Amperometric Biosensors

Rahul N

04/10/2014

Biosensor

❖ A **biosensor** is an analytical device, used for the detection of an analyte, that combines a biological component with a physicochemical detector.

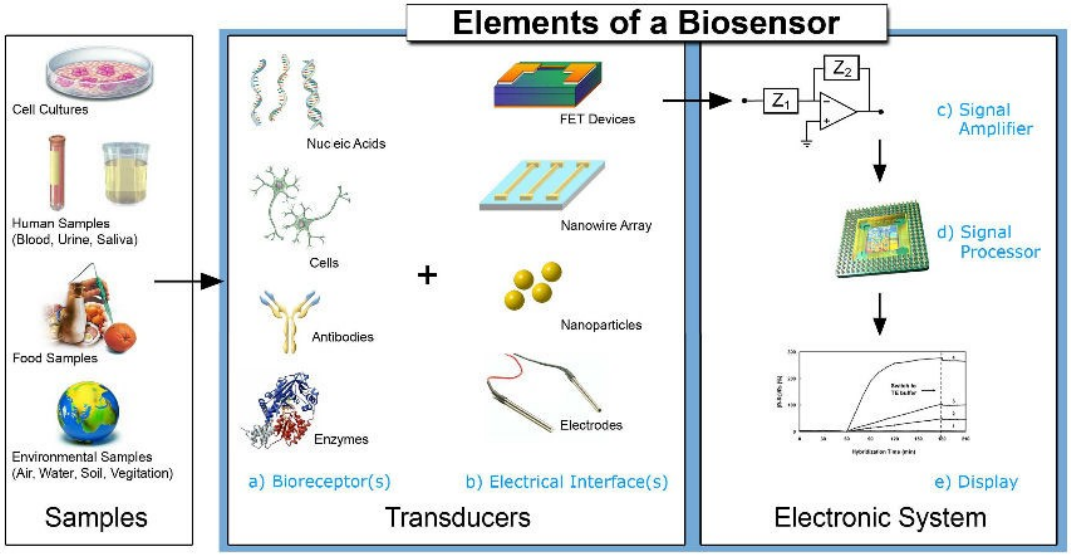
Bio-recognition component

❖ The *sensitive biological element* (e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc.), a biologically derived material or biomimetic component that interacts (binds or recognizes) the analyte under study. The biologically sensitive elements can also be created by biological engineering.

Biotransducer component

❖ The *transducer* or the *detector element* (works in a physicochemical way; optical, piezoelectric, electrochemical, etc.) that transforms the signal resulting from the interaction of the analyte with the biological element into another signal (i.e., transduces) that can be more easily measured and quantified.

❖ Biosensor reader device with the associated electronics



Professor Leland C Clark Jnr

History of Biosensors

1916 First report on immobilization of proteins : adsorption of invertase on activated charcoal

1922 First glass pH electrode.

1956 Clark published his definitive paper on the oxygen electrode.

1962 First description of a biosensor: an amperometric enzyme electrodre for glucose (Clark).

1969 Guilbault and Montalvo – First potentiometric biosensor: urease immobilized on an ammonia electrode to detect urea.

1970 Bergveld – ion selective Field Effect Transistor (ISFET).

1975 Lubbers and Opitz described a fibre-optic sensor with immobilised indicator to measure carbon dioxide or oxygen.

1975 First commercial biosensor (Yellow springs Instruments glucose biosensor).

1975 First microbe based biosensor, First immunosensor.

1976 First bedside artificial pancreas (Miles)

1980 First fibre optic pH sensor for in vivo blood gases (Peterson).

1982 First fibre optic-based biosensor for glucose.

1983 First surface plasmon resonance (SPR) immunosensor.

1984 First mediated amperometric biosensor: ferrocene used with glucose oxidase for glucose detection.

1987 Blood-glucose biosensor launched by MediSense ExacTech.

1990 SPR based biosensor by Pharmacia BIACore.

1992 Hand held blood biosensor by i-STAT.

1996 Launching of Glucocard.

1998 Blood glucose biosensor launch by LifeScan FastTake.

1998 Roche Diagnostics by Merger of Roche and Boehringer mannheim.

Current - Quantom dots, nanoparicles, nanowire, nanotube, etc

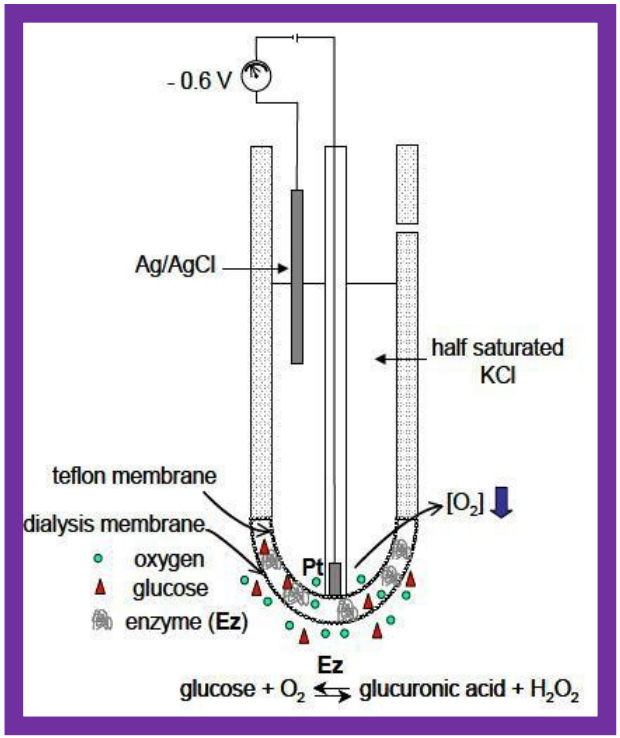
Types of Biosensors

- Biosensors are classified according to the parameter that is measured by the physicochemical transducer of the biological event as:
  - Optical,
  - Electrochemical ---- > **Amperometric**
  - Acoustic,
  - Thermal

Amperometry

- The detection of ions presence on solution on the basis of electric current or change in electric current is called as Amperometry.
  - **Working electrode (microelectrode)**
  - **Reference electrode**
  - **Auxiliary electrode**
- Apart from these electrodes, there is a voltage source and a devices for measuring current and voltage – voltmeters and ammeter.
- The method is based on the principle that the measurements of changes in time (τ) in the current (I) flowing through the system of electrodes in relation to potential (E) applied to the working electrode.

❖ **Amperometric Biosensors**: the oldest ones, which have led to the higher number of ready to- use devices, are based on the monitoring of electron-transfer processes.



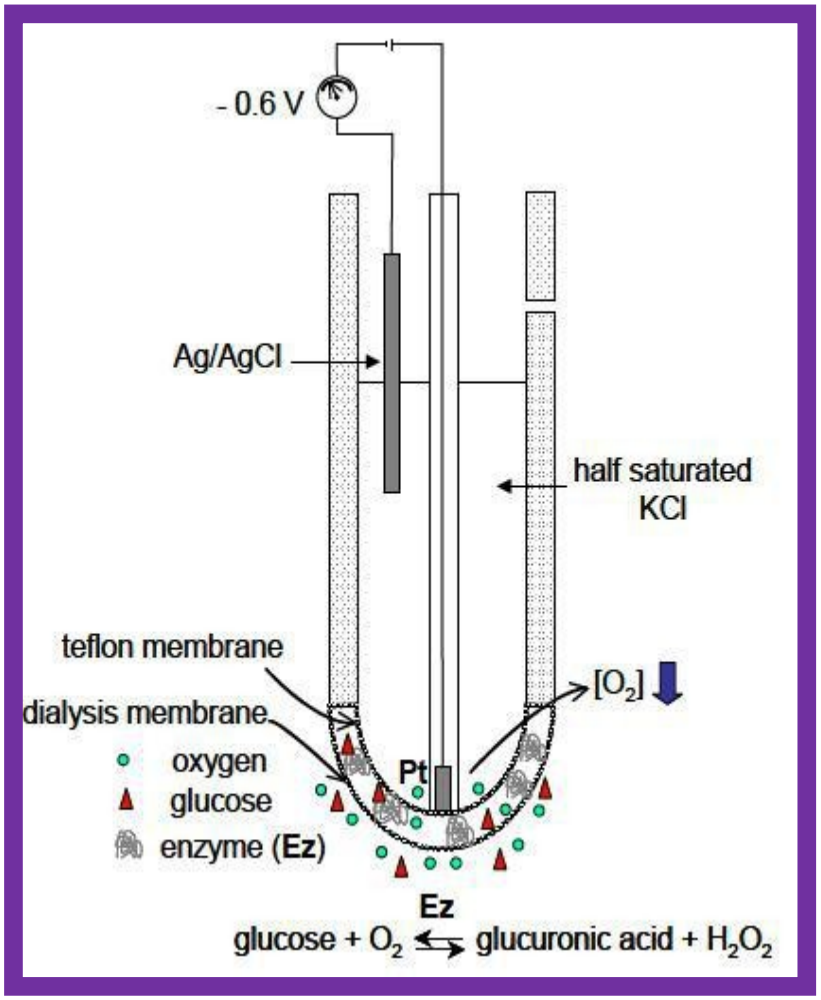
Amperometric Biosensors produce a current proportional to the concentration of the substance to be detected.

The most common amperometric biosensors use the Clark Oxygen electrode.

In the glucose Amperometric Biosensor, the Clark Oxygen electrode is separated from glucose by a membrane, that is permeable to oxygen.

A biocatalyst Glucose Oxidase(GOD) is housed between this membrane and another membrane that separates it from the glucose.

This membrane that separates GOD and glucose is permeable to both Oxygen and Glucose.



In effect, the enzyme GOD is immobilized between two membranes, the top being permeable only to oxygen and the bottom to both Oxygen and Glucose.

The Glucose that enters the membrane is Oxidised in presence of the enzyme GOD, to produce Glucuronic acid and Hydrogen Peroxide.

glucose + O2 – > glucuronic acid + H2O2

Hence the concentration of oxygen decreases as it moves up through the membranes to reach the cathode. This decrease in Oxygen concentration is reflected as a decrease in current between the electrodes.

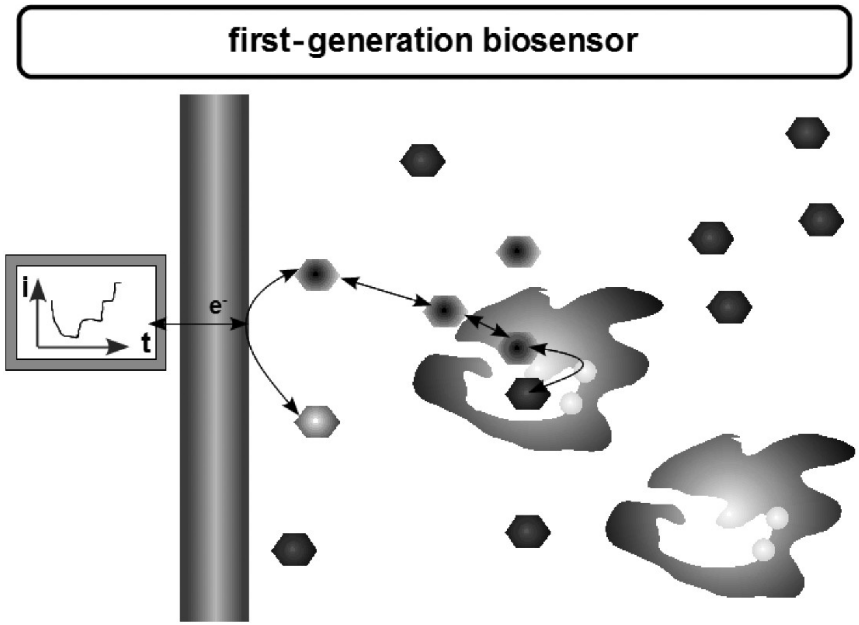
Alternatively,

the decrease in the concentration of Hydrogen Peroxide can also be used to find the concentration of glucose, by changing the voltage applied between the electrodes to +0.68 V relative to the Ag/AgCl electrode causing the reactions:

Pt anode H2O2 -- > O2 + 2H+ + 2e-

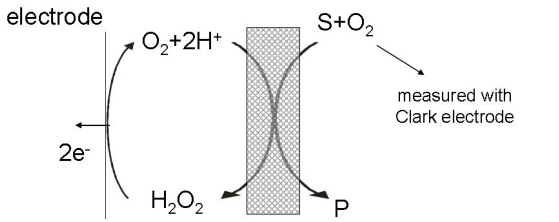
Ag cathode 2AgCl + 2e- -- > 2Ag0 + 2Cl-

1st generation: the normal product of the reaction diffuses to the transducer and causes electrical response



O_2 electrode-based sensors

1st generation

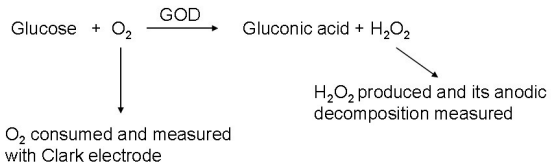


Alternatively:

Pt Anode, $H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$

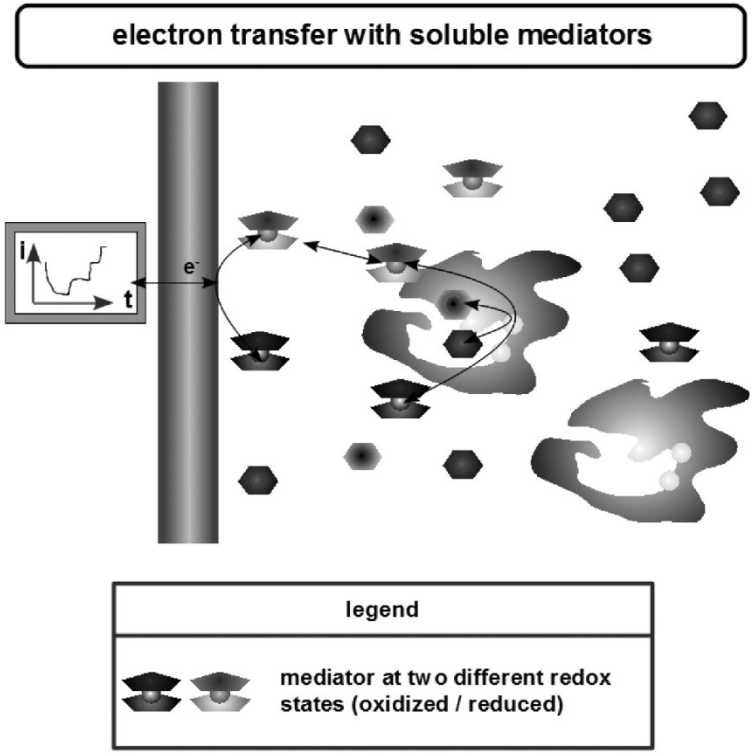
Ag Cathode, $2AgCl + 2e^- \longrightarrow 2Ag^o + 2Cl^-$

Glucose Sensor



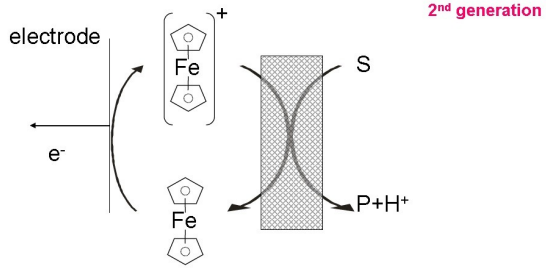
Dependence on dissolved O_2 concentration

How to control O_2 concentration?



2nd generation: involves specific mediators between reaction and transducer to generate improved response

Mediator-based sensors



$[Fe^{III}(Cp)_2]^+ + e^- \rightleftharpoons Fe^{II}(Cp)_2 \quad (E^o = +0.165\,V)$

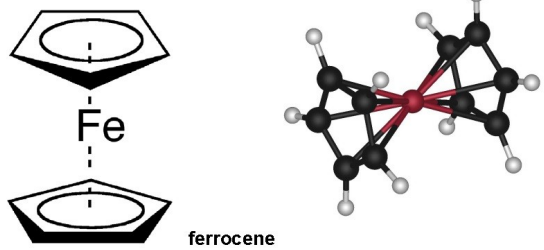
ferrocene

$E_p(Ox) = +0.193$ V

$E_p(Red) = +0.137$ V)

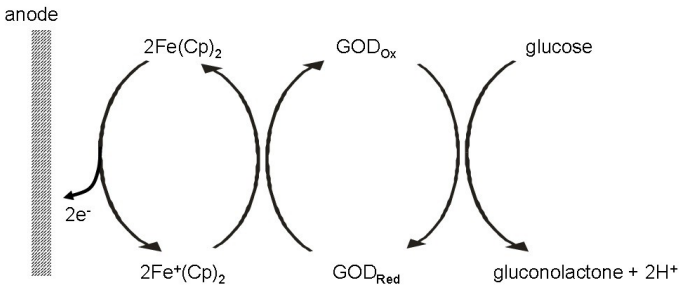
Ferrocene

- Rapid reaction with the reduced form of the enzyme
- Sufficiently soluble (Ox, Red) → rapid diffusion
- Small overpotential for the Ox and independent of pH
- The Red does not react with O_2
- Non toxic



ferrocene

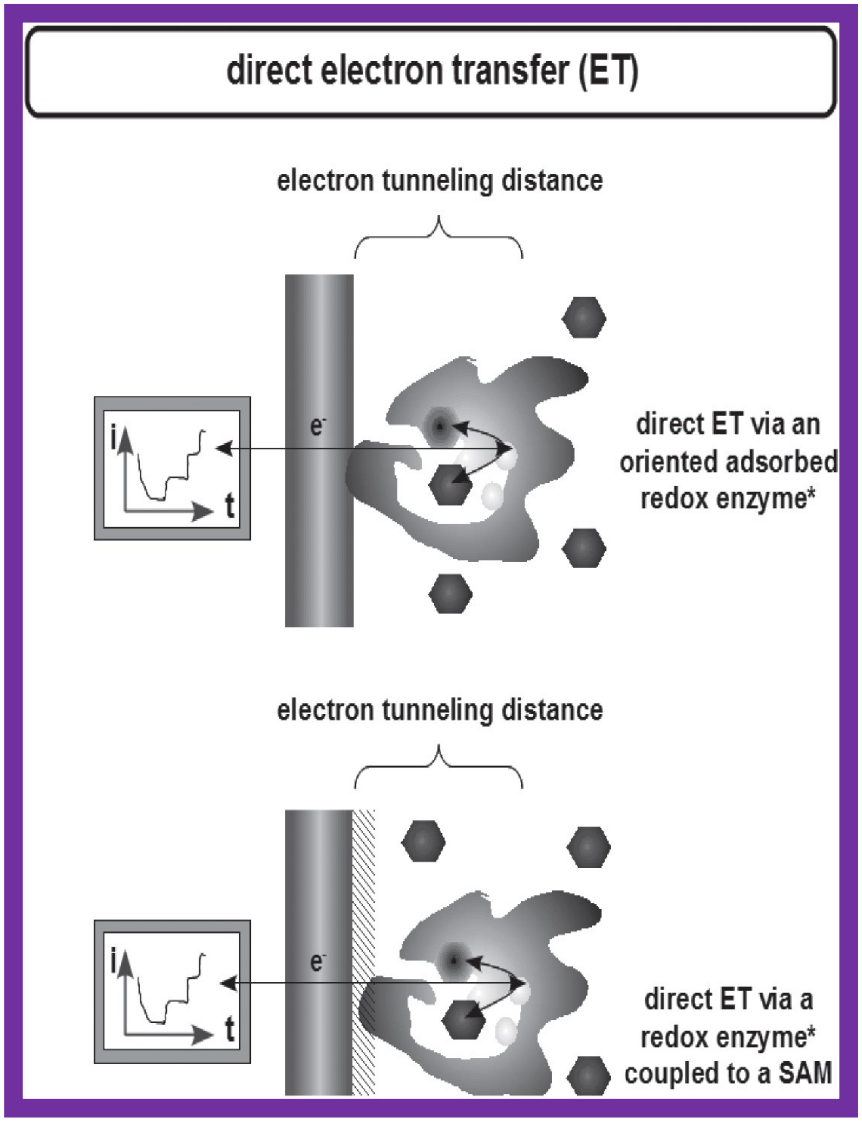
glucose



$glucose + GOD_{ox} \rightarrow gluconolactone + GOD_R + 2H^+$

$GOD_R + 2Fe^+ \rightarrow GOD_{ox} + 2Fe$

$2Fe \rightarrow 2Fe^+ + 2e^-$



3rd generation: reaction itself causes the response

Perfomance factors

- Selectivity
- Sensitivity
- Accuracy
- Response time
- Recovery time
- Lifetime

(i) *Selectivity*. This is the most important characteristic of sensors – the ability to discriminate between different substances. Such behaviour is principally a function of the selective component, although sometimes the operation of the transducer contributes to the selectivity.

(ii) *Sensitivity range*. This usually needs to be sub-millimolar, but in special cases can go down to the femtomolar (10^{-15} M) range.

(iii) *Accuracy*. This needs to be better than ±5%.

(iv) *Nature of solution*. Conditions such as pH, temperature and ionic strength must be considered.

(v) *Response time*. This is usually much longer (30 s or more) with biosensors than with chemical sensors.

(vi) *Recovery time*. This is the time that elapses before the sensor is ready to analyse the next sample – it must not be more than a few minutes.

(vii) The *working lifetime* is usually determined by the stability of the selective material. For biological materials this can be a short as a few days, although it is often several months or more.

Application of Biosensor

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

applications of biosensors - examples

clinical	non-clinical
• single-use → glucose monitoring (by the patient at home) → lactate (sport event/training)	• single analysis → glucose, alcohol, aldehyde monitoring (food industry)
• multi-analysis → glucose monitoring (hospital) → pathogen detection (pathology, Internal medicine)	• continuous monitoring → glucose, other small molecules, pathogens, pollutants (food & water industry, fermentation, quality control)
• short-term invasive → glucose monitoring (hospital, bedside)	• environmental monitoring → pathogens, e.g. plaque, anthrax (ecological agencies, military, quality control)
• long-term implantable → glucose monitoring (artificial organs)	