal results, lot-to-lot reliability, and well-to-well reproducibility. Polystyrene as Sorbent Material

Polystyrene is a long carbon chain .

ELISA kits are commercially available.ELISAs tests are typically performed in 96-well (or 384-well) polystyrene plates, which bind antibodies and proteins. On polystyrene it easy to separate bound from non-bound material during the assay.

I. Surface flavoring are introduced to change the binding properties of the matrix.



ELISA plates

II. Different functional groups are attached for binding with Bio molecules.
III. Surface chemistry can also be changed by UV treatment.
IV. Plate coating is achieved through passive adsorption of the protein to the plastic of the assay microplate.
V. This process occurs though hydrophobic interactions between the plastic and non-polar protein residues. Although individual proteins may require specific conditions or pretreatment for optimal binding.

Mechanism

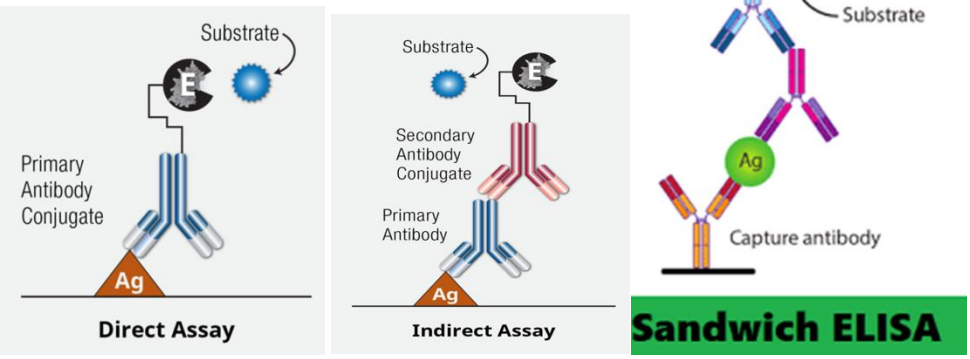
An antigen is immobilized on a solid surface. Antigen forms a complex with an antibody that is linked to an enzyme. The enzyme is incubated with a substrate to produce a measurable coloured product. Highly specific antibody-antigen is very important for interaction.

Types of ELISA

There are at least five types of ELISA assay,

1. Direct ELISA Assay
2. Indirect ELISA Assay
3. Sandwich ELISA Assay
4. Competitive Assay
5. Multiplex ELISA Assay

Other variations are also available binder, and detection methodology



Sandwich ELISA Test

The Sandwich ELISA is a sensitive and robust method which measures the antigen concentration in an unknown sample.

The Antigen of interest is quantified between two layers of antibodies:

1. Capture Antibody
2. Detection Antibody**.**

Summary

ELISA is a useful method for the detection. The signals are amplified by the enzyme HRP.
The enzyme is suitable for conjugation due to the availability of lysine residues and thiol groups. .
The modified form of polystyrene is used for immobilization.
There are several types of ELISA methods.

83. . Monocolonal Antibody

Introduction

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Monoclonal antibodies (mAb or moAb) are antibodies that are made by identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies can have monovalent affinity, in that they bind to the same epitope (the part of an antigen that is recognized by the antibody). Monoclonal antibodies (mAb) are important reagents used in biomedical research, in diagnosis of diseases, and in treatment of infections and cancer.

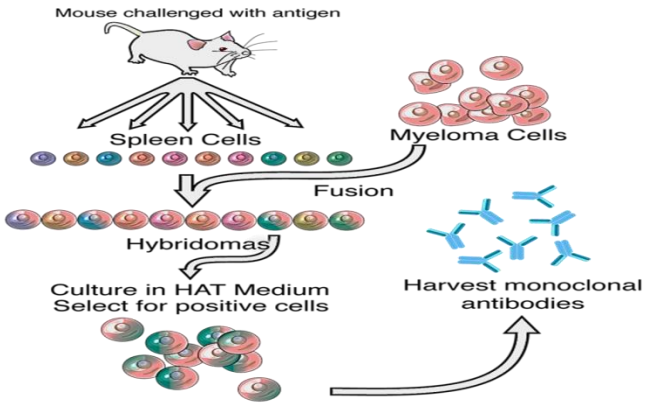
Each Monoclonal antibody is produced against a single epitope. An epitope, is an antigenic determinant, is the part of an antigen that is recognized by the immune system.

Production of Monoclonal Antibody

There are three general ways to produce monoclonal antibodies.

1. Hybridoma technique
2. Ascites method

Hybridoma antibody production method involves the in vivo and in vitro methods while Ascite method involve in vivo synthesis of MABs., which involve the use of animals. Monoclonal antibodies (mAbs) are produced by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells.



The resulting hybridoma cells are cultured and continue to produce antibodies to the antigen.

Ascite Method

Monoclonal antibodies can be produced by growing Hybridoma cells within the peritoneal cavity of a mouse (or rat). The Hybridoma cells are injected into the mouse, multiply and produce fluid (ascites) in its abdomen. This fluid contains a high concentration of antibody which can be harvested.

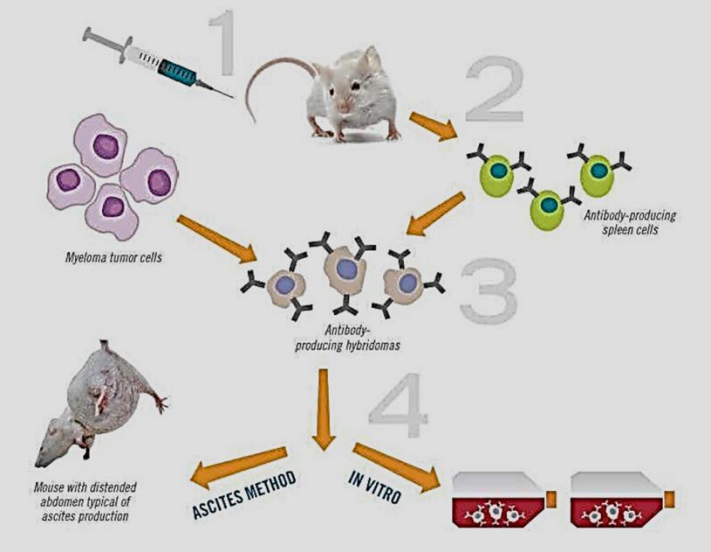


Fig illustrates the Ascite method and Hybridoma technique for MAB production.

Diagnostic Applications

MAB are used RIA and ELISA in the laboratory.

Pregnancy Test . MABS produced against human chorionic gonadotropinare used for detecting the pregnancy, using urine sample.

1. Cancer diagnostics
2. Hormonal Disorders
3. Infectious Diseases.
4. Cardiac Troponin test

Therapeutic Applications

Monoclonal antibodies have a wide range of therapeutic applications.

1. Treatment of cancer
2. Transplantation of bone marrow
3. Transplantation of organs

4. Autoimmune diseases
5. Cardiovascular diseases
6. Infectious diseases.

Monoclonal antibody therapy is a form of immunotherapy that uses monoclonal antibodies (mAb) to bind monospecifically to certain cells or proteins. The objective is that this treatment will stimulate the patient's immune system to attack those cellsThe four types of applications are: (1) Diagnostic Applications (2) Therapeutic Applications (3) Protein Purification and (4) Miscellaneous Applications.

Diagnostic tests based on the use of MAbs as reagents are routinely used in radioimmunoassay (RIA) and enzyme-linked immunosorbent assays (ELISA) in the laboratory. These assays measure the circulating concentrations of hormones (insulin, human chorionic gonadotropin, growth hormone, progesterone, thyroxine, triiodothyronine, thyroid stimulating hormone, gastrin, renin), and several other tissue and cell products (blood group antigens, blood clotting factors, interferon's, interleukins, histocompatibility antigens, tumor markers). In recent years, a number of diagnostic kits using MAbs have become commercially available. For instance, it is now possible to do the early diagnosis of the following conditions/diseases.

Summary

Monoclonal antibodies are produced by a single types of cells. Each MAP is generated against a specific epitope. There are two methods for the production of MABS. Now a days Ascite method (in vivo) is used for MAB production.MAB has major uses in the field of diagnostics and Therapeutics.

84. Antibody Fragments

For many applications antibody fragments are preferable.

Antibody fragments can be generated by biological, chemical or genetic methods.

Chemical fragmentation utilizes reducing agents to break the disulfide bonds within the hinge region and digestion of the antibody with proteases including pepsin, papain, and ficin.

Chemical and Biological methods.

Chemical and Biological fragmentation utilizes reducing agents to break the disulfide bonds within the hinge region.

Chemicals

Dithotherotol, Cyanogen bromide is also used for initially digesting the protein to get its fragments.

Biological.

Protease enzyme digestion of the antibody with proteases pepsin, papain, and ficin

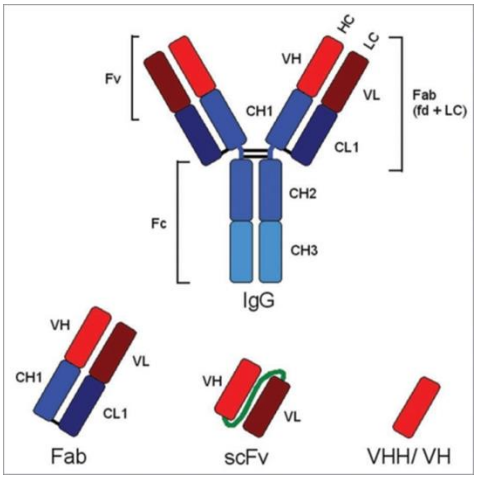
Antibody Fragmentation Process

Antibodies may be enzymatically digested to produce either an Fab or an F(ab') 2 fragment of the antibody. To produce an F(ab') 2 fragment IgG is digested with pepsin, which cleaves the heavy chains near the hinge region.

One or more of the disulfide bonds that join the heavy chains in the hinge region are preserved, so the two Fab regions of the antibody remain joined together, yielding a divalent molecule (containing two antibody binding sites), hence the designation F (ab') 2. The light chains remain intact and attached to the heavy chain. The Fc fragment is digested into small peptides. Fab fragments are generated by cleavage of IgG with papain instead of pepsin. Papain cleaves IgG above the hinge region containing the disulfide bonds that join the heavy chains, but below the site of the disulfide bond between the light chain and heavy chain. This generates two separate monovalent (containing a single antibody binding site) Fab fragments and an intact Fc fragment. The fragments can be purified by gel filtration, ion exchange, or affinity chromatography. IgG antibodies are immunoglobulin homodimers of heterodimers; they contain 2 heavy chains (50 kDa) each paired with a light chain (25 kDa). Heavy and light chains, as well as their dimerization, are stabilized through covalent disulfide bridges. Enzymatic cleavage of specific disulfide bonds results in fragments of differing composition.

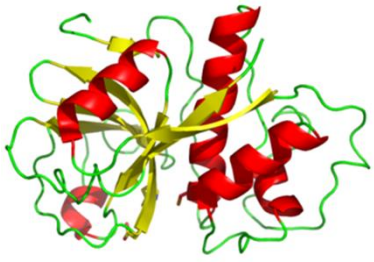
The use of papain, an enzyme derived from papaya, results in reduction of exposed disulfide bonds in the hinge region of the antibody. This separation, or fragmentation, breaks the antibody into three segments; 2 Fabs consisting of equal parts heavy chain and light chain, and 1 Fc region.

1. Antibody fragment types.
2. CH constant heavy chain
3. CL constant light chain
4. Fab antigen binding fragment
5. scFv single chain variable fragment
6. VH variable heavy chain;
7. VL variable light chain.

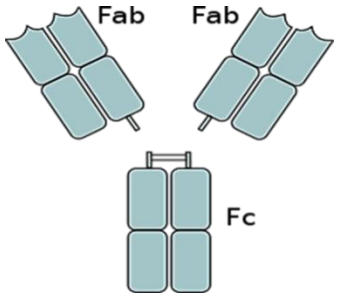


Papain Enzymatic digest

1. Papain is a Cys protease.
2. It cleavage results in reduction of exposed disulfide bonds in the hinge region of the antibody.
3. 2 Fabs consisting of equal parts heavy chain and light chain,
4. 1 Fc region.



protease



Antibody fragment by Prptease

Fragmentation by Pepsin

Pepsin digestion results in A F(ab')2 fragment composed of two Fab fragments still linked together by the remaining portion of their respective heavy chains at the hinge region. The Fc fragment is extensively degraded into small fragments.

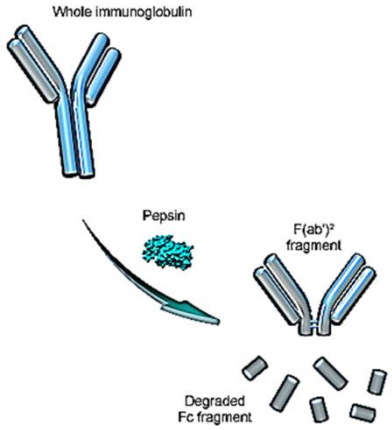


Fig shows fragmentation by pepsin.

Fragment crystallizable region

Fragment crystallizable region (Fc region) is the tail region of an antibody. It interacts with cell surface receptors called Fc receptors and some proteins of the complement system. This property allows antibodies to activate the immune system.

Recombinant Fragments of antibody

1. Well characterized and selected regions of antibody are produced by recombinant DNA technology.
2. Target specific Antibody fragments are designed and produced for best efficiency.Recombinant antibodies offer several advantages over both traditional monoclonal and polyclonal antibodies:
3. Improved consistency and reproducibility. Because recombinant antibodies are developed from a unique set of genes, antibody production is controlled and reliable..
4. With recombinant technology, it is easier to improve both antibody specificity and sensitivity through antibody engineering.
5. The selection process for the desired clone occurs at both the hybridoma and recombinant cloning stages, allowing us to select the most favorable antibody qualities. they can generate tailored antibodies in weeks rather than months.

6. Once the antibody-producing gene is isolated, animal-free in vitro production can be implemented.
7. Purification post-fragmentation is performed to remove unwanted Fc region, accomplished through protein A/G affinity or size exclusion chromatography.

85. Lateral Flow Assay (LFA)

Different industry sectors and different countries use varying terminology to describe a lateral flow immunoassay. Common names include:

1. Lateral flow test (LFT)
2. Lateral flow device (LFD)
3. Lateral flow assay (LFA)
4. Lateral flow immunoassay (LFIA)
5. Lateral flow immunochromatographic assays
6. Dipstick
7. Express test
8. Pen-side test
9. Quick test
10. Rapid test
11. Test strip

Basically, it is a simple to use diagnostic device used to confirm the presence or absence of a target analyte, such as pathogens or biomarkers in humans or animals, or contaminants in water supplies, foodstuffs, or animal feeds. The most commonly known type of lateral flow rapid test strip is the pregnancy test.

LFDs typically contains a control line to confirm the test is working properly, along with one or more target or test lines. They are designed to incorporate intuitive user protocols and require minimal training to operate. Lateral flow tests are widely used in human health for point of care testing. They can be performed by a healthcare professional or by the patient, and in a range of settings including the laboratory, clinic or home. In the medical diagnostic industry, there are strict regulatory requirements which must be adhered to for all products developed and manufactured and Abingdon Health can guide you through this process.Due to their versatile nature, lateral flow rapid tests are used across a number of other industry sectors including pharma, environmental testing, animal health, food and feed testing, and plant and crop health.

Types of lateral flow tests

Lateral flow assays can be developed to be used in a dipstick format or in a housed cassette. Both dipsticks and housed tests will work in a similar way, it is just dependent on the industry, sample matrix, and the market requirement, as to which format is suitable.

1. Sandwich assays – A positive test is represented by the presence of a coloured line at the test line position.
2. Competitive assays – A positive test is represented by the absence of a coloured line at the test line position.

Sample matrices

The target analyte and the market requirements will determine the type of sample that will be used in the assay. Some samples require running buffer to aid sample delivery e.g. animal feed. Other samples such as blood, serum, urine, or saliva may be able to be placed directly onto a test, while there are occasions where a dilution buffer is required.

LFDs are versatile enough to be developed to detect target analytes in sample matrices including:

1. Milk
2. Whole blood
3. Serum
4. Saliva
5. Urine
6. Tissue samples
7. Food
8. Drink
9. Animal feed
10. Plant material
11. Water
12. Fuel

Label types

Typically, lateral flow assays utilise conjugated gold, carbon, or coloured latex nanoparticles within the conjugate pad. Other labels include magnetic beads or coloured polystyrene beads. Regardless of the label types, they all perform the same

function to create a three-way bond with antibodies and targets in order to make visible the control and test lines. Labels will be chosen during lateral flow assay development depending on several factors such as the target, sample matrix and antibody.

Multiplexed lateral flow assays

Both sandwich and competitive assays can be developed to include one or more test lines. For example, our nucleic acid lateral flow immunoassay, PCRD, is a multiplexed sandwich assay containing two test lines and one control line. In addition, by using complementary reader technology, sandwich and competitive multiplexed assays can produce quantitative results.

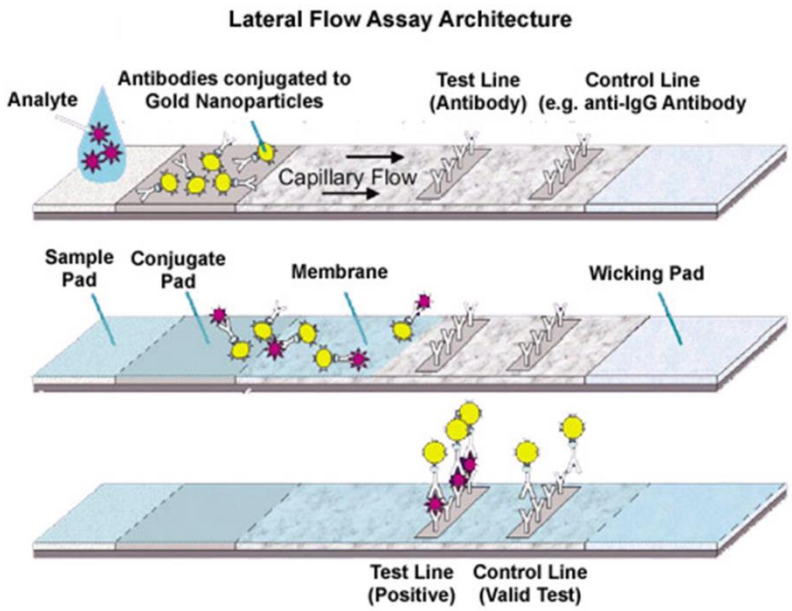
Quantitative rapid lateral flow devices

Using names such as rapid test or quick test can lead to myths about lateral flow devices that they are limited in their capability. However, lateral flow devices are compact, easy-to-use, and offer considerable flexibility.

Early versions of LFDs were predominantly qualitative assays. However, improvements in reagents, component materials, and reader technologies along with manufacturing processes mean quantitative results are achievable.

Working

LFDs use immunoassay technology using nitrocellulose membrane, coloured nanoparticles (or labels), and typically antibodies, to produce results. Antibodies binding on lateral flow immunoassay test lines. When a sample is added, the sample will flow along the test device passing through the conjugate pad into the nitrocellulose membrane and then onto the absorbent pad.



The steps in the working of LFA are given below.

1. The sample pad acts as the first stage of the absorption process, and in some cases contains a filter, to ensure the accurate and controlled flow of the sample.
2. The conjugate pad, which stores the conjugated labels and antibodies, will receive the sample. If the target is present, the immobilised conjugated antibodies and labels will bind to the target and continue to migrate along the test.
3. As the sample moves along the device the binding reagents situated on the nitrocellulose membrane will bind to the target at the test line. A coloured line will form and the density of the line will vary depending on the quantity of the target present. Some targets may require quantification to determine target concentration. This is where a rapid test can be combined with a reader to provide quantitative results.
4. The sample will pass through the nitrocellulose membrane into the absorbent pad. The absorbent pad will absorb the excess sample. The specification of the absorbent pad will have an impact on the volume of sample the test can incorporate

Summary

Lateral flow assay is the simplest testing method. It uses the immunological techniques to detect the molecule. The dipstick designed is used for the test. LFA is in heavily used in industry, diagnostics, environment and other fields of life.

86. Optical Immunosensors

In optical biosensors, the interaction between the AB (BRE) and AG (Analyte) is marked by formation of AB-AG complex, leading to a change in the optical properties of the wave.

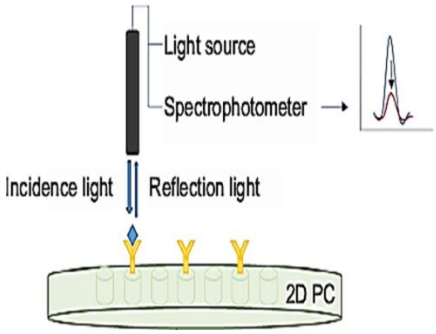
1. Reflectance
2. Absorbance
3. Refractive index
4. Optical path
5. Emission of Light.

Geometric Design of optical Platform

There are several geometric layouts, including

1. Strips
2. Waveguide fibers
3. Planar optical waveguide systems
4. Capillary sensors
5. Arrays.

Key point is the position of light source to detect the Biorecognition process.



Planar waveguide platforms

Planar wave guided platform is a popular deign as Planar geometry is compatible with micro fabrication technologies For example Lab on chip.The planar waveguide platform is based on different materials

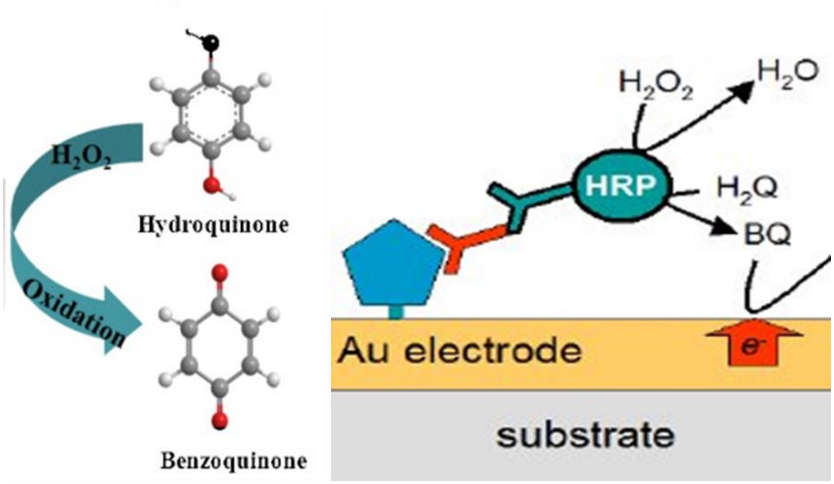
1. Glass
2. Plastic
3. Silicon

Some Methods or Techniques employed in optical immunosensors

Optical Signal	**Methods/Transducing technique**
Absorbance	Light intensity measurement (Different absorbance for different colours)
Reflectance	Light intensity measurement
Fluorescence	Total internal reflection fluorescence
Refraction index	(SPR) Total internal reflection

Use of mediator in Optical sensors

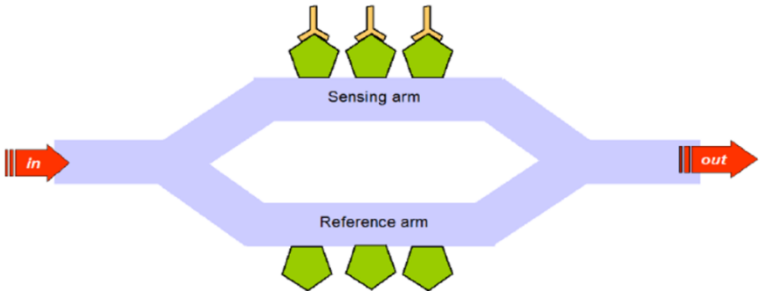
Hydroquinone is an organic compound and used as a mediator in optical Biosensors. With reference to biological organisms, It is present in abdomen of **Bombardier beetle** and employed as defensive agent.
Hydroquinone is reduced to 1,4-benzoquinone by the enzymatic reaction.



Use of Hydroquinone as mediator in Optical sensors . To detect the signals a medicator is used. Hydroquinone is converted into benzoquinone (bright-yellow c

Refractometric sensors

The refractive index of a material is defined as the ratio of velocity of light in a vacuum to that in a defined material. In a special interferometer the optical power is transported by a single-input waveguide. It a Y shape splitters. both branches are coated with the biological sensitive element. rystals) that is electrochemically reduced at the electrode surface.



At the sensing layer the formation of Ab-Ag complex leads to a change in the refractive index. For reference layer there is no change in the refractive indexUpon recombination, interference of the two optical signals occurs.

Summary

The optical immunosensors looked for the change in the optical properties, after the bio-recognition process. The Geometric Design of optical Platform is the pivotal in immunosensors. Hydroquinone is widely used for change in colour as an optical signal

87. **Surface Plasmon Resonance Immunosensor**

Surface plasmon resonance (SPR) is a spectroscopic method. The Biorecognition element is immobilized on the surface of thin metal. A wave is used to detect the ligand on a thin metal film. The change in refractive index upon binding of the analyte, is measured.

pH and Type of Buffer solution

Antigen antibody complex formation is a physiological phenomenon and it occurs at pH 7.4 and 10mM strength. PBS (Phosphate buffer saline) antigen antibody complex formation. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)

Metals and protein interactions

The Protein-metal interaction depends on functional groups of amino acids and the position of metal in the periodic table.

1. Alkali metals interact wealy.
2. Alkaline earth Metals interacts most strongly.
3. Transition earth Metals due to the availability of d-orbital stands a good chance for forming a protein metal complex.

Amino acids in metal interaction

Four groups of amino acids are mainly involved in interaction with metals..

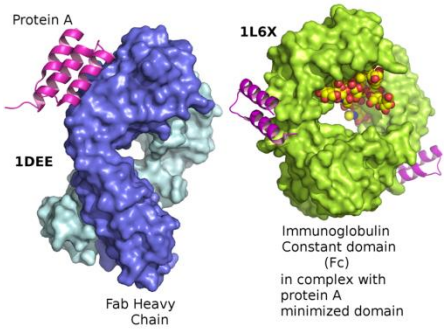
1. Aspartic acid and Glutamic acid involve the carboxylate chain is involved.
2. Cysteine and Methionine involve the Thiol and Thioether groups.
3. Histidine in which the Imidazole group is involved
4. Serine, Threonine and Tyrosine involve Hydroxyl groups

Immobilization of Fc Antibody fragment using Bacterial Proteins

There are three types of proteins produced by bacteria *Staphylococcus aureus*.

1. Protein A is a 42 kDa
2. Protein G 65-kDa
3. Protein G 58 kDa

They have specific binding affinity has binding affinity with the Fc region and Fab region of the Immunoglubulins. Immobilization of Fc Antibody fragment using Proteins



Kinetic parameters for Antigen Antibody complex

Kinetic parameters give information about affinity of the complex formed. Association rate constant (ka) is indicative of number of AG-Ab complex formed per second'. The dissociation rate constant (kd) is the rate of decay of complex formation per second. For ahigher affinity complex the rate of complex formation is high.

Use of flouropore in SPR immune sensors

A fluorophore is a fluorescent chemical compound that can re-emit light upon light excitation. The secondary antibody is conjugated with the fluorescence probe. The fluorescence probe enhanced the sensitivity of the SPR Immuno Sensor.

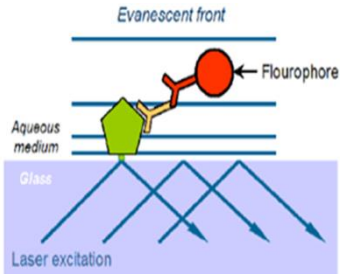


Fig illustrating flouropore in SPR immune sensors

Summary

SPR immune sensor uses a spectroscopic method for the detection of Anlyte. A thin metal film is used for the immobilization of the Biological element. Proteins has a biniding affinity for proteins. In SPR Fluorophore molecules are used to enhance the signals. Physiological pH is used in SPR Immuno sensors.

88. Electrochemical Immuno Biosensors

In electrochemical immunosensors, the event of the formation of antigen-antibody complex is converted into an electrical signal: an electric current (amperometric immunosensors), a voltage difference (potentiometric immunosensors), or a resistivity change (conductimetric immunosensors).

Magnetic beads for immobilization

The magnetic beads for the immobilization of BRE.

The magnetic beads are fixed onto the surface of electrodes by applying the magnetic field. Antigen can be immobilized onto the surface of magnetic beads. After running a test beads can be removed from the electrode, and electrode can be reuse.

The most common type of amperometric immunosensors can be regarded as ELISA tests with electrochemical detection, where redox species generated by a redox enzyme (enzymatic label) are converted into a measurable current.

An usual strategy is to immobilize the antigen onto the surface of a conductive electrode such as gold through adequate molecular linkers, for instance amino or carboxylic acid thiols. Thiols strongly bond to the gold surface, forming a self-assembled monolayer and providing the amino or carboxylic groups at the end of a small hydrocarbonated chain to which proteins can be covalently bonded.

The formation of the antigen-antibody labeled antibody complex is detected after the addition of the enzyme substrate and a proper redox mediator (cofactor).

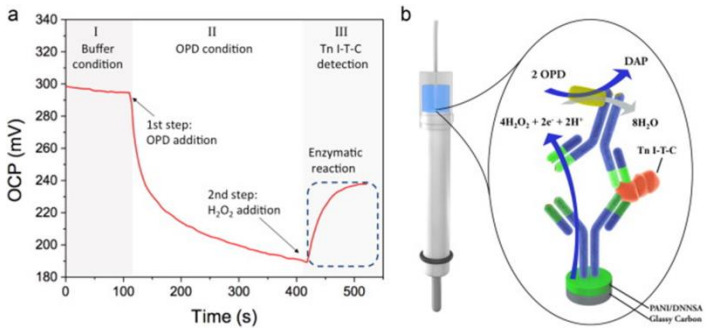
In the case of HRP, the substrate is hydrogen peroxidase and the redox mediator must be an adequate electron donor (a reduced species such as hydroquinone). HRP enzymatic activity converts the reduced redox mediator (hydroquinone) into an oxidized one (benzoquinone), which is further electrochemically reduced at the electrode surface.

Thus, a steady-state current is established in a process. For negative sera, no antigen-antibody-enzyme labeled antibody complexes are formed in the first place so that the measured current values are considerably lower. Thus, high current values are indicative of a positive result. In order for the immunosensor to work properly, it is necessary that the enzyme employed as a label must be close to the electrode surface. If the antigen is immobilized onto the electrode surface, this requisite is complied.

Potentiometric Sensors

Polyaniline based matrix is used in potentiometric immune sensors. The colour of PANI is pH dependent. The colour changes from green (acidic solution) to blue (basic solution). Ultrasensitive immunosensor based on potentiometric ELISA was developed to detect troponin I–T–C.

Potentiometric Cardiac Immuno Biosensor



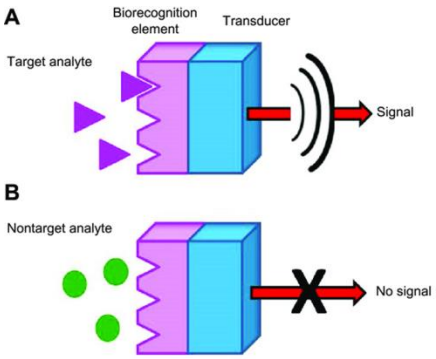
An enzymatic reaction between o-phenylenediamine (OPD) and hydrogen peroxide was catalyzed by HRP labeled on the secondary antibodies. The polymer transducer charged state was mediated through electron (e−) and charge transfers between the transducer and charged species generated by the same enzymatic reaction.

Summary

Electrochemical immune sensors detect the event of Antibody and Antigen complex for the generation of a signal, for the detection of Analyte.Immobilization of Analytes in conductivity electrodes is very important.The biological recognition event produced current that is converted into potentiometric, amperometric, conductometric signals.

89. .Impedance Immuno sensor

1. Impedance is a measure of the opposition to electrical flow, that is measured in ohms.
2. The nature of impedence sensors is all electrical.
3. Impedance biosensors are simpler than other methods because they lack optical or acoustic components.
4. Therefore it offers significant advantages for portable applications**.**



Immobilization

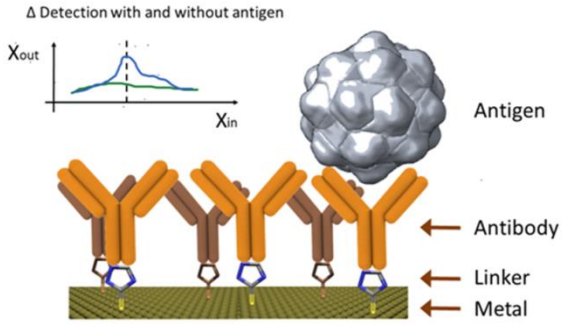
Immobilization of the BRE on the sensing platform is an important step.

Gold Nano-materials are coated on copper electrodes, gold and silver layers for immobilization.

I. Magnetic particles
II. Hydroxyapatite powder
III. ion-exchange membranes
IV. Polymeric films

Working

I. A BRE is immobilized on -functionalized surface, of the impedance Immnuno sensor.
II. When an AG-AB complex formed changes occur in the electrical properties of the surface such as dielectric constant or resistance.
III. These changes could be detected by the transducer.
IV. In immuno impedance sensors no label is required for impedance sensing.



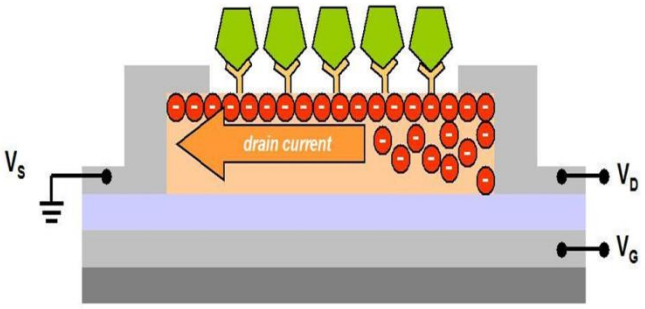
Most common configuration of electro-immuno sensor devices and their response with and without the presence of the antigen.

Field Effect Transistors

I. A FET consists of three terminals, called gate, drain, and source.
II. N-type transistors are used in impedance biosensors.
III. The gate controls the current between the source and the drain.

IV. The BRE is immobilized on the gate.

The formation of antigen-antibody complex at the gate terminal.



Merits and Demerits

Advantages of Impedance immuno sensor are, small instrument size, low cost and speedy.

Suitable for use at a bedside, in an ambulance, or during a clinic visit.

Disadvantages are the use of small analytes, complexity in detection, nonspecific adsorption, and problems stability of biomolecule immobilization.

Summary

impedance immuno biosensors biosensors are based on the determination of resistance as a signal for the detection of Analyte. FET are also used as impedance sensors when an immunogenic molecule is immobilized on the sensing platform of the sensor. Impedance seniors are small in size and have several advantages and disadvantages.

90. Piezo Electric Immuno Sensor

Introduction

1. Piezoelectric materials are used in the physical component of the PZ immuno sensors.
2. PZ materials has a unique ability to generate current when stress is applied.
3. In all type of Immuno sensors Ab-AG complex is considered as a Biorecognition process.
4. This process is determined by the transducers , using a number of techniques.

PZ material in sensors

1. Piezo electric crystals are used in Piezo electric immune sensors, in the physical component of the sensor.

2. In PZ immuno sensor, mass changes due to the formation of antigen-antibody complex, is detected by piezo electric crystal present in the transducer.
3. Generally Quartz Crystals are used .

Immobilization

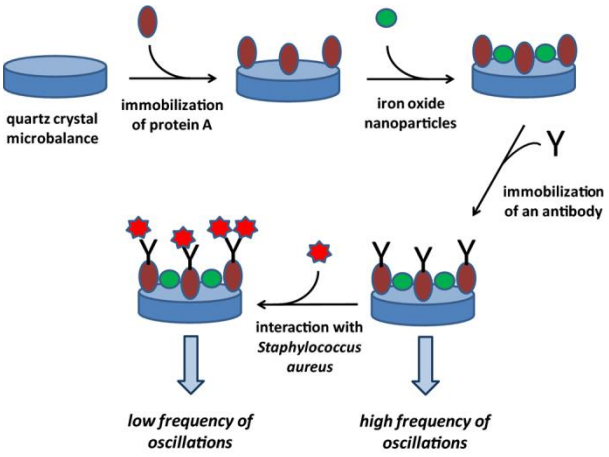
Antigens or antibodies can be immobilized onto the surface of piezoelectric sensing platform.
It is required to modify the sensing platform, for functionalize the surface to facilitate the immobilization.
A variety of organic and inorganic materials are used for coating the sensing platform. On the coated platform Bioreceptor is immobilized.

Coating materials

The coating materials include

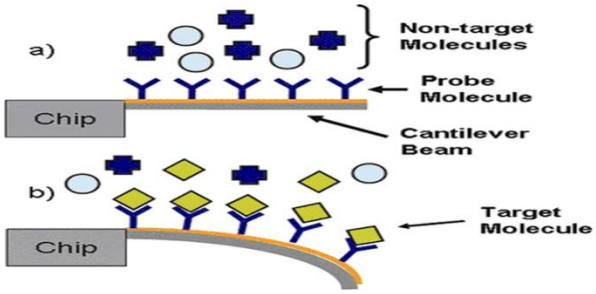
1. Silane reagents
2. Modified silica/HRP based membrane
3. Chitosan-/Multi Walled Carbon Nanotubes
4. ZnO micromechanical membrane
5. Protein A based film

are used for covalent attachment to the crystal surfaces.



Microcantilevers

A cantilever is a rigid structural element, such as a beam or a plate.
It is attached at one end to a support and the other end is free.
For use in Biosensors cantlever are commercially available made up of different natural and synthetic PZ materials such as , silicon nitride, polymers or quartz.



Microcantilever working in static mode. The formation of the antigen-antibody complexes provokes a surface stress and, consequently, a deflection of microcantilever, which is detected optically.

Summary

Piezoelectric material is used in immune sensors for the detection of target molecule.

BOR is coated on the membrane attached with the piezo electric material.

The formation of Ab and AG complex induce a vibration that is detected by QMC balanace or cantilever based physical component.

The technique is highly sensitive.

Biosensor Chapter 08

Cell Based Biosensors

Reading Materials and contents

91. An Introduction to Cell Biosensors
92. Bacterial cell sensors for pathogen Detection
93. Cell Biosensor with reporter gene
94. Cell Biosensor with regulatory protein
95. Selection of Host cell for cell Biosensors
96. Tissue Based Biosensors

97. Detection of Bacterial Pathogen by Cell Biosensors

98. Cell Membrane Protein Based Biosensors

99. Biosensors for plant pathogen detection

100. Plant tissue Biosensors to determine Stress

Learning Outcome.

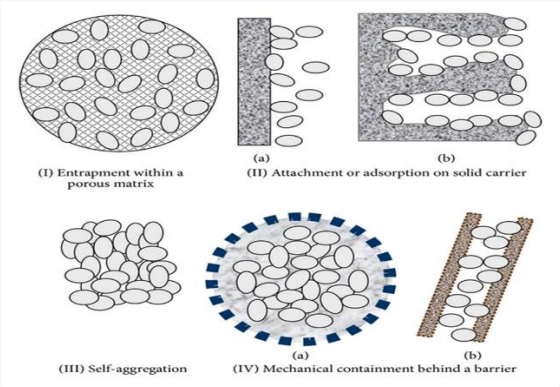
1. Students will be able to learn the basic concept of the designing of the Cell based Biosensor.
2. Students will learn a number of techniques that are required for fabricating cell based Biosensor.
3. Students can design a cell based biosensor depending upon the need.

91. Cell Biosensor

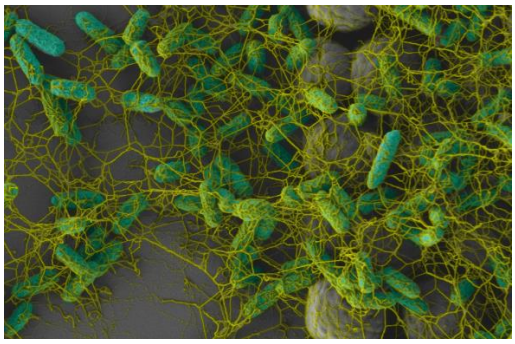
Biosensors based on tissue structures in living animals can be used to detect and measure hormones, drugs, and toxins. The potential use of tissue-based biosensors extends to such diverse fields of biomedical science as physiology, pharmacology, and biodefense. In general, tissue-based biosensors can be formed from genetically modified cells or by direct genetic modification in order to introduce biosensor proteins into a tissue in the animal. A biosensor that employee whole cell in viable or non viable form is known as a cell Biosennsor.

There are at least four types of cells that can be used as a Biorecognition element in cell Biosensors including Microbial cell, Organelle, Tissue ells and Stem cells. Immobilization

The most important step is development of biosensor is the immobilization of whole cell on a matrix. Mild conditions are used to attach the cell on the matrix. The methods of immobilization are already discussed in previous section. Here a brief discussion is given 1.Entrapment, it by passes the use of drastic chemical conditions. 2. Self Aggregation. 3. Adsorption on solid support and Mechanical confinement.



Biofilm formation is a property of a number of bacterial strains. For immobilization of biological Cells. Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers. Many bacterial species form biofilms, and their study has revealed them to be complex and diverse. Many different bacteria form biofilms, including gram-positive (e.g. Bacillus spp, Listeria monocytogenes, Staphylococcus spp, and lactic acid bacteria, including Lactobacillus plantarum and Lactococcus lactis) and gram-negative species (e.g. Escherichia coli, or Pseudomonas aeruginosa). This process takes a total of two to four days, with initial attachment occurring in a ma.ter of hours. As the biofilm progresses through these stages. A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface. These adherent cells become embedded within a slimy extracellular matrix that is composed of extracellular polymeric substances (EPS), produced by cells. It is composed of extracellular polysaccharides, proteins, lipids and DNA.

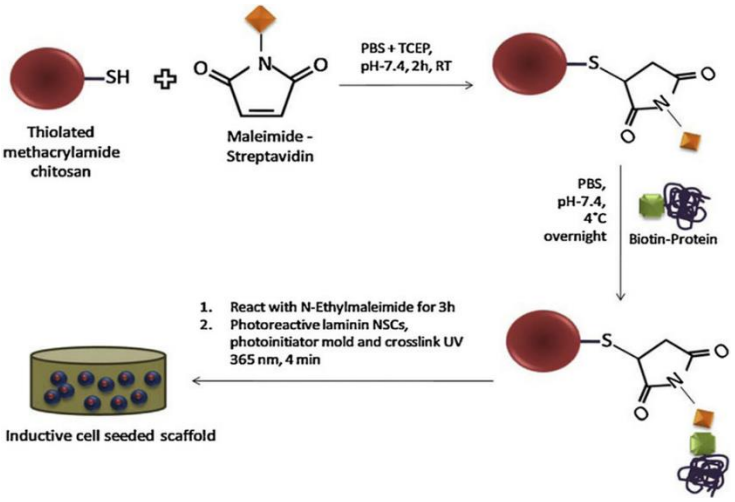


E.coli produce biofilms and with the help of pili and flagella they form weak bonds and survive the harsh environment

Biofilms are formed by bacterial aggregates. These aggregates held together by a mucus like matrix of carbohydrate which adheres to a surface. A biofilm has a nutrient

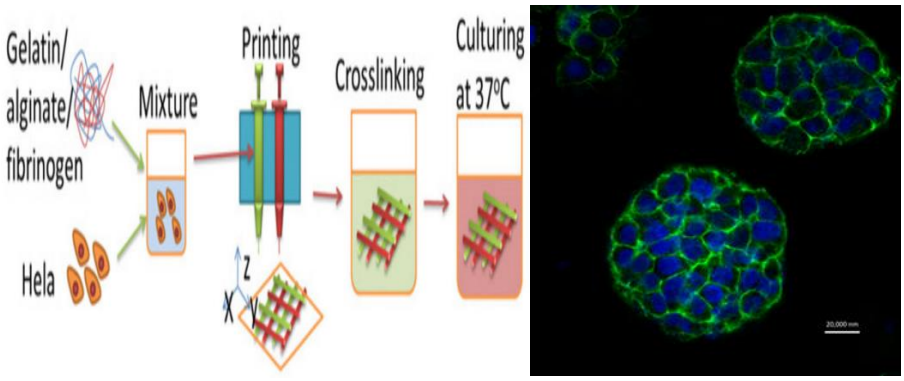
rich environment complex 3 dimensional structure that created an internal protective environment for bacterial cells.

In biology **scaffold is a structure** providing support. In labs Scaffolds are specifically designed for the immobilization of whole biological cells for different purposes. A variety of natural and synthetic materials is available and used for the fabrication of novel scaffolds. Three D printing of Hella cells.



Fabrication of Scaffold based on Thiolates methacrylamide chitosan and Maleimide Streptovidin and cross linked Biotinalted protein

Culturing of cells in petri plate is called 2D printing.



3D printing of Hella cell lines

A 3 D model consists of a scaffold made up of three fibrous proteins (gelatin, alginate, and fibrin) corresponding to the extracellular matrix (support structure) of a tumor. A 3D model to mimics the environment for tumour. Advances in three-dimensional (3D) printing have enabled the direct assembly of cells and extracellular matrix materials to form in vitro cellular models for 3D biology, the study of disease pathogenesis and new drug discovery. For 3D printing for Hela cells a gelatin/alginate/fibrinogen hydrogels was fabricated for in vitro cervical tumor models. Cell proliferation, matrix metalloproteinase (MMP) protein expression and chemoresistance can be measured in the printed 3D cervical tumor models and compared with conventional 2D planar culture models. Example is discussed in lecture.

92. BIOSENSORS FOR WHOLE BACTERIAL CELL DETECTION

Introduction

Biosensors Generally two types of cell Biosensors are used for Cell Biosensors.

1. Impedimetric Biosensors
2. Optical methods

Food-borne pathogens cause an estimated

1.7 million hospital-associated infections

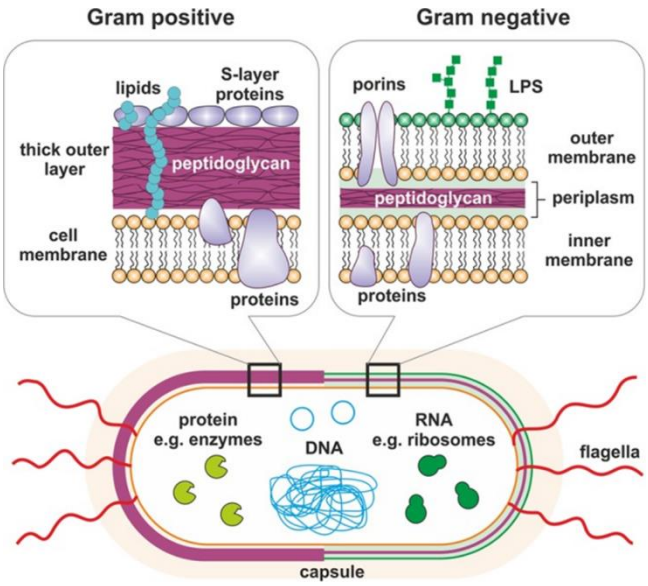
3.0 Million hospitalizations

0.1 Million deaths each year.

Examples: E. coli , Salmonella The development of biosensors for whole microorganisms is challenging because it requires detection of analytes that are much larger (micrometer scale) than typical molecular analytes such as proteins (nanometer scale), and bacteria display many surface epitopes that can lead to nonspecific interactions with the sensor surface.

Bacteria are typically between 0.5 and 5 µm in size, displaying different morphologies, including spherical cocci, rod-shaped bacilli, and spiral-shaped spirilla or spirochetes, among others. Unlike eukaryotic cells, most bacteria are encapsulated by a cell wall which is present on the outside of the cytoplasmic membrane.

The cell wall comprises mainly peptidoglycan, a negatively charged polymer matrix comprising of cross-linked chains of amino sugars, namely, N-acetylglucosamine and N-acetylmuramic acid. Bacteria can be classified as either Gram positive or Gram negative depending upon the architecture and thickness of the cell wall. Gram-positive bacteria retain the violet Gram stain due to their thick peptidoglycan layer on the outside of the cell membrane.



In contrast, Gram-negative bacteria do not take up the stain, as their thinner peptidoglycan layer is sandwiched between two cell membranes. The outer lipid membrane of Gram-negative bacteria also contains lipopolysaccharides (LPS), which act as endotoxins and elicit a strong immune response in humans, as well as various proteins, including porins. The thick peptidoglycan wall surrounding Gram-positive bacteria contains extra components such as lipids, surface proteins, and glycoproteins. Pathogenic Gram-negative bacteria include *Escherichia coli, Salmonella, Shigella, Legionella, Haemophilis influenzae, Neisseria gonorrhoeae, and Neisseria meningitides.* Examples of pathogenic Gram-positive bacteria include *Streptococcus, Staphylococcus, Bacillus, and Clostridium*.

Bacterial architecture and targets for biosensing. The cell wall of Gram-positive bacteria comprises a thick layer of peptidoglycan, which also contains lipids and other protein components, surrounding a lipid membrane. In contrast, Gram-negative

bacteria possess a much thinner peptidoglycan layer sandwiched in between two cell membranes. The outer membrane contains proteins, such as porins, as well as lipopolysaccharides (LPS), also known as endotoxin. The inner membranes of both types of bacteria contain various proteins. Both types of bacteria may have flagella. Intracellular targets for biosensing include proteins, DNA, and RNA.

Target for Biorecognition

A variety of surface antigens presented on the cell envelopes of whole bacteria, including proteins, glycoproteins, lipopolysaccharides, and peptidoglycan, can act as targets for bio-recognition.

Polyclonal antibodies raised against specific bacterial strains are the most commonly used for whole bacterial cell detection.

A variety of surface antigens presented on the cell envelopes of whole bacteria, including proteins, glycoproteins, lipopolysaccharides, and peptidoglycan, can act as targets for biorecognition. Certain bioreceptors have been developed to target a specific one of these moieties; for example, lectins, a type of carbohydrate binding protein, can be employed as bioreceptors for specific cell envelope sugars (. Bacteriophages, viruses which bind to specific bacterial receptor proteins in order to infect the host cells, have also been employed for bacterial detection. Polyclonal antibodies raised against specific bacterial strains are the most commonly used bioreceptors for whole bacterial cell detection, where the binding targets on the cell envelope are usually unknown.

93. Cell Biosensor with a reporter Gene

1. A reporter gene is a regulatory sequence of an other gene that is attached to the gene of interest. The product of the reporter gene can easily identified and measured. The most commonly used reporter gene systems include:
2. LacZ Reporter Gene
3. Luc Reporter Gene
4. Gfp Reporter Gene
5. Egfp gene (Enhanced Green Fluorescence Protein)
6. CAT Chloramphenicol Acetyltransferase Reporter gene

Commonly used reporter genes that induce visually identifiable characteristics usually involve fluorescent and luminescent proteins. Examples include the gene that encodes jellyfish **green fluorescent protein (GFP)**. The protein is coded for by a single gene.Green fluorescent protein (GFP) is a protein composed of 238 amino acid residues. It has a molecular weight of (26.9 kDa).

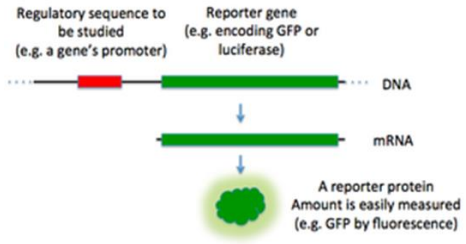
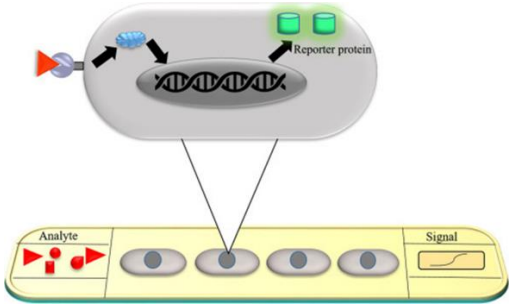


Fig shows the functioning of reporter gene.

The protein exhibits bright green fluorescence when exposed to light in the blue to ultraviolet range. It causes cells that express it to glow green under blue light, the enzyme luciferase, which catalyzes a reaction with luciferin to produce light, and the red fluorescent protein from the gene dsRed.



A schematic diagram for a Biosensor using a reporter gene.

A common reporter in bacteria is the ***E. coli* lacZ gene**, which encodes the protein beta-galactosidase. This enzyme causes bacteria expressing the gene to appear blue when grown on a medium that contains the substrate analog X-gal. Luc gene and lac Z gene requires specific substrates to generate an optical signal. lacZ reporter gene for beta galactosidase, requires chemiluminescent and electrochemical substrates An example of a selectable-marker which is also a reporter in bacteria is the chloramphenicol acetyltransferase (CAT) gene, which confers resistance to the antibiotic chloramphenicol.

A reporter gene crt A is responsible for carotenoid synthesis. When applied to a sample, the crtA-based whole cell-based biosensors.The change color of culture media from

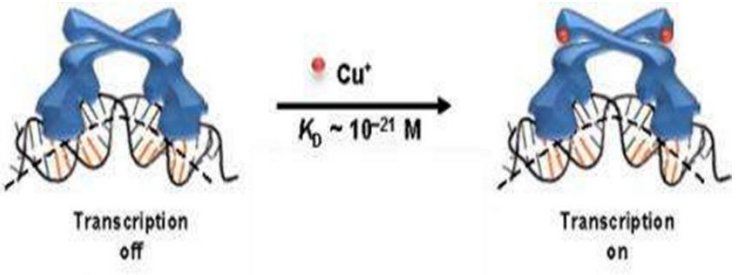
yellow to red without the addition of a supporting substrate, is a signal for biorecognition. It is considered as a good choice for rapid detection.

The firefly luciferase (luc) reporter has high sensitivity can detect 2fg. Luc gene has a linear broad range for detection.

Gene name	Gene product	Assay
lacZ	β-galactosidase	Enzyme assay, Histochemical
cat	Chloramphenicol acetyltransferase	Chloramphenicol acetylation
gfp	Green fluorescent protein	Fluorescent
rfp	Red fluorescent protein	Microscopical, Spectrophotometry
luc	Luciferase enzyme	Bioluminescence

94. Cell Biosensor with Regulatory proteins

The regulatory protein, which possesses complex interactions with the target analytes of the contaminants of interest, is essential for the specificity and sensitivity of the whole cell-based biosensors. In recent years, with the discovery of metallo-regulatory proteins, there are many reports elsewhere that utilized these biosensors for the detection of heavy metals in water and soil samples.



Functioning of a metallo regulatory protein.

These have shown higher selectivity, expanded detection ranges and enhanced sensitivity when compared to conventional biosensors. Regulatory proteins regulate the level of expression of a proteins. There are two types of regulatory proteins.

Repressor proteins bind to operators or promoters, preventing RNA polymerase from transcribing RNA.

Activator proteins bind to a site on the DNA molecule and causes an increase in transcription of a nearby gene.

The Metallo regulators are proteins that bind metals .

A panel of metalloregulatory proteins controls the expression of genes encoding

1. Membrane transporters
2. Metal trafficking proteins.

These metal sensors manage

1. Metal homeostasis
2. Metal resistance

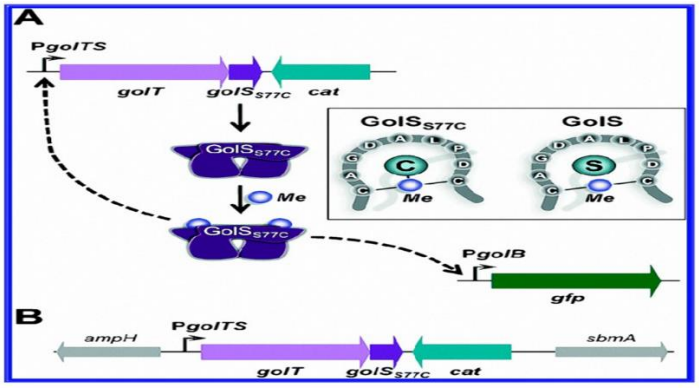
The metal ion(s) after binding with the gene promoter can induce different conformational changes.

i. Activates the promoter
ii. Inhibits the promoter

The binding could distort the promoter structure and can convert a poor promoter to a strong one.

Gold sensor Protein for metal detection

Gols is a gold sensor membrane bound protein. It is a regulatory gene GolS present in *Salmonella* for the detection of gold in water is employed for the detection of metal ions. The introduction of a single amino acid at the position 77 in the GolS protein.



The mutation increases the efficiency and protein was able to detection of

i. Mercury (Hg)
ii. Lead (Pd)
iii. Cadmium (Cd)

Summary

The expression of regulatory proteins is used as a signal for the detection of Analyte.

A regulatory gene GolS present in Salmonella for the detection of gold in water is employed for the detection of metal ions.

The mutated GolS gene was expressed in E.coli and the whole cell was used for the detection of Hg, Pb, Cd and Au ions in aqueous samples.

95. Selection of Host Cell

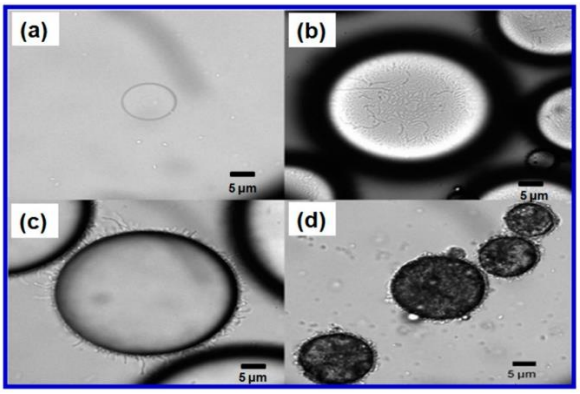
Whole microbial cells are selected on the basis of their proteomics and genomics for use in Biosensor as a sensing vehicle. The choice of host bacteria has a significant impact on the performance of a fully constructed whole cell-based biosensor. Microbial cell are tailored for the use as sensing vehicle in Biosensors. The selection of the type of host cell is important, for the performance of whole cell biosensor. It can effect many parameters including

i. Specificity,
ii. Sensitivity
iii. Reproducibility
iv. Reliability
v. Work load
vi. Time-response,
vii. Background noise

Biosensor for oil pollution.

For developing a biosensor to detect the oil pollution in sea, a bacterial strain should be selected that is a

1. A part of marine microbial Ecosystem
2. It could resist the composition of sea water.
3. it should have a gene for the detection of oil.
4. Acenatobacter baylyi is a natural adhesive to oil water interphase.
   Acinetobacter baylyi is found to tolerate seawater and have a special ability of adhering to an oil-water interface of 10-80 µm emulsified mineral and crude oil droplets. These properties make A, baylyi, an ideal bacterial chassis for constructing bioreporters that are able to actively search and sense oil spill in water and soils. Acinetobacter baylyi bioreporter ADPWH_alk are developed and applied to the detection of alkanes and alkenes in water, seawater and soils. Bioreporter ADPWH_alk are able to detect a broad range of alkanes and alkenes with carbon chain length from C7 to C36..



(a) Poor accessibility and emulsifying capability of E. coli (the circle) towards oil droplets. Good affinity and emulsifying capability of Acinetobacter baylyi (the circles) towards the surface of (b,c) the mineral oil droplets; and (d) the curde oil droplets

Genetic Engineering of the Host cells

Selection and engineering of host cell is based on the requirements for the detection of target molecule.

The tailored organism give a quantifiable signal in the presence of an Analyte. In Environment these bacterial metal-sensor are used for specificity and sensitivity.

E.coli for Ni ion detection

A biosensor was designed for the detection of metal (Ni) pollution in drinking water.

A series of different strains of E.coli was produced by recombinant DNA technology.

A cassette was prepared by fusing Ni/Co metallo-regulator, promoter and luc reporter genes.

E.coli (wild type) was proven best with ten times higher .

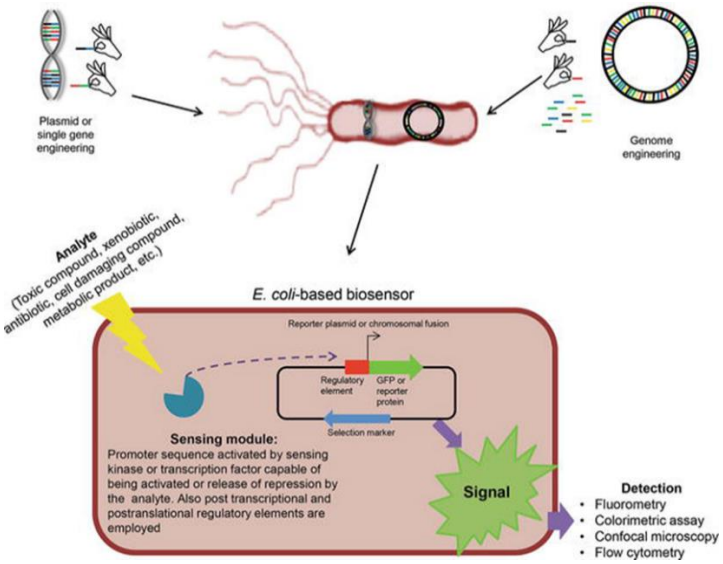


Fig shows a biosensor based on genetically engineered E.coli for the detectionof Ni,

Summary

1. Whole microbial cells are selected on the basis of their proteomics and genomics for use in Biosensor as a sensing vehicle.
2. The choice of host bacteria has a significant impact on the performance of a fully constructed whole cell-based biosensor.
3. Microbial cell are tailored for the use as sensing vehicle in Biosensors.

96. Animal cell Based Biosensors

In animal tissue based Biosensor animal tissues are used as BRE.Tissues are easy to immobilize and retain the biological molecules in their intact form.Biosensors are used for protein-drug interactions. BRET is a technique that is used to monitor molecular interactions in tissue based biosensors.

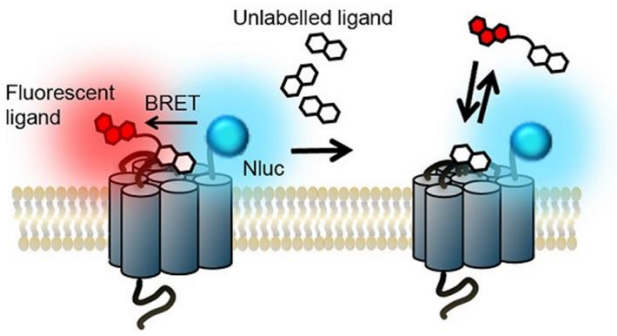
Biophotonics

Biosensor cells transduce the concentration of the molecule being detected into a physical signal, which can be precisely measured.

Biophotonics provides the most versatile basis for tissue-based biosensors. Light output from biosensor cells can be in the form of fluorescence or bioluminescence, and, of these two, bioluminescence offers advantages of not requiring an input source of light and having a more favorable signal to noise ratio in living animals than fluorescence. Protein-protein interactions can be used to detect almost any molecule, by means of fusion proteins that can be used to generate resonance energy transfer. **Bioluminescence resonance energy transfer (BRET)** has the potential to be used for the measurement of a wide variety of molecules in living animals. Bioluminescence resonance energy transfer (BRET), results from a naturally occurring phenomenon, involving

i. Resonance energy
ii. Enzymatic donor and
iii. Fluorescent protein acceptor.
iv. Range 1-10 nm range

BRET an ideal platform to study protein-protein interaction or structural changes in protein structure, in living cells. Two examples are discussed here: a tissue-based biosensor for the hormone vasopressin, and a biosensor for rapamycin, both based on The detection of the blue emission indicates the lack of binding between the ligand and the receptor. The detection of red fluorescence, which is caused by the excitation of the fluorescent indicates the specific binding of the ligand to the receptor. BRET.



Module No. 97. Detection of Pathogen by Cell Biosensors

Introduction

1. Rapid and accurate detection of pathogens is an important goal in the diagnosis of diseases.
2. Conventional microbiological methods can require several days to weeks.
3. Rapid and accurate detection of pathogens is an important goal in the diagnosis of diseases.
4. Rapid and accurate detection of pathogens is an important goal in the diagnosis of diseases.
5. Conventional microbiological methods can require several days to weeks.
6. Rapid and accurate detection of pathogens is an important goal in the diagnosis of diseases.

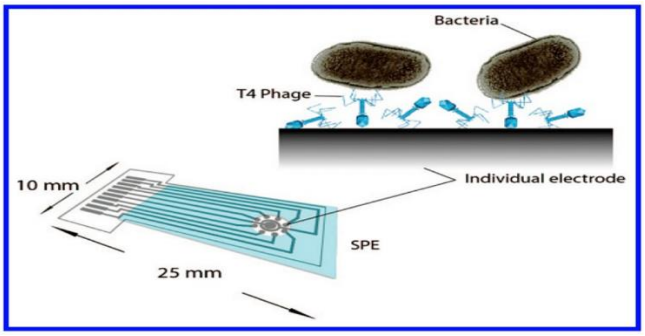
Virus as Bioreceptor Element

Specific and direct detection of bacteria was made by using a virus as a BRE.

The surface of electrode was functionalized by electrochemical oxidation in acidic media.

The virus was covalently immobilized on the surface of electrode.

Upon detection of bacteria, lysis occur.



Escherichia virus T4 is a species of bacteriophages that infect Escherichia coli bacteria. Lysis of Pathogenic E.coli cells indicates the presence of pathogen.

Quorum-Sensing

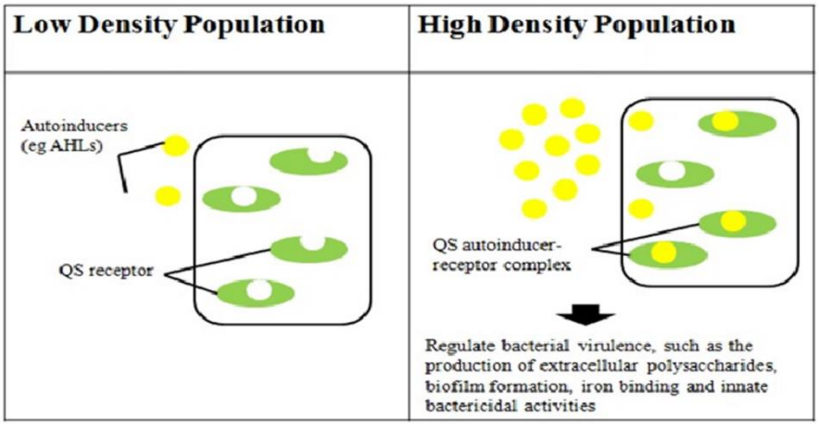
Quorum sensing is a process of intra cellular communication in bacteria.

It allows the release of auto inducible molecules (AIM) in the environment by the bacterium.

In low number of bacteria the conc. of AIM is too low to be detected.

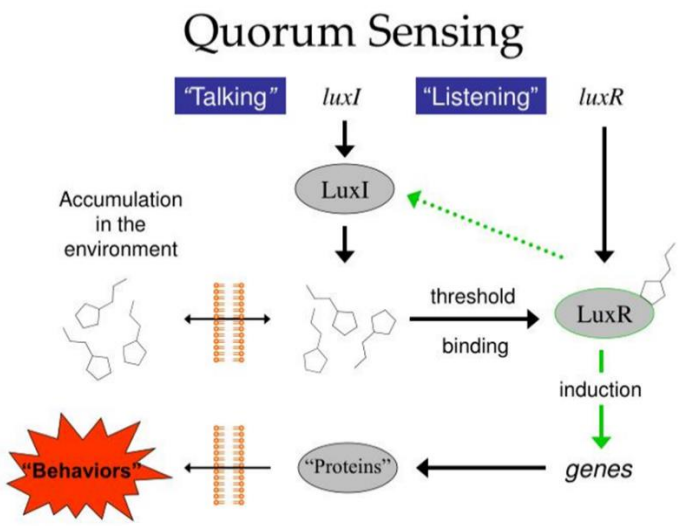
In higher bacterial population AIM reaches a threshold and bacteria express virulence gene.

N-Acylhomoserine Lactone Production by Clinical Multidrug Resistant Klebsiella pneumoniae



QSM and Biosensor

1. In Gram negative bacteria utilize N-Acyl homoserine lactone as auto inducible molecule.
2. A biosensor is designed having a cell containing a quorum sensing controlled promoter fused to a reporter gene (laz Z).
3. The functional activity of the promoter depends upon the presence of exogenous QSM.
4. On the detection of auto inducible molecule optical signals are generated.



Summary

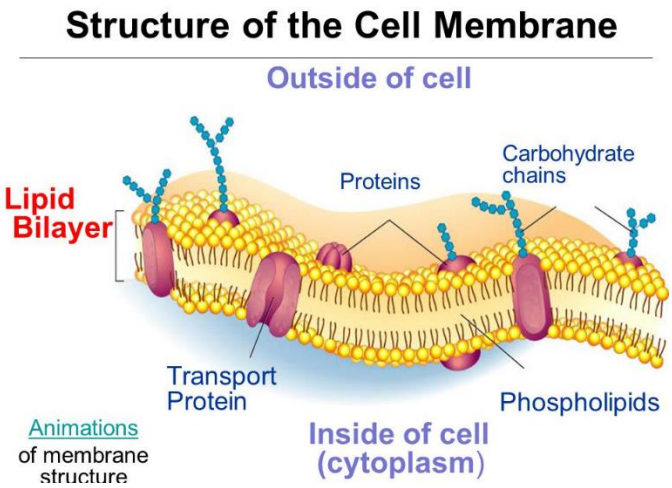
The whole cell biosensor are successfully used for the detection of bacterial pathogens, using virus and genetically tailored microbes.

Biosensors are designed to detect the quorum sensing in bacteria to analyse the virulence factor.

The whole cell-based biosensors are simple and can provide fast result for detection of pathogens.

Module No. 98 Cell Membrane Based Biosensor

Cell Membrane proteins are essential components of biological membranes. There are two types of proteins.



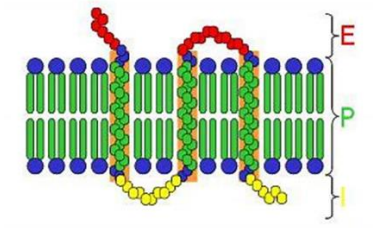
Peripheral (Extrinsic) Protein. They are rich in amino acids with hydrophilic side chains.. They interacts with the surrounding water and with the polar surface of the lipid bilayer. Extrinsic proteins often contain chains of sugars (glycoproteins).

Intrinsic membrane Proteins. Intrinsic membrane proteins contain both hydrophilic and hydrophobic regions. The hydrophilic portions of protein interact with the polar heads of the lipid molecules at each surface.The buried hydrophobic portion of protein in the lipid bilayer are rich in amino acids with hydrophobic side chains.

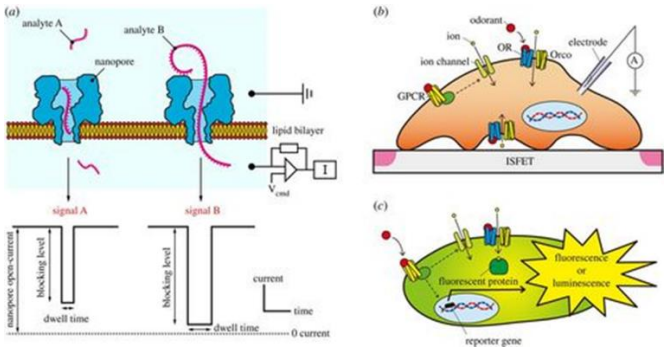
There are two types of Membrane Biosensor.

i. The lipid bilayer-based platform
ii. The cell-based platform

The Lipid bilayer platforms, the membrane proteins are embedded in a lipid bilayer. This layer forms a bridge between the protein and a sensor device.



Cell membrane protein.



Membrane protein-based biosensors on (a) a lipid-bilayer platform and (b,c) cell-based platforms. Membrane protein-based biosensors

Cell-based platforms, the membrane proteins are expressed in a cultured cell, which is then integrated in a sensor device.

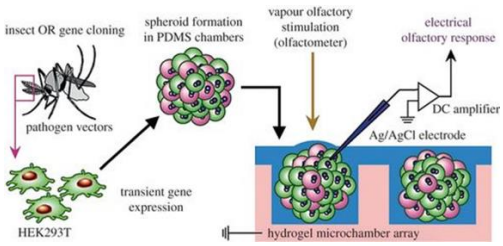
Biosensor based on Human embryonic kidney cell

HEK 293 cells have been widely used in cell biology research for many years.

1. The cell line shows reliable growth.
2. Human embryonic kidney cells are frequently used for foreign gene expression.
3. They have high transformation efficiency for the foreign cloned gene.

Olfactory Cell Biosenser

1. Insect olfactory receptor and OR co-receptor were expressed in HEK cells.
2. The cells were placed into a hydrogel microchamber maintaining the desired moisture conditions.
3. Vapour of the odorant was blown using Olfactometer.
4. The extracellular field potential of the HEK spheroid was recorded using an amplifier.



Insect olfactory receptor (OR) and OR co-receptor (Orco) were expressed in human embryonic kidney (HEK) cells that subsequently formed spheroids in polydimethylsiloxane (PDMS) chambers.

Summary

1. The membrane bound protein receptor are ideal receptors for targeting.
2. The receptor proteins are highly specific in their reception for the Analyte.
3. The membrane bound are cloned in HEK cell lines.
4. The cell membrane bound protein biosensor are fabricated with high specificity.

99 Biosensors for plant pathogen detection.

When plants are attacked by pathogen, they become under stress, as discussed in previous modules. Plants secrete a number of volatile compounds, as an indicator of s Plant microbial pathogens attack crops. These microbes include virus, bacteria and fungi.

A rough estimate shows that 20–30% of the field crops are lost annually. An estimate shows that in Georgia USA cost the economic loss estimates to up to 821.85 million dollars in 2013. tress. In lecture a number of examples are described to make the concept clear.

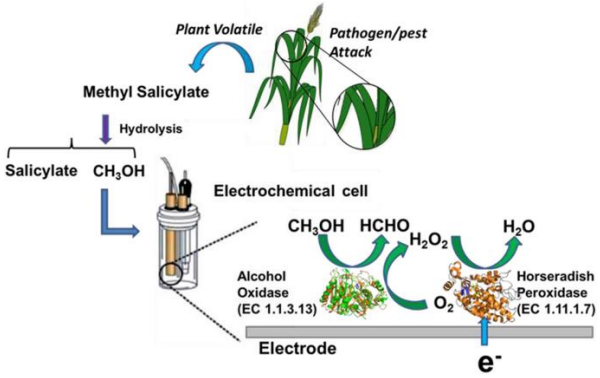
Traditional Detection Microbial Pathogens

1. It involves interpreting visual symptoms of disease,.
2. It is followed by pathogen diagnosis using microscopy techniques to confirm the data.
3. This approch cannot be used the syptoms start appearing.
4. It is not suitable for on-site diagnosis of plant pathogens.
   Methyl salicylate Biosensor
   A biosensor is designed to detect methyl salicylate
   It is a volatile organic compound released by pathogen-infected plants via systemic response.

Alcohol oxidase and HRP enzymes were immobilized on to a CNT matrix.
Amperometric method was used in the development of sensor.

Working of Biosensor

I. Hydrolysis of methyl salicylate to form salicylate and methanol.
II. AOD converts methanol into formaldehyde and simultaneous reduction of Oxygen and to hydrogen peroxide.
III. Reduction of hydrogen peroxide by HRP to water.
IV. Generation of amperometric signals.

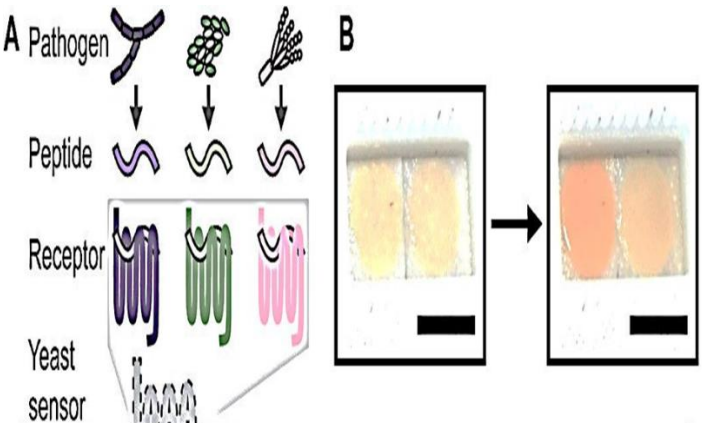


Methyl salisylate Bi- enzyme Biosensor.

I. Biosensor for pathogenic fungi
II. A biosensor is designed to target the mating peptides secreted by pathogenic fungi, as an indicator for the fungal infection.
III. Formation of sex organs in fungi is often induced by specific organic substances (pheromones).
IV. Sex pheromones or chemicals produced by one partner can elicit a sexual response in the other.

Working of Yeast biosensor

- Highly specific fungal receptors provide sensitive response to mating peptides secreted by pathogenic fungi.
- The response leads to the activation of the downstream mating signaling pathway.
- This pathway induces transcriptional activation of genes for production of red lycopene pigment visible to the naked eye.



Yeast biosensor for detection of fungal pathogens. After the detection of target molecules expression of lycopene gene shows pink color signal paper-based dipstick

Summary

Biosensor can provide point of care testing of the plants.

Use of Biosensor can help to reduce the loss due to pathogen attack.

It is essential to select a highly specific target molecule before designing a biosensor.

Methyl salicylate and pheromone peptides have to be proven excellent target molecules.

Module No. 100. Plant Biosensors

Introduction

In plants drought conditions and deficiency of essential elements like Nitrogen, phosphorus and potassium can badly effect crop.

For a healthy crop it is required to maintain all the nutritional requirements.

For the detection of any such deficiencies in time could give a chance to the farmer to provide the deficient element for a healthy crop.

Water shortage and Plants

When soil dries out, plants slow down their

I. Slow down Growth
II. Reduce photosynthesis
III. Tissues Damage
IV. Wilting

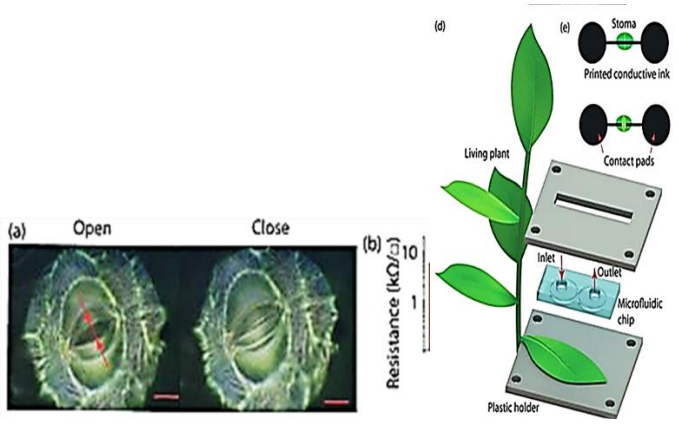
Some no visible signs of trouble until they have already experienced significant harm. Plant leaf stoma plays main role in the evaporation of water.

Biosensor to detect Water shortage

1. Tiny hollow tubes of carbon that conduct electricity.
2. A solution was prepared by mixing with SDS.
3. The ink is printed onto the leaf surface. It can create an electronic circuit.
4. When the pore is closed, the circuit is intact and .
5. When the pore opens, the circuit is broken and the current stops flowing.

Working and Advantages

Opening and closing of circuit was measured by the flow of current tested the sensors on a plant called the peace lily, which they chose in part because it has large stomata. it opens up the possibility of directly printing electronics onto plant life for long-term monitoring of plant physiological responses to environmental factors, such as drought.



Biosensor for N Fixation

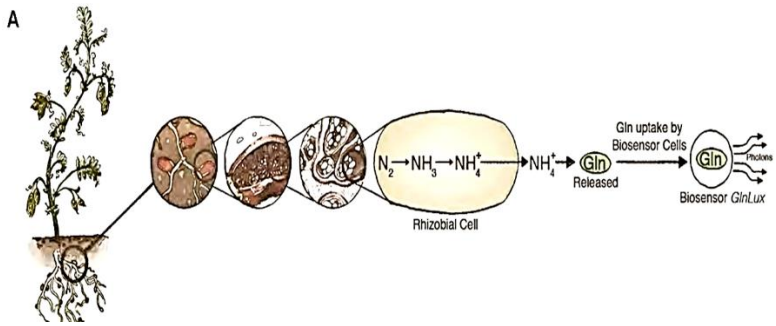
Plant can assimilate atmospheric N into amino acids including glutamine (Gln), by the virtue of rhizobia.

Gln is a for export to shoots as the major fraction.

During the export Gln is released into the atmosphere.

The release of less amount of Gln could be an indicator of N deficiency.

When Gln is released from plant tissues to the medium, it is taken up by GlnLux biosensor E.coli cells, causing them to become metabolically active and divide, which proportionally activates a lux operon tophoton capture devices release photons that are measured using



Summary

Biosensor designed for plant tissues are very helpful in identifying the nutritional deficiencies in plants.

A biosensor is designed to detect the drough conditions in plants. The sensor used the opening and closing of stomata to detect the water condition in the plant.

Another Biosensor is designed to detect the N deficiency using Gln Lux promoter.

Biosensor Chapter 09

DNA Biosensor and Microchip

101 An introduction to DNA Biosensors

102 DNA and PNA as Bioreceptor in a Biosensors

103. Immobilization in DNA Biosensors

104. Electrochemical behavior of DNA

105. Sensing Techniques in DNA Biosensors

106. Label Free Electrochemical detection in DNA Biosensors

107. Labeling of DNA by Physical methods

108. Molecular Beacons

109. Enzyme Sensing Techniques in DNA Biosensors

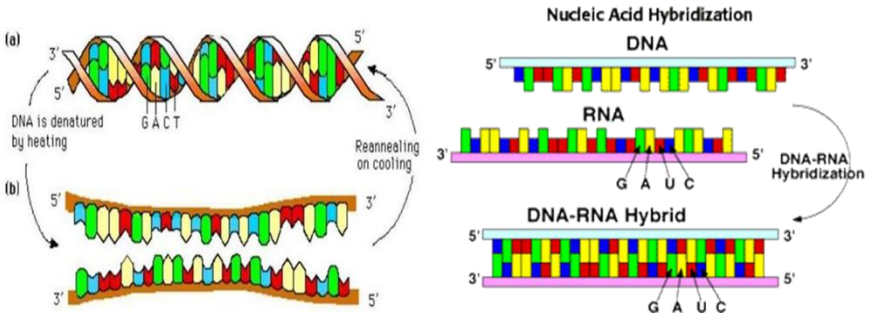
110. Aptamers Based DNA Sensors

Learning out come.

1. Students will learn about the basis of DNA Biosensors.
2. Students will learn about the electrochemical behavior of DNA and sensing techniques.
3. Students will be able to describe the fabrication of aptamer based sensors.

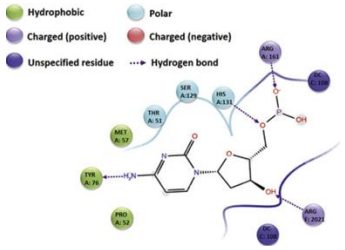
101 An introduction to DNA Biosensors

A ssDNA molecule is used as a BRE in a DNA based biosensor. DNA as BRE can bind with DNA and RNA target molecules. Single stranded DNA of the sensor interacts flexibly with target protein. Different types of weak forces paly a major role in the detection of target molecule. DNA biosensors are based on the employment of DNA molecule as a Biorecognition element.



Binding of DNA with single stranded DNA molecules RNA a

There is a selective binding between a DNA, that is immobilized on the electrode surface and target molecule in the sample. Generally in these Biosensors Optical, electrochemical and Piezo electric sensors are used. The binding of DNA the complementary DNA or RNA sequence is called Hybridization. The binding of receptor DNA with the proteins or other molecules is called inter action. DNA molecule can also inter act with other molecules such as toxins, Vitamins.



Protein DNA interaction. Single strand of DNA flexibly interacts with the proteins to adjust their position for binding. There are two types of forces are involved in the ssDNA and protein inter action. Hydrogen bonding Electrostatic interactions There are certain factors that can alter these forces are Ionic strength, pH and temperature. Common example Histones and DNA interaction.

Hydrophobic Amino acids and Protein DNA interaction

Hydrophobic amino acid play an important role in the binding of ssDNA with the protein. Amino acids are grouped according to what their side chains are like. The nine amino acids that have hydrophobic side chains are glycine (Gly), alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), proline (Pro), phenylalanine (Phe), methionine (Met), and tryptophan (Trp).

Role of Electrostatic forces in DNA- Protein complex Formation

Electrostatic force or Vander wall forces are the attractive or repulsive interactions due to charges between two molecules. A salt bridge (Biology) is a combination of two non-covalent interactions such as hydrogen bonding and electrostatic interaction within the two biological molecules.

Histones.

In biology, histones are highly basic proteins found in eukaryotic cell nuclei that pack and order the DNA into structural units called nucleosomes. Histones are abundant in lysine and arginine. Histone are the chief protein components of chromatin, acting as spools around which DNA winds, and playing a role in gene regulation. In order for very long DNA molecules to fit into the cell nucleus, they wrap around complexes of histone proteins, giving the chromosome a more compact shape.

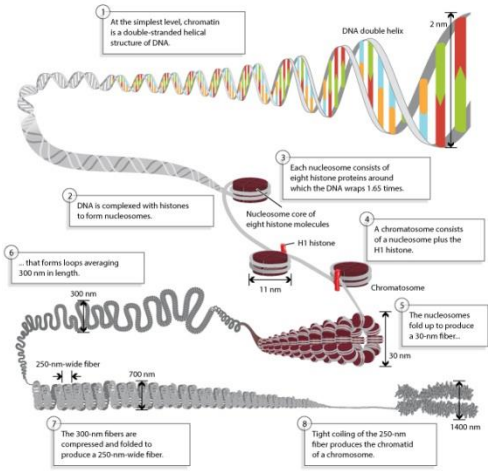


Figure 1: Chromosomes are composed of DNA tightly-wound around histones. Chromosomal DNA is packaged inside microscopic nuclei with the help of histones. These are positively-charged proteins that strongly adhere to negatively-charged DNA and form complexes called nucleosomes. Each nuclesome is composed of DNA wound 1.65 times around eight histone proteins. Nucleosomes fold up to form a 30-nanometer chromatin fiber, which forms loops averaging 300 nanometers in length. The 300 nm fibers are compressed and folded to produce a 250 nm-wide fiber, which is tightly coiled into the chromatid of a chromosome.

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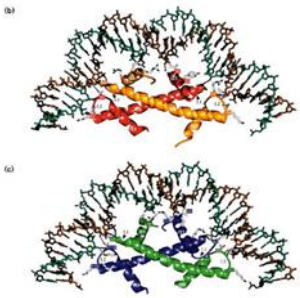
Histones are basic proteins, and their positive charges allow them to associate with DNA, which is negatively charged. Without histones, the unwound DNA in chromosomes would be very long (a length to width ratio of more than 10 million to 1 in human DNA). For example, each human diploid cell (containing 23 pairs of chromosomes) has about 1.8 meters of DNA; wound on the histones, the diploid cell has about 90 micrometers (0.09 mm) of chromatin.



Binding of DNA with histones.

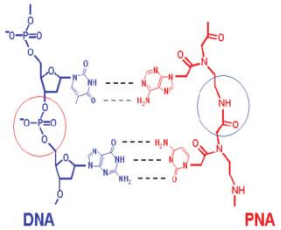
1. Polar interaction between protein helix and Phosphate groups of DNA.
2. Non polar interaction between ribose sugar and histones
3. Hydrogen bond between DNA and Amide group
4. Salt bridge between basic Amino acid residues and Phosphate group.
5. Non specific interactions between the N-terminal tail of Histone minor groove of DNA.

102 DNA and PNA as Bioreceptor in a Biosensors

DNA as Bio Receptor In DNA biosensors Small size fragments of DNA ranging from 10-100 nucleotides are used as DNA probes. ss DNA prepared by a series of chemical reactions producing a known sequence of nucleotides. Different strategies are used to improve the efficiency of the DNA biosensors. DNA biosensor are also known as Genosenors. Peptide nucleic acid (PNA) is a DNA mimic in which the nucleo-bases are linked by an N-(2-aminoethyl) glycine backbone. The chemical composition of PNA contains elements of DNA and peptides.

PNA as a receptor molecule

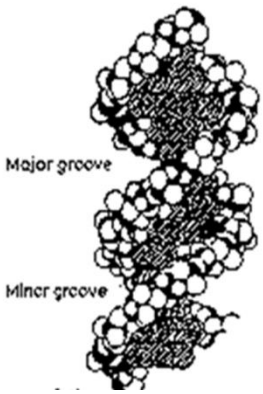
To increase the stability and efficiency of the DNA based biosensors a DNA derivative PNA is use in DNA.PNA can bind with the complementary DNA/RNA target molecule. PNAs can bind to complementary DNA strands in both parallel and antiparallel orientations. There is no internal repulsion in DNA-PNA dulpex as compared to DNA DNA duplex, that is due due to the absence of Phosphate groups.



PNA and DNA Hybrid

PNAs bind DNA and RNA with high specificity and selectivity, forming Watson–Crick base pairs and leading to PNA–RNA and PNA–DNA hybrids that are more stable than the corresponding nucleic acid

complexes . Because of their high thermal stability and resistance to proteases and nucleases, PNAs are ideal candidates as antisense or antigene therapeutic agents and are currently used as powerful tools in molecular biology and in diagnostics.

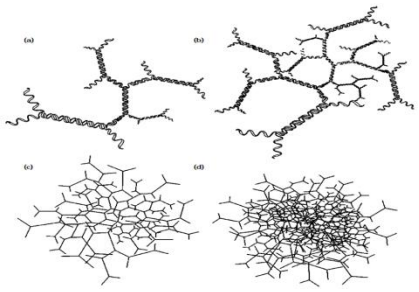


DNA-DNA Hybrid DNA=PNA hybrid

The rate of hybridization of PNA-DNA duplex increases upto 50,000 times as compared to DNA-DNA duplex. The stability of DNA/PNA hybrid increased with an increase in number of bases. A increase of one base pair in DNA-PNA duplex increases the thermal stability of the complex at least upto 1°C , as compared to DNA/DNA hybrids.

DNA Dendrimers

1. Dendrimers are tree like super structure.
2. Each DNA Dendrimer consists of several similar branched DNA units.
3. DNA units connected to each other using DNA ligase.
4. A dendrimer is typically symmetric around the core, and often adopts a spherical three - dimensional morphology.



DNA denrimers

103. Immobilization in DNA Biosensors

DNA probes are short DNA oligonucleotides (12-40-mer) able to hybridize with specific target sequences.

The immobilization step for the DNA probe is essential to develop a whole range of biosensors and microarrays. The achievement of high sensitivity and selectivity requires minimization of nonspecific adsorption and stability of immobilized biomolecules. The control of this step is essential to ensure high reactivity, orientation, accessibility, and stability of the surface-confined probe and to avoid nonspecific binding.

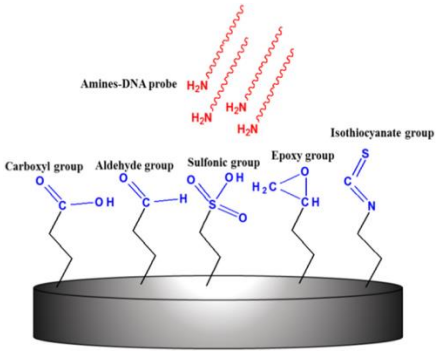
DNA can be immobilized on sensor surfaces with methods similar to those used for enzyme-based biosensors: adsorption, covalent immobilization, and avidin (or streptavidin)-biotin interaction.These immobilization techniques also canbe used to develop DNA microarrays.

Adsorption

Adsorption is the simplest immobilization method becauseit does not require any nucleic acid modification.Immobilization has been reported based on ionic interactions occurring between the negatively charged groups present on the DNA probe and positive charges covering the surface. For instance, a chitosan film was used for the immobilization of ssDNA on a glassy carbon electrode(GCE). Chitosan is a cationic polymer that can form a stable complex with the negatively charged phosphate groups of the DNA.

Covalent bonding

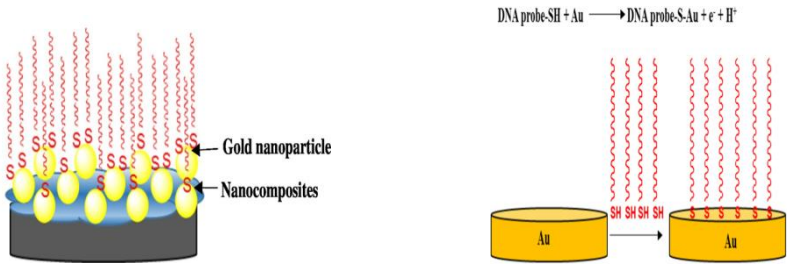
Unlike adsorption technique, DNA probes immobilization technique via covalent bonding demonstrated a good stability, flexible, highly binding strength and prevent desorption of DNA probe monolayer from the electrode surface. In covalent bonding technique, the synthesized DNA probe is typically linked with the group of thiols (SH) or amines (NH2) at the end of 3′ or 5′ to bind covalently to the metal surface or specific functional group introduced to the electrode surface.



A schematic of the covalent immobilization methods of amine-terminated DNA probe on the different functionalized group-modified electrode.

This procedure led to high specific attachment of DNA probe onto the electrode surface and can prevent non-specific binding. Chemisorption and covalent attachment are frequently used in the covalent immobilization of DNA probe.

The chemisorption technique is frequently used for covalently immobilization of DNA probe between thiol-modified DNA probes and gold (Au) surface forming a self-assembly monolayer (SAM) formation of DNA probes on gold electrode surface . This is because there is a strong affinity interaction between the thiol group and gold surface forming a covalent bonding of gold (Au)-sulfur (S) . Using this chemisorption



A B

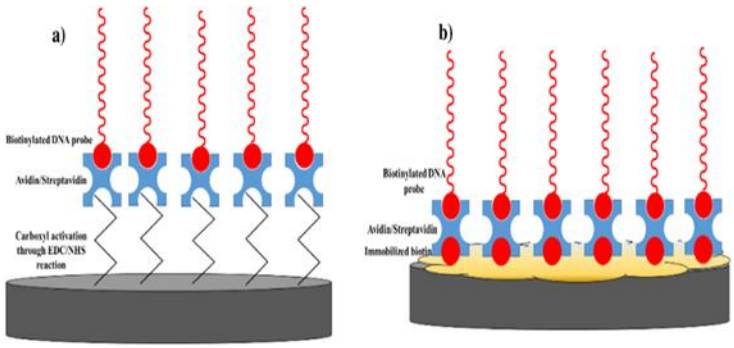
Binding of DNA based probe with the (A) gold nanoparticles and gold surface (B).

principle, AuS bond is always exploited in the fabrication of electrochemical DNA biosensor owing of its strong binding strength, easy preparation, highly stable and reproducible of DNA probe monolayer.

Besides planar gold electrode, the chemisorption of DNA probes onto gold nanoparticles (AuNPs) surface have gained an interest in electrochemical DNA construction due to increase surface area of electrode to enhance amount of immobilized DNA probe.

Avidin/streptavidin–biotin interaction

Another strategy for non-covalent immobilization of DNA probes on the electrode surface is based on the formation complex of avidin (either streptavidin)-biotin. This is due to the fact that small biotin molecule (molecular weight = 2.44.31 g/mol) could interact with the binding site of avidin/streptavidin with very high affinity (Ka = 1 × 10− 15 M) nearly to a covalent binding. Consequently, the binding avidin/streptavidin to biotin molecule is highly stable and resistant to the extreme of temperature, pH, denatured detergents and organic solvents . Moreover, avidin/streptavidin, a large tetrameric protein (70 kDa) could provide four binding sites of biotin molecule and these tetrameric interactions could be exploited to immobilize DNA probes onto the solid electrode surface. This could be done by modifying the end of 3’ or 5’ of DNA probes sequence with biotin molecule and later introducing it to the avidin/streptavidin-modified electrode.



A schematic diagram of the immobilization strategies of biotinylated DNA probe; a) avidin/streptavidin-functionalized electrode through carboxyl group activation; b) biotin/avidin (streptavidin)/biotin sandwiches technique

104. Electrochemical behavior of DNA

DNA is capable of showing electrochemical properties. Guanine is oxidizes more easily as compared to the other bases of DNA. The convenient oxidation of guanine base is helpful in the detection of target molecule under ambient conditions.

The efficiency of DNA base biosensor could be improved by carbon/metal base electrodes .

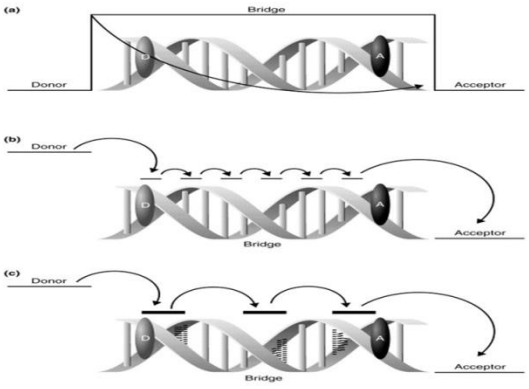
DNA can transport charge in solution and on the surface where it is immobilized.

DNA-mediated Current Transport requires electronic coupling to the base pair stack. There are three possible mechanisms are given for charge transport by DNA.

a. Superexchange
b. Hopping
c. Domain hopping.

Mechanism of Charge Transport in DNA

As a macromolecular assembly in solution, bases of DNA are constantly undergoing dynamic motion on timescales from picoseconds to milliseconds, and these motions, though subtle, are sufficient to facilitate or interrupt CT. DNA Charge Transport is attenuated by large and small perturbations in π-stacking. DNA CT represents powerful chemistry that permits redox reactions to be activated over long molecular distances, enabling sensing of small perturbations to the DNA base pair stack with high sensitivity, and potentially providing a means to communicate across the genome.



Three different mechanisms for charge transport in DNA includes Superexchange, Hopping and Domain hopping.

Superexchange: the charge tunnels from the donor (D) to the acceptor (A) through the bridge.

Hopping: charge occupies the bridge in travelling from donor to acceptor by hopping between bridge.

Domain hopping: charge occupies the bridge by delocalizing over several bases, or a domain

. **Electrochemical properties of DNA bases**

All four bases of DNA can be oxidized and produce their oxidation signals at highly positive potentials on carbon electrodes. The one electron reduction potentials of DNA nucleosides are

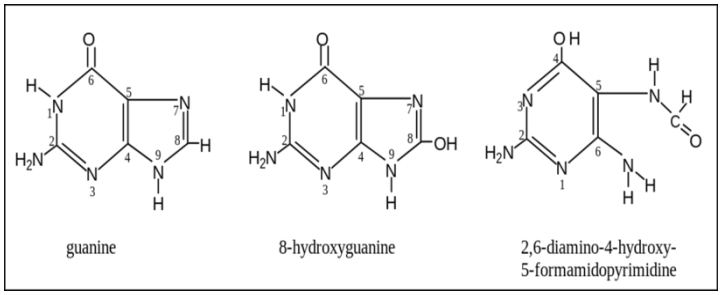
1. Guanine 1.29
2. Adenine 1.42
3. Cytosine 1.6
4. Thymine 1.7

DNA Damage

1. DNA can be damaged by oxidizing agents, alkylating agents and high-energy electromagnetic radiation such as ultraviolet light and X-rays.
2. DNA is damaged due to excessive levels of Reactive Oxygen Species. Normal level of ROS , may be necessary for memory and learning.
3. DNA damaged is related to cancer and other diseases.

Oxidation of Guanine

The oxidation of Guanine is pH dependent. At pH 7.4 the rate of oxidation potential is low. When human DNA is exposed to γ rays and heavy particles, there is an increase in the two main degraded products of guanine . The convenient oxidation of Guanine is used as a tool in biosensors for the fabrication of reagent less biosensors.



When DNA undergoes oxidative damage, two of the most common damages change guanine to 8-hydroxyguanine or to 2,6-diamino-4-hydroxy-5-formamidopyrimidine.

Biosensor based on Guanine Electrochemical Oxidation

1. Adsorption of guanine could be increased by adding metal ions Aluminum on carbon electrode.
2. Guanine can be oxidized electrochemically on carbon based electrodes using transition metal complex.
3. There will be a difference in the number of Guanine available before and after, the detect the target molecule.

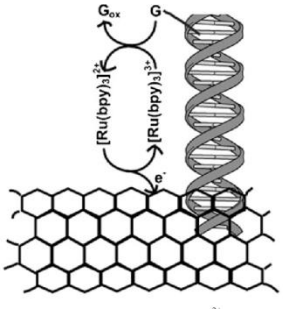


Fig shows an electrochemical DNA biosensor using graphene. The mechanism is based on the oxidation of Guanine.

Summary

DNA is capable of showing electrochemical properties. Guanine is oxidizes more easily as compared to the other bases of DNA. The convenient oxidation of guanine base is helpful in the detection of target molecule under ambient conditions. The efficiency of DNA based biosensor could be improved by carbon/metal based electrodes .

105. Sensing Techniques in DNA Biosensors

In DNA biosensors the process of Bio-recognition relies on the hybridization/interaction of receptor and target molecule. Direct and indirect methods could be used to detect the detection of target

molecule.Suitable techniques includes electrochemical methods, optical methods acoustic methods and mass sensitive methods. Sensing techniques are already discussed in detail in previous modules. In DNA biosensors, hybridization or protein interaction is the main event for the detection of target molecule.

The process of bio-recognition is detected by suitable techniques such as

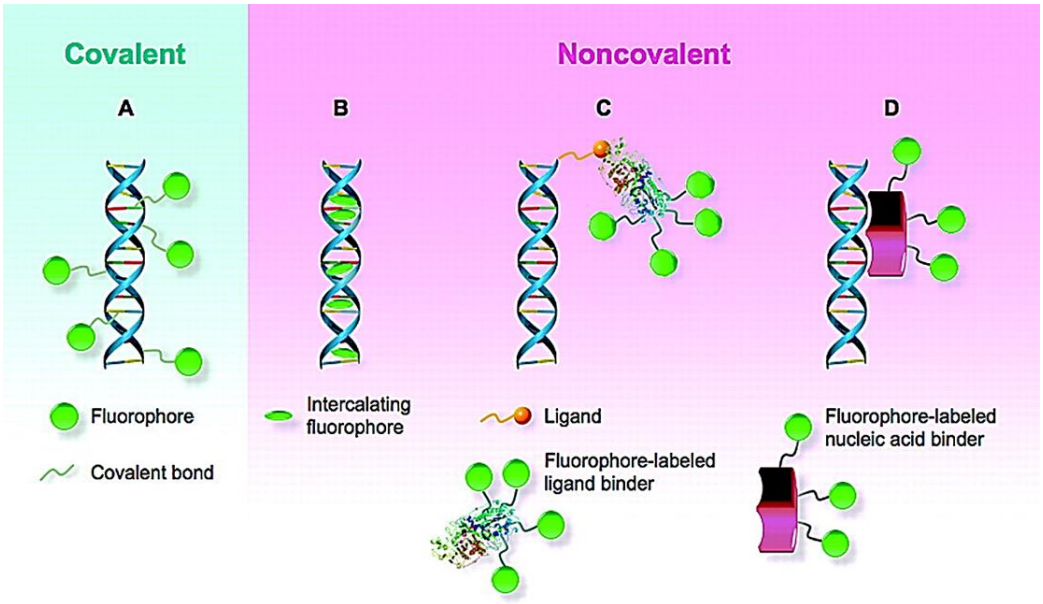
1. Optical methods
2. Electrochemical methods
3. Piezoelectric methods

These methods includes direct and indirect detection of the target molecule.

Optical methods

In optical methods the BRE DNA is labelled with a suitable reporter molecule to detect the biorecognition event.

1. The BRE could be labelled at DNA 3' end and DNA 5' end using terminal transferase enzyme.
2. Generally bacteriophage T4 Polynucleotide kinase (PNK) is used for labeling.
3. The reporter molecule includes Biotin, radio labelled or florescent molecules.



Different methods of DNA labeling including covalent and non-coval;ent methods are shown in Fig.

Mass sensitive Detection in DNA Biosensors

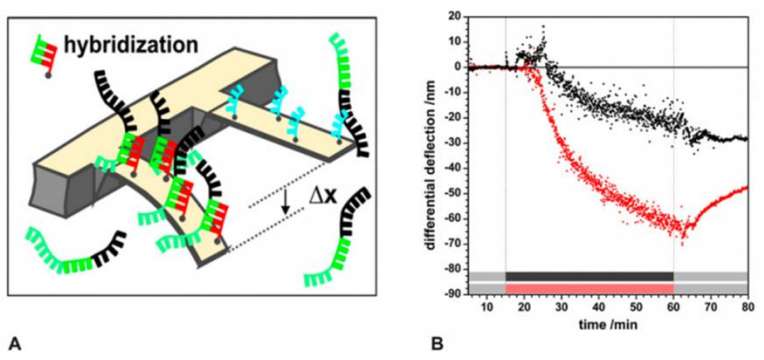
Unlike the standard DNA detection methods that involve DNA labeling, a cantilever biosensor platform involves adsorption of biomolecules on a micromechanical layer. As the biomolecules adsorb onto the surface of the cantilever, the reaction causes a decrease in the surface free energy. A differential surface

stress is generated between either side of the cantilever beam as a result of adsorption of biomolecules occurring at one side of the cantilever.

For DNA detection, the hybridization that occurs between the target probes changes the intermolecular interactions within a monolayer at one side of the cantilever layer. This further induces surface stress that bends the cantilever bean and initiates a motion . The deflection of the cantilever caused by surface stress change, the range of several nanometres, is measured using a piezoelectric readout.

Working.

1. Piezo electric crystals are used in the fabrication of highly sensitive mass sensors.
2. They are able to distinguish mass changes down to the level of single molecules.
3. Cantilever-based biosensors measure mass change on the basis of the changes of resonance frequency of the excited piezoelectric film.
4. After the detection of target molecule, DNA molecule hybridize with the target molecule.
5. The hybridization to immobilized oligonucleotides leads to molecular crowding, which creates a surface stress resulting in cantilever bending.
6. differential deflection can be measured with the help of a reference cantilever.



Mass sensitive detection of target DNA using a cantilever.

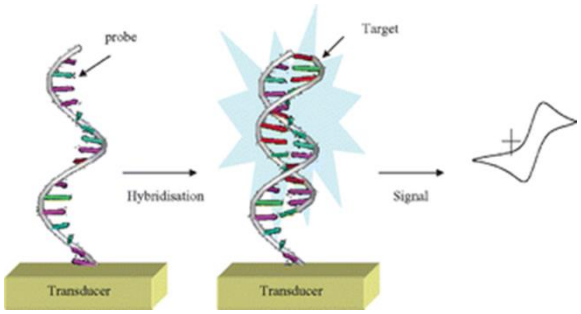
Summary

1. In DNA biosensors the process of Bio-recognition relies on the hybridization /interaction of receptor and target molecule.
2. Direct and indirect methods could be used to detect the detection of target molecule.
3. Suitable techniques includes electrochemical methods, optical methods acoustic methods and mass sensitive methods.

106. **Label Free Electrochemical detection** in DNA Biosensors Label free electrochemical detection is one of the suitable method for the detection of target molecule. No enzyme or mediator are required to detect the target molecule at neutral pH. Electrochemical DNA biosensors exploit the affinity of ssDNA for complementary strands of DNA/RNA.

The method is relied on the electrochemical properties of DNA and the all four bases of DNA.

In label free Electrochemical DNA sensors the biorecognition process is detected by oxidation /reduction.



Label free electrochemical detection.

The method is relied on the electrochemical properties of DNA and the all four bases of DNA.The electrochemical signals are detected on theoxidation/Reduction of bases, without any labelling. DNA and RNA are electroactive compounds producing reduction and oxidation signals after hybridization. Signals of adenine, cytosine, and guanine can be observed on oscillopolarograms of ssDNA, whereas these signals are absent with dsDNA. Guanine is described as the most redox-active nitrogeneous base in DNA. Immobilized guanine-free probes can be used to develop a label-free electrochemical DNA hybridization biosensor.

Drawbacks .

1. It requires high potential to detect the Guanine oxidation.
2. High background signals.
3. Non-specific adsorption of DNA target containing Guanine bases.

Strategy In label free Electrochemical DNA sensors modified electrodes are used to amplify the signals produced by hybridization. Glassy carbon is a good example.

Features of Glassy Carbon (Allotropic form of Carbon)

1. High-purity
2. Excellent Stability as high as at 3,000 °C in vacuum

3. Stable at 500 °C in the air
4. Inert against Chemical erosion
5. Impermeability to Gas and Solution
6. Significant Hardness / Strength
7. Favorable electric conducting property
8. Highly Resistant against Inorganic and Organic salts
9. Nonporous
10. Good Bio-Compatibility

Determination of DNA Bases using glassy carbon electrode.

A biosensor is fabricated to study the direct electro-catalytic activities of DNA bases using volta-metric techniques. In the biosensor using Cu and Cesium. Copper is a transition metal and a good conductor of electricity. Cesium is a chemical element with the symbol Cs and atomic number 55. It is a soft, silvery-golden alkali metal with a melting point of 28.5 °C, which makes it one of only five elemental metals that are liquid at or near room temperature.

1. A modified electrode glassy carbon electrode is used with 3%Cu/CeO2.
2. The oxidation of DNA bases is carried out near pH 7.4 using phosphate buffer solution.
3. Biosensor was successfully detected different bases.

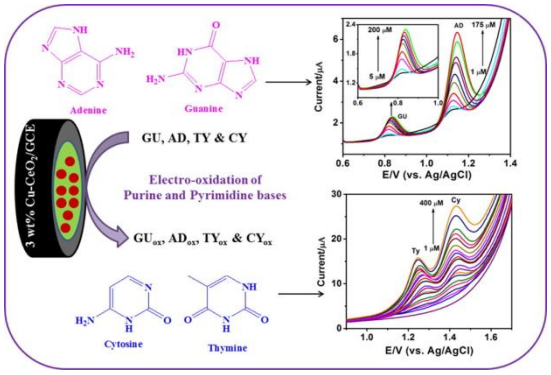
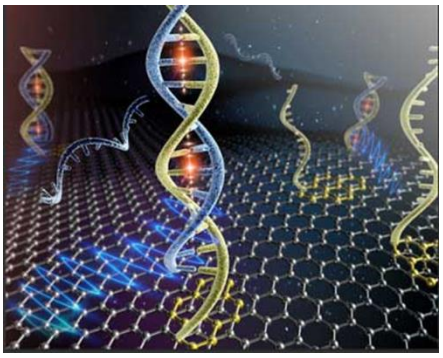


Fig shows Separation of A and G within the range of 0.8-1.2 while C and T were detected around 1.3 and 1.5.in glossy carbon based biosensor.

Electrochemical Detection HIV target DNA

A single-stranded PNA molecule is non-covalently linked the graphene surface with a pyrene linker group. The complementary DNA-strand binds with HIV target DNA. 2D grephene with excellent electronic properties help to detect this hybridization event at a very low noise level.



Use of PNA Based electrochemical Detection complementary HIV DNA molecules binding to PNA molecules that are noncovalently anchored on the graphene surface. The sine wave represents the sensing signal from the molecules being amplified by the low-noise graphene electronic sensor.

Advantages of label free DNA Biosensors

The changes in oxidation and reduction (with and without hybridization) are bases of the detection.

Label-free electrochemical DNA hybridization method is

1. Simple
2. Rapid
3. Highly sensitivity
4. Reproducibility
5. Long-term stable.

Summary

I. Label free electrochemical detection is one of the suitable method for the detection of target molecule.
II. It has some drawbacks.
III. Modified electrode are used to amplify the signals and reduce the noise.
IV. Glassy carbon electrode and grephene modified electrodes are used for specific detection of target molecule.

107. <u>Labeling of DNA by Physical methods</u>

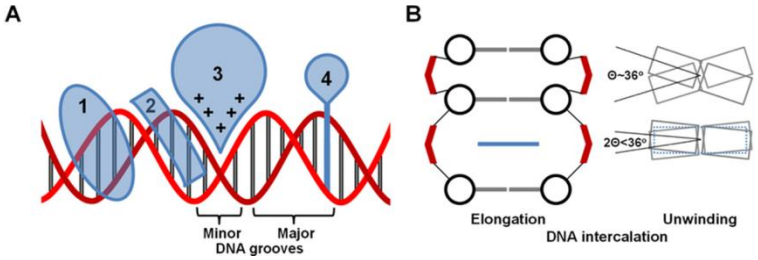
DNA molecule is used as BRE in DNA based Biosensors. The efficiency of DNA biosensor is improved by the employment of reported molecules. These molcules are attached by different physical mechanisms; These interactions are based on the physical methods. The reporter molecules can bind using different physical mechanisms to bind with the DNA molecule.

The different modes of DNA interactions depends on the nature of reporter molecule. Different types of physical interactions are discussed in the lecture

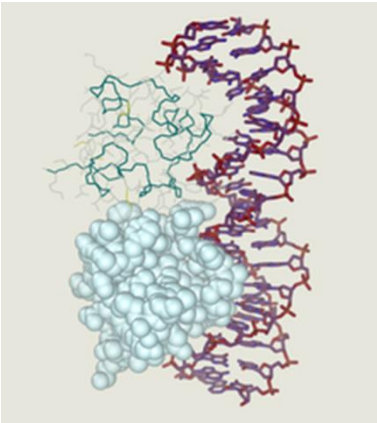
1. Electrostatic interaction
2. Intercalation binding
3. Binding with minor Grooves
4. Binding with major Groove
5. High affinity to a specific DNA sequence

DNA probe is physically labeled with different molecules to improve the performance of the sensor. The binding helps to monitor the DNA hybridization events via an electrochemical method.A number of molecules are used as an indicator such as

I. Organic Dyes
II. Metal complexes
III. Cancer Drugs



(A) Schematic diagrams of different DNA binding modes: 1 is a major groove binder, 2 is a minor groove binder, 3 represents electrostatic/allosteric binding and 4 is an intercalator. The DNA backbone is in red and the base pairs in gray.

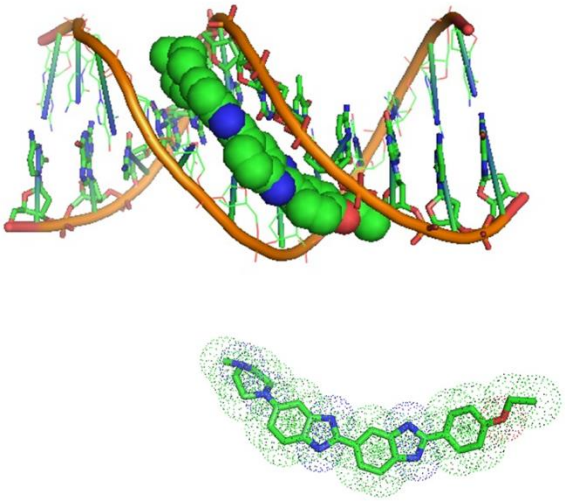


There are In general, proteins bind to DNA in the major groove; however, there are exceptions

Minor Groove Binders.

1. Minor Groove Binders are crescent-shaped molecules that selectively bind non-covalently to the minor groove of DNA.

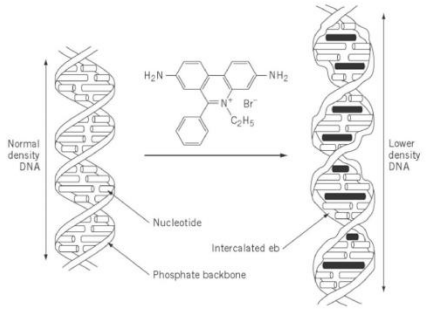
2. Binding to DNA with specific sequences usually takes place by directed hydrogen bonding to base pair edges.
3. Duocarmycin A is an antitumor agents, that bind to the minor groove of DNA and alkylate Adenine at the N3 position.



Binding of DNA with Minor Groove Binder .

<u>Binding by Intercalation</u>

1. Intercalation is the insertion of molecules between the planar bases of DNA by Vander wall forces.
2. Ethidium bromide intercalated between two adenine-thymine base pairs.
3. Ethidium is capable of forming close van der Walls contacts with the base pairs and that's why it binds to the hydrophobic interior of the DNA molecule.



Intercalation of Ethidium bromide with DNA.

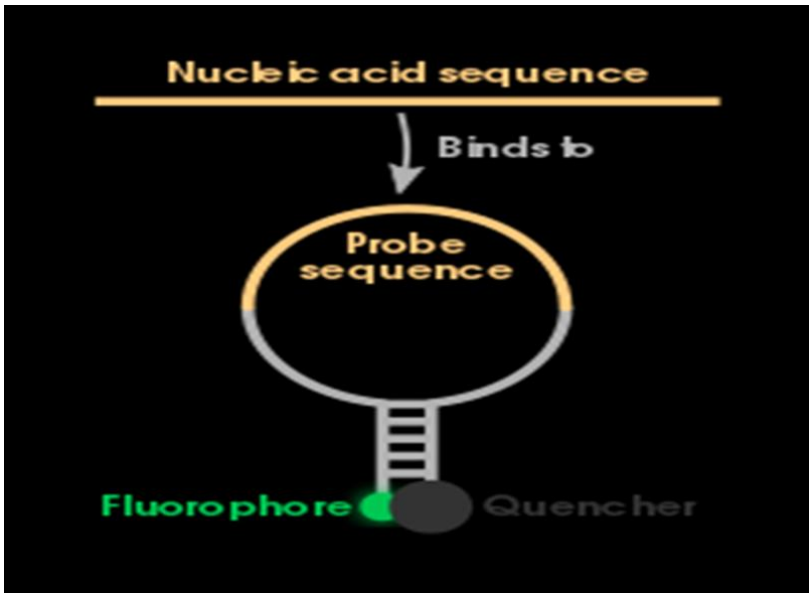
Summary

The efficiency of DNA biosensor is improved by the employment of reporter molecules. These molecules are attached with DNA molecule by Intercalation, Binding at minor groove , Binding at major groove, electrostatic interaction. These physical mechanism are based on the structure of DNA**.**

108. Molecular Beacons

Molecular beacons, or molecular beacon probes, are oligonucleotide hybridization probes that can report the presence of specific nucleic acids in homogenous solutions. Molecular beacons are hairpin-shaped molecules with an internally quenched fluorophore whose fluorescence is restored when they bind to a target nucleic acid sequence.

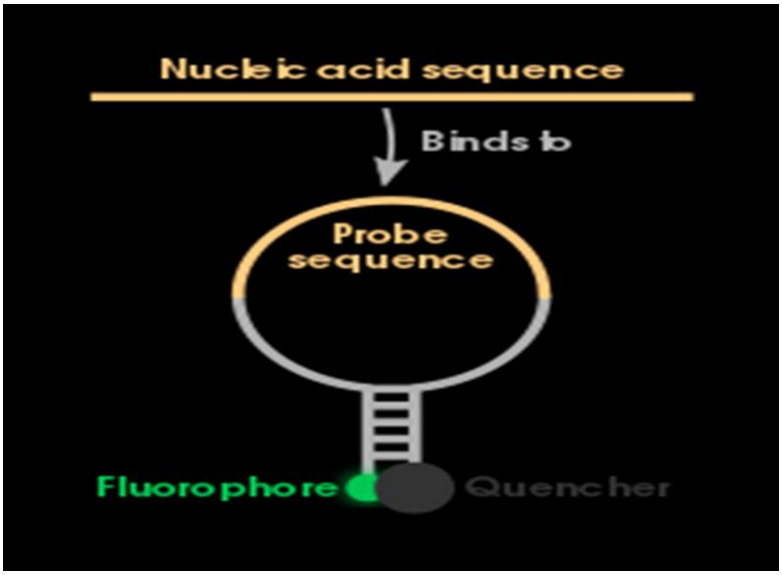
This is a novel non-radioactive method for detecting specific sequences of nucleic acids. They are useful in situations where it is either not possible or desirable to isolate the probe-target hybrids from an excess of the hybridization probes.



Structure of a molecular beacon.

A typical molecular beacon probe is 25 nucleotides long. The middle 15 nucleotides are complementary to the target DNA or RNA and do not base pair with one another, while the five nucleotides at each terminus are complementary to each other rather than to the target DNA. A typical molecular beacon structure can be divided in 4 parts: 1) loop, an 18–30 base pair region of the molecular beacon that is complementary to the target sequence; 2) stem formed by the attachment to both termini of the loop of two short (5 to 7 nucleotide residues) oligonucleotides that are complementary to each other; 3) 5'

fluorophore at the 5' end of the molecular beacon, a fluorescent dye is covalently attached; 4) 3' quencher (non fluorescent) dye that is covalently attached to the 3' end of the molecular beacon. When the beacon is in closed loop shape, the quencher resides in proximity to the fluorophore, which results in quenching the fluorescent emission of the latter.



If the nucleic acid to be detected is complementary to the strand in the loop, the event of hybridization occurs. The duplex formed between the nucleic acid and the loop is more stable than that of the stem because the former duplex involves more base pairs. This causes the separation of the stem and hence of the fluorophore and the quencher.

Fluorophore and Quencher

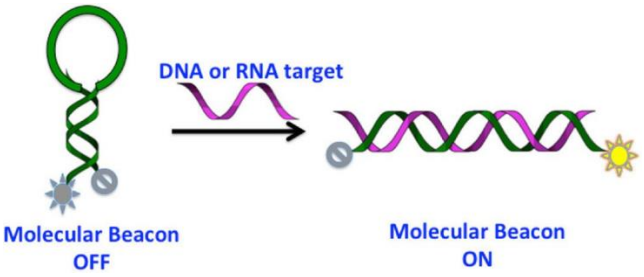
1. Fluorophore is attached at one end of one arm and Quencher is attached at the end of other arm.
2. Quenching is a process which decreases the fluorescence intensity of a substance.
3. When the fluorophore and quencher are in close proximity, the quencher absorbs the energy emitted from an excited fluorophore, thereby suppressing its emission.

Designing and principle

Molecular beacons do not fluoresce in their free form.
In the absence of targets, the probe is dark, because the stem places the fluorophore close to the non-fluorescent quencher.
Quencher and fluoropore transiently share electrons, eliminating the ability of the fluorophore to fluoresce.
Example of Quencher Cl, Acrylamide, NaI.

Working

When the probe encounters a target molecule, it forms a probe-target hybrid.
The hybrid is longer and more stable than the stem hybrid.
After hybridization the quencher moved away from the fluorophore.
As the quencher is away from the fluorophore, the fluorescence of the fluorophore is the restored.



.Working of a molecular beacon in a Biosensor.

Once the fluorophore is no longer next to the quencher, illumination of the hybrid with light results in the fluorescent emission. The presence of the emission reports that the event of hybridization has occurred and hence the target nucleic acid sequence is present in the test sample.

Summary

1. Molecular beacons are used for the detection of hybridization process.
2. Beacon is composed of a stem and a loop.
3. The beacons are designed with having two types of sequences.
4. A flouropore and a quencher at attached at each arm of the stem.
5. Hybridization allows the restoration of fluorescence.

109. **Enzyme Sensing Techniques in DNA Biosensors**

Introduction

1. Enzymes are used in DNA based Biosensors to increase their sensitivity.
2. Generally the enzymes are used in electrochemical based DNA biosensors.
3. Electrochemical signals of the DNA biosensors are amplified by a chromogenic substrate is used to detect the enzyme activity.

The DNA molecule act as a reporter DNA probe. The biological recognition element(DNA) attach with is the specific enzymes for signal amplification. Three enzymes are discussed in the lecture. These enzyme are also discussed in other modules.

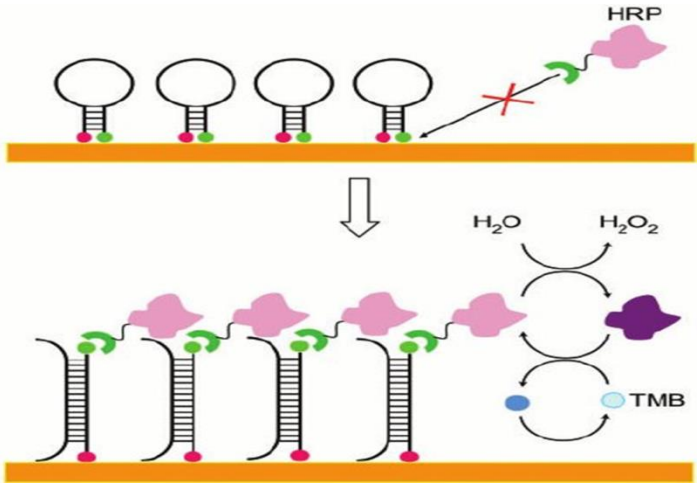
1. Horseradish peroxide
2. Alkaline phosphate
3. Glucose oxidase
4.

Hairpin DNA probe (HDP)

1. Hairpin DNA probes (HDPs) or stem loop pbobes are specially designed ss DNA and have excellent sensing specificity. HDP is immobilized on the electrode surface.
2. Hairpin DNA probe does not bind with the enzyme probe until it hybridize with the target molecule.
3. Binding of target molecule with the DNA leads to the generation of signals by the enzyme.

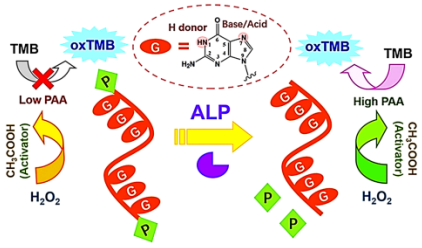
Biosensor based on HDP for Enzyme HRP

One end of the HDP is tagged with SH group for binding with the electrode and the other end is linked with the fluorescent probe for HRP.
When there is no hybridization, the hairpin is closed. HRP could not form a complex with the probe.
After hybridization with target, the hairpin opened up and the HRP complex is formed with the probe.



Alkaline Phosphatase reporter enzyme

In a DNA based Biosensor ALP is not effectively used because Phosphates significantly retard the oxidation of TMB (substrate).
The use of per acetic acid in the biosensor can improves the signals.
Guanine bases of DNA acts as a proton donor and electro-activity improves the signals.



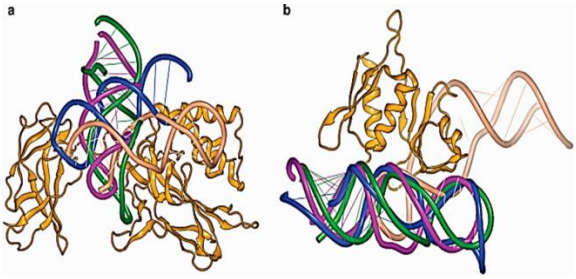
Summary

1. Enzymes are employed in DNA based Biosensors to increase the sensitivity of the DNA based biosensors.

2. Different enzymes including HRP, GOX and alkaline phosphatases are used.
3. All the enzymes have chromogenic substrates to amplify the signals for the detection of target molecules.
4. A number of strategies are used for signal amplification by the enzymes

110. <u>Aptamers Based DNA Sensors</u>

Aptamers are single stranded DNA or RNA molecules that fold into defined secondary structures and bind to targets with high affinity and specificity. Aptamers are developed using both RNA and DNA, as well as non-natural bases, backbones, and small molecules.



Shape dependant interaction of Aptamer with target molecules.

Aptamers have been used in a variety of applications; detection molecules in ELISA-like assays, protein-specific tissue staining, targeted drug delivery, and as an FDA-approved treatment for macular degeneration.

SELEX Techniques

Aptamer development is accomplished in 3 phases:

1. Selection: DNA sequences that bind to the target are partitioned from sequences that do not. Binding sequences are amplified via PCR and brought forward for additional rounds of SELEX (generally 5-10).

2. Sequencing: RayBiotech uses NGS and data analysis to determine which aptamers to evaluate as opposed to randomly selecting individual clones.

3. Candidate Evaluation: Aptamers are evaluated for binding and the best aptamer is supplied to the customer

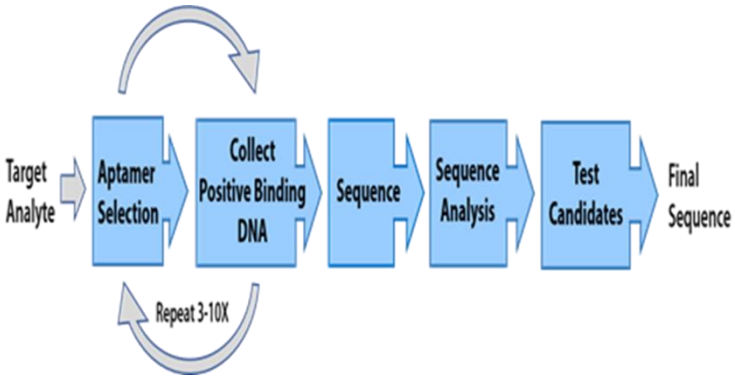


Fig shows Process of Sequential Evolution of Ligands by Exponential Enrichment (SELEX).

Aptamers are developed using a process known as **SELEX** (Systemic Evolution of Ligands by Exponential Enrichment). During SELEX, trillions of random DNA oligos are mixed with the target molecule; sequences that bind to the target are then collected and amplified. After multiple rounds of selection, the DNA is sequenced and individual sequences are evaluated for binding. Using SELEX aptamers with affinity for a variety of targets can be developed, including proteins, peptides, and small molecules.

The Aptamer Advantage

Due to their robust binding affinities for specific biomolecules, aptamers have been explored as an alternative to antibodies. Aptamers offer the following advantages:

- Increased thermal stability and shelf life
- Aptamers can undergo multiple freeze-thaw cycles and retain activity
- Aptamers can be designed to increase enzyme resistance for in vivo and in vitro use
- Aptamers can be modified with fluorescent dyes or biotin for detection without post synthesis modification.
- Aptamers can be easily conjugated to proteins, peptides, drugs, and other small molecules using a variety of chemistries without concern
- Time to completion of custom aptamers is significantly faster than antibody development, generally 3 months
- Eliminates need for hybridoma storage and maintenance. Aptamers can be "stored" digitally and are produced synthetically based on their sequence.
- Easily scalable; can be generated and replenished without the use of animals. Aptamers can be synthesized in under a week.

Designing and production of High affinity ligand

High affinity can be created by ligand-induced structural changes in the aptamers.

It resulted in the formation of unique secondary structures that are responsible for high affinity ligand binding.

For example the introduction of Abasic site in the binding site of the target molecule introduces changes.

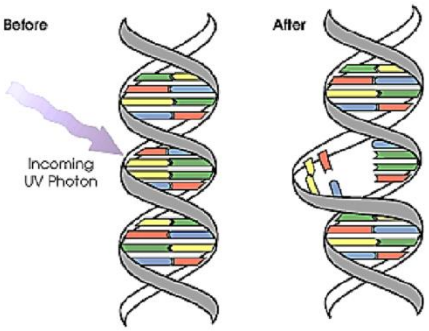


Fig shows the introduction Introduction of Apurinic/Apyrimidinic site in DNA molecule for aptamer dsigning by UV radiation.

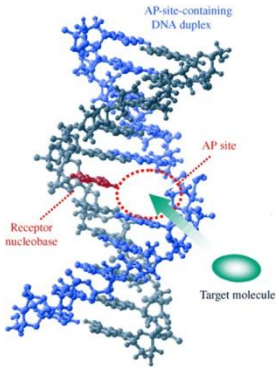


Fig shows The binding motif of an AP-site-containing DNA duplex as an aptamer for small ligands. The pale-blue strand represents the AP-site-containing strand and the gray strand represents the complementary strand with the receptor nucleobase (red).

Advantages/Applications.

1. Aptamers are single stranded nucleic acids or their derivatives small molecule.
2. They are chemically synthesized therefore easily scalable.
3. They have Increased thermal stability and shelf life.
4. Aptamers can undergo multiple freeze-thaw cycles and retain activity.

Summary

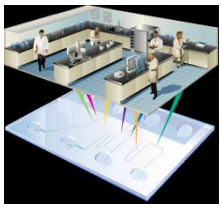
Aptamers are ssDNA/ RNA or their derivative molecules used as a BRE.
Selection of Aptamer is the most important step in the fabrication of a Biosensor.
Aptamers bind to their target by determining the tertiary structure.
Aptamers are cost effective, reliable tools for biosensors.

Biosensor

Chapter 10 Lab on Chip

111. An Introduction to Lab on Chip

A lab-on-a-chip is a device that an perform the functions of a laboratory on a single chip of only millimeters to a few square centimeters in size. LOCs deal with the handling of extremely small liquid volumes upto pico liters.Lab-on-a-chip devices are a subset of micro-electro-mechanical systems (MEMS) devices. LOC technology was established by defense purposes. A small chip can perform the functions of a laboratory. It is mainly used for diagnostic purposes. LOC is one of the fastest growing technology.



Development of LOC technology

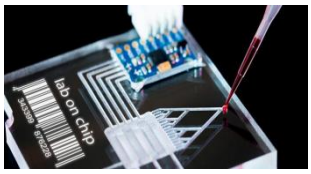
The development of LOC technology is related to human genome sequence project, where fast running technologies were established to sequence the whole human genome. Later this approach was used to establish LOC by Defense Advanced Research Project Agency (DARPA) of USA for military applications. The ability to perform laboratory operations on a small scale using miniaturized (labon-a-chip) devices is very appealing. Small volumes reduce the time taken to synthesize and analyse a product; the unique behaviour of liquids at the microscale allows greater control of molecular concentrations and interactions; and reagent costs and the amount of chemical waste can be much reduced. Compact devices also allow samples to be analysed at the point of need rather than a centralized laboratory. construct highly integrated compact devices. Chemists are using such tools to synthesize new molecules and materials, and biologists are using them to study complex cellular processes. Furthermore, labs on chips offer point-of-care diagnostic abilities that could revolutionize medicine. Such devices may find uses in other areas, including a range of

industrial applications and environmental monitoring. Commercial exploitation has been slow, but is gaining pace, with some products now on the market. The advantages are compelling, but designing and making devices of reduced size that operate effectively is challenging. The pioneers recognized the huge financial input and research effort needed to realize the full potential of the concept.

Structure of LOC

A small chip made up of silicon, glass or ceramics.

1. Micro channels that are engraved or molded on the surface of the chip for the introduction of samples and reaction.
2. A reagent cartridge that contains the required reagents for the test.
3. Components for sample introduction and mixing.
4. Electronic circuits.



Features of LOC

1. Safe Platforms.
2. The LOC platforms use very minute amount of samples and reagents, there is no need for any proper training and no specific safety precautions are required.
3. Lower fabrication cost. It allows mass production for cost effective disposable chips.
4. Quality control. The quality of the result is automatically verified.

Components of LOC

For understanding the LOC technology it is require to study the following in detail.

1. Materials for chip
2. Lithography
3. Capillary Electrophoresis

4. Microfluidics
5. Introduction of sample/Reagent onto LOC
6. Mixing of Reagents in LOC
7. Electronic circuits

Advantages.

Required less sample volumes for diagnostics

1. Low fluid volumes consumption
2. Less waste
3. Lower reagents costs
4. Fast response
5. Fast Analysis
6. Small heat capacity
7. Fast heating

Summary

A lab-on-a-chip (LOC) is a device of several square millimetres to centimetres that comprises several analytical steps. Technology uses analytical processes after miniaturization to enhance mobility and efficiency. This makes LOC applications suitable for clinical diagnostics and 'near-patient' or 'point-of-care' (POC) testing. In many ways, the features of LOC devices fulfil the requirements for a POC diagnostic device: low consumption of reagents and sample, miniaturization of device and fast turn-around time for analysis.

112. Microfludics

Microfluidics is the technology of manipulating and controlling fluids and particles at micron and submicron

dimensions and the technology associated with the development of methods and

devices to undertake such operations. Using building blocks

to form microfluidic platforms enables the implementation of assay

miniaturization. Such platforms, characterized by fluidic channels and chambers,

will enable the miniaturization, integration, automation and parallelization, as in

performing multiple tests at the same time, of (bio)chemical processes.

Microfluidics is defined as the manipulation of the flow of very small quantities of fluid within channels in the micrometre range - nanofluidics takes this a stage further, and often deals with the movements of individual macromolecules in solution. Understanding and commercializing this area of physics has been crucial to development of viable lab-on-a-chip devices.

The discipline of microfluidics developed as a consequence of increasingly accurate analysis techniques such as high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), which are able to obtain extremely accurate results from a small sample size. As the capabilities of these techniques progressed, it was a natural step to try and perform them in as compact a form factor as possible.

The study behavior of fluids at micro level 100 nanometers to 500 micrometersis known as Microfluids. Microfludics is the study of the behavior of fluids at micro level.

Different physical forces effect the flow of liquid in an electric field.

A number of factors play important role in Microfluids.

1. Surface tension,
2. Energy dissipation, and
3. Fluidic resistance.

Due to flow of blood in capillaries, microfluidics is of great importance.

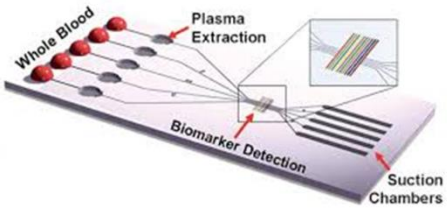
Applications

. Microfluidic-based LOC devices are particularly useful for applications in drug discovery, life sciences, ecology and clinical (in vitro) diagnostics .

Elements/Requirements of Microfluidics.

1. The elements of microfluidic chip are required to accurately manage the fluid inside.

2. The components can be embedded inside the chip or outside the chip.
3. The Quake pneumatic microvalves could be fillted inside.
4. The pressure controllers are fitted outside the microfluidic chip.



A microfluidic chip showing the component.

Reynolds Number

1. Reynolds number is a dimension less quantity.
2. Reynolds number compares the effect of the momentum of a fluid to the effect of viscosity.
3. The Reynolds number is the ratio of inertial forces to viscous forces.
4. It can predict if a flow condition will be laminar or turbulent.
5. Reynolds Number = Inertial Force / Viscous Force
6. V = velocity of the fluid
7. μ = viscosity of fluid
8. L = length or diameter of the fluid. The fluid flow is turbulent when the value of Reynolds number is greater than 4000.
9. The fluid flow is laminar when the value of Reynolds number (Re) is less than 2000.

The application of Reynolds number is helpful to predict the behavior of fluid in a system. he Reynolds number is the ratio of inertial forces to viscous forces within a fluid which is subjected to relative internal movement due to different fluid velocities. A region where these forces change behavior is known as a boundary layer, such as the bounding surface in the interior of a pipe. A similar effect is created by the introduction of a stream of high-velocity fluid into a low-velocity fluid, such as the hot

gases emitted from a flame in air. This relative movement generates fluid friction, which is a factor in developing turbulent flow. Counteracting this effect is the viscosity of the fluid, which tends to inhibit turbulence. The Reynolds number quantifies the relative importance of these two types of forces for given flow conditions, and is a guide to when turbulent flow will occur in a particular situation.

This ability to predict the onset of turbulent flow is an important design tool for equipment such as piping systems or aircraft wings, but the Reynolds number is also used in scaling of fluid dynamics problems, and is used to determine dynamic similitude between two different cases of fluid flow, such as between a model aircraft, and its full-size version. Such scaling is not linear and the application of Reynolds numbers to both situations allows scaling factors to be developed.

Types of flow in LOC

With respect to laminar and turbulent flow regimes: laminar flow occurs at low Reynolds numbers, where viscous forces are dominant, and is characterized by smooth, constant fluid motion;turbulent flow occurs at high Reynolds numbers and is dominated by inertial forces, which tend to produce chaotic eddies, vortices and other flow instabilities

1. Laminar Flow: the flow of a fluid when each particle of the fluid follows a smooth path, that never interfere with one another.
2. The velocity of the fluid is constant at any point in the fluid.
3. Turbulent Flow: In turbulent flow the speed of the fluid at a point is continuously undergoing changes in both magnitude and direction.

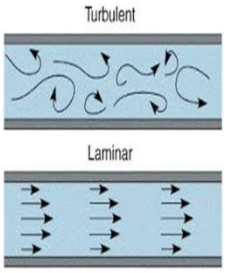


Fig showing the types of flow.

Types of forces that are involved in Microfluidics.

Generally the flow of fluid in a microchip is laminar due to small diameter and small distances.

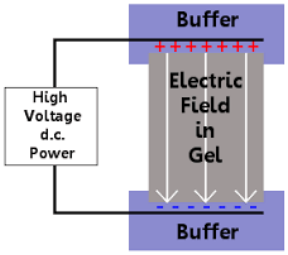
1. It is essential to identify the forces acting at solid-liquid interphase in an electric field, for controlling the processing of sample.
2. There are two basic electrokinetic phenomena.
3. Electrophoresis motion of charged particle relative to surrounding. Electrophoresis is a general term that describes the migration and separation of charged particles (ions) under the influence of an electric field. An electrophoretic system consists of two electrodes of opposite charge (anode, cathode), connected by a conducting medium called an electrolyte.
4. Electrosmosis flow of liquid under the influence of electrical potential applied. Electroosmosis is the convective solvent flow resulting from the application of an electric field across a solution next to a charged surface. For electroosmosis across a charged porous membrane, the fixed charges on a pore wall in the charged membrane lead to an excess of counterions in the solution adjacent to the surface of the pore (to maintain charge neutrality). This leads to the formation of an electrical double layer in the pores of the membrane.

113. **Capillary Electrophoresis**

Capillary electrophoresis is a types of electrophoresis, in which a capillary is used biomolecules are separated on the basis of their charge. The method is very efficient and quick for the separation of Biomolecules requiring very little amount of sample. It is used in the automation in the DNA sequencing methods.

Electrophoresis is an analytical technique that separates ions based on their electrophoretic mobility with the use of an applied voltage. The mobility is dependent upon the charge of the molecule, the viscosity, and the atom's radius. The rate at which the particle moves is directly proportional to the applied electric field. Development of capillary array electrophoresis (CAE) has provided an impressively high-speed, high-throughput sequencing method.

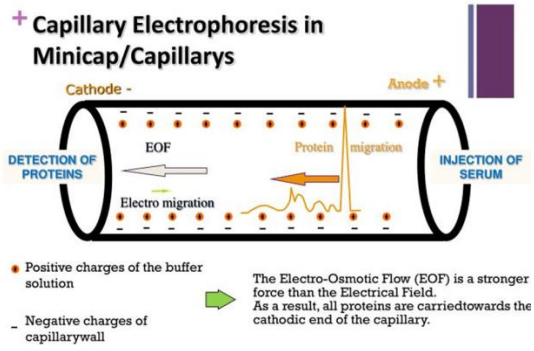
Sequence analysis by capillary electrophoresis (CE) essentially involves the separation of fluorescently-labeled sequencing samples on hair-thin, 30-50cm long capillary gels. Recent advances in the type, composition and quality of the separating polymer, method of sample preparation, dye chemistry and automation have refined the process such that >1000 bases can be sequenced per hour in a single capillary.



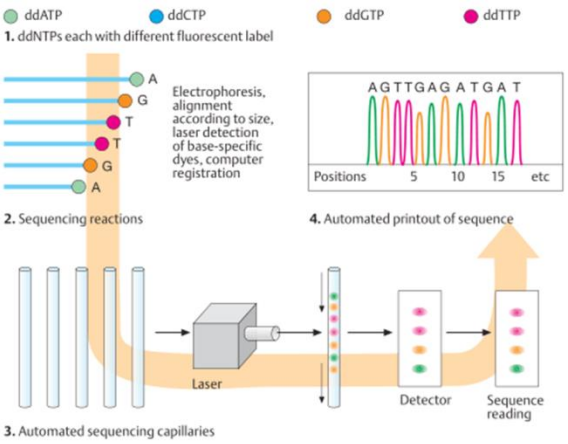
A typical electrophorsis apparatues

Capillary Electrphoresis Apparatus

1. Capillaries are typically of 50 mr l inner diameter and 0.5 to 1 m in length.
2. The applied potential is 20 to 30 kV. Electroosmotic flow is from negative to positive electrode.
3. A small volume of sample (10 nL) is injected at the positive end of the capillary and the separated components are detected near the negative end of the capillary.



A standard CE unit includes a sample injector assembly, the capillary, a power supply, and a detector. Samples are introduced into the capillary through gravity, electric current , or pressure. Common high-sensitivity detectors include UV or visible wavelength and fluorescence detectors. Example. Automated DNA sequencing



114. Photolithography

Photolithography is an optical means of transferring a pattern on a substrate. Photolithography has been one of the main methods used for the patterning of polymers over the past three decades. It is the process of transferring geometric shapes on a mask to the surface of a silicon wafer. Patterns are generated by selectively exposing a light sensitive substrate coated silicon wafer to photoirradiation through a patterned mask, and by subsequently removing selected area of the film through dissolution in an appropriate solvent. At the micron scale, photolithography is relatively easy to create structure of any desired shapes rapidly and reproducibly. However, the traditional photolithography is inherently limited by the wave length of the light used and the typical resolution obtained in laboratories can go down to 100 nm. With deep ultraviolet light it is possible to fabricate the feature sizes down to 50 nm. A recent report demonstrated that ultrahigh resolution periodic patterns can be formed in a photoresist down to 15 nm by surface plasmon interference and the findings open up an avenue to push the half-pitch resolution of photolithography towards 10 nm. Photolithography is an important step in the fabrication of Lab on chip. It requires a low wavelength light source generally UV light is used.Two types of Photoresistor are used to transfer the geometrical pattern on the silicon based wafers.The unwanted resister is removed by etching.

Main points .

1. Photolithoraphy is a technique for transferring a geomatric pattern from a mask.
2. A light source is used to transfer the pattern on silicon chip.
3. This technique is used for printing circuit board and microprocessors.

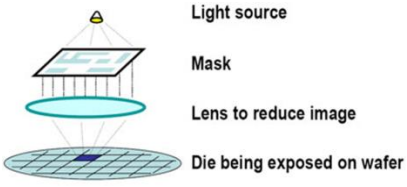
4. The process is also called as optical lithography.

   In photolithography light wave with short wave length is used. Light source is the main constituent of the process. In the technique UV light from gas-discharge lamps using mercury. UV light is also used in combination with noble gases such as xenon. These lamps produce light across a broad spectrum with several strong peaks in the ultraviolet range. In photolithography light wave with short wave length is used. In the technique UV light from gas-discharge lamps using mercury. UV light is also used in combination with noble gases such as xenon. These lamps produce light across a broad spectrum with several strong peaks in the ultraviolet range.

   Steps involved in Photolithography are

   There are a number of steps that are involved in photolithography.

1. Wafer cleaning A solutions based on hydrogen peroxide, trichloromethane, acetone or methanol are used to clean the surface.
2. The wafer is then heated to remove any moisture and stored for further use.
3. An adhesion promoter such as hexamethyl-disililazane (HMDS) is applied to form a highly water repellant layer.
4. Barrier layer formation
5. Photoresist application
6. Soft baking
7. Mask alignment
8. Exposure and development
9. Hard-baking



Cleaning and Preparation

A solutions based on hydrogen peroxide, trichloromethane, acetone or methanol are used to clean the surface. The wafer is then heated to remove any moisture and stored for further use.

An adhesion promoter such as hexamethyl-disililazane (HMDS) is applied to form a highly water repellant layer.

A photoresistor is a type of resistor whose resistance decreases when the intensity of light increases.

Positive photoresisters

On the exposure of light positive photo resister are dissolved.

Negative photo resisters

These photoresistor are not dissolved on exposure to light.

Example. Epoxy based polymers

Etching

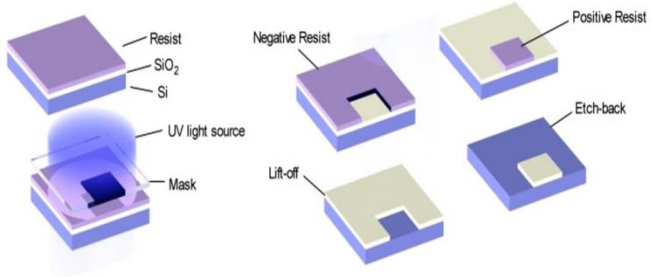
Etching in microfabrication is the removal of unwanted photoresister from the wafer. There are two methods of etching.

Wet Etching

Hydroflouric acid is used to bath the treated silicon wafers for wet etching.

Plasma Etching

Standard conditions for such as pressure under vacuume is created for etching.



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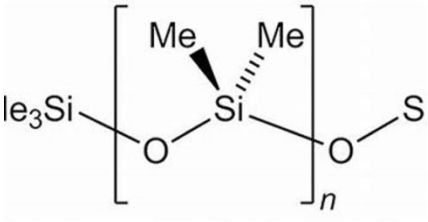
Application of positive and negative photoresistor.

First, the photoresist is placed on the substrate. Then, the substrate is exposed to electromagnetic radiation which modifies the molecular structure followed by a change in the solubility of the material, while placing the mask of pattern. After exposure, etching is carried out. Then this substrate is immersed in a developer solution. Developer solutions are typically aqueous and dissolve away areas of the photoresist exposed to light.

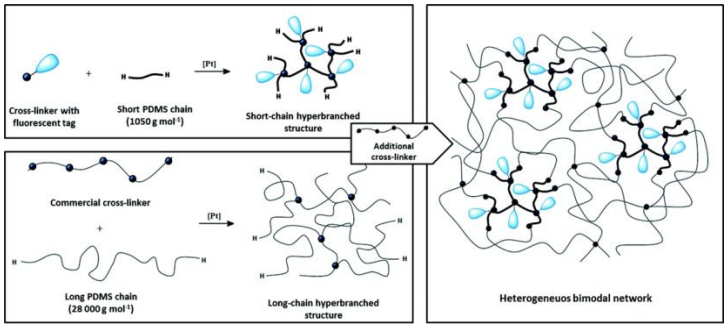
115. Soft Lithography

Soft lithography is a technique for the fabrication of lab on chip, when biological samples are used.Method uses a silicon based elastomer PMDS.The technique is used for the fabrication of microfluidic channels. It is a cost effective techniques for uneven surface. Soft lithography is an extension of photolithography. Soft lithography, extends the possibilities of conventional photolithography. Soft lithography can process a wide range of elastomeric materials, i.e. mechanically soft materials. This is why the term “soft” is used. For instance, soft lithography is well suited for polymers, gels, and organic monolayers. A novel soft material polydimethylsilioxane (PMDS) is the basic material used in the technique.

Soft lithography is used to construct features measured on the micrometer to nanometer scale for non even surfaces. PDMS, however, has been the most widely used material for the applications of soft lithography because of its useful properties including low cost, biocompatibility, low toxicity, chemical inertness, versatile surface chemistry insulating, as well as mechanical flexibility and durability,the PDMS can also be easily manipulated and doing PDMS device can require only few equipments.



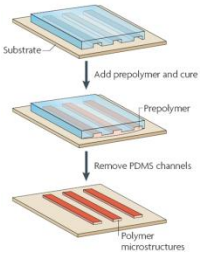
Cross linking of PDMS



.Soft lithography is all based on using a patterned layer of PDMS. PMDS is transparent.It is biocompatible with some restrictions and is easy to mold.It forms strong bond with other PMDS layer of glass by simple plasma treatment.It is gas permeable. It enables cell culture by controlling the amount of gas through PDMS.

Method

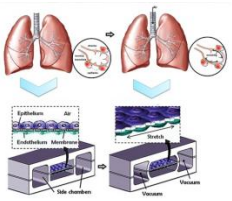
1. Preparation of the mold with the silanization
2. Scaling and mixing of the PDMS and the curing agent.
3. PDMS pouring on the mold
4. The PDMS baking
5. The PDMS peeling off the mold
6. Cutting and piercing
7. The PDMS bonding by plasma.



Advantages.

1. Well-suited for applications involving large or nonplanar surfaces.
2. Smaller details than photolithography in laboratory settings (~30 nm vs ~100 nm).

3. Human alveolar epithelial and pulmonary microvascular endothelial cells cultivated in a PDMS chip to mimick lung functions.



PDMS SOFT LITHOGRAPHY FOR MICROFLUIDICS

A first essential feature of soft lithography is the possibility to obtain a sealed microfluidic device. Typically, the microchannels imprinted in the PDMS layer are simply closed with a glass slide. Alternatively, another piece of PDMS can be used.

PDMS bonding is required to obtain a strong permanent bonding between the layer of PDMS and the glass slide. This bonding is usually made by plasma bonding. When the microchannels are properly sealed, fluids can be pumped at pressures as high as ~350 kPa without failure.

Types of PDMS SOFT LITHOGRAPHY:

1. **REPLICA MOLDING**

In replica molding, a patterned layer of PDMS is used as a soft mold where a polymer is poured. After curing, the polymer is separated from the PDMS mold. In replica molding can duplicate 3D structures in a single step and the same PDMS mold can be reused many times.

2. **CAPILLARY MOLDING**

Capillary molding is a second technique where a patterned PDMS is used as a mold. The patterns of the PDMS layer must first be brought into contact with a substrate (e.g. a glass slide). As the name of the technique implies, capillarity is exploited to progressively fill the patterns. After having cured the polymer, the PDMS can be gently removed, leaving solid microstructures at the surface of the substrate.

PDMS soft lithography micromolding in capillaries

3. **MICROTRANSFER MOLDING**

In microtransfer molding the patterned surface of a PDMS layer is filled with a liquid polymer. When the excess polymer has been removed, the PDMS layer is inverted and

brought into contact with a substrate. After the curing of polymer, the PDMS layer is cautiously peeled away, leaving a solid structure with a feature size down to 1µm on the surface of the substrate.

.116**. Optical Fibers in LOC**

Fiber optics, or optical fiber, refers to the medium and the technology associated with the transmission of information as light pulses along a glass or plastic strand or fiber, surrounding the glass fiber core is another glass layer called cladding. Light travels down a fiber optic cable by bouncing off the walls of the cable repeatedly. Each light particle (photon) bounces down the pipe with continued internal mirror-like reflection. The light beam travels down the core of the cable. Optical fiber is used as a medium for telecommunication and computer networking because it is flexible and can be bundled as cables. It is especially advantageous for long-distance communications, because infrared light propagates through the fiber with much lower attenuation compared to electrical cables.

Using optical fibers to perform detection on-chip is an excellent example of two completely different technologies merging together to produce a synergistic result. In this entry, on-chip refers to a lab-on-a-chip (LOC) or micro total analysis system (µTAS). The optical fiber or fibre is a thin, transparent strand, usually made of glass or plastic, for transmitting light. Optical fibers integrated with an LOC may be used to detect particles, chemicals, biomaterials, or other analytes, typically in an aqueous medium. The fibers are used to deliver source light to the chip and to collect emitted light from the sample located in the detection zone of the chip. The optical fiber is integrated into LOC for the delivery the light source. Application of fiber optics in LOC in an advancement in the technology. Fiber optic detects signals and prevent the signal loss. Optical fiber can be embedded within the proximity of the LOC. False positive signals are not detected and the sensitivity of the device is increased manifold. It is used for the detection of signals at a precise location.

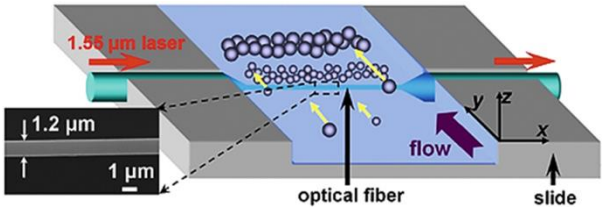
Lab on chip devices show poor sensitivity due to three apparent reasons.

1. Loss of detectable signals
2. Presence of Ambient signals
3. Use of small sample Volume

Detection limit is the lowest concentration of the Analyte that is determined by a LOC with statistical signification.

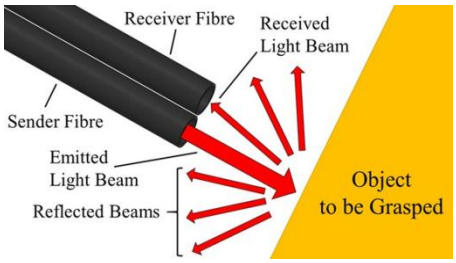
There are two ways under which optical fibre can be integrated into the LOC. he fiber optics belong to the finest class of smart materials.

Embedded optical fibers are incorporated into the Lab on chip with physical contact to the micro-channel structures. This setting offers best performance because there is very little loss of signals.The disadvantage is the complicated designing and fabrication process.



Embedded optical fibre in LOC

The proximity optical fibers are located in close proximity of Lab on chip but they do not touch the chip. The advantage of this system is that they are convenient to design and fabricate. The proximity optical fiber may introduce additional notice to the system. Fiber optic proximity sensors are used to detect the proximity of target objects using light. Light is supplied and returned via fiber optic cables. Fiber optic cables can fit in small spaces, are not susceptible to electrical noise, and exhibit no danger of sparking or shorting.



117. Diagnostic targets in LOC

LOC in Clinical diagnostic are used for the detection of early indicators of disease. LOC are used for the detection of different Analytes in Biological samples.

1. Proteins or their derivatives
2. Metabolites
3. Nucleic Acids

4. Cells
5. Pathogens

Proteins

Current LOC devices utilize immunoassay technology, including antigen-antibody binding.

1. Examples.
2. Troponin I test
3. Troponin T test
4. Prostate-specific antigen for prostate cancer,
5. Pregnancy test to detect HCG (Human Chorionic Gonadotropin)

The best-known protein-detection device, the pregnancy test kit, measures the pregnancy hormone human chorionic gonadotropin. A pregnancy test is around 99 percent reliable.It works by measuring levels of a hormone (HCG). HCG can be present in the blood and urine approximately 10 to 14 days after conception.

Glucose (a type of sugar) molecules in the blood normally become stuck to hemoglobin molecules - this means the hemoglobin has become glycosylated (also referred to as hemoglobin A1c, or HbA1c). The glycosylated hemoglobin test shows what a person's average blood glucose level was for the 2 to 3 months before the test. The Glycated hemoglobin is a form of hemoglobin that is chemically linked to a sugar.

It indicates the presence of excessive sugar in the bloodstream, indicative of diabetes.

It is measured primarily to determine the three-month average blood sugar level and can be used as a diagnostic test for diabetes mellitus. Glycated haemoglobin, intermediary compound is reversible but after some internal rearrangement of the compound, a stable HbA1c is formed. Of the three types of HbA1 namely, HbA1a, HbA1b, and HbA1c. HhA1c represents the most prevalent glycated species.

Cells.

The identification and enumeration of specific (human) cells in blood and other samples is a rapidly expanding field in POC diagnostics. In addition to basic blood cell counting, it has been widely recognized that POC cell assay-based devices could implement diagnostic and prognostic testing for infectious diseases, cancers, inflammatory responses and haematological parameters The cell assay-based devices could implement diagnostic and prognostic testing for

1. Infectious diseases
2. Cancers
3. inflammatory responses
4. Haematological parameters

Metabolic products Metabolites are products of chemical processes that generate energy, nutrients or wastes. Because of the similarities in their physiological transport and detection approaches for LOC assays, they are grouped together with blood ions (Na^+, K^+, Cl^- , etc.) and small-molecule organic substances, including non-protein hormones, e.g. epinephrine and cortisol. Levels of these molecules are often diagnostic indicators of disease

Nucleic acids Nucleic acid diagnostics, often referred to as molecular diagnostics, measure DNA or various types of RNA in order to assay particular genomic or genetic details of a patient or to assay nucleic acid sequences unique to invading pathogens. Polymerase chain reaction (PCR) and numerous other methods of selectively copying ('amplifying') preselected nucleic acid sequences are often part of such assays

PCR on chip.

The polymerase chain reaction (PCR) is a technique for copying specific DNA sequences. The three basic steps in the process - splitting a DNA template into its two single strands (denaturation); adding short segments of complementary DNA called primers to initiate replication of a chosen DNA sequence (annealing); and adding DNA polymerase to synthesise the complementary strand (extension) - are repeated again and again to amplify the sequence. Each of these steps occurs optimally at a different temperature, so heating and cooling is carried out with an instrument called a thermocycler.

Small volume operation and rapid thermal cycling have been subjects of numerous reports in micro reactor chip development. Sensitivity aspects of the micro PCR reactor have not been studied in detail, however, despite the fact that detection of rare targets or trace genomic material from clinical and/or environmental samples has been a great challenge for microfluidic devices. PCR on chip is one of the most challenging assays to develop due to additional steps required for sample. It is used to assay particular genomic

details of a subject. It requires

1. Cell sorting
2. Isolation
3. Lysis
4. DNA extraction
5. Signal amplification
6. Detection of end product

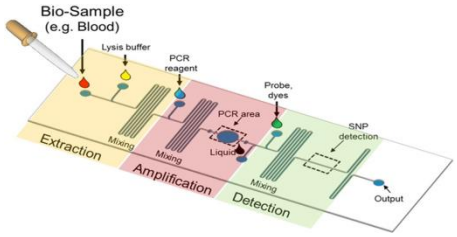


Fig shows PCR in Lab on Chip.

118. Detection principles in Lab on Chip

The detection principles for sensors on microfluidic-based LOC devices are classified into several types, including optical, electrochemical, magnetic and mass sensitive methods. The trend in the development of detectors has been to pursue two key qualities: sensitivity and selectivity, aiming to minimize the numbers of false negatives and false positives. There are several techniques that are used in the detection of target molecule on LOC.

The objective is to maximize the sensitivity, selectivity and minimize the positive and negative false.

Apart from other techniques magnetic methods are used efficiently.

Optical detection

Conventional optical detection methods, including absorbance, fluorescence and chemiluminiscence, have all been applied in LOC devices. Miniaturizing devices that use optical detection is generally difficult because of the expensive hardware it requires. Furthermore, due to the shorter optical paths through the sample, sensitivity is reduced and increased noise from non-specific adsorption

to the walls of the chamber can be caused by a lower surface-to-volume ratio. To address these issues, many integrated optical systems are being explored in which new techniques are integrated onto the microfluidic device to reduce costs and increase sensitivity.

Electrochemical detection

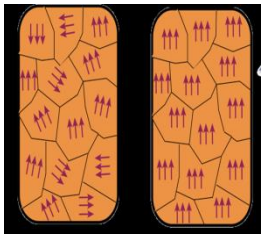
Electrochemical detection methods can be divided into three types of measurements, namely amperometric, potentiometric, and impedimetric measurements. The most commonly used biosensors are amperometric ones. Typically, they generate current in proportion to the concentration of the detected analyte, used for instance in glucose assays. Potentiometric detection examines the difference in potential between two reference electrodes separated by a selective permeable membrane. Impedimetric biosensors operate by measuring the change in impedance caused by changes in resistance at the Sensor.

Magnetic detection

Magnetic particles can be used to concentrate and localize analytes. Moreover, they can be used as labelling technology for detection without the requirements of fluorescent dyes. Stimulated by advances in memory devices, magnetic particle detection technology has evolved rapidly, the most promising and sensitive methods now using the giant magnetoresistance (GMR) effect, with detectors based on so-called spin valves or magnetic tunnel junction methods.

Magnetic field can be applied to detect the magnetic material. An everyday example of ferromagnetism is a refrigerator magnet used to hold notes on a refrigerator door.

A material is “ferromagnetic” if all of its magnetic ions add a positive contribution to the net magnetization.



Ferromagnetic materials have a positive susceptibility to an external magnetic field.

They exhibit a strong attraction to magnetic fields. They are able to retain their magnetic properties after the external field has been removed.Example Iron, nickel, and cobalt. Magnetic particles can be used to concentrate and localize analytes. Magnetic particles can be used as labeling technology for detection without the requirements of fluorescent dyes. The magnetic particle detection technology is used for the separation and other operations in LOC.

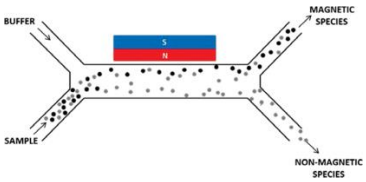


Fig shows the Application of magnetic nanoparticles in the Lab on chip for the separation and detection of Analyte.

Superparamagnetism. It is a form of magnetism which appears in small ferromagnetic or ferrimagnetic nanoparticles (10 nm-150 nm in diameter).NP magnetization can randomly flip direction under the influence of temperature. Example Superparamagnetic iron-oxide nanoparticles (SPION).

.119. Dry Reagent Storage

Introduction.

Storage of chemicals is one of the important elements of the Lab on chip.. For performing the test particular chemical and reganets are required. It is essential to store these chmicals and reagent in a form that are immediately available for performing the test. Reagents or chemicals are stored in dry or liquid form.

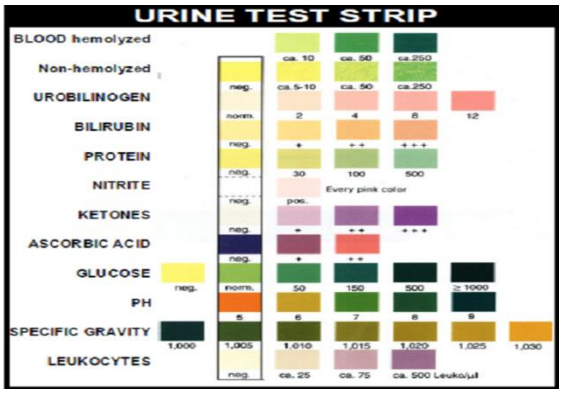
Dry Storage

Chemicals and reagents are successfully stored in the dried form on the absorbent paper pads.

Urine test strip is an excellent example of dry storage of reagents.

These strips with pads allow several determinations simultaneously.

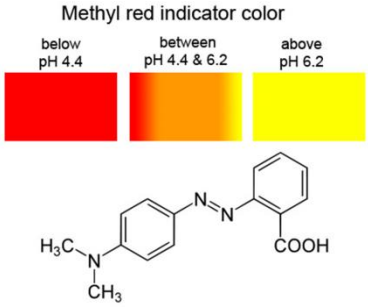
The result provided can be quantitative, qualitative or a positive or negative.

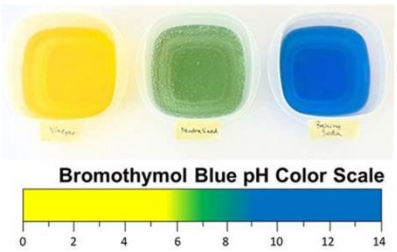


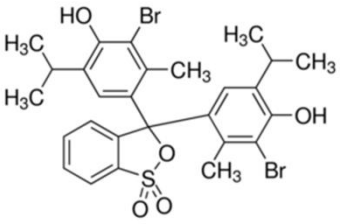
Urine dipstick is a good example of dry storage of reagents.

<u>Determination of pH urine using dried chemical</u>.

The range 5 to 9 the strips show colours that change from orange at pH 5, passing through yellow and green to dark blue at pH 9. Methyl red produces a colour change from red to yellow in the range of pH 4 to 6. Bromothymol blue changes from yellow to blue between pH 6 and 9.







Colours and structure of Bromothymol blue.

Detection of Blood in Urine

1. Reagent strip tests can detect conc. as low as five RBC cells per microliter.
2. The RBCs red are lysed on the pad and liberate hemoglobin.
3. Fe present in the hemoglobin acts as a nanozyme and catalyse the conversion of per oxide into water with the release of electrons.
4. Reagents peroxide, and tetramethyl-benzidine.

Detection of Bilirubin

1. The detection of urinary bilirubin is an early indication of liver disease.
2. The test strips use a diazotization reaction in order to detect bilirubin.
3. The bilirubin combines with a diazonium salt in an acid medium to produce an azo dye
4. The color varies from pink to violet.

Detection on Lab On Chip

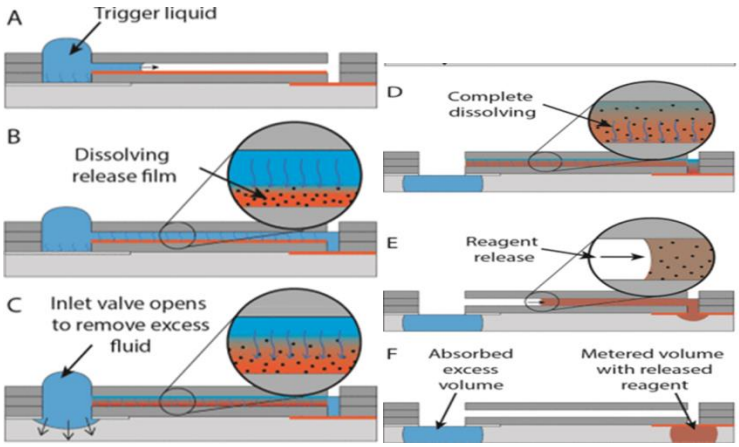
Applying a liquid to the inlet

Reagent release layer starts dissolving.

Complete -volume absorption.

Reagent release layer completely dissolved.

The liquid mixed with the particles from the release layer is absorbed by the paper under the outlet.



A scheme for the detection on LOC.

Summary

In Biosensor technology the storage of dry reagent is one of the important element.

A number of reagents could be stored in dry form on a storage pad in strip.

The stored reagents reconstitute their chemical activity in aqueous environment.

The Dry agents are successfully used for many diagnostic tests.

120. Dry Storage of Biological molecules

Introduction

Biological molecules like enzymes and Antibody are used as a diagnostic tool in several modern hand held devices in clinical Biochemistry. It is require to use dried and stable biological molecules that retain their biological activity after mixing in aqueous environment. Trehalose acts as is a good stabilizer.

Freeze drying

This technique is also known as lyophilization. It involves the removal of water molecules from the sample by

1. Freezing the sample
2. Lowering the pressure creating a vacuum.

3. The biological molecules such as enzymes retained their shape and biological activity when reconstituted in water.
4. The biological samples dried by using this technique.

Sugars as stabilizer

1. Sugars in general protect proteins against dehydration by hydrogen bonding to the dried protein by serving as water substitute.
2. Sugars protect proteins against loss of activity , chemical and thermal denaturation.
3. Trehalose has also been found to be very effective in the stabilization of labile proteins during lyophilization and exposure to high temperatures in solution.

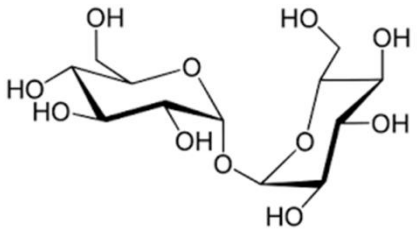
Stabilizer

Trehalose is a disaccharide composed of two glucose molecules joined by an alpha-alpha (1,1) glycosidic bond.

Trehalose occurs naturally in small amounts in mushrooms, honey, yeast

Trehalose is a white crystalline substance. It is a non reducing sugar like sucrose. This molecule is produced by insects.

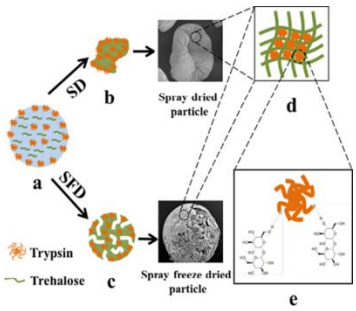
It has low hygroscopicity and it does not readily absorb water.



Structure of molecules of trehalose.

Stabilization of Trypsin enzyme activity.

Trypsin enzyme activity was stabilized in the presence of Trehalose and was immolbilized by using two different techniques.



Stabilization of Trypsin by trehalose.

Spray dried immobilization Freeze dried Immobilization.

In each case trehalose act as a stabilizer by forming hydrogen bond with the enzyme and the glass matrix that was used for immunization.

Summary

Biological molecules atr dried by using freeze drying as they they could retain their activity. Biological molecules are heat labile.

Trehalose can be expected to work as a universal stabilizer of protein conformation due to its exceptional effect on the structure and properties of solvent water compared with other sugars.

Biosensors

Lecture contents

Module No 121- 130

121. Wet Reagent Storage on LOC

Introduction

Storage of liquid reagents is one of the major challenges for the real time application for LOC devices.Micro-dispenser is a device that stores and dispenses reagents/soln. across different microfluidics platforms. It protects stored reagents against external factors, such as humidity and contamination for extents their shelf life.

Liquids storage on-chip describes a reagent storage concept for disposable pressure driven Lab-on-Chip (LoC) devices, which enables liquid storage in reservoirs without additional packaging. ... The liquids are collected in reservoirs, which are made of high barrier polymers or coated by selected barrier layers.

Microdispensing is the technique of producing liquid media dosages in volumes of less than one microlitre. A well-known example of micro dispensing is is inkjet printing. In this application, the volume of a small dispensing chamber with adjoining nozzle becomes reduced through a short impulse, whereby the ink is ejected through the nozzle. Microdispensing systems are based on state-of-the-art piezo technology. The microdispensing system may include fast valve modules with electronic controllers.. Generally systems are designed for easy handling and maximum adaptability in order to enable a vast variety of dispensing requirements.

Dispensing material

The micro-dispenser are manufactured with a number of materials based on

1. Aluminum
2. Glass
3. Polymers

The material used should be inert.

The material can be coated with different materials to induce the desired properties.

Methods for producing Microdispenser

There are two method for producing microdispenser.

Coldforming method in which no heat is applied and aluminum is used as a substrate.

Thermoforming It is manufacturing process. In this method plastic polymers are used and heat is applied.

Cold Forming

In cold forming, an aluminum-based laminate film is simply pressed into a mold by means of a stamp.

Al is elongated and maintain the formed shape.

Advantage of cold form foil blisters is that a complete barrier for water and oxygen.

It allows an extended product expiry date.

Thermoforming Method

In thermoforming method a plastic sheet is heated to a pliable form that is a thermoformed plasticing temperature.

The warm plastic is treated under pressure (4 to 8 bar) will form the cavity into a negative mold.

The mold is cooled such that the plastic becomes rigid again and maintains its shape when removed.

Storage Devices

Several type of packaging materials are used for different storage devices for the liquid reagents.

1. Reagents in Blisters
2. Glass ampoules
3. Stick packs
4. Thermally actuated paraffin film
5. Hydrophobic Chamber
6. Hydrogel microvalve

Blister Packing

The primary component of a blister pack is a cavity or pocket made from a "formable" web, usually a thermoformed plastic.

Thermoforming is a manufacturing process.

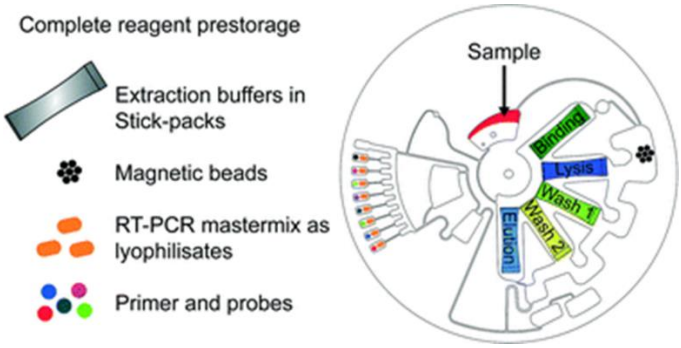
In this method Plastic sheet is heated to a pliable forming temperature.

It forms a specific shape in a mold, and trimmed to create a usable product.

Diagnostics Reagent Blisters comprised of cold-formed foils filled with reagents

Stick Packs

1. Tubular bags, so-called stick packs, are widely used in the packaging industry.
2. It is used in LOC pressure driven devices.
3. Glass Ampules
4. Glass ampoules are also used for reagent pre storage , which are then sealed into a cartridge.
5. Cartridge has flexible lid and can be disrupted on the demand of chemical.



Stick-packs for liquid Reagent pre-storage in a Lab on Chip.

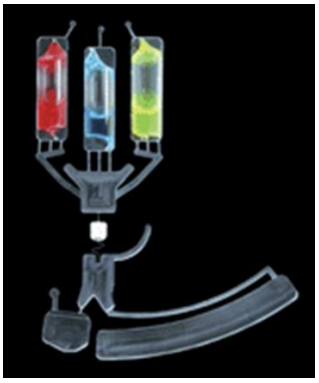
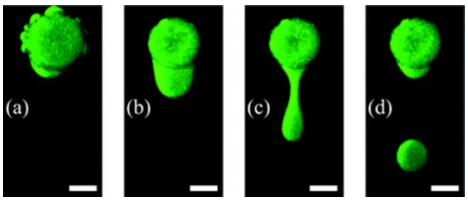


Fig shows Glass ampules for reagent storage in Lab on chip.

Smart Hydrogels

Smart hydrogels, or stimuli-responsive hydrogels, are three-dimensional networks composed of crosslinked hydrophilic polymer chains that are able to dramatically change their volume and other properties in response to environmental stimuli such as temperature, pH and certain chemicals.

1. Smart hydrogels are useful elements in microfluidic systems.
2. The swelling and shrinking capabilities allow them to act as storage elements for reagents absorbed in the swelling process.
3. Smart hydrogels respond to environmental stimuli (pH Temp) .
4. They are capable of storing and releasing aqueous reagents.



Smart Hydrogels Release of reagent under the influence of increase in temperature

Dispensing process including the heating step. After heating water comes out of the pores of the hydrogel forming small satellite water reservoirs.

Summary

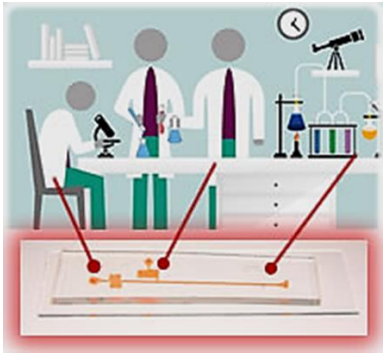
The storage of liquid reagent is required for the use of a Lab-on-a-Chip in a real point-of-need application that the reagent may be immediately available .Several packaging materials polymers, glass and aluminum are used. A number of packing devices and smart gels are used for safely storing in liquid reagent in LOC.

122. Sample introduction a in LOC

In the functioning of Lab on chip there are main four steps.

1. Sample introduction
2. Preparation of sample
3. Testing of sample
4. Detection of signals.

The introduction of sample is very important. The sample has to be properly loaded that it can processed accordingly

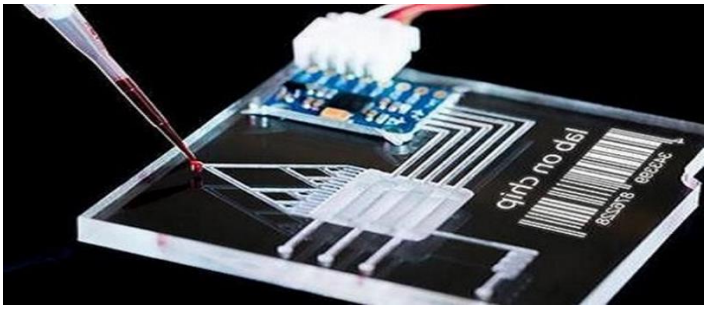


The process of sampling is shown in Fig. Sampling is the process of collecting, preparing, and introducing an appropriate volume element (voxel) into a system.

There are two methods of sample introduction. Active method of sample introduction and active emethod of sample introduction. Sample is introduced into a micro channel in a lab on chip.. Microchannel in microtechnology is a channel with a hydraulic diameter below 1 mm. Microchannels are used in fluid control (see Microfluidics) and heat transfer (see Micro heat exchanger).

Passive Methods for sample introduction.

1. Passive method In this method the gravitational force is the main force.
2. The sample application can be improved by using hydrophobic internal surface of the micro channels.
3. It allows the non absorption and fast mobility of the aqueous samples.



Passive method for sample introduction.

Active Methods for sample introduction.

Active method for sample application external forces are applied and micro devices like micro valve or pressure pumps are used.

Forces used by these devices include

1. Electric current
2. Change in pressure
3. Change ionic strength
4. Change in Mass

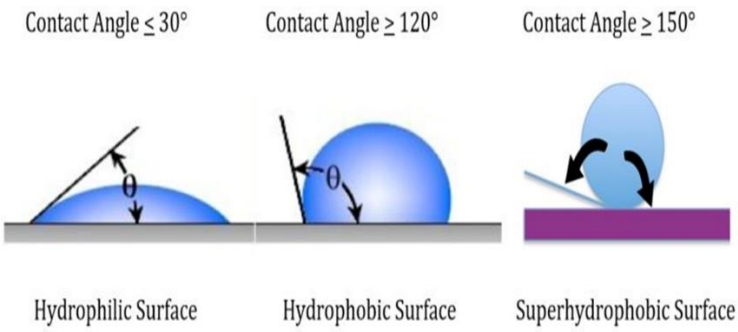
Functions of Micro devices

Microfluidic analytical systems require micropumps and microvalves for active sample application. Microdevices are required for the precise control of the following applications. The functional components include: injector, transporter, preparator, mixer, reactor, separator, detector, controller, and power supply.

- Sample Application
- Addition of buffer
- Reagent flow
- Mixing
- Delivery

Super-hydrophobic coating of Micro channels

A hydrophobic coating is a thin surface layer that repels water. Droplets of samples hitting superhydrophobic coating can fully bounce.It minimize the surface bioadhesion of the blood. The blood clotting time is increased. Loss of Analyte molecules is prevented. This coating facilitate the functioning of the lab on chip.



The superhydrophobic coating increased the contact angle of the drop with the surface and increase the mobility of the fluid. As shown in fir in hydrophilic surface is less then 30 while in case of superhydrophobic surface ange could be more then 150.

Example of Micro devices

1. An integrated operation of microfluidic pumps and valves can be controlled by finger actuation.
2. A single button can be used to control the flow and path of the reactants.
3. Poly dimethylsiloxane (PDMS) membrane are used for controlling the pneumatic valves, flow direction and pumps.

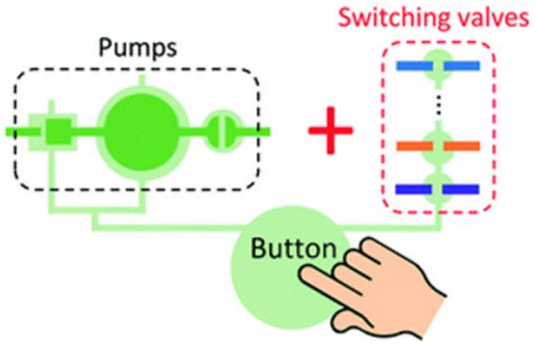


Fig shows a Lab on chip with micro devices lke pumps, switching valve and push button.

Summary

Application of sample is the first step in the testing of a biological sample in a Lab on chip.

Active and passive methods and used for sample application.

Micro valve and micro pumps are used in active sample application.

Superhydrophobic coatings are used to properly introduce the sample in microchannels

123. Mixing in LOC

Introduction.

Mixing is an important function in microfluidics, which combines various reagents with the sample for analysis. Small-scale mixing is of uttermost importance in bio- and chemical analyses using micro TAS (total analysis systems) or lab-on-chips. Many microfluidic applications involve chemical reactions where, most often, the fluid diffusivity is very low so that without the help of chaotic advection the reaction time can be extremely long.

As the fluid is confined in the microscale domain, mixing becomes difficult due to the low Reynolds number with laminar flow.

Diffusion is the only process that is operated in such conditions.

Types of microfluidic mixing

In a LOC Controllable and fast mixing techniques are required.

There are two types of mixing strategies.

1. Passive Mixing in this approach the designing of the microchanels facilitate the mixing without any external force. passive mixers employ no external energy input and judicial selection of device geometry play dominant role for getting effective mixing. These designs account for several advantages such as cost effectiveness, reduced complexity, ease of fabrication etc.
2. Active mixing. In this case, mixing efficiency is increased by external forces applied to the samples. Active mixers demand external force fields, such as thermal , acoustic , magnetic and electro kinetic to strengthen mixing efficiency.
3. The main aim of microfluidic mixing devices is to acquire complete and optimummixing of two or more samplesin micro-scale devices. However,achieving effectivemixing of more than one fluidis very challenging task, owing to the limitation induced by reduced convection in micro-channels.

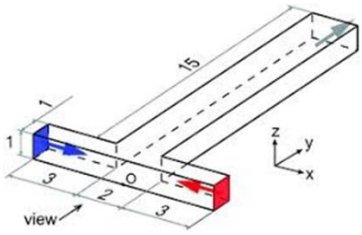
Working of Passive microfluidic mixing

Controllable and fast mixing techniques are required for processing the sample.
Passive Microfluidic mixing involves two approaches.

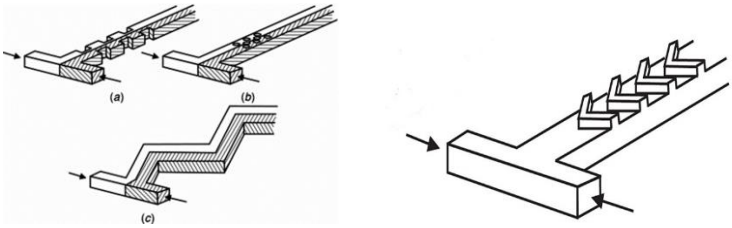
The samples may be flowed through various holes incorporated or multichannels into the microfluidic chip.
The channels are designed to increase the contact area or/and the contact time.

T Shaped micro-mixer

In T-shaped micro mixer consist of two inlets and one outlet.
The microchannels with two inlets for samples are flowed perpendicularly to each other. A T-shaped micro mixer has two characteristic flow regimes that dependent on the Reynolds number and the geometry. The mixing of the fluids entering in the two channels is determined by diffusion or by convective transport. Mixing efficiency can be increased by adding some barriers and obstacles in mixing channels, which creates additional perturbations.



Example of T shaped microfluidic passive mixer. Fluid 1 and Fluid 2 enters from two separate inlets. Mixing occurs while flowing in the common channel.



Introduction of grooves and different patterns in the mixing channel to increases mixing efficiency and reduces mixing time.

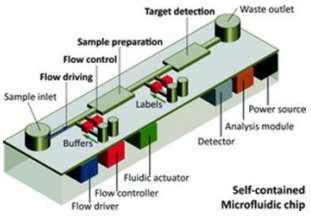
Active Micro fluidic mixer

To implement “active” fluid mixing and influence mixing process, different physical forces can be involved:

1. Acoustic waves
2. Pressure perturbations
3. Magnetic field
4. Thermal methods
5. Propagation of ultra sound waves
6. Fluctuation of Electric field

Working

Active microfluidics refers to the defined manipulation of the working fluid by active (micro) components such as micropumps or microvalves .
Micropumps supply fluids in a continuous manner or are used for dosing.
Microvalves determine the flow direction or the mode of movement of pumped liquids.



A lab on chip with micro devices.

Summary

Mixing of reagents and sample is important for the functioning of the LOC.
There are two approaches that are Passive and Active Microfluidic mixing.
In Passive mixing main force is the diffusion that is amplified by geometrical deigns.
In active microfluidic method different micro-devices are used.

124. Magnetic NanoParticles in LOC

Introduction

Magnetic nanoparticles are a class of nanoparticle that can be manipulated using magnetic fields. Such particles commonly consist of two components, a magnetic material, often iron, nickel and cobalt, and a chemical component that has functionality.Magnetic nano particles are used in Lab on chip to facilitate a number of operations. Separation and concentration of analyte in the sample from the other molecules is required. MNP are especially designed for use in LOC to assist and perform different functions.

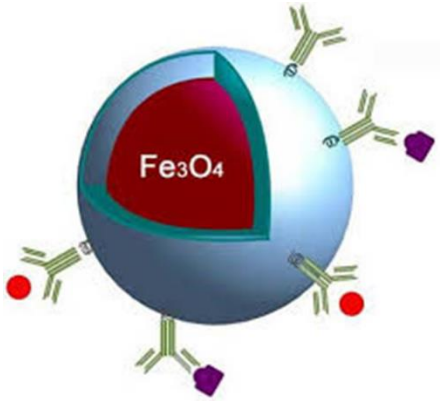
Magnetic nanoparticles

The magnetic nanoparticles are smaller than 1 micrometer in diameter (1–100 nanometers), the larger microbeads are upto 500 micrometer in diameter.

Magnetic nanoparticles commonly consist of two components.

A magnetic material, often iron, nickel and cobalt.

A chemical component that has functionality.



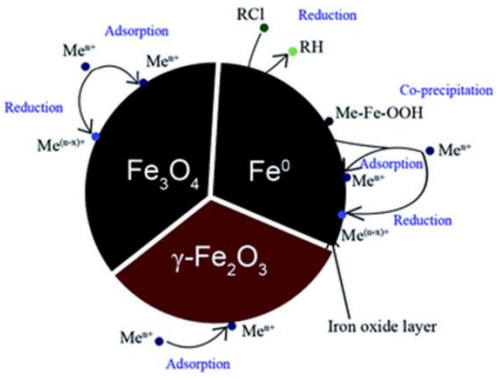
aMNP is shown with core and shell. Antibodies are attached on the shell.

Functional Properties

1. The functional properties of magnetic nanoparticles largely depend on the synthesis method, size and chemical structure.
2. The chemical component that is attached to the MNP determines their functionality.
3. An important size dependent property of MNP (1 to 100 nm) is superparamagnetism

Properties of Fe based NP

1. Iron based nano particles contributes to chemical properties of the NP.
2. The zero-valent iron in the core mainly provides the reducing power for the reactions.
3. The oxide shell provides sites for sorption.
4. Adsorption also occurs on the iron oxides surface, while Ferric oxide possesses reducing power.



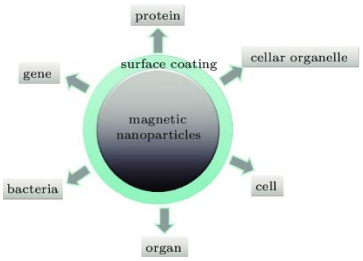
Adsorption and catalytic properties of iron based MNP.

Surface Modifications of NP

The surface modification of the magnetic materials is essential for most of the chemical and biological applications.

Many properties may be induced.

1. Biocompatibility
2. Biostability
3. Vehicle for targeted Drug delivery
4. Catalytic properties



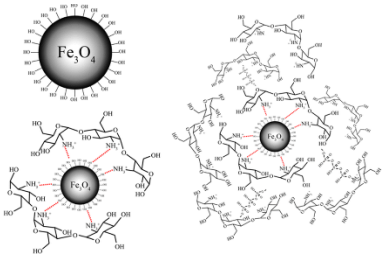
Surface modification of MNP.

MNP as a carrier

1. The core and shell are the potential carriers. The active agents are attached to the shell can be adsorbed into the magnetic core,
2. The active agents can interact with the shell via hydrophobic or hydrophilic interactions.
3. Active agents can be distributed between the two components of the core and the shell structures.

Capping Agents

i. Salicylic acid allows the formation of strong hydrogen bonds.
ii. Glutamic acid based interactions involve hydrogen bonds and electrostatic inter actions.
iii. In each case phenolic and carboxylic groups are involved.
iv. Trichloroacetic acid, induced hydrophobic and dipole based interactions.



Coating of MNP.

Summary

Magnatic nano particles play an important role in the functioning of the Lab on chip.

MNP are capped with different functional molecules to introduce certain properties.

In Lab on Chip MNG are mostly used for the separation and concentration of Analytes.

125. Sample processing LOC

i. Sample processing and sample pretreatment are the essential components of Lab on chip Operations.
ii. Sample processing and pre-treatment can be of different types depending on the nature of the sample.
iii. Often an analyte of interest is present within an extremely complex matrix (for example blood).

Processing

The isolation and 'clean-up' of a particular analyte or set of analytes is required under for processing the sample. A lab-on-a-chip integrates and implements multilaboratory functions in a miniaturized microfluidic device that deals with extremely small volumes of fluid, enables rapid sample screening, analyzes targeted agents, and manipulates fluid or analytes. Processes, normally carried out in a laboratory, are miniaturized on a single chip in order to enhance efficiency and mobility as well as reduce sample and reagent.

The processes may include

1. Sample filtration
2. Centrifugation
3. Dilution
4. Concentration
5. Target amplification
6. Extraction.

Use of Superhydrophobic membrane

Precise control over the cell microenvironment during separation procedures and the ability to scale down the analysis to very small volumes of blood are among the most attractive capabilities of the new approaches.
Use of super hydrophobic membrane coating could be the first step in the blood sample processing.
It minimize the surface bio-adhesion of the blood.
The blood clotting time is increased.
Blood cell hemolysis is reduced.

Loss of target molecules is prevented.

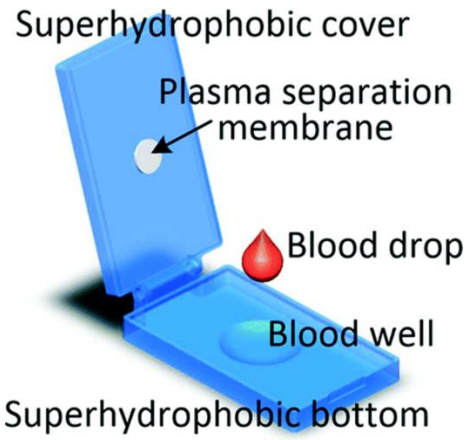
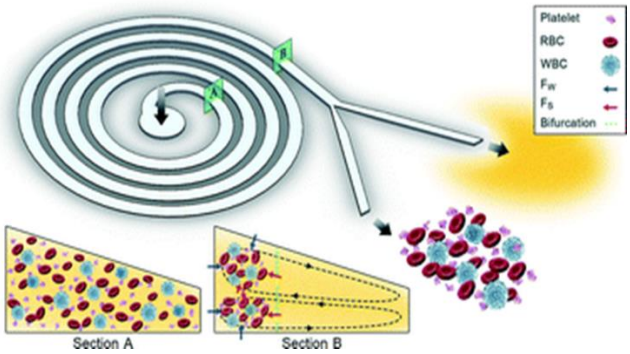


Fig shows the application of superhydrophobic membrane in plasma separation.

Multiplex slanted spiral microchannels

1. Multiplex slantes Spiral microfluidic channel are used for the pretreatment of blood samples.
2. When blood sample is applied the forces in the system drive diiferent particles in different directions depending on size.
3. Smaller particles will migrate near the outer wall of the channel.



Multiplex slanted spiral channel.

Summary

Processing of loaded sample in lab on chip is one of the important elements.

In clinical samples blood is a common test sample that needs separation of and concentration of target molecule.

A number of techniques are used including filtration, use of hydrophobic membrane and use of spiral slanted slanted channels.

126. LOC Platforms

Microfluidic platforms Lab on chip is developed on the basis of a proper design. The architect of the LOC facilities and supports the placement of functional units on a substrate.There are several LOC platforms available in literature. The selection of platform is dependent on objectives and goals of proposed LOC

A microfluidic platform provides a set of fluidic unit operations which are designed for easy combination within a well-defined manufacturing technology. There are different types of architectural design are available for LOC platforms.

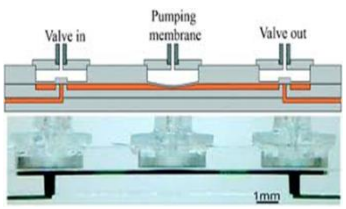
1. Lateral flow platform
2. Linear actuated platform
3. Microfluidic large scale integration platform
4. Centrifuged microfluidics platform
5. Electrowetting platform

Lateral flow tests

In lateral flow tests, also known as test strips, the liquids are driven by capillaryforces. Liquid movement is controlled by the wettability and feature size of theporous or microstructured substrate. All required chemicals are pre-stored withinthe strip. Typically, the readout of a test is done optically and is oftenimplemented as colour change of the detection area that can be seen by thenaked eye. A common example of this type of test is the pregnancy test strip.

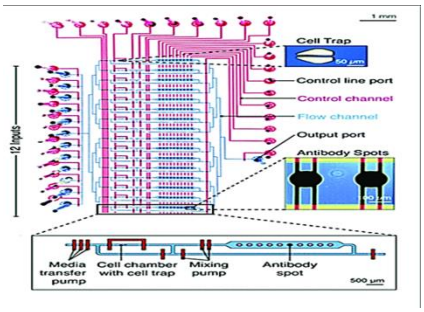
Linear-actuated devices

The liquid movement is controlled by mechanical devices e.g. by a plunger.Liquid flows in one-dimension in a linear fashion without branches or alternative liquid pathways. The reagents and reaction buffers are pre-stored in pouches.Linear-actuated devices control liquid movement by mechanical displacement of liquid, e.g. by a plunger. Liquid control is mostly limited to a one-dimensional liquid flow in a linear fashion without branches or alternative fluid pathways. . The blood sample is introduced into the cartridge and placed inside the analyser.



Microfluidic large-scale integration

Microfluidic large-scale integration describes a microfluidic channel circuitry with chip-integrated microvalves based on flexible membranes between a liquidguiding layer and a pneumatic control-channel layer. The microvalves are closed or open corresponding to the pneumatic pressure applied to the control channels. Just by combining several microvalves, more complex units such as micropumps, mixers, multiplexers, etc., can be built up with hundreds of units on a single chip.



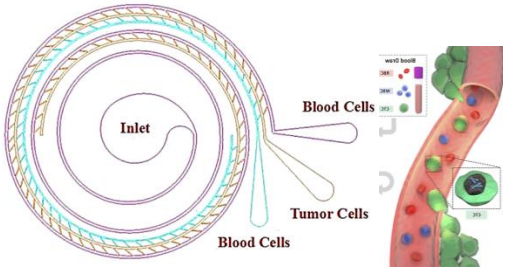
Segmented flow microfluidics

Segmented flow microfluidics describes the principle of using small liquid plugs

and/or droplets immersed in a second immiscible continuous phase (gas orliquid) as stable micro-confinements within closed microfluidic channels. Those micro-confinements are in the picolitre to microlitre volume range. They can be transported by pressure gradients and can be merged, split, sorted and processed without any dispersion in microfluidic channels.

<u>Centrifugal microfluidics</u>

All processes in centrifugal microfluidics are controlled by rotating a microstructured substrate. This provides several relevant forces for liquid transport; centrifugal force, capillary force, Coriolis force and Euler force. Assays are implemented as a sequence of liquid operations arranged from radially inward positions to radially outward positions. Spinning CD-like fluidic disks transport samples and reagents by the interplay of the above mentioned forces. Example. A triplet microfluidic chip is used for the separation of tumor cells in blood sample. Channels with tilted slits are organized along the flow direction for separation.Different sized cells are separated by Spiral-Slits Chip in different channels of the chip



Centrifugal Platform for the Continuous Separation of Tumor Cells using a Spiral-Slits Chip

Electrokinetics

In electrokinetics platforms microfluidic unit operations are controlled by electricfields acting on electric charges, or electric field gradients acting on electricdipoles. Several electrokinetic effects such as electro-osmosis, electrophoresis, dielectrophoresis and polarization superimpose each other and can be used in the same LOC, dependent on buffers and/or sample.

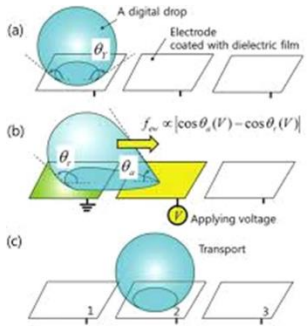
Electrowetting

Electrowetting platforms use droplets immersed in a second immiscible continuous phase (gas or liquid) as stable micro-confinements. The droplets reside on a hydrophobic surface that contains a one or two-dimensional array of individually addressable electrodes. The voltage between a droplet and the electrode underneath the droplet defines its wetting behaviour. By changing voltages between neighbouring electrodes, droplets can be generated, transported, split, merged and processed. Electrowetting is the modification of the wetting properties of a surface with an applied electric field.

Electrowetting is the reduction of contact angle by an applied voltage.

The operations include

1. Creation of a droplet
2. Transportation
3. Mixing and
4. Splitting of droplets.



Steps involved in the Process of electrowetting, when an electrode is coated with a dielectric material that is a hydrophilic material. The contact angle between the droplet and surface greatly increased and facilitate the transport or mixing of the fluid in the microchannels.

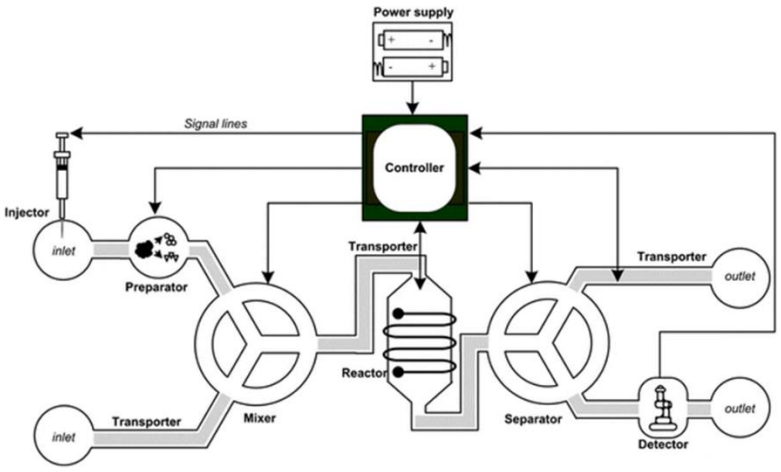
127. **Reactors in Lab On Chip**

Microreactors, are used in lab on chip. They can easily be optimized and closely monitored for the reaction rate and to get the reaction endpoint. The microfluidic reactors will allow for more efficient, and consequently more rapid chemical processes, due to diffusion.

There are several components of a Lab onchip assembly

The main components of LOC

1. Injector
2. Transporter
3. Preparator
4. Mixer
5. Reactor
6. Separator
7. Detector
8. Controller
9. Power supply



.Fig shows Components of Lab on chip.

Reactors

Reactor is like a test tube/flask of LOC where all the reactions take place.

Reactor processes and controls a chemical or biological reaction in a controlled environment.

The reactor is usually equipped with

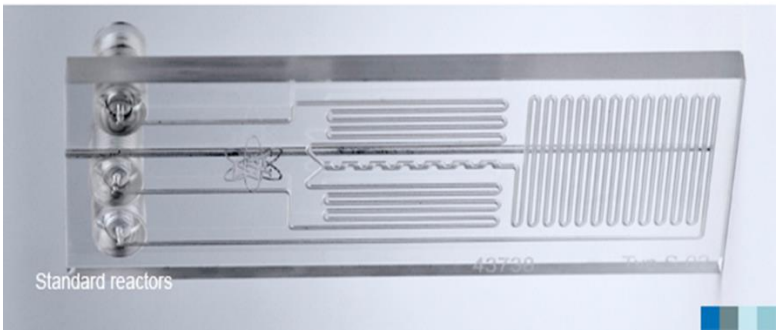
1. Heaters
2. Sensors
3. Actuators

Micro-Reactors

Micro-reactors are useful tools for optimizing and studying chemical reactions.
Micro reactor reduce the chemical amounts.
It enables higher reaction velocities.
Main objective is to collect information.
Micro-reactor has a better control of the process parameter

Advantage

The cost and risk is significantly reduced as only minimal volume of sample and reagent is required for the reaction process.
It provides improved heat and mass transfer properties in micro-fabricated structure.
Reactors in LOC provide in a high yield.



A standard reactors for microreaction technology prepared from glass.

Classification

Reactors can be classified into three types.

The classification is based on the types of reaction conducted in the reactor.

1. Gas-phase Reactor
2. Liquid phase Reactor for liquid sample and reagents.
3. Packed bed Reactor for surface contact.

Liquid phase reactor

1. The components and the end product that is monitored in liquid in each case.
2. Reagents and samples are continuously fed into the reactor.
3. Pumps are used for continuous supply at a constant speed.
4. The resultant products are immediately separated.

Packed-bed Reactor

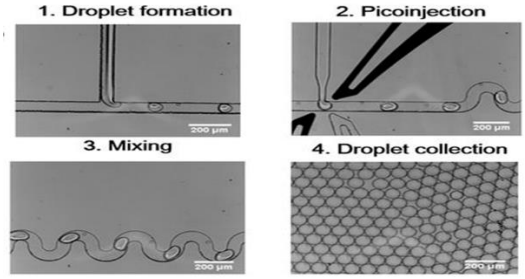
1. The chemical reaction takes place on the surface of the catalyst.

2. Catalyst NPs have been extensively synthesized using microfluidics.
3. The properties on the NP are related to the shape and size of the particles.
4. The shape and size variations could be controlled by checking the contact time

Droplet-based Microfluidics Platform

The method of drop-let microfluidics processes involves splitting a fluid stream into small, discrete droplets, and provides the added benefits of removing the effects of Taylor dispersion and the subsequently increased ease of droplet transport (as shown in fig). Reactions performed in droplets have been shown to have significantly affected reaction kinetics, showing increased equilibrium and forward rate constants as the droplet radius decreased. Droplet-based techniques, by isolating reactions from the microfluidic channel walls, can also prevent microfluidic channel fouling, a problem that can sometimes occur in continuous flow methods.

Optical images of a device for gold nanoparticle synthesis; (1) Droplet formation, (2) Picoinjection, (3) Mixing and (4) Droplet collection.



Summary

All the biological or chemical reactions are being carried out in the Reactor.

Reactor is equipped with different micro devices such as heater, mixer and sensors.

Microreactors are efficient and safe because the problems due to leakage are minimized.

128. Detectors in LOC

Detector is one of the necessary components of the Lab on chip. Optical detectors are the most popular as they are simple to integrate, low cost and easily available. The camera and light source provided in the smart phones is being successfully used in a number of devices as an optical detector.

Detectors in a Lab on chip perform the two functions

i. Identification
ii. Quantification

It consists of a Transducer receives the signals for the detection of Analyte

Transducer converts them into electrical signals using detection methods optical, electrochemical, mass spectrometry and magnetic methods.

Optical methods

Optical methods are simple to integrate.

Organic LEDs (OLEDs) and dye lasers are the most widely used optofluidic light sources. The method used following techniques foe detection.

i. Absorbance
ii. Fluorescence
iii. Chemiluminescence
iv. Surface Plasmon Resonance
v. Interferometer based Techniques

Types of Optical Detector

The conventional Optical detectors are

1. Photo multiplier tube
2. Charged coupled devices CCDs are also used to perform multiplexing.
3. Silicon or organic-OPDs organic Photodiode are used as Integrated detectors.
4. Complementary metal oxide sensors permit lens-free imaging.

Smart phone as Optical Detector

The smart phones are equipped with powerful white flash and high resolution camera.

Smart phone cameras can be used as a powerful optical detector and light source.

Smartphone-based microfluidic platforms allows fast, low-cost and simple analysis

Cell phone-based sensor for detection of E. coli

A cell phone–based fluorescent imaging can detect the presence of Escherichia coli in samples.

Antibody functionalized glass capillaries with quantum dots can detect 5 CFU/mL of E. coli in liquid samples.

The system uses a lightweight, compact attachment to cell-phone camera.

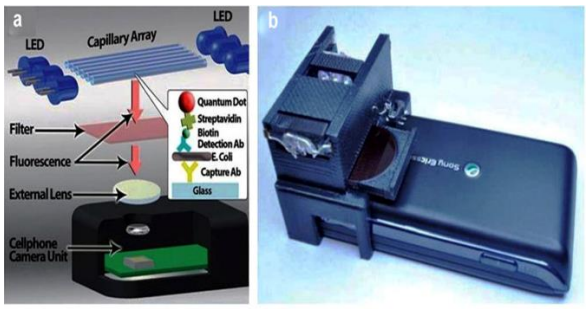


Fig shows the use of cellphone as an optical detector. It shows capillary array , LED, Filter, flourescence produced and external lens. The signals are detected by the vellphone camers.

Mobile Water Kit (MWK)for the detection of coliform bacteria and E. coli

A smartphone compatible low-cost water monitoring system is developed for rapid detection of total coliform and E. coli.A part from other components a smartphone operating on android equipped with a custom built mobile application called mHealth E. coli App.

Fir shows a low cost water quality monitoring system, using a cellphone camer as an optical detector.

129. Controller and Power supply on Supply on LOC

Controller in a Lab on chip is composed of software modules.These modules conduct all the operations in a control environment.

The high tech operations (PCR) require constant and high voltage power supply.

There is a WHO criteria for the development of low cost diagnostics.

A typical controller consists of certain software modules.

These modules include.

1. Microcontroller
2. Communication interface
3. Programmable memory
4. Control circuits
5. Signal preconditioning circuits
6. Power interface circuits

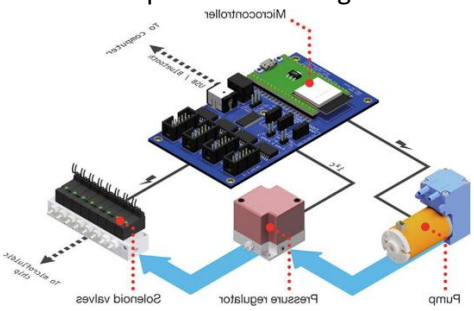
Types of Controller system

The are two types of controller systems in LOC.

- Controller system on Chip All the necessary components are integrate all on-chip.
- Controller system off Chip

There is a main system to control the functioning of the chip.

The control panel is not integrated into the chip.



Power supply

Power supply is an essential unit in LOC systems.

LOC devices utilize batteries as their power source.

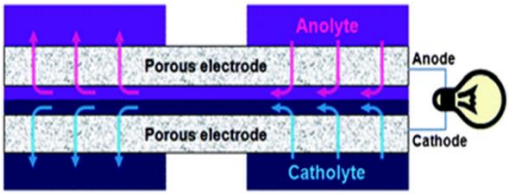
Power supplies energize on-chip components including

1. Transducers
2. Actuators
3. Electronic circuits.

   The power supply could powered by an onboard cell battery.

   For Hi tech operations on LOC such as electrophoresis and PCR, self-containable and adjustable batteries are used.

   The power supply provides power to the system to operate.



A microchip is shown, two elelctrode and anode and cathode are used for the generation of power supply.

World Health Organization ASSURED criteria for low cost diagnostics developments

There is a criteria set by World Health Organization for low cost diagnostic development that is lab on chip. The World Health Organization (WHO) stipulates quality-Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, Deliverable (ASSURED) criteria as a benchmark for point-of-care (POC) tests that are ideal for healthcare facilities in rural and resource-limited settingsThe criteria is abbreviated as ASSURED.

1. Affordable
2. Sensitive
3. Specific
4. User friendly
5. Rapid and Robust
6. Equipment-free
7. Deliverable to end users

130. RT-PCT on LOC

Reverse transcription polymerase chain reaction (RT-PCR) is a common technique used to quantify specific ribonucleic acid (RNA) molecules within cells or tissues, which can be applied in molecular biology, genetic disease diagnosis, and forensics.

The extraction of high-quality messenger RNA (mRNA) from biological samples is critical for accurate and efficient RT-PCR performance. mRNA is destroyed readily by ribonucleases (RNases) from bacteria and molds present on human skin, as well as from dust in the environment. Because the ubiquitous RNases are highly active, it is difficult to purify high-quality mRNA without contamination and degradation.

Due to the necessity for time-consuming sample preparation with conventional RT-PCR methods, RNA degradation by RNases is difficult to avoid. Thus, performing an accurate RT-PCR assay using small quantities of biological samples is challenging. Closed, RNase-free, rapid RNA extraction is essential to avoid RNA degradation and so achieve high-precision RT-PCR analysis. Microfluidic with a closed, integrated format to have been developed prevent RNase contamination and achieve high-quality genetic assays.

Isolation of RNA in microfluidic devices

Microfluidic devices with a closed, integrated format is developed to prevent RNase contaminations.

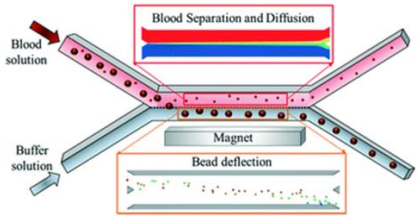
It can achieve high-quality mRNA within 1 min.

The RNA microchannel, are made up of up of Fe and Ni ferromagnetic wires.

Magnetic oligo-dT beads are used for RNA purification.

Magnetic separation can be achieved through a magnetophoresis process, using different types of magnetic particles. In a continuous flow, magnetic particles are deflected from the direction of laminar flow by a perpendicular magnetic field depending on their magnetic susceptibility, size, and flow rate . Through this process, only the target molecules or cells are bonded to the magnetic beads and, therefore, the magnetic sorting process is highly specific.

Magnetophoresis has a wide range of applications since magnetic particles are commonly used in bioanalysis as a support material for different biological samples: antigens, antibodies, DNA, and cells



Magneto-phoresis for the separation of Target molecule.

Working

The microchip is composed of PDMS and glass.

There are two separate units for mRNA and DNA in LOC. a. RNA microchannel b. DNA microchamber

cDNA is synthesized using mRNA template in a micro chamber.

Gene is amplified by using the newly synthesized cDNA and stored reagents.

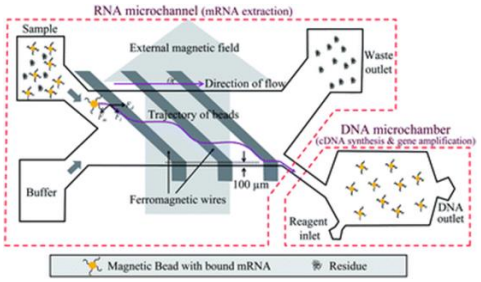


Fig shows working of a microchip for coding DNA sequence from RNA.

Summary.

RT-PCR microchip, consisting of an RNA microchannel and a DNA microchamber.

The ferromagnetic wire (Fe and Ni) array is inlaid into the RNA microchannel to introduce magnetism.

mRNA is used for cDNA synthesis and gene amplification in DNA chamber.

Modules 131-135 Paper Based LOC

Introduction.

Paper-based diagnostics have already revolutionized point-of-care approaches for health and environmental applications, by providing low-cost, disposable tools that can be utilized in remote settings. These devices typically consist of microfluidic, chemical, and biological diagnostic components implemented on paper substrates, towards addressing the ASSURED (Affordable, Sensitive, Specific, User friendly, Rapid and Robust, Equipment free and Deliverable to end users) principles set out by the World Health Organization. Paper-based diagnostics primarily contribute to the affordable, equipment-free, and deliverable-to-end-user aspects. However, additional functionality must be integrated with paper-based diagnostic devices to achieve truly ASSURED solutions.

Paper based lab on chip use cellulose paper or nitrocellulose as substrate.A number of techniques are used for printing the microfluidic channels on the substrate. The biological component and required chemicals are deposited on the paper. Paper is as an platform for disease diagnostics and environmental monitoring. It has been successfully used for quantitative analysis of several analytes from biological samples such as glucose, lactate and uric acid from urine.

They do not require expensive

Paper based technology for is a cost effective.

Paper-based LOC are madeup of cellulosic materials.

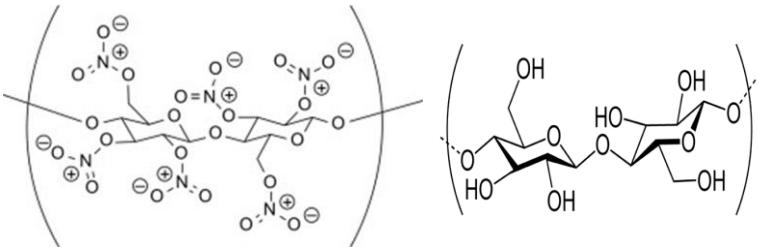
The cellulosic fiber guide liquid from an inlet to a desired outlet by imbibition.

Imbibition is a special type of diffusion that takes place when water is adsorbed by solids-colloids causing an increase in volume.

Substrate.

Paper-based microfluidics provide a favourable platform on which to develop solutions that address these requirements, with paper exhibiting a number of useful properties that are either inherent or that can be achieved through straightforward modifications. Cellulose is a natural polysaccharide, composed of glucose units. Nitrocellulose is a derivative of cellulose. Nitrocellulose is a highly flammable compound formed by nitrating cellulose through exposure to nitric acid or another powerful nitrating agent.

They contains a number of functional groups on the surface.



Components.

The paper based lab on chip has a proper design.

1. Inlet
2. Microfluidics
3. Storage of reagents
4. Transporter
5. Mixer
6. Reactor
7. Detector
8. Electronics
9. Batteries

Impact on flow rate.

The movement of fluid through paper is governed by the physical properties of the substrate and the fluid.

Physical properties of substrate that can change the flow rate include

1. Permeability
2. Geometry
3. Evaporation effects.
4. Pore s

Impact of physical properties of the fluid of the flow rate.

The movement of fluid through paper is governed by the physical properties of the fluid include.

1. Viscosity

2. Density
3. Contact angle
4. Temperature
5. Surface tension

Paper Chromatography

1. Paper Chromatography is a method of separating mixtures by using a moving solvent on filter paper.
2. A drop of mixture is spotted at one end of the paper and then dried.
3. A solvent system is used for the separation of different compounds on the basis of their mobility

Flow Control on LOC.

There are various ways to control the fluid flow in the channels.

1. Changing the channel width and length.
2. Altering the wettability of the paper.
3. Diverting some fluid through a parallel channel.
4. Changing the viscosity of the fluid.

Flow Control on LOC.

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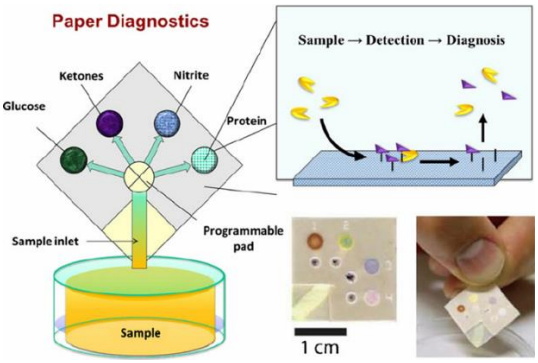
Response factor.

Every compounds has a unique Rf value in a solvent system.

RF value for specific compound can be obtained by dividing the peak value of the spot by solvent front.

Separation of compounds is done on the basis of difference in mobility of cellulosic paper.



Paper-based diagnostics. Using the capillarity of a fibrous paper mat and immobilized dry regents to produce colorimetric responses to analytes.

132. Sample processing in paper based LOC

Introduction

Paper based lab on chip are fully equipped with the elements for the detection of target molecule.

Highly sophisticated technology is developed to control the system.

Elements like, electronics, electrical sensors, thermal sensors, chromogenic sensors are successfully integrated on paper chip. Paper based LOC are fully equipped with the elements to function as a diagnostic tool.

Paper based LOC has gained popularity in the consumer markets due to their compactness and portability.

Paper-based microfluidics is considered a low-cost, lightweight, and disposable technology.

Elements on paper based LOC

1. Control system
2. Microfluidics
3. Electronics
4. Sensing element
5. Data processing
6. Readouts

7. Display unit
8. Connectivity
9. Energy storage

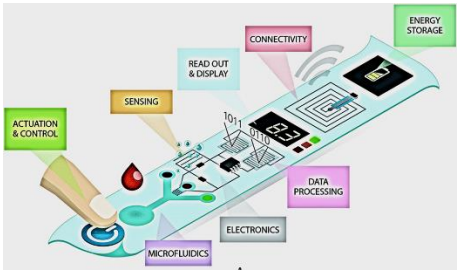


Fig shows the Elements for the functioning of Paper based LOC,

Sample processing modules

1. Standard lateral flow test strips with sample pad, conjugate pad, test and control lines and wicking pad,
2. 2D paper-based diagnostics using hydrophobic fluidic barriers (e.g. wax printing)
3. 3D paper-based diagnostics using stacking of multiple layers folding or origami paper devices.

Paper tests such as pH strips and home-based lateral flow pregnancy tests are examples of the first and most common paper-based microfluidic devices. Enhanced functionality such as multiplexing and multi-dimensional fluidic handling can be garnered from advances in paper-based microfluidics, also referred to as microfluidic paper-based analytical devices (µPADs),20 through multi-channel designs within a paper substrate, or by stacking and folding of paper in to so-called three-dimensional or “origami” paper-based microfluidics. Wax printing of fluidic channels is often implemented using hydrophobic barriers to contain and guide fluids through a paper device. Inkjet printing, screen printing and flexographic printing have also been utilized. Modifications in the composition of the paper substrate allow for heightened functionality of paper-based diagnosticsand printing of paper substrates themselves are currently being explored, for example by using printable nanocellulose pulp. The use of nanocellulose for implementing printed electronics has gained attention6 and along with advances in paper-based microfluidics and diagnostics, forms the foundation on which to further develop augmented printed functionality for paper-based diagnostics.

Standard Paper-based 3D microfluidic device

The device consists of 2 layers

The test solutions are loaded to injection zone.

The chemical reaction take place in folded paper-based 3D microfluidic device.

Paper based microfluidic device is then air dried.

Image of result is visible after unfolding the paper device

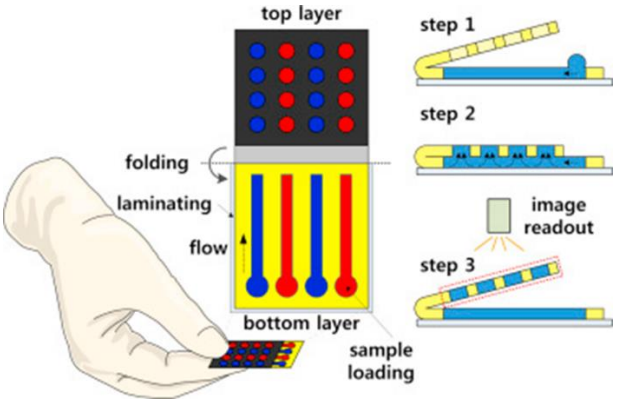
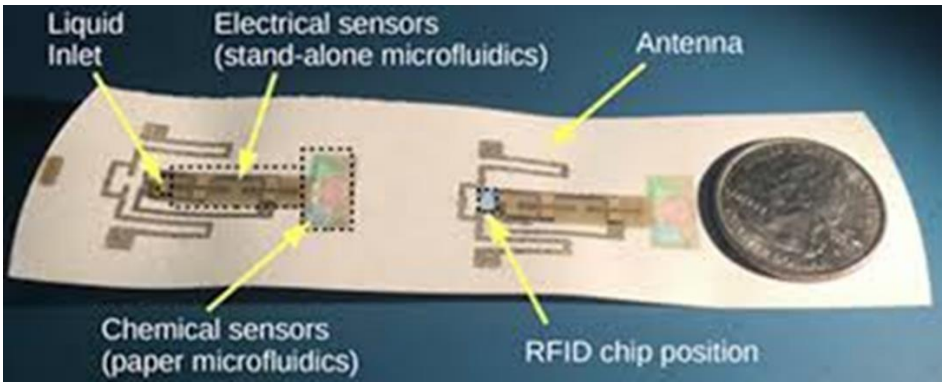


Fig shows Microfluidic on paper.

Control of system Control of system requires switches, valves, heating and energy source.

Sensing For detection of target molecule different types of sensors could be placed on paper chip.

1. Electrochemical
2. Temperature
3. Chromogenic
4. Light sensors



Chemical Sensor and Electrical sensors on Paper Chip

Electronics and Data Processing Units

For the function of LOC a number of microelectronic equipment's are required such as Transistors, electrodes, capacitor, inductors.

A proper data processing units composed of different electronic circuits and memory units are also integrated onto the paper chip.

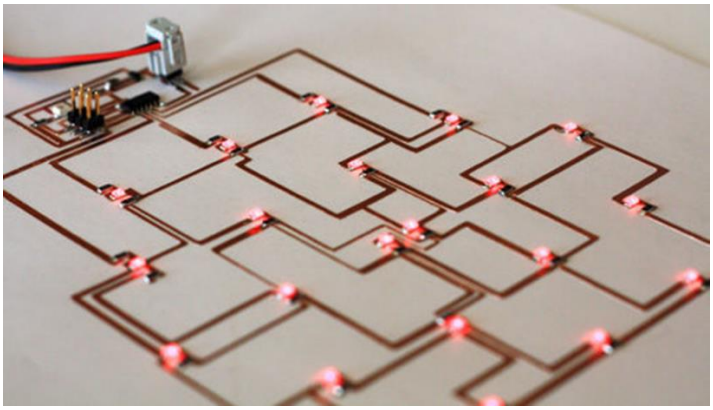
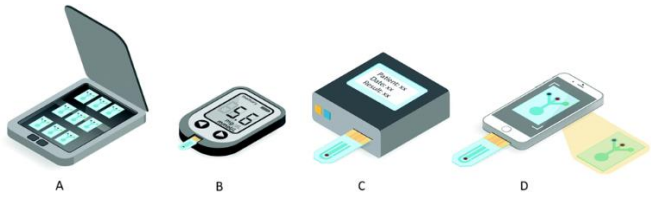


Fig shows Circuits on paper

External Instruments

A number of external instrumentation solutions that can be used with paper-based diagnostic tests.

1. Desktop scanner
2. Portable glucose meter used with paper-based diagnostic electrochemical sensors
3. A phone camera for image capture and processing of a paper-based device



133. Conductive ink Technology

Introduction

Electronic components are the main components.

Electronics are required to be integrated on the surface of the paper.

Conductive ink technology is introduced to print the electronic components on the substrate.

The ink contained conductive materials and is used in conductive pen, as paint and in printers.Electronics are the main elements of the Physical component of Lab on chip.

Electronics are integrated on the paper chip using conducting ink technology.

Ink is infused with a conductive material, like

- Graphite
- Silver

These materials enables ink to conduct electricity.

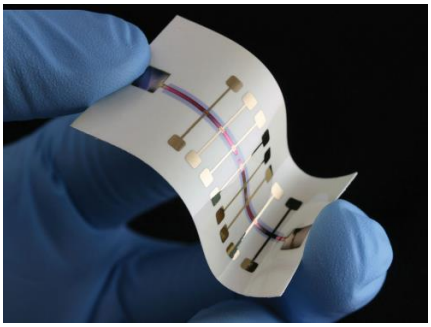


Fig shows Circuit on paper

Printing Material

Several conductive materials could be considered for preparing conductive ink, such as

Conductive polymers

1. Carbon
2. Organic Metallic compounds
3. Metal precursors
4. Metal NPs

Conductive Ink

Conductive ink is being used for drawing circuits for flexible electronic , displays. Conductive silver, copper or carbon-based inks are typically used to print electronic tracks, electrodes and antennas.

Transparent conductors such as indium tin oxide (ITO) and more recently poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) and graphene are utilized in printing of components such as solar cells and organic light-emitting diodes (OLEDs). Both inorganic and organic materials can be printed, and various commercially available functional inks enable printed functionality to be implemented.

A novel conductive metal ink made of copper nanosheets coated with silver nano particles is developed.

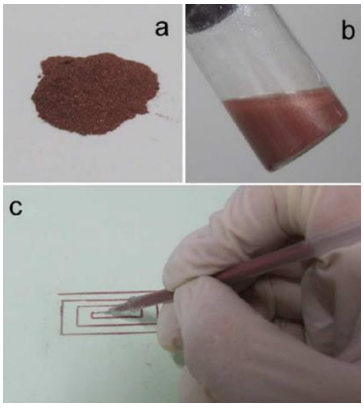
The novel ink can be used in a pen to draw a functioning, flexible electric circuit on regular printer paper.

Preparation

Aq soln. of silver nitrate is combined with polyacrylic acid and diethanolamine, which serve as the capping agent and the reducing agent.

A size of about 400 nm is achieved after processing , and ethanol to convert their liquid state.

After centrifugation, the hydroxyethyl cellulose is mixed, to act as a binder.



Conductive INK Pen

A conductive pen is a liquid polymer delivery system a standard ink pen.

The polymer is electrically conductive and is rapid to dry.

They are used to create or repair conductive traces on printed circuit boards.

Silver-infused conductive inks used in modern transit tickets.

NP and conductive Ink

Most conductive inks are based on metal NPs

NPs are inserted into a solvent at a certain loading ratio to provide the wanted viscosity.

It results in a liquid solution, called the “the metal ink,” with the NPs in suspension.

Average size of the particle varies between 10 and 100 nm.

How to check the functioning of conductive ink.

To show that the ink could conduct electricity, the scientists added small LED chips (lights) to the drawing.

When circuit is connected to a battery LED lits up and show the working of the conductive ink on a paper.

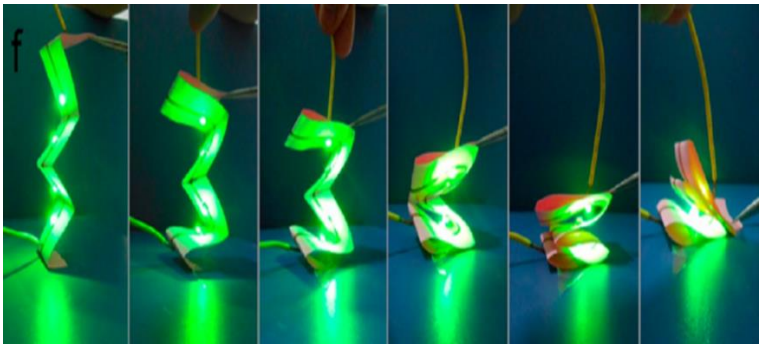
Fig shows a picture drawn with conductive ink glow with green LED.

Conducting Ink functionality on a folded paper. and LED

To test the ink's flexibility, of the conductive ink the paper green LED were integrated on to the paper.

The paper was folded several times and conductivity was checked.

It was that the ink maintained 80 to 90 percent of its conductivity.



Bending process of copper-ink-based circuits under stress

134. Paper Batteries

Paper battery is a thin flexible energy storing device employing cellulose or cellulose based compounds as substrate. Paper battery can be used by folding, cutting, and rolling. In paper based LOC or microfluidics batteries are integrated and used as energy source.

A typical paper-based battery consists of two electrodes made from metal or carbon deposited on to paper substrates, with an electrolyte-filled paper in between to connect the two electrodes by a salt bridge or ion exchange membrane and demonstrates the usefulness of paper in these devices. Foldable or stacked paper designs with wax patterned wells for the various reagents have been implemented, with higher porosity papers tending to yield higher current densities. Printed batteries can either be in sandwich or parallel formats, with several printing techniques having been utilized to realize customized batteries. Commercially available printed primary (non-rechargeable) batteries have been under development for a few decades, commonly utilizing the well-known manganese dioxide/zinc battery chemistries and with scale up possible for roll-to-roll manufacture

Lithium based batteries are used because they are proven as superior storage device and are light weight.

Li is used in the form of lithium oxide and lithium cobalt oxide.

Paper batteries uses lithium and CNTs.

It functions as a battery as well as a capacitor.

It is a modern storage device with ultra-thin in size.

Paper battery can generate electrical energy of 1.5V.

The output voltage of paper battery can be customized.

Used as both battery and capacitor.

1. It is ultra thin energy storage device.
2. Long lasting.
3. Non toxic.
4. Steady power production.

Lithium based batteries

Lithium based Batteries are more energy dense compared to both Lead acid and Nickel Metal Hydride.

Due to their novel characteristics Li-ion cells are widely used in

- Automotive
- Laptops
- Cameras
- Electronics equipment

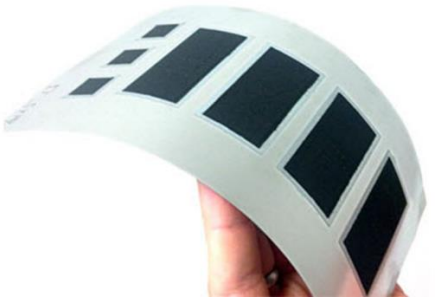
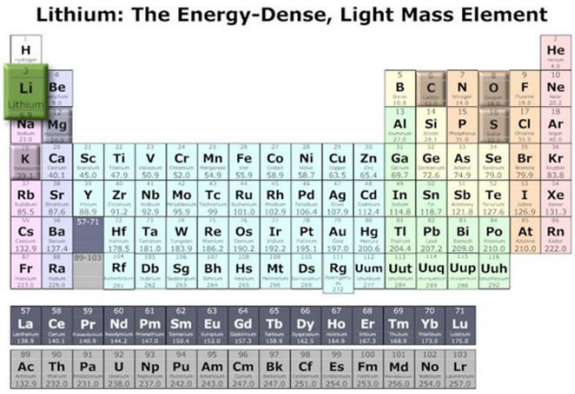


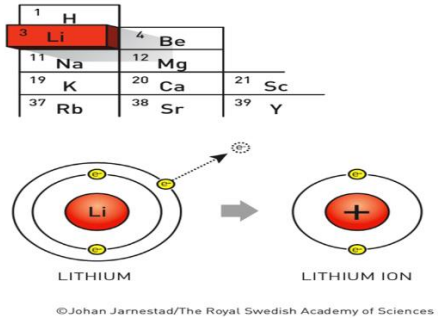
Fig shows a paper battery

Lithium

1. Lithium is an alkali metal with an atomic No. of 3.
2. It has one electron in the outer most shell.
3. Lithium metal is used in paper batteries.
4. Lithium is a light weight alkali earth metal
5. It hash density comparable to pine wood.
6. It is the least reactive of the alkali metals.



Periodic Table



Atomic structure of lithium

Components

The major components are :

1. Carbon Nanotube (CNT) used for cathode terminal
2. Lithium metal (Li+) used for anode terminal.
3. Li is used as Lithium oxide and Lithium cobalt oxide.
4. Different types of electrolytes that include blood, urine, and sweat.
5. Cellulose acts as a Separator

Properties of Carbon Nanotubes

1. Low mass density
2. Flexibility
3. High packing density
4. Lightness
5. Better electrical conductivity than silicon,
6. Thin
7. Low resistance.

Properties of Cellulose

1. Excellent porosity
2. Biodegradability
3. Non-toxic
4. Recyclability
5. High-tensile strength
6. Good absorption capacity
7. low-shear strength.

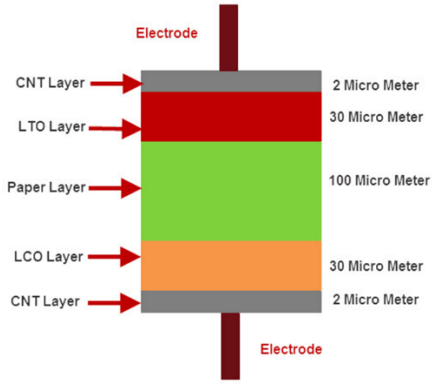


Fig shows structure of a paper battery

Working of Paper battery

The working principal of paper battery is same as for the conventional battery.

1. Battery is dipped in ion-based liquid, then the battery starts working.
2. There is a chemical reaction between the electrodes and liquid.
3. Electricity is generated by the movement of electrons from cathode terminal to anode terminal.

Lithium ion batteries are well suited to applications requiring high power and energy densities and show promise as rechargeable and durable power sources. Ions are transported between the anode and cathode through an ion-conductive electrolyte that is electronically insulating. Typical achievable voltages are around 3 V with energy capacities of 2250 mA h Paper-based lithium ion batteries typically make use of commercial papers that are coated with carbon nanotubes to create highly conductive, easily manufacturable battery components which can be foldable.

135. Printing on Paper Chip

Introduction

For paper LOC the circuits and electrodes could be integrated by printing on the paper surface.Printed functionality can be incorporated with these devices, stemming from printed materials development and printed electronics fields to result in devices with enhanced user-friendliness through automated result read-out and communication. This also removes the need for training or external instrumentation to correctly perform a test. Building blocks from the field of printed electronics, such as sensors, processors, displays, connectivity and power have the potential to be incorporated to extend the functionality of these devices. The printing technology successfully integrates the electronic elements on the substrate.

The printing technology includes wax printing, ink jet printing and Digital light processingA number of techniques are used for producing desired patterns on the paper.

These patterns are based on the original architecture of paper chip.

Biological and chemical reagents are then deposited along the device by either dipping the substrate into a reagent or spotting a reagent onto the substrate.

Fig shows printing of electronics on paper chip;

Development of Paper LOC

To develop a paper LOC hydrophobic physical barriers are created on hydrophilic paper.

These barriers passively transport aqueous solutions.

Biological and chemical reagents must then be deposited selectively along the device by

1. Dipping
2. Locally spotting.

Objective

The objective is to create hydrophobic physical barriers on hydrophilic paper that passively transport aqueous solutions.

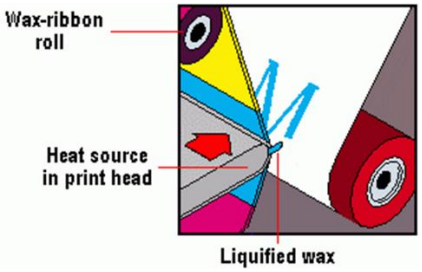
1. Wax printing
2. inkjet printing
3. Photolithography
4. Flexographic printing
5. Laser treatment

6. Digital light processing (DLP)

More sophisticated result read-out generally requires some form of external instrumentation to perform processing and communication of the result from the paper-based test. Common methods include the use of existing devices such as scanners, portable glucose meters and mobile phones, as well as custom-developed systems such as portable potentiostat.

Wax printing

- Wax printing, a simple and inexpensive method for fabricating microfluidic devices.
- Thousands of heating elements on the print head cause the wax to melt on the roll.
- Wax adhere to specially coated paper and microchannels are integrated on the paper surface.



Wax printing on a paper

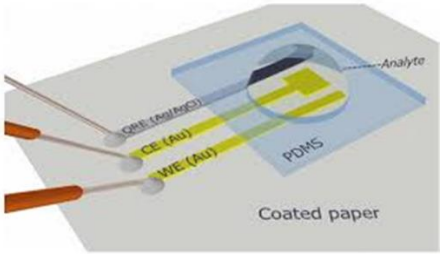
Inkjet printing

Inkjet printing requires a paper in a hydrophobic polymer.

This technique is low cost with high resolution.

It is well suited for low-viscosity, soluble materials like organic semi-conductors.

With high-viscosity materials, nozzle clogging could occurs.



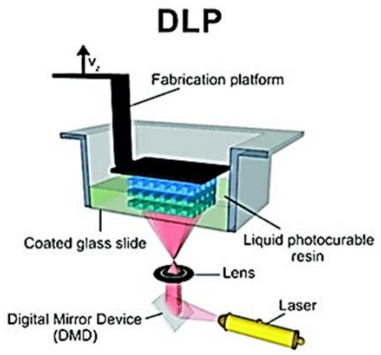
Ink jet printing

Digital Light Processing

This technology uses LASER light source and micro mirrors.

Digital mirror devices are used for printing.

It employs a photo curable resin for printing.



DLP printing

Digital Light Processing (DLP) is based on optical micro-electro-mechanical technology. In DLP projectors, the image is created by microscopically small mirrors laid out in a matrix on a semiconductor chip, known as a Digital Micromirror Device (DMD). These mirrors are so small that DMD pixel pitch may be 5.4 µm or less. Each mirror represents one or more pixels in the projected image.