

BIOSENOSRS

BIO 580

Electrochemical Biosensors - theory part 3

Amperometric & Conductimetric Biosensors

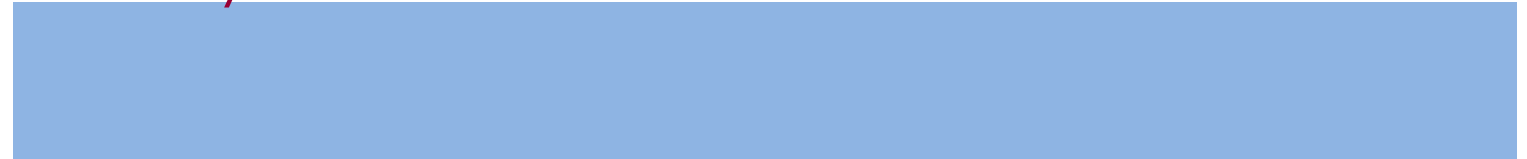
WEEK-3

Fall Semester

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Faculty of Engineering & Natural Sciences

Sabanci University



Topics that will be covered in the course

- ❑ History of biosensor development, applications and requirements of biosensors and classification
- ❑ Principles of molecular recognition and transduction signal acquisition
 - ✓ Sources of Biological Recognition elements – enzymes/proteins, ssDNAs, antibody and Others
 - ✓ Design considerations for use of recognition elements in biosensors
 - ✓ Modeling of reactions for various biosensor applications- electrochemical, optical, piezoelectric, colorimetric, fluorometric and others.
- ❑ Modification of sensor surfaces and immobilization techniques
 - ✓ Covalent modification of surfaces using surface chemistry
 - ✓ Self Assembled Monolayers (SAM) and adsorptions
 - ✓ Other ways to immobilize biological macromolecules on various solid surfaces
- ❑ Detection methods and Physical Sensors
 - ✓ Electrodes/transducers – electrochemical (amperometric, potentiometric, and conductimetric transductions)
 - ✓ Other sensors - for e.g., optical sensors (colorimetric/fluorimetric/luminometric sensors), Surface Plasmon Resonance (SPR) sensors, and piezoelectric resonators.
- ❑ Fabrication of biosensors
 - ✓ Miniaturization-application of nano-materials, nanoparticles, carbon nanotubes (CNTs) and others
 - ✓ Biocompatibility – stability, reproducibility and repeatability of biomolecules on transducer surfaces
- ❑ Data acquisition, statistical and error analysis
 - ✓ Inter and Intra-assays and Coefficient of variation (CV)
 - ✓ Signal to noise ratio
 - ✓ Normalization/optimization and signal retrieval
- ❑ Examples of commercial biosensors

Electrochemical Biosensor

There are three basic electrochemical processes - useful in transducers for biosensors:

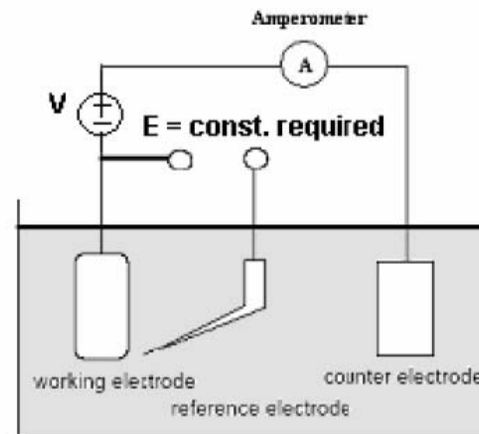
- i. **Potentiometry** - the measurement of a cell potential at zero current
- ii. **Voltammetry (amperometry)** - an oxidizing (or reducing) potential is applied between the cell electrodes and the cell current is measured
- iii. **Conductimetry** - the conductance (reciprocal of resistance) of the cell is measured by an alternating current bridge method.

Electrical measurements on a solution of the analyte using two or more electrodes (electronic conductors)

POTENTIOMETRY	AMPEROMETRY	VOLTAMMETRY
♦ Measure potential difference (E) at zero current ♦ Two electrodes are used – indicator and reference ♦ Carried out under equilibrium conditions ♦ Quantitative analysis – E is related to the concentration of ions in the sample	♦ Measure current at fixed potential ♦ Two or three electrodes are used ♦ Coulometry – when the current is integrated to give total charge	♦ Measure current as a function of scanned potential ♦ Three electrodes are used – working, reference and counter (auxiliary) ♦ Non-equilibrium measurement – gives kinetic information ♦ Qualitative and quantitative analysis ♦ Polarography – use of a dropping Hg electrode

Three electrode cell

- Requires a precise control of the potential at the electrode.
- Three electrodes:
 - Working electrode (WE),
 - Counter electrode (CE)
 - Reference electrode (RE).
- No current through RE ideally.
- RE is used to provide precise control of potential at the WE, and the current from WE to CE is measured.



Reference Electrode

An electrode which has reproducible potentials in solution with low coefficient of variation with temperature. Many varieties of these electrodes have been devised.

Most common and available commercially:

- Standard hydrogen electrode (SHE) ($E=0.000\text{V}$)
- the silver-silver chloride electrode ($E=0.225\text{V}$ saturated)
- the saturated calomel electrode (SCE) ($E=+0.242\text{V}$ saturated)

Auxiliary electrode

The auxiliary electrode is also known as the counter electrode.

Its purpose is to conduct electricity from the signal source into the solution, maintaining the correct current.





Working electrode

The most important electrode in CV is the working electrode. It can be made from a variety of materials including: platinum, gold, silver, glassy carbon, nickel and palladium.

Redox of the analyte takes place here.

Votammetry

Voltammetry: An increasing (decreasing) potential is applied to the cell until oxidation (reduction) of the substance to be analyzed occurs and there is a sharp rise (fall) in the current to give a peak current.

The height of the peak current is directly proportional to the concentration of the electro active material.

If the appropriate oxidation (reduction) potential is known, one may step the potential directly to that value and observe the current

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



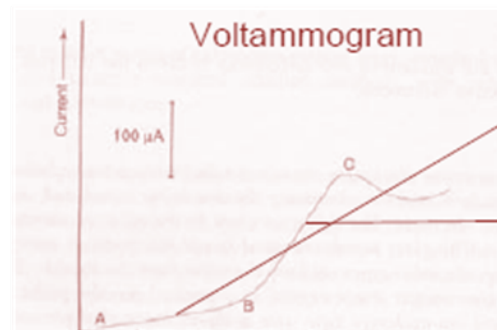
- Capacitive current: charging of double layer

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

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

linear-sweep voltammetry

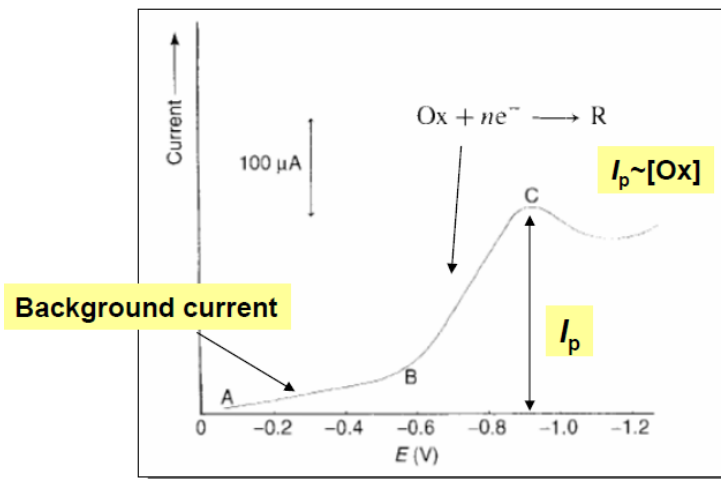
Application of a linearly varying potential between a working electrode and a reference electrode in an electrochemical cell containing a high concentration of an indifferent electrolyte to make the solution conduct (supporting electrolyte) and the electroactive species (an oxidizable or reducible species--- $\text{Ox} + ne^- \rightarrow \text{R}$).



A-B Insulating interface

B Reduction potential of the oxidized species

B-C Increase of cell current due to the flow of electron to the oxidized species for reduction

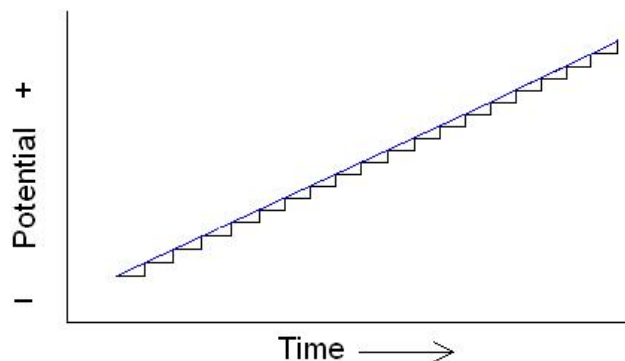


$$E_p = E^0 + 0.056/n$$

Stairwave voltammetry

A derivative linear sweep voltammetry

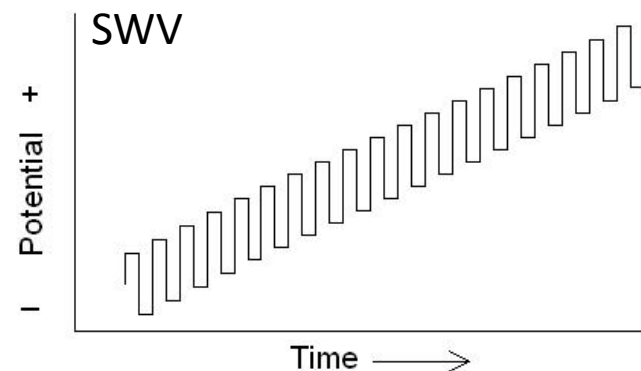
- 1.
- 2.
3. In StWV, voltammetry the potential sweep is a series of stair steps.
4. The current is measured at the end of each potential change, right before the next, so that the contribution to the current signal from the capacitive charging current is minimized.



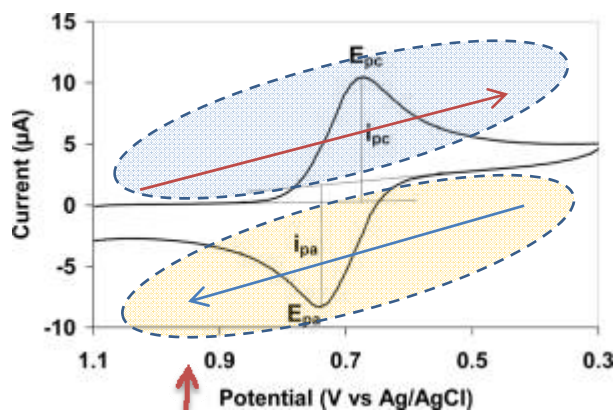
Squarewave voltammetry

A derivative linear sweep voltammetry

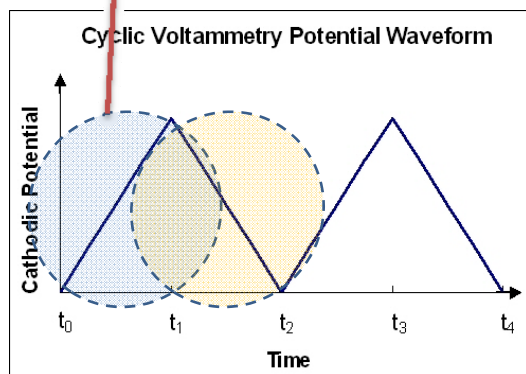
1. The current at a working electrode is measured while the potential between the WE and a RE is swept linearly in time- linear
2. Oxidation or reduction of species is registered as a peak or trough in the current signal at the potential at which the species begins to be oxidized or reduced.
3. In SWV, a squarewave is superimposed on the potential staircase sweep.
4. The current is measured at the end of each half-wave, just prior to potential change.
5. The differential current is then plotted as a function of potential, and the reduction or oxidation of species is measured as a peak



Cyclic Voltammetry

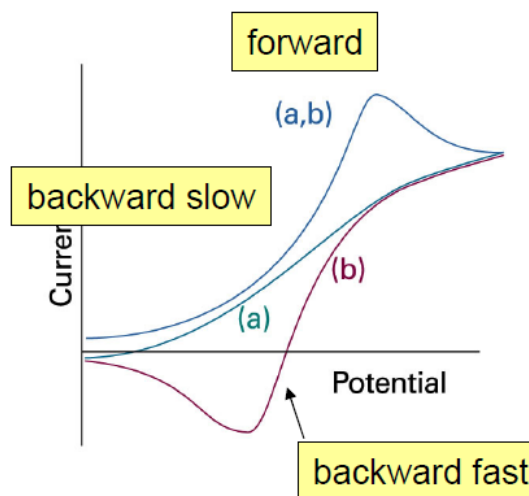
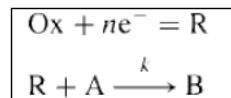


The average of the two peak is E^0

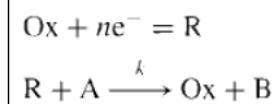


1. Working electrode potential is ramped linearly Vs time in linear sweep voltammetry
2. CV uses a set potential and the cyclic nature reaches a set potential, the working electrode's potential ramp is reversed.
3. This reversion can happen multiple times during a single expt.
4. The Current at the WE is plotted Vs the applied V to give a cyclic voltammogram.

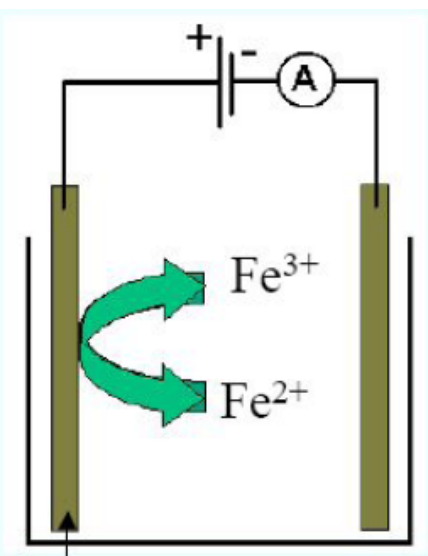
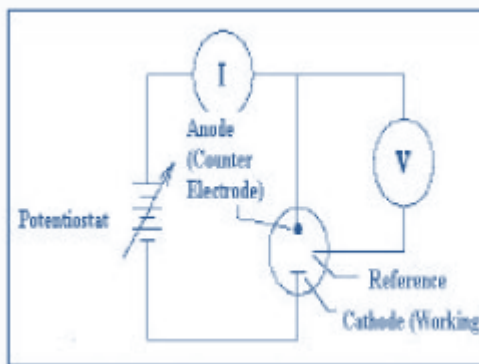
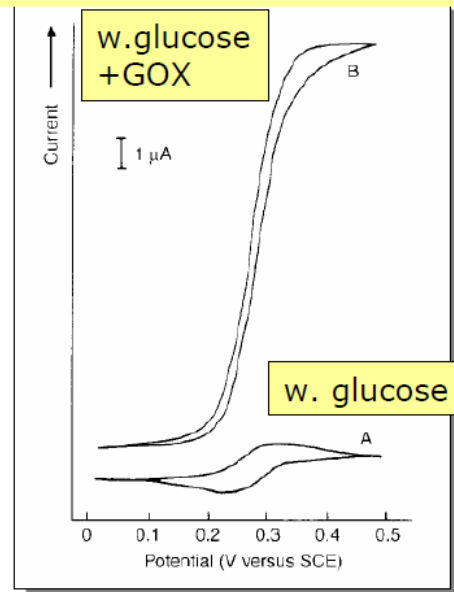
- usually, there is another chemical reaction coupled to the electron transfer
 - consumption of reduced product



–regeneration of the oxidized reagent



Voltammogram of ferrocene

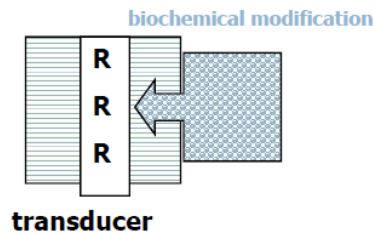


Amperometric Biosensors

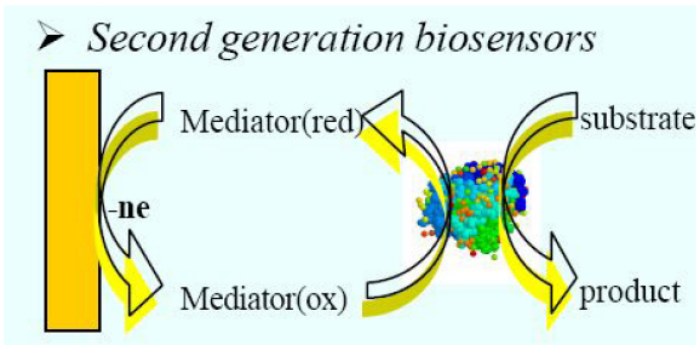
- First Generation – oxygen electrode based sensors
- Second Generation – mediator based sensors
- Third Generation – directly coupled enzyme electrodes

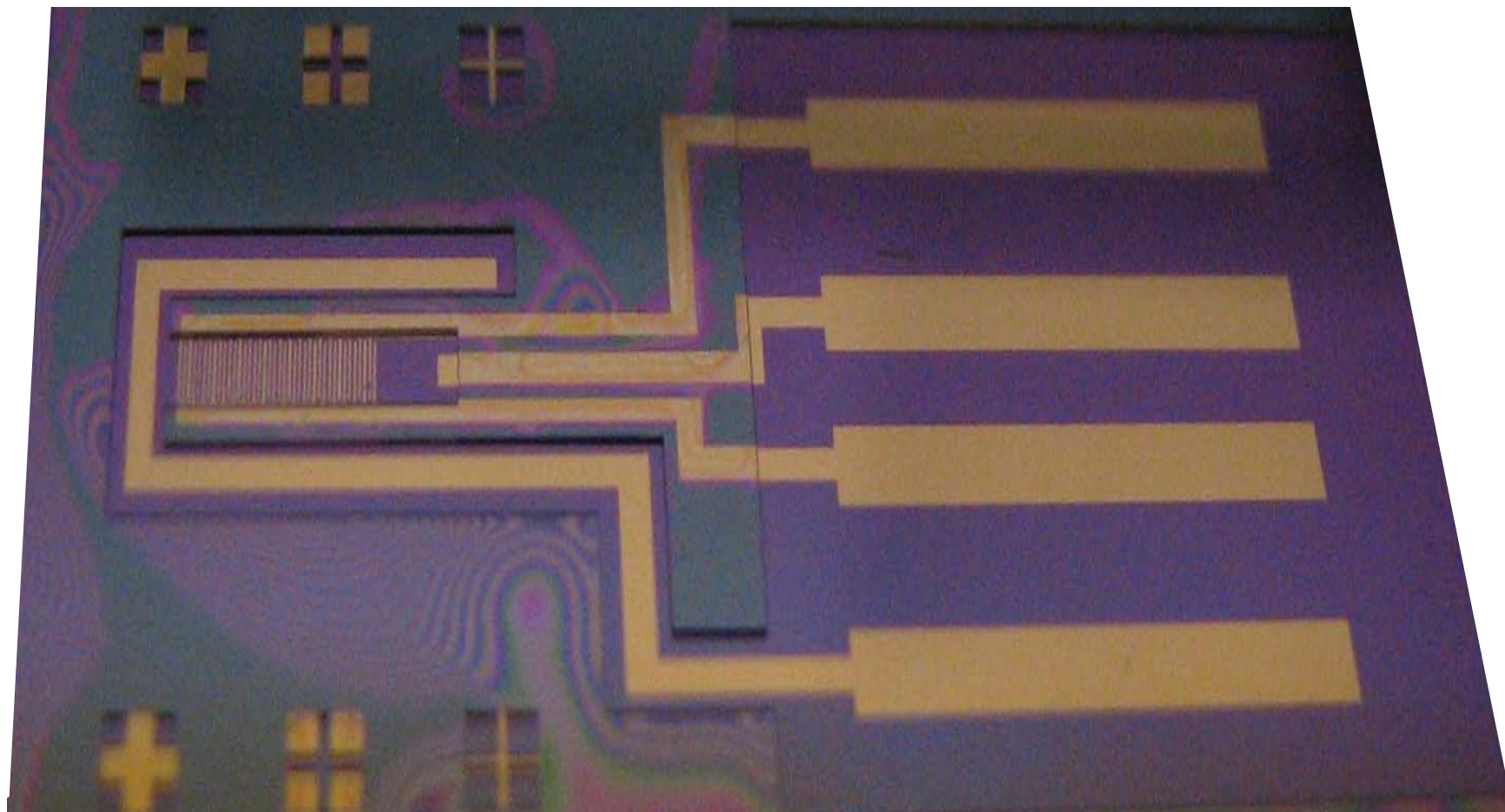
2nd generation:

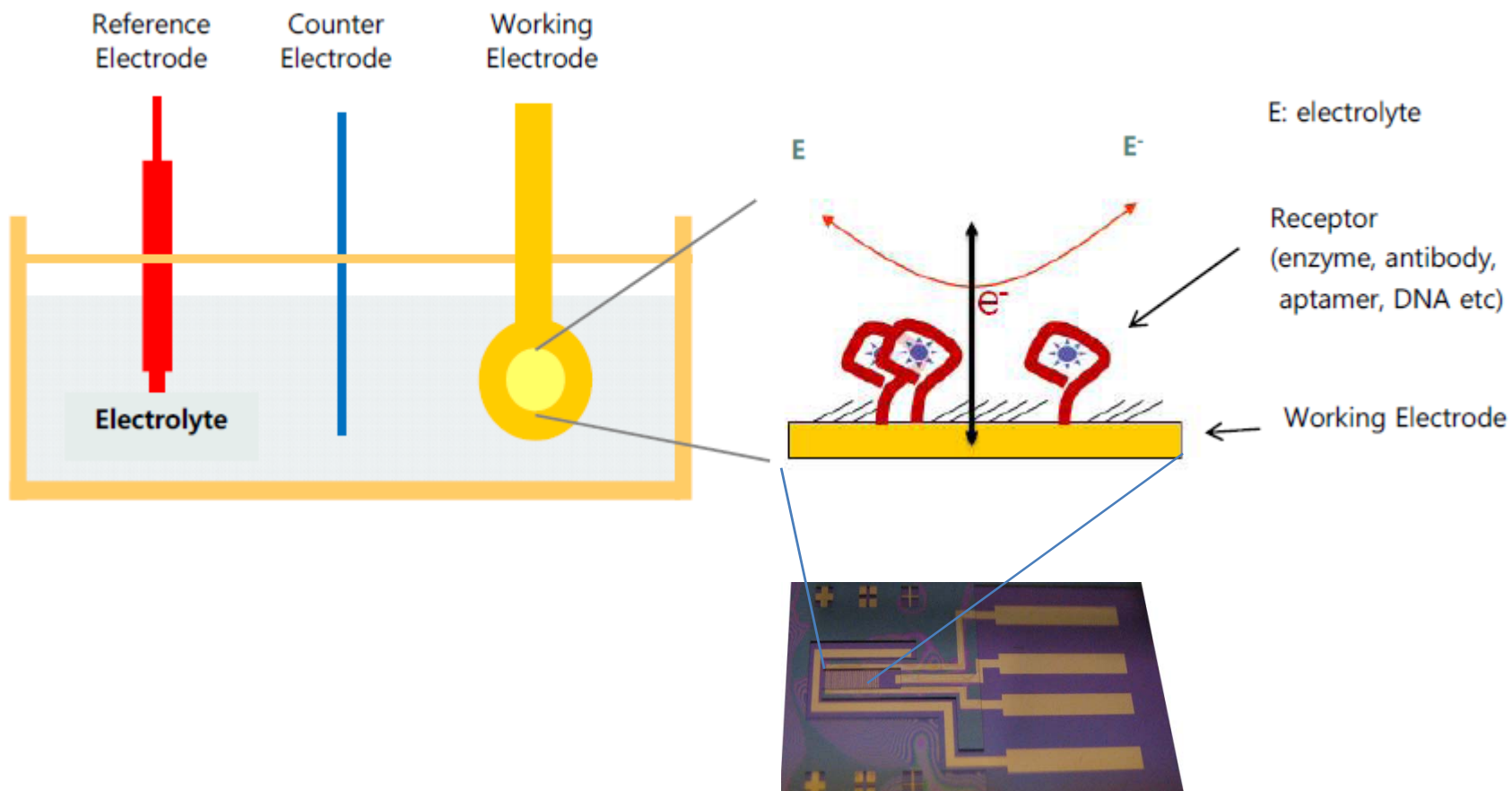
the **covalent fixation** of biologically active component to the transducer's surface permits the elimination of the semi permeable membrane



Introduction of mediators







Role of the potentiostat:

- ✦ Control the potential at the working electrode relative to the reference electrode
- ✦ Adjust the current between the working and counter electrodes
- ✦ Accord negligible current through the reference electrode

Consequence of good potentiostat and cell design:

- ✦ Only the electrochemistry at the working electrode need be considered
- ✦ Because of negligible current draw through the reference electrode, this value of potential remains constant
- ✦ The area of the counter electrode should be large (ca. 1,000 times) that of the working electrode

$$E = E_{\text{applied}} + iR_u$$

Where

E = the actual impressed electrode potential

E_{applied} = the apparent impressed electrode potential

R_u = resistance

$$i = i_{\text{faradaic}} + i_{\text{capacitive}}$$

Where

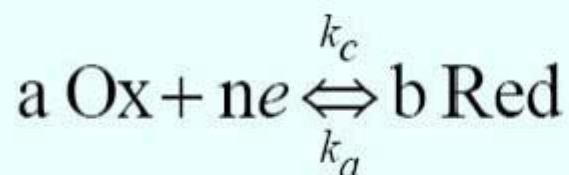
i_{faradaic} = the current arising from charge transfer reactions on or at the electrode. Arises from serial coupling of both mass transport and electron transfer. One or other may be rate limiting.

$i_{\text{capacitive}}$ = the current associated with the charging and discharging of the double layer

R_u = resistance

◆ Thermodynamics

- /// Guides us as to whether a redox reaction should occur and the direction and extent of reaction
- /// Governed by the Nernst Equation



$$E = E^\theta + \frac{RT}{nF} \ln \left(\frac{a_{\text{Ox}}^a}{a_{\text{Red}}^b} \right)$$

- ◆ Electric potential forces the electrochemical equilibrium to shift from the oxidized (reduced) form of the analyte to the reduced (oxidized) form; the resulting charge transfer across the electrode/solution interface is observed as current.

$$i_{\text{cathodic}} / nFA = k_c C_{\text{Ox}}(x = o, t)$$

$$i_{\text{anodic}} / nFA = k_b C_{\text{Red}}(x = o, t)$$

$$i_{\text{total}} = i_{\text{cathodic}} - i_{\text{anodic}}$$

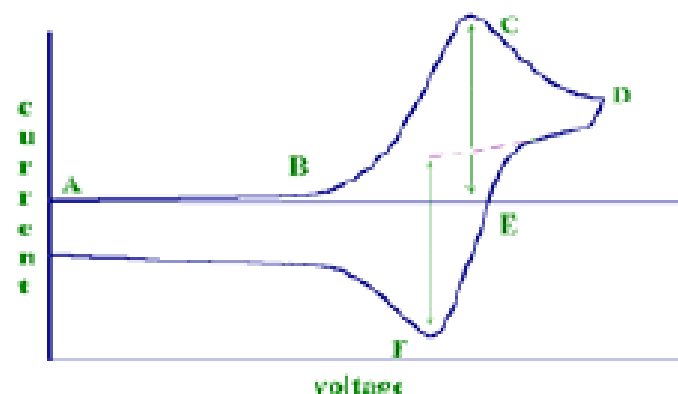
Voltametry – Mass Transport

- ◆ **Diffusion:** The movement of species arising from a difference in chemical potential
 - /// Consumption or appearance of a specie will change its chemical potential.
- ◆ **Convection:** Movement of the medium, e.g via stirring or from temperature gradients
 - /// Stirring will alter the boundary layer thickness
- ◆ **Electro-migration:** Movement of ions under the influence of an electric field. (Not normally important because of the use of a large background of supporting electrolyte)
 - /// Background electrolyte of KCl
 - /// K^+ and Cl^- have similar transference numbers

Analysis of an Example

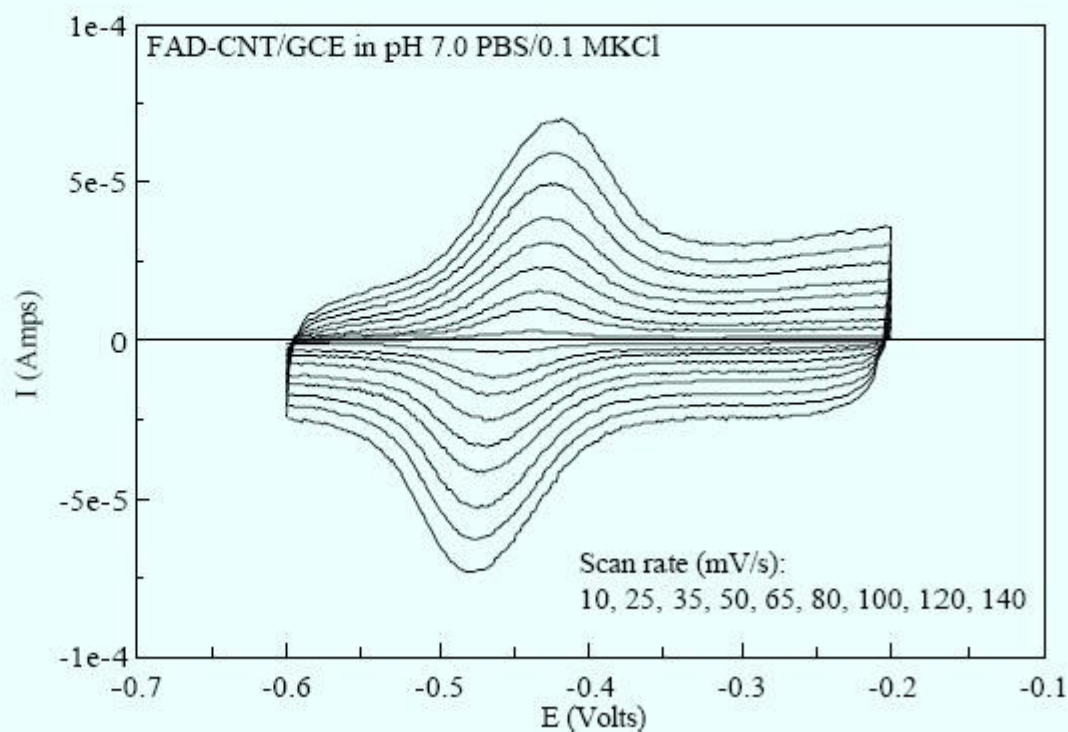
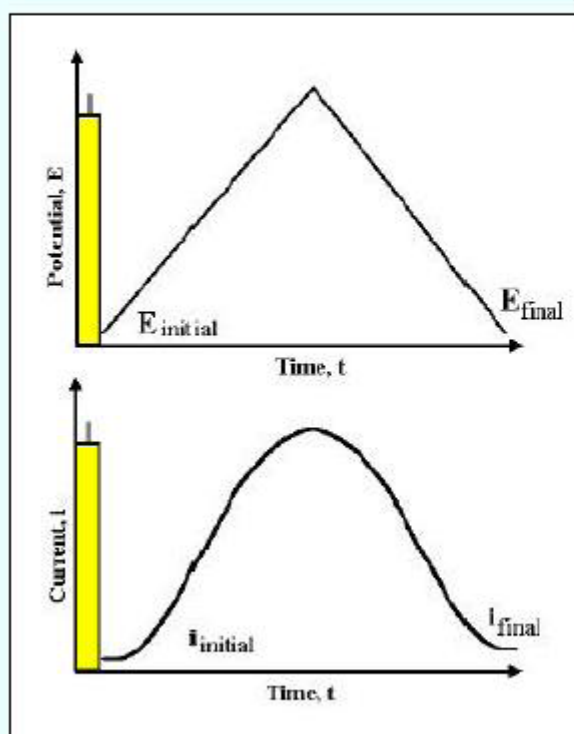
$\text{K}_3\text{Fe}(\text{CN})_6$

- Starting at an initial voltage (A), the potential is scanned in the negative direction.
- At B, the potential has become negative enough to start a cathodic current between the species, reducing the analyte at the working electrode.
- The reaction continues at the electrode until most of the species has been reduced, peaking the cathodic current at (C).
- The current then decays for the rest of the forward scan until the potential scan is reversed (D). The scan in the positive direction proceeds similarly to that of the negative scan.



- The cathodic current continues to slowly decay until the potential reaches a point to start the oxidation of the analyte (E).
- The anodic current is then measured as the concentration of the reduced species is significantly diminished (F).
- The anodic current then decays from this peak, and the potential completes its cycle.

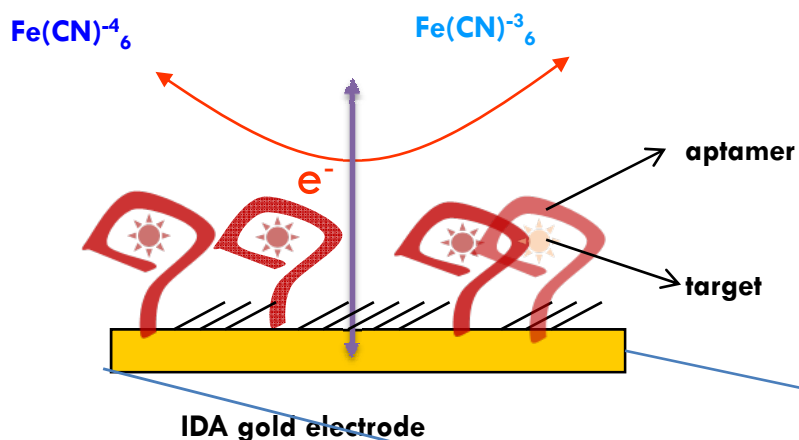
Cyclic Sweep Volatmetry



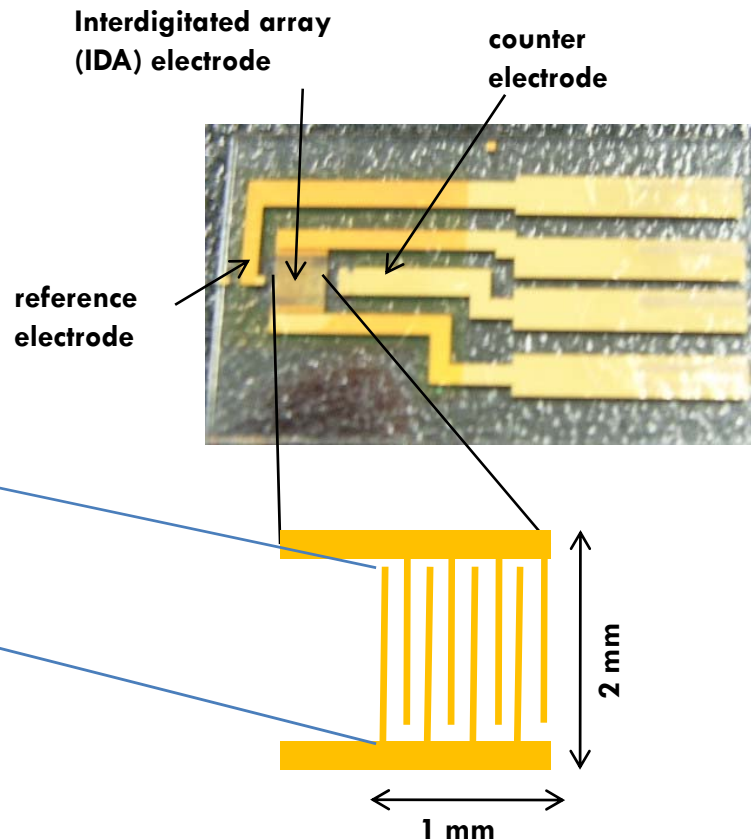
Cyclic Voltammetry - a case study

Electrochemical detection of OTC using aptamer immobilized gold IDA electrode chip

A typical electrochemical reaction



PGstat 12 (Autolab): Electrochemical Analyzer

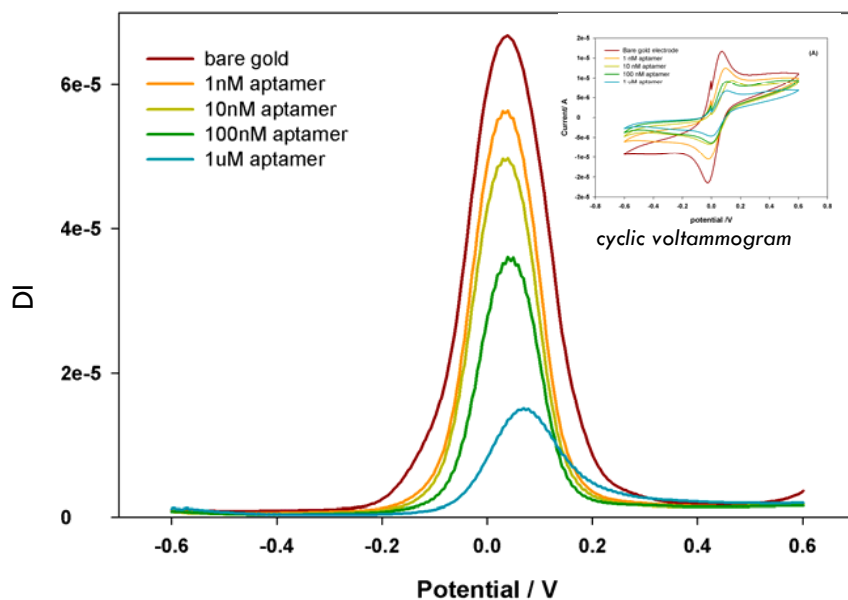


$$(\Delta I = (I_0 - I_1) / I_0 \times 100)$$

ΔI - relative current change

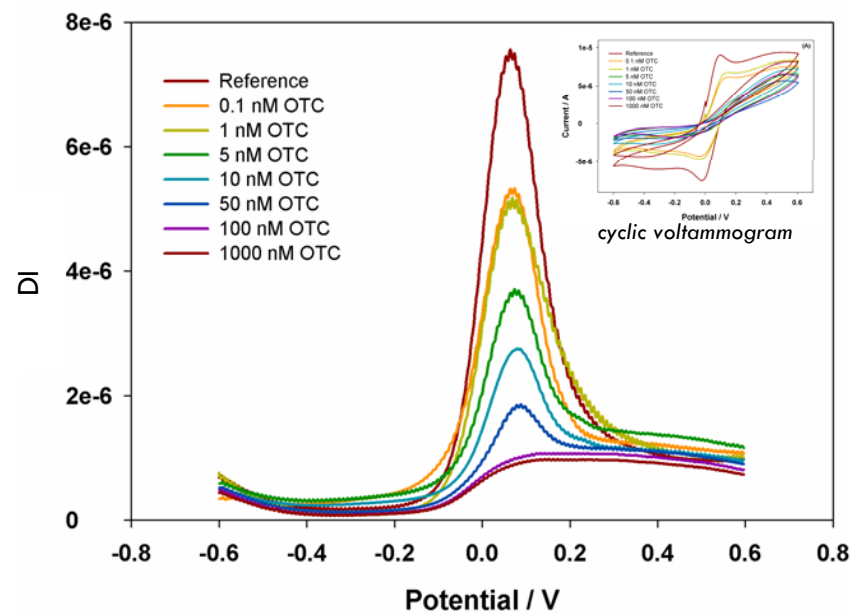
I_0 & I_1 - the current before and after sample application

Electrochemical analysis for immobilization of thiol-modified aptamer on IDA gold electrode

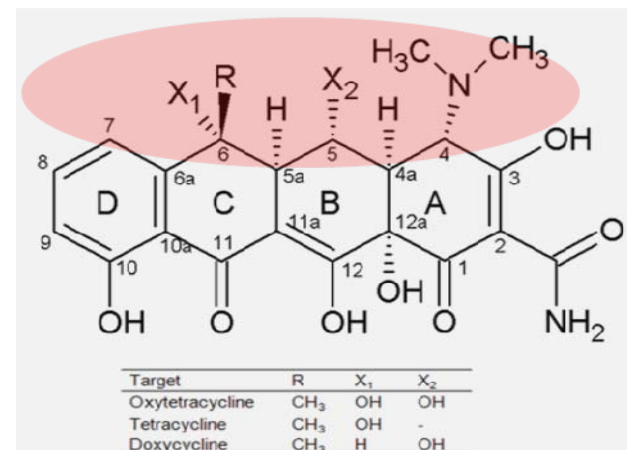
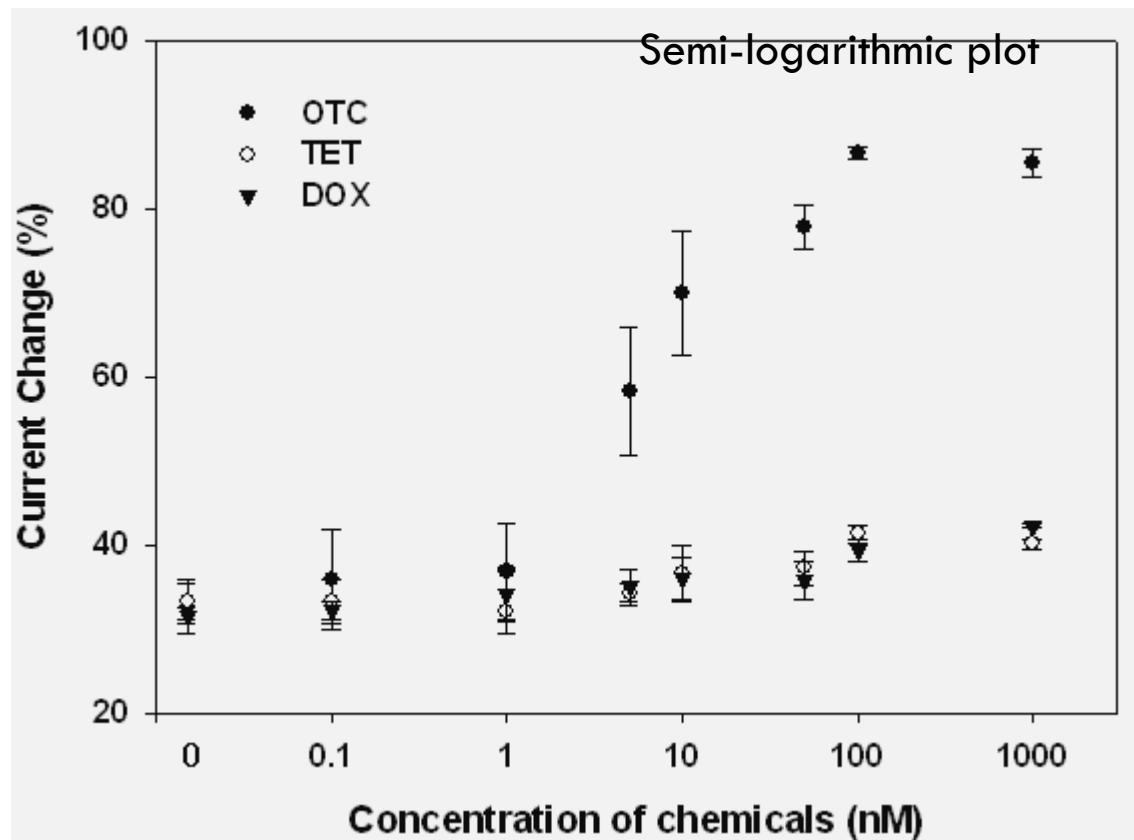


Current decrease depended on the concentration of ssDNA aptamer in square wave voltammogram (SWV)

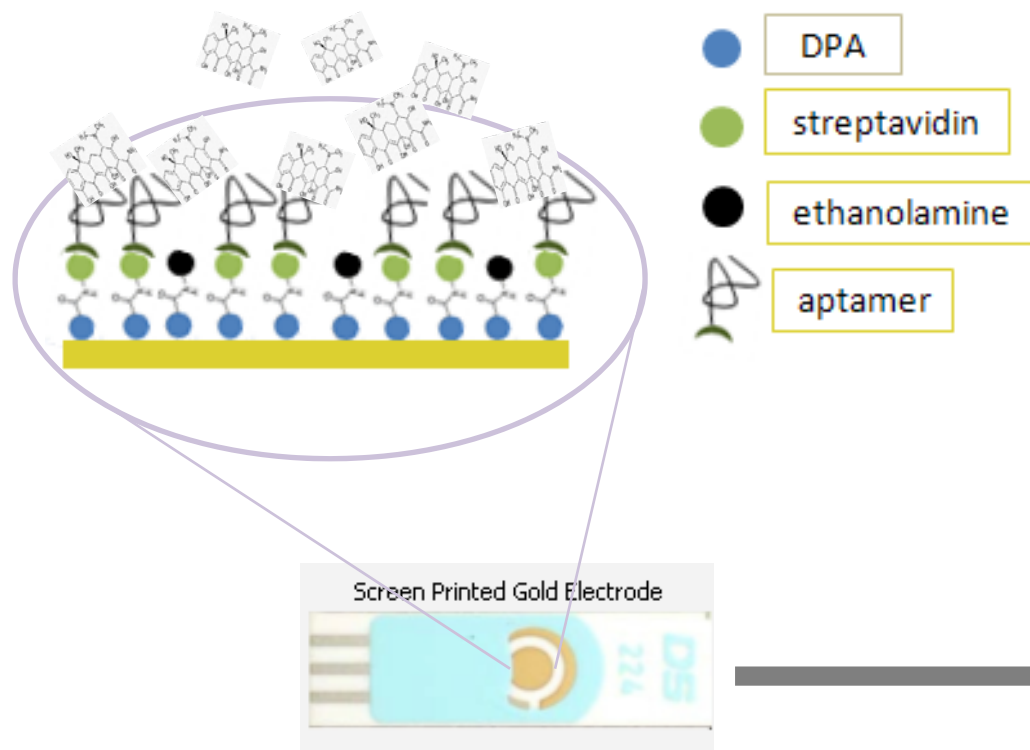
Electrochemical analysis for OTC using 1 nM aptamer immobilized IDA gold electrode



Current drop- depended on the concentration of OTC in SWV

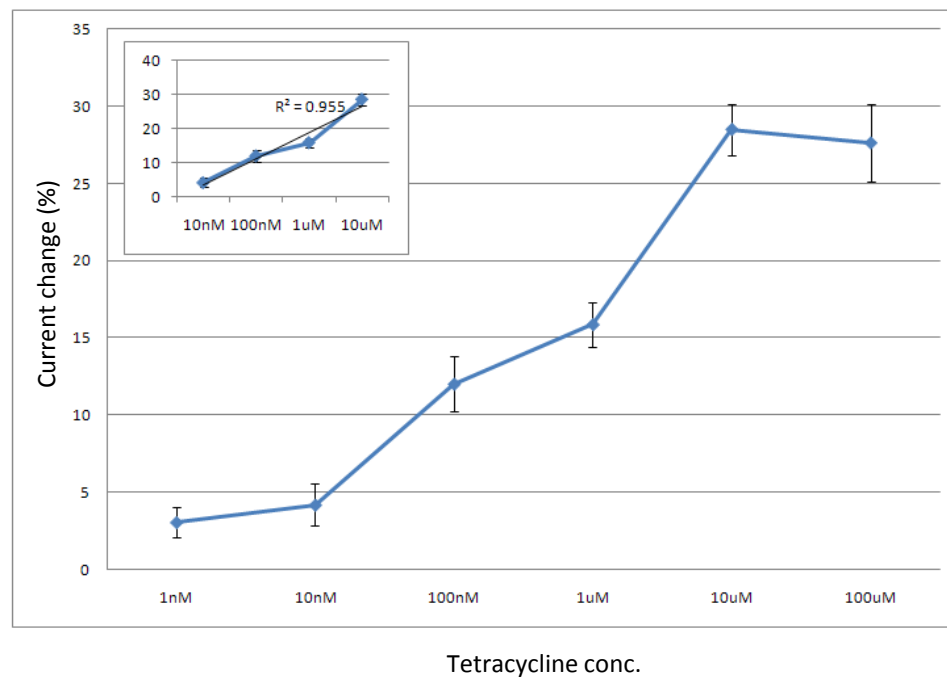
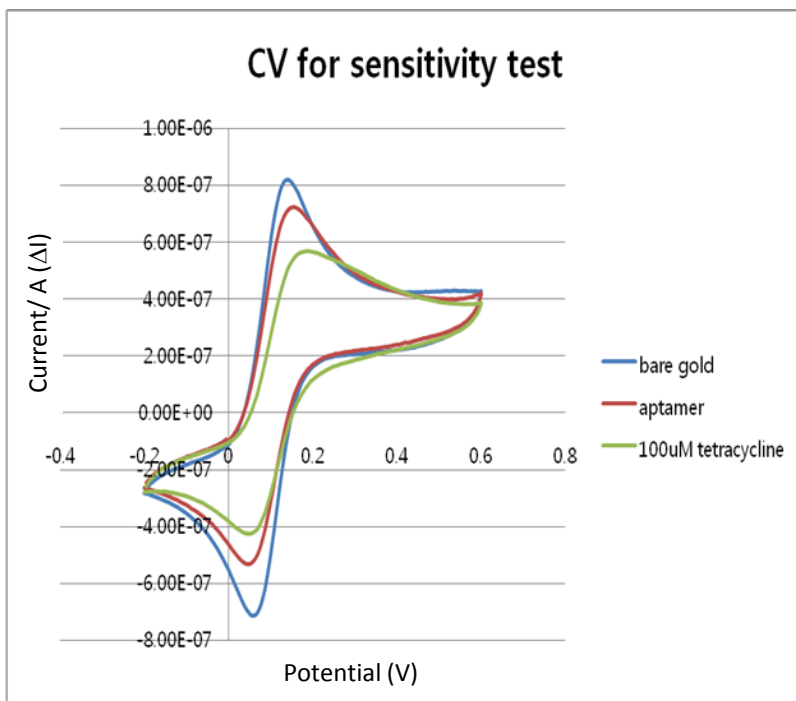


Eg., 2) Electrochemical aptasensor for TET detection



Screen printed gold electrode (DropSens Inc.)

- WE: Gold, CE: Gold, RE: Silver
- Inner diameter of WE: 1.6 mm



Sensitivity in a range 1 nM -10 μ M with preferential selectivity toward TET