

Electrochemistry of Biomolecules

Literature:

- 1. Bioelectrochemistry: Principles and Practice** (88WD 2400 C244) G. Lenaz, G. Milazzo, Birkhäuser Verlag Basel, 1997
- 2. Electroanalytical Chemistry A Series of Advances** (86VG 8000-3) A Series of Advances, Allen J. Bard, M. Dekker, NY
- 3. Electrochemical Methods** (86VE 6300 B245 E3(2)) A. J. Bard, I. R. Faulkner, J. Wiley, NY, 2001
- 4. New Instrumental Methods in Electrochemistry** (86VE 6300 D333) P. Delahay, C. N. Reilley, J. Wiley, NY, 1966
- 5. Laboratory Techniques in Electroanalytical Chemistry** (86VG 8000 K61(2)) P. T. Kissinger M. Dekker, NY, 1996
- 6. Experimental Electrochemistry for Chemists** (86VE 6300 S271) D. T. Sawyer, J. L. Roberts, J. Wiley, NY, 1974
- 7. Instrumental Methods in Electrochemistry** (86VE 6300 G793) Southampton Electrochemistry Group, J. Wiley, NY
- 8. Encyclopedia of Electrochemistry**, A. J. Bard, M. Stratmann, Vol. 9, **Bioelectrochemistry**, edited by G. S. Wilson, Wiley VCH, 2002

Aims

- Understanding of Natural Electron Transfer Processes
- Thermodynamic and Kinetic Information

Electrochemical Potentials in Redox Cascades
(i. e. in Photosynthesis or Respiratory Chain)

Number of Electrons being transferred

Diffusion Constants

Mechanistic Studies

Information of Combined Chemical Reactions, Rate Constants
(e. g. Proton transfer)

- Design of Biosensors
- Development of Fuel Cells

Electrochemical Parameters

Potential E [V]

Amount of electrical force in a system. As potential increases more force is available to make a reaction happen

Current i [A]

magnitude of electron flow in a system

Time t [s]

Measurement of duration

Charge $Q = it$ [1C = 1As]

Measure of number of electrons being transferred

Resistance $R = E/i$ [1 Ω = 1V/A]

Opposite of conductivity

Capacity $C = Q/E$ [1F = 1C/V]

Ability to store charge

The Electrochemical Potential

The electrochemical potential E is directly related to the free energy ΔG :

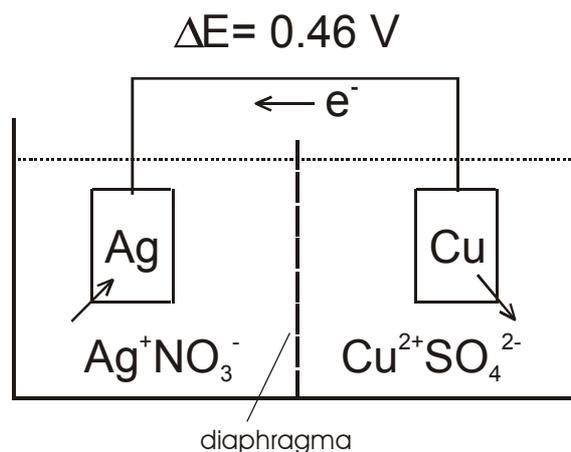
$$\Delta G = -nF\Delta E$$

n : number of electrons

F : faraday constant $F = N_A e$

e : elementary charge

N_A : Avogadro's Number



Galvanic Element consisting of two half cells:
 $\text{Ag}/\text{Ag}^+//\text{Cu}/\text{Cu}^{2+}$

Concentration Cells:

An electrochemical potential is also present between two half cells containing the same components in different concentration.

Nernst Equation

The potential of every half cell is given by the **Nernst Equation**:

$$E = E_0 + \frac{RT}{zF} \ln \left(\frac{a_{\text{Ox}}}{a_{\text{Red}}} \right)$$

a: activities of oxidised or reduced species

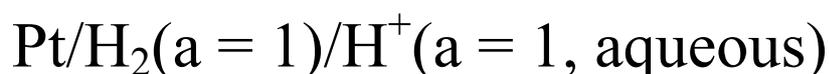
z: number of electrons

Reference Electrodes

Lit.: **Reference Electrodes** Theory and Practice, D. J. G. Ives, G. J. Janz, Academic Press, 1969

Electrochemical potentials can only be measured relatively to each other. Therefore one has to apply a reference system.

The internationally accepted primary reference is the normal (also termed standard) hydrogen electrode NHE (SHE) which has all components at unit activity:



Because this electrode is not easy to handle other reference electrodes are common:

SCE: Hg/Hg₂Cl₂/KCl(sat. in H₂O), 0.242V
vs. NHE

Ag/AgCl: Ag/AgCl/KCl(sat. in H₂O), 0.197V
vs. NHE

$$E_{\text{Ag/AgCl}} = E_{0\text{Ag/AgCl}} + \frac{RT}{F} \ln(L_{\text{AgCl}}) - \frac{RT}{F} \ln(a_{\text{Cl}^-})$$

Redox Titration

The electrochemical potential of redox active compounds can be determined in solution by applying a redox titration. The absorption spectrum of the investigated compound is observed. The potential of the solution is changed by adding a reducing or oxidising agent. If the potential in solution is negative (positive) enough to reduce (oxidise) the investigated compound the optical spectrum will change until the reduction (oxidation) is complete.

Heterogeneous Electron Transfer

Electron transfer **ET** between the solid electrode and the redox active compound in solution is called heterogeneous electron transfer. **Involved Processes:**

Mass Transfer

Electron transfer at the electrode surface

Chemical reactions preceding or following the electron transfer

Other surface reactions (e. g. adsorption, desorption)

The rate of **ET** is determined by the slowest step.

When the concentration of the oxidised and reduced compounds are governed by the Nernst equation the electron transfer is called nernstian or reversible. The electron transfer can be described by the **Nernst Plank equation** if mass transport is rate determining:

$$J_i(x) = -D_i \frac{\partial C_i(x)}{\partial x} - \frac{z_i F}{RT} D_i C_i \frac{\partial \Phi(x)}{\partial x} + C_i v(x)$$

$J_i(x)$: flux of the species i at distance x from surface

D_i : diffusion coefficient [cm/s]. A measure of how fast an ion moves in solution using only diffusion.

$\frac{\partial C_i(x)}{\partial x}$: concentration gradient at distance x

$\frac{\partial \Phi(x)}{\partial x}$: potential gradient

$v(x)$: velocity of movement of a vol. element in solution

Mass Transport in Solution

Migration: Movement of a charged body under the influence of an electric field

Diffusion: Movement of a species under the influence of a gradient of chemical potential

Convection: Stirring or hydrodynamic transport (e. g. caused by density gradients).

Many electrochemical experiments are diffusion controlled. A supporting electrolyte is added to avoid migration.

Fick's laws are differential equations describing the flux [$\text{mol s}^{-1} \text{cm}^{-2}$] of a substance and its concentration as functions of time and position:

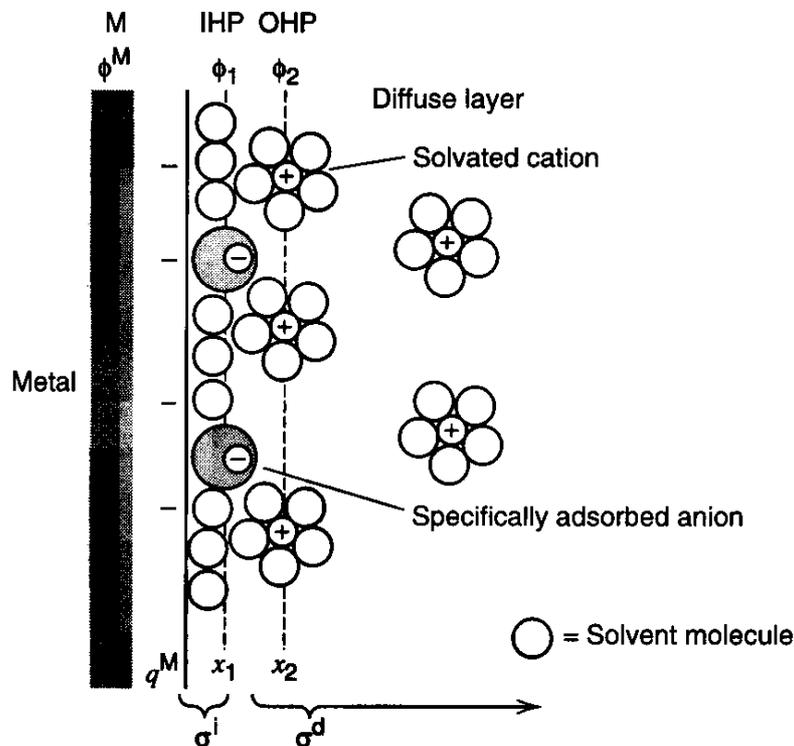
$$J_i(x,t) = -D_i \frac{\partial C_i(x,t)}{\partial x} \quad \text{Fick's First Law}$$

$$\frac{\partial C_i(x,t)}{\partial t} = D_i \frac{\partial^2 C_i(x,t)}{\partial x^2} \quad \text{Fick's Second Law}$$

By solving these equations with respect to the experimental boundary conditions several electrochemical methods can be described.

Processes at the Electrode Surface

Electrical/Nernstian Double Layer



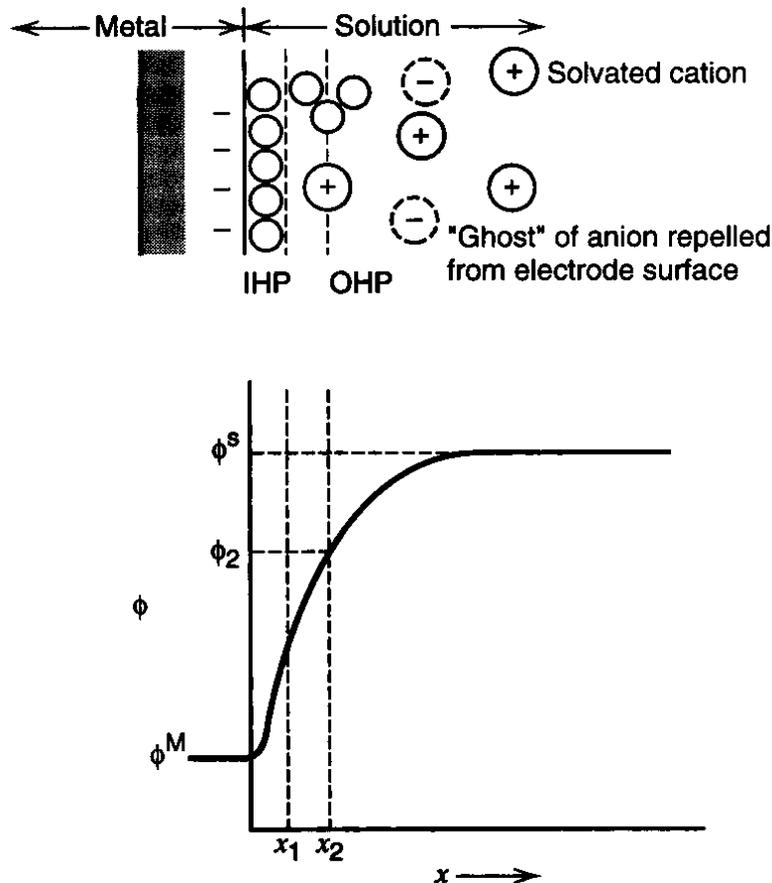
graphic from Lit. 3

Inner Helmholtz plane: The electrode surface can directly only be reached by solvent molecules.

Outer Helmholtz plane: Because anions and cations are surrounded by solvent molecules these charged species can only approach the electrode up to a certain distance.

The Electrical Double layer can be treated as a capacity.

Concentration Profile



graphic from Lit. 3

Common electrochemical reactions occur at the electrode surface in the nernstian double layer. When the concentration of a redox active species at the electrode is changed due to electron transfer there is an area near the electrode in which a concentration gradient occurs.

This area due to the electrode surface is called diffusion layer.

In semiinfinite conditions there exist a part of solution volume that is not affected by the concentration gradient.

This part is called the bulk of solution.

$$C_i(r,0) = C_i^* \quad (r > r_0)$$

$$\lim(x \rightarrow \infty) C_i(r,0) = C_i^*$$

Modes of Diffusion



graphics from Lit. 1

Linear Diffusion: If the electrode surface of a planar electrode is large compared to the thickness of the diffusion layer (that is usually up to 100 μm) the diffusion is linear.

Spherical diffusion: If the electrode surface of a planar electrode is about the thickness of the diffusion layer the diffusion is spherical.

Electrode Kinetics

ET does not require direct contact between a redox center and a electrode surface. In the simplest case the heterogeneous ET rate constant, k_{obs} , is exponentially dependent on the distance between the electrode surface and the redox center of the electroactive compound.

$$k_{obs} = k_0 e^{-\beta r}$$

where r is the distance between the electrode surface and the redox center. The electron may tunnel through an adsorbed spacer with decreasing k_{obs} . The nature of the spacer layer effects β that is also called tunneling constant. k_0 is the rate constant without spacer.

Electrochemical Methods

The Uncompensated Resistance

When a current flows through an electrochemical cell the applied potential E_{appl} is¹:

$$E_{\text{appl}} = E_{\text{redox}} + \eta - iR_s$$

E_{redox} : potential of the observed redox process

η : Overpotential at the electrode surfaces

iR_s : Potential drop due to the current flow through the solution

The values of η and iR_s can cause an experimental error.

When there is no current flow or the current is very small (e. g. due to the use of ultramicroelectrodes) in an electrochemical experiment and the resistance of the solution is very small a two electrode system can be used. A working electrode is used together with a second electrode that serves as counter and reference electrode.

In all other potential controlled experiments one has to use a three electrode setup. The current flow occurs only between working and counter electrode. The potential of the working electrode is applied against a nonpolarisable reference electrode. Working and reference electrode have to be placed very close together. Because this is not possible in a perfect way in some experiments a remaining resistance R_s often termed R_u (u = uncompensated) has to be taken into account.

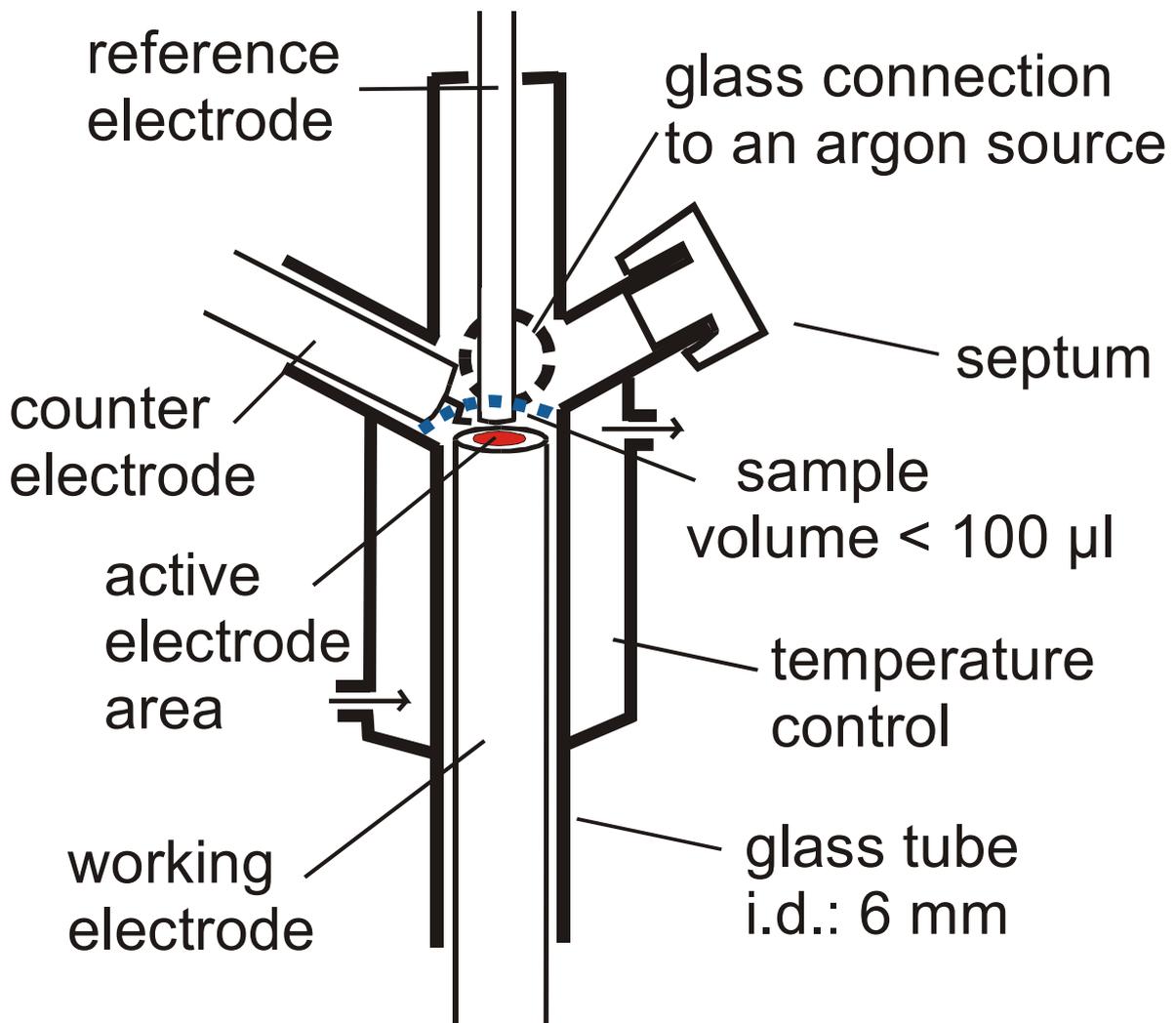
¹ The polaric convention is used where negative potentials result in a positive current.

The Cell

Our cell is constructed to measure small sample volumes less than $100\ \mu\text{l}$ as described in: E. T. Smith, D. W. Bennett, B. A. Feinberg, *Analytica Chimica Acta*, 1991, 251, 27-33.

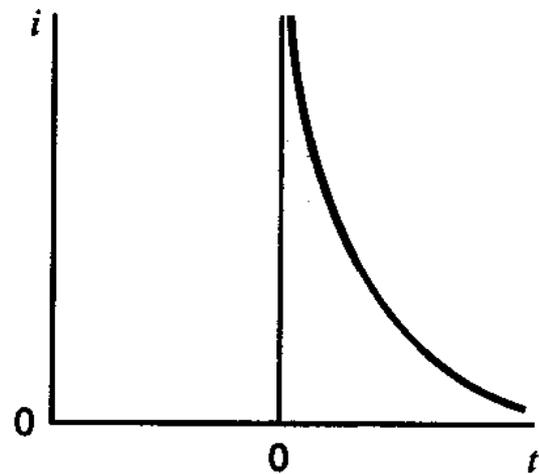
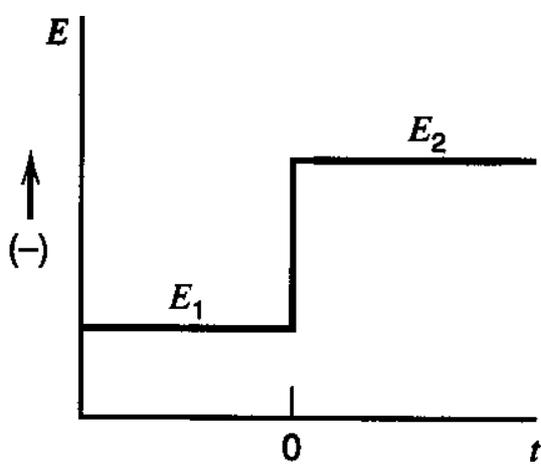
A common three electrode setup is used:

Ag/AgCl reference electrode, Pt counter electrode, working electrodes with different electrode materials such as platin, gold, pyrolytic graphite, carbon paste and glassy carbon are available.



Chronoamperometry

For many applications it is necessary to know the active area A of an electrode surface that differs from the macroscopic one. Also very often one has to determine a diffusion coefficient. This can be done by chronoamperometry if one of both parameters is known. A constant potential is applied and the resulting current i is measured.



graphics from Lit. 3

Applied Potential vs. time

Current response vs. time

Because the experiment is diffusion controlled after a certain time almost all molecules that are able to reach the electrode are reduced (oxidised). Therefore the current decreases proportional to $1/\sqrt{t}$ as described by the following Cottrell Equation:

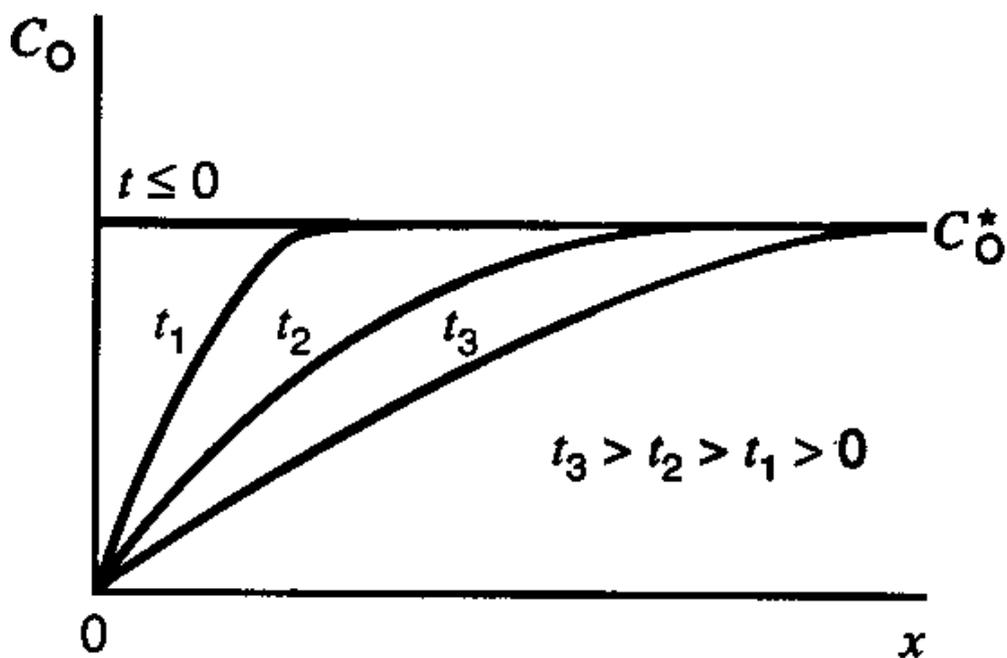
$$i = \frac{nFAD^{1/2}C^*}{\pi^{1/2}t^{1/2}}$$

By plotting the current vs. $1/\sqrt{t}$ the slope of the resulting straight line contains the desired information.

By chronoamperometric measurements also undergoing chemical reactions can be investigated in some cases.

Concentration Profile

In the following concentration profile the growth of the diffusion layer with increasing time is shown for a chronoamperic experiment. The applied potential in chronoamperometry has to be sufficient for all molecules at the electrode surface being immediately reduced.

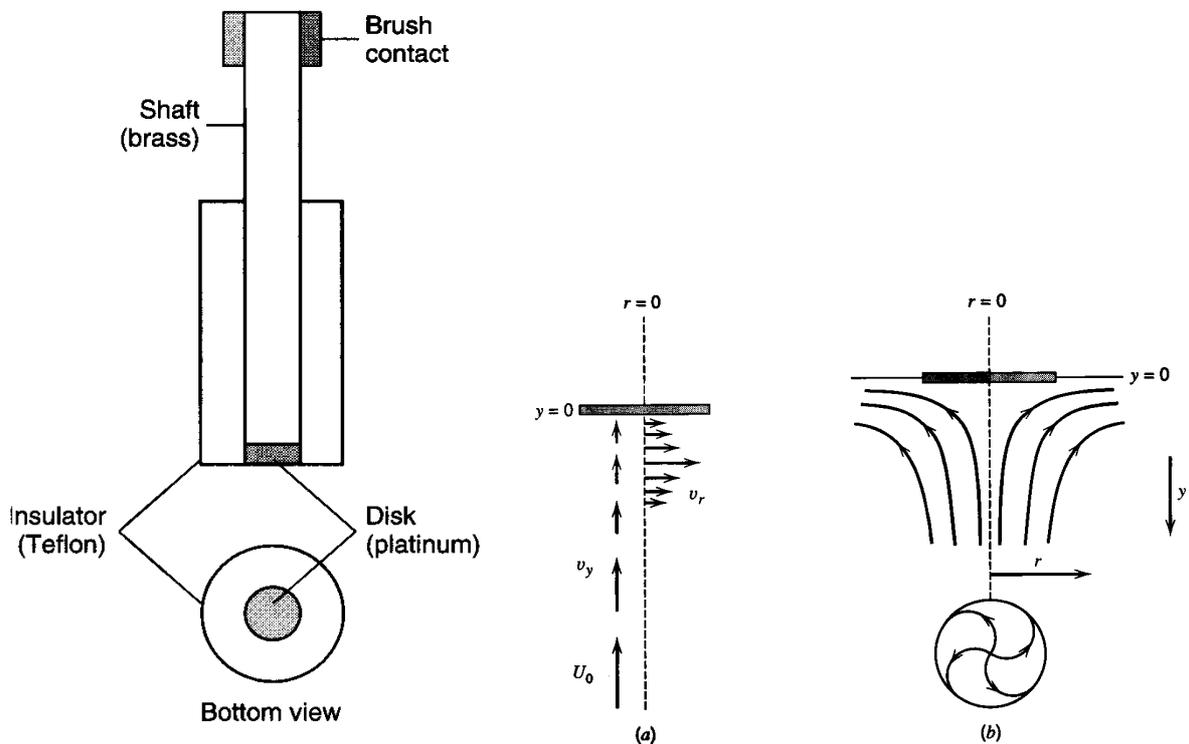


graphic from Lit. 3

Concentration of O being reduced at the Electrode vs. distance x from the planar electrode at different times t .

Lit.: Elektroanalytische Methoden I: Elektrodenreaktionen und Chronoamperometry, B. Speiser, Chemie in unserer Zeit **1981**, 15, 21-26.

The Rotating Disk Electrode



graphics from Lit. 3

Although the electrode is rotating the resulting mass transport is diffusion controlled. At totally mass transfer limited conditions the limiting current i_l is proportional to C^* and $\omega^{1/2}$ (angular velocity $\omega = 2\pi f$) as described by the Lewich equation:

$$i_l = 0.62nFAD^{2/3}\omega^{1/2}\nu^{-1/6}C^*$$

ν : kinematic viscosity of the solvent

Diffusion coefficients can be determined by plotting i_l vs. $\omega^{1/2}$ for different ω . A rotating disk electrode can also be used for various other applications. Lit.: The Rotating Disc Electrode, Yu. V. Pleskov and V. Yu. Lilinovskii, 1976, Consultants Bureau, NY.

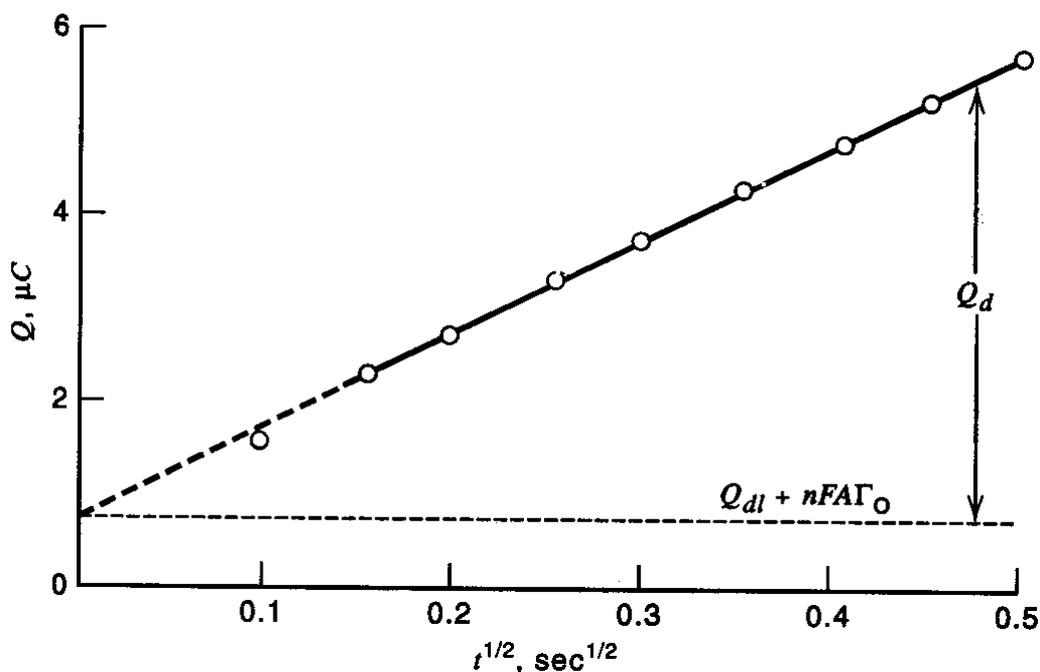
Chronocoulometry

In chronocoulometric experiments a constant potential is applied like in chronoamperometry. Not the resulting current but the resulting charge is measured. If a compound is adsorbed at the electrode surface the charge at the initial point of the experiment will be larger than without adsorbed species.

The charge Q_{dl} is needed to charge the double layer, the additional charge $nFA\Gamma$ is due to the reduction (oxidation) of the surface excess Γ [mol/cm²] of an adsorbed compound.

Many experimenters have used the value of the charge due to adsorption to calculate adsorption layer thickness.

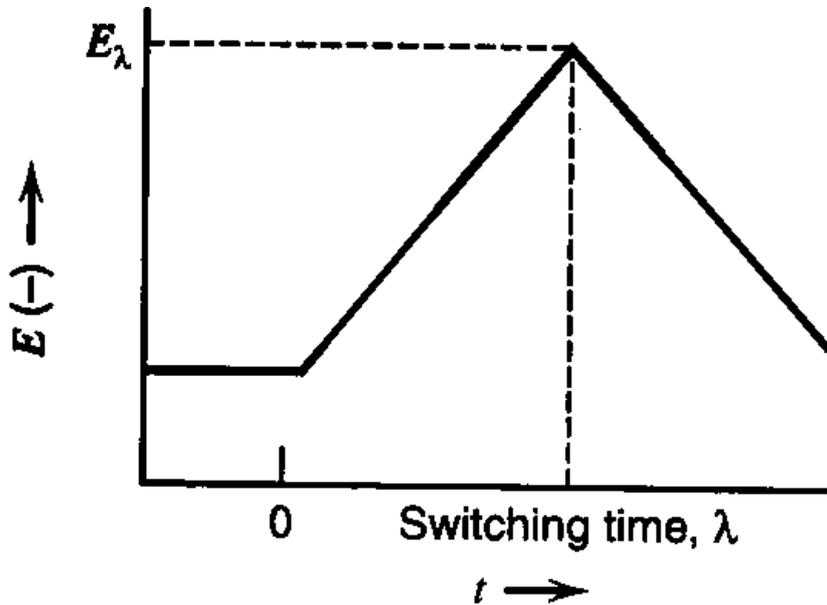
$$Q = \frac{2nFAD^{1/2}C^*t^{1/2}}{\pi^{1/2}} + Q_{dl} + nFA\Gamma$$



graphic from Lit. 3

Plot of Q vs. $\text{sqrt}(t)$

Cyclic Voltammetry



graphic from Lit. 3

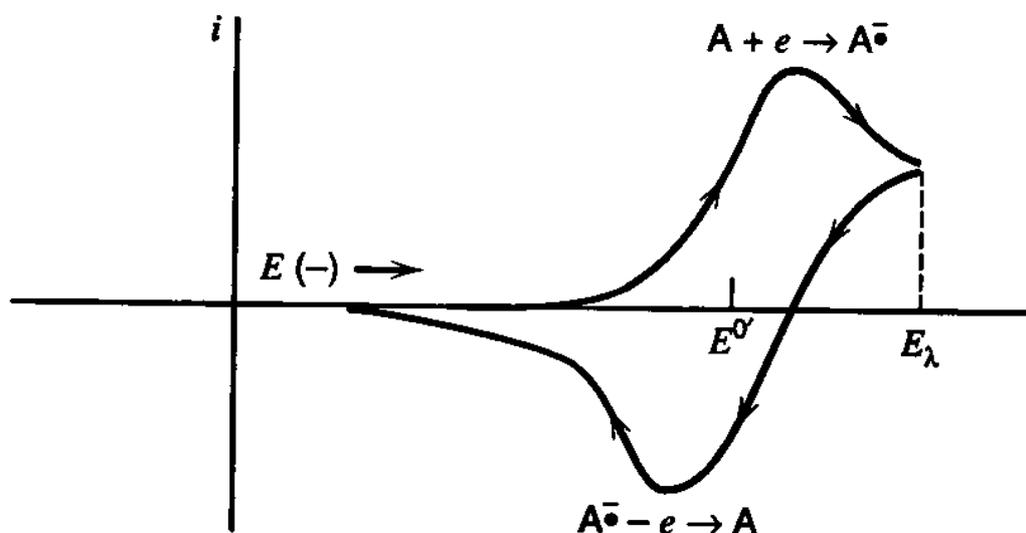
Applied Potential for CV vs. time.

Two different conventions for i vs. E plots are common:

Polaric convention: Reducing negative potential is plotted at the right side of the x-axis, the corresponding reducing current is positive and is plotted at the upper (positive) side of the y-axis.

IUPAC: Reducing negative potential is plotted at the left side of the x-axis, the corresponding reducing current is negative and is plotted at the lower (negative) side of the y-axis.

A: Semiinfinite Conditions



graphic from Lit. 3

Resulting current for a single electron reduction vs. applied current: When the negative potential is applied first only a very small current flows due to charging of the electrode. The charging current is proportional to the scan speed. At a certain potential the reduction begins to occur and the current rises with increasing negative potential. On the other hand at a sufficient potential the current starts to decrease like in chronocoulometry due to the growth of the diffusion layer into the solution. These contrary processes overlap to a resulting wave with a maximum current at a peak potential often called E_p . The current of the redox process (also called faradaic current) is proportional to the square root of the scanning speed v . By changing the direction of the potential (i. e. scanning back to the starting value) the reduced compound is re-oxidised again if the redox reaction is electrochemically reversible. This results in a second wave. The (half way) Potential (often called $E_{1/2}$) of a reversible redox process is equal to E_0 . $E_{1/2}$ is determined by adding the peak potentials E_p of the forward and backward scan and dividing by two.

Electrochemical Reversibility:

Electrochemical reversibility has nothing in common with the chemical or even thermodynamic meaning of reversibility. It just means that no side reaction occurs within the time scale of the electrochemical experiment. Therefore it is strongly depending on the reaction conditions e. g. scanning speed.

For reversible diffusion controlled CV-measurements one should get two symmetric waves (with the same area under the curves) in the i vs. E plot with a potential difference of about 60 mV due to diffusion.

B: Ultramicroelectrodes

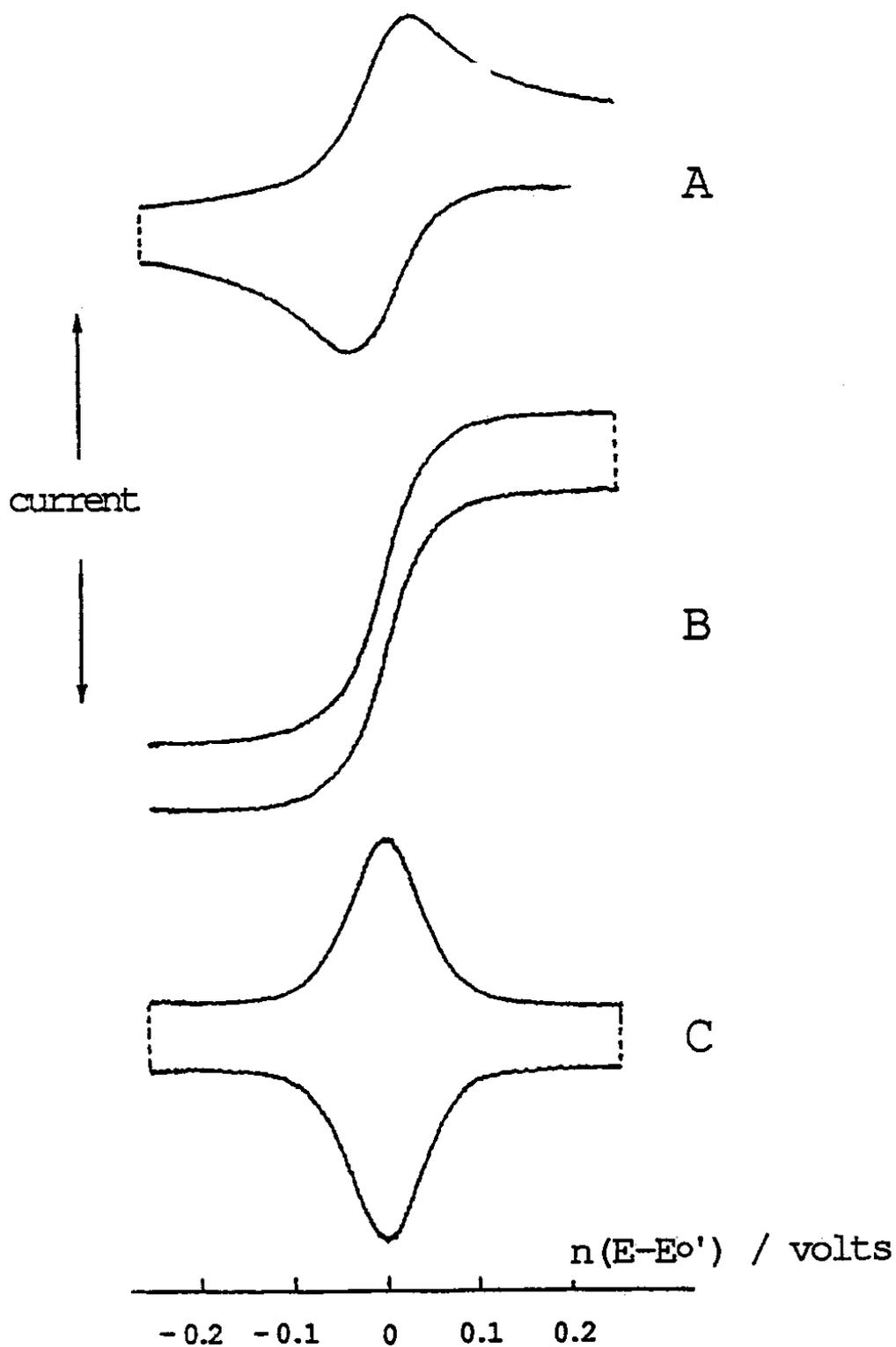
By having spherical diffusion (due to the use of ultra micro electrodes) the current would not decrease but keep its maximum constant value. This current is called the (diffusion) limited current and is independent of the scanning speed. One gets a sigmoidal curve for i vs. E .

C: Thin layer measurements/measurement of compounds adsorbed to the electrode surface

When a cyclic voltammetric experiment is done in a thin layer cell $<$ diffusion layer or with a redox active compound adsorbed at the electrode surface the process is not diffusion controlled any more. Therefore there is no peak separation between E_p of reductive and oxidative wave any more. The current is direct proportional to the scan rate v .

Obtained results of CV experiments can be proofed by fitting the results or by digital simulation.

Plot of i vs. E for different boundary conditions A, B, C.

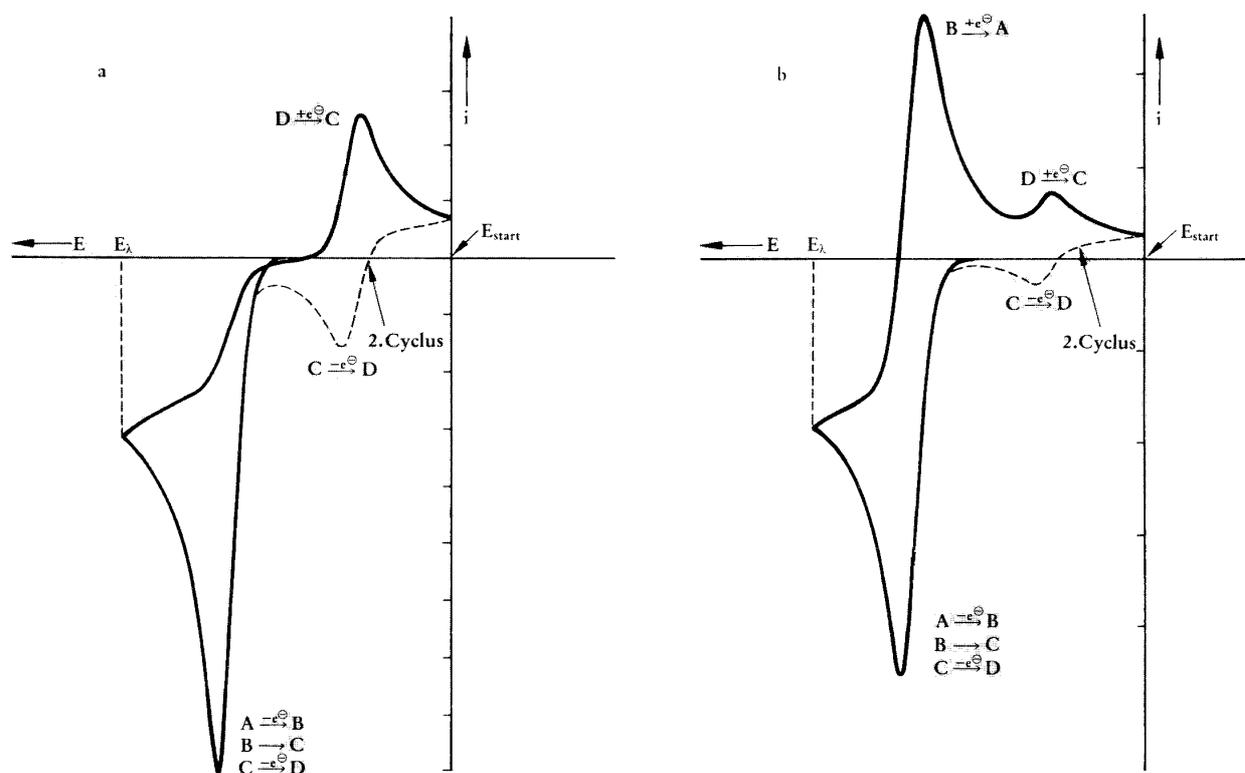
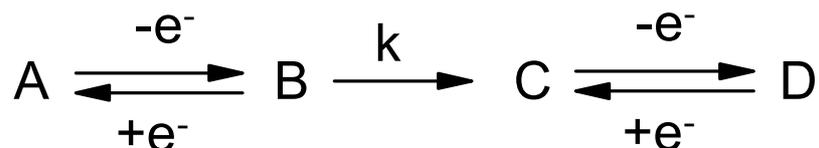


graphic from Lit. 1

note that here IUPAC convention is used.

Mechanistic Studies

Cyclic voltammetry is very a useful tool to study kinetic processes. One example for an ECE process (electrochemical reaction, chemical react., electrochem. react.) at two different scan rates is shown above.



from Lit:

Elektroanalytische Methoden II: Cyclische Voltammetrie, B. Speiser, Chemie in unserer Zeit **1981**, 15, 62-67.

Square Wave Voltammetry

Many pulse techniques have been originally developed to enhance the sensitivity of polarographic methods e. g. to avoid charging currents. Polarographic experiments are very powerful for the determination of ions in low concentration. A dropping mercury electrode is used.

The most powerful pulse technique that is also applicable for solid electrodes is the square wave technique. This is mainly due to the elimination of charging current. SWV can be used for analytical, kinetic and mechanistic work.

Advantages over CV:

Faster scan rates are possible

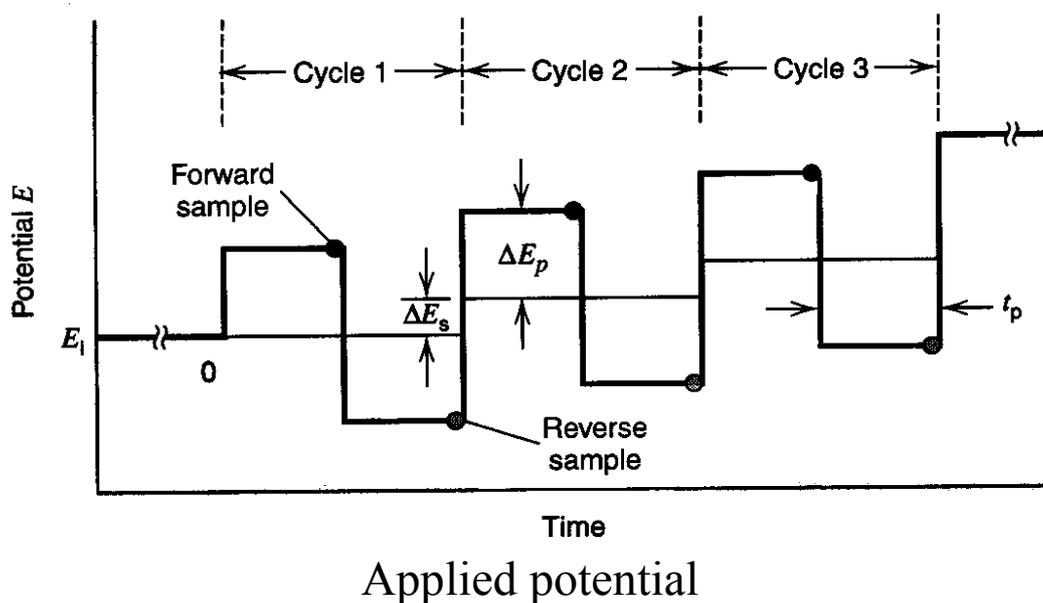
(faster reactions can be studied)

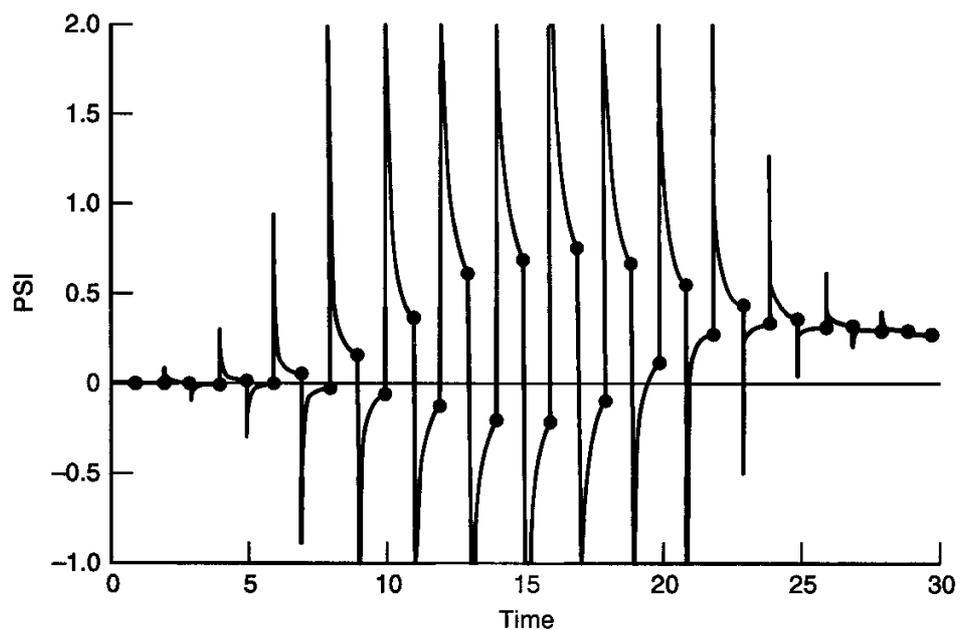
Higher sensitivity

(lower concentrations can be used)

Higher Dynamic range

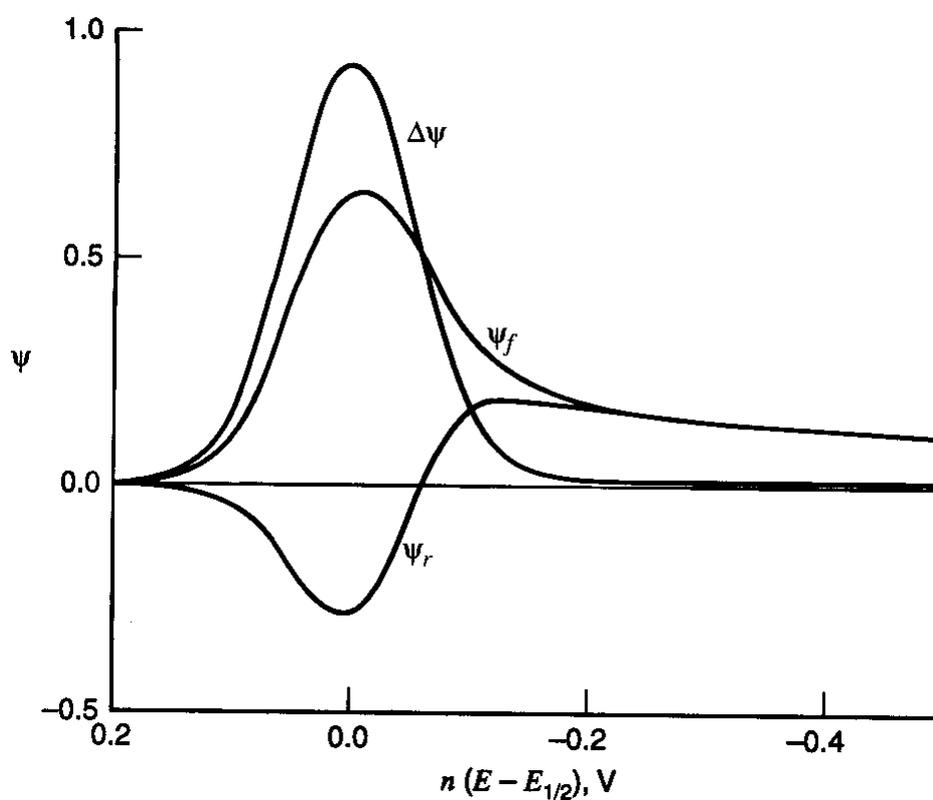
(a larger range of concentrations can be investigated)





graphic from Lit. 3

resulting current vs. t



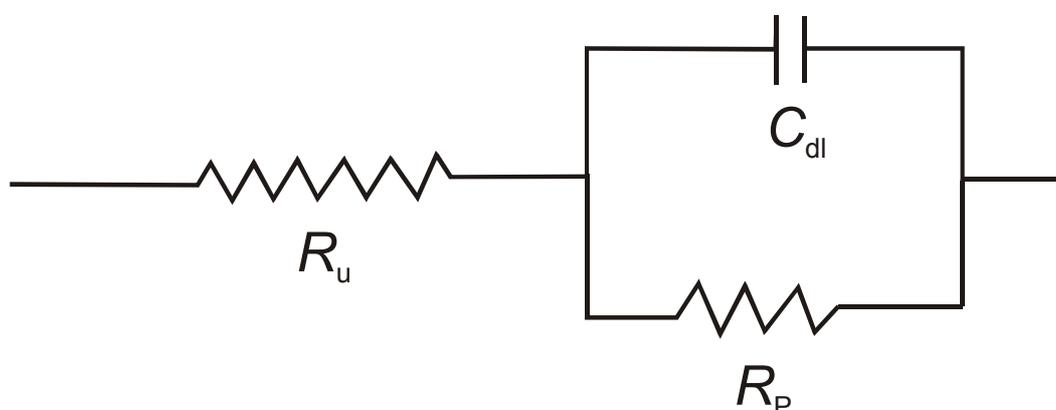
graphic from Lit. 3

three different current plots vs. E
the current is proportional to the scanning speed.

Impedance Spectroscopy

The terms impedance and resistance both denote an opposition to the flow of electrons or current. In direct current **dc** circuits only resistors produce this effect. In alternating current **ac** circuits two other elements, capacitors and inductors, impede the flow of electrons. The impedance Z can be expressed as a complex number, where the resistance is the real component and the combined capacitance and inductance is the imaginary component.

A simple electrochemical cell can be represented by the following equivalent circuit, where R_p is the polarisation or charge transfer resistance at the electrode/solution interface:



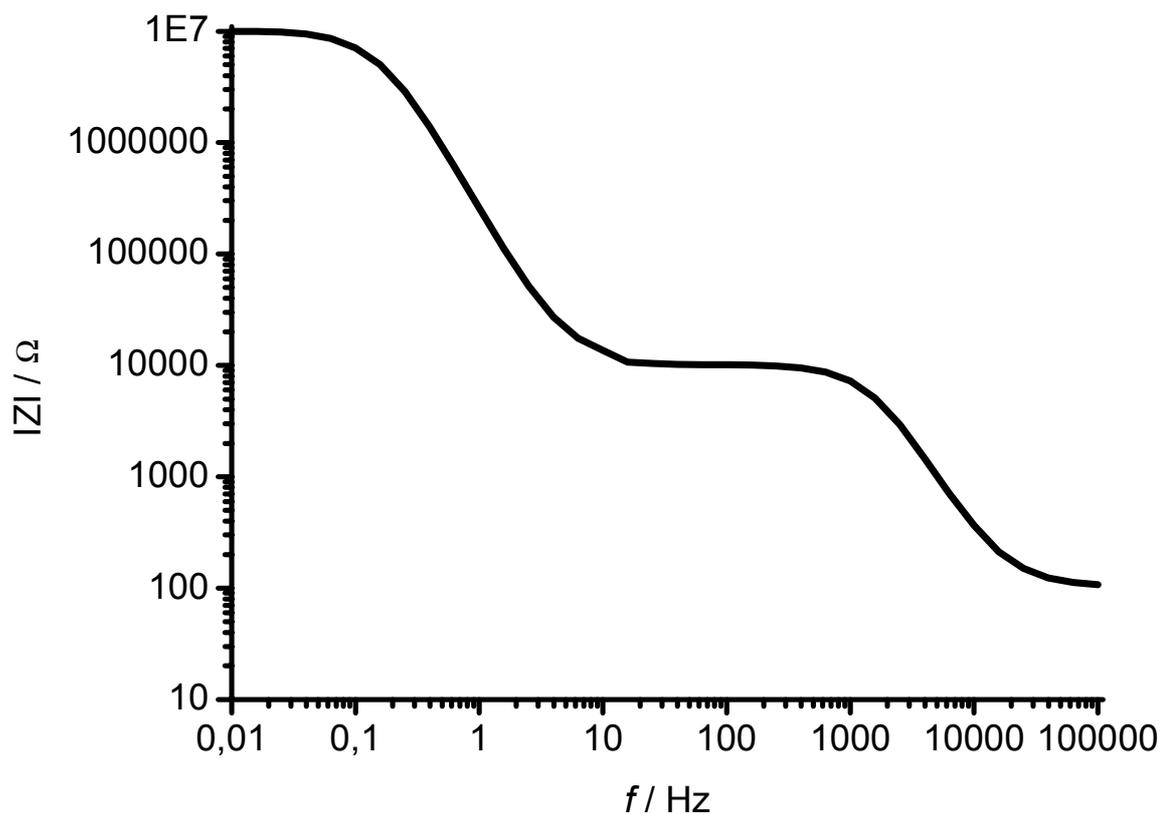
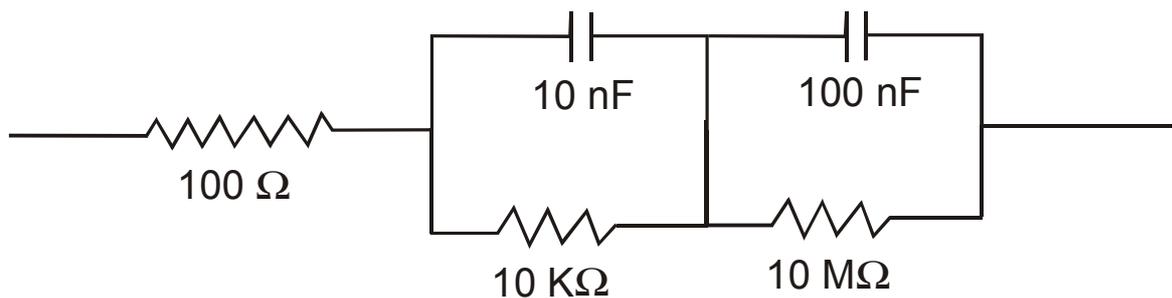
At low frequencies the impedance of the cell is the combined resistance values of R_{Ω} and R_p , the capacitor acts as open circuit.

Above a certain frequency the impedance of C_{dl} becomes much smaller than R_p and removes R_p from the circuit.

The impedance of the capacity diminishes as the frequency increases. At high frequencies the impedance is controlled almost entirely by R_u .

The impedance of an equivalent circuit is depending on the frequency of ac. Therefore the impedance is measured with respect to the frequency, a usual range is 10^{-3} Hz to 10^4 Hz. At the beginning of impedance spectroscopy the impedance at different frequencies have been measured separately.

Today also pulse techniques can be used to reduce the time of measurement. To interpret the experiment a lot of different plots are usual. One possibility is to plot $\log(|Z|)$ vs. $\log(f)$:



Impedance measurements are done quite easy. The difficulty is to apply the right open circuit model that represents the experimental conditions.

Electrochemistry of redox Proteins

Redox active proteins contain redox active groups (e. g. FAD, FMN or heme groups) as part of a coenzyme. These coenzymes are surrounded by the major non redox active part of the protein, the so called apoenzyme.

Coenzyme and apoenzyme can be covalently linked in some proteins but they don't have to.

Therefore one irreversible process in protein electrochemistry could be the loss of a cofactor.

Problems in protein electrochemistry

Slow rates of electron transfer to or within proteins

A special orientation Protein-electrode has to be achieved (e.g. modification of the electrode surface is necessary.)

Adsorption at the electrode surface

Therefore protein samples should be purified very carefully. Special modified electrode surfaces have to be applied.

Slow diffusion of the protein

Some redox active electron carriers i. e. mediators have to be used. Also modification of the electrode surface is helpful.

ET reactions often are coupled with proton transfer

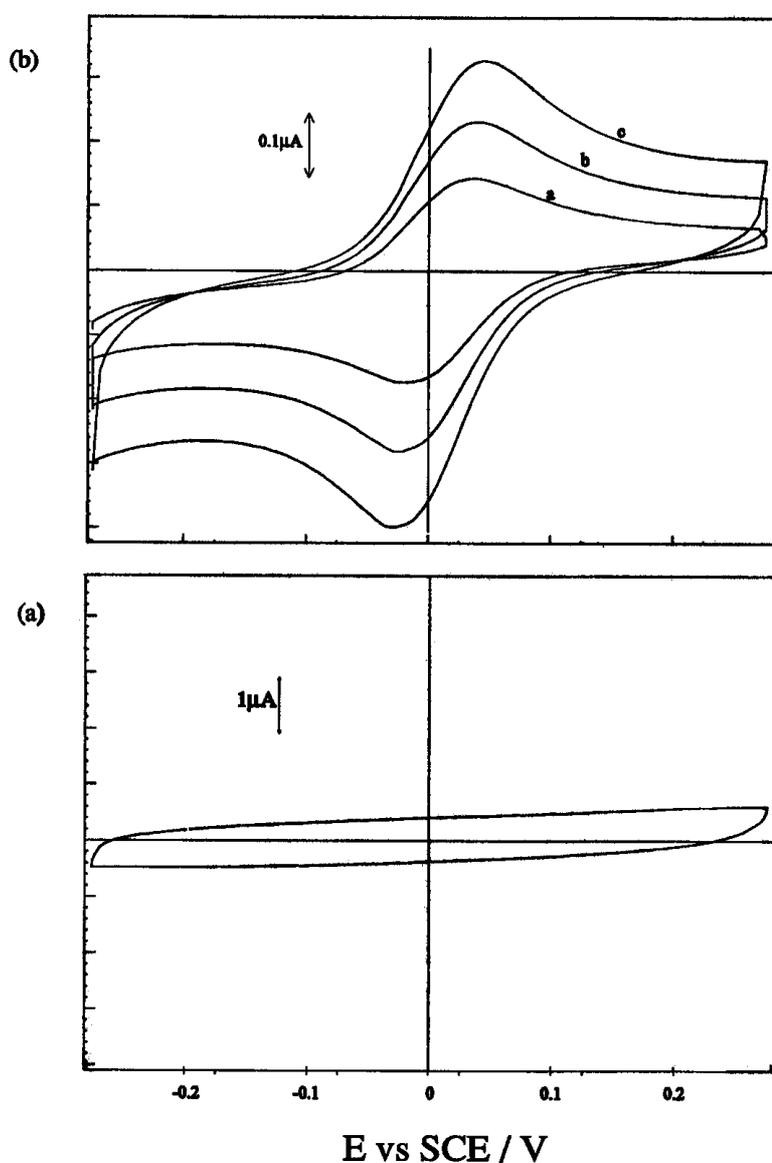
The redox potentials are strongly pH dependent. ECE mechanisms have to be taken into account.

Lit.: Electrochemical behaviour of FAD at a gold electrode studied by electrochemical quartz crystal microbalance, Y. Wang, G. Zhu, E. Wang, Anal. Chim. Acta 1997, 338, 97.

Electrode Materials

Common electrode materials are: Mercury, Gold, Platin, Silver, Nickel, Metaloxides (e. g. ito), carbon materials.

In 1977 a well defined one electron reduction Fe(III)/Fe(II) in cytochrome *c* was observed at a gold electrode modified by 4,4'-bipyridyl serving as a promoter at the electrode surface:



Lit.: M. J. Eddowes, H. A. O. Hill, J. Chem. Soc., Chem. Com. 1977, 45, 771.

MODIFIED ELECTRODES

ADSORPTION OF
THIOLS/DISULPHIDES
ON Au/Pt

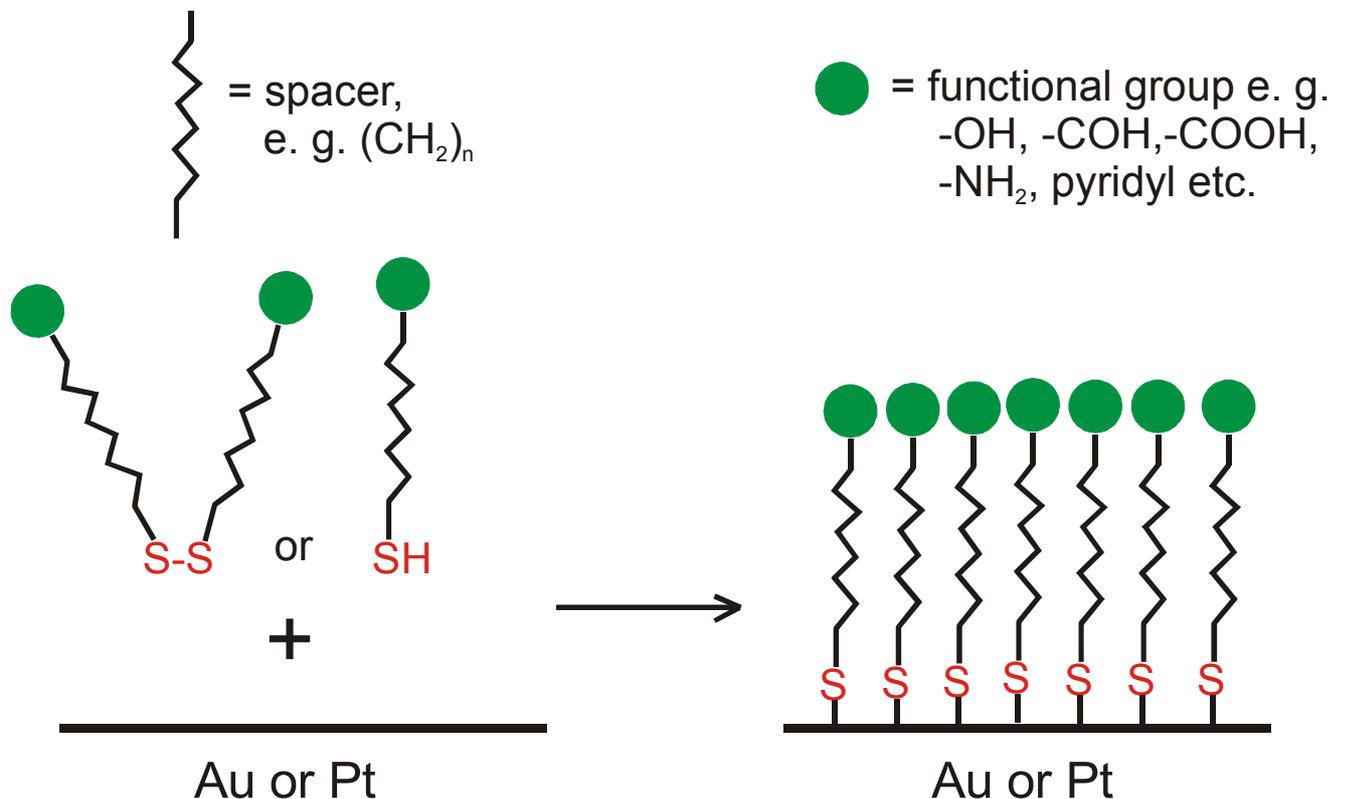
COATING WITH LIPID
BILAYERS CONTAINING
IMMOBILISED PROTEIN

ELECTRO-
POLYMERISATION

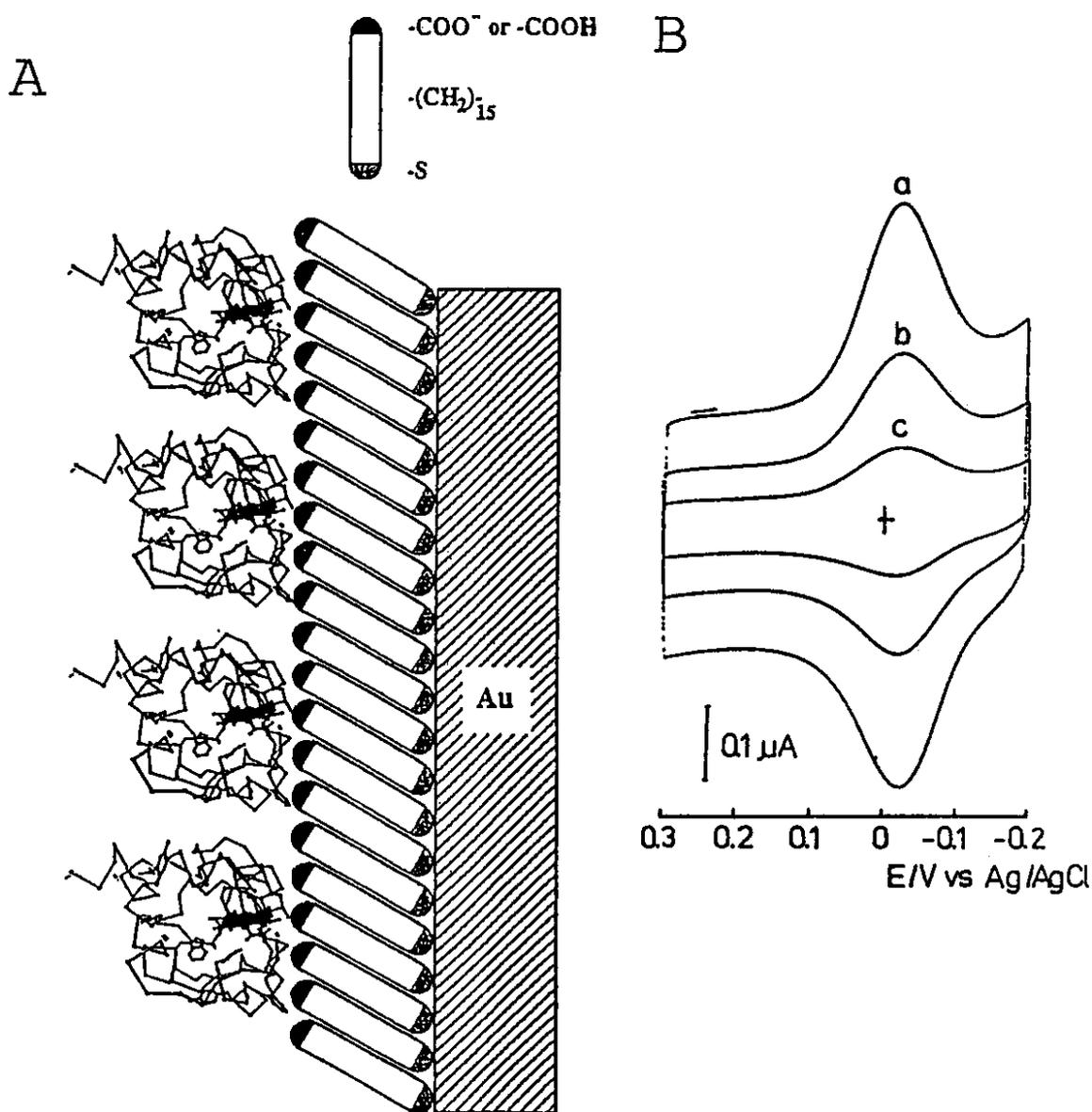
COVALENT
ATTACHMENT

Adsorption of Thiols/Disulphides

Surface modification of the electrode by adsorption of thiols or disulphides generally yields monolayer coverages. Various functional groups can be introduced by this technique:



This technique has been applied to investigate cytochrome *c*:



A: Hypothetical structure of an ideal ordered and oriented composite layer of cytochrome *c* molecules bound to a monolayer of alkanethiol carboxylic acid.

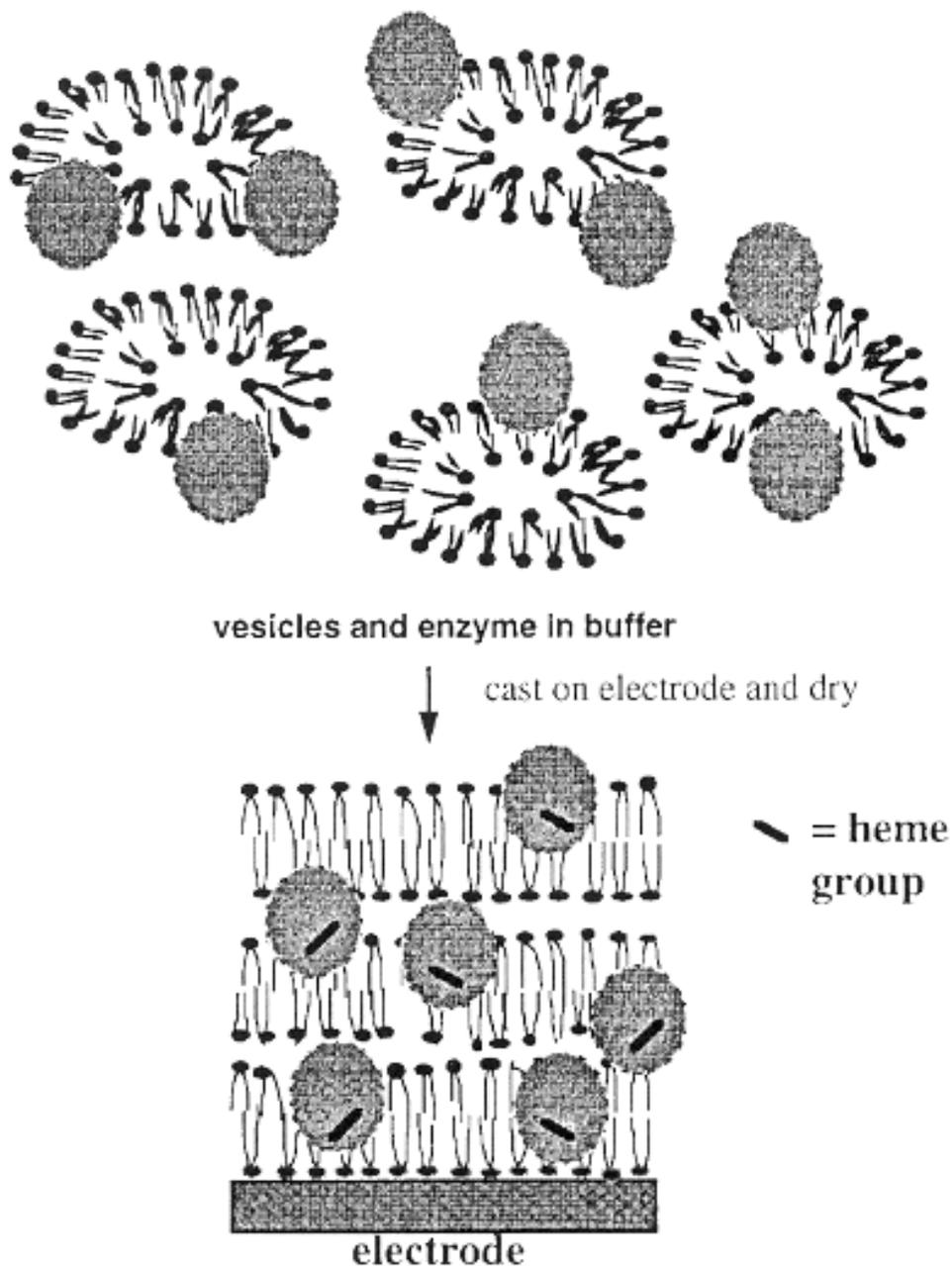
B. Corresponding voltammograms, $v = 200, 100$ and 50 mV/s.

from Lit.: S. Song, R. A. Clark, E. F. Bowden, M. J. Tarlov, J. Phys. Chem. 1993, 97, 6564-6572.

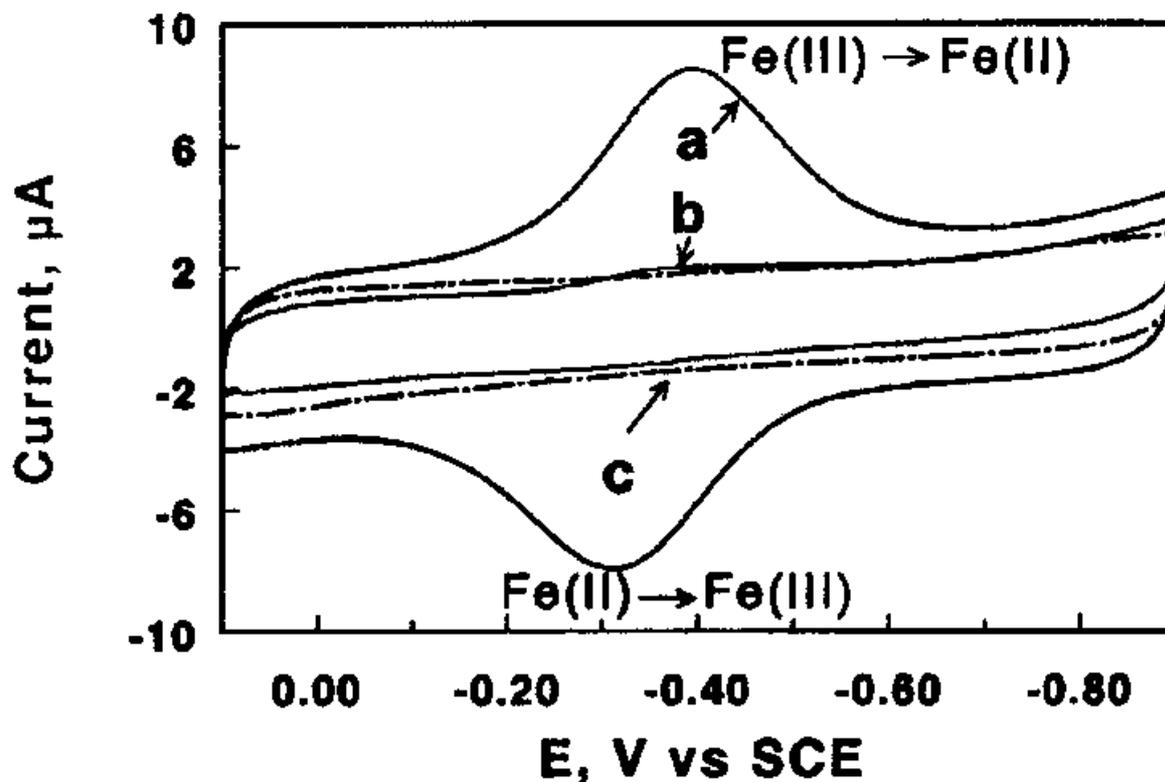
Coating electrode surfaces with lipid bilayers containing immobilised protein

Some insoluble surfactants used for cast films:

Lauric acid, DDAB: didodecyldimethylammonium bromide, PSS: polystyrenesulfonate, DHP: dihexadecyl phosphate, DMPC: dimyristoylphosphatidylcholine



Representation of the cast vesicle method for film preparation.



corresponding CVs

- a: cytP450-DMPC film on basal plane PG electrode in solution without cytP450
- b: bare electrode in solution containing cytP450
- c: DMPC film in solution without cytP450

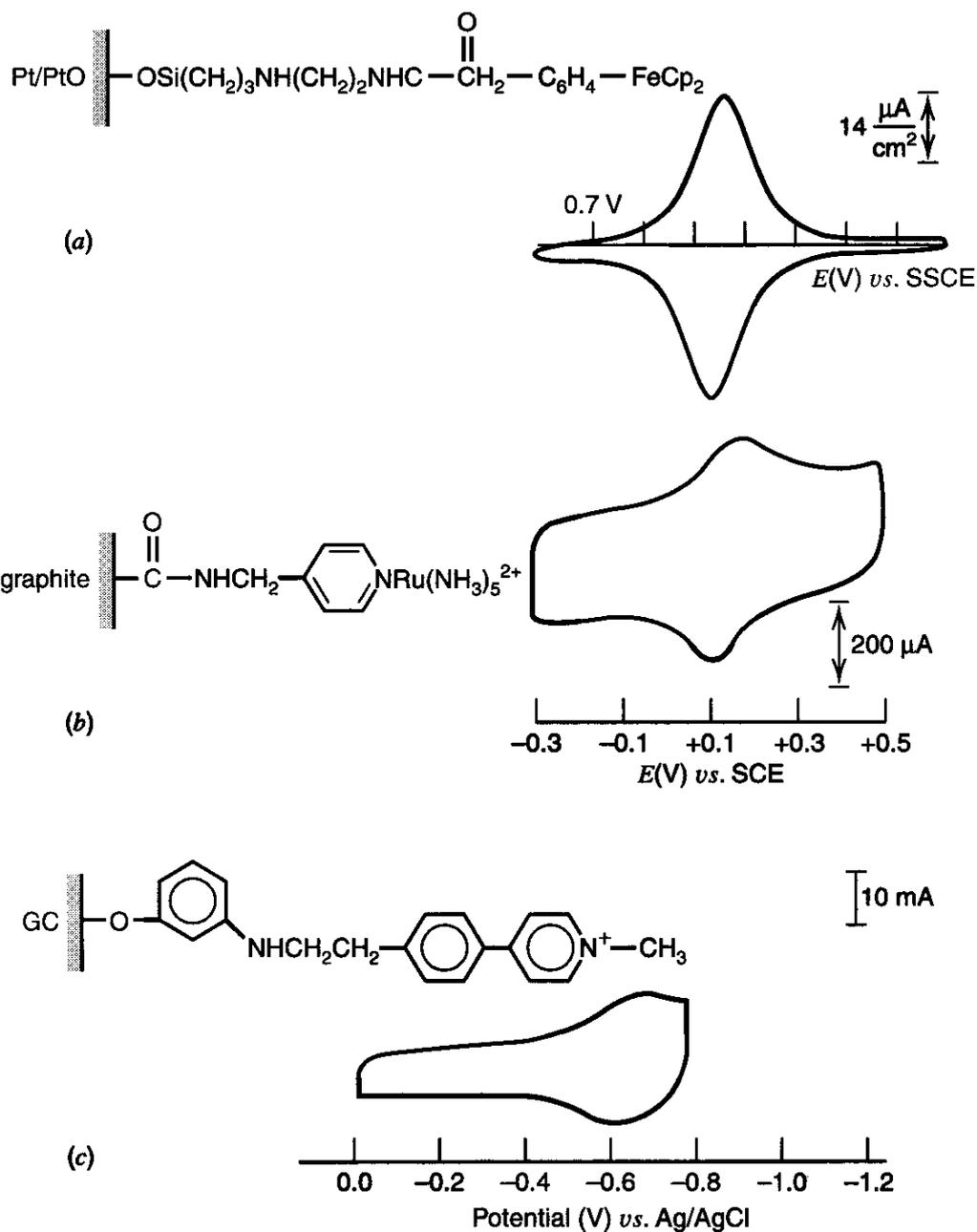
from Lit.: Enzyme Bioelectrochemistry in Cast Biomembrane-like Films, J. F. Rusling, Acc. Chem. Res. 1998, 31, 363-369.

Electropolymerisation

Fairly thick films, containing many more electroactive sites than a monolayer, can be formed. Several different types of polymers have been used. Electroactive polymers contain redoxactive groups covalently linked to the polymer backbone e. g. Polyvenylferrocene, Poly(xylylviologen), Poly(4-vinylpyridine), Polyaniline etc.

Covalent Attachment

Covalent attachment can be done by the use of cyanuric chloride, carbon functionalisation or silanisation. Some examples are shown above:



a: Pt electrode with attached ferrocene, $v = 200 \text{ mV/s}$.

b: Graphite with attached $\text{py-Ru}(\text{NH}_3)_5$, $v = 5 \text{ mV/s}$.

c: Glassy carbon with attached viologen, $v = 100 \text{ mV/s}$.

graphic from Lit. 3

Carbon Electrodes

Carbon paste:

Graphite powder is used together with certain binders. Electrode characteristics can be modified by additives.

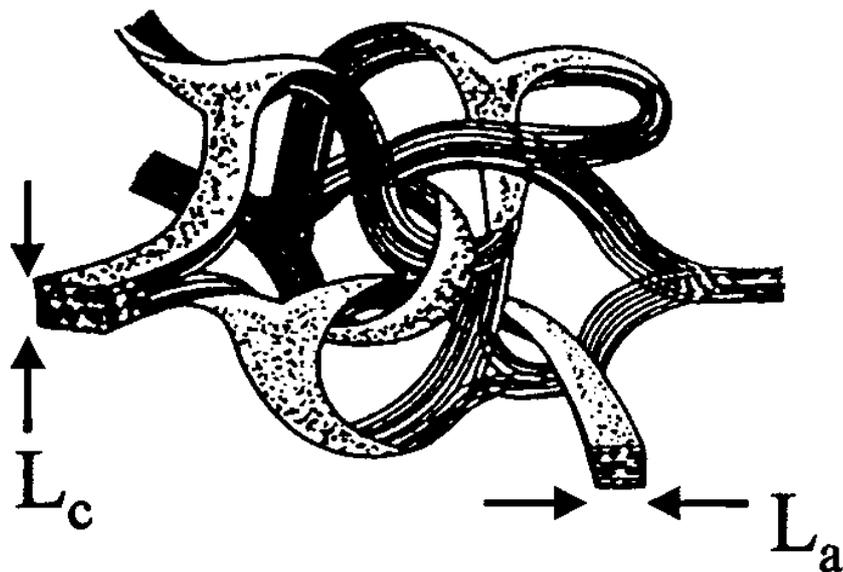
Glassy carbon:

GC is made by heat treating an organic polymer, often polyacrylonitrile or phenolic resin.

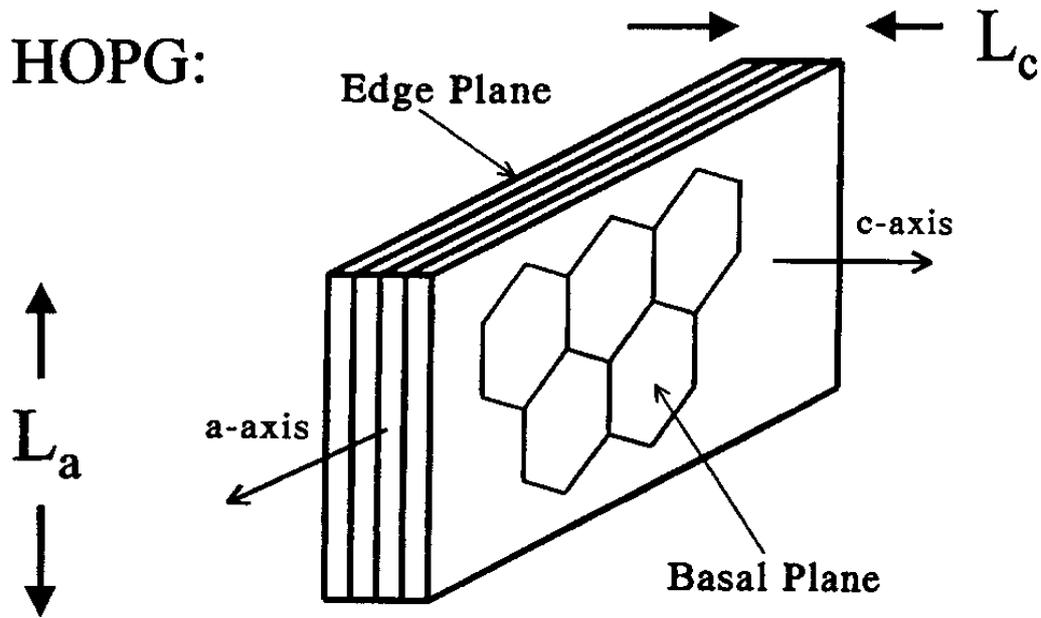
(Highly ordered) pyrolytic graphite HOPG

HOPG is made of hydrocarbons by a pyrolytic process. The material and its conductivity is strongly anisotropic. It can be used at the edge or at the basal plane.

Glassy carbon:

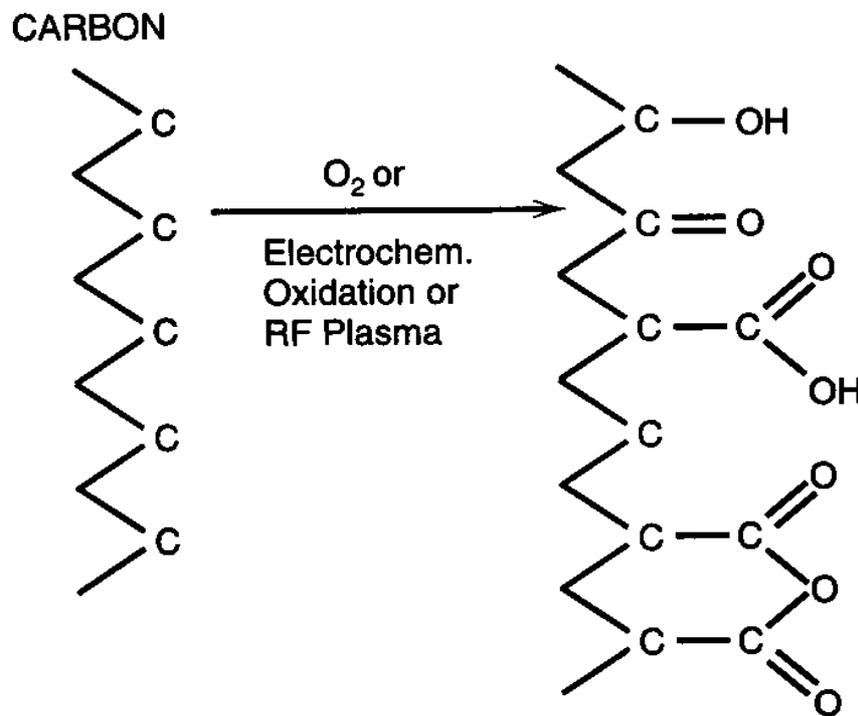


HOPG:



graphics from Lit.:
Interfacial Chemistry, A. Wieckowski 1999, M. Dekker, NY

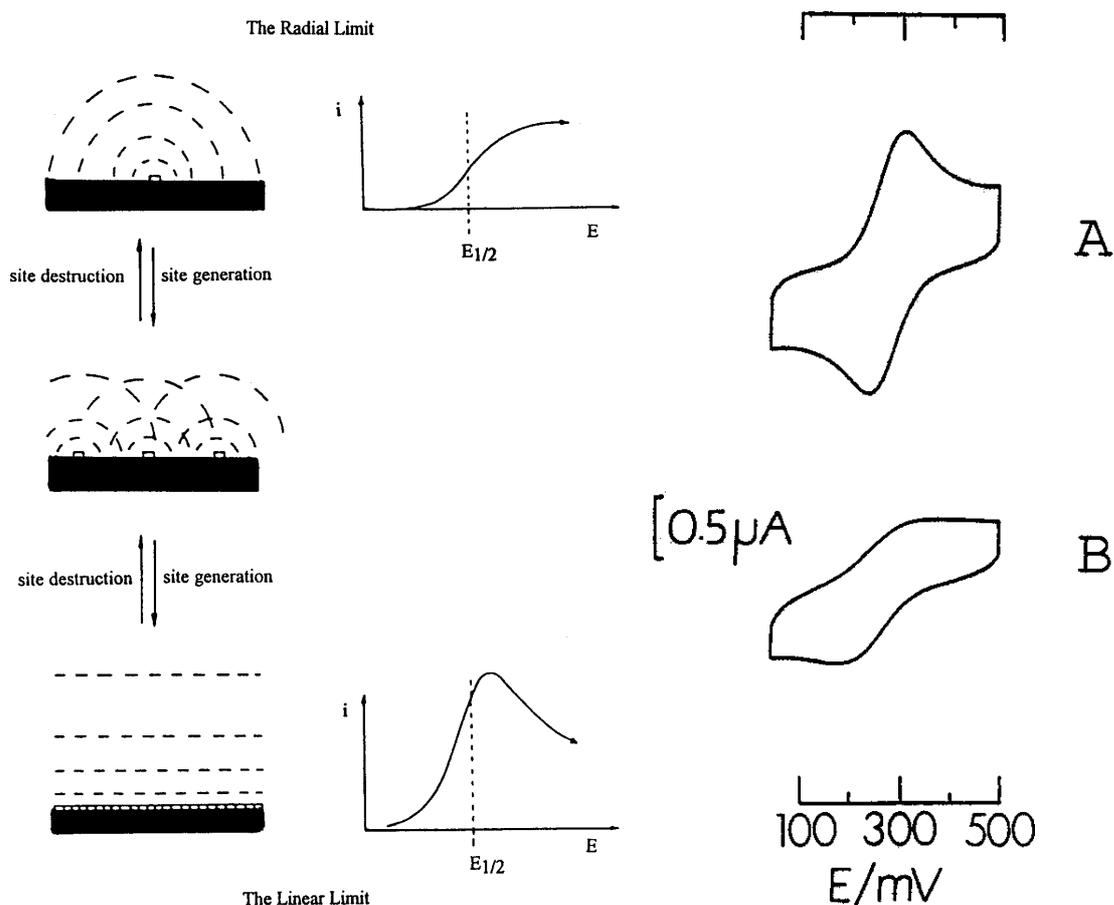
Functional groups are available by heating, polishing or the use of oxidating acids:



graphic from Lit. 3

An electrode of HOPG edge oriented is much higher in conductivity (~ 1000 times) than with basal plane orientation.

A basal plane oriented HOPG electrode behaves like an isolator containing conducting “islands”. The diffusion is in between linear and spherical diffusion.



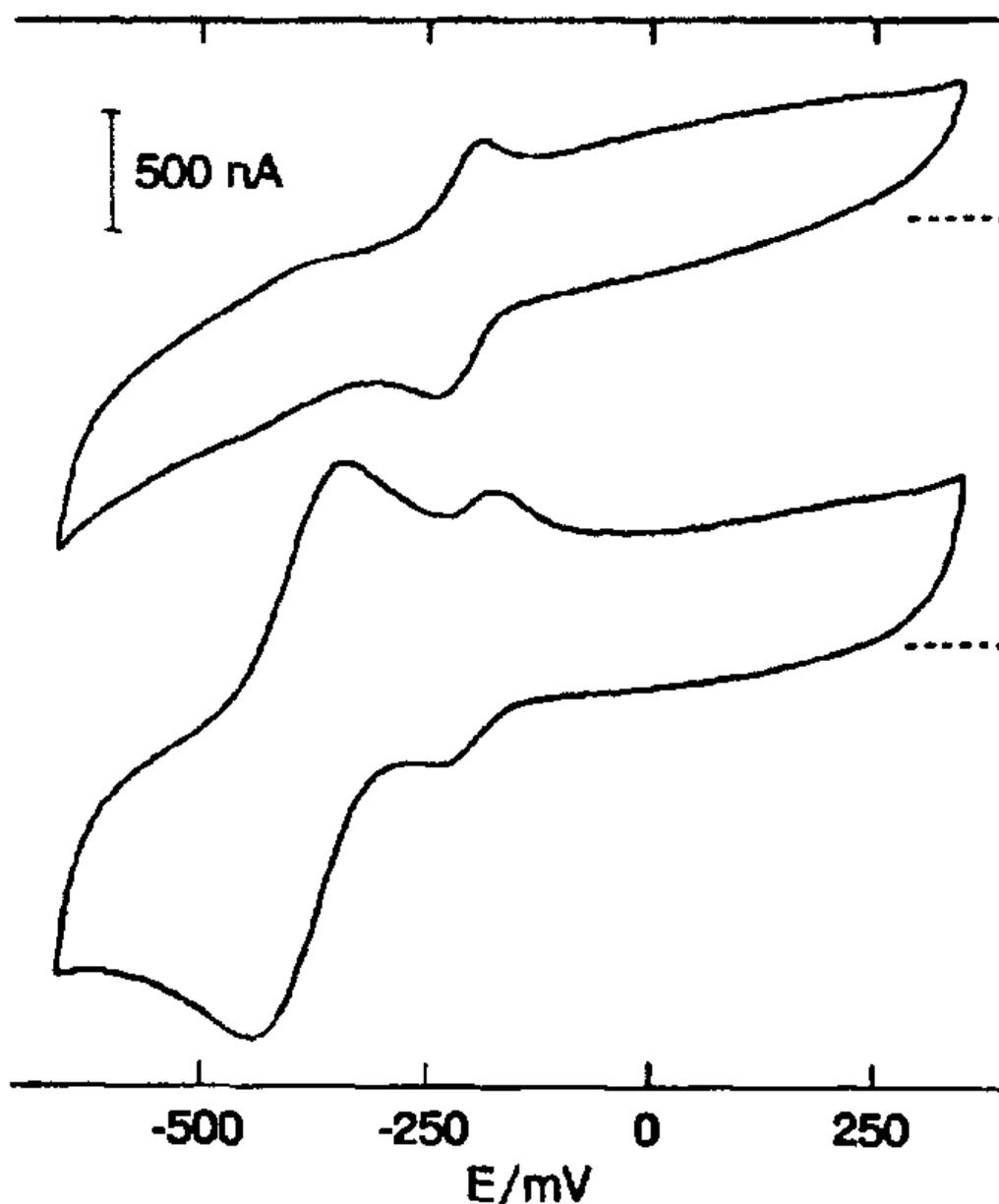
CVs of cytochrome *c* at PG electrodes

a: freshly polished edge oriented PG

b: freshly cleaved basal plane PG

graphics from Lit. 1

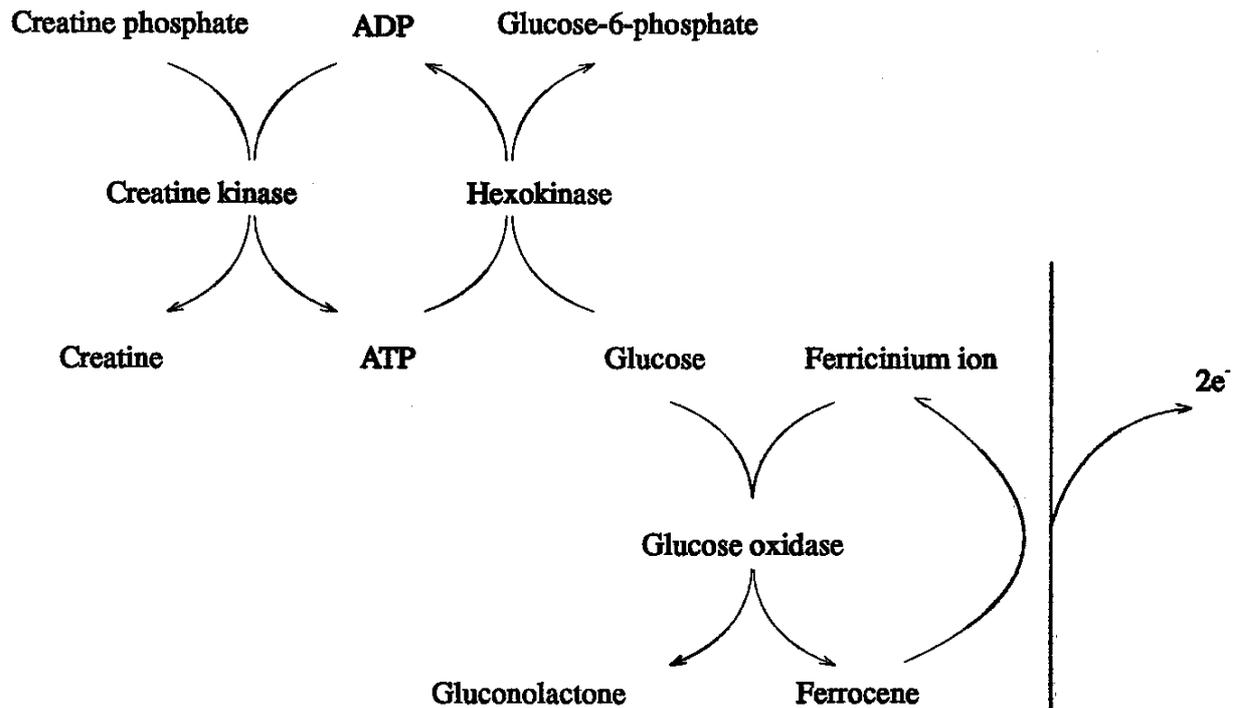
Complex electrochemistry of flavodoxin at carbon-based electrodes: Results from a combination of direct electron transfer, flavin-mediated electron transfer and comproportionation, H. A. Heering, W. R. Hagen, *J. Electroanal. Chem.* 1996, 404, 249-260.



CV of flavodoxin without and with promoter.

Biosensors

Reaction scheme depicting the creatine kinase assay via ferrocene mediated glucose oxidase.



graphic from Lit. 1