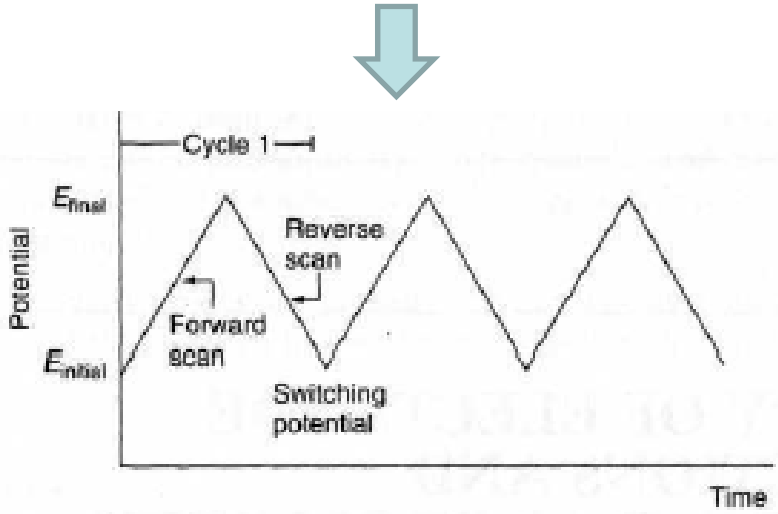
Lecture 3

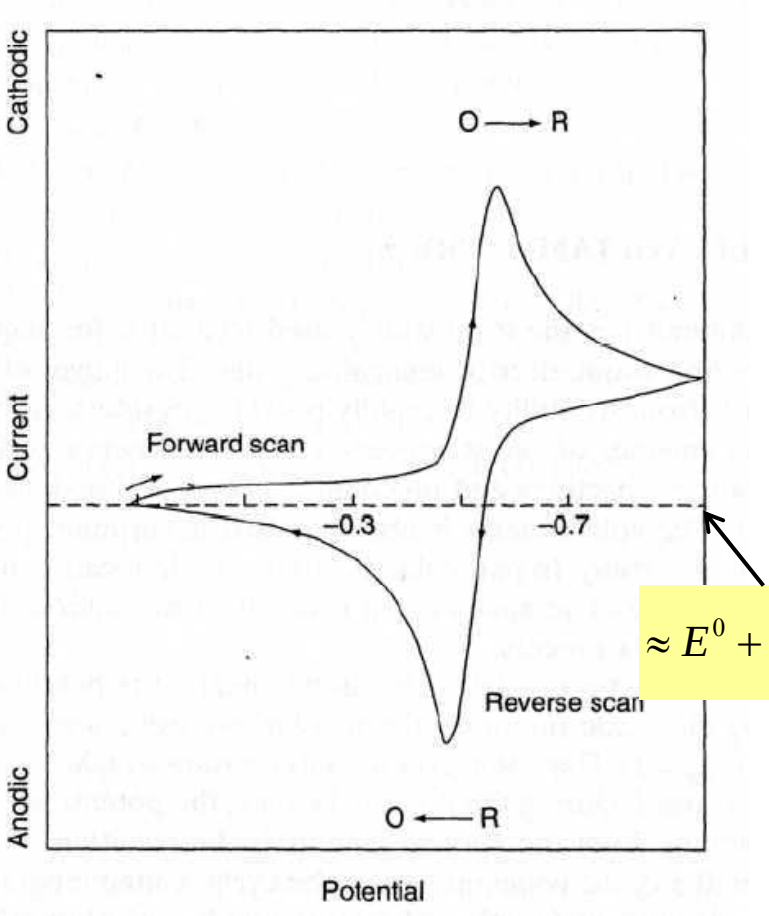
Potential-Controlled Techniques in Electrochemical Sensing.
Enzymatic Electrodes.

Cyclic voltammetry

- The most widely used technique for acquiring **quantitative** information about e/chemical reaction
- Involves linear scanning of the working electrode potential



cyclic voltammogram



- for reversible reaction

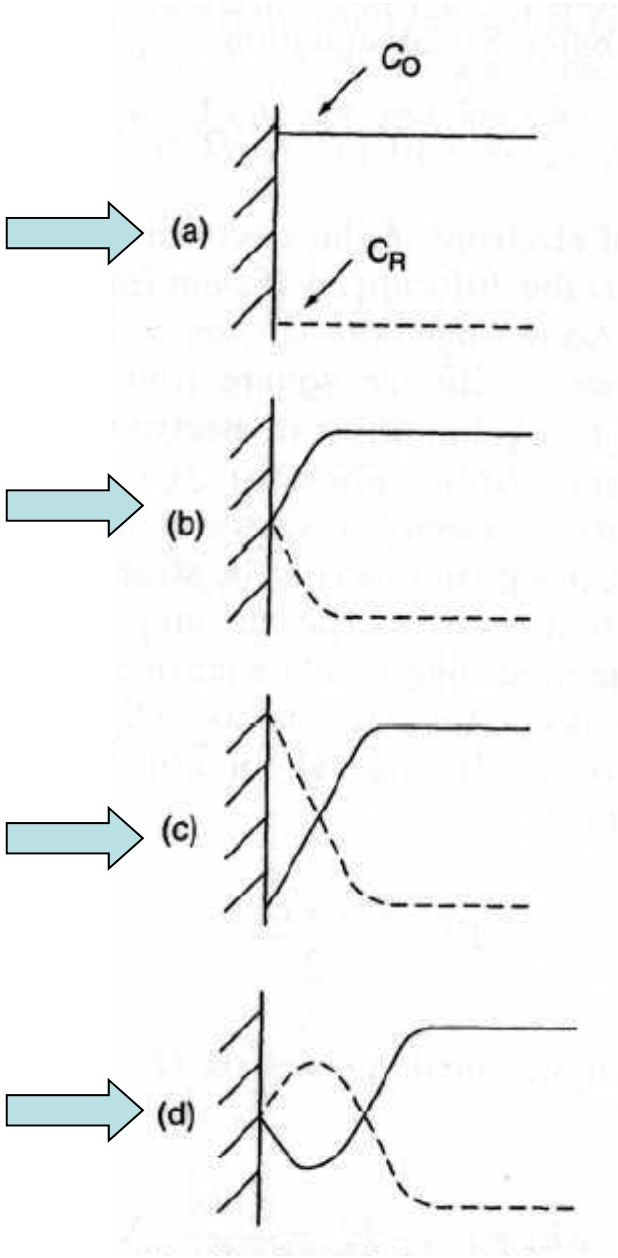
$$\approx E^0 + \frac{90}{z} mV$$

Cyclic voltammetry

- initial situation
- formal potential reached in forward scan

$$E = E^0 + RT \ln\left(\frac{Ox}{\mathrm{Re}}\right)$$

- max. current
- formal potential reached in reversed scan



Cyclic voltammetry

- The peak current for a reversible system is given by Randles-Sevcik equation:

$$i_p = \left(2.69 \times 10^5\right) n^{3/2} A C D^{1/2} v^{1/2}$$

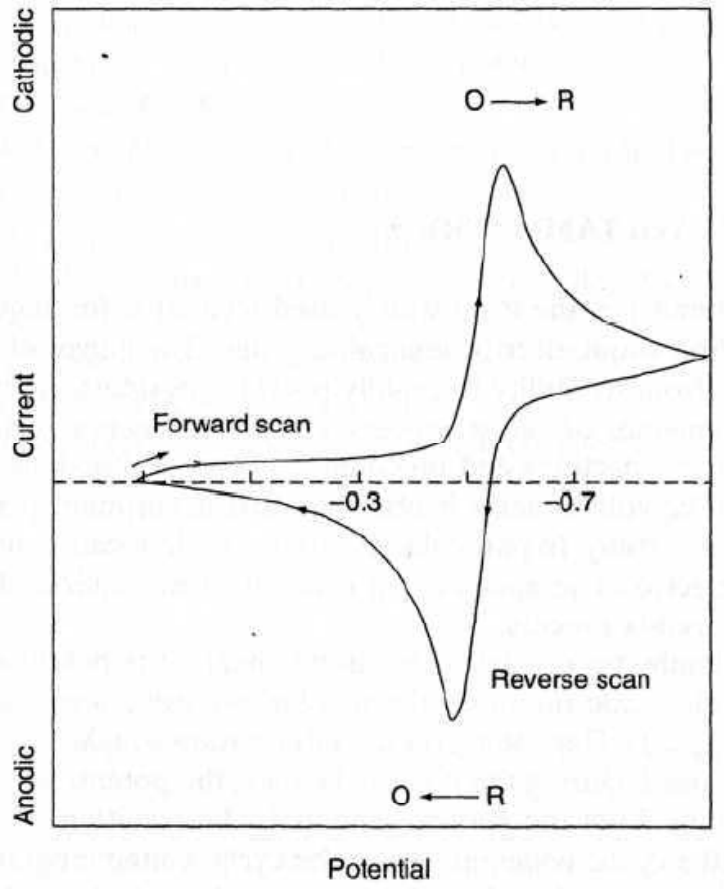
A in cm^2, ***C*** in mol/cm^3, ***D*** cm^2/s, ***v*** in V/s, at 25ºC.

- The formal potential for a reversible couple:

$$E^0 = \frac{E_{p,a} + E_{p,c}}{2}$$

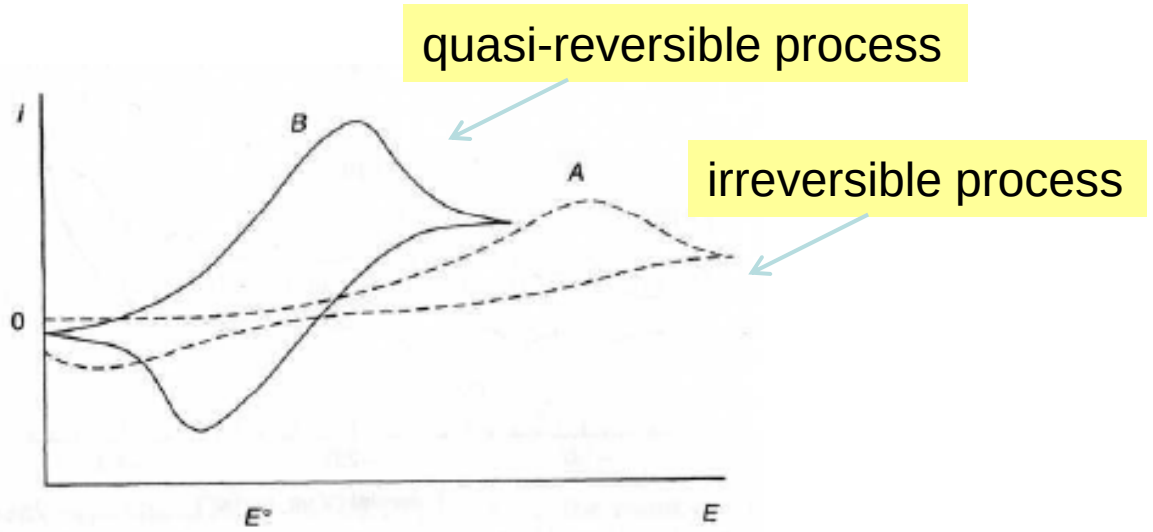
- The separation between the peaks gives information on the number of electrons transferred (for a reversible couple)

$$\Delta E_p = E_{p,a} + E_{p,c} = \frac{59mV}{z}$$



Cyclic Voltammetry

- For irreversible processes (where electron transfer is a limiting factor) peak potential will depend on the scan rate



$$E_p = E^\circ - \frac{RT}{\alpha n_a F}\left[0.78 - \ln\frac{k^\circ}{D^{1/2}} + \ln\left(\frac{\alpha n_a F v}{RT}\right)^{1/2}\right]$$

where α is electron transfer coefficient

$$i_p = (2.99 \times 10^5) n (\alpha n_a)^{1/2} A C D^{1/2} v^{1/2}$$

at α=0.5 peak height drops to 80%

Cyclic voltammetry

- Cyclic voltammetry in the presence of competing chemical reaction can be used to study kinetics

$$O + ne^- \rightleftharpoons R \longrightarrow Z$$

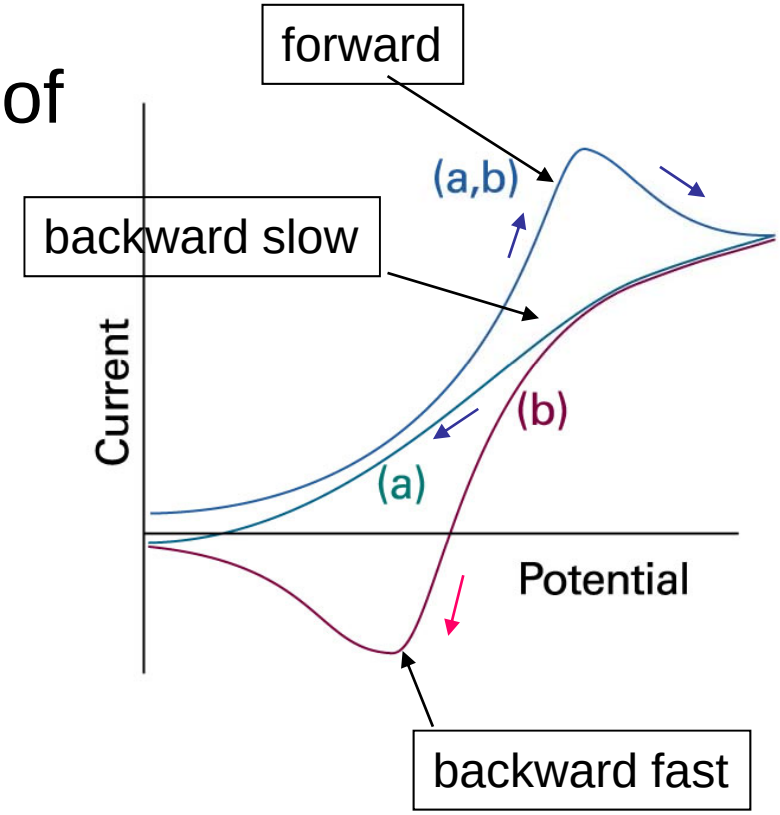
- Example: electro reduction of p-bromonitrobenzene

$$BrC_6H_4NO_2 + e^- \longrightarrow BrC_6H_4NO_2^-$$

$$BrC_6H_4NO_2^- \longrightarrow \bullet C_6H_4NO_2 + Br^-$$

$$\bullet C_6H_4NO_2 + e^- \longrightarrow C_6H_4NO_2^-$$

$$C_6H_4NO_2^- + H^+ \longrightarrow C_6H_5NO_2$$

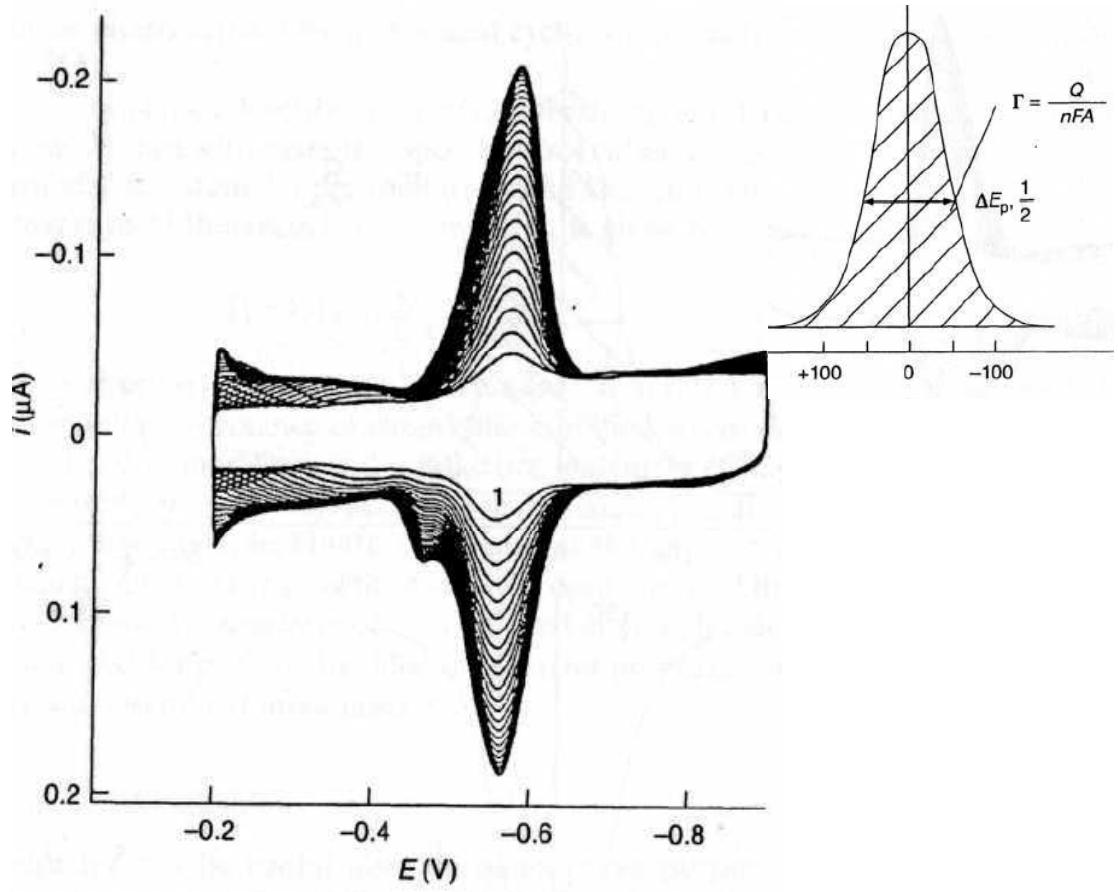


Cyclic voltammetry

- In the case of adsorption process on the electrode, the separation between the peaks will be smaller and current will be proportional to the adsorption

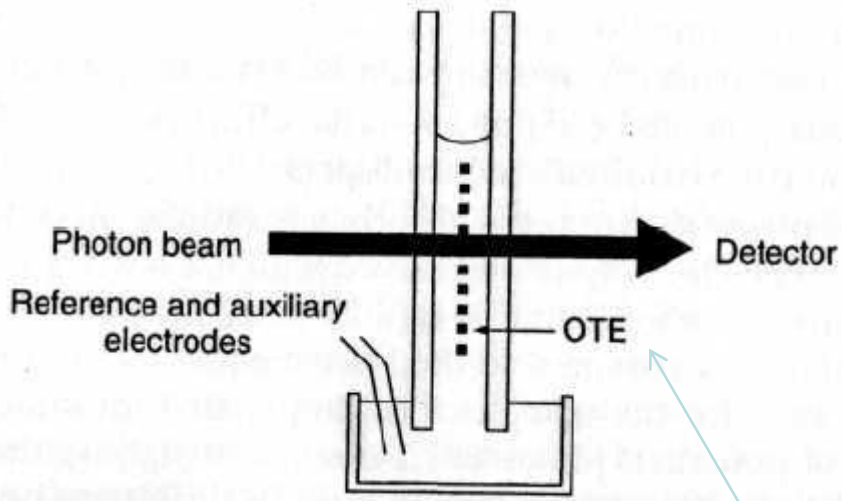
$$i_p = \frac{n^2 F^2 \Gamma A v}{4RT}$$

$$Q = nFA\Gamma$$

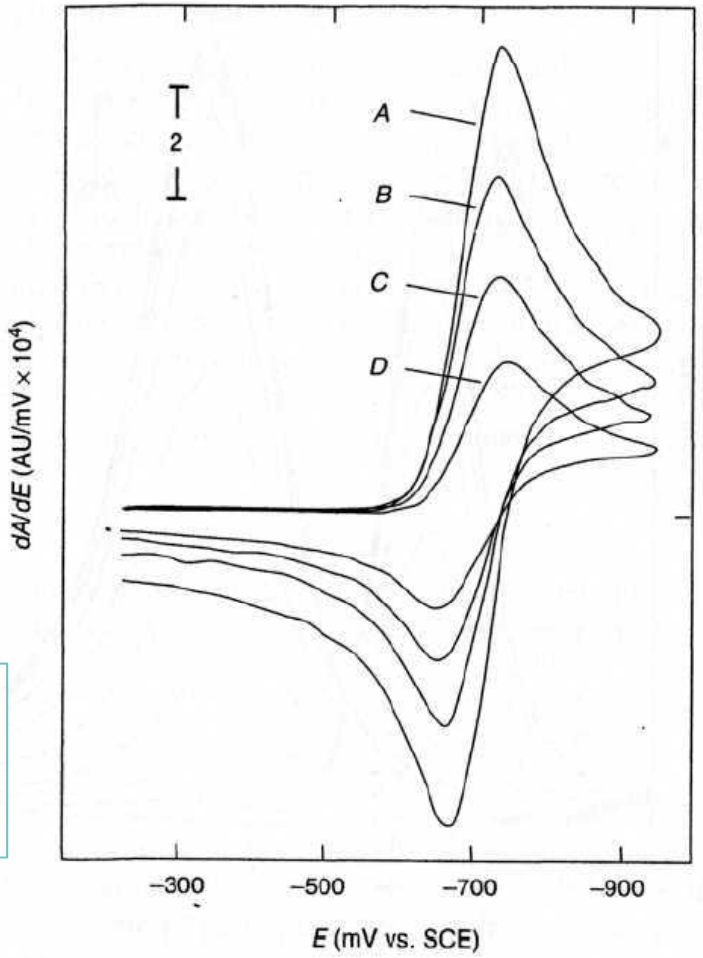


Spectroelectrochemistry

- Optical techniques, e.g. spectroscopic adsorption can be coupled to e/chemical methods



optically transparent electrode (e.g. metal grid, thin metal layer, ITO etc.)



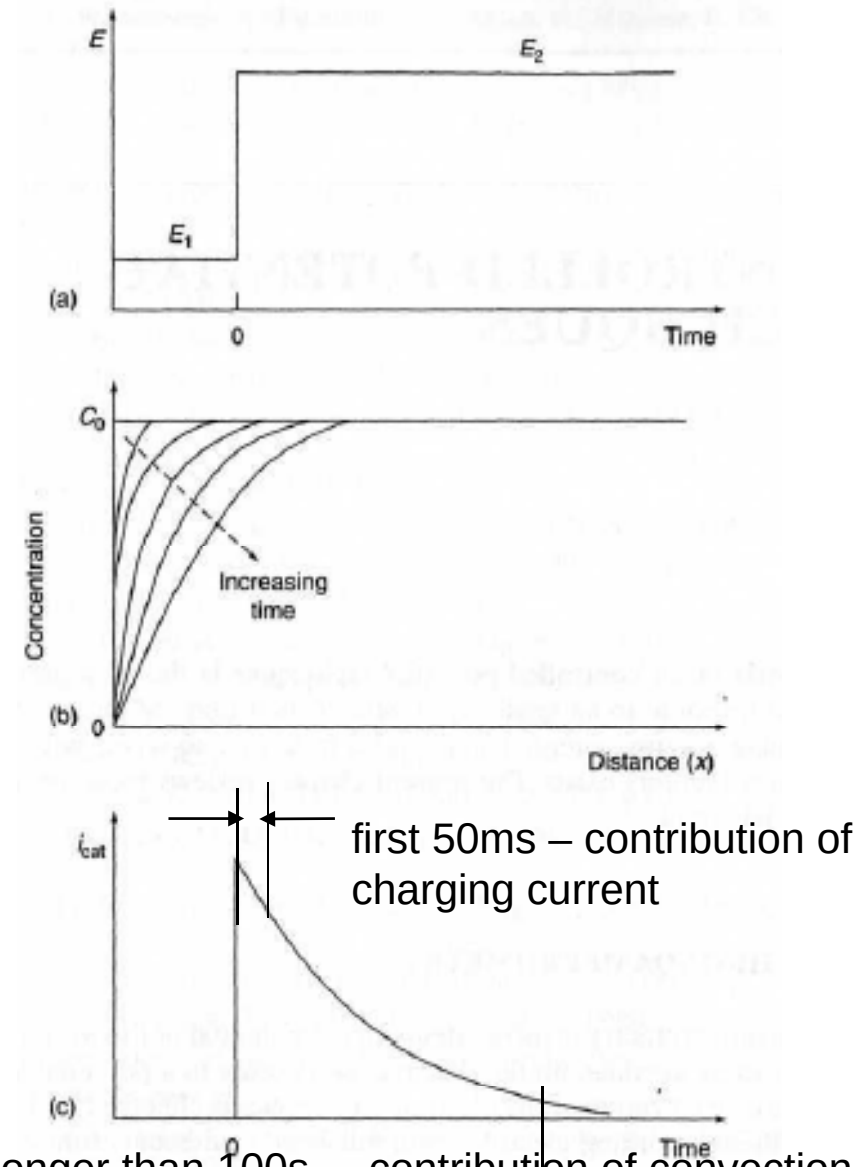
Chronoamperometry

- involves stepping potential of the working electrode from a value when no faradaic current occurs to a potential at which the concentration of electractive species becomes zero
- Response described by Cottrell equation:

$$i(t) = \frac{nFAD_O C_O(b)}{\sqrt{\pi D_O t}} = kt^{-1/2}$$

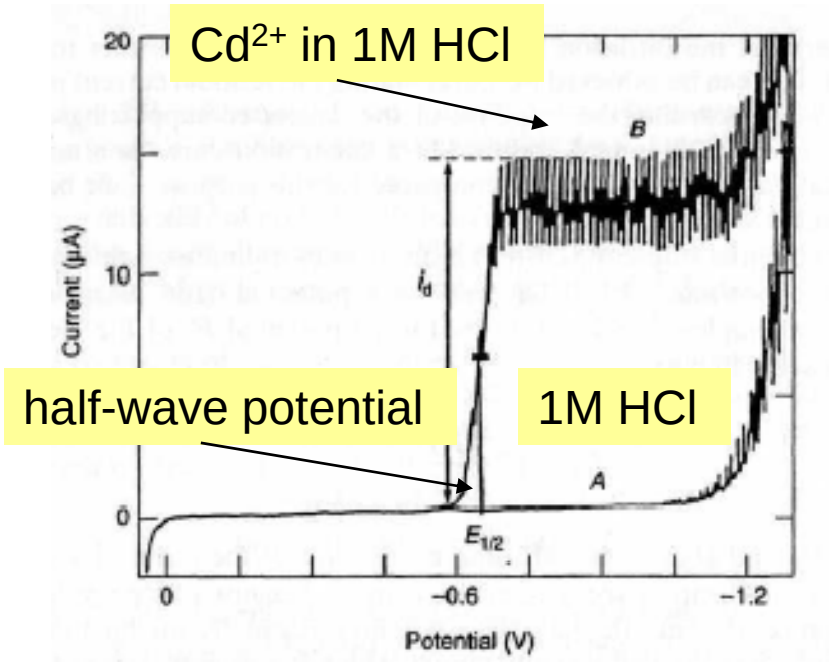
- Anson plot: $Q(t^{1/2})$

$$Q(t) = \frac{nFAC_O(b)\sqrt{D_O t}C_O(b)}{\sqrt{\pi}} + Q_{dl} + Q_i$$



Polarography

- subclass of voltammetry when dropping mercure electrode (DME) is used as a working electrode
- due to the impact of the technique on the electroanalysis its inventor J.Heyrovsky was awarded a 1959 Nobel price in Chemistry



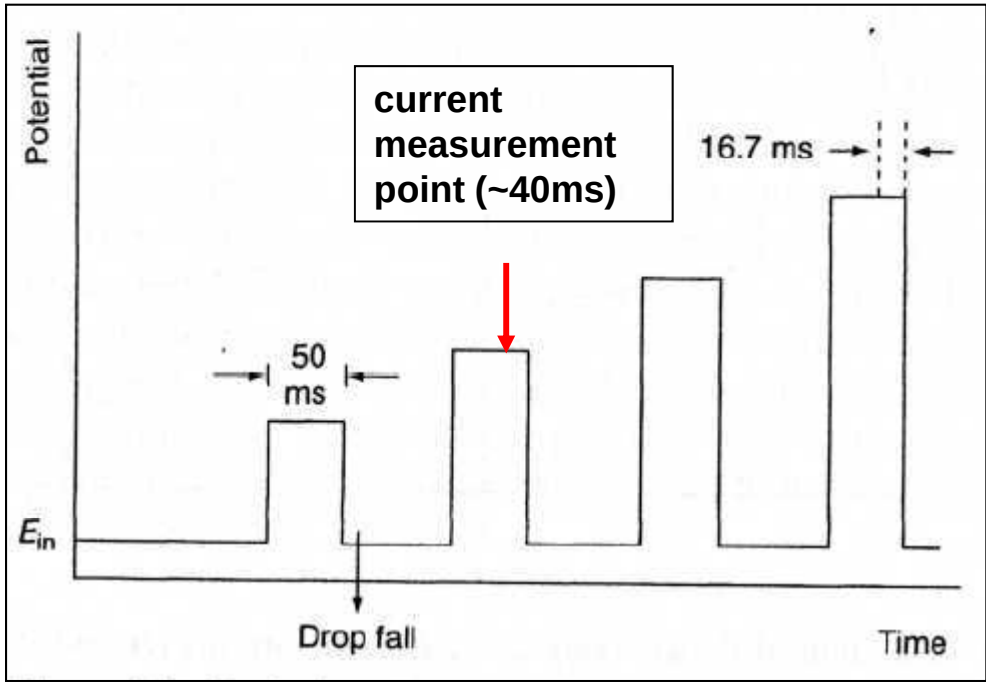
Pulse Voltammetry

- Pulse voltammetry techniques are aimed at lowering the detection limits (down to 10^{-8}M!)by reducing the ratio between faradaic and non-faradaic currents
- The difference between the different pulse techniques:
  - excitation waveform
  - sampling of current

Normal-Pulse Voltammetry

- consists of series of pulses with increasing amplitude (in case of DME applied to successive drops near the end of the drop lifiteme)

$$i(t) = \frac{nFAD_O C_O(b)}{\sqrt{\pi D_O t_m}}$$

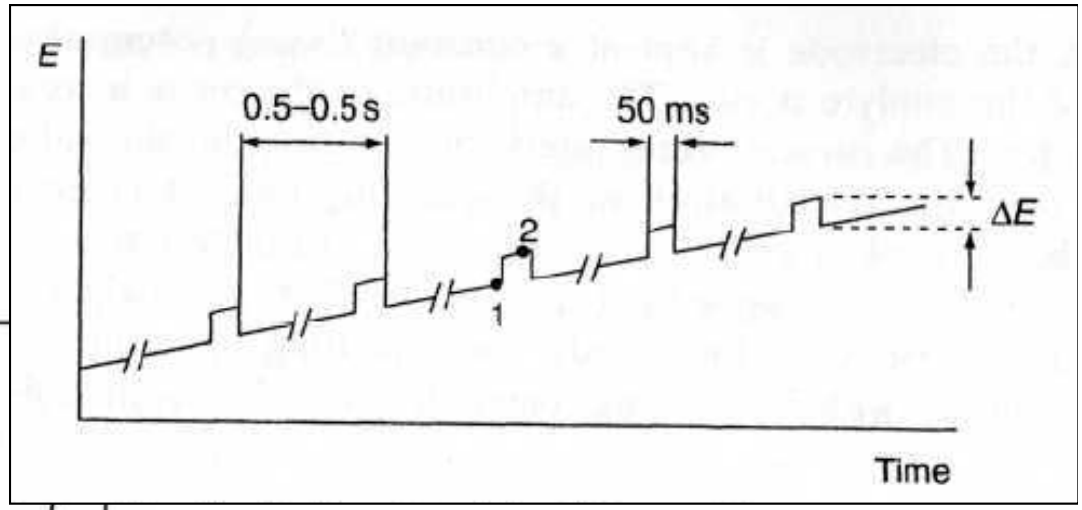


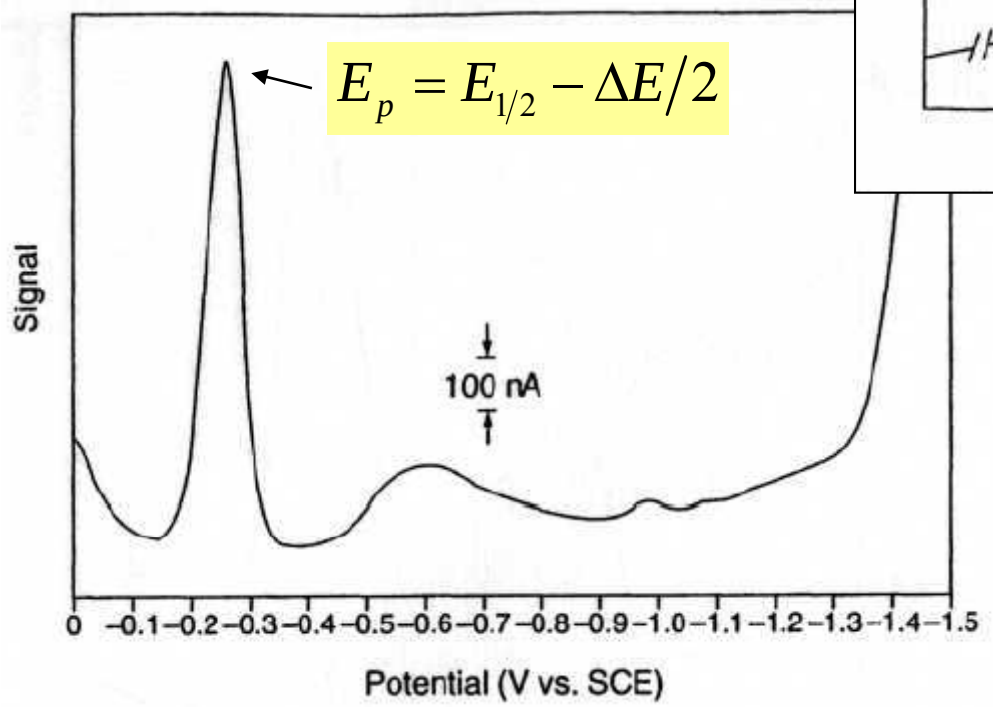
- Advantages:
  - due to short pulse duration, the diffusion layer is thinner and therefore higher faradaic current
  - almost zero charging current

Differential Pulse Voltammetry

- fixed magnitude pulses are superimposed on the linear potential ramp
- current sampled twice: before the pulse (1) and 40ms after the pulse begins

$$\Delta i = t(t_2) - t(t_1) \;\; vs. \;\; V$$



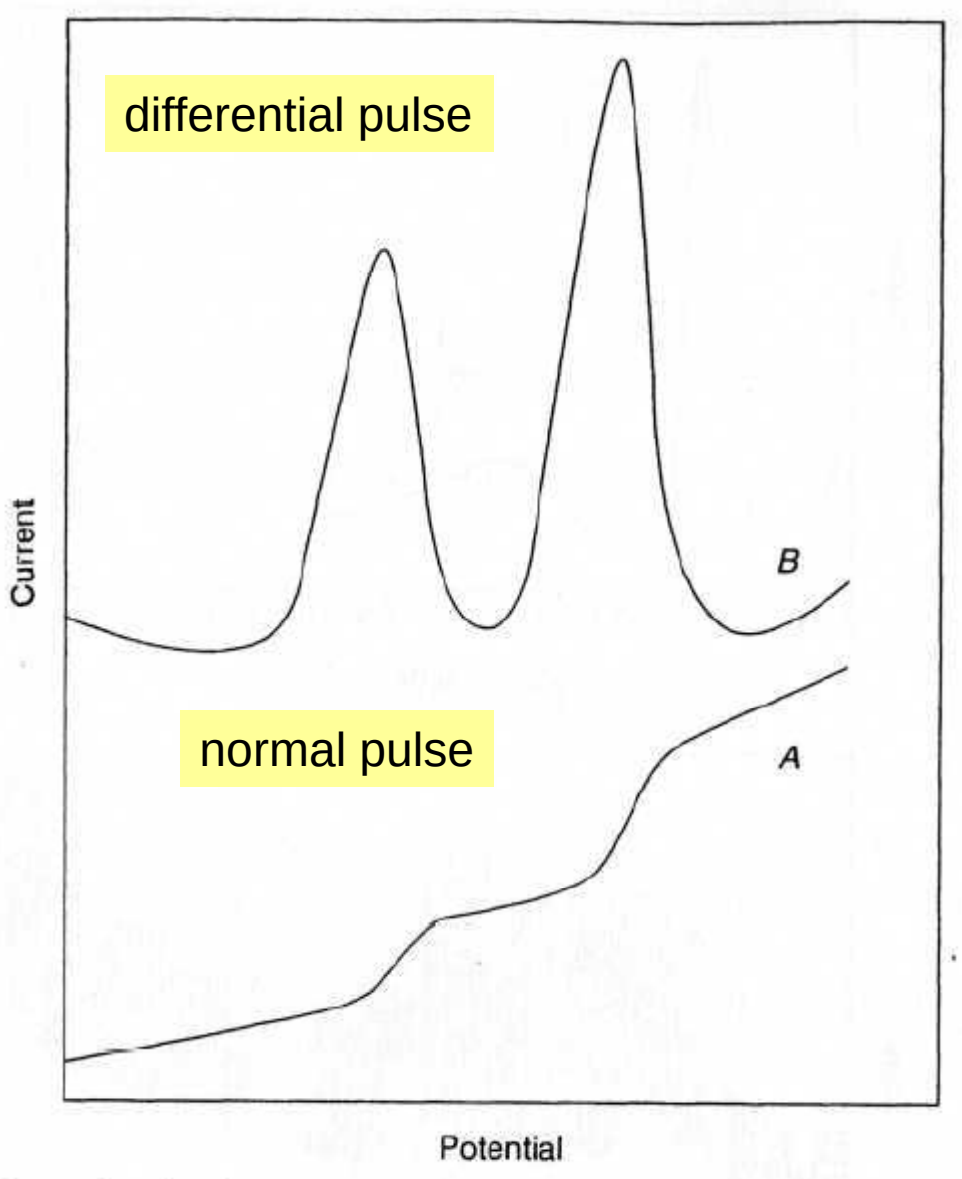


$$i_p(t) = \frac{nFAD_O C_O(b)}{\sqrt{\pi D_O t_m}} \left(\frac{1-\sigma}{1+\sigma} \right)$$

$$\sigma = \exp\left[\left(nf / RT \right) / \left(\Delta E/2 \right) \right]$$

Differential Pulse Voltammetry

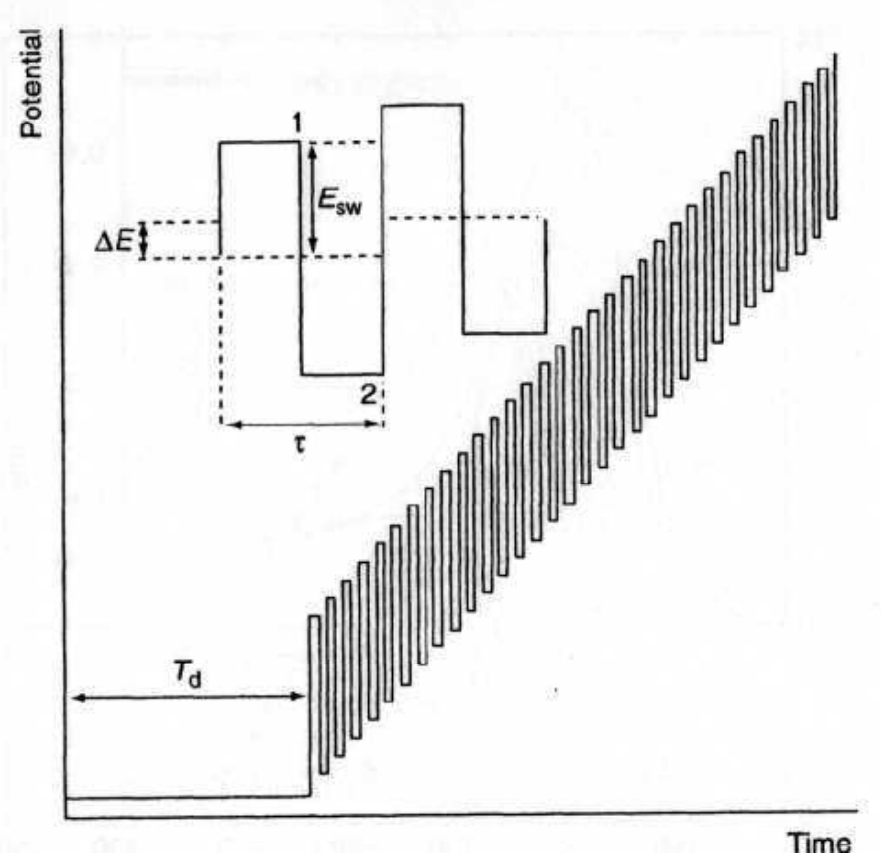
- allows measurement down to 10-8 M concentration
- improved resolution between the species with similar potential (down to 50 mV)
- typical parameters:
  - pulse 25-50 mV
  - scan rate 5mV/s



mixture of Cd^{2+} and Pb^{2+} in 0.1M HNO_3.

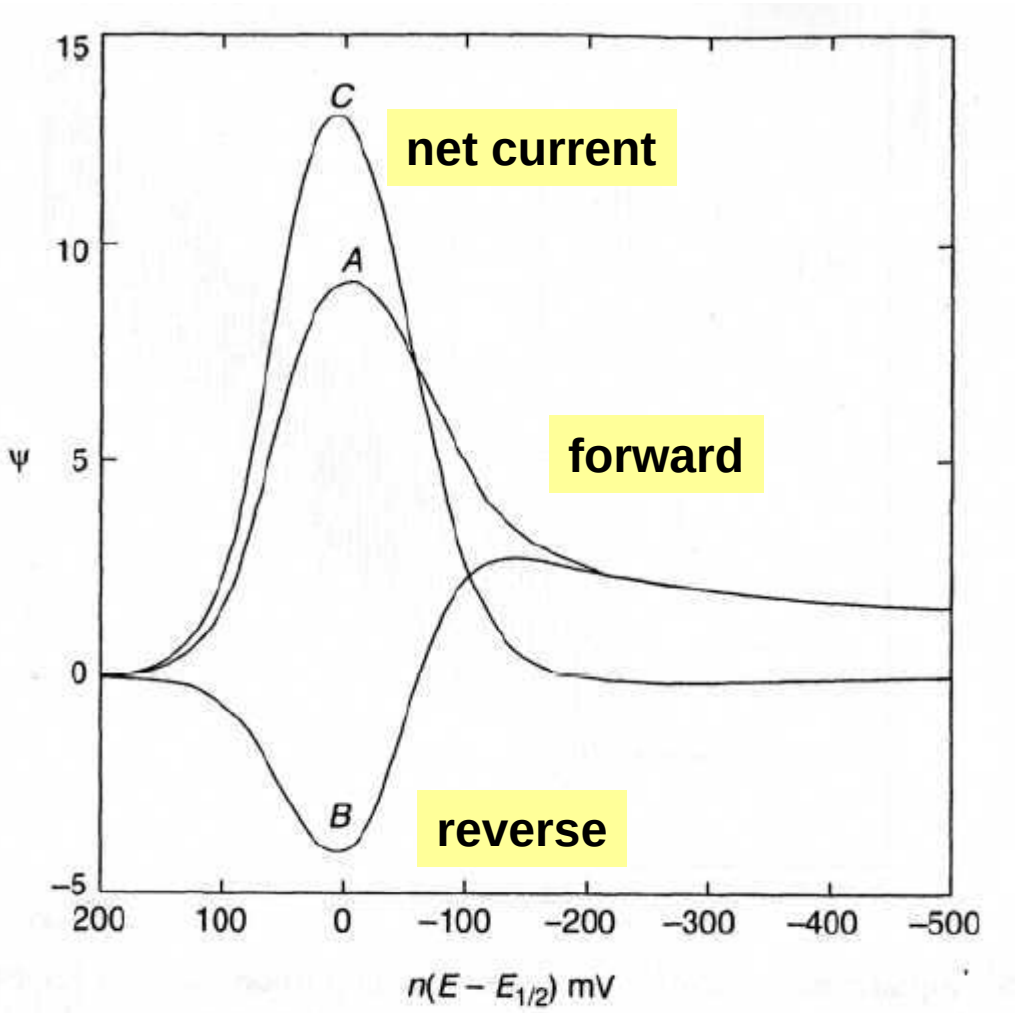
Square-Wave Voltammetry

- large-amplitude differential technique, the reverse pulse causes the reverse reaction of the product
- the current is sampled twice: at the end of the forward pulse and at the end of the reversed pulse



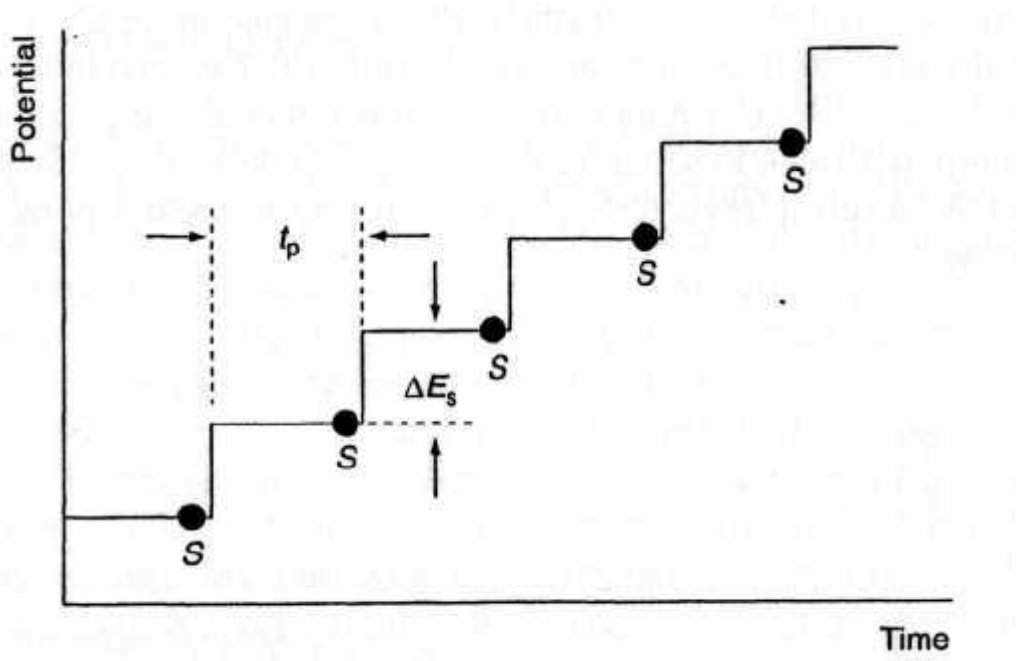
Square-Wave Voltammetry

- major advantage – speed, complete voltammogramm can be recorded within a couple of seconds
- advantageous in batch and flow analytical operations, can resolve neighboring peaks in chromatography and capillary electrophoresis



Staircase Voltammetry

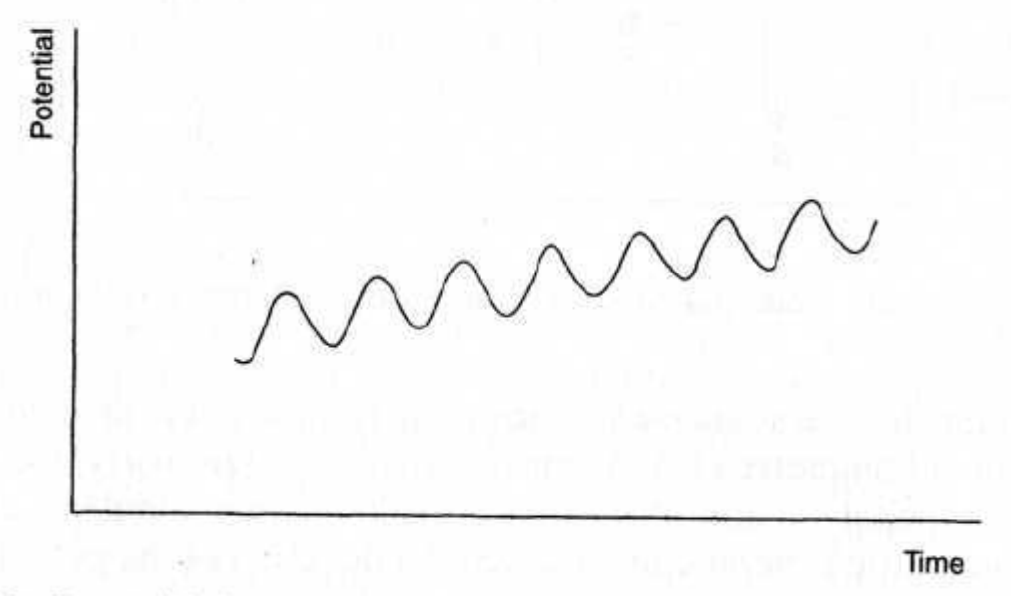
- voltage is increased in steps of ~10mV with 50ms delay
- response similar to cyclic voltammetry but with reduced charging current



AC Voltammetry

- small amplitude of AC is superimposed on linear ramp
- for a reversible system the response is similar to derivative of the DC response
- detection of AC components allows separation of faradaic current (45º with excitation) and charging (90º with excitation)
- detection limit ~5×10^{-7} M
- large amplitude AC (>50mV) allows identification of specific components via higher harmonics “fingerprinting”
- the height of the peak is proportional to the concentration, amplitude and sq.root of frequency

$$i_p = \frac{n^2F^2A\omega^{1/2}D^{1/2}C\Delta E}{4RT}$$



Stripping analysis

- the idea:
  - first pre-concentrate the analyte on the surface of the electrode
  - then strip (dissolve) the analyte and measure
- detection levels down to 10^{-10} M is feasible
- varios variations exists:
  - anodic stripping voltammetry
  - potentiometric
  - adsorptive stripping
  - cathodic stripping
  - abrasive stripping

Anodic Stripping Voltammetry

- pre-concentration is done by amalgaming the metal in question in small volume mercury electrode

$$M^{n+} + ne^- + Hg \rightarrow M(Hg)$$

- the concentration can be calculated from the pre-concentration current measured

$$C_{Hg} = \frac{i_1 t_d}{nFV_{Hg}}$$

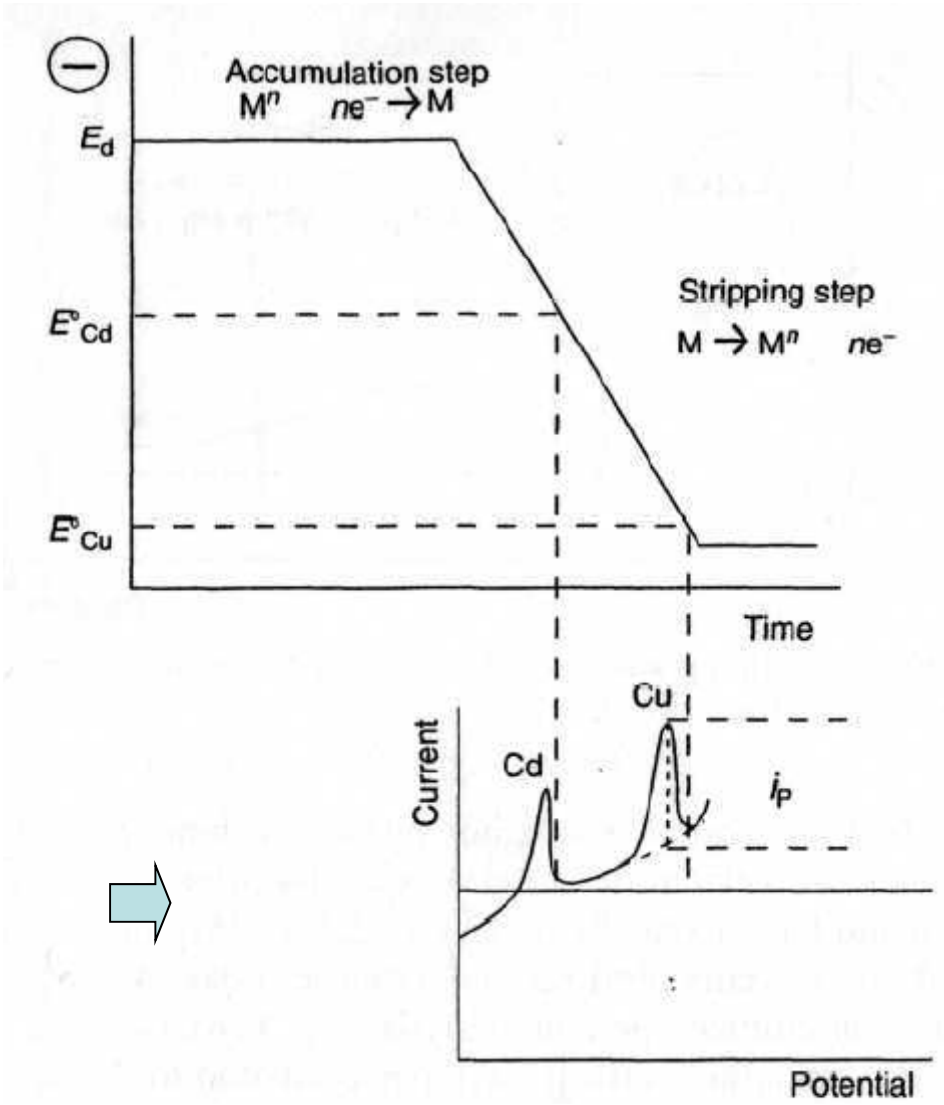
- during the anodic scan the metal is re-oxidated and stripped from the electrode

$$M(Hg) \rightarrow M^{n+} + ne^- + Hg$$

Anodic Stripping Voltammetry

- potential scan

- voltammogram

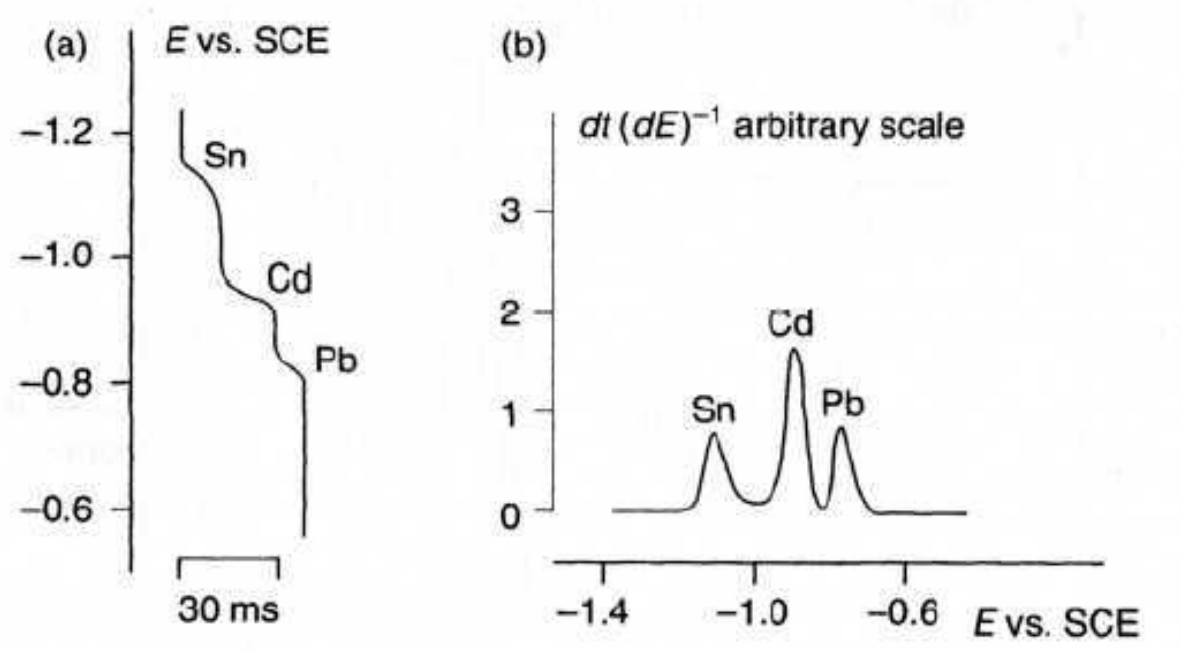


Potentiometric Stripping Analysis

- the oxidation step is done using an oxidation agent (O2, Hg(II) etc.) present in the solution

$$M(Hg) + oxidant \rightarrow M^{n+}$$

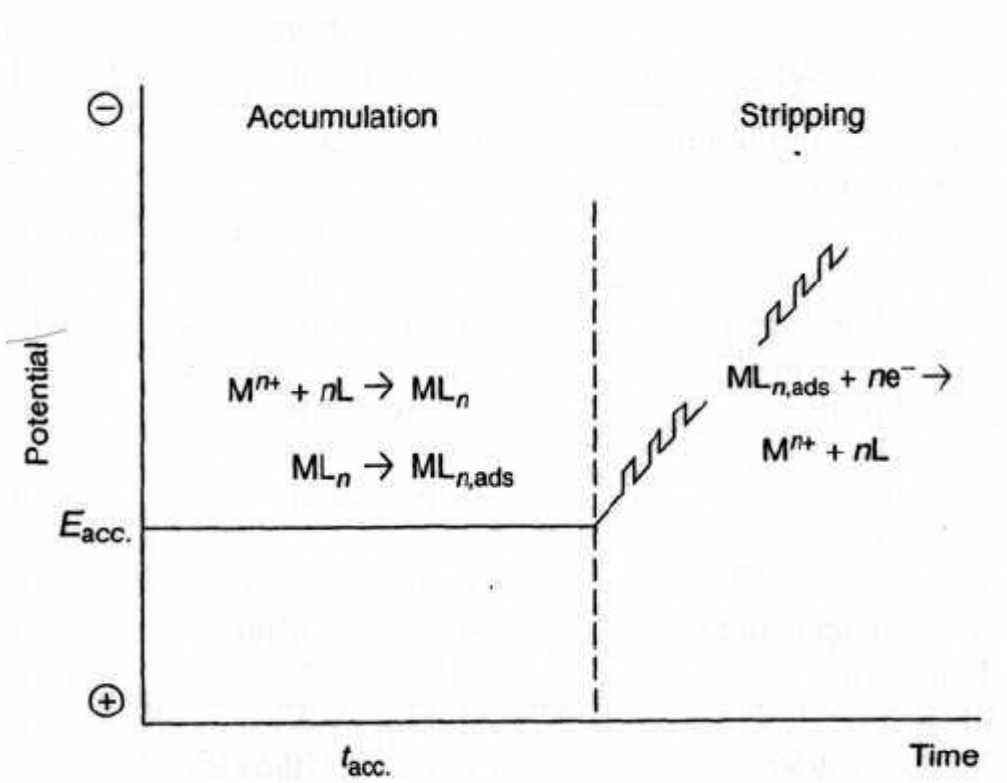
- potential of the electrode is measured vs time



Adsorptive Stripping Voltametry

- pre-concentration goes via adsorption of a metal ion in a surface bound complex (instead of amalgaming)

- Langmuir kinetics of adsorption vs time
- extremely low detection limits can be achieved (down to 10^{-12} M)



Cathodic stripping voltammetry

- involves anodic deposition of analyte followed by negative-going potential scan for detection of anions in the solution

$$A^{n-} + Hg \underset{stripping}{\overset{deposition}{\rightleftharpoons}} HgA + ne^{-}$$

- suitable for a wide range of compounds forming insoluble salts with mercury (halide ions, thiols, penicillins etc.)
- silver and copper can be used in a similar manner

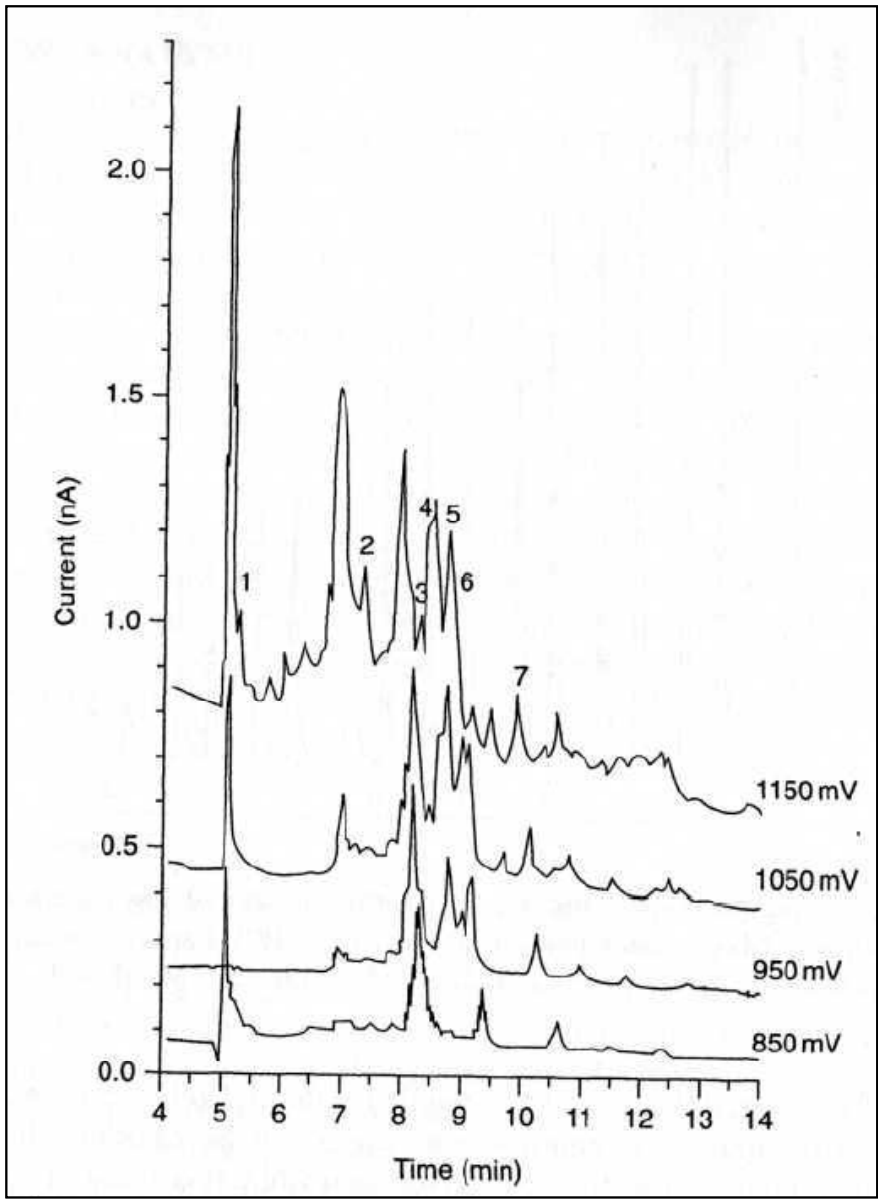
Abrasive stripping voltammetry

- mechanical (abrasive) transfer of solid material onto an electrode surface (e.g. paraffin coated graphite)

Flow analysis

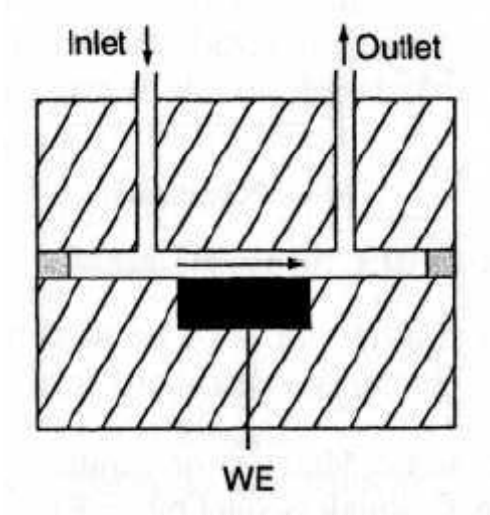
- Electrochemical techniques can be combined with chromatography (flow) analysis to identify the components present

Capillary electrophoresis/amperometric analysis of Bud Ligh beer

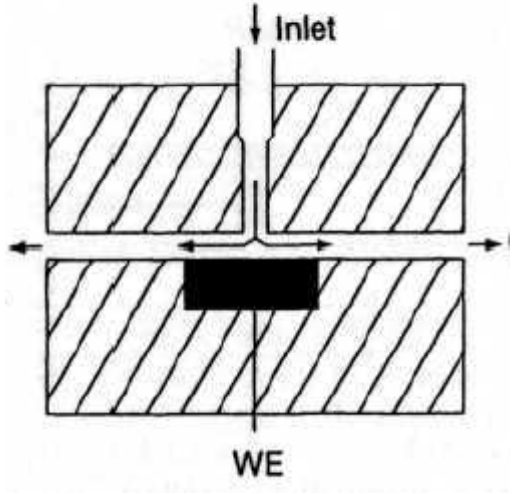


Flow analysis

- thin layer cell design



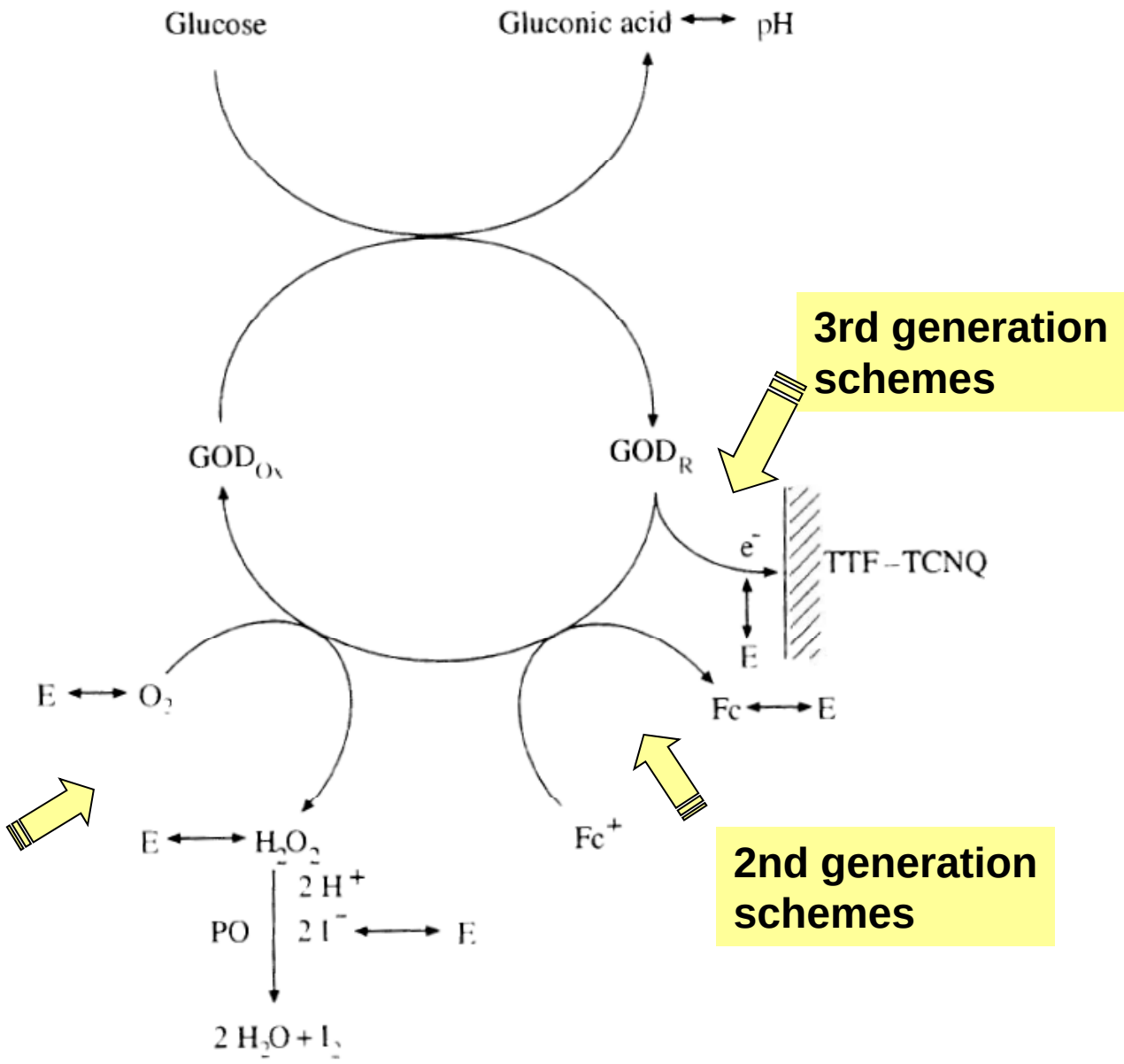
- thin layer cell design



Amperometric Biosensors with Enzyme Electrodes

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

Possible glucose detection schemes



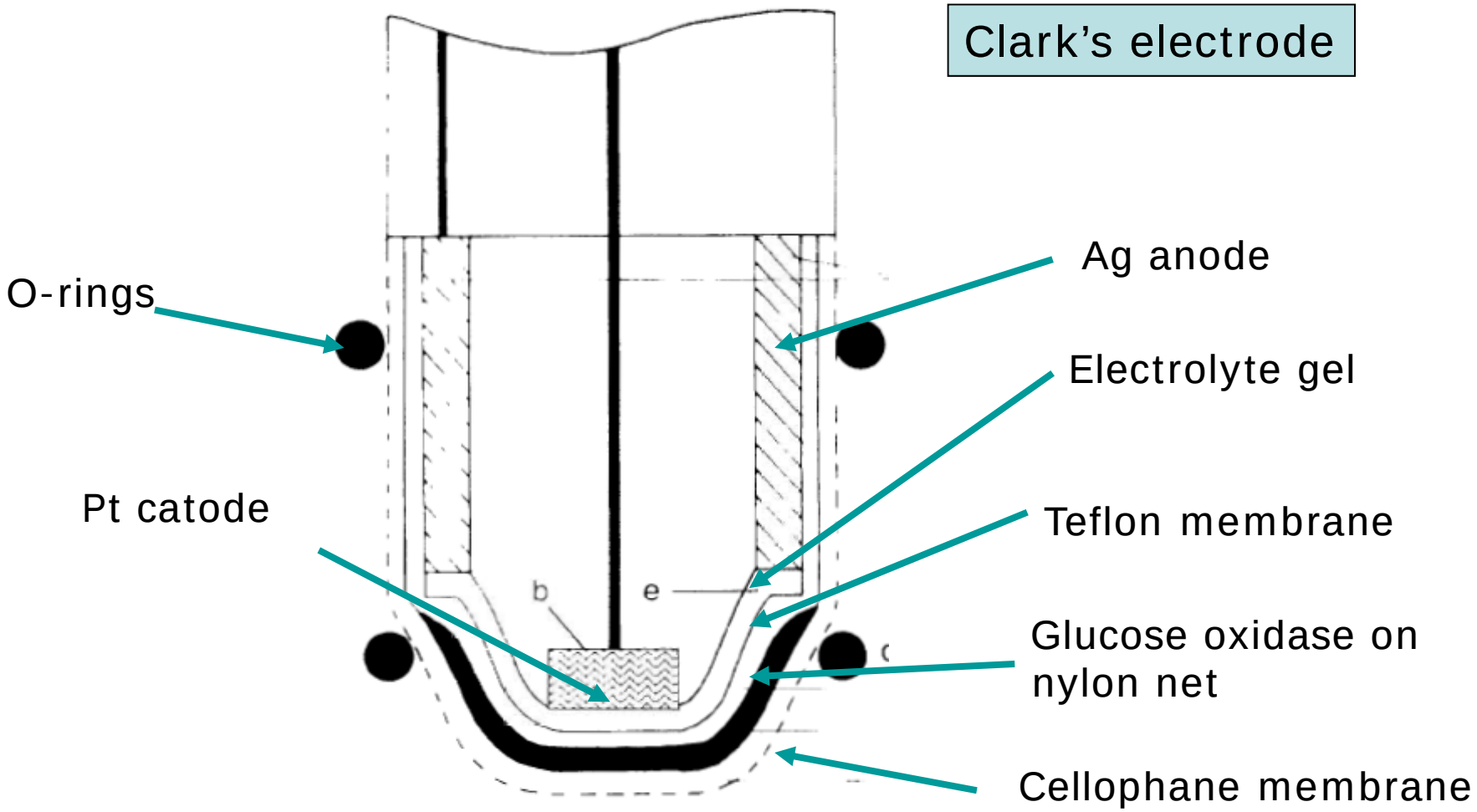
3rd generation schemes

1st generation schemes

2nd generation schemes

Clark-type glucose electrode

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



Reactions at the Oxygen electrode (1st generation)

- Reaction at the enzyme electrode

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

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, by pumping oxygen containing buffer, re-cycling H_2O_2 using catalaze etc.)

Measuring hydrogen peroxide:

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

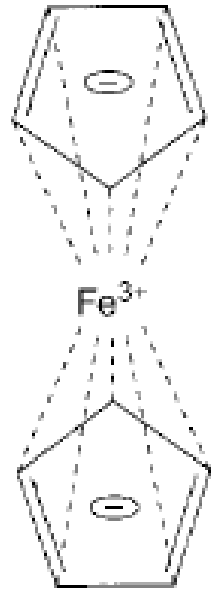
Problem: still fairly high potential (interference from e.g. ascorbic asid)

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	—	—

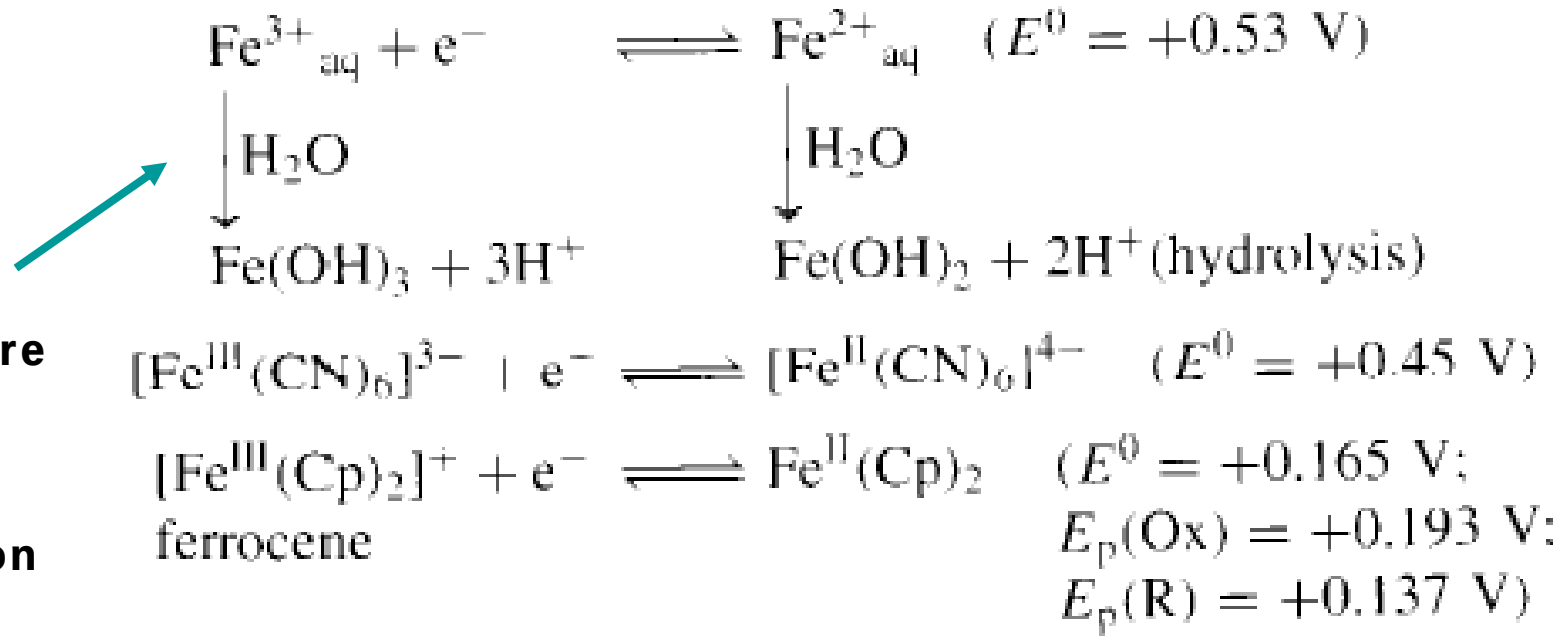
Mediator Based Sensors

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

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



Fc

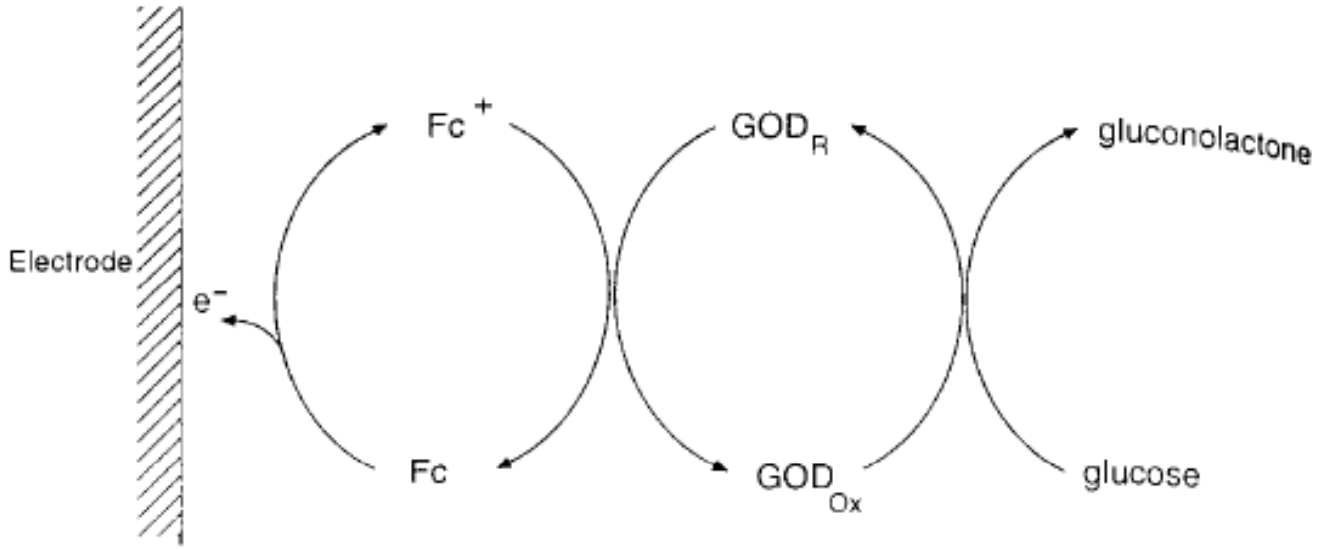


Free Fe^{3+} are subject to hydrolysis and precipitation

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

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

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



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

Rate constant for electron transfer to the enzyme

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] Parent (unsubstituted) material.

Compare with -0.7V required for Clarks electrode

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		

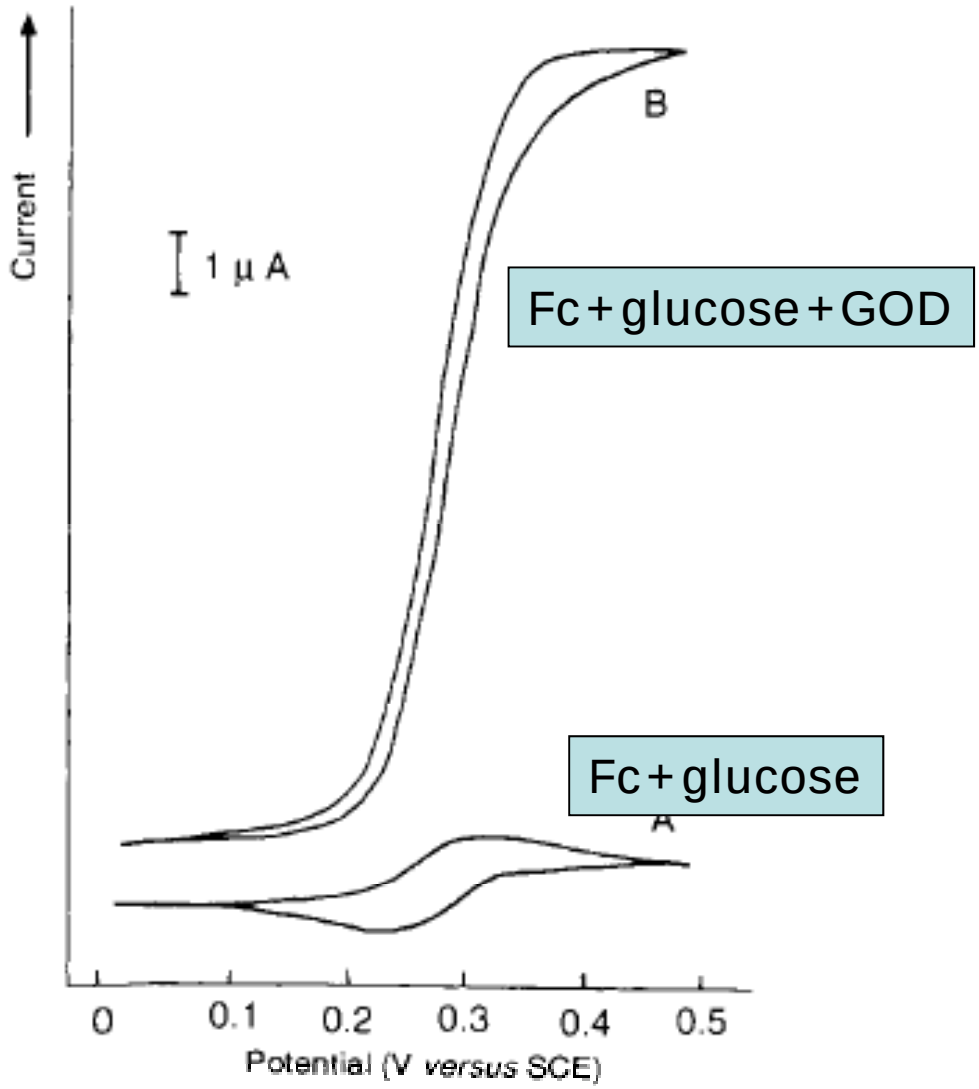
[a] Versus the standard hydrogen electrode (SHE).

How it works...

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

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

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

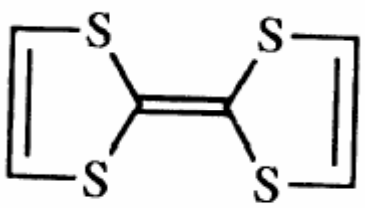


In real biosensors both GOD and Fc are immobilised

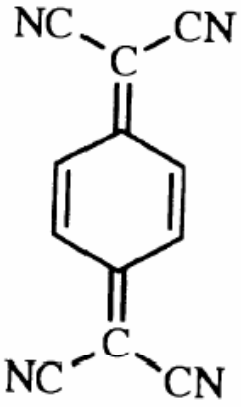
Directly Coupled Enzyme

- 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

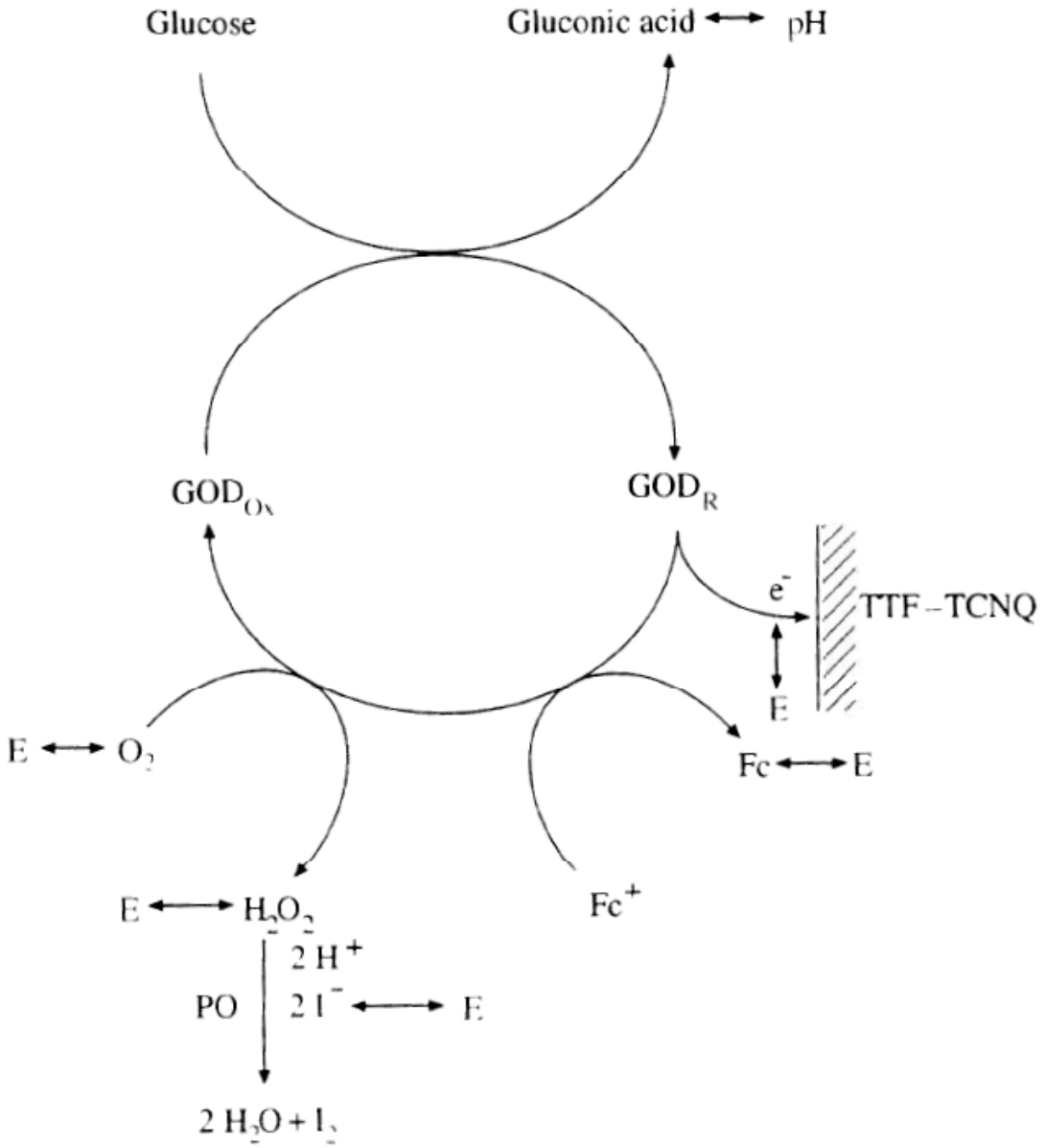


TTF



TCNQ

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