AMPEROMETRIC BIOSENSORS

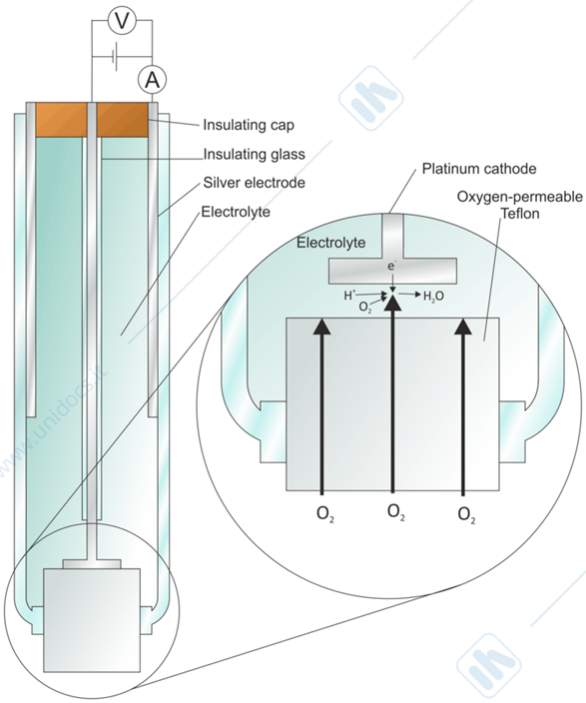
The only difference between these biosensors and the potentiometric ones is that here we work under current probing, so it's a sensor not working in equilibrium conditions. That means that the techniques and the instruments that I have to use to measure this signal have to be more sophisticated.

Clark cell

Originally the Clark cell is of non-bio nature and was created for detecting O_2. The cell itself consists of 2 electrodes: a working Pt electrode (where O_2 reduction reaction occurs) and a reference electrode (Ag/AgCl). Both electrodes are plunged into an in-cell saturated solution of KCl (as you know, it's necessary to have the Cl^- ions in solution in order to have an exact Ag/AgCl reference electrode). All that is separated from the measured solution by a Teflon or PTEF membrane permeable to O_2 only (if possible), so we can avoid interferences. When the potential of 0.6V is applied by an automatic system, the reduction reaction occurs and we obtain a signal that is proportional to the amount of oxygen that is present in the solution (this is why the reaction environment has to be closed, because otherwise oxygen in the air would interfere). The reactions occurring on electrodes are:

Anode: $4Ag^0_{(s)} + 4Cl^-_{(aq)} = 4AgCl_{(s)} + 4e^-$ (1)

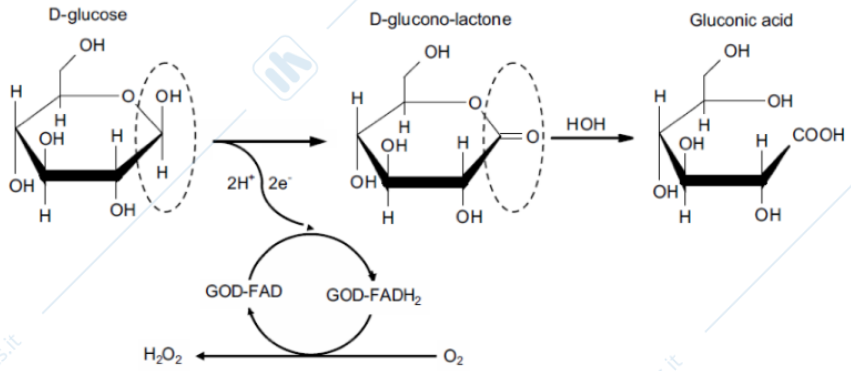
Cathode: $O_{2\,(g)} + 4H^+_{(aq)} + 4e^- = 2H_2O$ (2)



Pic.1 Clark cell

As mentioned before, this electrochemical cell served as a basis for the first glucose biosensor, only by making some simple changes. The basic idea is that the membrane is covered with glucose oxidase (the enzyme that oxidizes glucose with the production of gluconic acid O_2) and a signal

(electro-chemical potential of the working electrode) proportional to the amount of produced oxygen is measured. Anyway, this kind of biosensor in the beginning had some problems, because it strongly depends on the amount oxygen in the solution. This problem was overcome later, we will see how.



Reaction of glucose oxidation

Amperometric cell

In amperometric cells we apply voltage and then measure the produced current (these two variables are connected by Ohm's law). The rule is: the higher the concentration, the higher the current, in a linear way (**$i \propto C$**).

We saw that in potentiometric cells we just have to use 2 electrodes, because we simply measure the difference in potential. The amperometric cell consists of 3 electrodes:

- Working
- Auxiliary (counter)
- Reference

The voltage (difference of potentials) is applied to working and reference electrodes while the current flows between working and counter electrodes. In this type of cell there is no equilibrium.

Why do we need an auxiliary electrode? Because the main purpose of a reference electrode is to stay at the constant potential, which is impossible if the current flows through it, so we need one more electrode for a current flow i.e., an auxiliary one. In meantime, on the reference electrode there is a constant reaction:

$Ag^0 + Cl^- = AgCl$ (3)

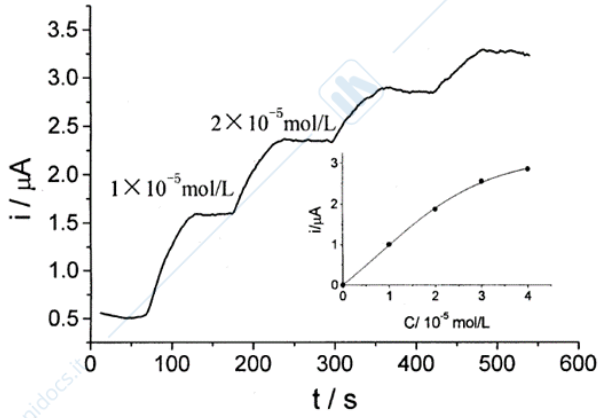
which is one of the drawbacks - over (very-very long) time the reference electrode will be worn out (consumed to form AgCl).

Amperometry

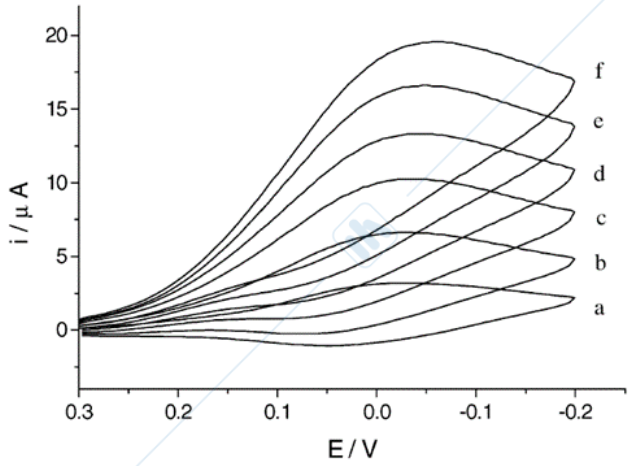
There are two methods of amperometry depending on how we apply the difference of potentials:

Voltammetry is at variable voltage. It is mostly used in laboratory work as not only it gives info about the concentration, but also about the presence of impurities in a solution (there will be more than one peak)

Chronoamperometry is at constant voltage. It is mostly used as a tool to make calibration curves by adding a known amount of measured electrolyte into the solution. The higher the concentration, the more time needed for the same voltage to induce the reaction (as shown on the upper graph).



Pic. 2 Chronoamperometry



Pic. 3 Voltammetry

Chronoamperometry

When the constant voltage is applied, the reaction needs some time to occur and that time is to be defined naturally (by the solution nature, etc.). Why does reaction take time? Because species need to come closer (diffuse) to the electrode surface and the higher the concentration, the more time needed for that -> causes the slope in the graph with the rise of C.

Mostly there are 3 main **stages in electrode kinetics**:

- Diffusion (usually a limitation step)
- Charge transfer
- Relaxation

Mass transport plays an important role in kinetics of the reaction and has many minor aspects in it like concentration gradient, its changes in space and time, etc. And reaction rate is a variation of moles of reaction species over time (4).

v_e – velocity (rate) of an electro-chemical reaction;

A – area in which the reaction occurs;

$N_{O/R}$ – number of molecules in oxidized or reduced forms;

F – Faraday constant = 96 485,3 C/mol;

n – number of electrons participating in the reaction.

$$v_e = -\frac{1}{A}\frac{dN_O}{dt} = \frac{1}{A}\frac{dN_R}{dt} \quad (4)$$

$$i = nFAv_e = -nF\frac{dN_O}{dt} = nF\frac{dN_R}{dt} \quad (5)$$

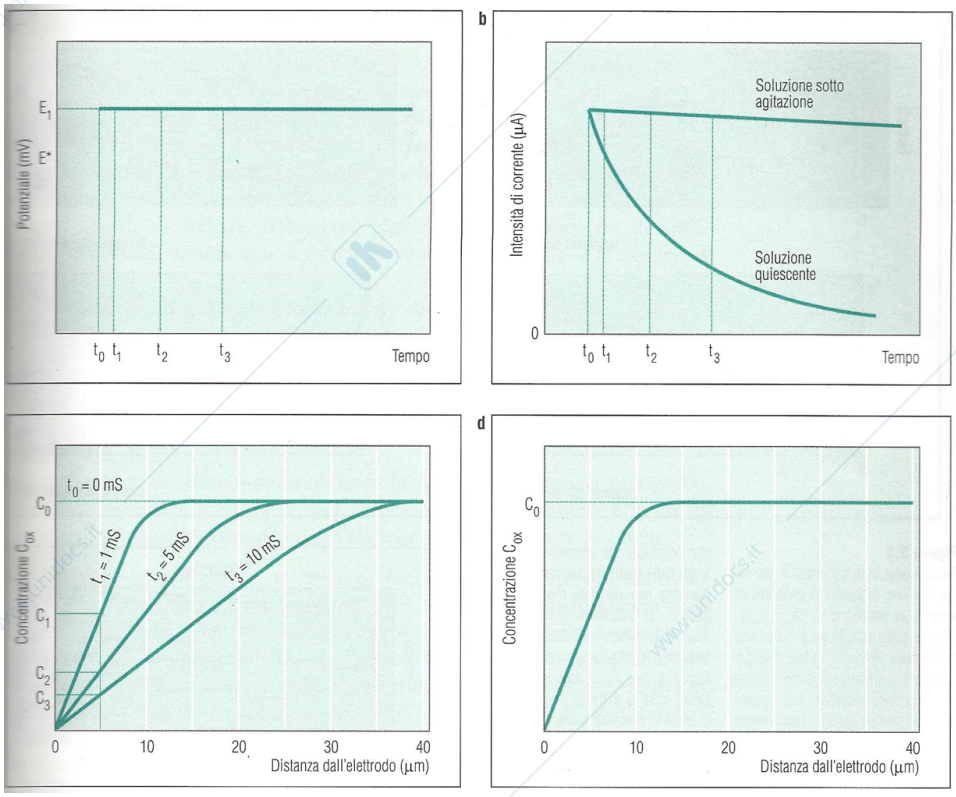
The space close to the electrode where most of the diffusion occurs is called **diffusion layer**. The law that determines the behavior of the solution in a diffusion layer is Fick's law, but we don't study this one in terms of our course. What we should know is that concentration of species changes not only over time, but also in space. Moreover, in Nernst model concentration profile the curve of concentration is deemed as a linear function (6) though out of the diffusion layer there's no linear trend.

$C_{O,\,b/i}$ - concentration of the oxidized species on the bulk/near the electrode;

δ - thickness of the diffusion layer.

$$\frac{dc_O}{dx} = \frac{c_{O,b} - c_{O,i}}{\delta_O} \quad \text{and} \quad \frac{dc_R}{dx} = \frac{c_{R,b} - c_{R,i}}{\delta_R} \quad (6)$$

What's shown on the upper right graph means that when we have an unstirred solution, we can't favor the movement of oxidized species towards the electrode (so make the current flow well, at high numbers) and hence, the current is decreased.



Pic.4 (upper right) The dependence of current in stirred and unstirred solutions on time; and (lower left) The dependence of thickness of diffusion layer on concentration in stirred and unstirred solutions.

The thickness of the diffusion layer differs in stirred and unstirred solutions too. First of all, we can determine the thickness with the help of the graph when the correlation stops to be linear (until bended region). And when the solution is unstirred this layer is thicker than in the same solution but stirred.

Faraday current (i_f) is a result of the reaction that occurs while charge transfer reaction on the surface of the electrode.

D – diffusion coefficient;

t – time.

$$i_f = nFA\sqrt{\frac{D}{\pi t}}c_b \qquad (7)$$

Capacitive current (i_c) is the current of formation of a double layer. When the voltage is applied there is a separation of charge, causing the formation of a double layer on the electrode that happens really fast and makes the curve steeper.

R – resistance of the system, Ohm;

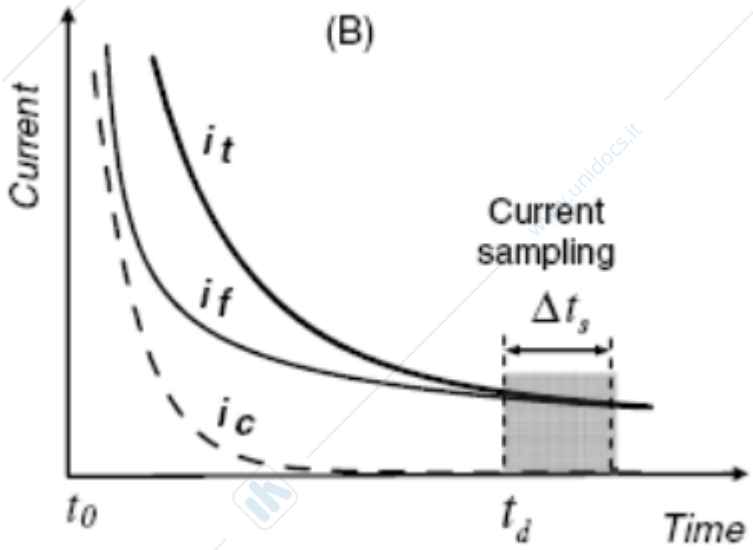
C – capacitance, F (depends on a);

l – distance between electrodes.

$$i_C = \frac{E_s - E_i}{R_s} \exp\left(-\frac{t}{R_s C}\right) \quad (8)$$

$C = e\ A/l$ (9)

What we should memorize is that **dependence of the capacitive current over time is exponential.**



For the exam: no precise formulas; we should know that current is proportional to the concentration; what are the main parameters that affect this rule (answer: diffusion coefficient); why do we have to wait before taking the measurement?