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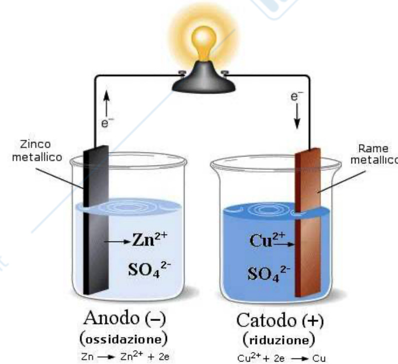
BASIS OF ELECTROCHEMISTRY

Electrochemical cell

An **electrochemical cell** is a cell with electrodes and an ionic conductor, so it is a solution that contains some salt, and there are reactions. There are primary cells and secondary cells. These are cells that simply discharge, and the reactions are spontaneous. There are other cells that can be recharged so can produce the reverse reaction by applying power from an external source. We are going to focus our attention on the first type.



In the first kind of sensors (biometrical) the reaction occurs in the electrodes. There is an **equilibrium solution**, which does not mean that there is no reaction at all, it means that the current of oxidation is the same as the current of reduction.

In other kind of sensors, we will see something that is more similar to what is called electrolytic cell. In this case, you have to provide a volt potential in order to make the reaction possible.

Whatever is the shape of our cell, for example the glucose test, we have three elements: **the working electrode, the reference electrode, and a conductor wire.**

POTENTIOMETRIC BIOSENSORS

Introduction

Potentiometric biosensors are specific biosensors formed by a membrane and there is an activity which is known inside the reference electrode and an activity that is unknown in the working electrode which is the analyte we want to measure. The potential difference between two electrodes is measured under equilibrium conditions.

The equation when reaction is Ox+ ne⁻ ⇋ Red

$$V = V^0 - \frac{RT}{nF} * \ln\frac{a_{red}}{a_{ox}}$$

V= potential of electrode

R= gas constant= 8,314 J/K*mol

T= temperature (Kelvin)

n= number of electrons

F= constant= 96485 C/mol

a= activity of the reduced and oxidated species

Equation for a cell when the reaction is A + B ⇋ C + D

$$E = E^0 - \frac{RT}{nF} * \ln\frac{a_C * a_D}{a_A * a_B}$$

Sometimes E_0 is not present because we have equal electrodes

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Ion selective electrodes (ISE)

An **ion-selective electrode interacts selectively with an analyte** in solution. In **equilibrium conditions**, the potential difference through the membrane depends on the difference between the ion activity in the internal solution and in the sample.

$$Em = -\frac{RT}{nF} * \ln\frac{a_1}{a_x} = -\frac{RT}{nF} * \ln a_1 - \frac{RT}{nF} * \ln\left(\frac{1}{a_x}\right)$$

$$Em = \underbrace{-\frac{RT}{nF} * ln\, a_1}_{\text{constant}} + \frac{RT}{nF} * \ln a_x$$

E_m= membrane potential

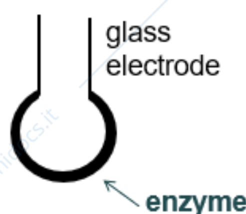
The activity I want to know is a_x

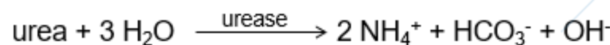
ISE membranes can be solid (glass, polymer, inorganic crystal) and liquid (usually hydrophobic solvents).

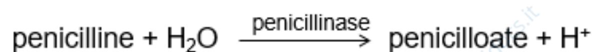
How to make a biosensor starting from an ISE

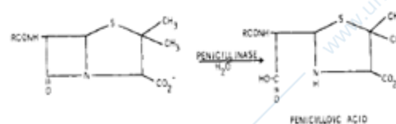
A biosensor is a sensor in which I have a bioelement. The simplest way to make a biosensor is to **select an enzymatic reaction** of the analyte that **produces an ion detectable** by an ISE (like an enzyme) and a **technique that is suitable to fix the bioelement** to the ISE.

Example









I have a reaction where the enzyme urease can produce $NH4^+$ or OH^-. In this case, I can use electrodes of pH detection or electrodes to detect ammonium.

In the case of penicillin, if I use penicillinase, I produce H+ so I can use the glass electrode.

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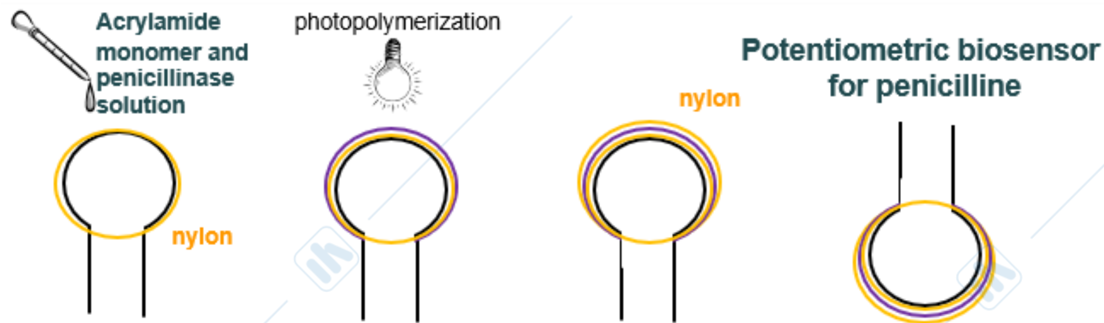


This is the potentiometric biosensor for penicillin.

What I want to do now is attach the enzyme to the glass of the electrodes. The first step is to cover the final part of the electrode with a **nylon layer** and then, **add the monomer** which is acrylamide that can be **polymerized** with radiation. The **solution of the monomer contains the enzyme as well.**

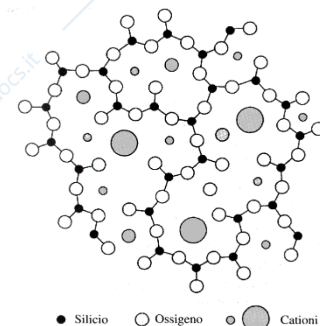
After performing the polymerization, I obtain a polymer layer with the enzyme. Then I cover it **again with a nylon layer** and finally we obtain the biosensor.

With this procedure we can create a lot of biosensors with different enzymes and analytes that need to be detected.

The nylon layer maintains the structure and the acrylamide monomer better. The second layer has the same function because in this way we prevent the enzyme from go in the solution.

MEMBRANES

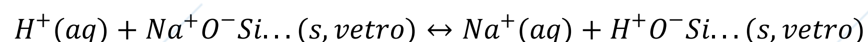
Glass membrane (pH)



The **glass membrane** is made of a specific kind of glass that is **able to exchange small ions** like protons. The structure of the image is a silicate glass a 3D structure where all the ions can move.

This kind of glass to have a good movement **should absorb water**. At some point it converts into a kind of gel which is important to exchange ions. If it is dry, the exchange does not happen.

We have the Exchange of H^+ with Na^+. You have to be careful to not add strong alkaline solutions because otherwise we will have some errors.



$$H^+(aq) + Na^+O^-Si...(s, vetro) \leftrightarrow Na^+(aq) + H^+O^-Si...(s, vetro)$$

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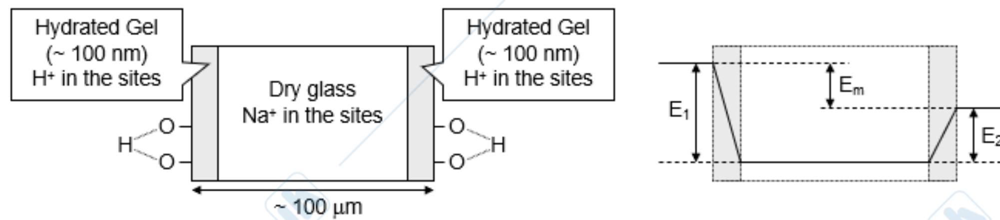
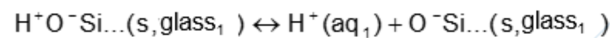
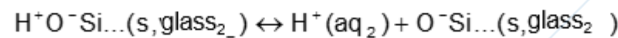


E_m is, indeed, the sum of two different potentials, E_1 ed E_2, that originate at the two surfaces because of the dissociation of H^+ from the hydrated gel layer:



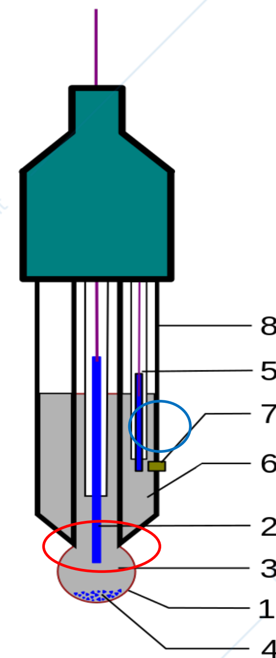


The two hydrated gel layers become negatively charged and the amount of their charge is determined by the concentration of H^+ in the contact solutions.

The potential is different in the external and inside the membrane because of the concentration of H^+ and potential

Glass electrode for pH measurement

It is called electrode, but it is not, it is a **cell that combines two electrodes** (a glass and reference electrode)



1- Sensing part of electrode made of specific glass that can exchange ions.
2- Internal electrode made of a silver chloride electrode.
3- Solution of HCl at a specific pH we know
4- An amount of AgCl can precipitate inside the glass electrode
5- External electrode. It is the reference electrode, and it is a silver electrode same as type 2.
6- Reference internal solution, usually saturated KCl
7- A junction made of porous ceramics that connects the external part (the solution we want to measure) to the inner solution of reference so there are two connections. It is important because the reference electrode needs to be connected to the solutions as well.
8- Body of electrode, made from non-conductive glass or plastics

The **permeation of the solution should be fast** because if not there can be a deformation due to an excess of iron and have another potential. We should have a good exchange of ion just to maintain low the junction.

Specific glass for pH porous frit

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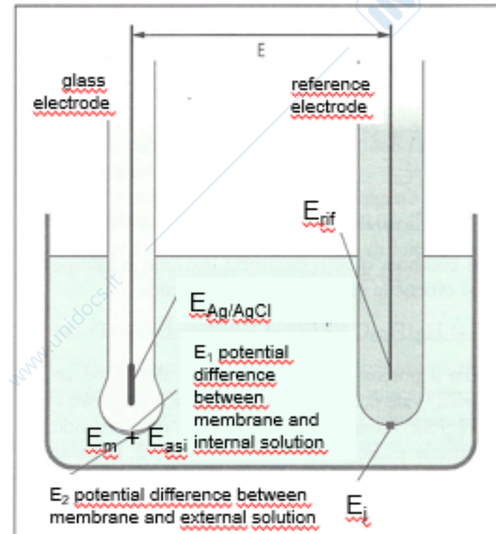
In this example we have the same electrode in both sides. The important thing is that **in the second type of electrode a well-known amount of Cl- is needed.**

Before there were combined electrodes, we have to use the glass electrode with an external reference electrode. Combined are more practical.

How it Works

We have the working electrode (indicator), the E measures the difference between the potential of the working and the reference electrode, and we have a junction potential where all the ions can move.



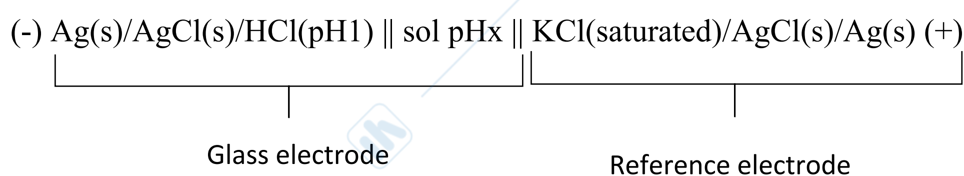
On the left side is the membrane potential and the **asymmetric potential** which is a potential that is **due to the fact that you have a membrane**. Even if you have the same concentration inside and outside, the potential is not zero because there is some difference in the glass. However, asymmetric potential **is extremely low so do not consider.**

If you want to **measure the difference in potential between two electrodes**, we have to measure the difference potential between the indicator minus the potential of the reference and plus the potential of the junction if present.

$$E = (E_{ind} - E_{rif}) + E_j$$

The **indicator potential** is the sum of the internal reference potential (Ag/AgCl), the potential of the membrane and the asymmetry potential.

$$E_{ind} = E_{Ag/AgCl} + E_m + E_{asi}$$



The potential of my electrode is **$E=V_+-V_-$** (reference potential - glass potential)

In the glass, negative electrode (anode), there is a spontaneous reaction, so occurs an oxidation: $Cl^-_{(M1)} + Ag_{(s)} \leftrightarrow AgCl_{(s)} + 1\ e^-$

I consider that the ionic transport is the trans-membrane transport of H^+: $H^+_{(M1)} \leftrightarrow H^+_{(Mx)}$

M1 > Mx

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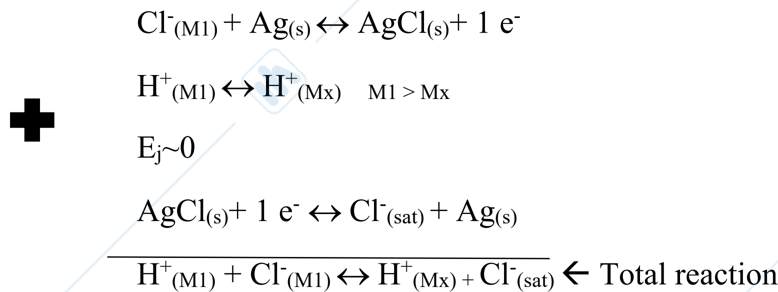
At the positive electrode there is a reduction: $AgCl_{(s)} + 1\ e^- \leftrightarrow Cl^-_{(sat)} + Ag_{(s)}$

sat mens saturated solution

The reactions are summed and finally we have the total reaction of our cell.



So, the potential is (n=1):

$$E = -\frac{RT}{F} * \ln\left(\frac{[H^+]_x * [Cl^-]_{sat}}{[H^+]_1 * [Cl^-]_1}\right)$$

Transform ln into log

$$E = -2{,}303\frac{RT}{F}\log\,[H^+]_x + 2{,}303\frac{RT}{F}\log\frac{[H^+]_1[Cl^-]_1}{[Cl^-]_{sat}}$$

Second term is constant

$$\boldsymbol{E = cost + 2,303\frac{RT}{F}\,pHx}$$

If 25ºC

$$\boldsymbol{E = cost + 0,0592 * pHx}$$

From another point of view:

$$E_{ind} = E_{Ag/AgCl} + E_m + E_{asi}$$

E_{ind}= -(RT/F) ln ($[H^+]_x[Cl^-]_{sat}/([H^+]_1[Cl^-]_1)$) = -2,303 (RT/F) log ($[H^+]_x/[H^+]_1$) + 2,303 (RT/F) log $[Cl^-]_1/[Cl^-]_{sat}$

E_m — constant — $E_{Ag/AgCl}$

The membrane potential E_m is related to the concentration (or, better, the activity) of H^+ in the two solutions in contact with the membrane

$$E_m = 0{,}0592\,\log\frac{a_1}{a_x} = 0{,}0592\,\log a_1 - 0{,}0592\,\log a_x$$

(constant: $0{,}0592\,\log a_1$)

where a_x is the activity of H^+ in the solution under test and a_1 is the activity of H^+ in the internal solution.

It has been observed that $E_m \neq 0$ even if $a_1 = a_x$. This is due to the **asymmetry potential** (E_{asy}), i.e. a contribution to potential which is due to differences between the inner and outer membrane surfaces and is independent from H^+ activity.

C. Arbizzani - Pharmaceutical Biotechnology AA 2021-2022 20

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Crystalline membrane (F^-)

The crystalline membrane is used for the **detection of anions** such as the chloride ion Cl^-

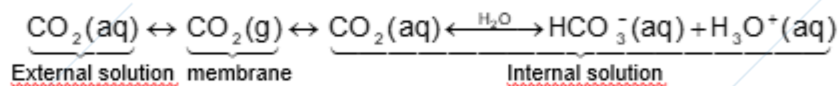
The **membrane is made of crystal LaF_3 doped with EuF_2** which improves the conductivity of the membrane

The **movement of the ions is between vacances** (empty places where the ions can move inside the crystal). **There is a balance** of these ions between the internal solution and the external one just like in the case of the glass membrane. You have in the same way an internal electrode and the reference electrode. Many different anions can be detected by this crystal membrane.

Gas-selective membrane (CO_2, O_2)

Other kind of membranes are those for the **detection of gases**. They are made **up of an ion-selective electrode and a membrane that has the function of isolating the species** detected by the electrode. As in the case of the combined pH electrode, they often also contain the reference electrode.

Example for the detection of CO_2. We have CO_2 in the external solution that come through the membrane, which is able to make possible the conversion to CO_2 gas. In the internal solution there is an equilibrium, so the reaction between the CO_2 and the water gives the carbonate ion and the H^+. In the internal solution there is an electrode that detects the increase or decrease of H^+ and this indirectly gives you the amount of CO_2 present in the solution.



This electrode is **used for environment gas detection and safety.**

Polymer membrane

This one uses a **conductive polymer in which a suitable molecule is impregnated.**

They are made of polymer carbonate but mainly of **PVC**. In this membrane sometimes there is an ion exchanger that gives the possibility to carry the ion from the sample outside to the internal part of the electrode. This kind of membranes are mainly useful for the **detection of pharmaceutical molecules**. With this kind of polymer membrane, you can detect almost every kind of molecules.

Example where we want to detect NH_4^+ ion. We have the internal reference electrode that is always Ag/AgCl, a well know concentration of NH4NO3 and KCl for the reference electrode. The membrane has an ion exchanger inside that helps to transfer the ammonium from outside to inside. The equation to find the concentration de Ammonium is:

$$Eind = cost - 0{,}0592 * log\ a_{NH4+}$$

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Ion- exchanger and ionophores

Ion exchanger characteristics:

- high lipophilicity and a strong affinity for the ion that must be determined
- low affinity for all the others so there is no interference
- rapid exchange kinetics and adequate constants of formation of the complexes;
- sufficiently solubility in the membrane and sufficiently lipophilicity to prevent its dispersion in the sample (generally aqueous) solution.

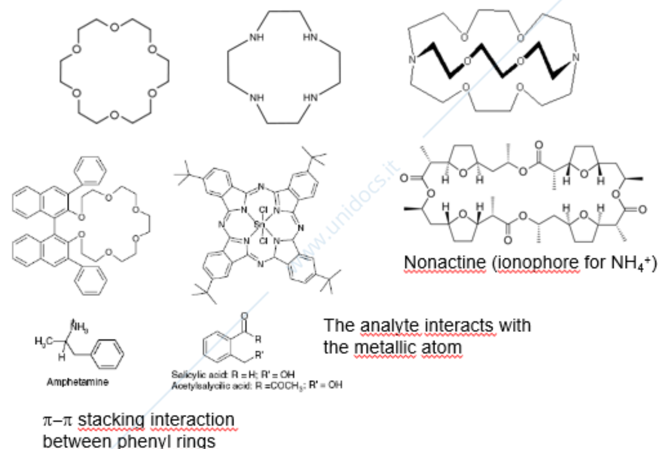
Analytes:

- Amino acids: alanine, leucine, aspartic acid, cysteine, tyrosine, phenylalanine
- Alkaloids: caffeine, nicotine, codeine, cocaine, heroin, etc.
- Antidepressants: amitriptillin, tiopental, nefazodone, etc.
- Anesthetics: benzocaine, lidocaine, etc.
- Vitamins: pyridoxine hydrochloride (B6), thiamine hydrochloride (B1) etc.
- Antibiotics: doxacillin, gentamicin
- Muscle relaxants, Antispasmodics, Antifungals, other drugs

Just know there are ethers, aza and thio-corona, criptands, thio-compounds, phosphometalates, ammonium salts, etc.

These are molecules which structure is a little bit complex and need to capture the molecule to transfer it.

They can have direct interactions or indirect ones like π-π that uses the ring structure to capture the molecule.



POTENTIOMETRIC BIOSENSORS: CASE OF STUDY

ION SELECTIVE ELECTRODE FOR THE DETECTION OF NEFAZODONE HYDROCHLORIDE, AN ANTIDEPRESSANT.

I have to build an ion exchanger that is suitable to detect this molecule. This ion exchanger is nefazodone phosphotungstate (NFN.PT) that is incorporated in PVC and also sometimes there is an addition of a plasticizer matrix just to maintain the membrane. When the polymer is ready with the molecule, I cut small disks of the membrane and I

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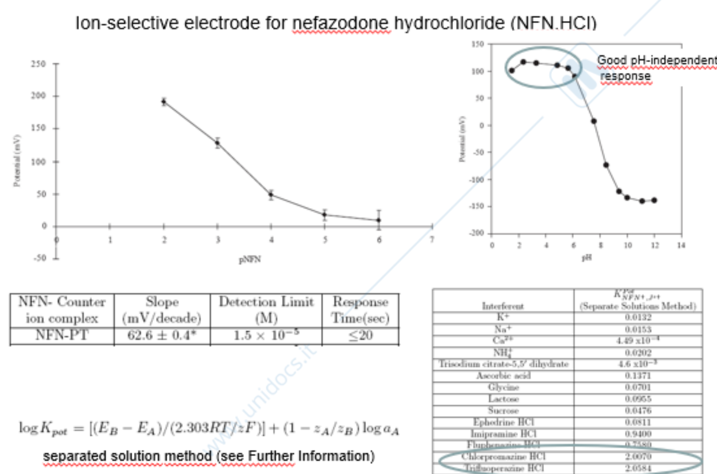
put it at the end of a tube (Tygon tube). This kind of electrodes are built with a solution of our molecule in a specific concentration. You also insert a small wire of Ag/AgCl inside the solution, and you have already prepared the electrode.

Results

There is good selectivity, at least up to 10^-5 of this molecule. There is a good response. Up to a certain level is quite independent from pH (up to pH of 6) so this is the range in which you can use. The response time is less than 20 seconds. In this case, it is an electrode that is used in the laboratory to check the presence of this molecule. The only problem could be the interference of antidepressants of the same family.

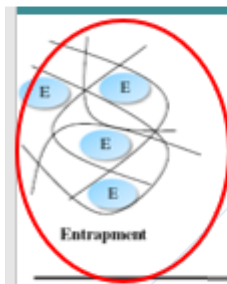


NFN- Counter ion complex	Slope (mV/decade)	Detection Limit (M)	Response Time(sec)
NFN-PT	62.6 ± 0.4*	1.5×10^{-5}	≤20

$$\log K_{pot} = [(E_B - E_A)/(2.303RT/zF)] + (1 - z_A/z_B)\log a_A$$

Interferent	$K^{Pot}_{NFN^+,J^{z+}}$ (Separate Solutions Method)
K^+	0.0132
Na^+	0.0153
Ca^{2+}	4.49 x10^{-4}
NH_4^+	0.0202
Trisodium citrate-5,5′ dihydrate	4.6 x10^{-3}
Ascorbic acid	0.1371
Glycine	0.0701
Lactose	0.0955
Sucrose	0.0476
Ephedrine HCl	0.0811
Imipramine HCl	0.9400
Fluphenazine HCl	0.7580
Chlorpromazine HCl	2.0070
Trifluoperazine HCl	2.0584

Immobilization in this kind of electrodes



Is a simple **entrapment:** I put the enzyme in a solution containing monomer, then I polymerize the monomer to get a polymer, so the **molecule is trapped** inside.

Advantages: There is **no chemical bond** formation between the monomer and the enzyme **that could affect the activity** (chemical bond between enzyme and monomer can reduce the affinity of the enzyme). You can immobilize whatever molecule. It is not too much specific.

Disadvantages: you can have leakage of your molecule, you can have big diffusion barrier (you can have a problem of function because the response is quite low), sometimes you need high concentration of a molecule.

DETERMINATION OF L- LYSINE IN COMMERCIAL CAPSULES AND TABLETS

You can do more or less the same procedure

You can use your enzyme (in this case is an oxidase) and immobilize it inside the membrane (PVC membrane because is easy to polymerize, is cheaper and is not problematic). You produce a layer that is selected for NH_4^+ and you prepare this polymer containing the enzyme, then cut the polymer and put it in the bottom of the cube filled with a solution of NH_4Cl and has an Ag wire.

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Results

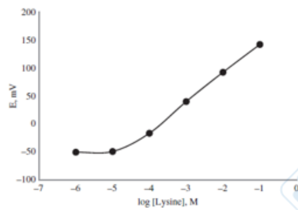

Figure 3. The calibration graphs of lysine biosensor. The study was carried out with 10^{-1}–10^{-6}M lysine calibration solutions in 10 mM TRIS buffer (pH 7.5).

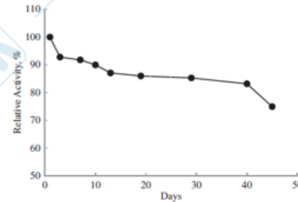

Figure 7. The lifetime of lysine biosensor for a period of 45 days. The study was carried out with 10^{-1}–10^{-5}M lysine calibration solutions in 1 [illegible]

Good response up to 10^-5 concentration of lysine. The relative activity is high, which means that you can use it for several time as there is not much release of solution. Enzyme is stable up to a month.

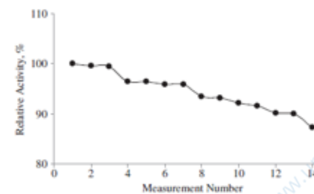

Figure 8. Reproducibility of the lysine biosensor within a day. The study was carried out with 10^{-1}–10^{-5}M lysine calibration solutions in 10 mM TRIS buffer (pH 7.5).

After 14 measurements, it is still above 80% of function

The main interference is tyrosine, so it is important to remove it from the sample.

Good response with a quite simple system.

Table II. Selectivity of lysine biosensor towards ascorbic acid and some amino acids.

Amino acid	Relative response %
Lysine	100
Tyrosine	96.0
Isoleucine	19.2
Glycine	14.0
Valine	13.7
Glutamine	8.0
Proline	7.9
Ascorbic Acid	7.5
Phenylalanine	6.4
Leucine	5.2
Histidine	4.2
Glutamic acid	3.3
Arginine	1.4
Methionine	1.2
Alanine	0.4
Cysteine	0.4
Serine	0

The only significant interference is that of tyrosine.
Interference with ascorbic acid, which may be present in real samples, has no effect on the biosensor.

Table III. The results of lysine content obtained from the proposed lysine biosensor in commercial lysine tablet and capsule.

	Reported value	Lysine biosensor	*Recovery, %
L-lysine tablet (Solgar), mg lysine/1 tablet	1000	989 ± 0.10	98.9
Amino 75 capsule (Solgar), mg lysine/ 1 capsule	75	73 ± 0.12	97.3

% Recovery = Cs/C × 100%.

Preparation of ammonium-ion selective membrane electrode (just to give an idea): In this case the enzyme is not put inside the PVC and the monomer. Just simply do the chemical immobilization of the enzyme in a membrane already prepared. It is a little bit different. You can perform a crosslinking because there is GAD and EDC.

PROS AND CONS OF POTENTIOMETRIC BIOSENSORS

PROS

- Quite simple to prepare
- There are many molecules and technology available
- Direct and simply measurements
- Different configurations (macro electrode, static electrode, Flow, micro electrode) can be used.
- The cost is low because preparation is not difficult.

Cons

- Some biosensors have not high selectivity, and we have to eliminate the interference before performing the measure.

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FURTHER INFORMATION: SIGN CONVENTION

This is just for our knowledge

Sometimes the sign in the equation is different from what we have been taught because of the selective ion (cation, anion).

If in the galvanic cell for the determination of the activity of a cation the ion-selective electrode is positioned on the left (negative electrode) as in the case of the pH-metry, and for the determination of an anion is positioned on the right (positive electrode), the equation for determining the activity of the ion at unknown concentration will be the same:

$$E = cost - (2.303\ RT/z_iF) \log a_{i,x}$$

However, some manufactures indicate to use the following equation (an operational equation) where the sign is different because there is Z which is the charge of the ion considered with the sign. If the ion is a cation (H^+) you have to put +1, but if is an anion (Cl^-) you have to put -1. This can cause confusion. We use n= nº electrons involved in our reaction.

$$E = cost + (2.303RT/z_iF) \log(a_{i,x})$$

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