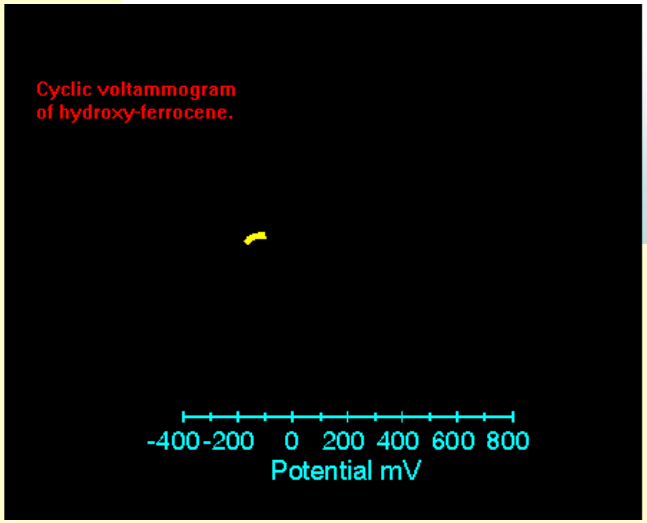
VOLTAMMETRY in the service of PHARMACY; MEDICINE AND CHEMISTRY

Rubin Gulaboski

УГД-ШТИП, 12. 10. 2017-Stip, Macedonia-

Faculty of Medical Sciences

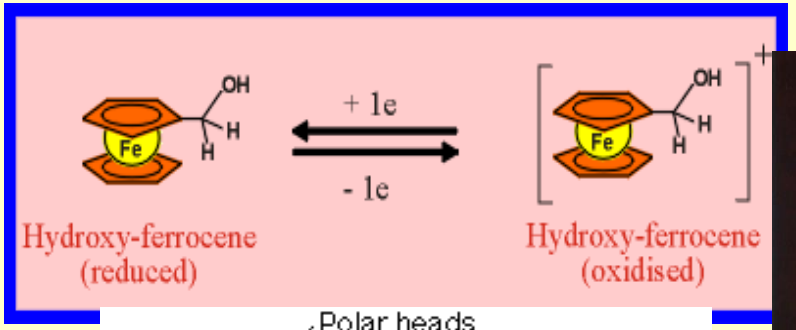
Goce Delcev University, Stip, Macedonia

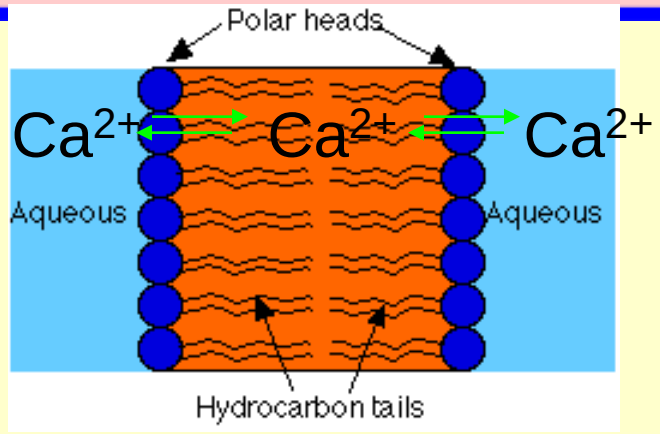


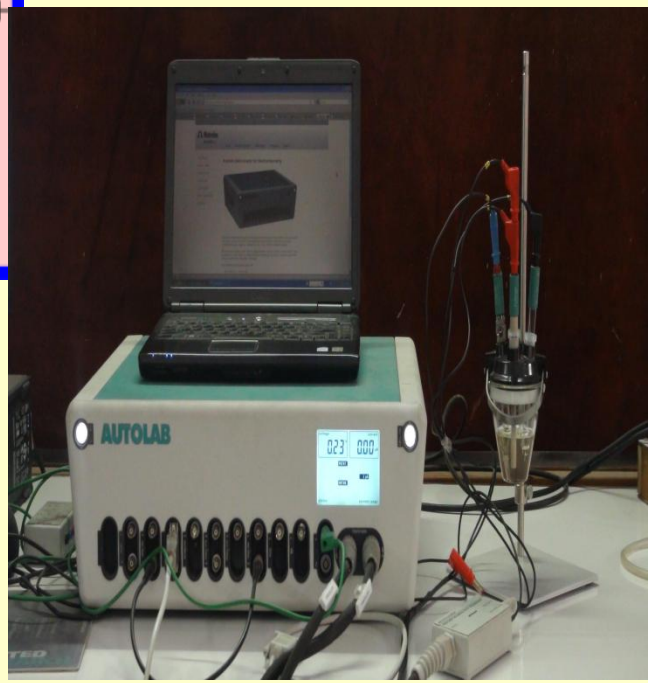
VOLTAMMETRY is a branch of *ELECTROCHEMISTRY*

-deals wity the processes of CHARGE transfer between two systems

-FLOW of electric charge=CURRENT

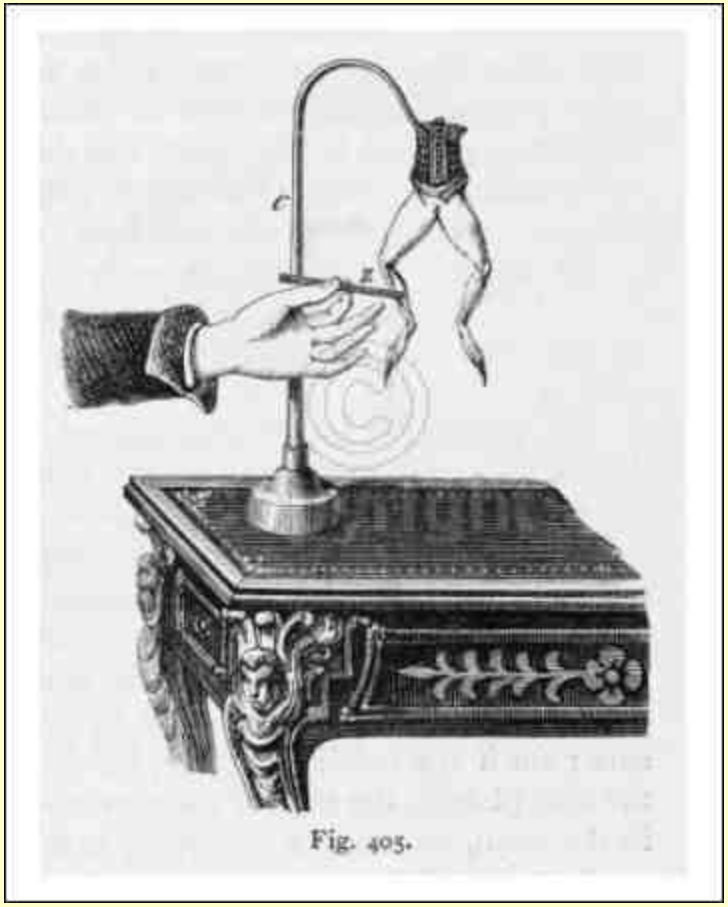


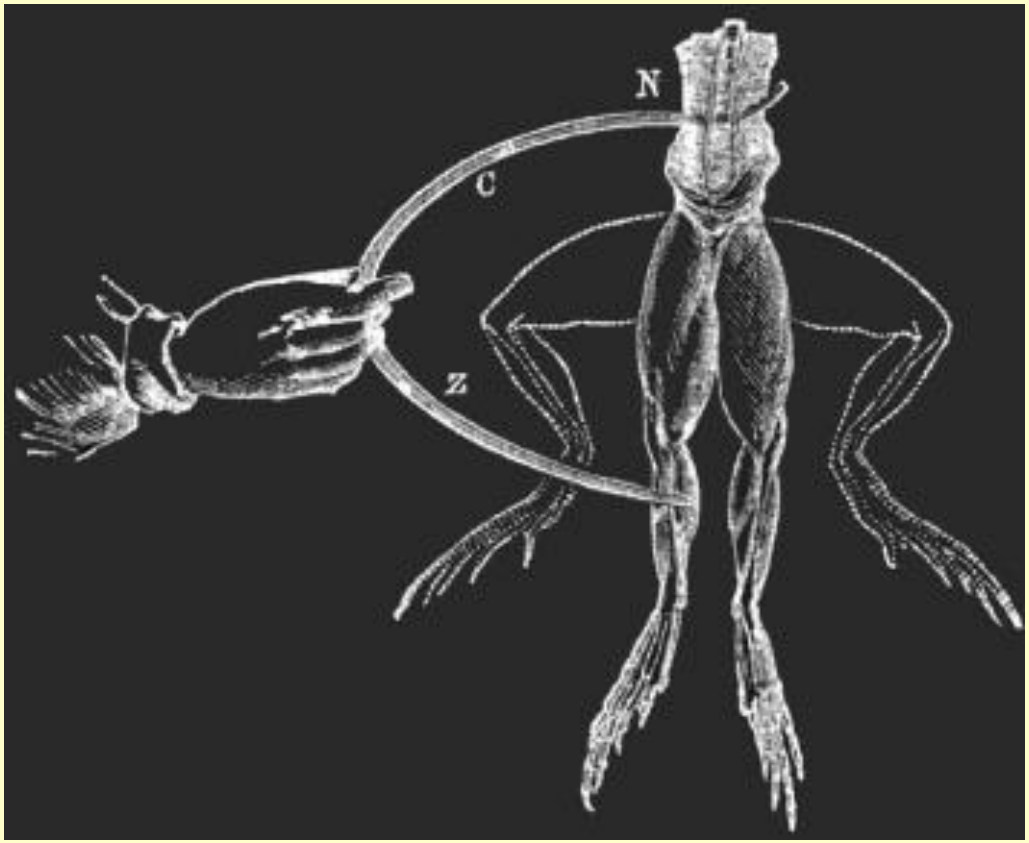


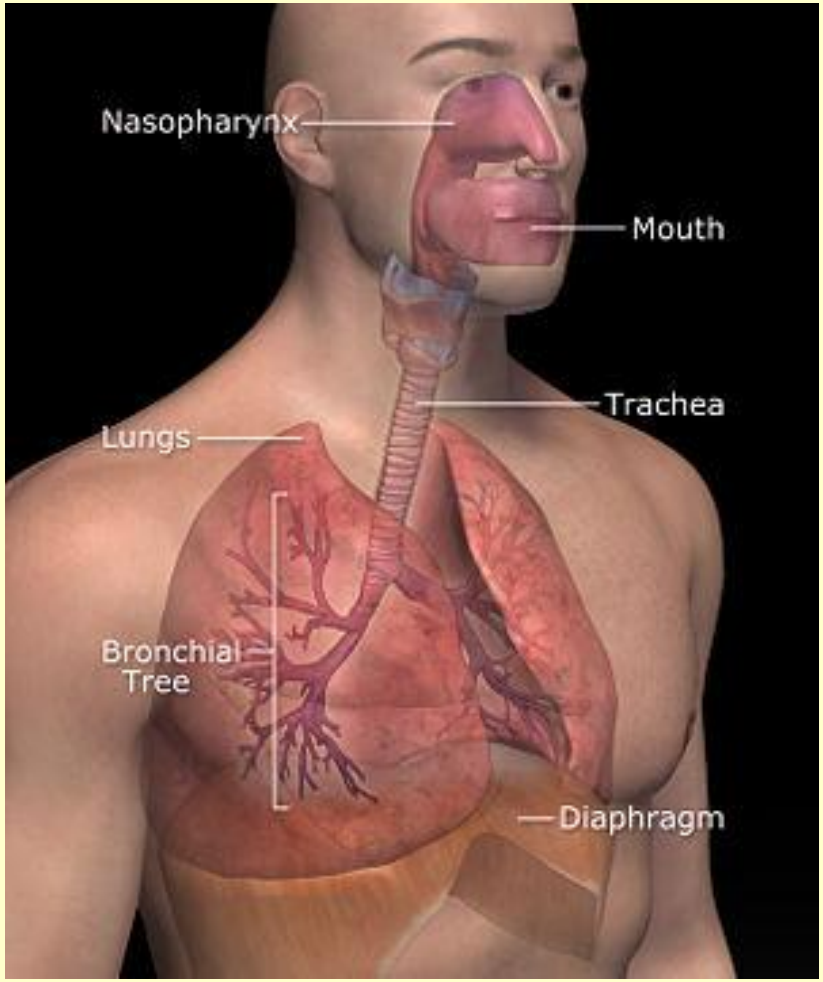


We can say-voltammetry deals MAINLY with Processes of oxidation and reduction

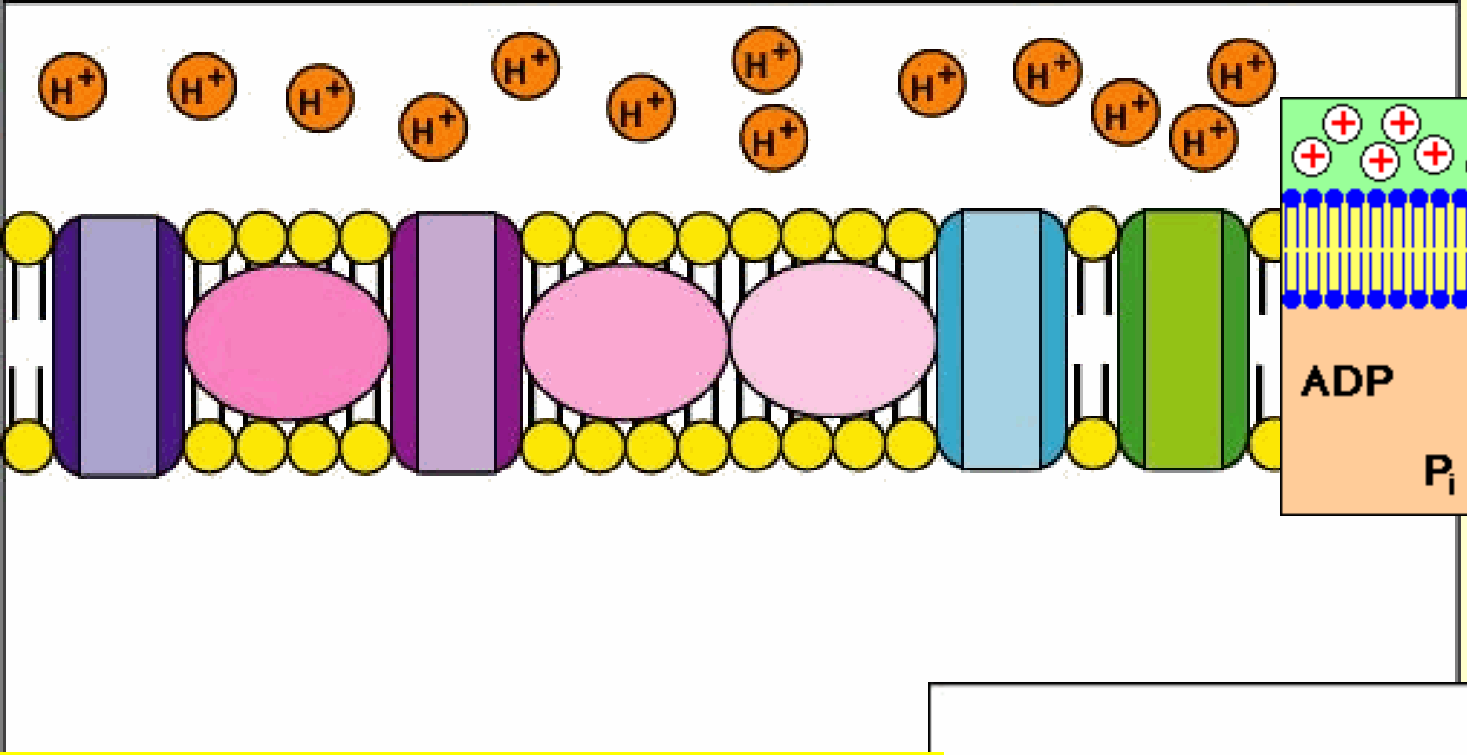
Galvani experiment on frog legs ---first unintentionally designed Electrochemical experiment

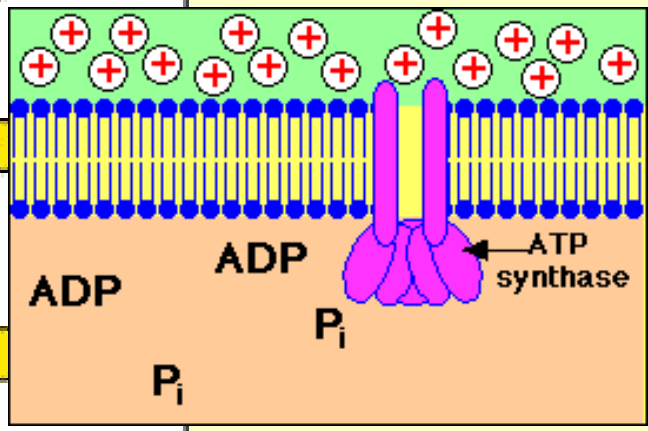




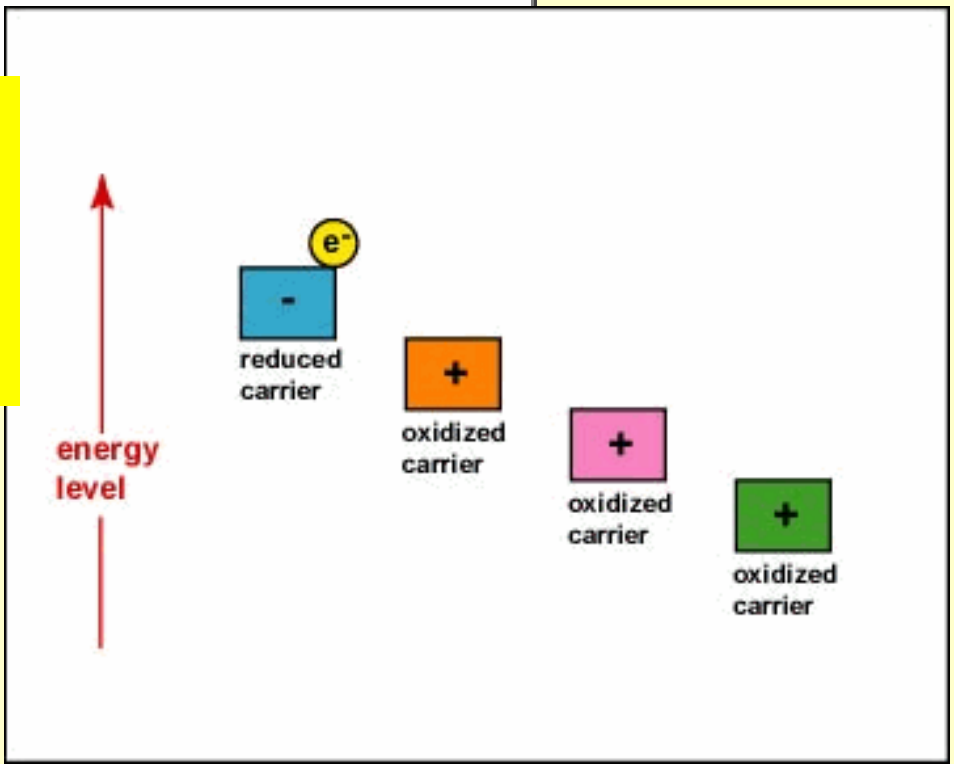


$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2880\ kJ/mol$$

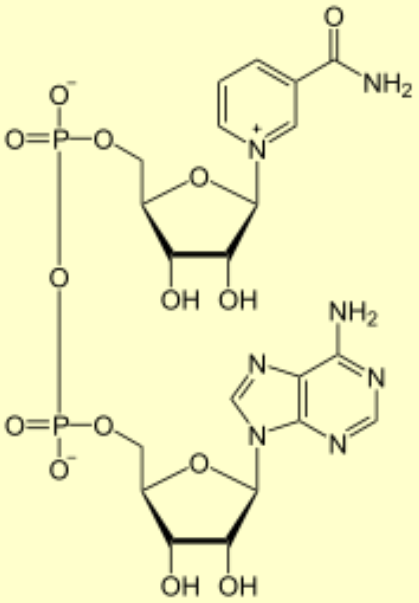




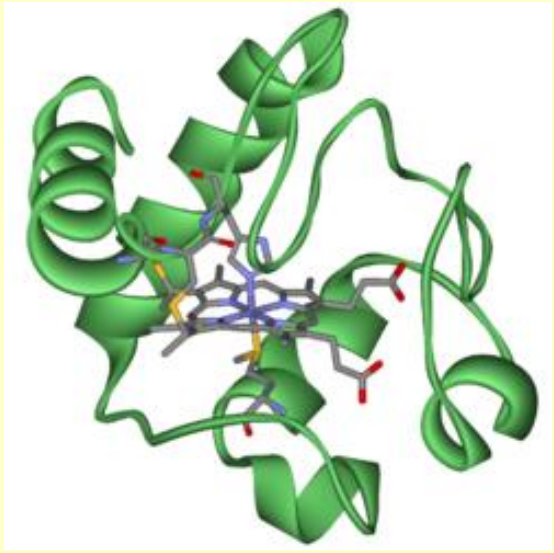
The Electron Transport Chain -most important Electrochemical process in living systems



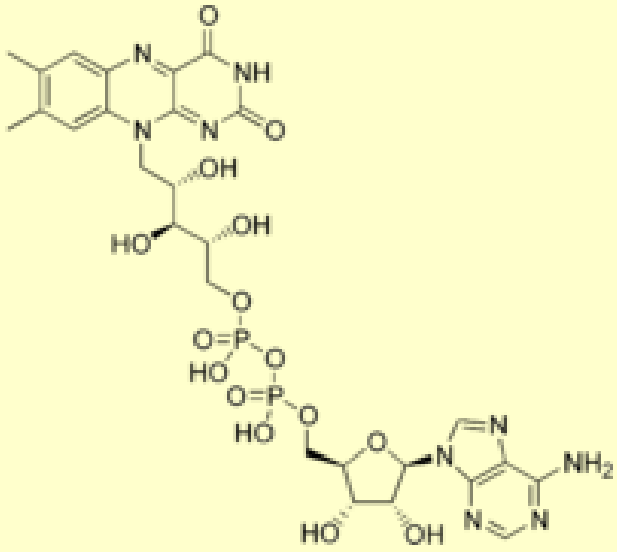
Most important compounds involved in the Mitochondrial Electron Transport Chain



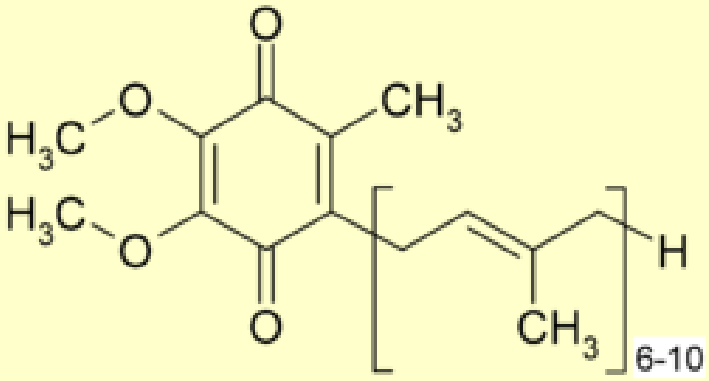
Nicotinamide adenine dinucleotide-
NAD^+ (redox enzyme)



Cytochrome C (with Hem)



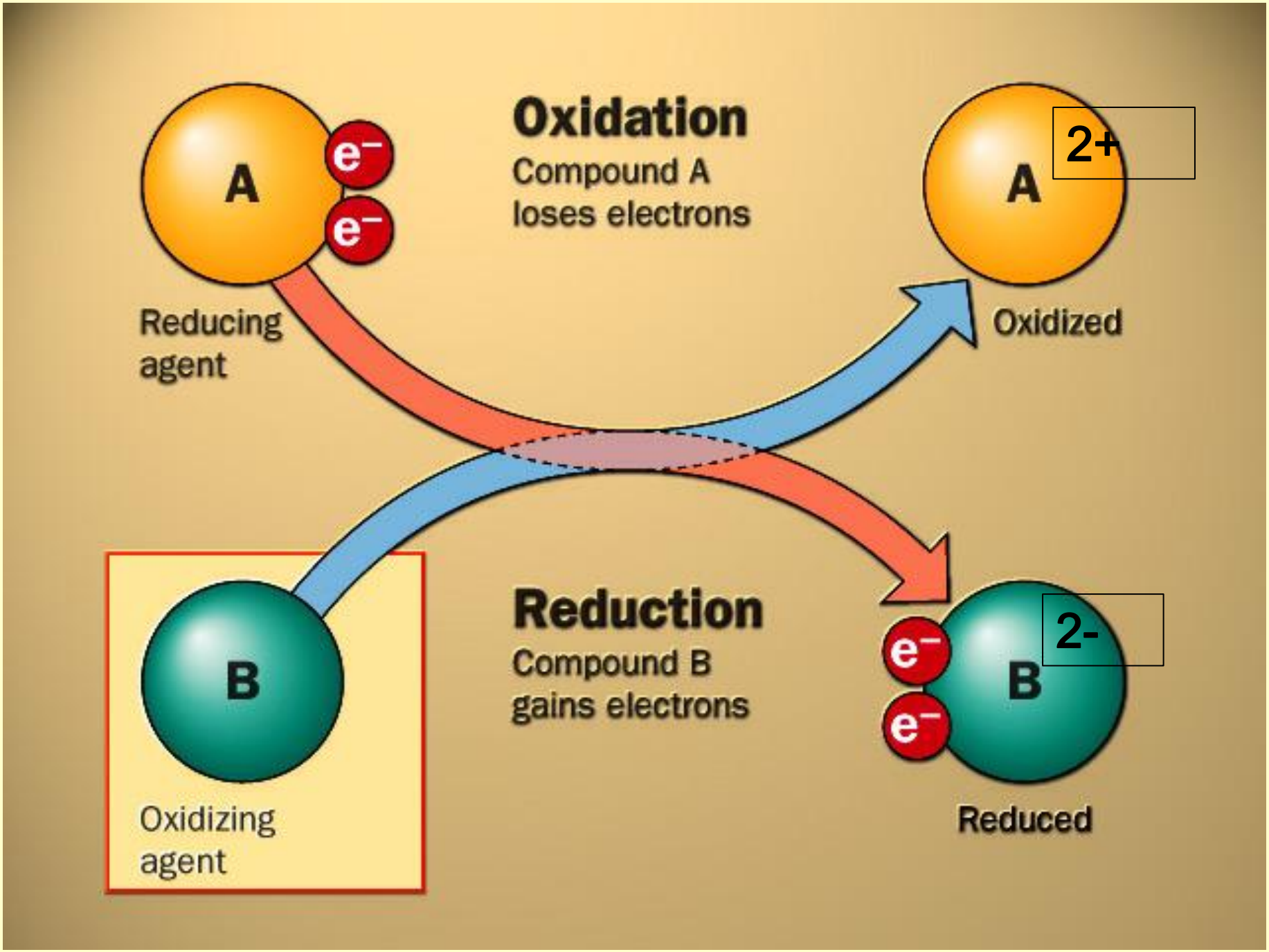
Flavin Adenine dinucleotide-
FAD

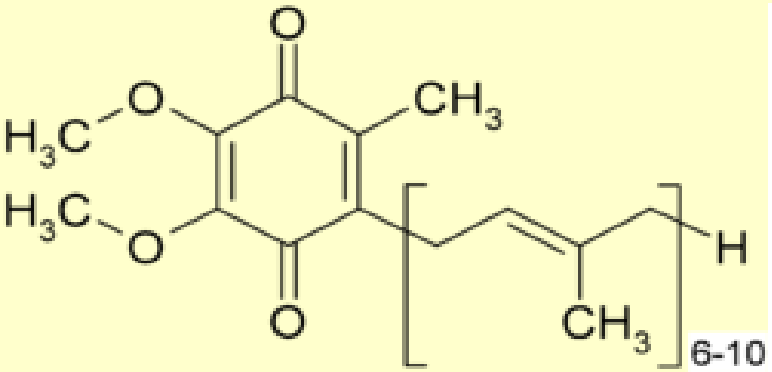


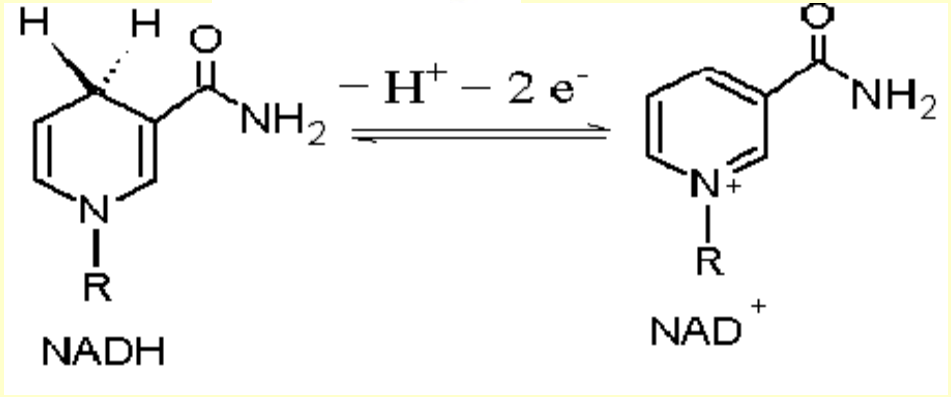
Coenzyme Q

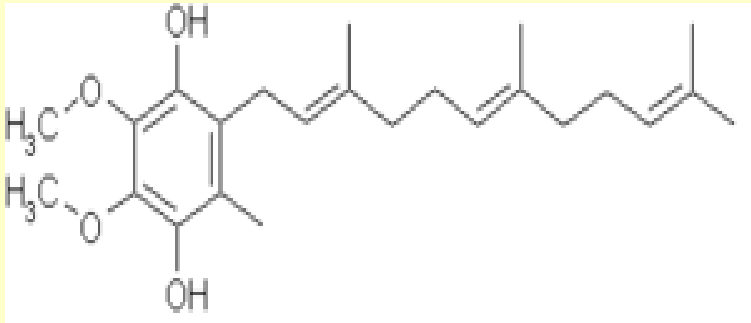
DEFINITIONS

- **Oxidation**: **Loss of electrons**.
- **Reduction**: **Gain of electro**ns.
- **Reductant**: Species that loses electrons.
- **Oxidant**: Species that gains electrons.
- **Valence**: the electrical charge an atom would acquire if it formed ions in aqueous solution.


Oxidation
Compound A
loses electrons
A
e⁻
e⁻
Reducing
agent
A
2+
Oxidized
Reduction
Compound B
gains electrons
B
Oxidizing
agent
e⁻
e⁻
B
2-
Reduced

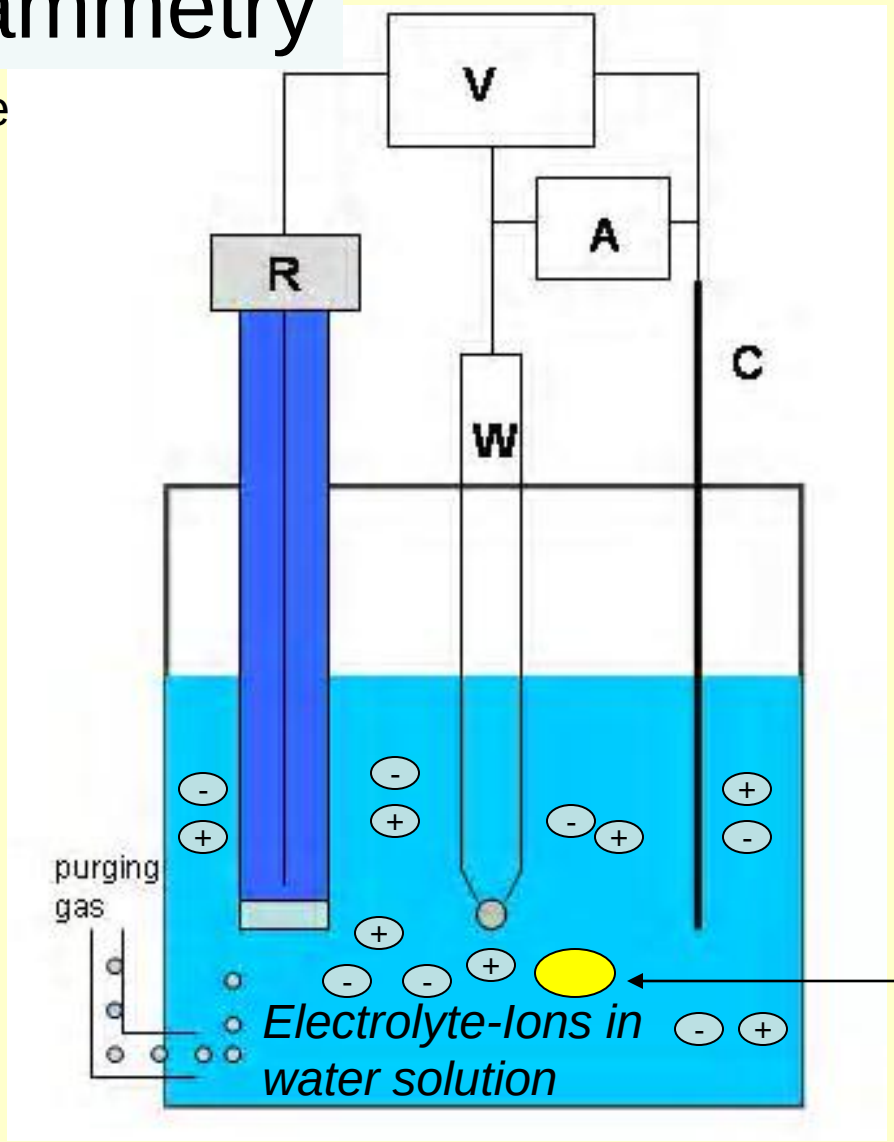

O
H3C
O
CH3
H3C
O
O
CH3
H
6-10


H
H
O
NH2
N
R
NADH
– H+ – 2 e-
O
NH2
N+
R
NAD+

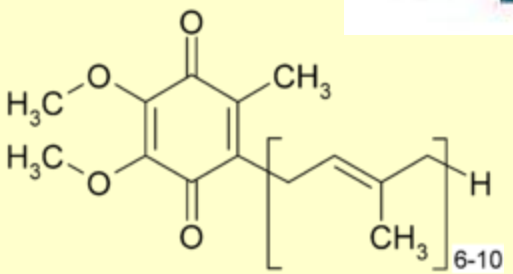

OH
O
H3C
H3C
O
OH

Voltammetry

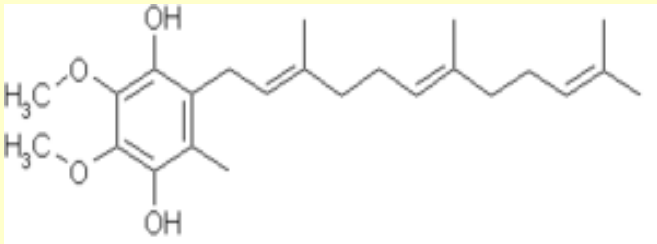
W-working electrode

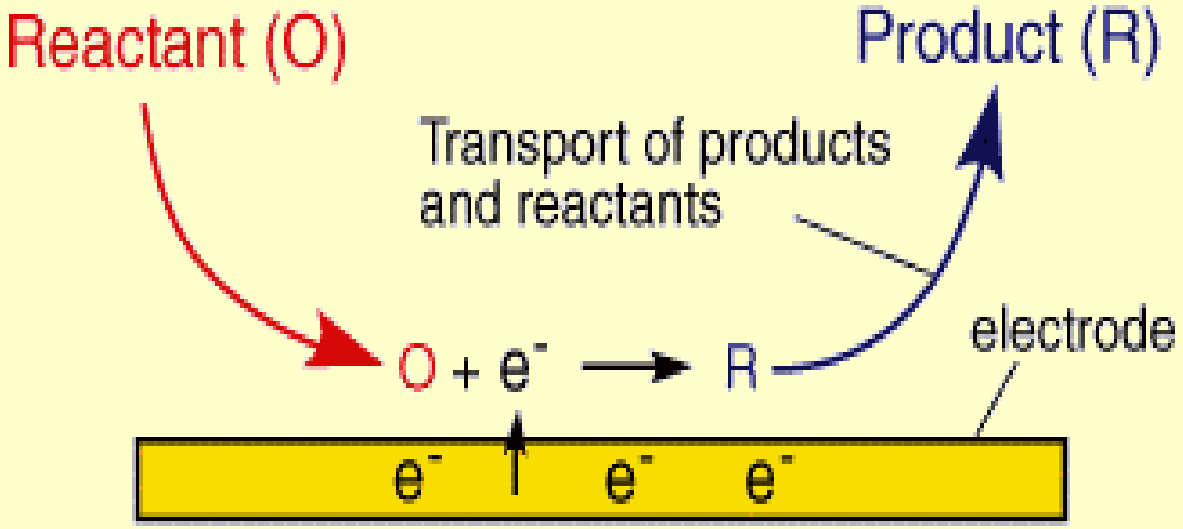


R-reference electrode

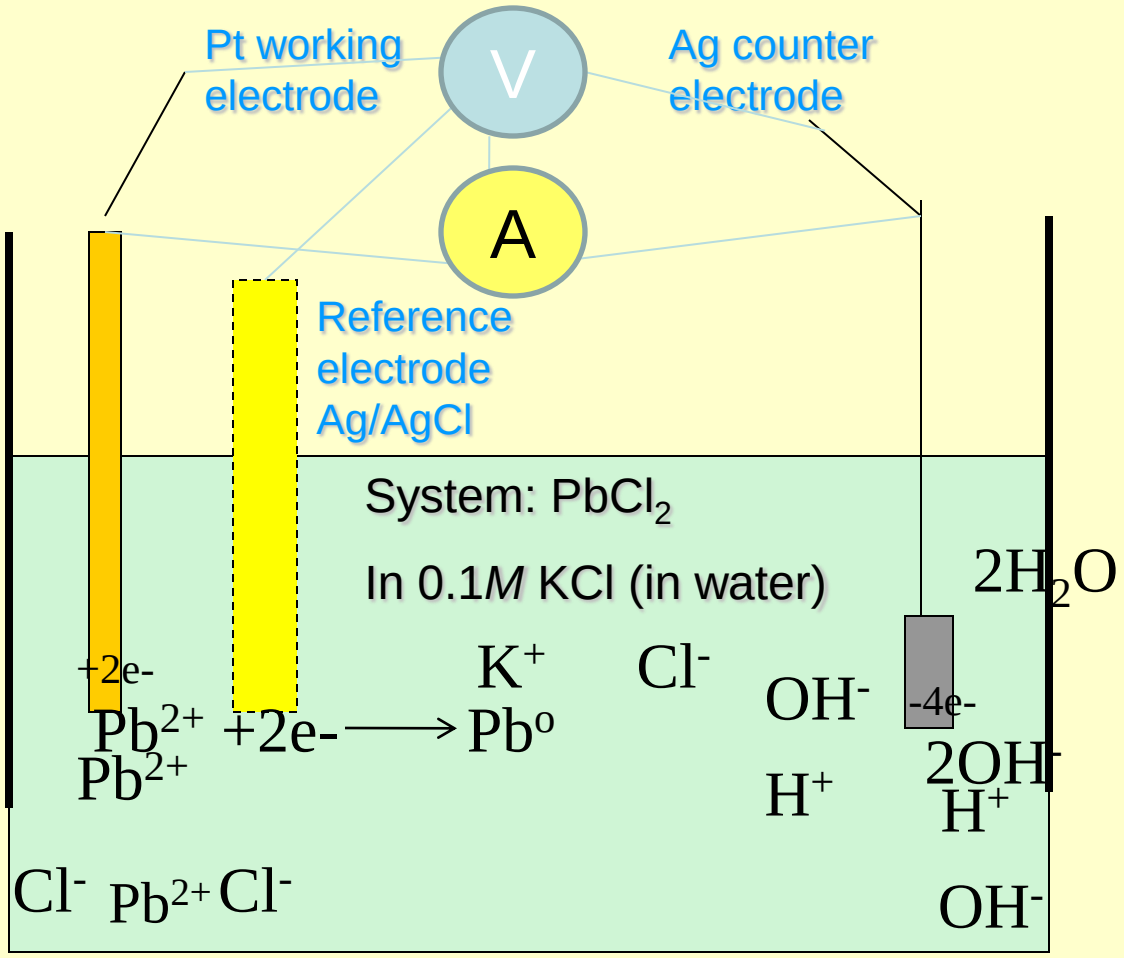


+2 H+ + 2e- ⇌

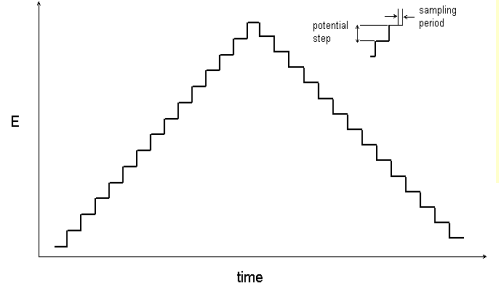



Reactant (O)
Product (R)
Transport of products
and reactants
O + e⁻
R
electrode
e⁻
e⁻
e⁻

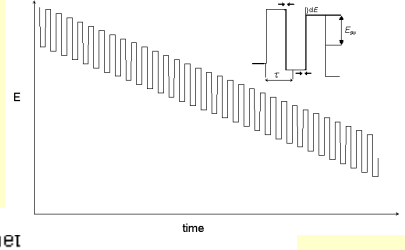
System: PbCl2 in KCl



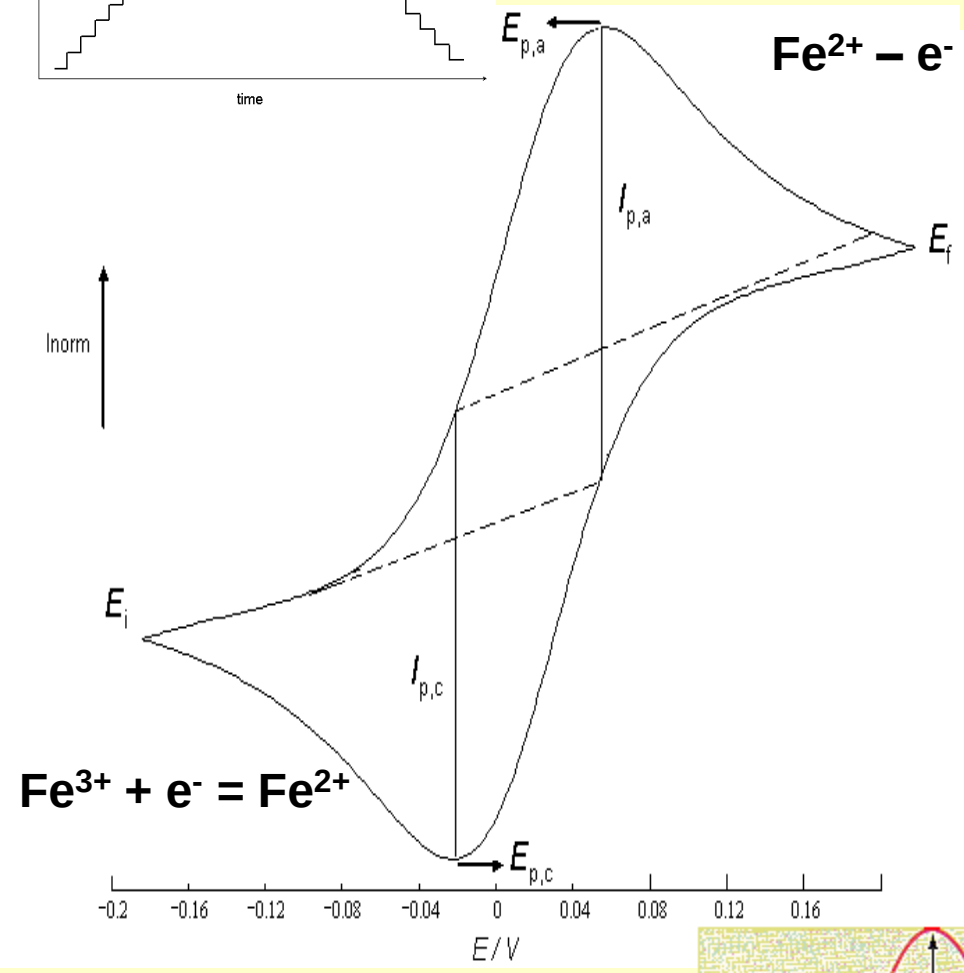
Cyclic voltammogram
of hydroxy-ferrocene.



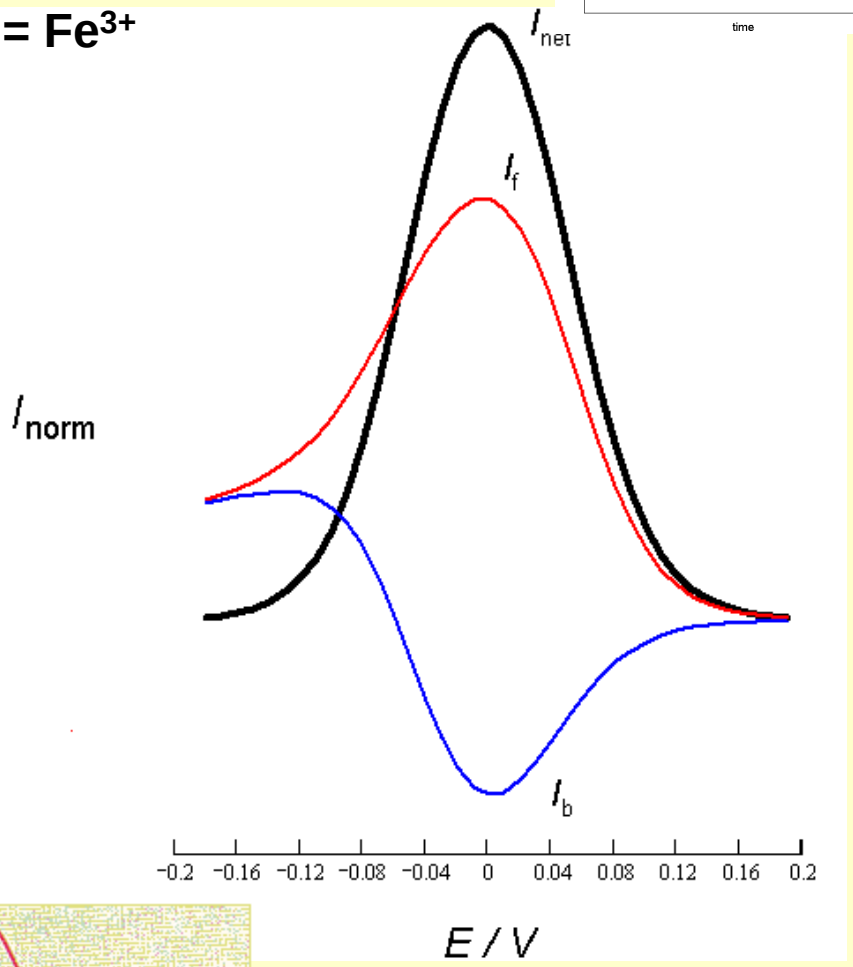
$$Fe^{3+} + e^- \rightleftharpoons Fe^{2+}$$



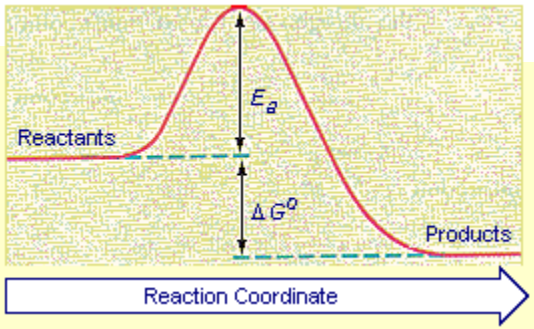
$Fe^{2+} - e^- = Fe^{3+}$



$Fe^{3+} + e^- = Fe^{2+}$



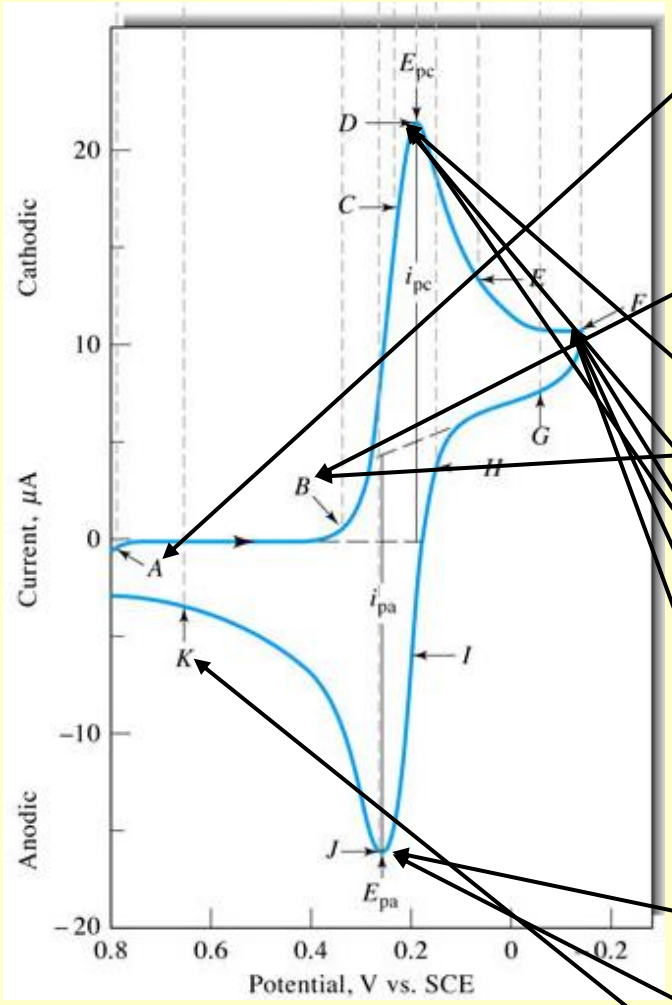
Cyclic voltammogram



Square-Wave Voltammogram

Работна електрода е Pt & референтна електрода е SCE

6 mM $K_3Fe(CN)_6$ & 1 M KNO_3



На почеток тече негативна струја поради оксидацијата на H_2O до O_2

Не тече струја помеѓу A & B (+0.7 до +0.4V) бидејќи во растворот нема честички што можат да бидат оксидирани или редуцирани во овој регион на потенцијали

B. При 0.4V, струјата почнува да расте како резултат на одвивање на следната рекација на катодата (т.е. На работната електрода):

$$Fe(CN)_6^{3-} + e^- \text{ » } Fe(CN)_6^{4-}$$

B.-D. Нагло зголемување на струјата како резултат на намалување на површинската концентрација на $Fe(CN)_6^{3-}$

D. Катоден пик потенцијал (E_{pc}) и катодна пик струја (i_{pc})

D.-F. Струјата почнува нагло да се намалува како што дифузониот слој се проширува кон внатрешноста на растворот

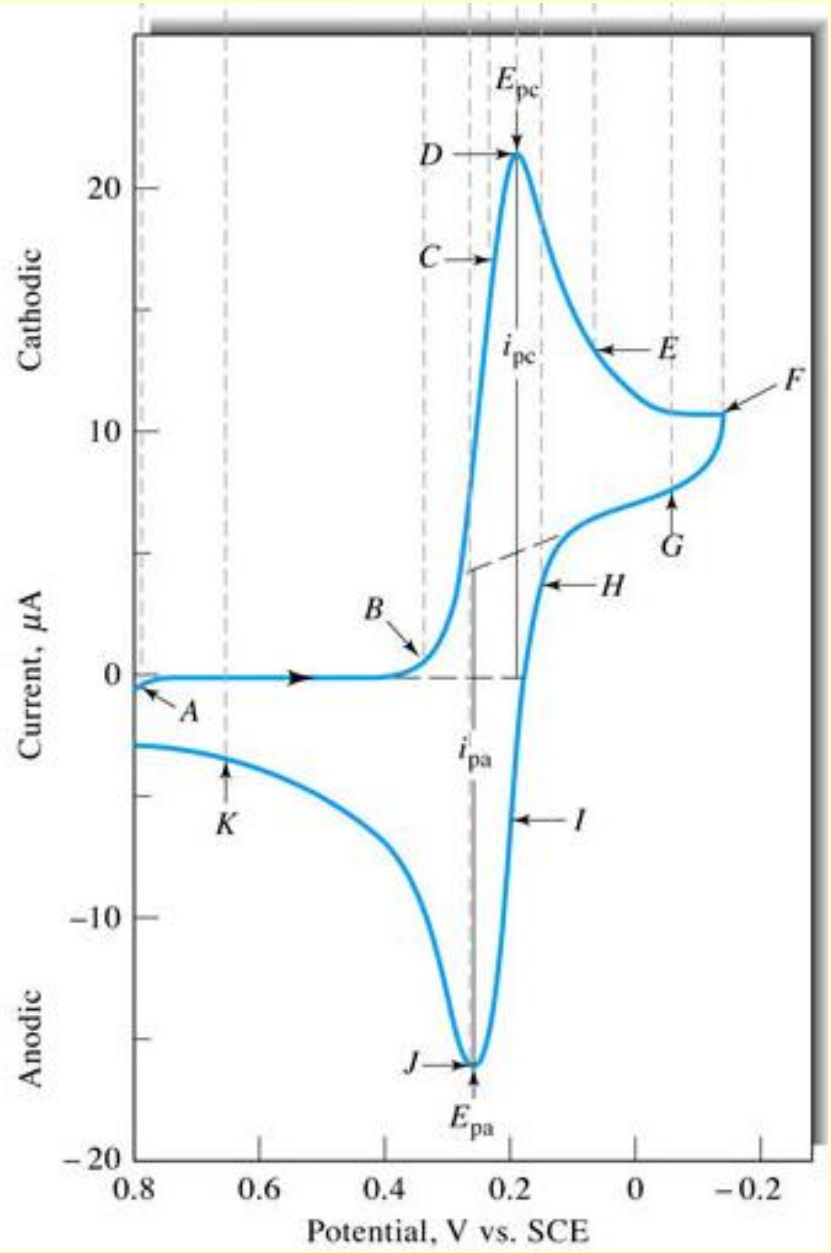
F. Се свртува потенцијалот (-0.15V), потенцијалот е се уште негативе за да овозможи редукција на електроактивните $Fe(CN)_6^{3-}$

F.-J. При овој момент нема повеќе да се случува редукција на $Fe(CN)_6^{3-}$ и ќе почне да тече анодна струја како резултат на реоксидацијата на $Fe(CN)_6^{4-}$

J. Аноден пик потенцијал (E_{pa}) и анодна пик струја (i_{pa})

K. Анодната струја се намалува како што акумулираниот $Fe(CN)_6^{4-}$ е употребен во реакцијата на оксидација

What do we get from cyclic voltammograms



- i_{pc} i_{pa}
- $\Delta E_p = (E_{pa} - E_{pc}) = 0.0592/n$, n = number of electrones exchanges
- E^0 = mid-peak potential E_{pa} → E_{pc}
- $I_p = 2.686 \times 10^5 n^{3/2} A c D^{1/2} v^{1/2}$

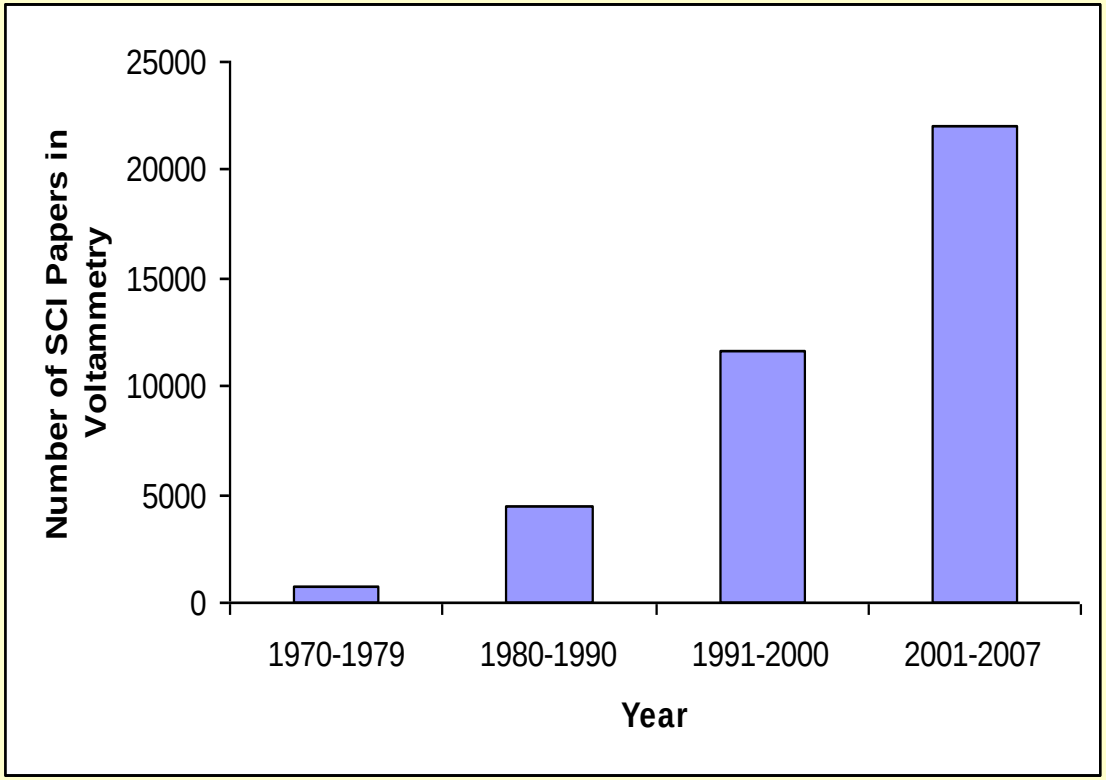
  - *A*: electrode surface
  - *c*: electroactive compound concentration
  - v: scan rate
  - *D*: diffusion coefficient

Application of the Voltammetry

-In Chemistry, Physics and Engineering

-In Biology and Biochemistry (biosensors)

-In Pharmacy



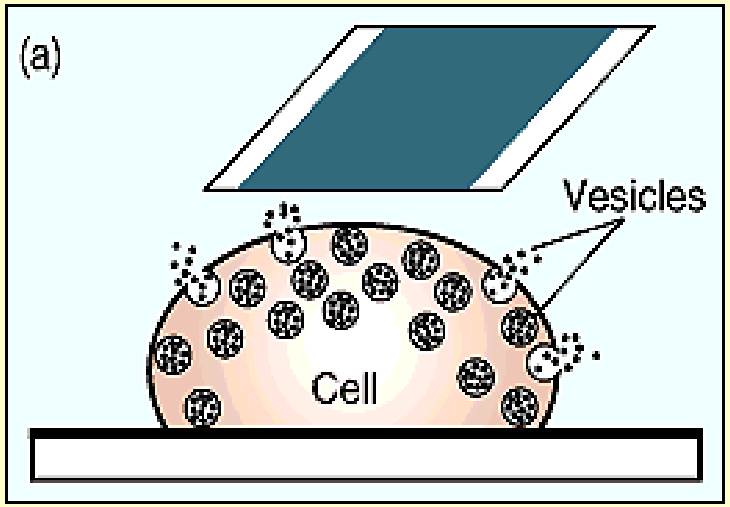
-In Medicine

-detection of reactive radicals nitroxides, superoxides,...

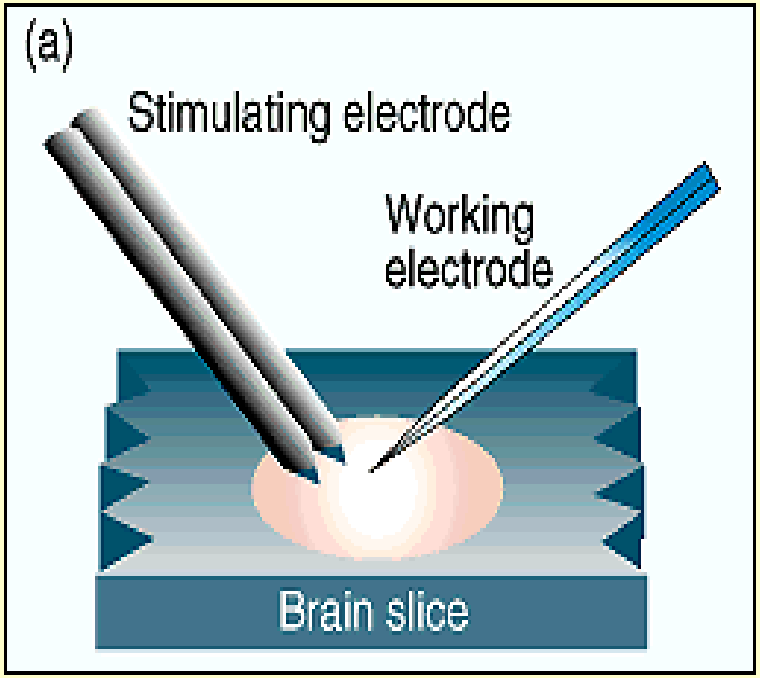
-determination of various active compounds

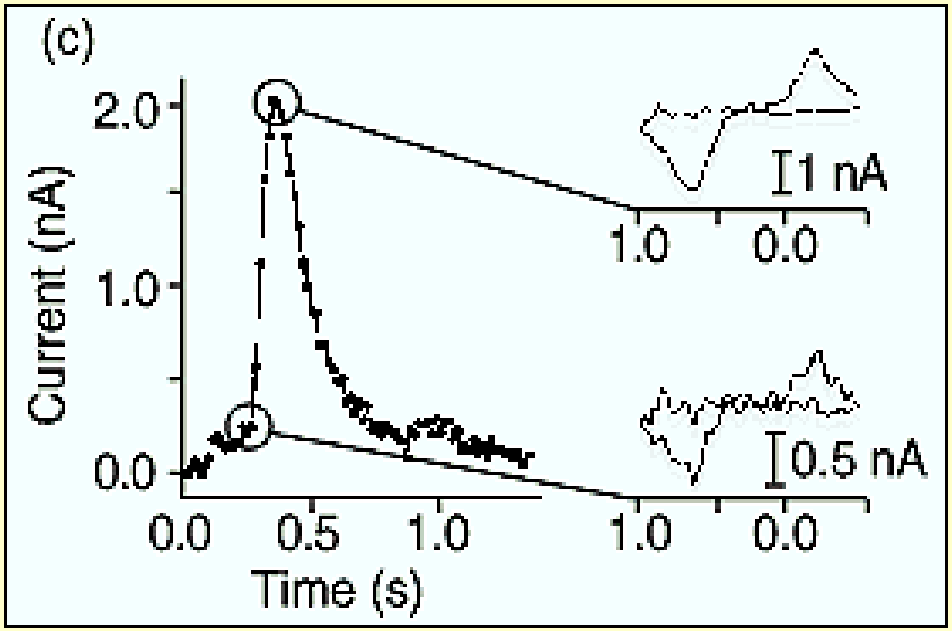
-following of protein-protein interactions

-medical sensors for various electron cariers and neurotransmiters



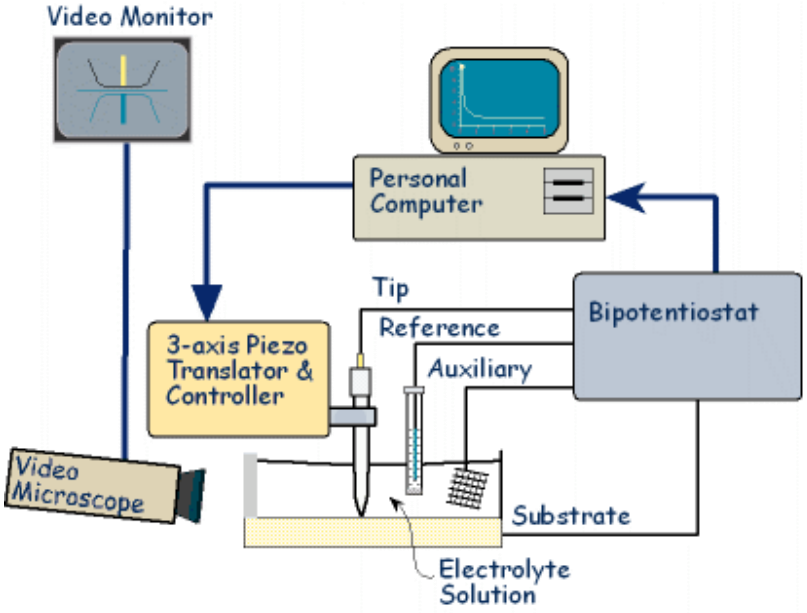
IN-VIVO voltammetric determination of catecholamine

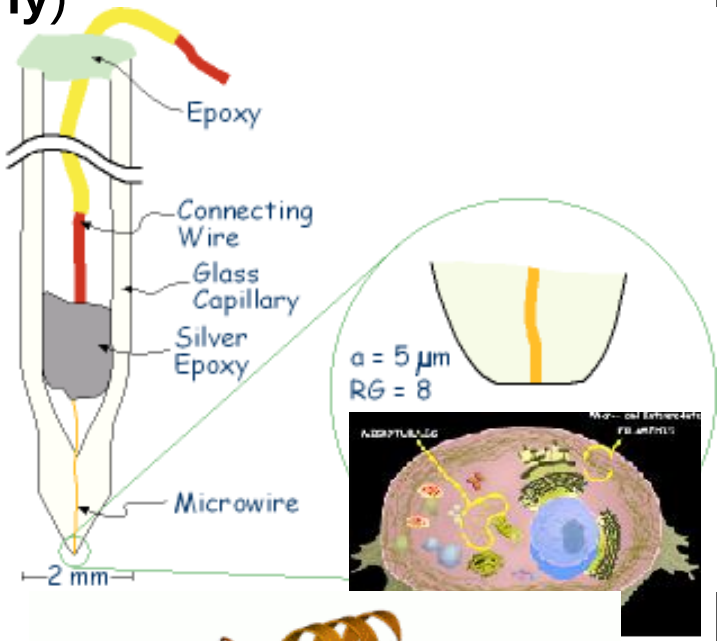




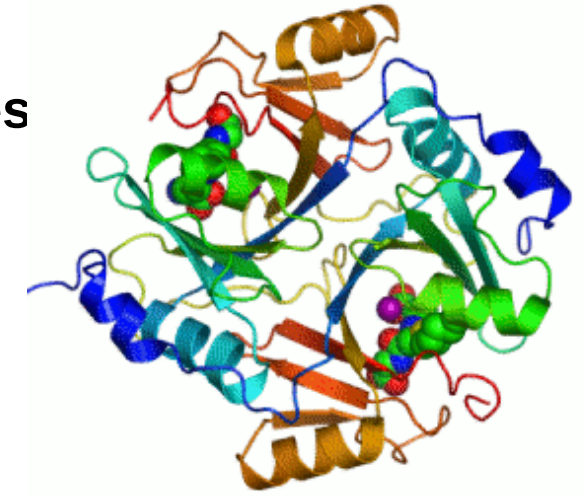
Voltammetry in service of the Scanning Electrochemical Microscopy

-Powerfull tool for probing the electrochemical activity of single living cells at different spots (**cell topography**)

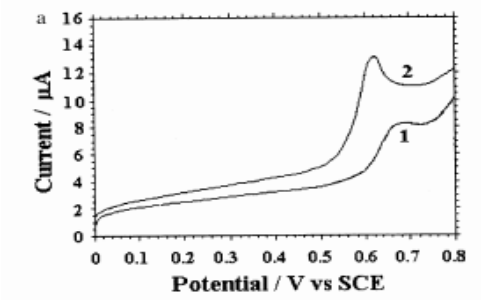




-Detection of **active sites of Enzymes**



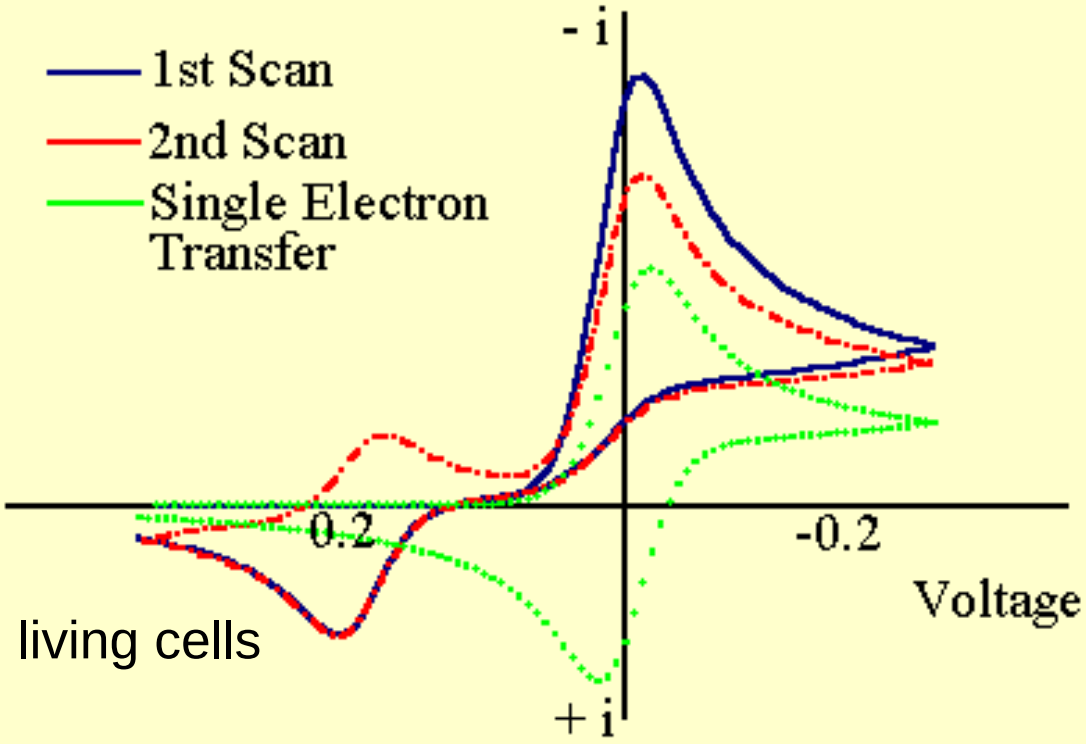
-Detection of **human breast cancer cells**



What kind of information can provide Voltammetry?

-Mechanism pathway

-detection of the **intermediates** and final products of the redox reactions



Peroxyde nitrite oxidation in living cells

ONO_2^- - e- = $ONO_2^{\cdot}$...NO_2^-

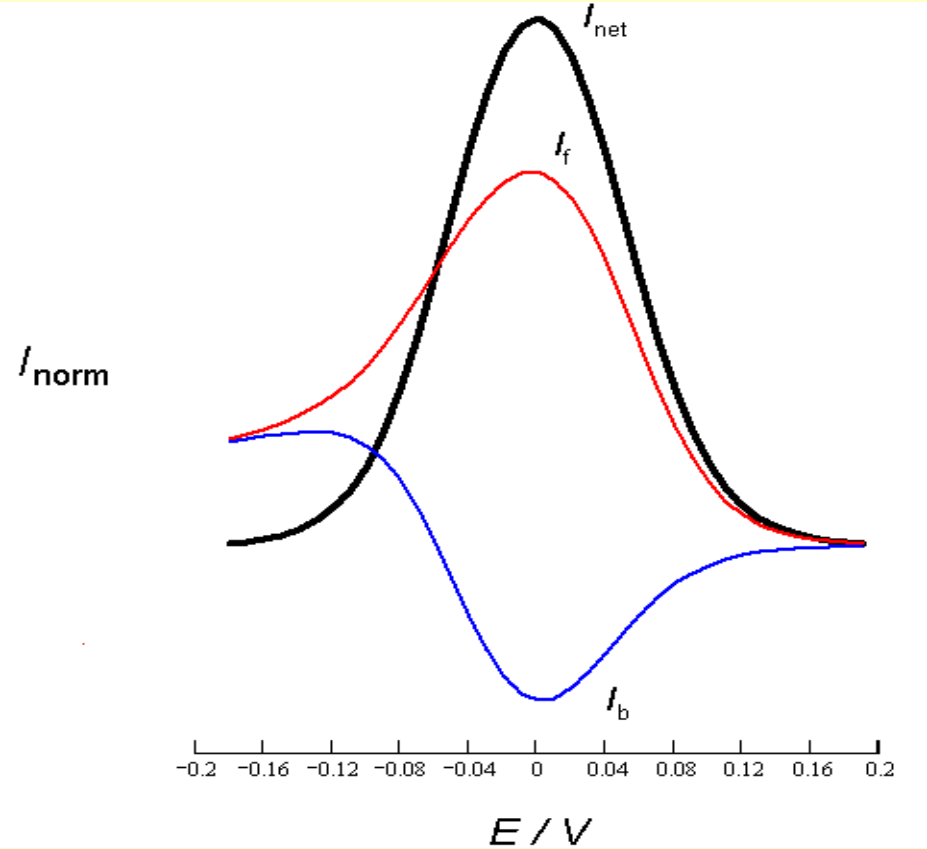
NO_2^- + 2OH- -2e- = NO_3^- + H_2O

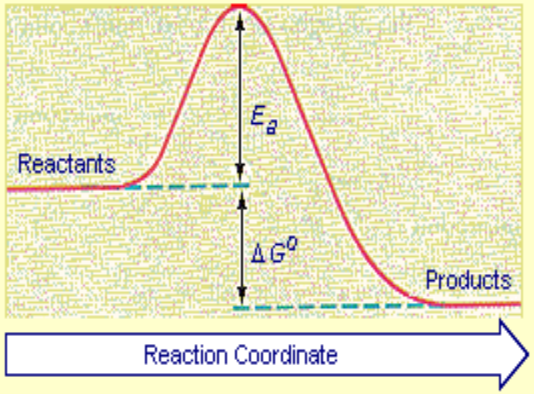
-Thermodynamic Parameters of Redox Reactions

Standard Redox Potential*-Energy of Activation*., Enthalpy, **Complexation Constants**...

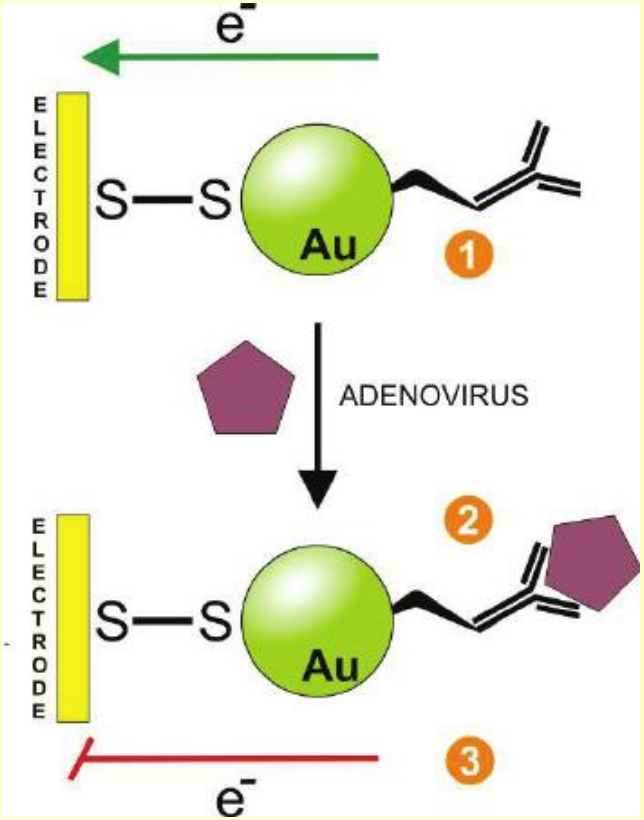
-Kinetic Parameters

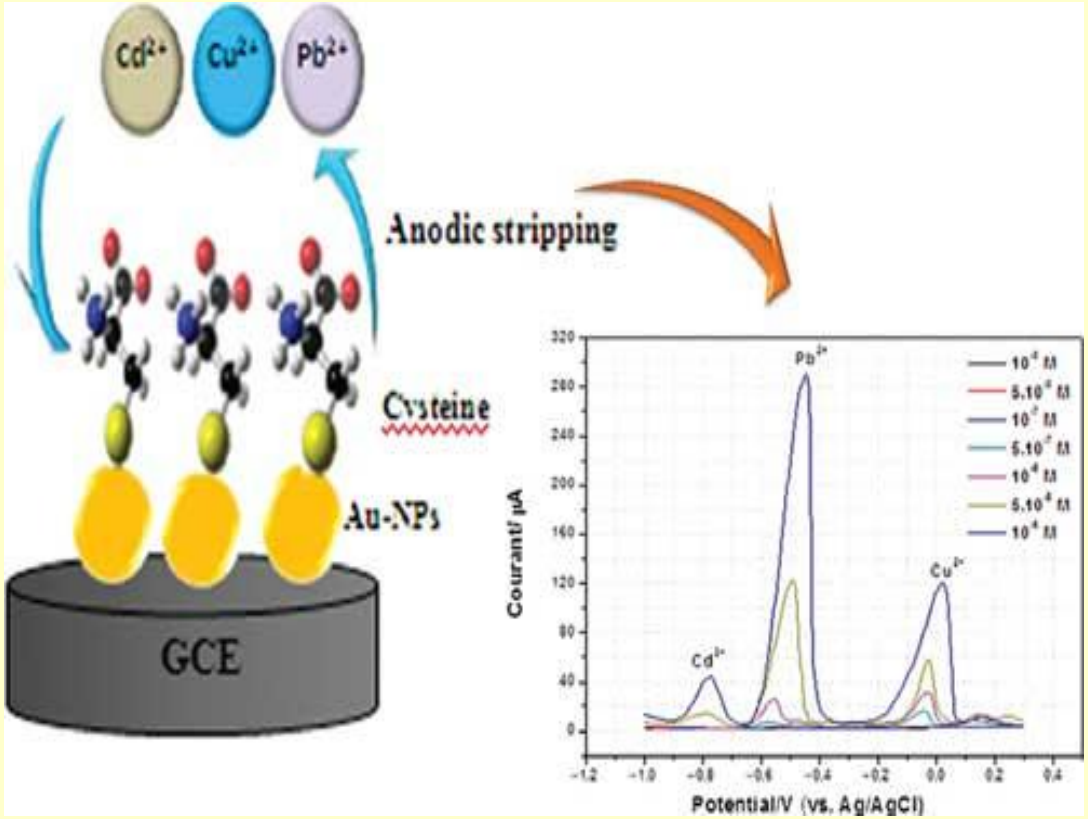
-standar rate constants of electron/ion transfers; kinetics of enzymatic reactions; kinetics of chemical reactions; ***pharmakokinetic parameters...***



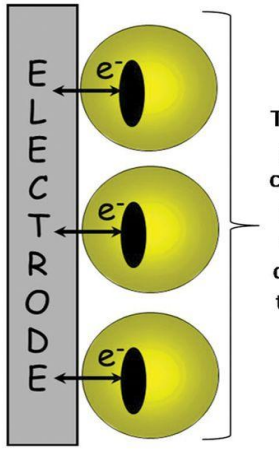


Voltammetry-NANOPARTICLES MODFICATION -new way of improving electrochemical signals-

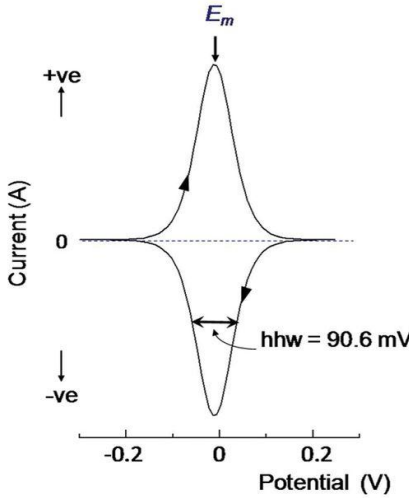


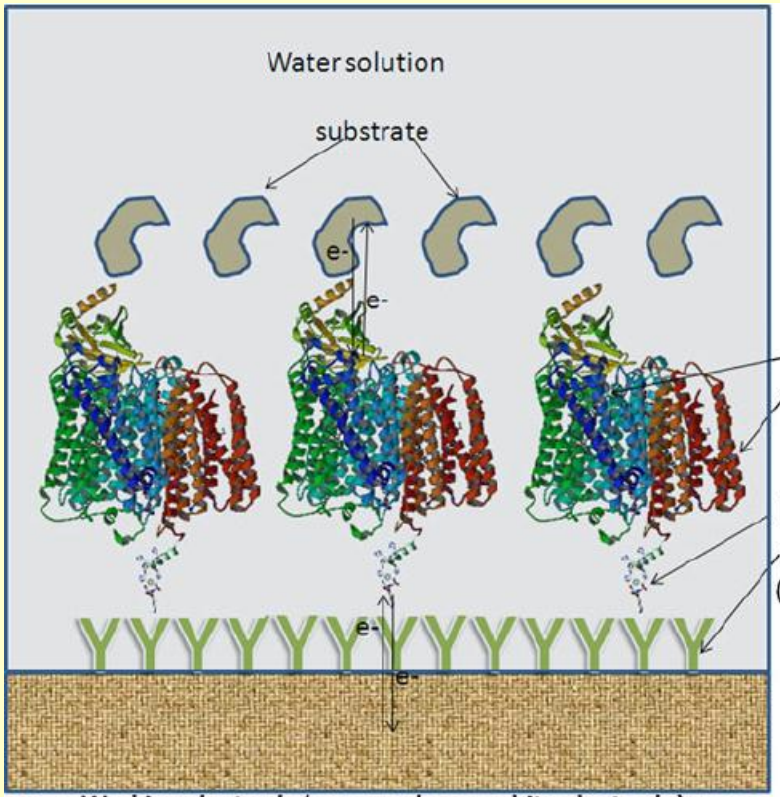


Протеин-филм волтаметрија -испитување на редокс својствата на ензимите со волтаметрија



The adsorbed protein film can be placed in solutions of different composition, temperature etc.



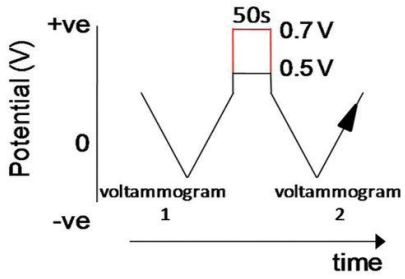


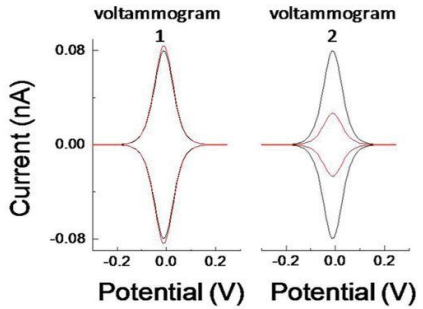
insulating parts of the protein

Protein redox active site

Modifiers of the electrode surface (nano particles, conducting polymers)

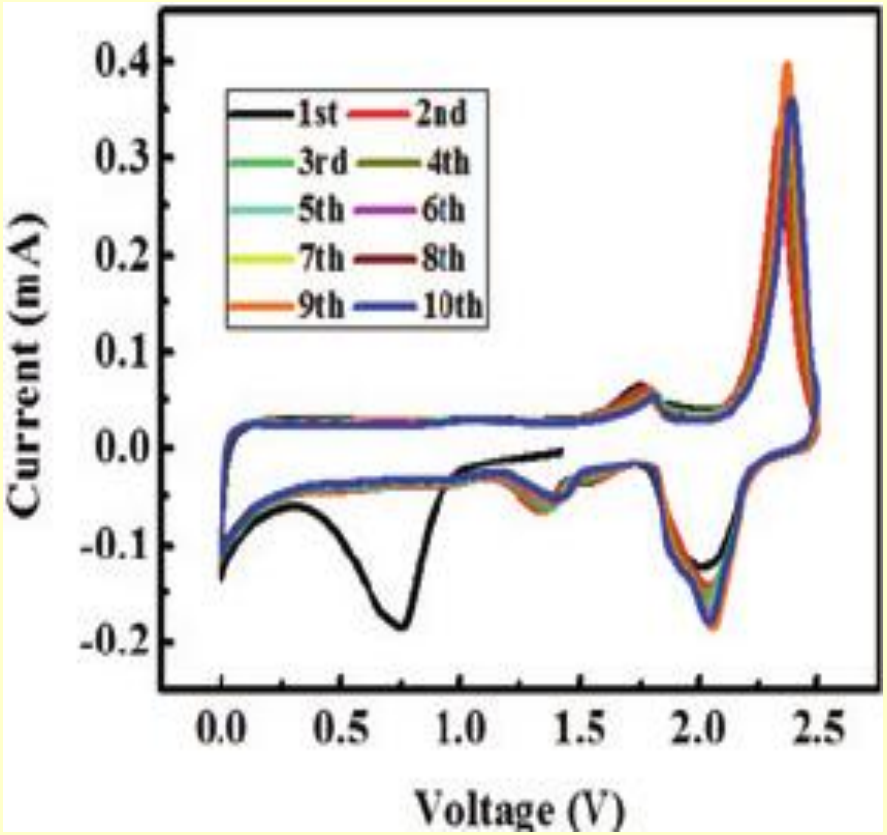
B

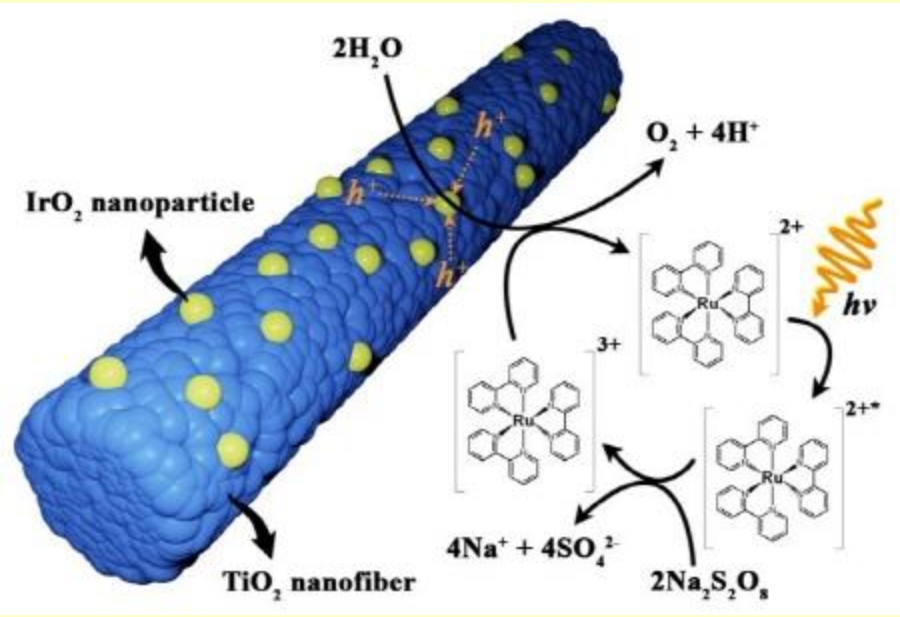




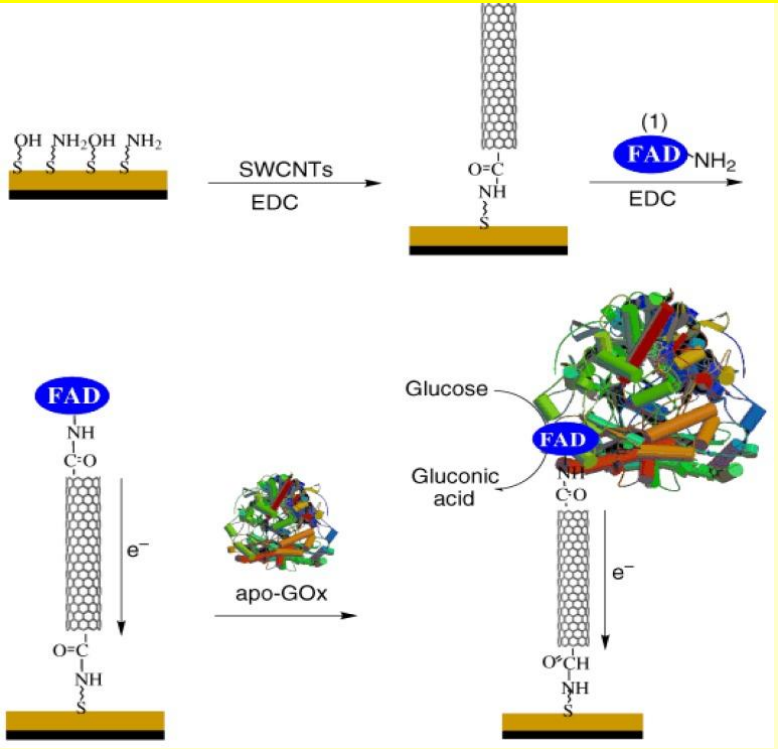
Волтаметрија на БИОМАТЕРИЈАЛИ

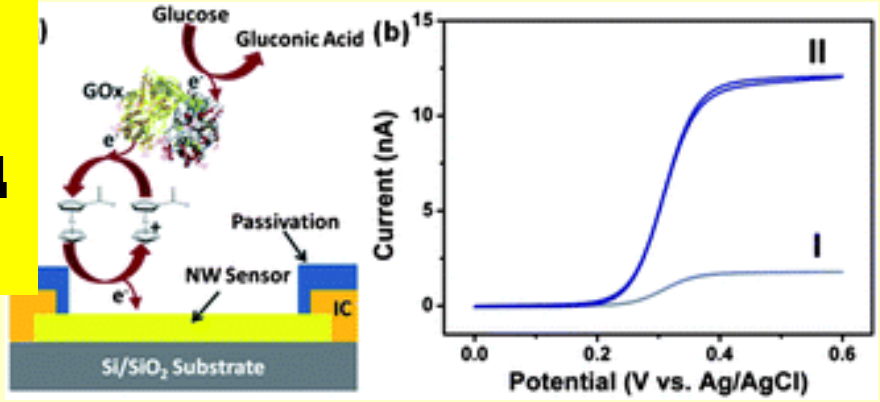
Стабилност на импланти





Волтаметрија-алатка за
Конструкција на
Амперометриски
БИОСЕНЗОРИ
За гликоза, водород пероксид
.,,,





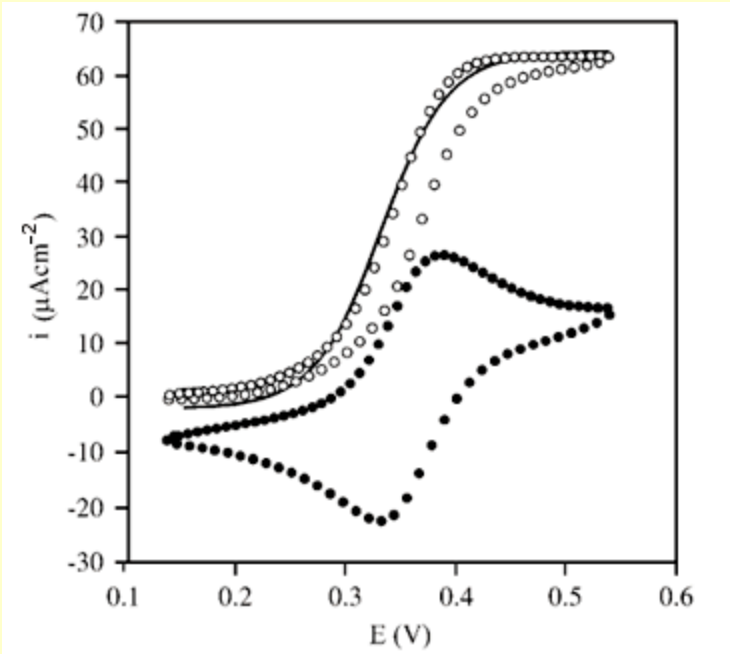
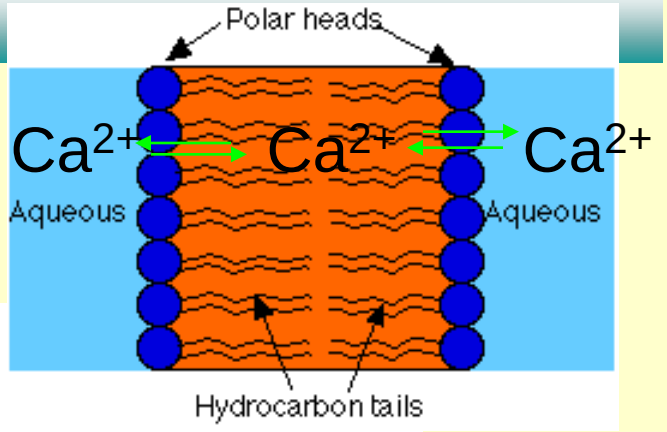
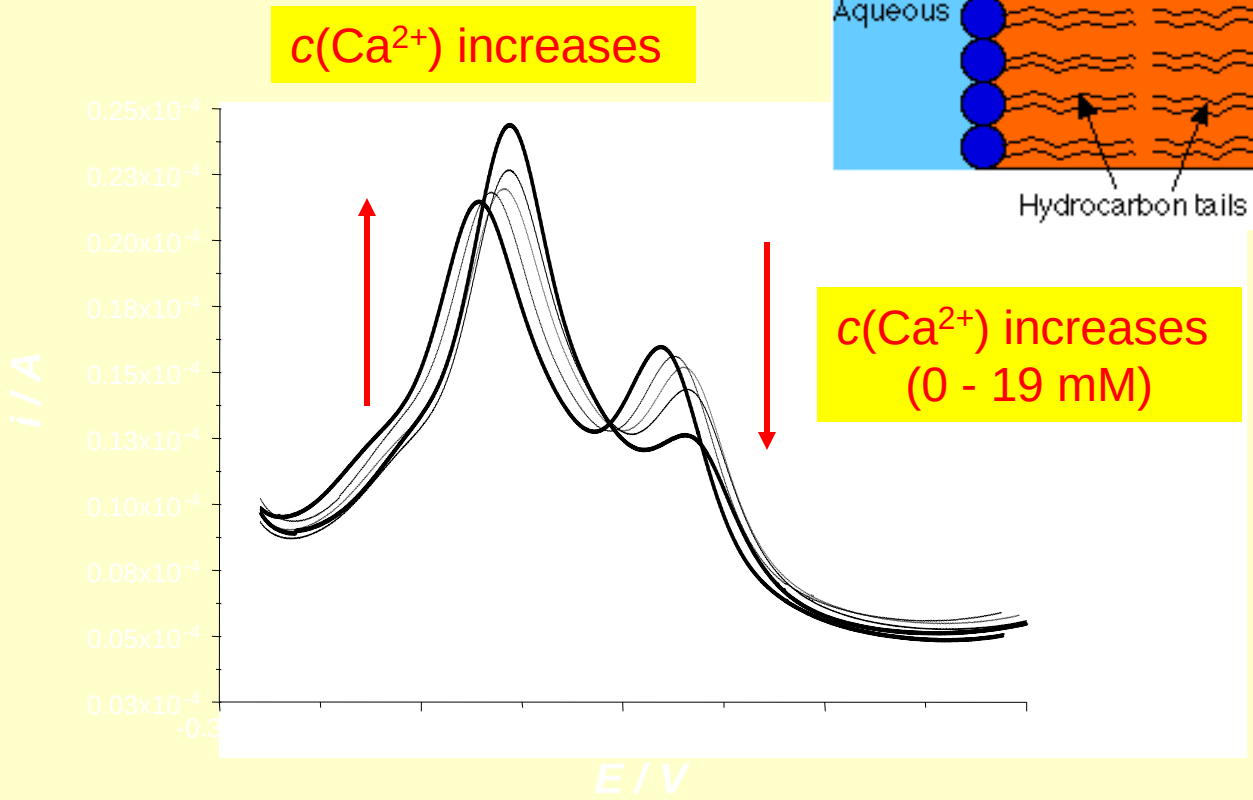


Figure 5. Catalytic wave for glucose oxidation in 50 mM phosphate buffer pH 7.1 and 0.1 M KNO_3 at a Fc-PAA-GOx hydrogel modified electrode (•) glucose free solution 10 mV s^{-1} and (o) 0.1 M glucose solution 5 mV s^{-1}. Solid line corresponds to best fit to Eq. 8. (see text).

Complexation of Quinone-like compounds and Ca^{2+} ions

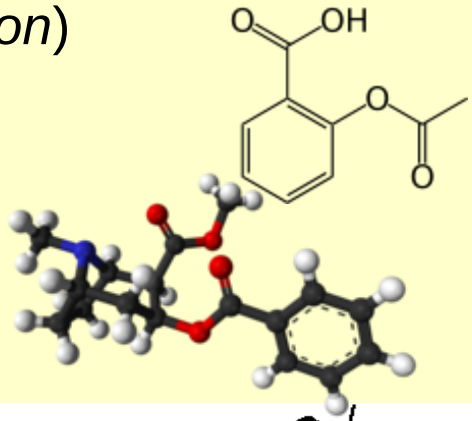


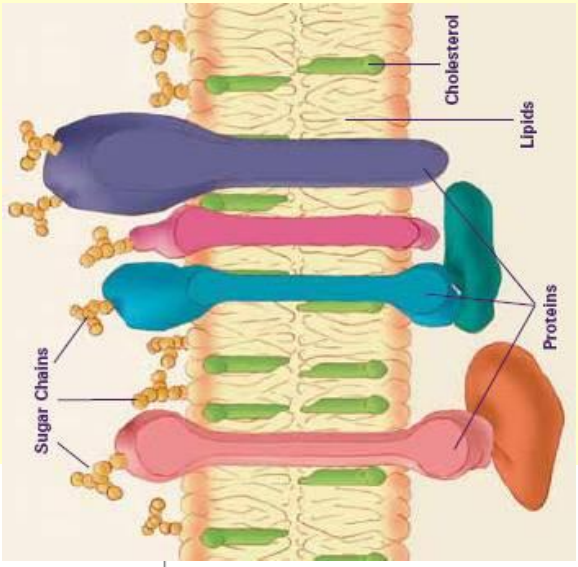


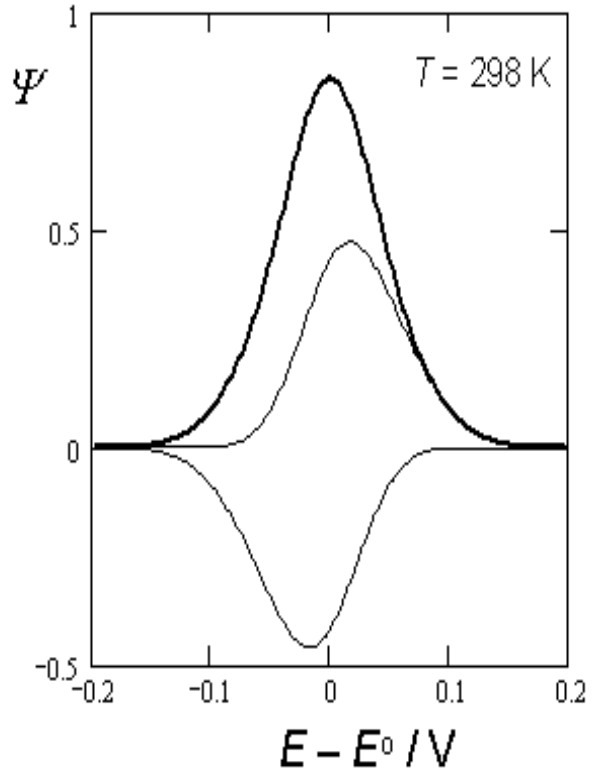
Cyclic voltammograms showing complexation of PalmytoilQuinone with Ca^{2+}

-***physical phenomena taking place in the system*** (*absorption, phase transformation*)

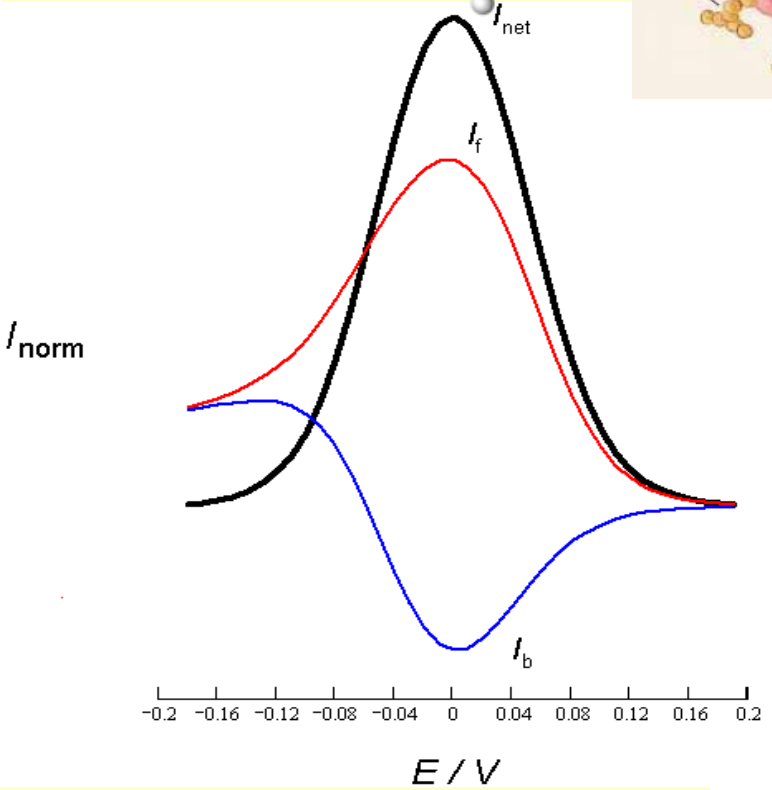
-way of mass transfer



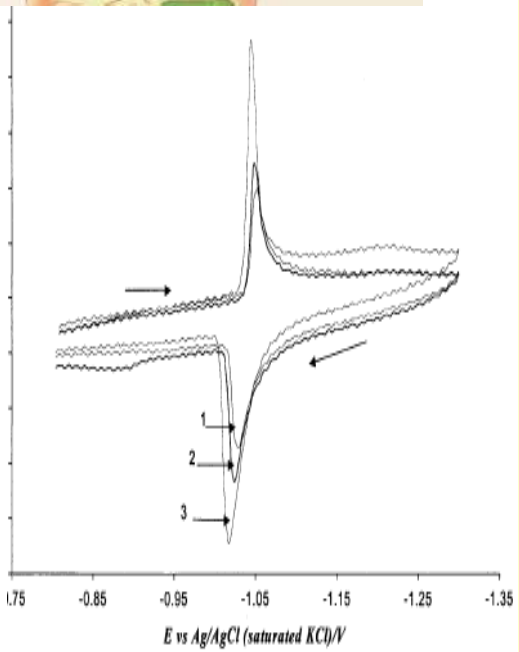




absorption



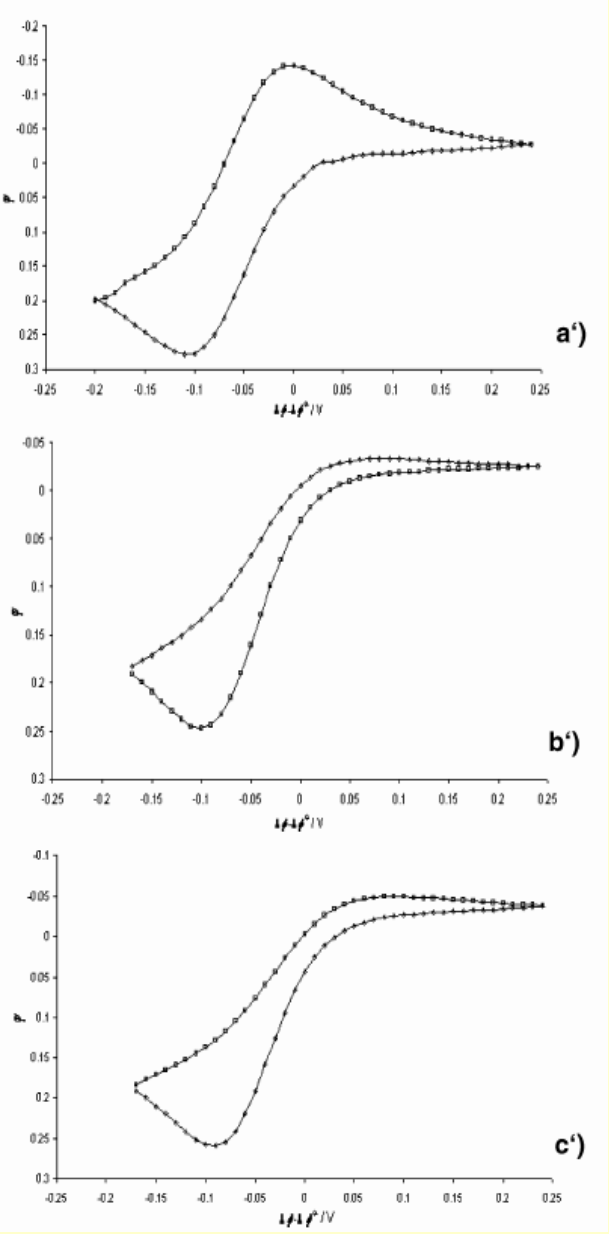
diffusion

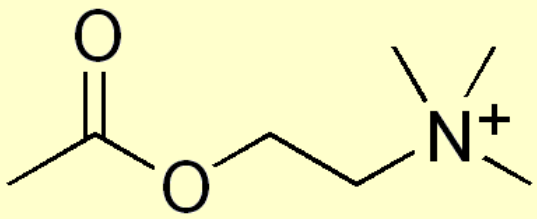


Phase-transformation

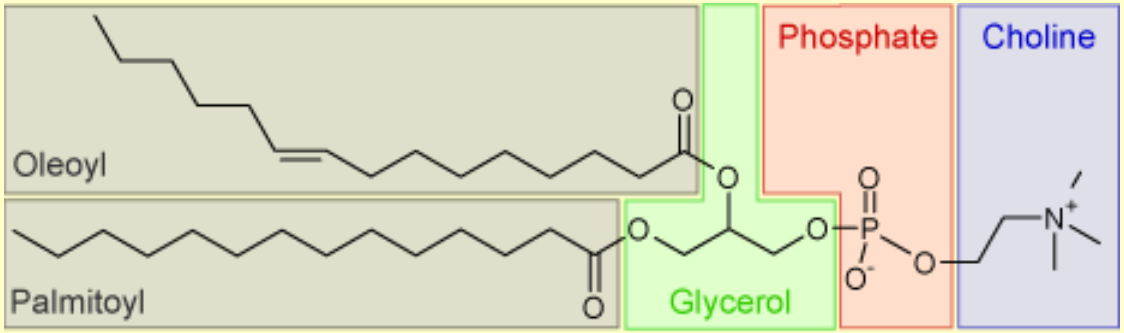
-thermodynamic and kinetic parameters related to the physical phenomena

-type and strenghts of interactions between various compounds





Acetylcholine



Phospholipids

TABLE 1: Determined Kinetic Parameters of the Ion Transfer of AcH^+ from Water to DCE (k_s and α) and for the Interactions between AcH^+ and DOPC (K, ϵ, k_f, and k_b)

measuring technique	k_s/cm s^{-1}	α	K	ϵ/s^{-1}	k_f/s^{-1}	k_b/s^{-1}
SWV	0.0030	0.50	0.44	13.10	4.00	9.10
EIS	0.0033	0.53	0.80	13.30	5.90	7.40

Which compounds can be investigated by Voltammetry?

Inorganic compounds, metals, alloys,

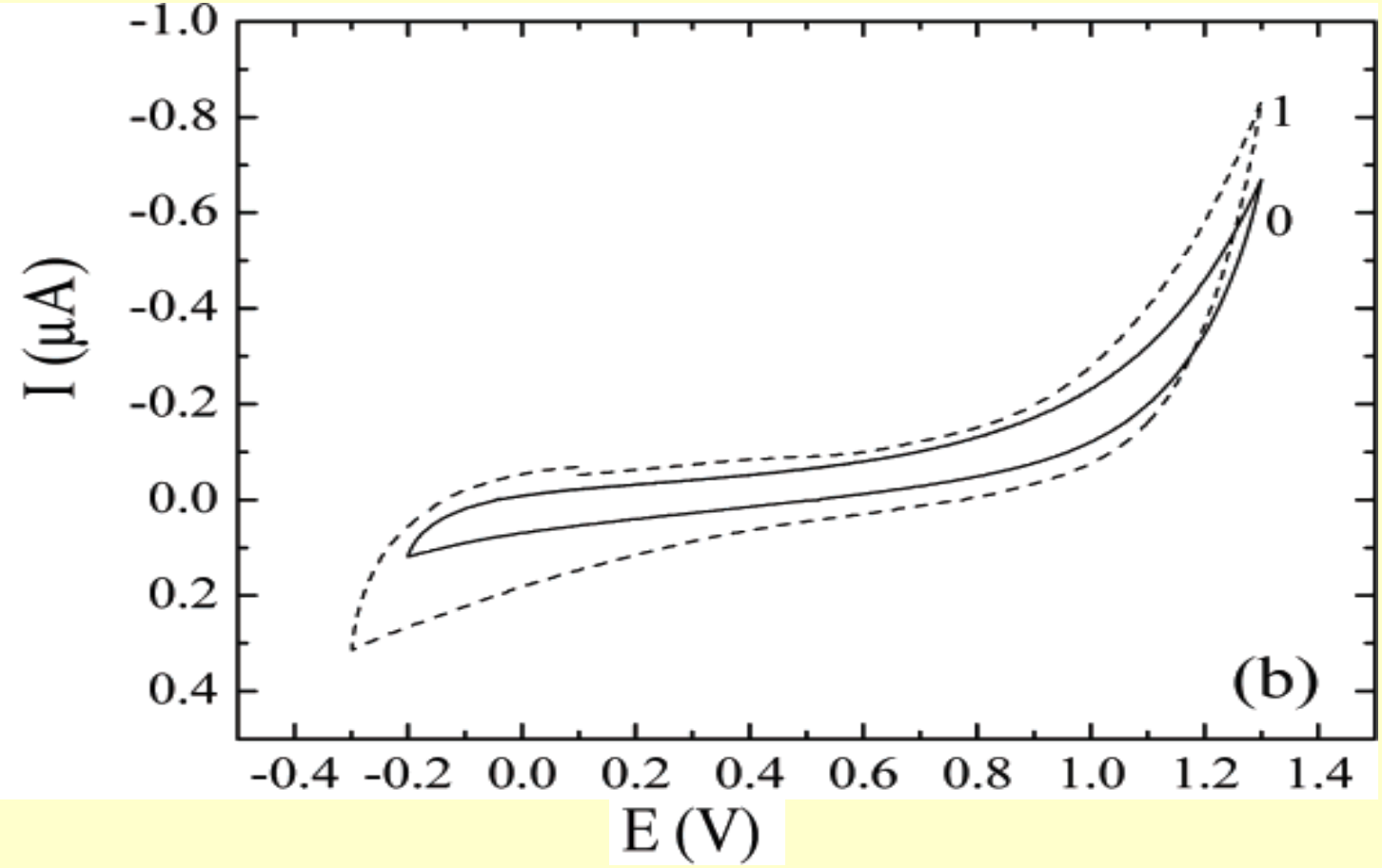
Organic compounds containing redox active groups:
C=O;Ar=O; N-R; “=“; N=N; S-H; Ar-OH: Ar-NO_2

Organic compounds containing incorporated metal ions (various **redox enzymes**

All medicaments and drugs containing “redox active” sites.

neurotransmitters dopamine, noradrenaline, adrenaline, serotonin…

Are there some limitations?

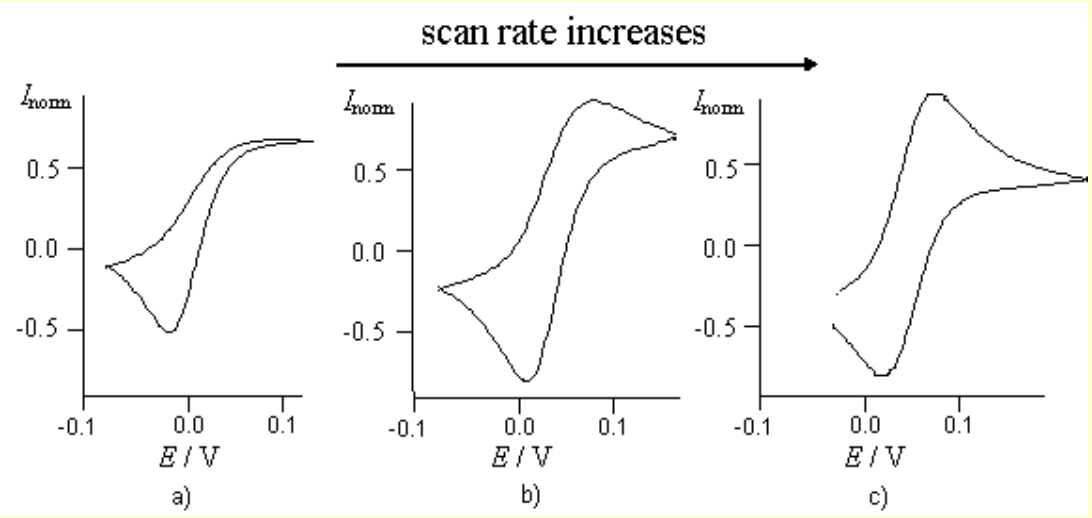


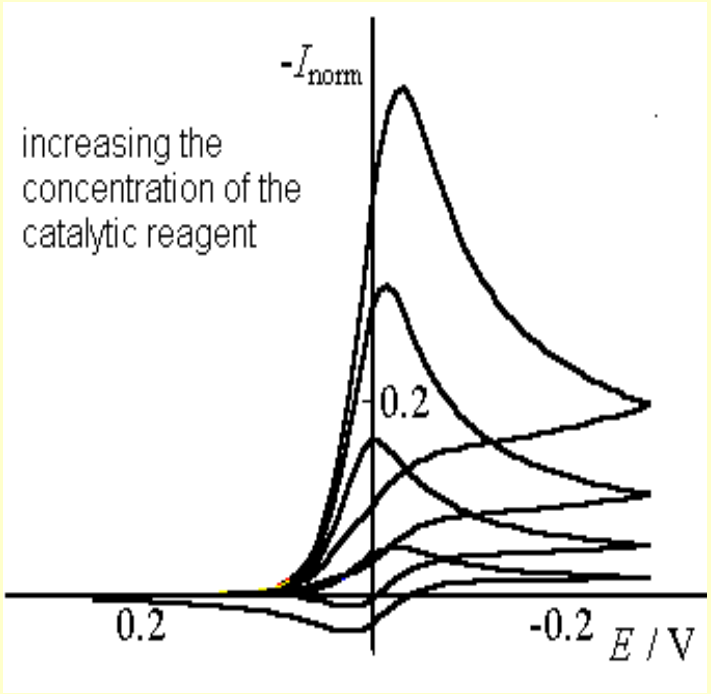
BLANK Cyclic voltammogram

-What can we make in the case if our compound does not show “electrochemical activity”?

transmitters such as GABA, glycine and glutamate are NOT electroactive

-to make coupled CHEMICAL reactions with redox active compounds

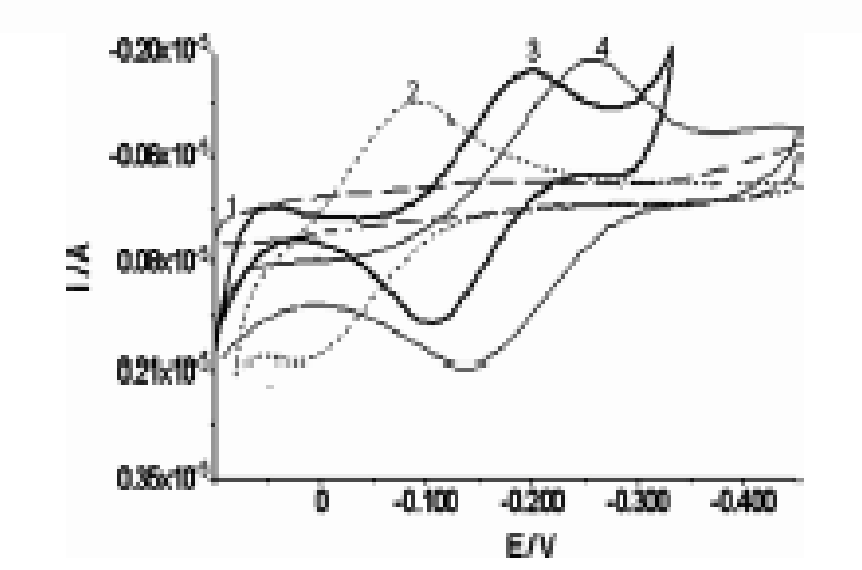


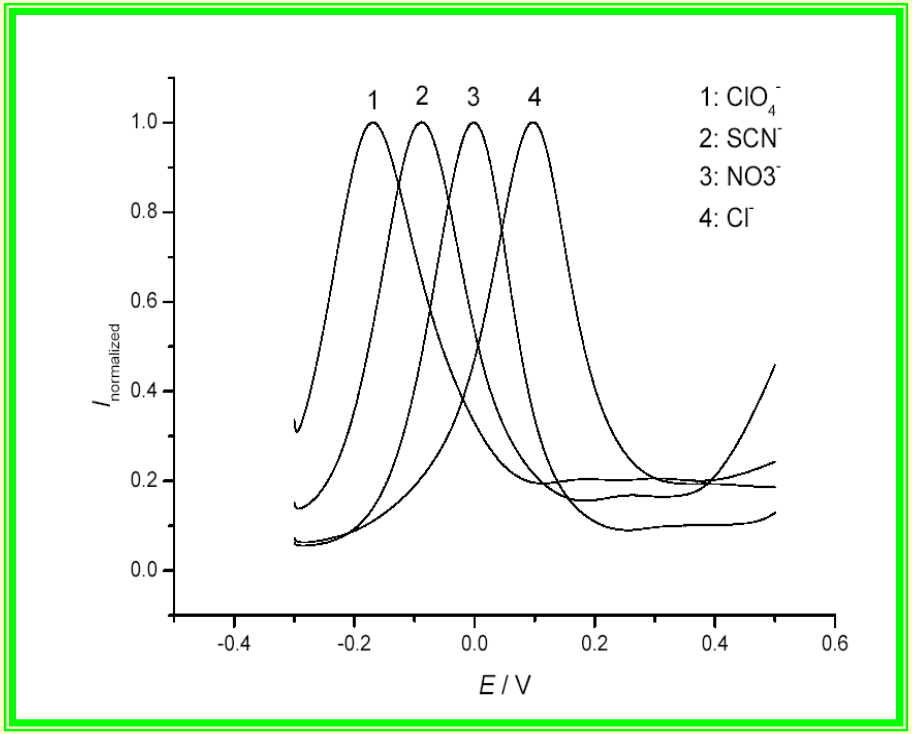


-to probe whether our compound can react with the material of the electrode

Is it possible to investigate only the "electron" transfer reactions with Voltammetry?

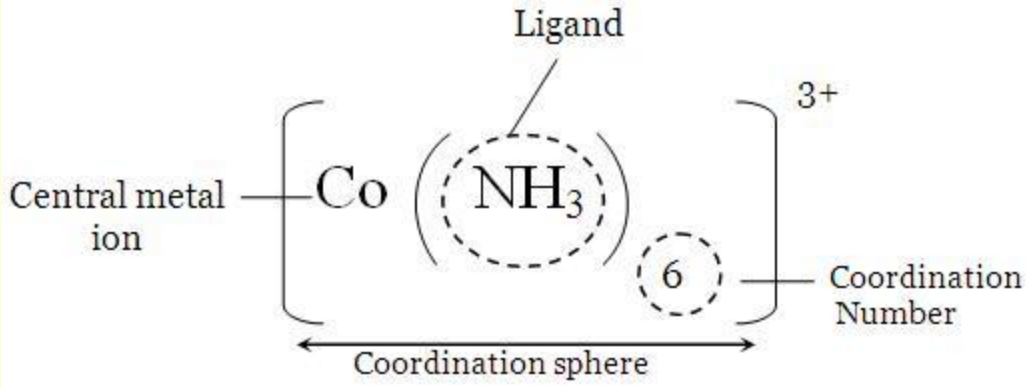
NO, it is possible to follow voltammetrically also reactions comprising only ION transfer, or COUPLED ELECTRON-ION transfer





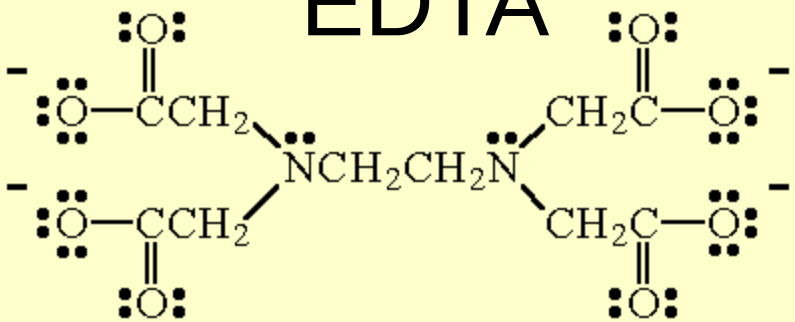
Transfer of Ionized Drugs-
heroin, cocaine and codeine
across biomimetic membranes

VOLTAMMETRY is Extremely efficient And powerfull toll in characterizing Coordination complexes!!!

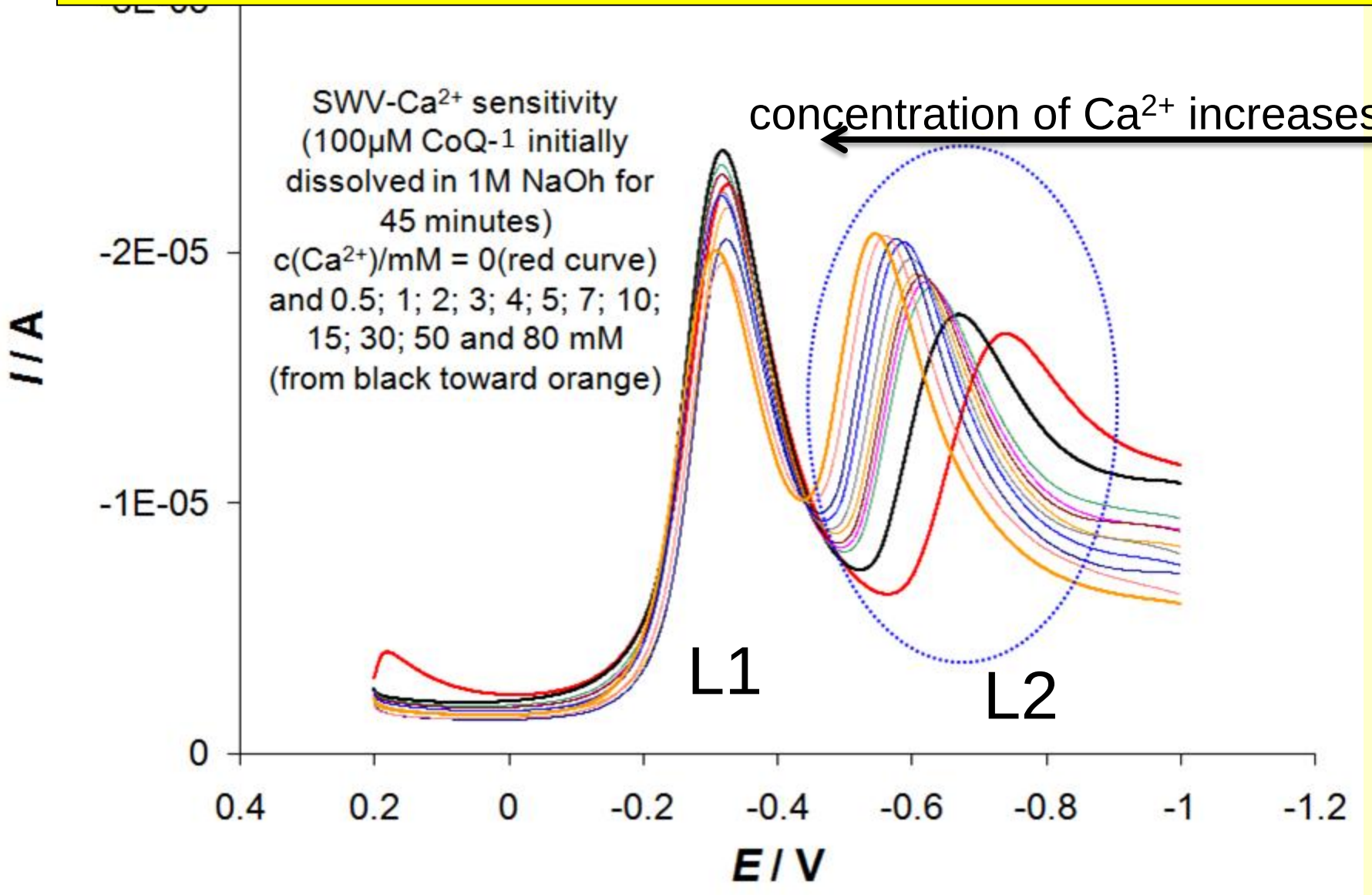


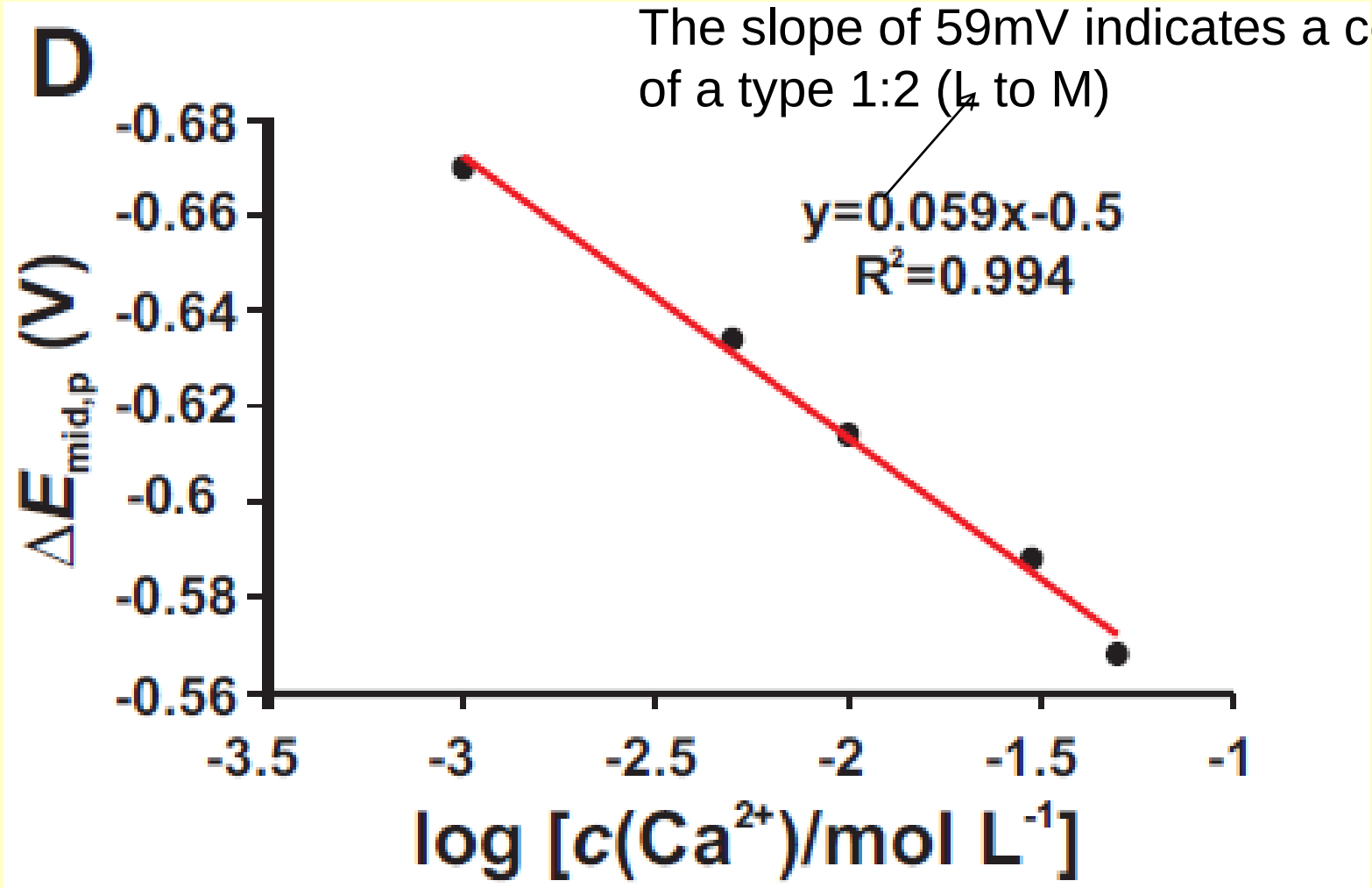
EDTA

Kstab

Lu^{3+} +  ⇌ Lu-(EDTA)x

TWO LIGANDS L1 and L2-One (L2) is able to bind Ca^{2+} ions in stochiometric ratio 1:2 ($L:M^{2+}$), while the other (L1) NOT





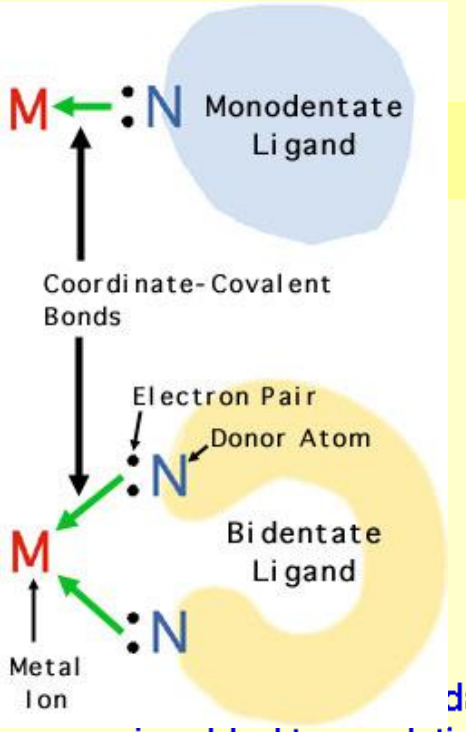
Dependence of the mid-peak potential of the cyclic voltammograms of the Ligands
on the logarithm of Ca^{2+} concentration

What can we evaluate from voltammetric Experiments?

Kst → Stability constant value

$$M^{X+} + yL = [MLy]^{X+}$$

→ Stochiometry of the complexes



dark blue tetraammine copper(II) ion when ammonia is added to a solution of copper(II) sulfate.

$$Cu^{2+}(aq) + 4NH_3 \rightleftharpoons Cu(NH_3)_4^{2+}(aq)$$

$$K_f = \frac{[Cu(NH_3)_4^{2+}(aq)]}{[Cu^{2+}(aq)][NH_3]^4}$$

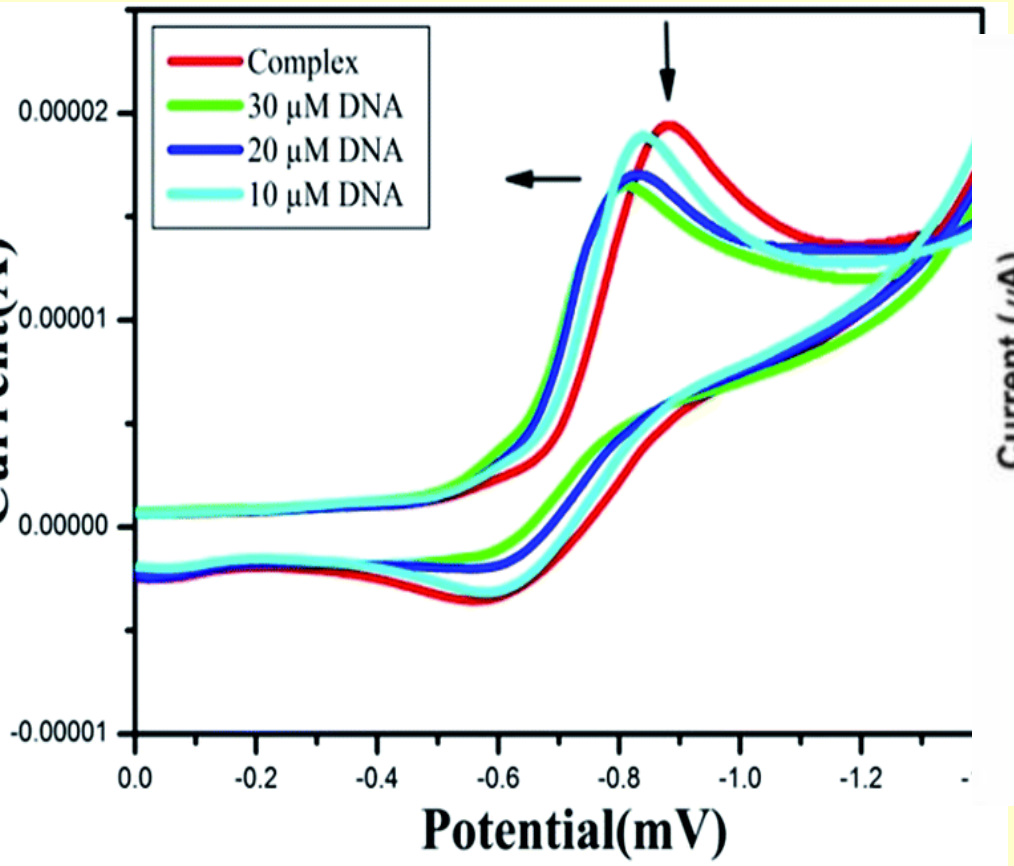
Stability constant / Formation constant

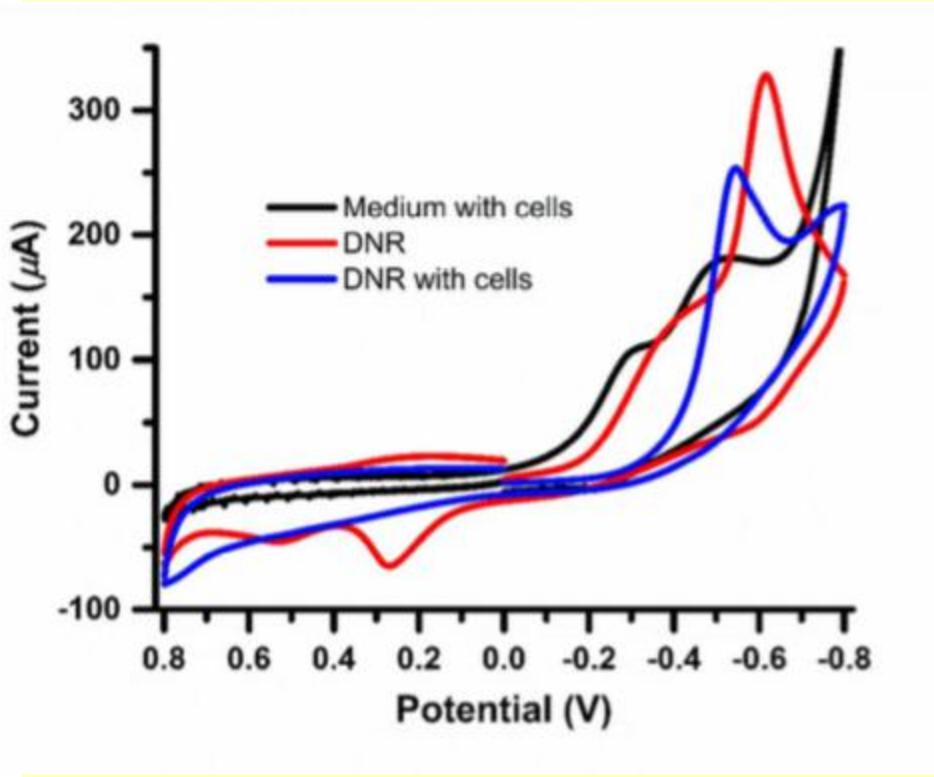
- According to Bjerrum formation of a complex in aqueous solution proceeds through a stepwise fashion with corresponding equilibrium constants

$M + L \overset{K_1}{\rightleftharpoons} ML$	ML	$K1 = [ML] / [M] [L]$
$ML + L \overset{K_2}{\rightleftharpoons} ML_2$	ML_2	$K_2 = [ML_2] / [ML] [L]$
$ML_2 + L \overset{K_3}{\rightleftharpoons} ML_3$	ML_3	$K_3 = [ML_2] / [ML2] [L]$
.....		
.....		
$ML_{n-1} + L \overset{K_n}{\rightleftharpoons} ML_n$	ML_n	$K_n = [ML_n] / [ML_{n-1}] [L]$

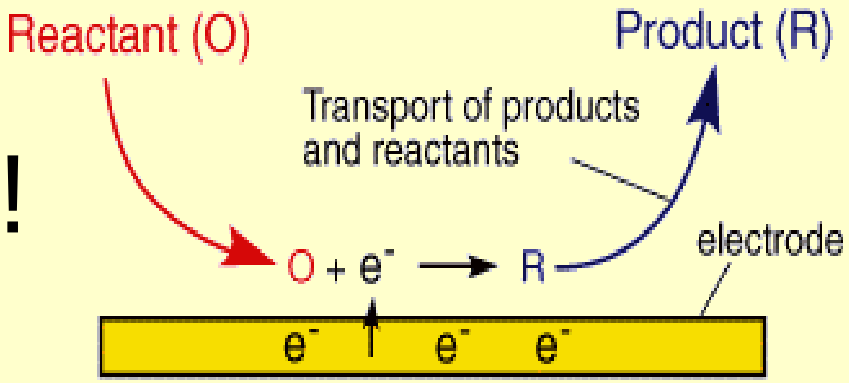
These $K_1, K_2, K_3 \ldots K_n$ are called stepwise formation constants

Voltammetry-tool for studying the strenght of the drug-drug interactions...it shows whether and how strong Two drugs interact between them when administrated... Gives hints whether two drugs should be taken together or not...



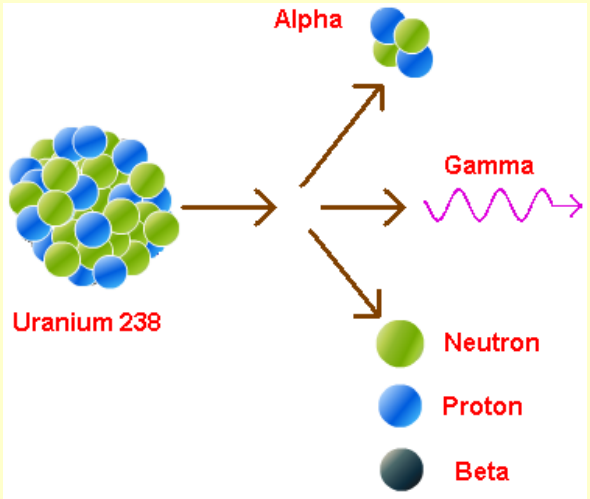


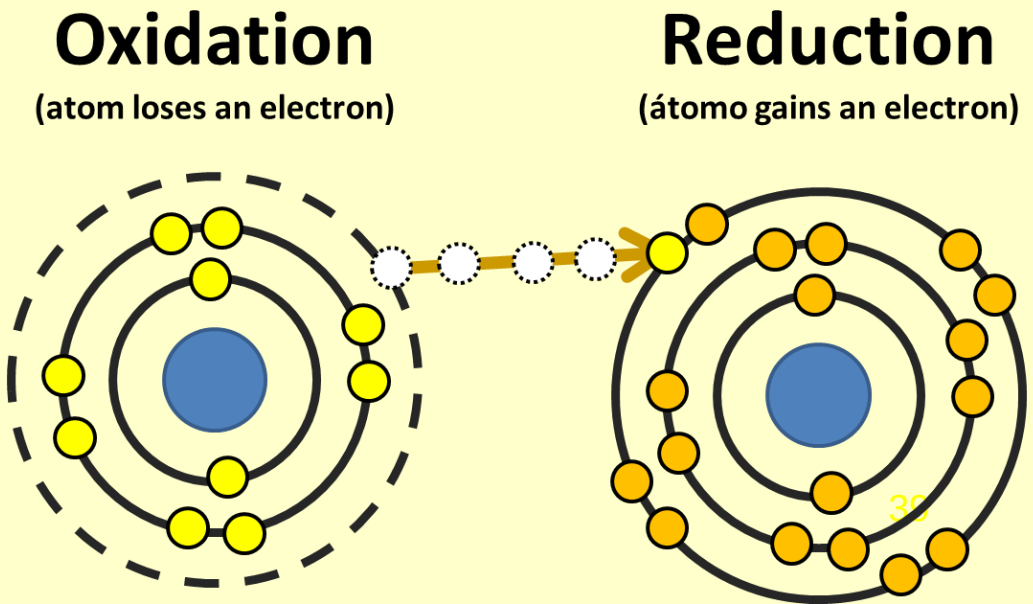
VERY IMPORTANT!!!



Electrochemistry deals with the electrons, NOT with the CORE particles of the atom!!!

So, whether we use RADIOACTIVE or „NORMAL“ non -radioactive isotopes, we should have (almost) the Same results!





Final Conclusions:

Voltammetry is a SIMPLE, CHEAP and powerful tool for:

-Quantitative determination (sensing) of various biologically active compounds

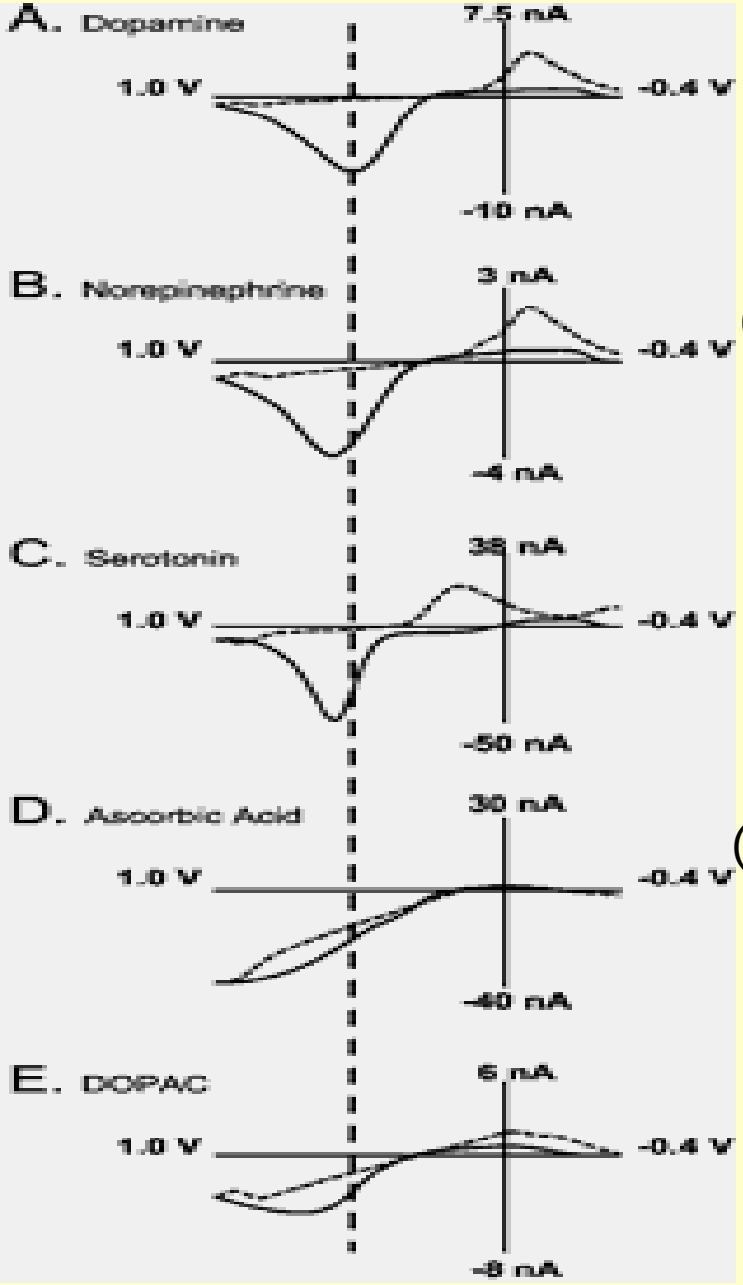
-simple technique for revealing the mechanistic pathways and The nature of **drug-drug, metal-drug interactions**

-effective tool **for thermodynamic and kinetic measurements**

-Inevitable technique in almost ALL research laboratories

In GENERAL: FOR EVERY SYSTEM (Compound) ONE CAN GET ELECTROCHEMICAL INFORMATION REGARDLESS OF ITS STRUCTURE

Cyclic Voltammograms of some NUEORTRANSMITTERS



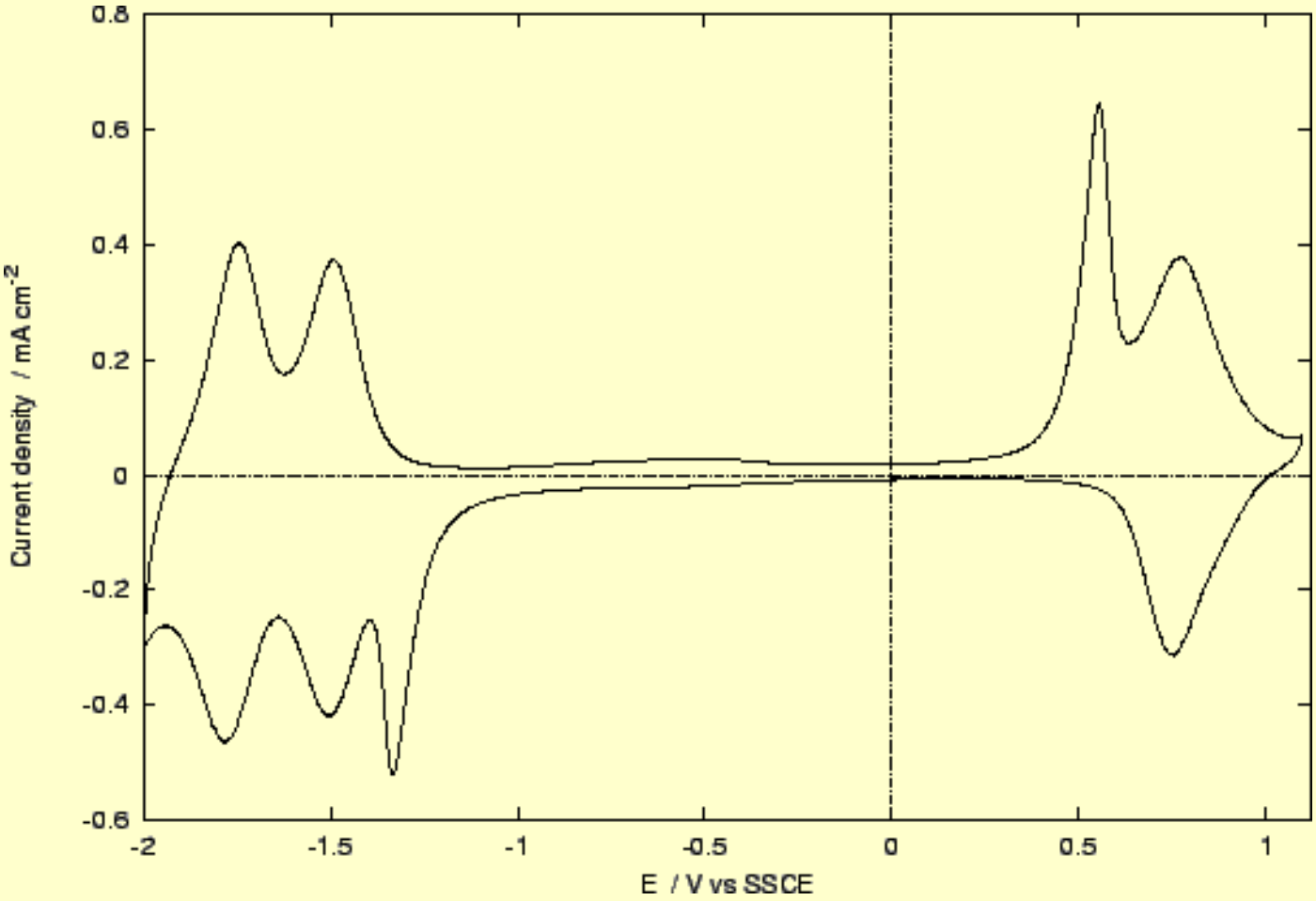
A), cyclic voltammogram for 2 µmol/L **dopamine**.

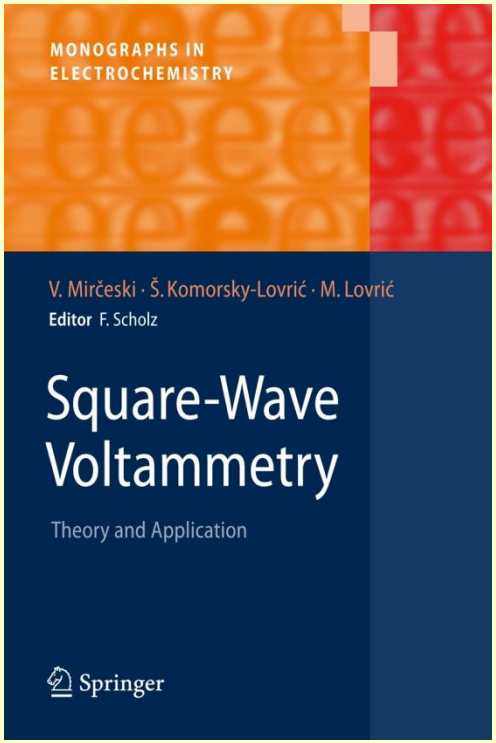
(*B*), cyclic voltammogram for 2 µmol/L **norepinephrine**

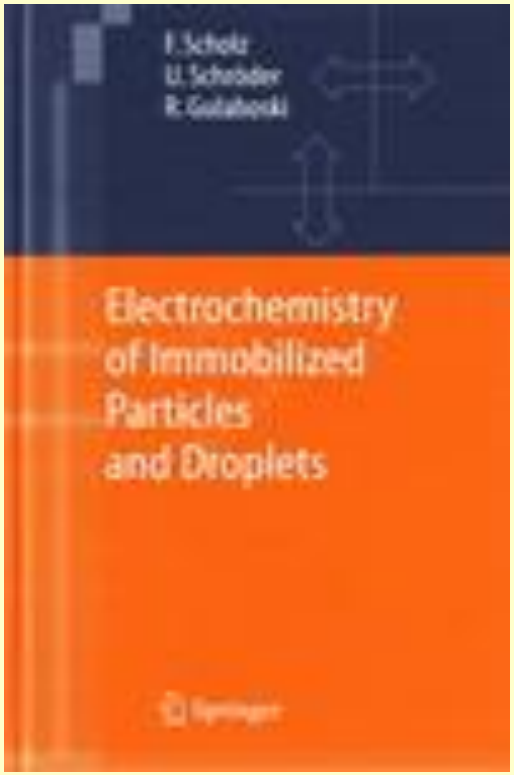
(*C*), cyclic voltammogram for 2 µmol/L **serotonin**

(*D*), cyclic voltammogram for 200 µmol/L **ascorbic acid**

(*E*), cyclic voltammogram for 20 µmol/L **DOPAC**


0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-2
-1.5
-1
-0.5
0
0.5
1
Current density / mA cm-2
E / V vs SSCE





Rubin Gulaboski, in
ELECTROCHEMICAL DICTIONARY (2008)
A. J. Bard, G. Inzelt, F. Scholz (editors)

F. Scholz, U. Schroeder, **R. Gulaboski**

R. Gulaboski, C. M. Pereira in
Handbook of Food Analysis Instruments (2008)
Semih Otles (Ed.)

Acknowledgments

A. v. Humboldt Foundation
Prof. Markus Hoth
Dr Ivan Bogeski

Prof. Fritz Scholz
Greifswald University

Prof. Valentin Mirceski
Macedonia University

Prof. Milivoj and Sebojka Lovric
Croatia

The speaker's attendance at this conference was sponsored by the Alexander von Humboldt Foundation.

- http://www.humboldt-foundation.de