ELECTROCHEMICAL BIOSENSORS

Electroanalytical methods commonly used in biosensor

Bulk methods (conductometry) Interfacial methods

Dynamic (I≠0)
(Voltammetry, amperometry, EIS, coulometry etc.)

Static(I=0)
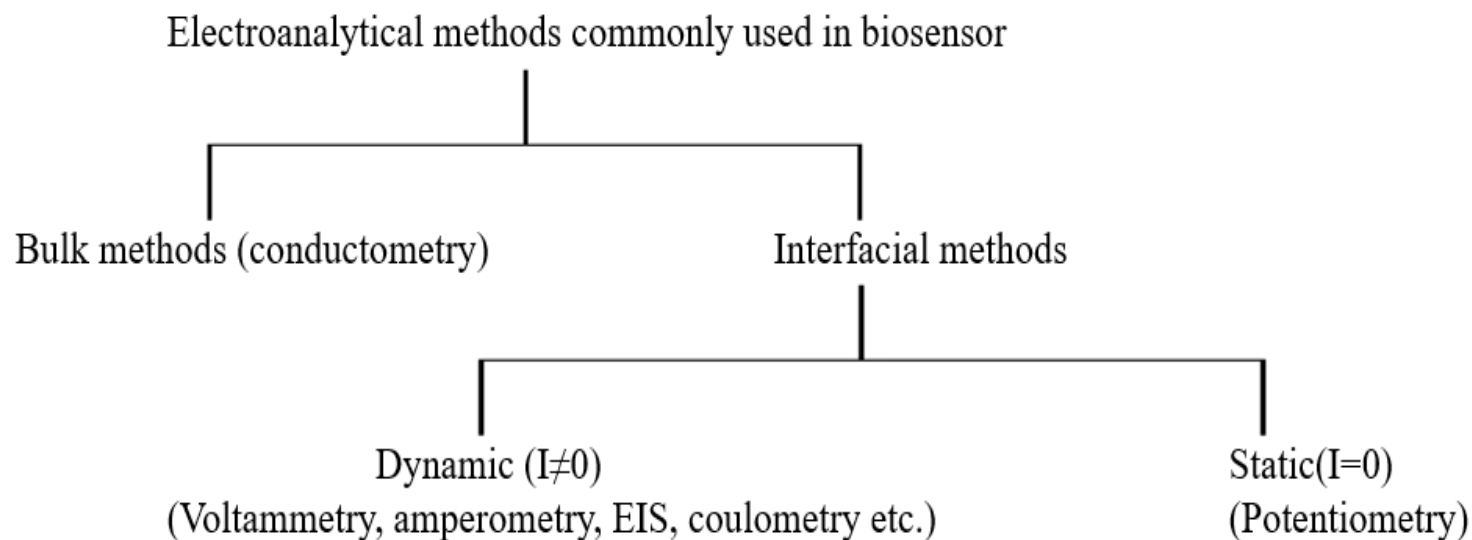(Potentiometry)

Classification of commonly used electroanalytical techniques for biosensors.

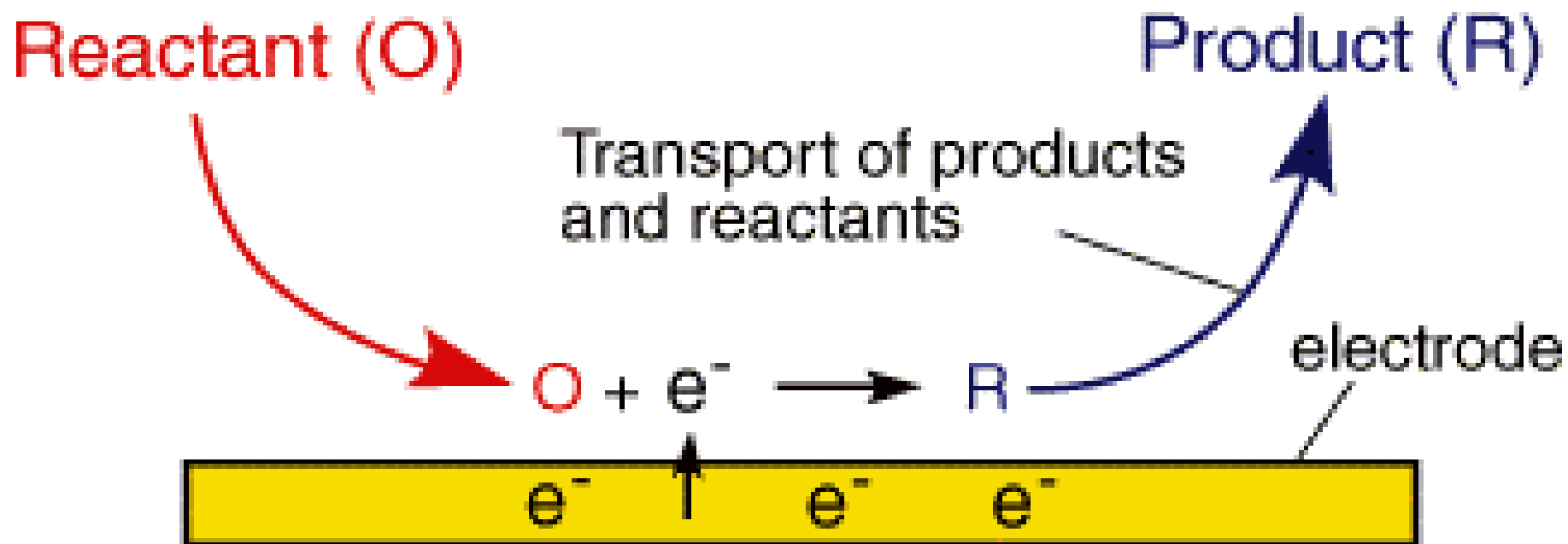Kalita, Gogoi, Minteer, Goswami, *ACS Measurement Sci*. Au, 3, (6) (2023)
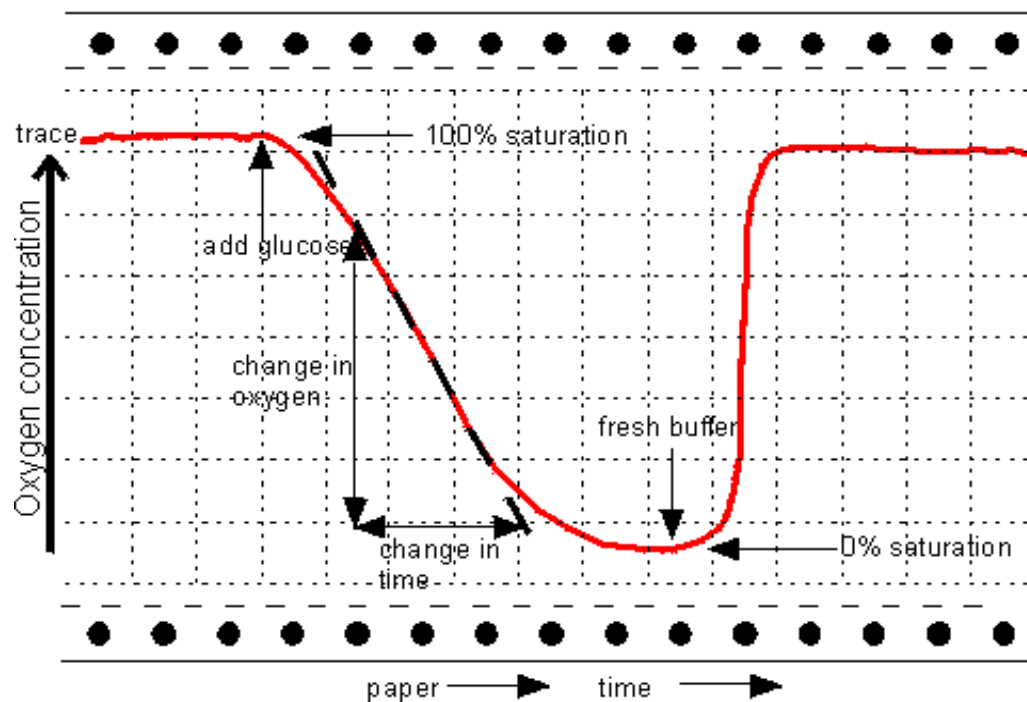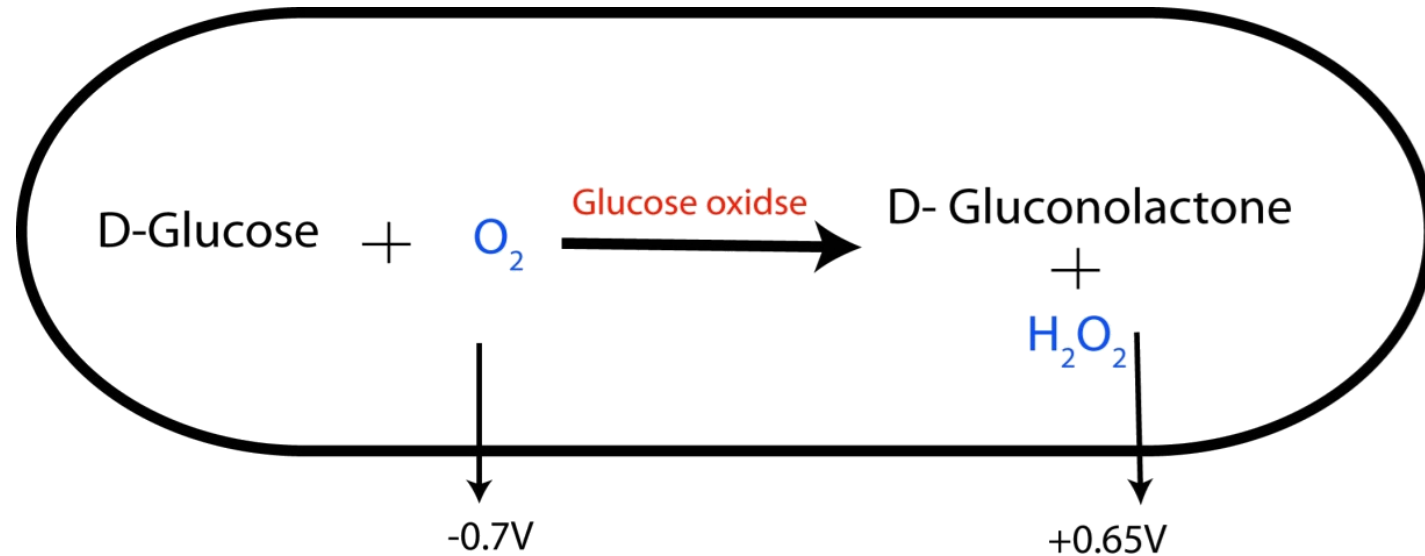
Dynamic methods :
- involve measurement of current by controlling the potential known as controlled-potential technique or opposite, the controlled-current technique.

- In the controlled potential technique, the current response is correlated to the analyte concentration. The resulting current is a combination of two components: the faradaic component, represents the redox events occurring at the interface (follows Faraday's law), and non-faradaic component (doesn't follow Faraday's law),

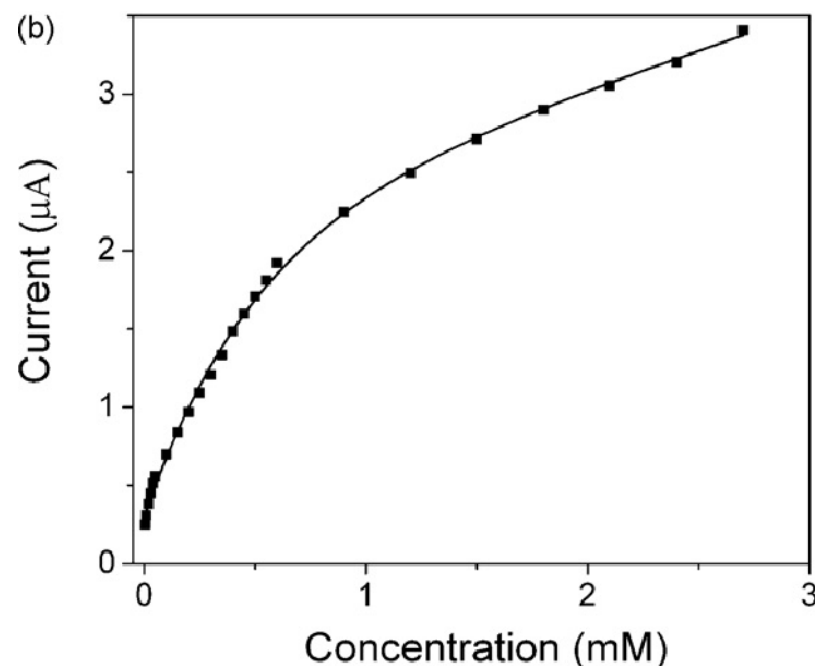$$I_{Total} = I_{Faradaic} + I_{Non\text{-}faradaic}.$$

- Many factors may contribute to the non-faradaic component. One of them is charging the electrical double layer during dynamic measurements.

AMPEROMETRIC BIOSENSORS

Reactant (O)

Product (R)

Transport of products and reactants

O + e⁻ → R

electrode

e⁻   e⁻   e⁻

D-Glucose $+$ O_2 $\xrightarrow{\text{Glucose oxidse}}$ D- Gluconolactone $+$ H_2O_2

$-0.7V$ $\qquad$ $+0.65V$

(b)

O$_2$ as redox entity $\qquad$ H$_2$O$_2$ as redox entity

What is volt?

- volt is simply the energy (*J*) required to move charge (*c*).
- Reactions are initiated by applying a potential,

$$V = Joule/Coulomb$$

- Application of a voltage to an electrode therefore supplies electrical energy.
- Since electrons possess charge an applied voltage can alter the 'energy' of the electrons within a metal electrode.

Which electron in a metal?

•Electrons of the Fermi-level (E_F).

•The Fermi-level corresponds to the energy of the highest occupied orbitals.

•This level is not fixed and can be moved by supplying electrical energy.

•Electrochemist's are therefore able to alter the energy of the Fermi-level by applying a voltage to an electrode.

•Depending upon the position of the Fermi level it may be thermodynamically feasible to reduce/oxidise a species in solution.

$$O \ + \ e \ \rightleftharpoons \ R$$

The figure below shows the Fermi-level within a metal along with the orbital energies (HOMO and LUMO) of a molecule (**O**) in solution.
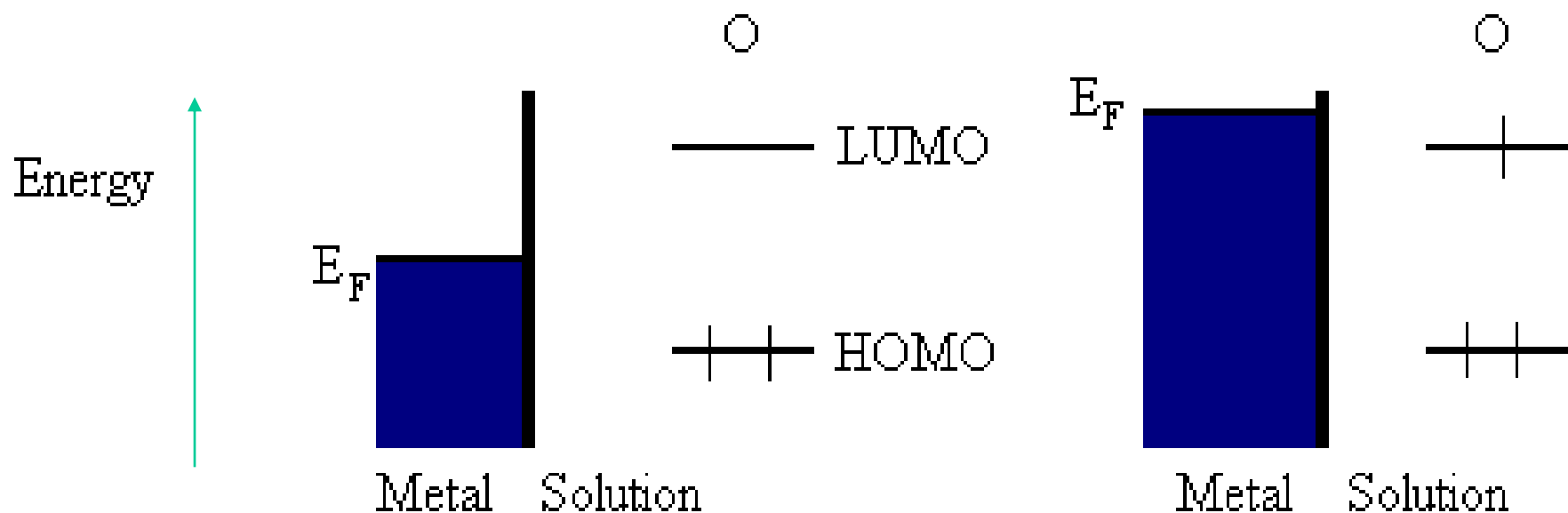
Voltage

- Left: E_F < the E of LUMO of (**O**), hence *thermodynamically unfavourable* for an e to jump from the electrode to O.
- Right: E_F > the E of LUMO of (**O**), it is *thermodynamically favourable* for the electron transfer to occur to O.

The fundamental relationship between the current flowing and the applied voltage is given by the following Butler-Volmer equation,.

$$i \;=\; i_o \left\{ \frac{[O]_o}{[O]_{bulk}} e^{\frac{-\alpha n F (E - E_s)}{RT}} \;-\; \frac{[R]_o}{[R]_{bulk}} e^{\frac{(1 - \alpha) n F (E - E_s)}{RT}} \right\}$$

This expression shows how the current will respond to changes in potential, the value of **alpha** and the quantity i_o which is called the exchange current (density). The term **alpha** reflects the sensitivity of the transition state to the applied voltage. If **alpha = 0** then the transition state shows no potential dependence. Typically **alpha = 0.5** this means that the transition state responds to potential in a manner half way between the reactants and the products response.

Here we wish to see how the voltage influences the current in the absence of concentration effects. To do this we will assume that the a solution is well mixed ie that the surface and bulk concentrations are identical which will be reasonable under condition of small current flow. Now the Butler-Volmer equation simplifies to

$$i \;=\; i_o \left\{ e^{\frac{-\alpha n F (E - E_s)}{RT}} \;-\; e^{\frac{(1 - \alpha) n F (E - E_s)}{RT}} \right\}$$

Without concentration and therefore mass transport effects to complicate the electrolysis it is possible to establish the effects of voltage on the current flowing. In this situation the quantity **E - E_e** reflects the activation energy required to force current **i** to flow.

- **Amperometry** is a subclass of voltammetry in which the electrode is held at constant potentials for various lengths of time.
- Voltammetry applies a constant and/or varying potential at an electrode's surface and measures the resulting current with a three electrode system.
- To conduct such an experiment requires at least two electrodes.
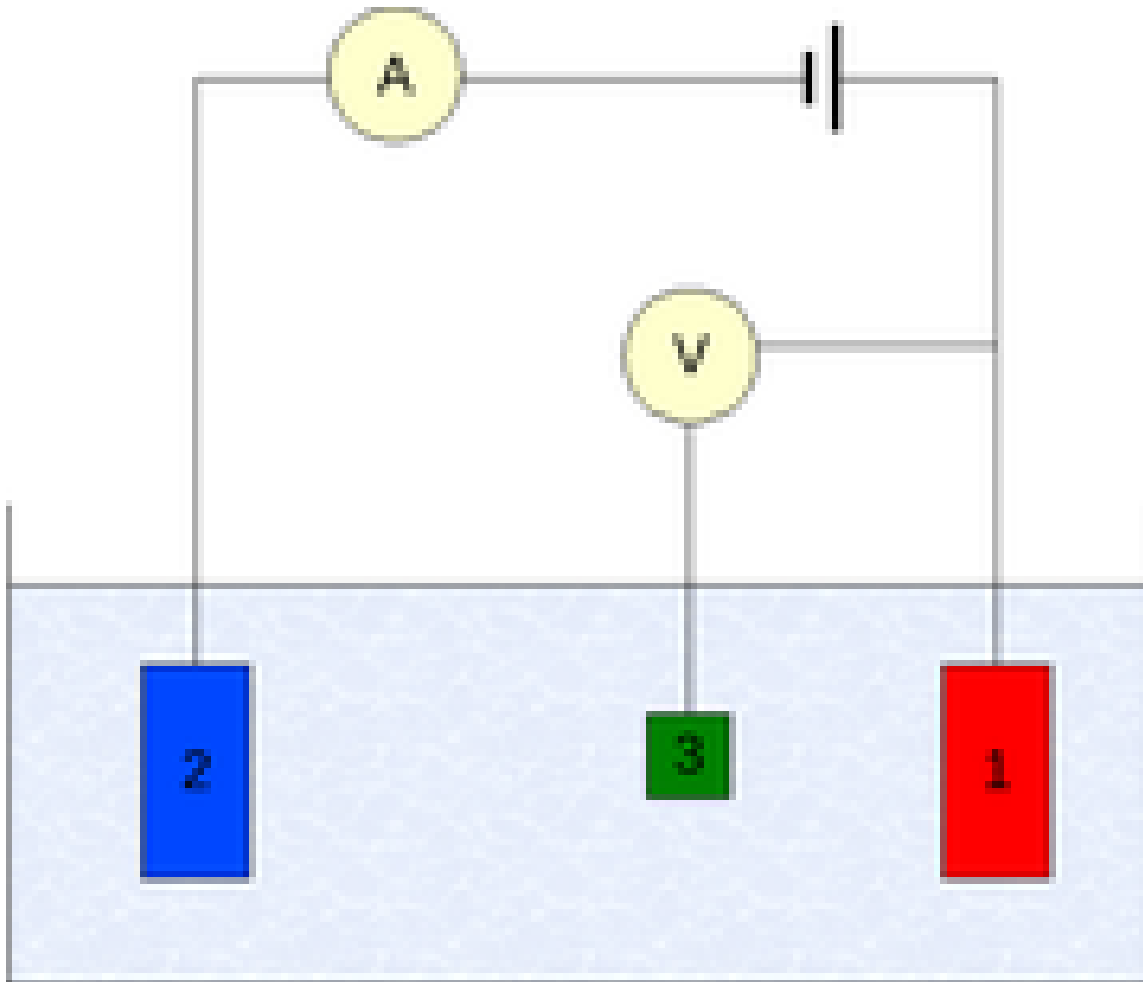
Three-electrode setup:
(1) working electrode;
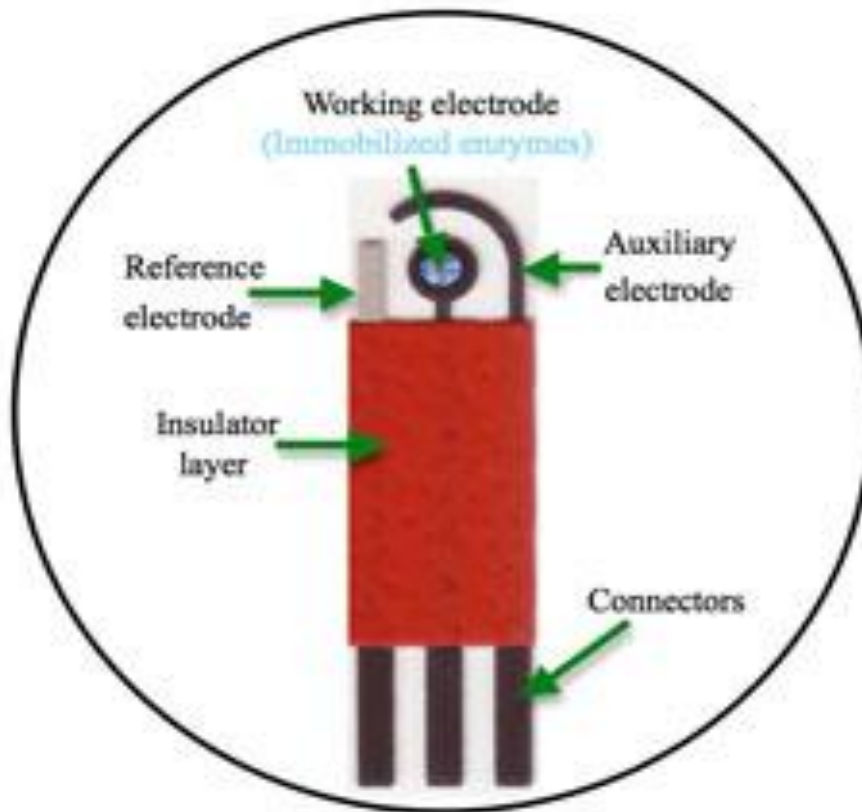(2) auxiliary electrode;
(3) reference electrode

<u>Working electrode</u>: It makes contact with the analyte, must apply the desired potential in a controlled way and facilitate the transfer of <u>electrons</u> to and from the analyte.

<u>Reference electrode</u>: It acts as the other half of the cell, has a known potential with which to gauge the potential of the working electrode.
It may use to balance the electrons added or removed by the working electrode. While this is a viable setup, it is extremely difficult for an electrode to maintain a constant potential while passing current to counter redox events at the working electrode. To solve this problem, the role of supplying electrons and referencing potential has been divided between two separate electrodes.

<u>Aauxiliary electrode</u>: <u>It</u> passes all the current needed to balance the current observed at the working electrode. To achieve this current, the auxiliary will often swing to extreme potentials at the edges of the solvent window, where it oxidizes or reduces the solvent or supporting electrolyte.

These electrodes, the <u>working</u>, <u>reference</u>, and <u>auxiliary</u> make up the modern three electrode system.

Working electrode
(Immobilized enzymes)

Reference electrode

Auxiliary electrode

Insulator layer

Connectors

Two steps
- 1st Blanc
- 2nd Sample

Layer

12 ppb
50% lnm

✓Fast
✓Sensitive
✓Selective
✓Label free
✓Min. or no sample loss
✓Small size-low cost