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RESEARCH ARTICLE

BIOSENSORS

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INTRODUCTION

Conventional physical methods for analysis do not involve the use of any living organisms or molecules of biological origin. However, for this purpose, biological molecules or living cells have been used to develop sensitive devices that are described as 'biosensors'. A biosensor is an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component. It consists of 2 parts:

- *Sensitive biological element* (biological material) (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.). The sensitive elements can be created by biological engineering.
- *Transducer* or the *detector element* that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transducers) that can be more easily measured and quantified. It detects, record and transmits information regarding a physiological change or the presence of various chemical or biological materials in the environment. An analyte can be a protein, toxin, sugar, antibiotic or vitamin present in the body fluid.

History: The history of biosensors started in the year 1962 with the development of enzyme electrodes by the scientist Leland C. Clark. He used platinum (Pt) electrodes to detect oxygen. The major events are summarized as follows:

Year	Achievements
1975	First commercial biosensor (glucose biosensor) by yellow springs instrument company
1975	First microbial biosensor and immunosensor
1980	First optic biosensor
1984	First amperometric biosensor
1987	Blood glucose biosensor launched by MediSense Exac Tech
1998	Blood glucose biosensor launched by LifeScan Fast Take
Current	Carbon nanotubes

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Principle of Biosensors

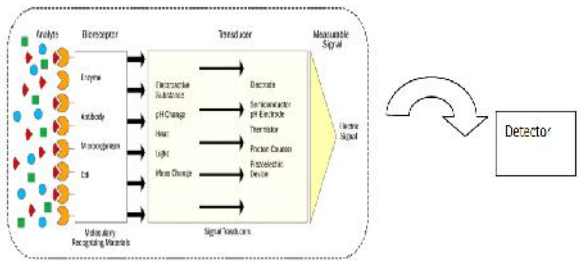
Immobilized biological material
⇩
Sample passed through the membrane
⇩
Interaction of analyte and immobilized biological material
⇩
Biological signal
⇩
Transducer
⇩
Electrical signal

Methods of immobilization

The biological material can be immobilized by following methods:

1. Membrane entrapment: Immobilization of biological active material within the semipermeable membrane.
2. Physical adsorption: Enzyme solution is simply mixed with inert carrier and it gets immobilized.
3. Matrix entrapment: The entrapment of biological material within the lattice of polymerised gel.
4. Covalent bonding: The formation of covalent bonds between biocatalyst and the carrier.

Components of a biosensor



1. Bioreceptor: An analyte present in the sample would bind to this component only. But it should be highly specific. e.g., glucose oxidase acts only on glucose to produce gluconic acid and hydrogen peroxide. Bioreceptor should be stable under storage conditions and it should be immobilized.
2. Transducer: acts as an interface since it is present between first and third component. It measures the physical change that occurs with the reaction at the bioreceptor then transforming that energy into measurable electrical output.
3. Detector: Signals from the transducer are passed to a microprocessor where they are amplified and analyzed. The data is then converted to concentration units and transferred to a display or/and data storage device.

Product	Sensor
Heat	Thermistor
Light	Optical transducer
Mass	Piezo-electric transducer
Current	Electrochemical transducer

Biological and Physical Components of some biosensors and their uses

Biological component	Physical component	Substance measured
Glucose oxidase	Oxygen electrode	Glucose
hCG catalase	Oxygen electrode	Human chorionic gonadotropin
NADH and dehydrogenase	Redox electrode	Ethanol
Nitrifying bacteria	Oxygen electrode	Nitrite and nitrate

Characteristics of Biosensor

1. Selectivity means that sensor detects a certain analyte and doesn't react to admixtures and contaminants.
2. Precision is a characteristic of any scientific device that makes quantitative measurements.
3. Signal stability shows the signal drift under constant conditions which causes an error in measured concentration.
4. Sensitivity (detection limit) shows the minimal amount (or concentration) of analyte that can be detected.
5. Working range is the range of analyte concentrations in which the sensor can operate. For example, glucose concentration in blood typically varies from 0.2mM to 20 mM.
6. Response time is time required to analyze the assay.
7. Regeneration time is the time required to return the sensor to working state after interaction with the sample.
8. Number of cycles is the number of times the sensor can be operated. Degradation of biological material is inevitable and it needs to be replaced. In some sensors (e.g. hand-held commercial glucose sensors) transducers are disposable, they need to be changed after each measurement.
9. Reproducibility is the accuracy with which sensor's output can be obtained.
10. Life time is the time period over which sensor can be used without significant deterioration in performance characteristics.
11. Biosensor should be independent of temperature and pH.
12. Biosensors should be economical.
13. The complete biosensor should be cheap, small, portable and capable of being used by semi-skilled operators.

Types of Biosensor

Electrochemical Biosensors: In this configuration, sensing molecules are either coated onto or covalently bonded to a probe surface. A membrane holds the sensing molecules in place, excluding interfering species from the analyte solution. The sensing molecules react specifically with compounds to be detected, sparking an electrical signal proportional to the concentration of the analyte. The bio-molecules may also respond to an entire class of compounds such as opiates and their metabolites. The most common detection method for electrochemical biosensors involves measurement of current, voltage, conductance, capacitance and impedance.

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

Ag anode $4Ag^0 + 4Cl^- \longrightarrow 4AgCl + 4e^-$

Pt cathode $O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$

The major problem with these biosensors is their dependence on the dissolved oxygen concentration. This may be overcome by the use of 'mediators' which transfer the electrons directly to the electrode. These mediators must possess a number of useful properties.

1. They must react rapidly with the reduced form of the enzyme.
2. They must be sufficiently soluble, in both the oxidised and reduced forms, to be able to rapidly diffuse between the active site of the enzyme and the electrode surface. This solubility should, however, not be so great as to cause significant loss of the mediator from the biosensor's microenvironment to the bulk of the solution. The mediator should generally be non-toxic.
3. The overpotential for the regeneration of the oxidised mediator, at the electrode, should be low and independent of pH.
4. The reduced form of the mediator should not readily react with oxygen.

Potentiometric Biosensors

Potentiometric biosensors: Potentiometric biosensor make use of ion-selective electrodes in order to transduce the biological reaction into an electrical signal. In the simplest terms this consists of an immobilised enzyme membrane surrounding the probe from a pH-meter, where the catalysed reaction generates or absorbs hydrogen ions. The reaction occurring next to the thin sensing glass membrane causes a change in pH which may be read directly from the pH-meter's display. Typical of the use of such electrodes is that the electrical potential is determined at very high impedance allowing effectively zero current flow and causing no interference with the reaction. A semi-permeable membrane (a) surrounds the biocatalyst (b) entrapped next to the active glass membrane (c) of a pH probe (d). The electrical potential (e) is generated between the internal Ag/AgCl electrode (f) bathed in dilute HCl (g) and an external reference electrode (h). Here, the measured parameter is voltage.

Potentiometric biosensors are of three types

1. Ion-sensitive electrodes
2. Gas-sensing electrodes
3. Field-effect transistors

1. Ion-sensitive electrodes: These are usually pH meter electrodes. Biosensors using these electrodes detect and measure many reactions which generate or use up H^+ ions.
2. Gas-sensing electrodes: detect and measure the amount of gas produced. These are also pH meter electrodes, but the electrode surface is covered by gas-permeable membrane selective for CO_2, NH_3 or H_2S. The diffusion of the gas through this membrane causes a change in pH of a sensing solution between the membrane and the electrode which is then determined
3. Field-effect transistors: These are usually solid state electrodes. The electrode surface is covered by a polymer layer. This polymer layer is selectively permeable for analyte ions. The ions diffuse through the polymer layer and in turn cause a change in the surface potential.

Optical Biosensors

Optical biosensors measure signal in the form of light. The major advantage of optical biosensors is that they do not have electrical interferences and therefore are biocompatible for in vivo and can detect changes which occur in the micro-environment that surrounds their surface. Optical biosensors are very useful for monitoring and testing down-well waters.

Piezo-electric Biosensors

In this mode, sensing molecules are attached to a piezoelectric surface in which interactions between the analyte and the sensing molecules set up mechanical vibrations that can be translated into an electrical signal proportional to the amount of the analyte. Example of such a sensor is quartz crystal micro or nano balance. Piezo-electric crystals (quartz) vibrates under the influence of electric field. The frequency of oscillation depends upon thickness and cut of crystal. The change in frequency is proportional to the mass of absorbed material.

Calorimetric Biosensors

Many enzyme catalysed reactions are exothermic, generating heat which may be used as a basis for measuring the rate of reaction and, hence, the analyte concentration. This represents the most generally applicable type of biosensor. The temperature changes are usually determined by means of thermistors at the entrance and exit of small packed bed columns containing immobilised enzymes within a constant temperature environment. Under such closely controlled conditions, up to 80% of the heat generated in the reaction may be registered as a temperature change in the sample stream. This may be simply calculated from the enthalpy change and the amount reacted.

Whole cell biosensors or microbial biosensors

Whole cell biosensors interact with many substrates and detect dozen of pathogens within few minutes.

Commonly used microbial biosensors are

BOD Sensor: A biosensor consisting of immobilised yeast *Trichosporon cutaneum* and an oxygen probe was developed for BOD estimation. The BOD biosensor includes an oxygen electrode that consists of a platinum cathode and an aluminium anode bathing in saturated KCl solution and a Teflon membrane. Yeast cells are immobilized on a porous membrane and are trapped between the pores and the Teflon membranes. Oxygen consumption by the immobilized microorganisms causes a decrease in current until a steady state is reached. The BOD biosensor measures BOD at 3-60 mg/.

Methane Biosensor: This biosensor consists of immobilized methanotrophic bacteria (*Methylomonas flagellata*) in contact with an oxygen electrode. The immobilized bacteria use methane as well as oxygen according to the following reaction.

$$CH_4 + NADH_2 + O_2 \longrightarrow CH_3OH + NAD + H_2O,$$

where NAD is nicotinamide adenine dinucleotide and $NADH_2$ is the reduced form of the coenzyme.

Ammonia and Nitrate Biosensors: Ammonia biosensor consists of immobilized nitrifying bacteria (*Nitrosomonas europaea*) and a modified oxygen electrode. This biosensor, with a lifetime of approximately 2 weeks was used for ammonia determination in waste waters based on the conversion of nitrate to N_2O by an immobilized denitrifying bacterium *Agrobacterium* sp. The nitrate biosensor has been used to measure nitrate profiles in biofilms in environment samples.

Carbon nanotubes: Carbon nanotubes are made up of carbon atoms which are rolled into sheet of 1 nanometer. In these nanotubes light is emitted after absorbing light in infrared region. As wavelength of infrared light are not blocked by body fluid, therefore this nanotubes are used to get information about the body.

Advantages

1. Biosensors can be implanted in the human body and are suitable for in vivo detection. For example - medical telesensors and artificial pancreas.
2. Biosensors are useful for measuring various substrates in a small amount of sample solution.
3. The commonly performed assays in hospitals for glucose monitoring require reagents at each step to treat the sample. However, glucose biosensors donot require reagent.
4. The main advantage of biosensor is its simplicity, speed of measurement and production of real data.
5. Biosensors can detect analytes present in micromolar to nanomolar range.
6. Biosensors are cheap, less labour intensive.

Disadvantages

Biosensors can't be heat sterilized since it will denature the biological part of biosensor.

1. Changes in pH and temperature can lead to signal error.
2. Biosensors have short life-time.

Applications

Biosensors in environmental field

1. Biosensors can be used for monitoring the pollution level in the environment including air, land and water. Small portable analysers can be developed for use in the field for on-line applications to monitor pollutants or dedicated biosensors can be developed for off-line monitoring in the laboratory.
2. Biosensors can be used to measure the concentration of various metal ions. For example, optical biosensors are used for the detection of cadmium ions in the milk.
3. Biosensors are used in BOD measurement during waste water treatment.
4. Biosensors are used in the detection of polyaromatic hydrocarbons present in the water. Polyaromatic hydrocarbons are the atmospheric pollutants. For example, naphthalene. They mix more easily with oil than water and are found in cooked food.
5. Biosensors can be used for monitoring the quality of water through tests for pollutants, chemical residues, pesticides, herbicides, toxins and microbes in water reservoirs.
6. Whole cell biosensors with immobilized cells of *Salmonella typhimurium* and *Bacillus subtilis* coupled with an oxygen electrode can be used for testing the mutagenicity and carcinogenicity of various chemicals.

Biosensors for medical diagnostics

***In vitro* diagnostic:** In vitro diagnostic tests fall in three categories.

Centralized tests in hospitals

These tests conducted in hospitals include tests for glucose, lactic acid, uric acid, viruses and a variety of pathogenic microbes. Amperometric biosensor devices for glucose include Satellite G (from Medisense), Glucose Analyser 23A (from Yellow Spring Instruments Co.) and Stat Profile 5 and 6 (from Nova Biomedical). DNA based biosensors are also being developed for diagnosis of hereditary diseases, viruses and cancer.

Tests in doctors clinics

Analysers to be used in the nursing homes or in private clinics of practising doctors are also being developed for testing glucose, lactic acid, creatinine and urea.

A recent example of these biosensors is the portable I-Stat system which can make use of unprocessed blood and can be operated by the doctor or a nurse. This can perform tests for glucose, urea, nitrogen, sodium, potassium and chloride.

Tests by consumers

Biosensors are now available, which can be used by individuals at their residence without any help from a doctor or a nurse. A popular example is ExacTech^{TM} blood glucose monitor (from Medisense).

In vivo diagnostics

The most important example of the application of biosensors for *in vivo* diagnostics is the idea of artificial pancreas (permanently implanted in skin of diabetic patients) which continuously monitors *in vivo* level of glucose by a control on the rate of insulin infusion from an implanted minipump as a potential cure for diabetes.

The normal pancreas continuously monitors blood sugar levels and promptly reacts to changes blood sugar levels by increasing or decreasing the production of insulin.

The artificial pancreas consists of a miniatured biosensor attached with a minipump containing insulin, so that the level of blood sugar will be estimated and a desirable quantity of insulin will be pumped into the patient's blood.

Biosensors for agriculture *and* food industry

1. Applications of biosensors in agriculture include detection of viral, fungal, bacterial diseases, fertilizers and pesticides.
2. The most viable use of biosensors in food industry will include rapid detection of total microbial contaminants and sugar quantification in soft drinks.
3. Biosensors have been developed to determine the freshness of fish and other food items including beef.
4. Biosensors are used in determining the quality of food.

Biosensors in fermentation industry

1. Biosensors can be used for monitoring the cultured micro-organisms producing drugs, hormones, vaccines, single cell proteins etc.
2. Biosensors have been developed for alerting workers for the presence of hazardous substances in the industrial atmosphere.

Biosensors for military and defense industry

In military and defense organisations, portable biosensors can be very useful for detection of toxic gases and the agents of chemical warfare such as mustard and nerve gas.

1. Use of biosensors in industry will improve manufacturing techniques.
2. Biosensors can be used to monitor the manufacturing of pharmaceutical compounds
3. Biosensors are used in biotechnological processes such as to determine the proteins .
4. Biosensors can be used in determining intracellular proteins and plasmids.
5. Mitomycin – cancer causing toxin in inborne infants can be detected by using biosensor.

CONCLUSION

Biosensors are cheap and small devices which can be operated by an individual at residence. They are highly reliable for determining the freshness and quality of food. They are less time consuming and highly useful for diabetic patients. However, implantation of artificial pancreas causes allergenicity and malfunctioning. Although a demand for biosensors in medical diagnostics may certainly rise, the new wave of biosensors that will be produced in 21st century will address applications in environment monitoring and the food and drink industry.

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