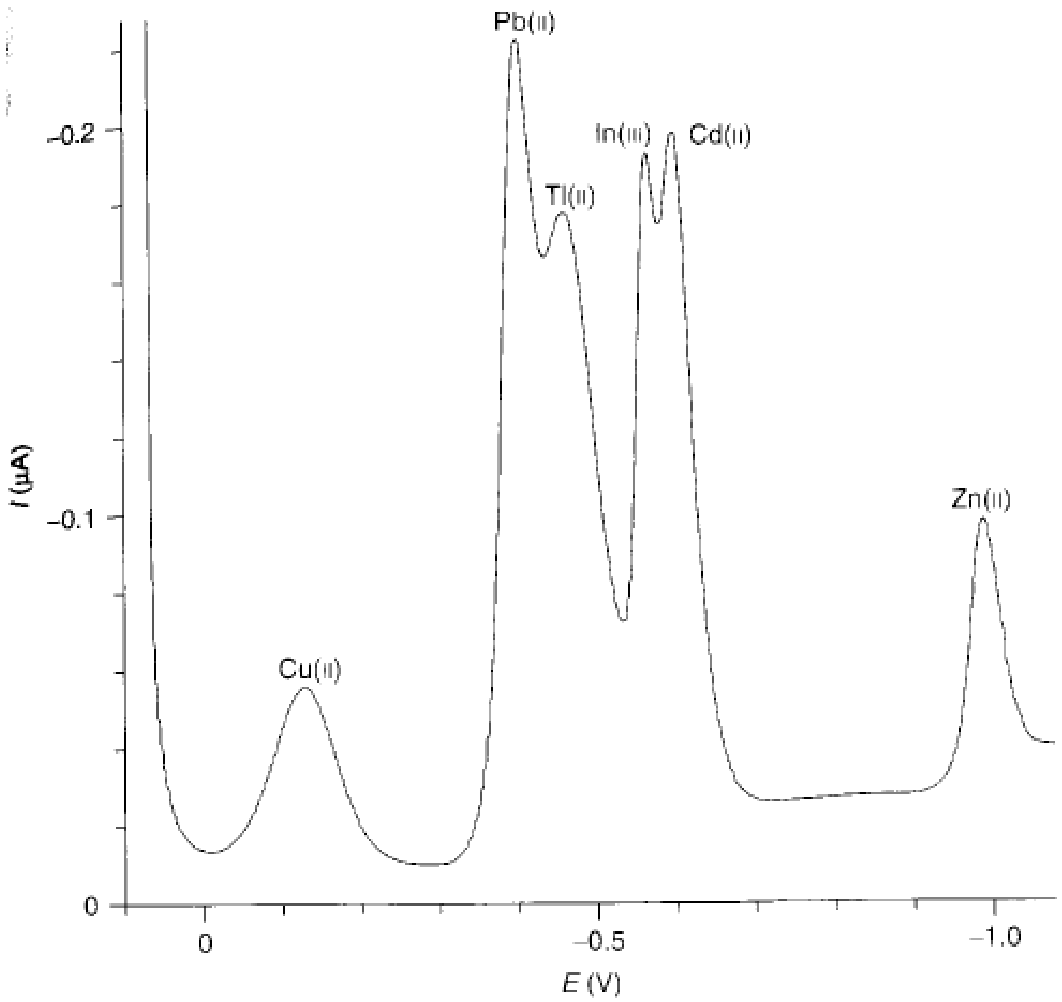
# Electrochemical biosensors II: Amperometric biosensors

## Lecture 2

# Amperometric Sensors: Problem formulation

- amperometric techniques have some selectivity as every RedOx reaction has it’s own characteristic potential



| | $E^0$, V |
|---|---|
| $Cu^{2+} + e \rightarrow Cu^{+}$ | +0.16 |
| $Pb^{2+} + 2e \rightarrow Pb$ | -0.13 |
| $Tl^{2+} + 2e \rightarrow Tl$ | -0.34 |
| $In^{3+} + 3e \rightarrow In$ | -0.34 |
| $Cd^{2+} + 2e \rightarrow Cd$ | -0.40 |
| $Zn^{2+} + 2e \rightarrow Zn^{+}$ | -0.76 |

# Electrode Reactions

- Current:
  - Faradaic current: current associated with Oxidation/Reduction of species of interest

$$\mathrm{A} + e \rightarrow \mathrm{B}$$

  - Capacitive current: charging of double layer

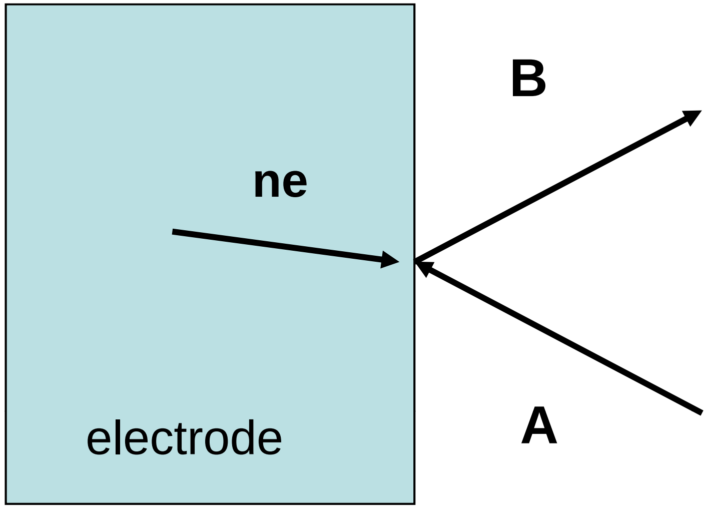
$$\frac{I_C}{A} = C' \frac{dE}{dt}$$

  - Other background currents due to presence of other species e.g. oxygen

# Electrode Reactions

- Faradaic current:

$$\mathrm{A} + ne \rightarrow \mathrm{B}$$

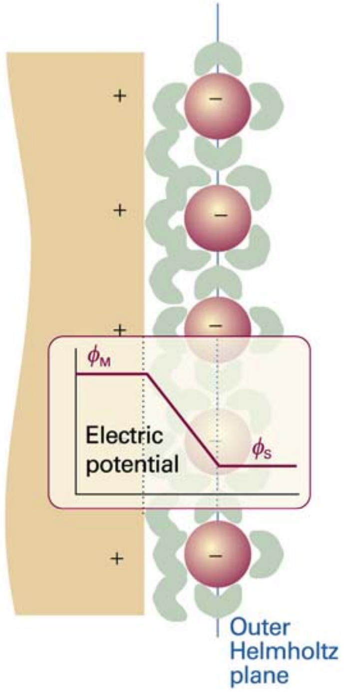


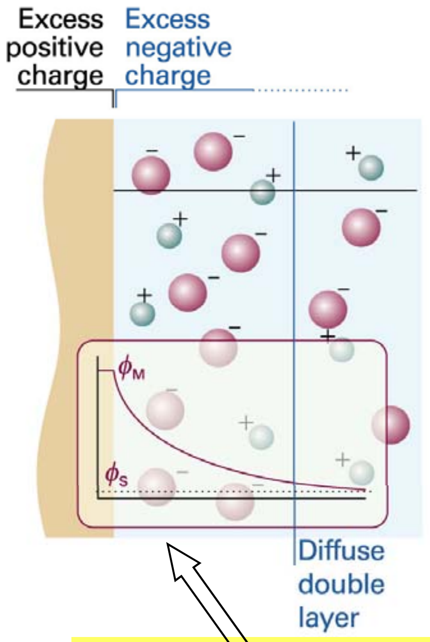
- Possible limiting steps:
  - – electron transfer
  - – mass transport

**rate of arrival of A = 1/n rate of e-transfer = rate of departure**

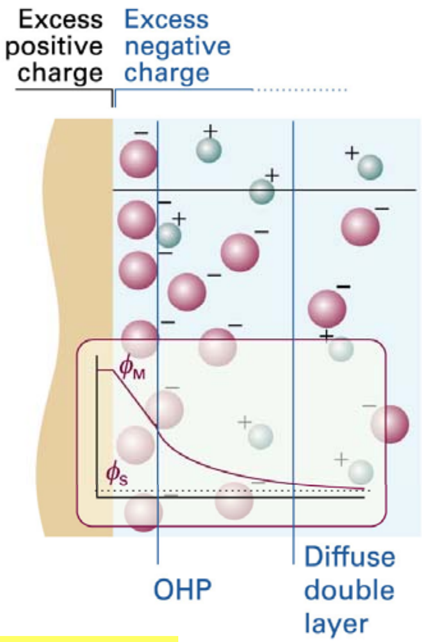
$$-J_A = \frac{1}{n}\frac{I}{AF} = J_B$$

# The electrode-solution interface





Different activity next to electrode!



Helmholtz layer model

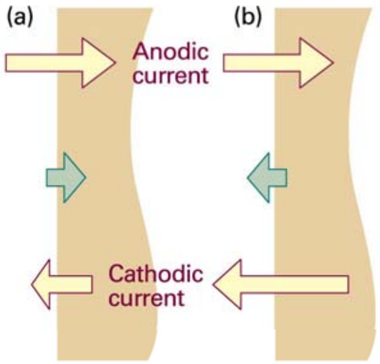
Gouy-Chapman model

Stern model

Graham model = Stern model + IHP

# The rate of charge transfer

$$\mathrm{Ox} + \nu e^{-} \rightarrow \mathrm{Red}$$



- First order reaction

the rate of reduction: $v_{Ox} = k_c[Ox]$ ⇒ $j_c = \nu F k_c[Ox]$

the rate of oxidation: $v_{\mathrm{Red}} = k_a[\mathrm{Red}]$ ⇒ $j_{\mathrm{a}} = \nu F k_a[\mathrm{Red}]$

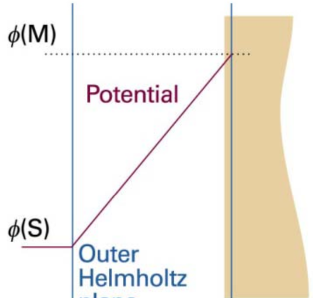
$$j = j_{\mathrm{a}} - j_c = \nu F k_a[\mathrm{Red}] - \nu F k_c[\mathrm{Ox}]$$

- The activation Gibbs energy

both processes involve activation $k = Be^{-\Delta^{*}G/RT}$

$$j = \nu F k_a B_a[\mathrm{Red}]e^{-\Delta^{*}G_a/RT} - \nu F k_c B_a[\mathrm{Ox}]e^{-\Delta^{*}G_c/RT}$$

# The Butler-Volmer equation



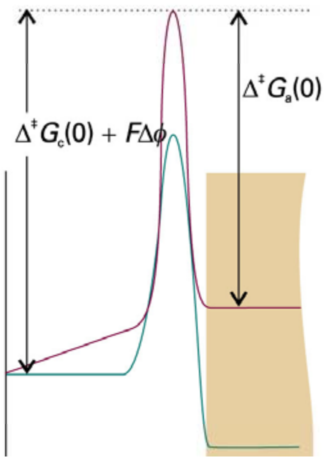
- Reduction reaction $\mathrm{Ox} + \nu e^- \rightarrow \mathrm{Red}$

  transition state is product like: $\Delta^* G_c = \Delta^* G_c(0) + F\Delta\phi$

  transition state is reagent like: $\Delta^* G_c \approx \Delta^* G_c(0)$

$$\Delta^* G_c = \Delta^* G_c(0) + \alpha F\Delta\phi$$

cathodic transfer coefficient
usually approx. 0.5

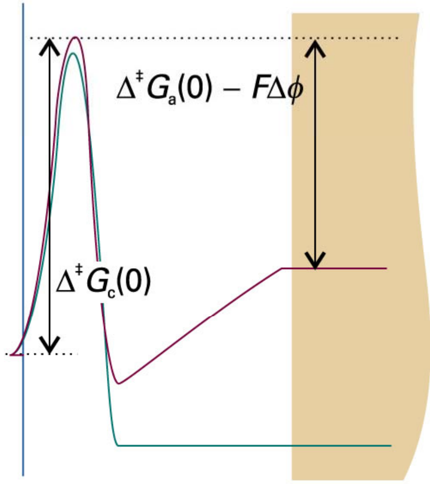


- Oxidation reaction $\mathrm{Red} - \nu e^- \rightarrow \mathrm{Ox}$

  transition state is product like: $\Delta^* G_c = \Delta^* G_c(0) - F\Delta\phi$

  transition state is reagent like: $\Delta^* G_c \approx \Delta^* G_c(0)$

$$\Delta^* G_c = \Delta^* G_c(0) - (1-\alpha) F\Delta\phi$$



# The Butler-Volmer equation

$$j = \nu F k_a B_a \left[\mathrm{Red}\right] e^{-\Delta^* G_a(0)/RT} e^{(1-\alpha)F\Delta\phi/RT} - \nu F k_c B_a \left[\mathrm{Ox}\right] e^{-\Delta^* G_c/RT} e^{-\alpha F\Delta\phi/RT}$$

if the cell is balanced (j=0) by an external source, E:

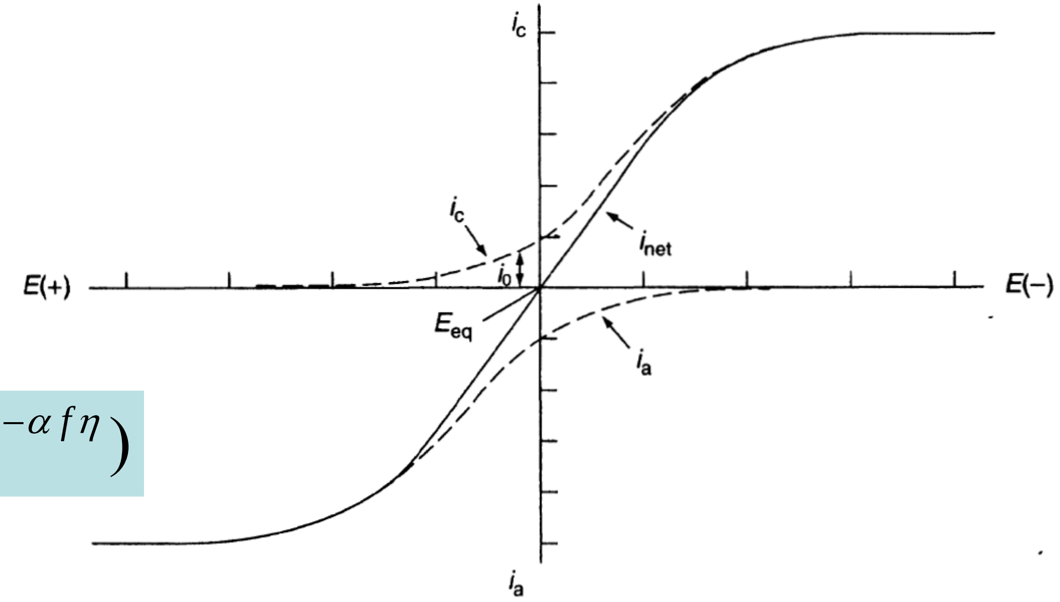
$$j_a = \nu F k_a B_a \left[\mathrm{Red}\right] e^{-\Delta^* G_a(0)/RT} e^{(1-\alpha) fE}$$

$$j_c = \nu F k_c B_a \left[\mathrm{Ox}\right] e^{-\Delta^* G_c/RT} e^{-\alpha fE}$$

$$j_{\mathrm{a}} = j_c = j_0, \quad f = \frac{F}{RT}$$

exchange current density

now, if a voltage is supplied:



**The Butler-Volmer equation**

$$\eta = E' - E$$

$$j = j_0 \left(e^{(1-\alpha) f\eta} - e^{-\alpha f\eta}\right)$$

# The Butler-Volmer equation

$$j = j_0 (e^{(1-\alpha) f \eta} - e^{-\alpha f \eta})$$

- The low overpotential limit $f\eta \ll 1$, in practice $\eta < 0.01\text{V}$

$$j = j_0 (1 + (1-\alpha) f \eta + \ldots - 1 - \alpha f \eta - \ldots) \approx j_0 f \eta$$

$$\eta \approx \frac{j}{j_0 f}$$ Ohm's law

- The high overpotential limit in practice $\eta \geq 0.12\text{V}$

positive overpotential: $j = j_0 e^{(1-\alpha) f \eta}$

negative overpotential: $j = j_0 e^{-\alpha f \eta}$

# Electrode polarizability

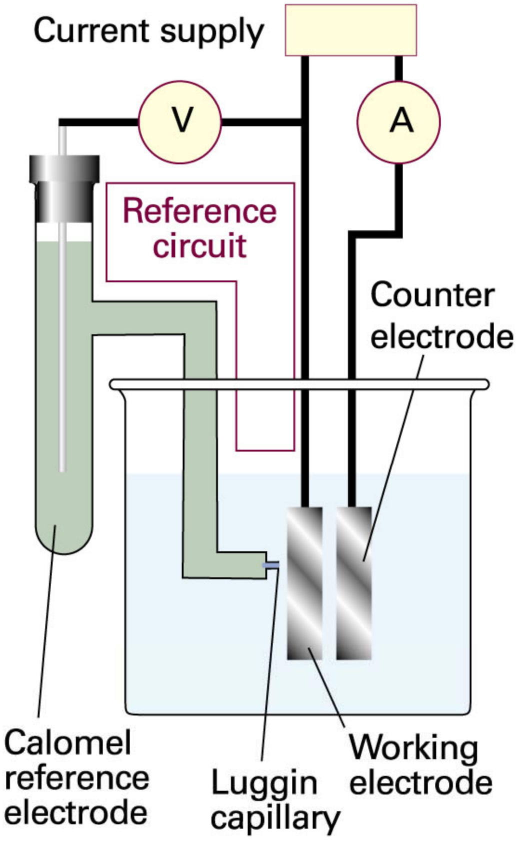
- **non-polarizable** electrodes: potential changes only slightly with current,
  **polarizable** electrodes: potential changes significantly with current
- reference electrodes are highly non-polarizable

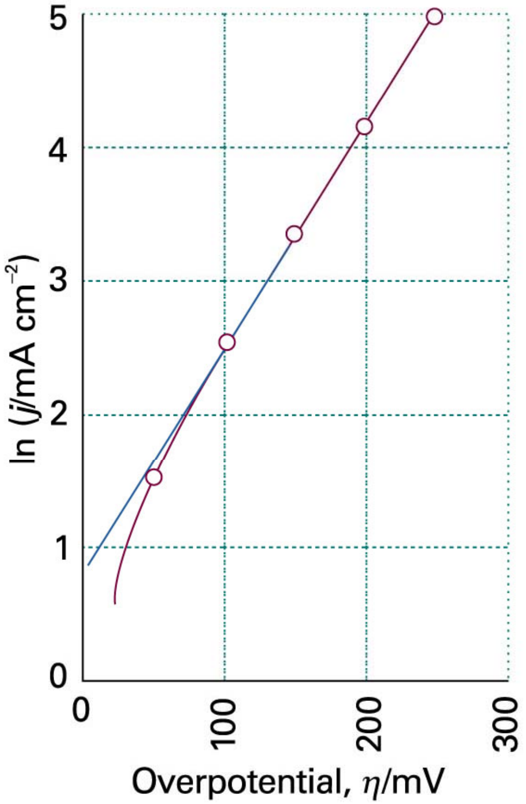
$$\eta \approx \frac{j}{j_0 f}$$

- high exchange current is benefitial for low polarizatbility

# Tafel plot

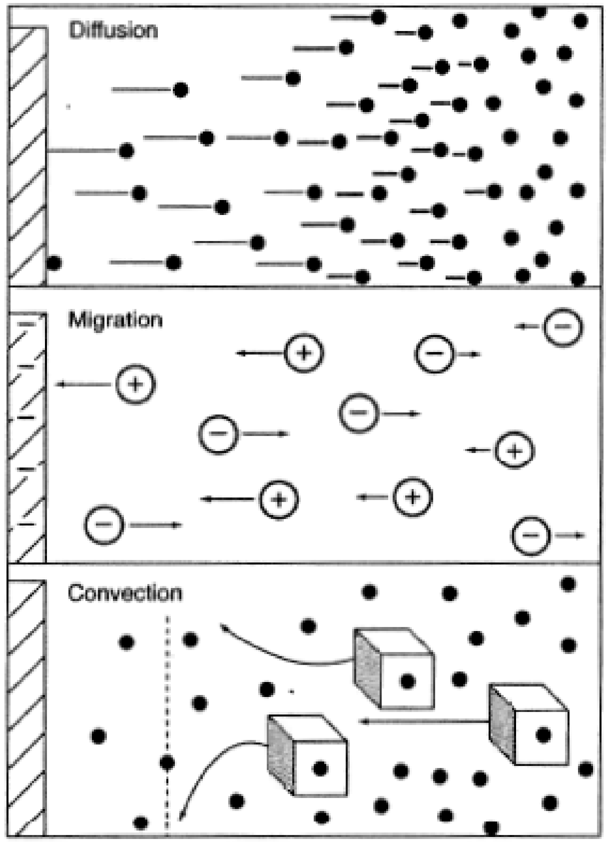
- a plot of ln(j) vs. overpotential is called Tafel plot





# Electrode Reactions

- Mass transport modes:
  - Diffusion: spontaneous movement due to concentration gradient
  - Convection: transport by gross physical movement, e.g. stirring or flowing the solution, or rotating/vibrating the electrode
  - Migration: movement of charged particles



$$J(x,t) = -D\frac{\partial C(x,t)}{\partial x} - \frac{zFDC}{RT}\frac{\partial \phi(x,t)}{\partial x} + C(x,t)V(x,t)$$

# Mass transport mechanisms

- Migration (for ions) in response to a gradient of potential

$$J_m = \sum_i \frac{-z_i F}{RT} D_i [i] \frac{\partial \varphi}{\partial x}$$

- Diffusion in response to a concentration gradient

$$J_d = -D_A \frac{\partial [A]}{\partial x}$$

- Convection in response to pressure gradient

$$J_c = [A] v$$

# Concentration polarization

- **concentration polarization** - phenomena related to consumption of the reactive species on the electrode

at zero current:

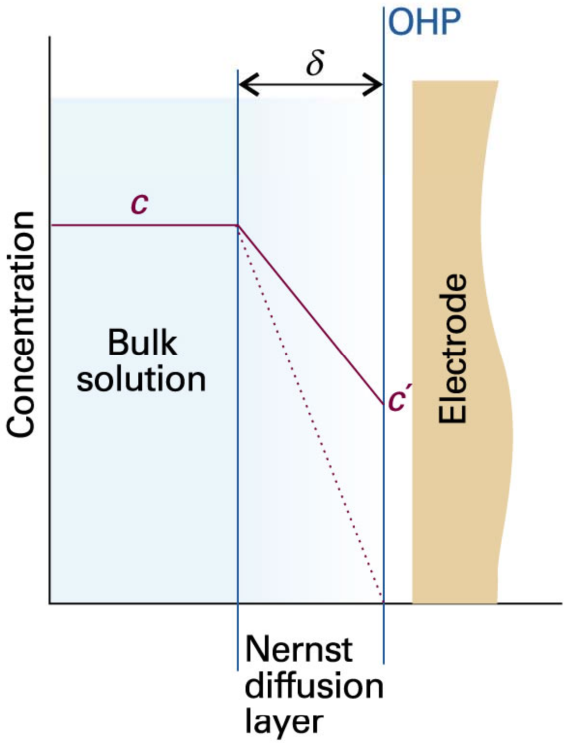
$$E = E^{\theta} + \frac{RT}{zF}\ln a = \boxed{E^{\theta} + \frac{RT}{zF}\ln\gamma} + \frac{RT}{zF}\ln c$$

$$E = E^{0} + \frac{RT}{zF}\ln c$$

with current:

$$E' = E^{0} + \frac{RT}{zF}\ln c'$$

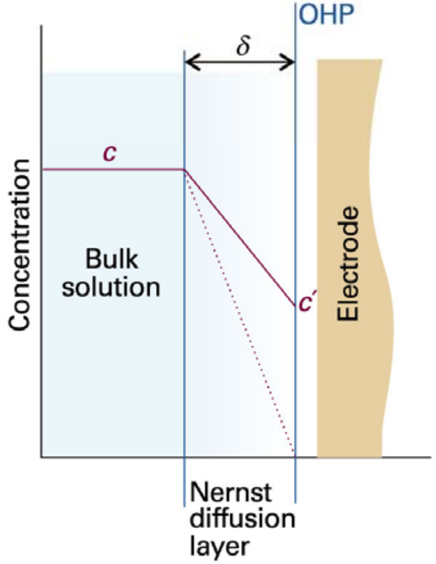
$$\eta^{c} = E' - E = \frac{RT}{zF}\ln\left(\frac{c'}{c}\right)$$



# Concentration polarization

$$\eta^c = E' - E = \frac{RT}{zF}\ln\left(\frac{c'}{c}\right)$$

- Mass transport through the Nernst layer:



First Fick's law: $J = -D\left(\frac{\partial c}{\partial x}\right) = D\frac{c - c'}{\delta}$

$$j = zFJ = zFD\frac{c - c'}{\delta}$$

**limiting current density** $j_{\lim} = zFD\frac{c}{\delta} = \frac{cRT\lambda}{zF\delta}$

using Nernst-Einstein equation: $D = \frac{RT\lambda}{z^2F^2}$

λ – ionic conductivity

conc. overpotential vs current:

$$\eta^c = \frac{RT}{zF}\ln\left(1 - \frac{j\delta}{zcFD}\right)$$

# Potential step experiment

$$\mathrm{O} + ne \rightarrow R$$

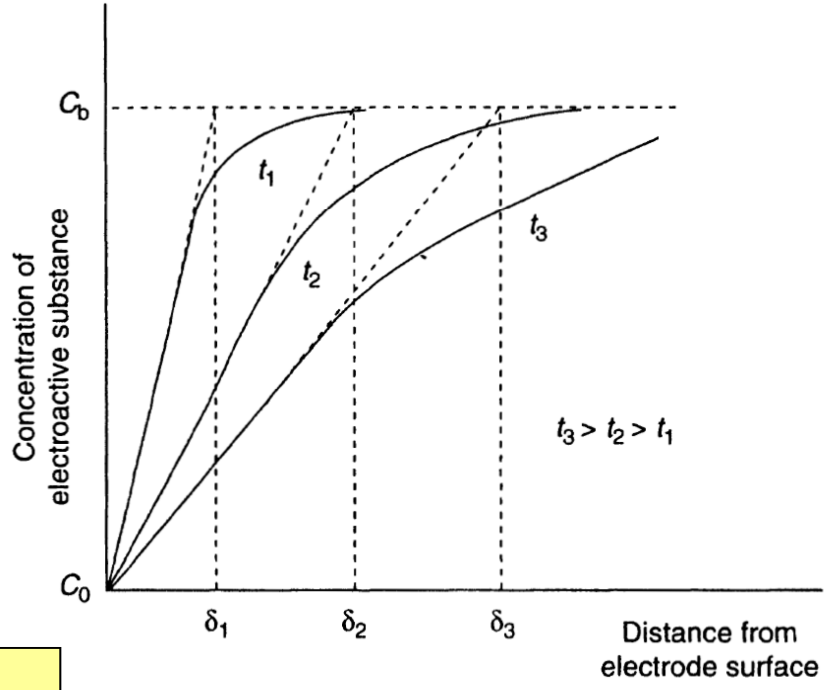
- Experiment: potential is increased stepwise to some value, only O is initially present.

- in a planar geometry:

$$C_O(x,t) = C_O(b)\left[1 - erf\left(\frac{x}{\sqrt{4D_O t}}\right)\right]$$

$$\frac{\partial C}{\partial x} = \frac{C_O(b)}{\sqrt{4D_O t}}$$

$$J(t) = -D\frac{\partial C}{\partial x} \Rightarrow \boxed{i(t) = nFAD_O\frac{C_O(b)}{\sqrt{4D_O t}}}$$



Cottrell equation

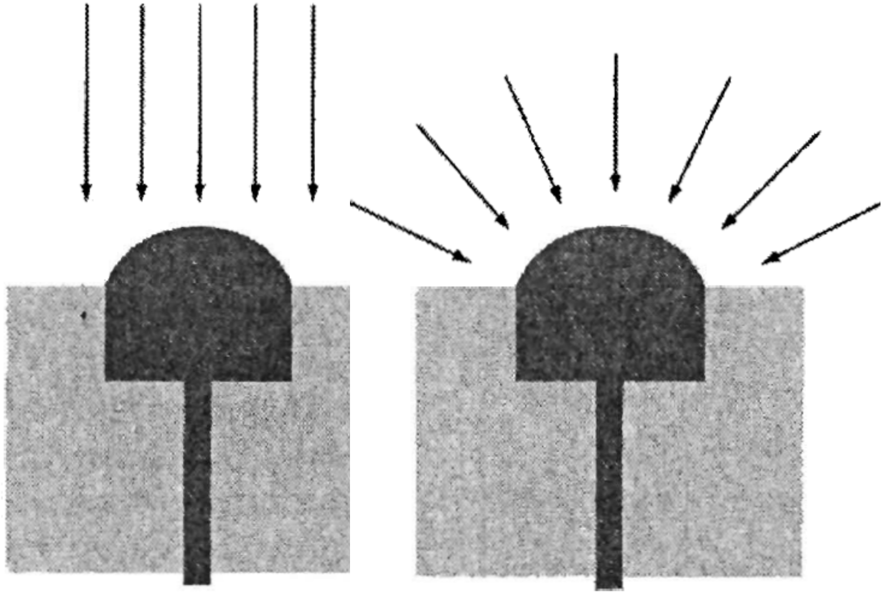
# Potential step experiment

- At a spherical electrode the situation is different as the diffusion equation will have another term:

$$\frac{\partial C(x,t)}{\partial t} = D\left[\frac{\partial^2 C}{\partial r^2} + \frac{2}{r}\frac{\partial C}{\partial r}\right]$$

$$i(t) = nFAD_O \frac{C_O(b)}{\sqrt{4D_O t}} + nFAD_O \frac{C_O(b)}{r}$$

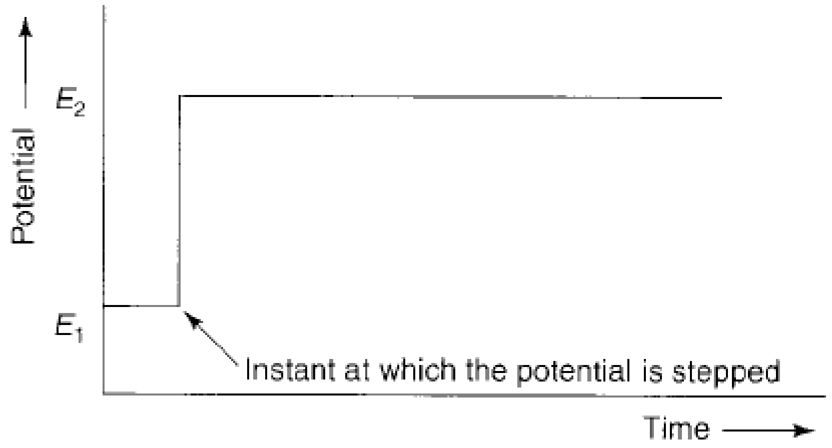
Time independent term

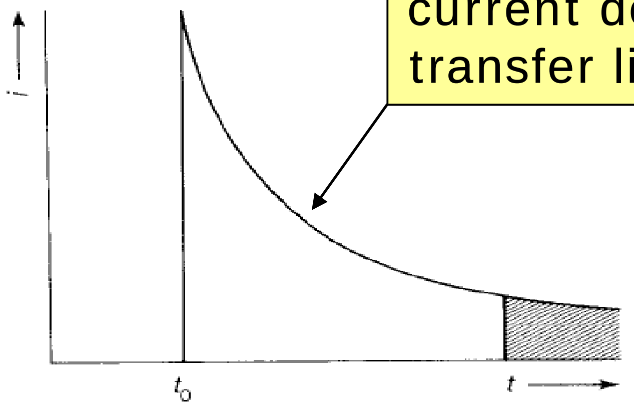


- This leads to unique transport properties of microelectrodes (due to their small radius)

# Chronoamperometry

- The potential is stepped to $E_2 > E_p$, current is monitored as a function of time





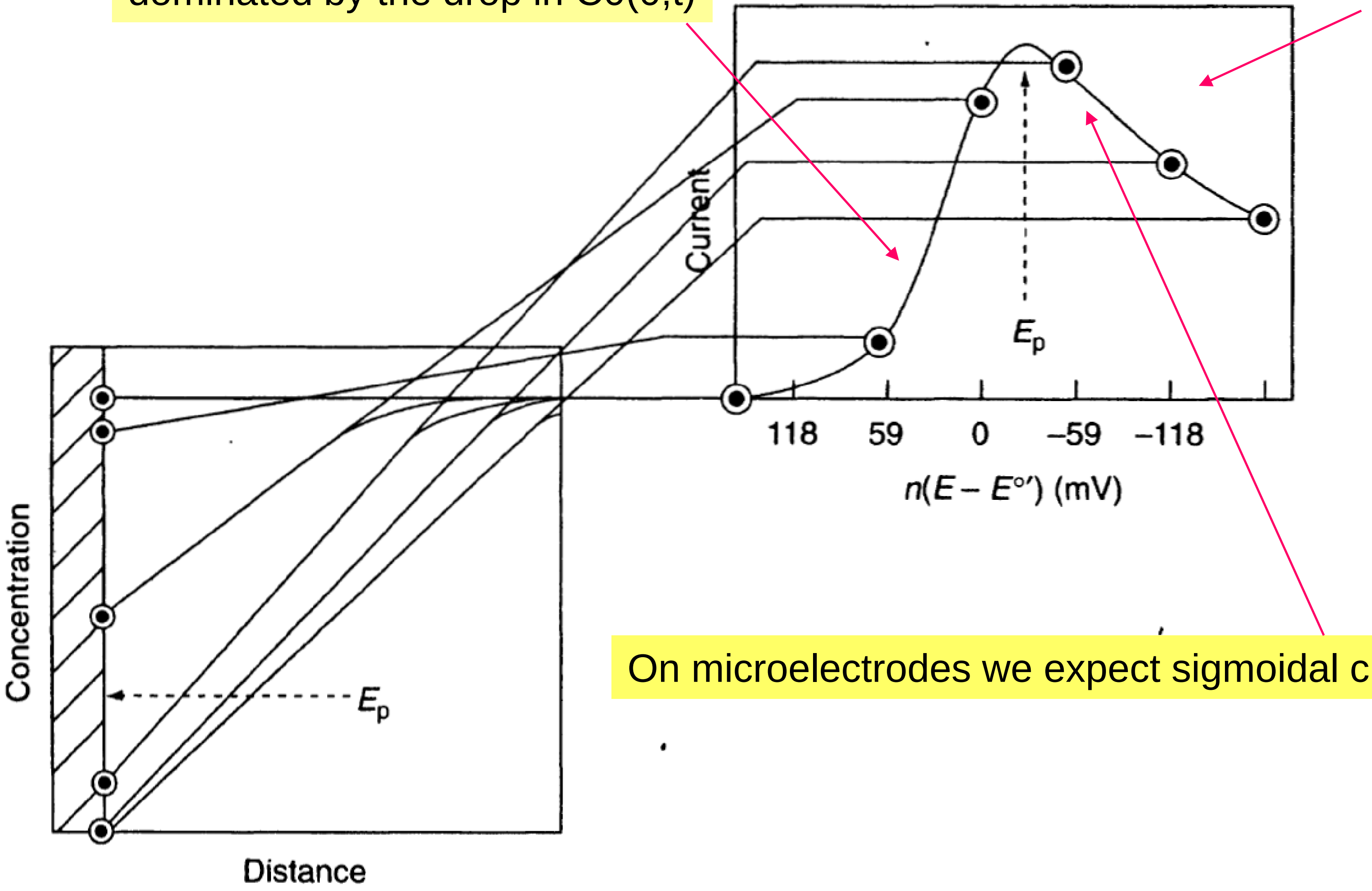
current decay due to mass transfer limitation

$$i_d = \frac{nFADC_{Ox}}{\pi^{1/2}t^{1/2}}$$

limiting value:

$$i = \frac{nFAD^{1/2}C_{ox}}{\delta}$$

# Potential sweep experiments



Current raise,
dominated by the drop in C0(0,t)

Current drop,
dominated by the increase in d.

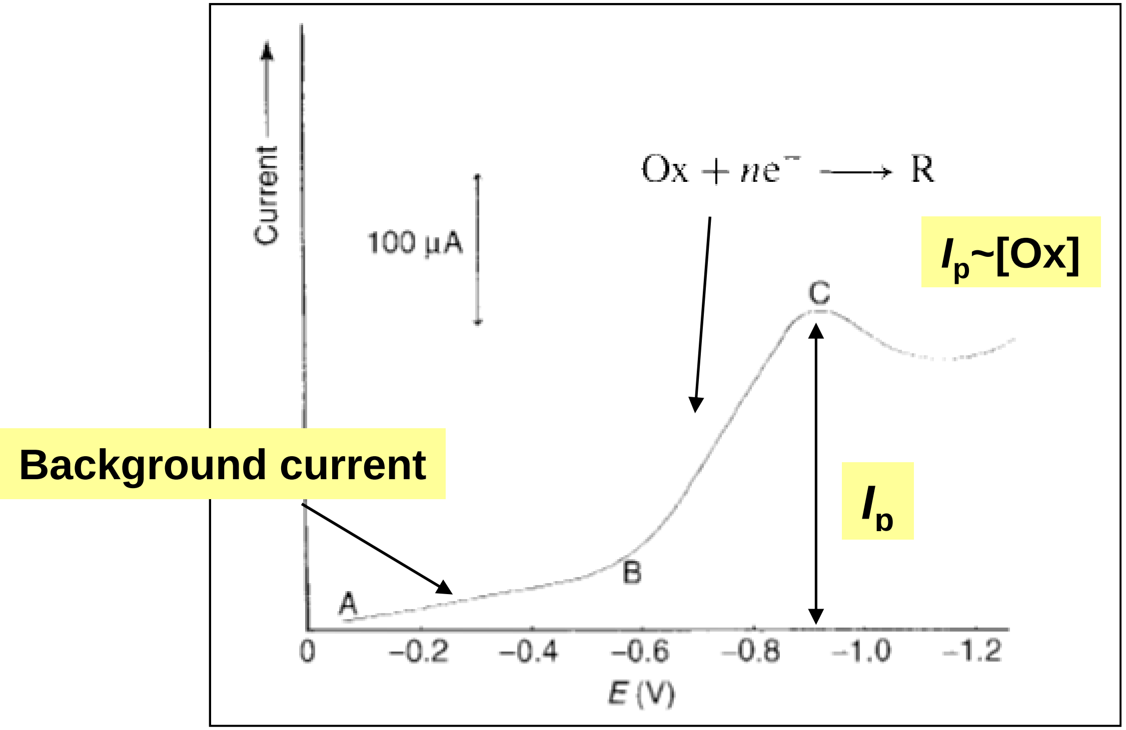
On microelectrodes we expect sigmoidal chape

# Potential sweep experiment

- In the case of stirring, the distance d is maintained;
- The voltammogram will be sigmoidal in the case of stirring
- In aqueous solution distance d is typically 10-50µm for electrode rotation and 100-150 µm for solution stirring

# Linear Sweep Voltammetry (LSW)

- Linearly varied potential is applied between working electrode and reference electrode while current is monitored.



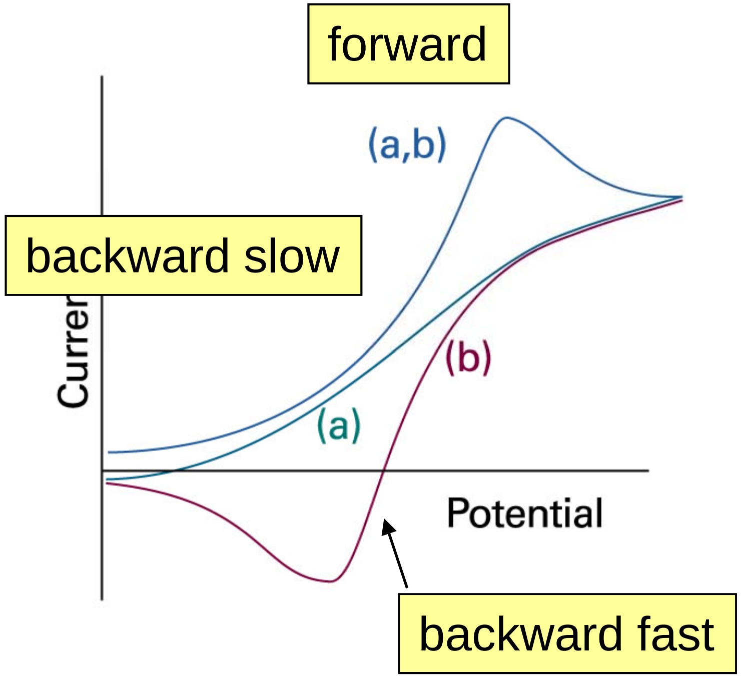
$$E_p = E^0 + 0.056/n$$

# Kinetic and Catalytic Effects

- usually, there is another chemical reaction coupled to the electron transfer
  - **consumption of reduced product**

$$\mathrm{Ox} + ne^- = \mathrm{R}$$

$$\mathrm{R} + \mathrm{A} \xrightarrow{k} \mathrm{B}$$



forward

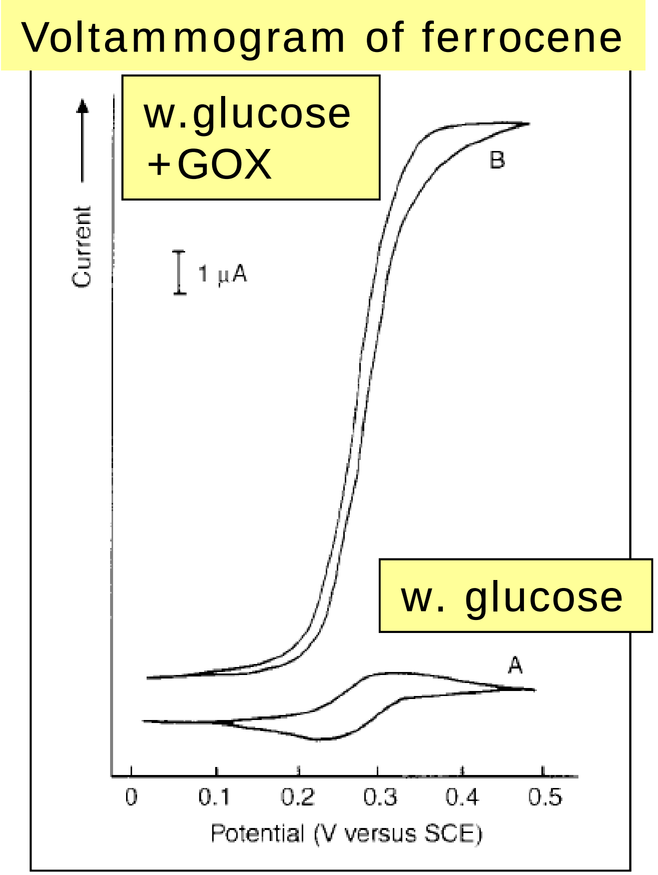
backward slow

backward fast

- **regeneration of the oxidized reagent**

$$\mathrm{Ox} + ne^- = \mathrm{R}$$

$$\mathrm{R} + \mathrm{A} \xrightarrow{k} \mathrm{Ox} + \mathrm{B}$$



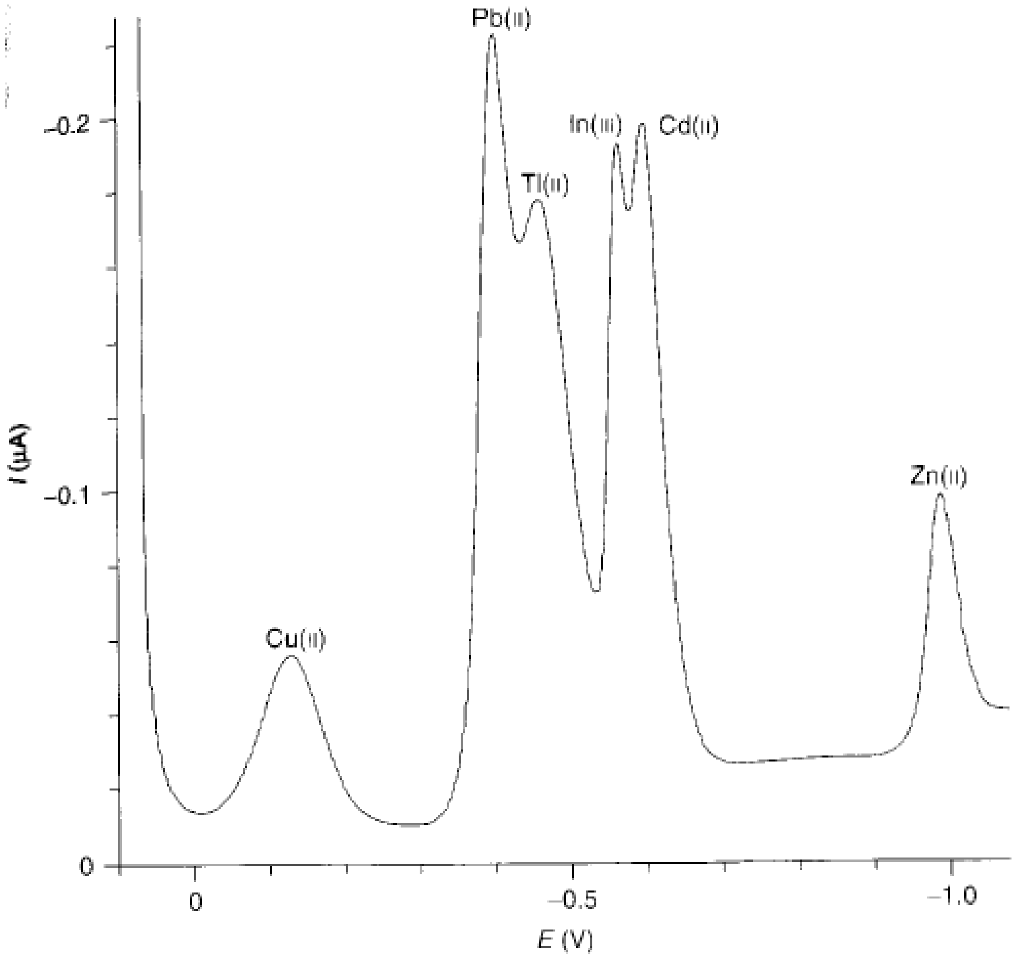
Voltammogram of ferrocene

w.glucose +GOX

w. glucose

# Amperometric Sensors

- amperometric techniques have some selectivity as every RedOx reaction has it’s own characteristic potential
- however the selectivity is limited unless modified electrodes are used

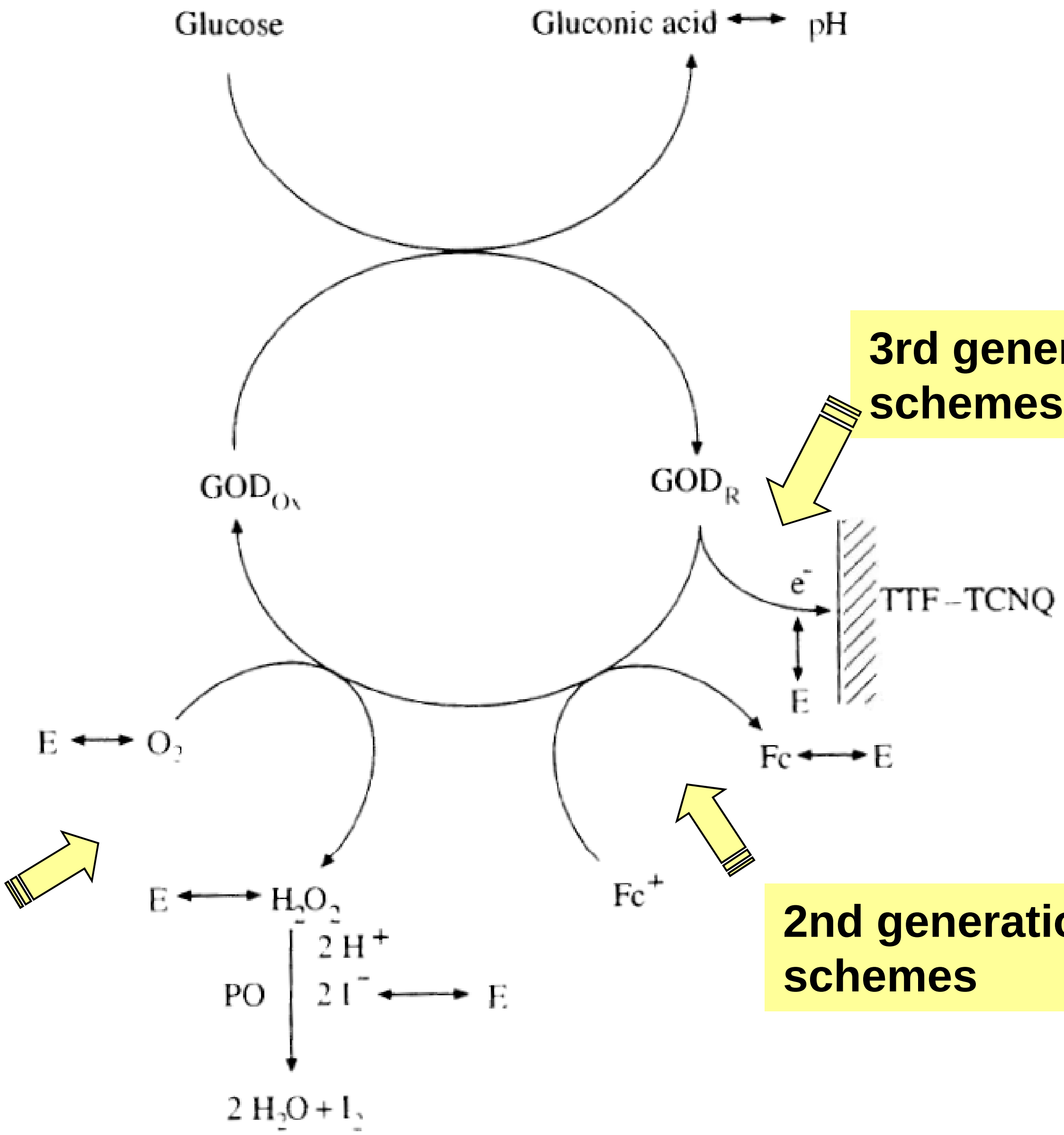


Differential pulse polarogram for a mixture of six cations

# Amperometric Biosensors

- First Generation – oxygen electrode based sensors
- Second Generation – mediator based sensors
- Third Generation – directly coupled enzyme electrodes

# Possible glucose detection schemes



**3rd generation schemes**

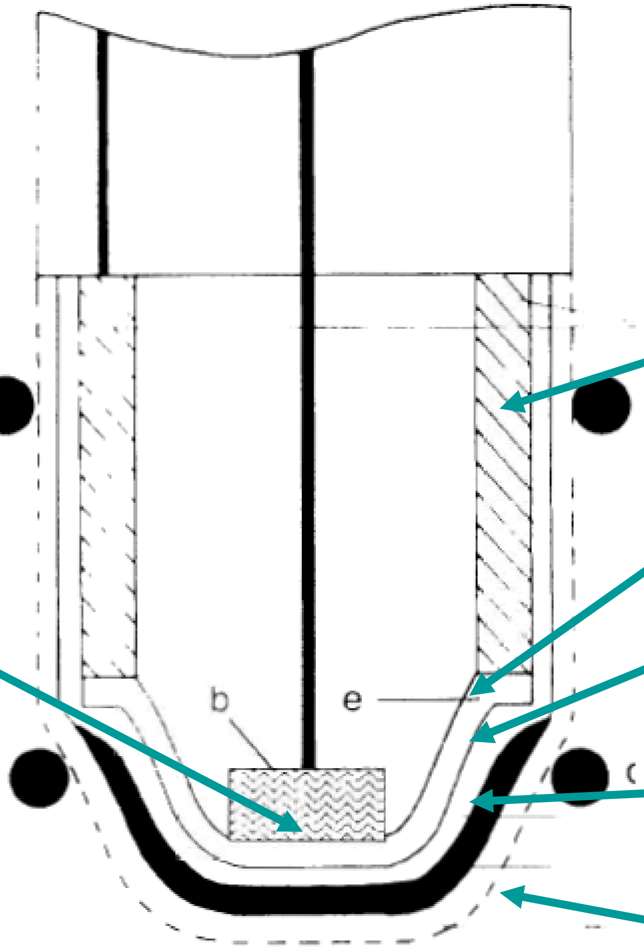
**1st generation schemes**

**2nd generation schemes**

# oxygen electrode based sensors

$$\text{glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconic acid} + H_2O_2$$

Clark's electrode



O-rings

Pt catode

Ag anode

Electrolyte gel

Teflon membrane

Glucose oxidase on nylon net

Cellophane membrane

| Analyte | Enzyme | Response time (min) | Stability (days) |
|---|---|---|---|
| Glucose | Glucose oxidase | 2 | >30 |
| Cholesterol | Cholesterol oxidase | 3 | 7 |
| Monoamines | Monoamine oxidase | 4 | 14 |
| Oxalate | Oxalate oxidase | 4 | 60 |
| Lactate | Lactate oxidase | — | — |
| Formaldehyde | Aldehyde oxidase | — | — |
| Ethanol | Alcohol oxidase | — | — |
| Glycollate | Glycollate oxidase | — | — |
| NADH | NADH oxidase | — | — |

Measuring oxygen:

$O_2 + e^- \longrightarrow O_2^-$ **E=-0.7V**

Problems: fairly high potential (interference is probable), oxygen needs to be controlled and replenished (e.g. By oxygen generating reaction or by pumping oxygen containing buffer)

Measuring hydrogen peroxide:

$H_2O_2 \longrightarrow 2H^+ + 2e^- + O_2$ **E=+0.65V**

Problem: still fairly high potential (interference from e.g. ascorbic asid)

# Mediator Based Sensors

- Oxygen is substituted with another oxidizing agent (electron transfer agent)
- Iron ions or complexes are most common mediators

$$\mathrm{Fe(III)} + e^- \longrightarrow \mathrm{Fe(II)}$$

**Fc**

**Free Fe3+ are subject to hydrolysis and precipitation**

$$\begin{tikzcd} \mathrm{Fe^{3+}}_{aq} + e^- \arrow[r, shift left] \arrow[d, "H_2O"] & \mathrm{Fe^{2+}}_{aq} \arrow[l, shift left] \arrow[d, "H_2O"] & (E^0 = +0.53\ \mathrm{V}) \\ \mathrm{Fe(OH)_3} + 3H^+ & \mathrm{Fe(OH)_2} + 2H^+ \text{(hydrolysis)} & \end{tikzcd}$$

$$[\mathrm{Fe^{III}(CN)_6}]^{3-} + e^- \rightleftharpoons [\mathrm{Fe^{II}(CN)_6}]^{4-} \quad (E^0 = +0.45\ \mathrm{V})$$

$$[\mathrm{Fe^{III}(Cp)_2}]^+ + e^- \rightleftharpoons \mathrm{Fe^{II}(Cp)_2} \quad (E^0 = +0.165\ \mathrm{V};$$

ferrocene

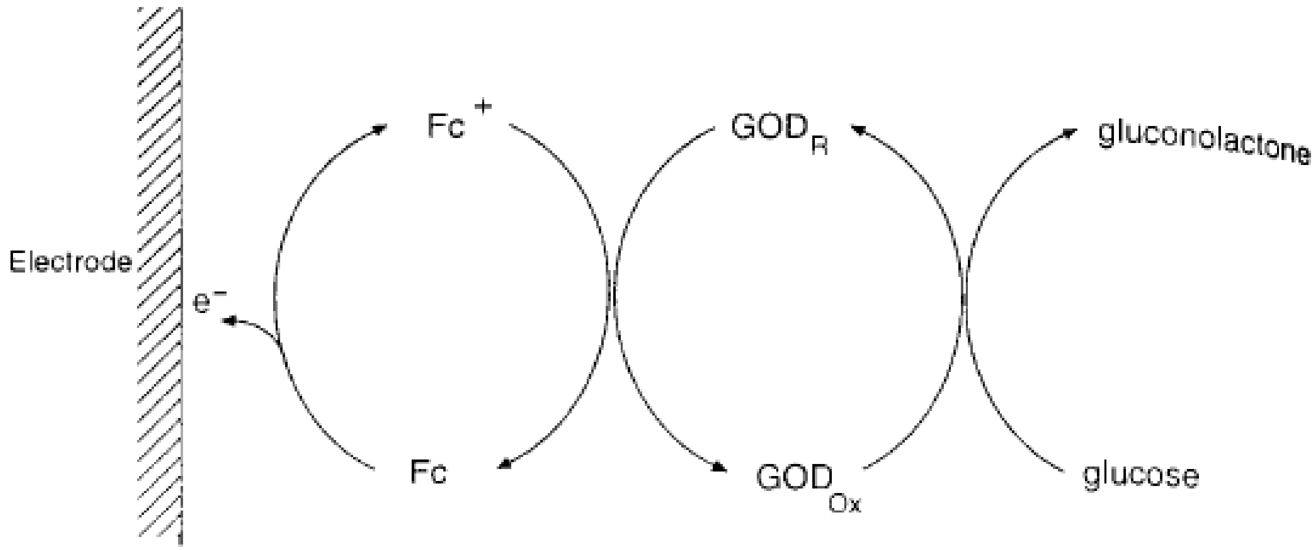
$$E_p(\mathrm{Ox}) = +0.193\ \mathrm{V};$$

$$E_p(\mathrm{R}) = +0.137\ \mathrm{V})$$

$$\text{glucose} + \text{GOD}_{\text{Ox}} \longrightarrow \text{gluconolactone} + \text{GOD}_{\text{R}} + 2\text{H}^+$$

$$\text{GOD}_{\text{R}} + 2\text{Fc}^+ \longrightarrow \text{GOD}_{\text{Ox}} + 2\text{Fc}$$

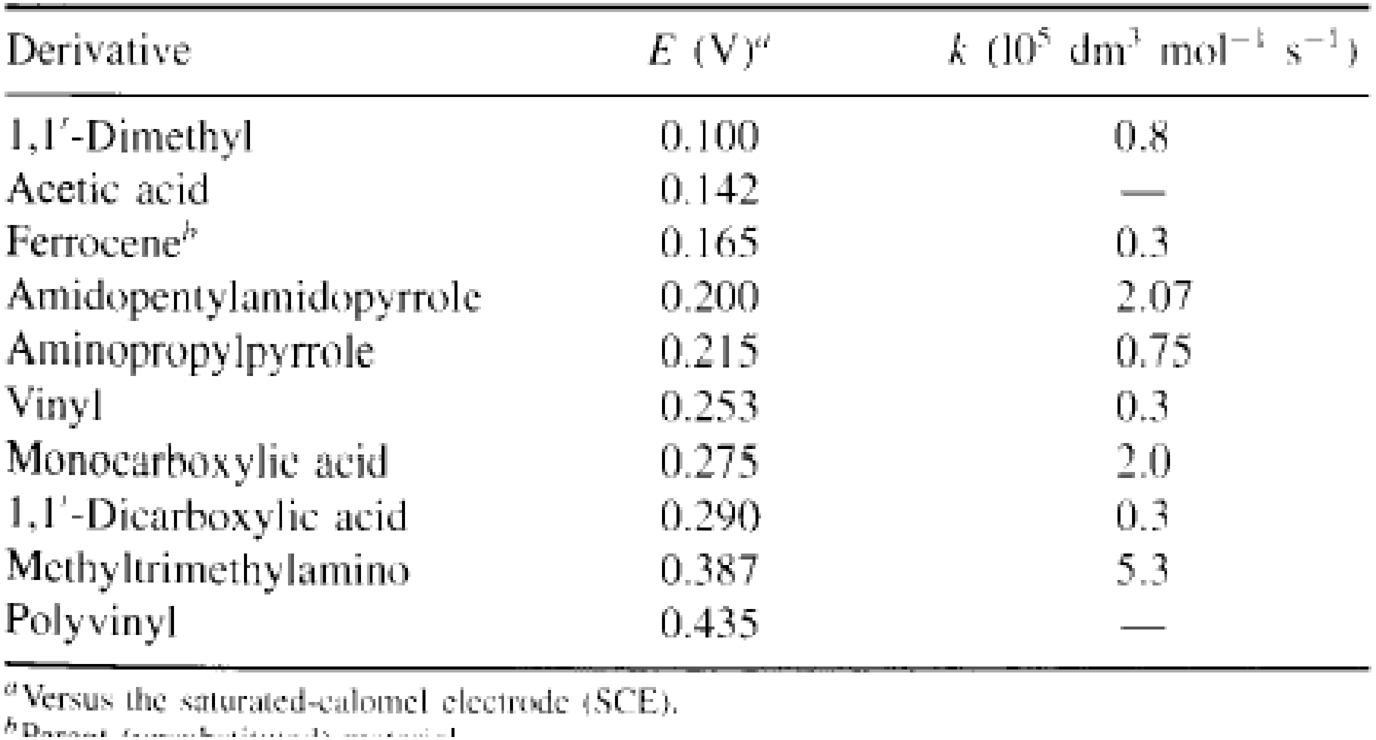
$$2\text{Fc} - 2\text{e}^- \longrightarrow 2\text{Fc}^+$$



# Good Mediator

- Rapid reaction with enzyme
- Fast electron transfer kinetics
- Low overpotential
- Independent of pH
- Stable in Ox and R forms
- Doesn’t react with oxygen
- Non toxic

# Fc derivatives

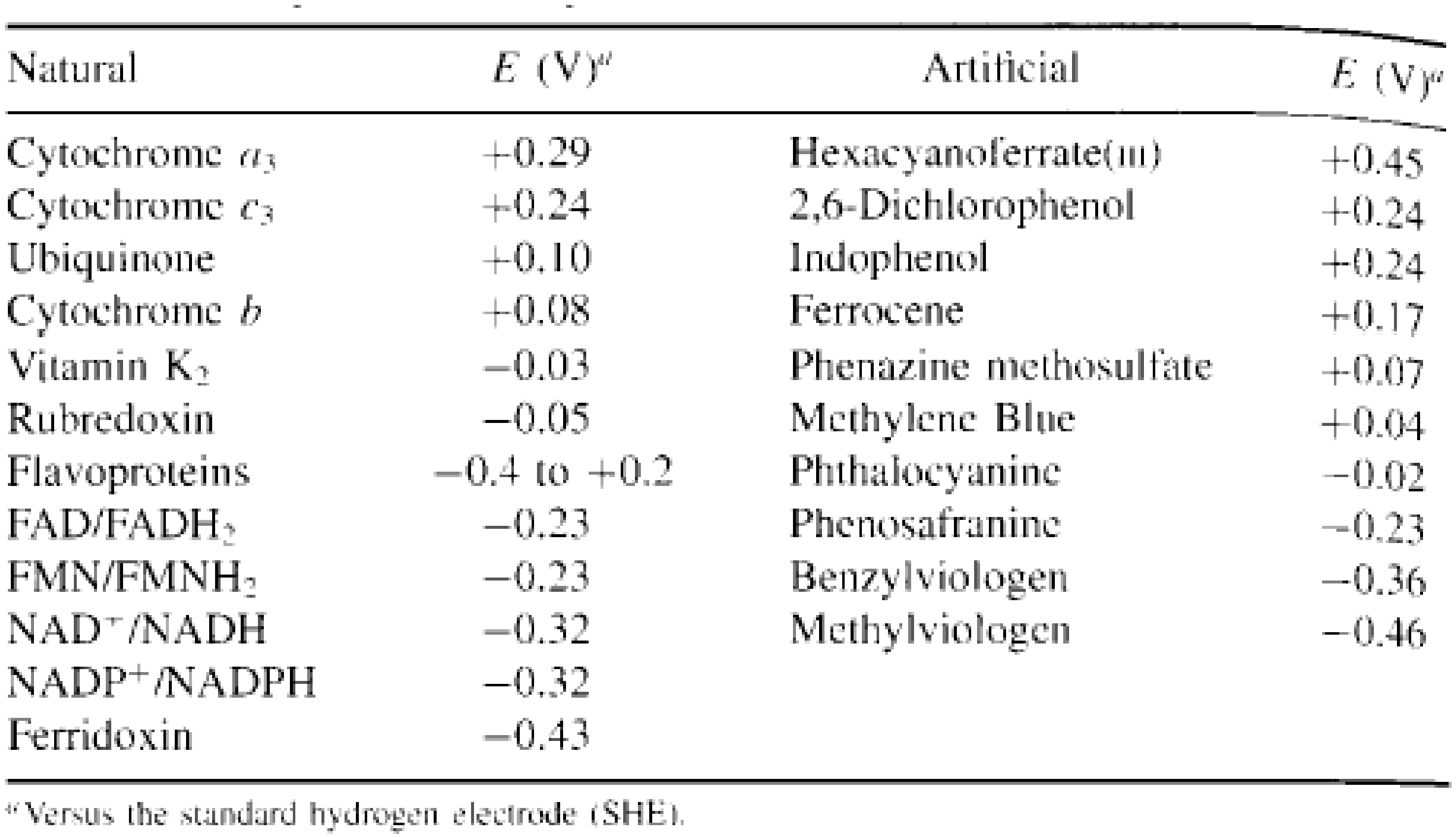


| Derivative | $E$ (V)[a] | $k$ ($10^5$ dm$^3$ mol$^{-1}$ s$^{-1}$) |
|---|---|---|
| 1,1′-Dimethyl | 0.100 | 0.8 |
| Acetic acid | 0.142 | — |
| Ferrocene[b] | 0.165 | 0.3 |
| Amidopentylamidopyrrole | 0.200 | 2.07 |
| Aminopropylpyrrole | 0.215 | 0.75 |
| Vinyl | 0.253 | 0.3 |
| Monocarboxylic acid | 0.275 | 2.0 |
| 1,1′-Dicarboxylic acid | 0.290 | 0.3 |
| Methyltrimethylamino | 0.387 | 5.3 |
| Polyvinyl | 0.435 | — |

[a] Versus the saturated-calomel electrode (SCE).

[b] [illegible]

# Various mediators (natural and artificial)



| Natural | $E$ (V)[a] | Artificial | $E$ (V)[a] |
|---|---|---|---|
| Cytochrome $a_3$ | +0.29 | Hexacyanoferrate(III) | +0.45 |
| Cytochrome $c_3$ | +0.24 | 2,6-Dichlorophenol | +0.24 |
| Ubiquinone | +0.10 | Indophenol | +0.24 |
| Cytochrome $b$ | +0.08 | Ferrocene | +0.17 |
| Vitamin $K_2$ | −0.03 | Phenazine methosulfate | +0.07 |
| Rubredoxin | −0.05 | Methylene Blue | +0.04 |
| Flavoproteins | −0.4 to +0.2 | Phthalocyanine | −0.02 |
| FAD/$FADH_2$ | −0.23 | Phenosafranine | −0.23 |
| FMN/$FMNH_2$ | −0.23 | Benzylviologen | −0.36 |
| $NAD^+$/NADH | −0.32 | Methylviologen | −0.46 |
| $NADP^+$/NADPH | −0.32 | | |
| Ferridoxin | −0.43 | | |

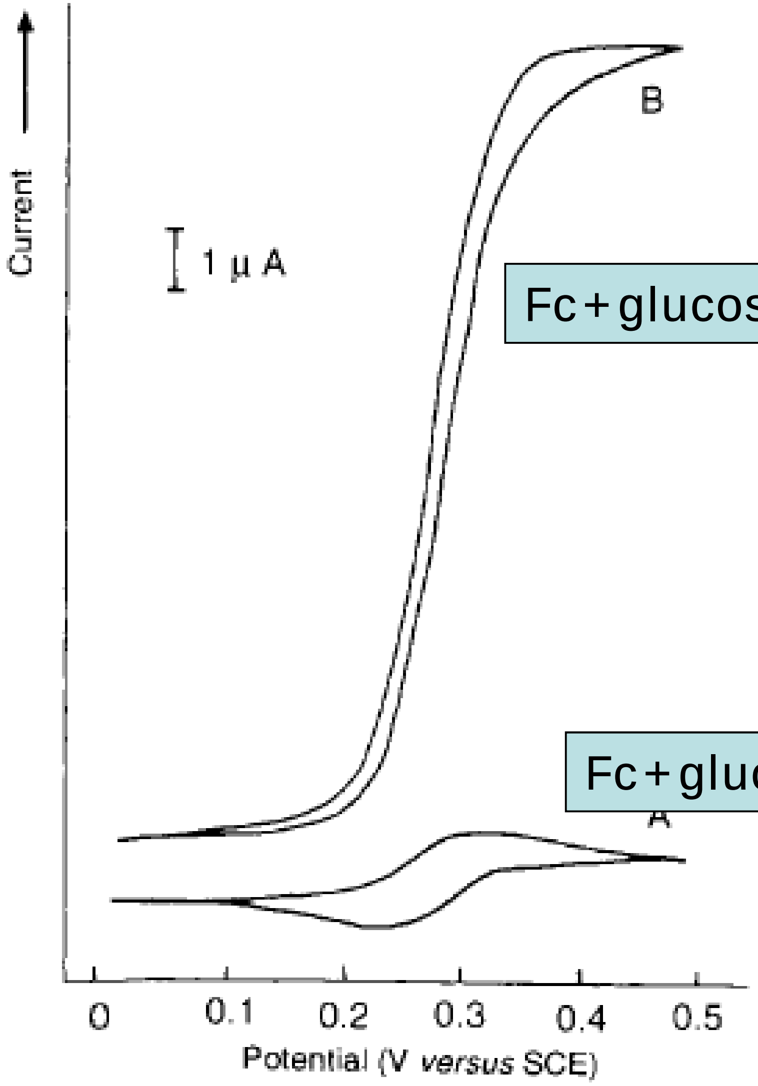
[a] Versus the standard hydrogen electrode (SHE).

# How it works...

$$R \rightleftharpoons Ox + e^-$$

$$E_R + Ox \xrightarrow{k_1} E_{Ox} + R$$

$$E_{Ox} + \text{glucose} \xrightarrow{k_2} E_{red} + \text{gluconolactone}$$



Fc+glucose+GOD

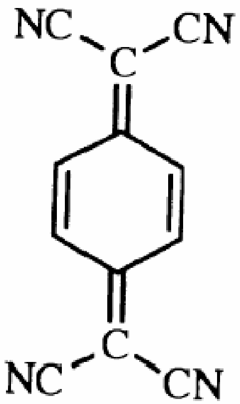
Fc+glucose

In real biosensors both GOD and Fc are immobilised

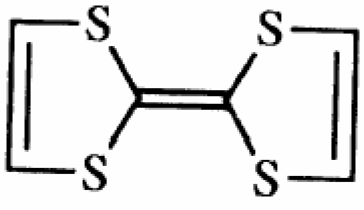
# Directly Coupled Enzyme

- Generally, the enzyme might denature on the electrode surface;
- electron transfer reaction might be slow
- Thus, the surface has to be modified...

- Enzymes can be directly wired to the electrode using organic conducting salts (e.g.TTF/TCNQ) or redox polymers
- Enzymes can be modified to facilitat electron transfer and attachement

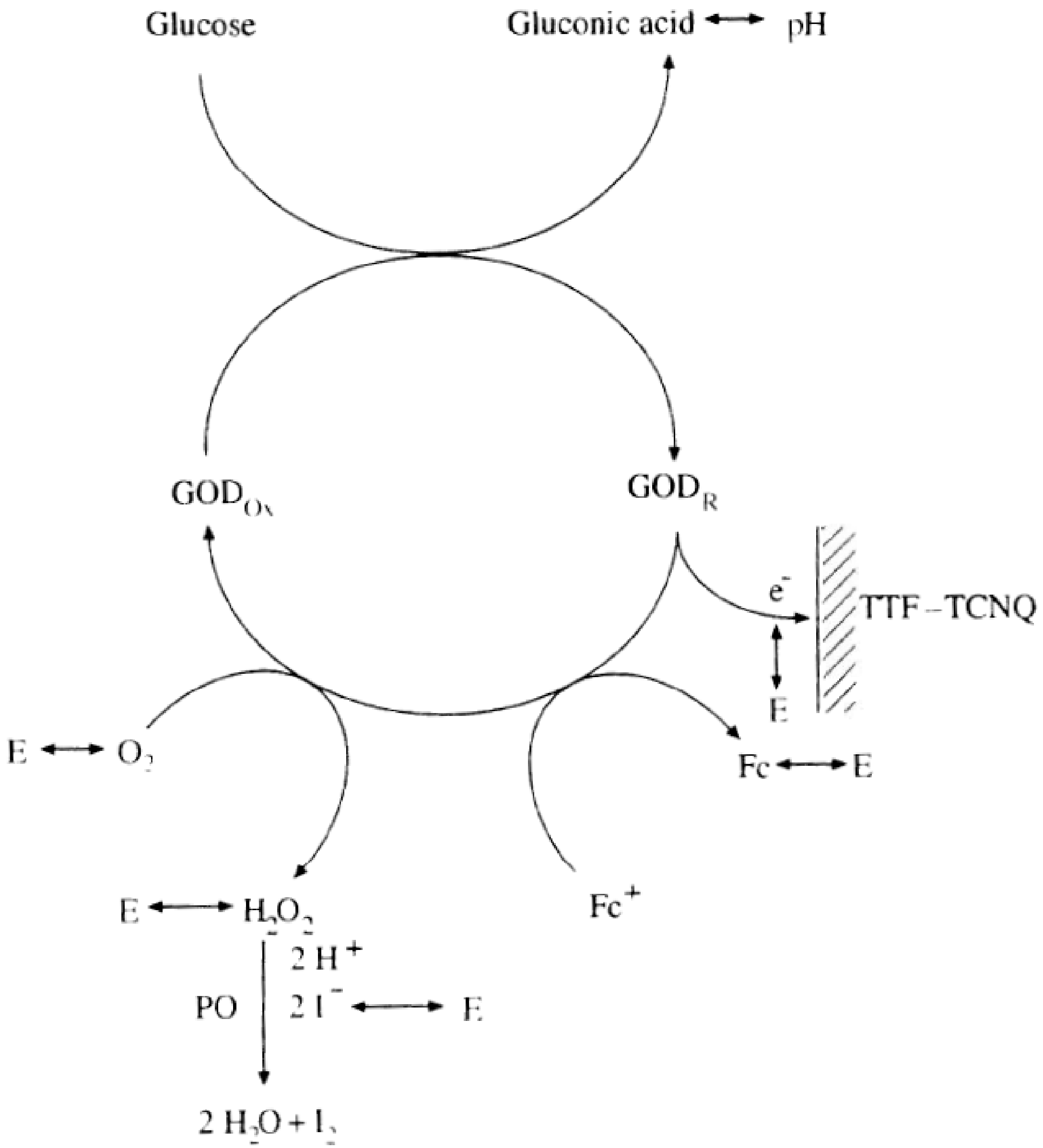


TCNQ



TTF

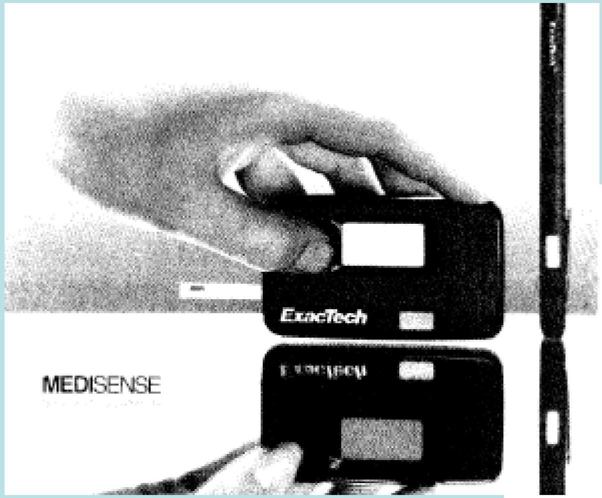
# Possible glucose detection schemes

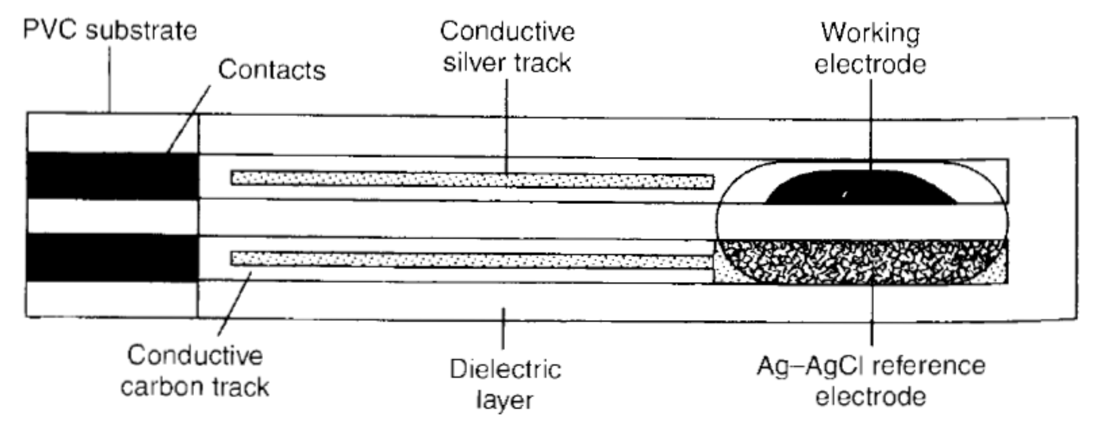


# Design example: Glucose sensor

- Aim: for use by patient at home (should be simple, reliable and cheap)
- Performance: blood glucose range 1.1-33.3 mM; precision 3-8%; test time 30s; life time 6 month.
- Selective element: Glucose Oxidase – inexpensive, stable over long period
- Transducer: Amperometric (GOD+Fc) – cheap, reliable, easy read-out with LCD.
- Immobilisation: covalent bonding for long life (graphite foil coated with Fc, GOD immobilised)

# ExacTech Glucose Sensor





# Problems

- **Atkins 25.16a**. The transfer coefficient of a certain electrode in contact with $M^{3+}$ and $M^{4+}$ in aqueous solution at 25°C is 0.39. The current density is found to be 55.0 $mA\cdot cm^{-2}$ when the overvoltage is 125 mV. What is the overvoltage required for a current density of 75 $mA\cdot cm^{-2}$ ?
- **Atkins 25.20a** Estimate the limiting current density at an electrode in which the concentration of $Ag^+$ ions is 2.5 mmol $dm^{-3}$ at 25°C. The thickness of the Nernst diffusion layer is 0.40 mm. The ionic conductivity of $Ag^+$ at infinite dilution and 25°C is 6.19 mS $m^2$ $mol^{-1}$.
- **Atkins 25.26a** What is the effective resistance at 25°C of an electrode interface when the overpotential is small? Evaluate it for 1.0 $cm^2$ (a) $Pt,H_2|H^+$ ,($j_0$=7.9x$10^{-4}$ A/$cm^2$), (b) $Hg,H_2|H^+$ ($j_0$=7.9x$10^{-13}$ A/$cm^2$) electrodes.