- Food Analysis (II year)
-Chemical Analysis of Foods and chemometrics (I year)

a.a. 2025/26

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Subjects

- Mass spectrometry, principles of instrumentation, ifenation of mass spectrometry with gas and liquid chromatography. Use of LC-MS-MS and GC-MS in food analysis.

- Confirmatory methods in food analysis, guidelines of Eu Legislation for food contaminants, maximum residue limits, antropic and natural contaminants, fitochemicals , control strategies, validation of methods.

- IR spectroscopy, basic theory, rotational and vibrational transitions. FT-IR instrumentation, samples and sampling signal in FT-IR, qualitycontrol, qualitative and quantitative application. FT-NIR in food analysis for quality and process control.

Previous knowledge required

- Spectroscopy:

electromagneticwaves, intensity, amplitude, wawelenght, Beer Law, UV-VIS classical spectrophotometer

- Sensors and Biosensors:

principles of potentiometry and voltammetry, enzyme kinetic (Michaelis-Menten)

- Confirmatory methods and hyphenated tecniques

Classical pretreatment of the samples, priciples of chromatography, gas and liquid chromatography

Examination

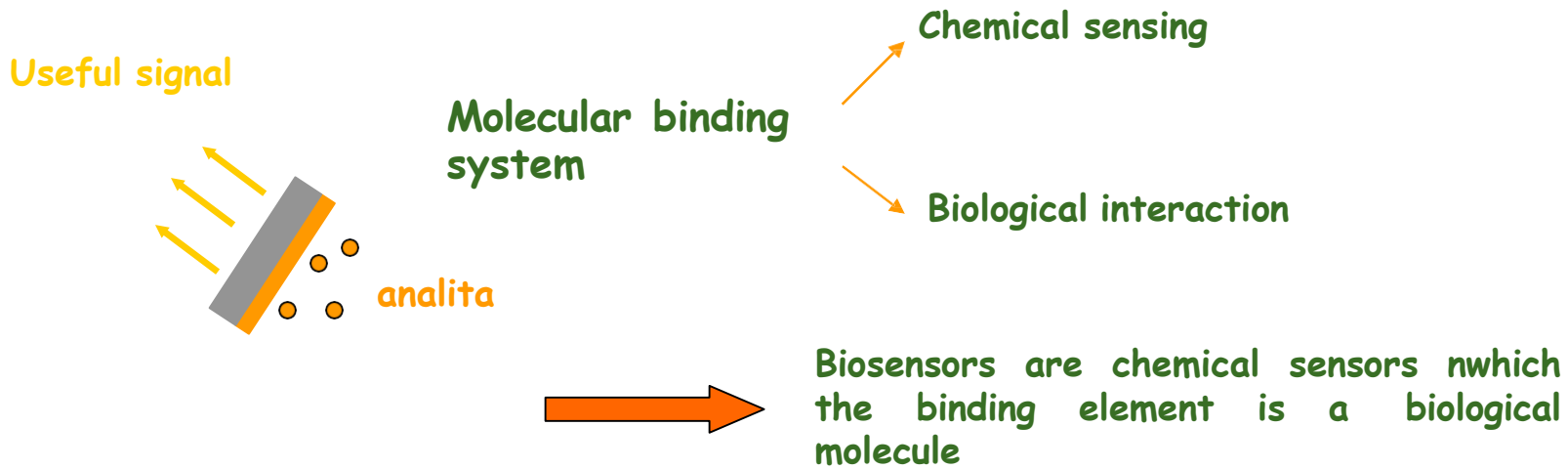
- Written examination (list of questions available)

What are sensors and biosensors?

"…a biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semiquantitative analytical information using a biological recognition element (biochemical receptor) which is retained in direct spatial contact with a transduction element."

Technical report " Reccomended definition and classification" IUPAC (Physical Chemistry and Analytical Chemistry Divisions) 2001

A sensor is a device able to transform a physical or chemical info (e.g. concentration of one or more compounds in a solution), into an analytical useful signal

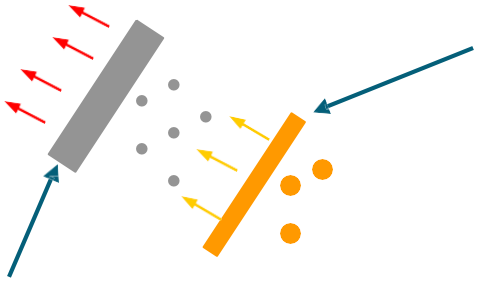


Self-testing

Numbers:

- 85% of the global market for biosensors
- 70 million tests per day worldwide
- 15 billion $ in 2015
- 30 billion $ in 2024 (exp.)

The biological element translates the info of the biochemical domain (e.g. concentration) into a chemical or physical signal with a certain selectivity



Transducing element

Biological binding system

- selectivity
- sensitivity

Tranducers

- electrochemical
- optical
- piezoelectric
- thermal

- potentiometric
- amperometric
- voltammetric
- conduttimetric,
- impedimetric

Classification of biosensors on the basis of the biological element

Catalytic biosensors

catalysis of a chemical reaction

ex: enzymes, cells, tissues

Affinity biosensors

formation of a stable complex with the ligand

ex: antibodies, DNA strands, proteic receptors

Biomimetic biosensors

affinity biosensors using a synthethic receptor

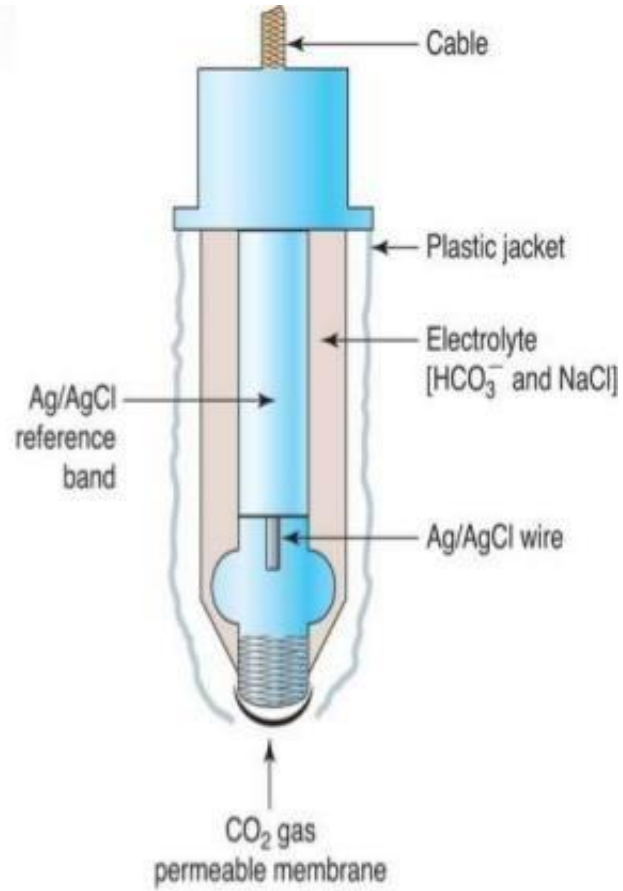
es: peptides, aptamers, molularly imprinted polymers

Ion selective electrodes for biosensors

The most used ISEs for the development of biosensors have been CO_2 and NH_3 probes.
This potentiometric gas-sensors are realised using a pH glass electrode and a reference behind a gas permeable membrane (polytetrafluoroethilene, polypropylene, etc.). A very thin film of a suitable electrolyte is present between the gas permeable membrane and the surface of the pH electrode.
Hydrolysis of NH_3 or CO_2 (diffused from the sample) in the electrolyte causes a change in H^+ that is measured at the electrode.
This is related to the partial pressure of the gas in the sample solution. Immobilisation of a suitable enzyme onto the surface leads to the detection of metabolites in the 10^{-5} – 10^{-2} mol/L range.

PCO_2 ELECTRODE

- Measurement of PCO_2 in routine blood gases
- A modified pH electrode with a CO_2 permeable membrane covering the glass membrane surface
- A bicarbonate buffer separates the membranes
- Change in pH is proportional to the concentration of dissolved CO_2 in the blood

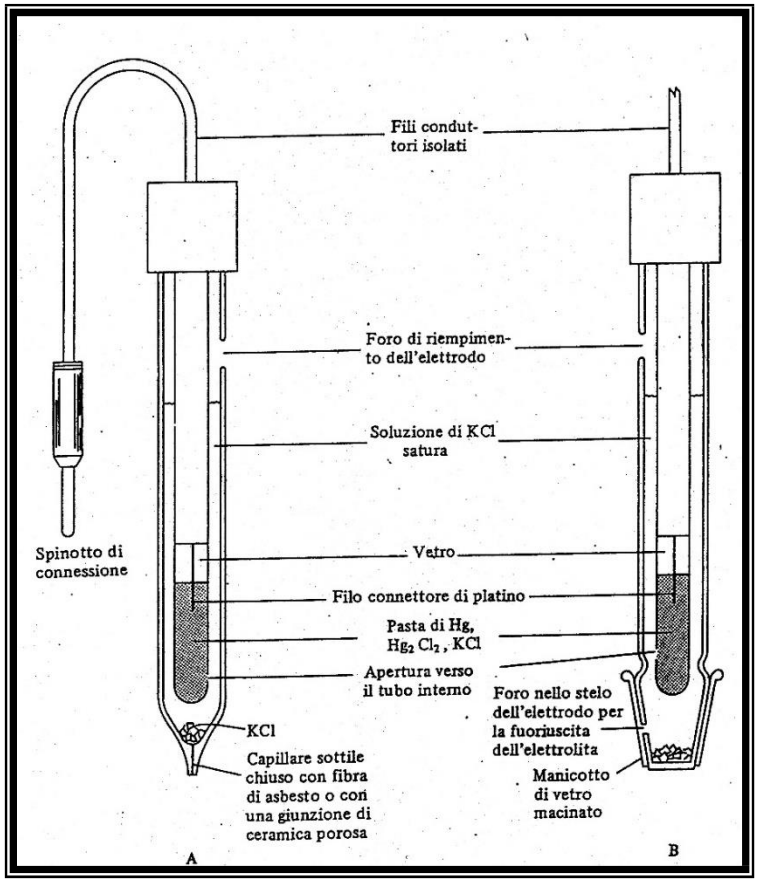


Reaction occuring in the electrolyte solution:

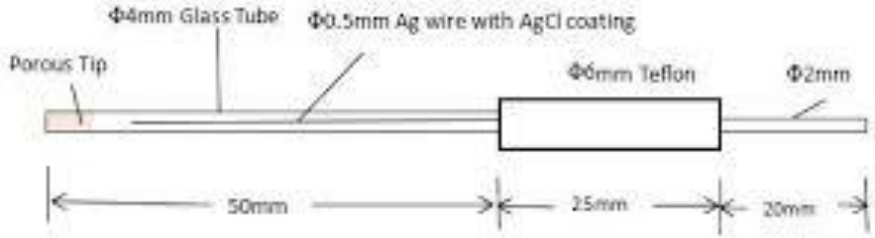
$$CO_2 + H_2O \longleftrightarrow H_2CO_3 \longleftrightarrow H^+ + HCO_3^-$$

Reference electrodes

calomel



Ag/AgCl



CS901 Ag/AgCl Reference Electrode

Amperometric sensors monitor the current flow when a selected fixed potential is applied at a working electrode with respect to a reference electrode. The current generated by the oxidation (or reduction) of a compound is dependent by:

- heterogeneous rate constant k
- diffusion (mass transfer) of the electroactive specie at the electrode surface
- preceding or following chemical reactions
- surface reactions (adsorption)

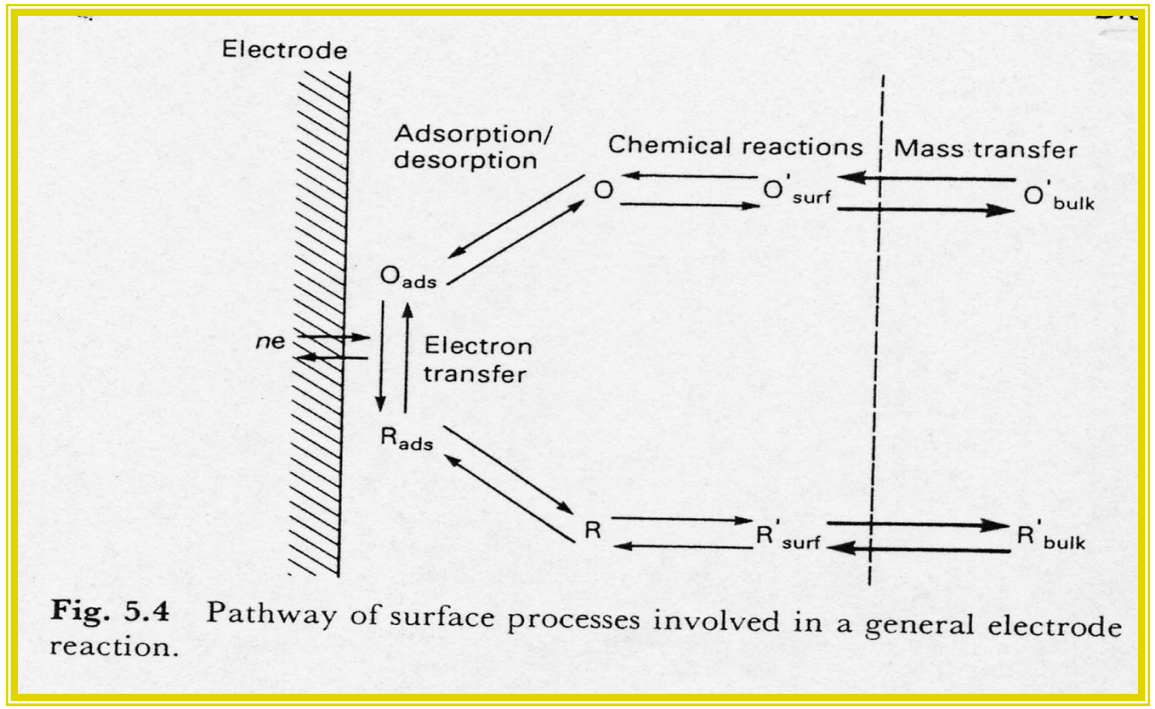
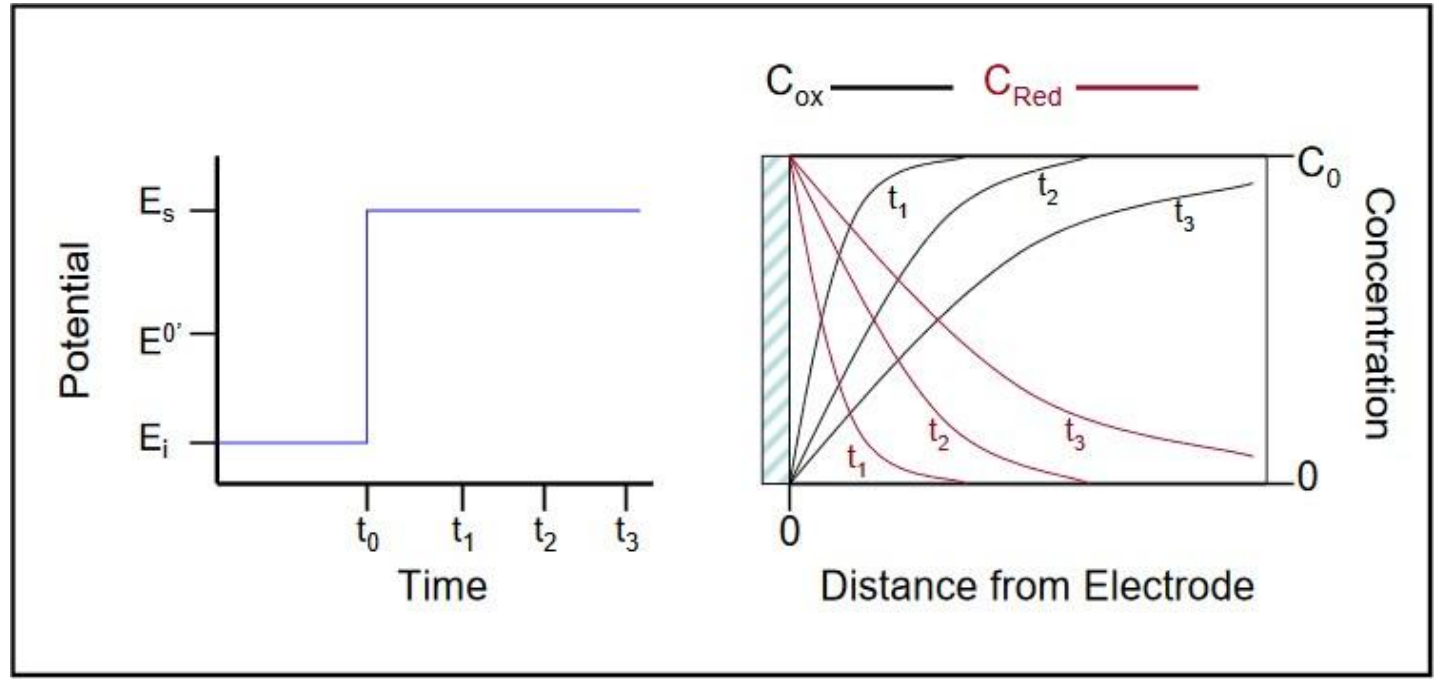


Fig. 5.4 Pathway of surface processes involved in a general electrode reaction.



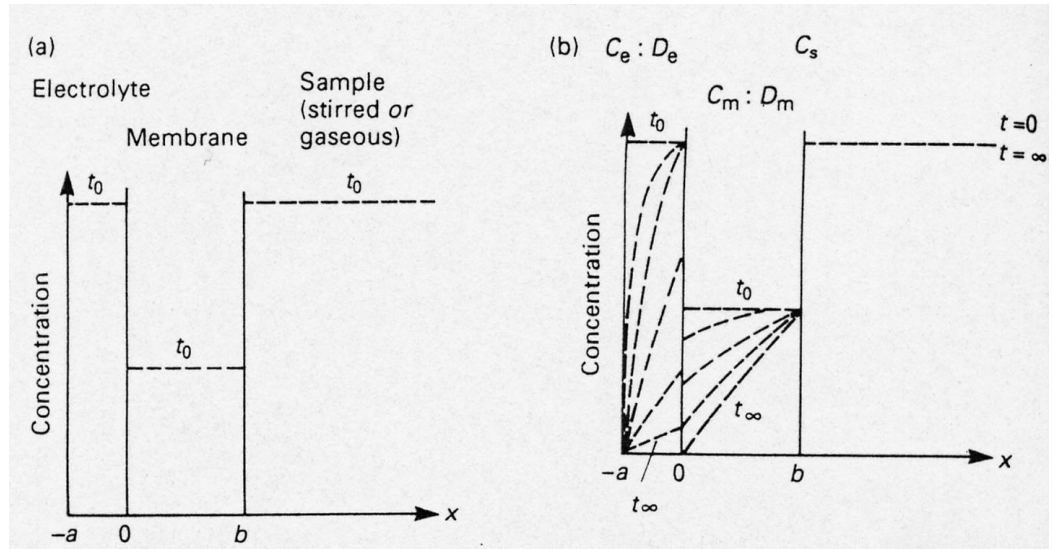
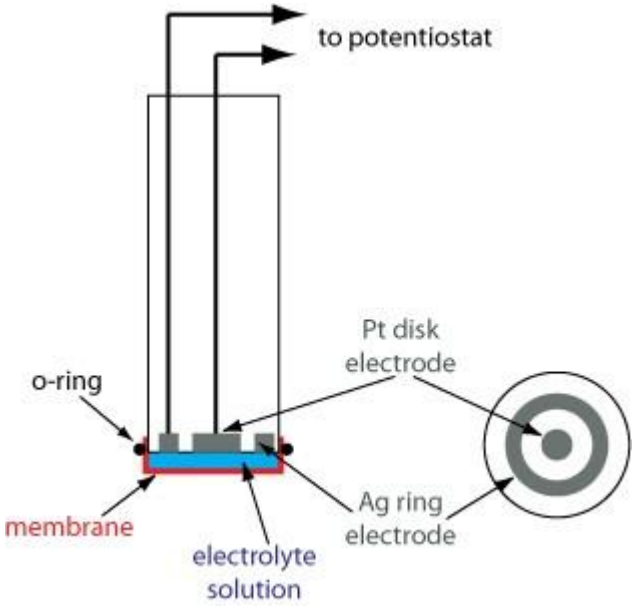
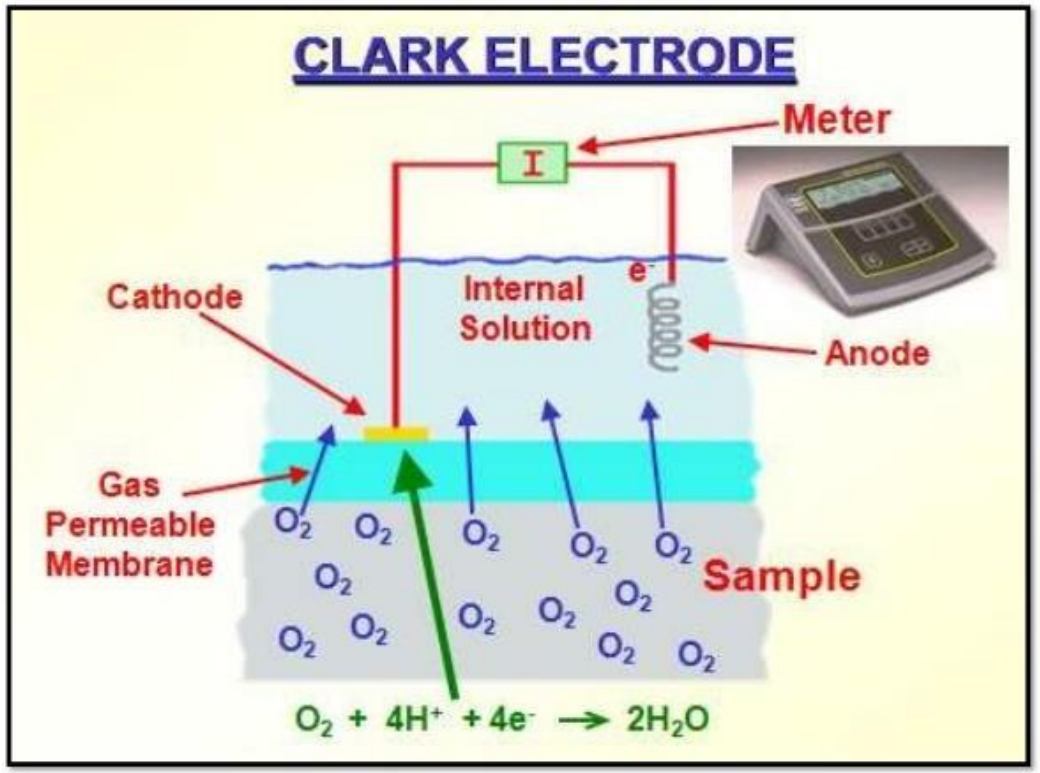


Fig. 5.8 Concentration profiles for a membrane-covered electrode. (a) The initial profile; (b) the development of concentration gradients through the different phases during attainment of steady state.





Applied potential
-0.7 V vs. Ag/AgCl

Dissolved Oxygen Electrode /Sensor
Industrial Type Model : MS DO 714

OXYGEN PROBE

The cathode should possess: high catalytic activity for the reduction of O_2 , (large exchange current) , sufficient electrical conductivity (low adsorption of organic impurities or O_2),it should be inert , it should exhibit a large overvoltage for the decomposition of water (no hydrogen liberation), it should permit the required construction operation (e.g. sealing into glass)

Pt and Au are the most used materials

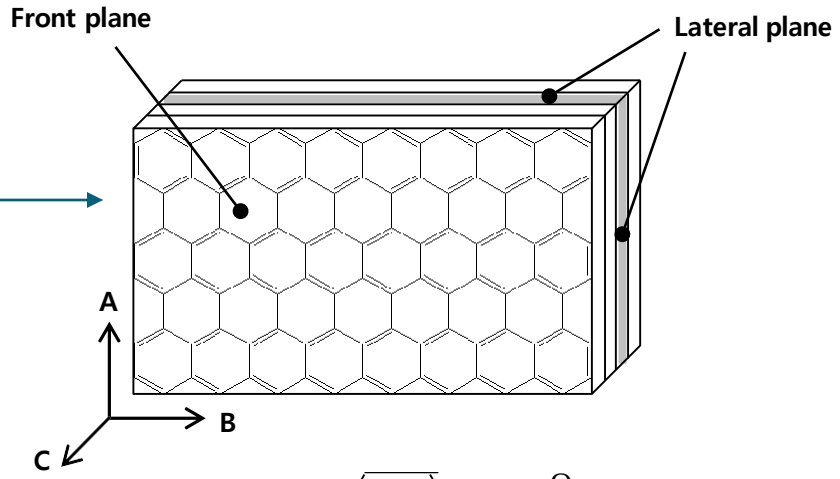
The gas permeable membrane protects the electrodes from contamination, provides for reproducible conditions of oxygen tranport and minimize undesirable changes in electrolyte composition. Ideally, it shoud be with low permeability and high diffusivity for oxygen.

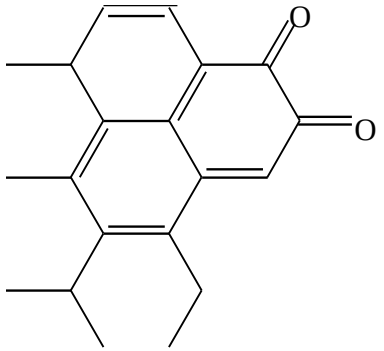
Non-hydratable polymers as polytetrafluoroethylene (PTFE), polypropylene (PP) and polyethylene (PE) are generally used. Alternatively elastic rubber or silicone are useful, even though less stable in alkaline medium.

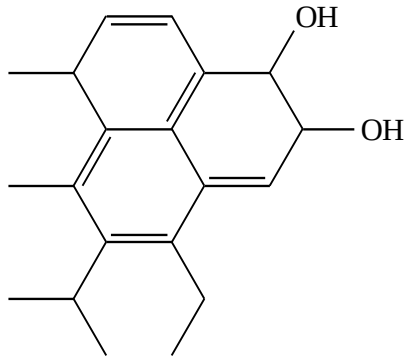
The anode is Ag/AgCl and a neutral or alkaline electrolyte is used in the final assembling.

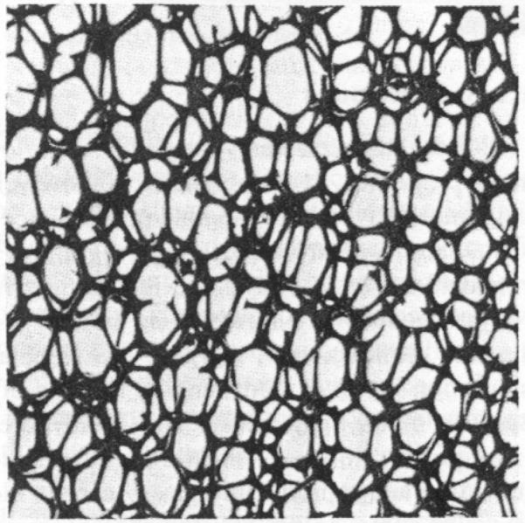
Conventional carbon based probes:

- graphite
- carbon paste
- pyrolitic graphite
- glassy carbon

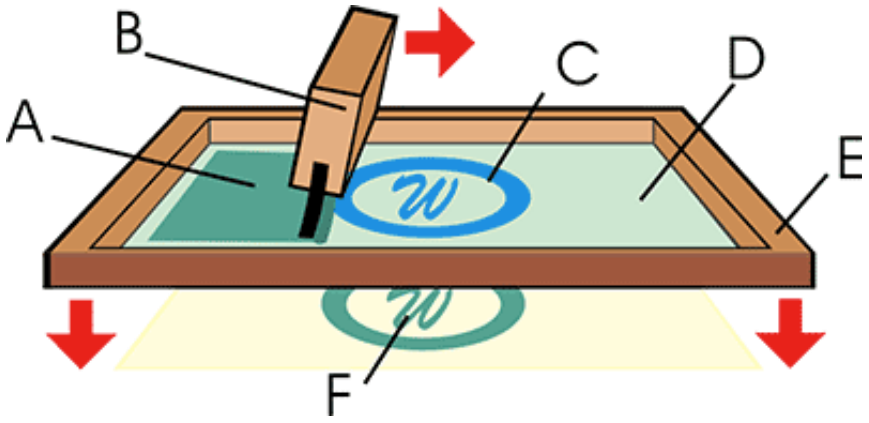








Printing electrodes: serigraphy

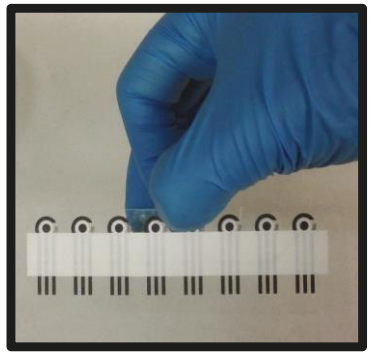


A. ink; B. squeege;

C. printing mask;

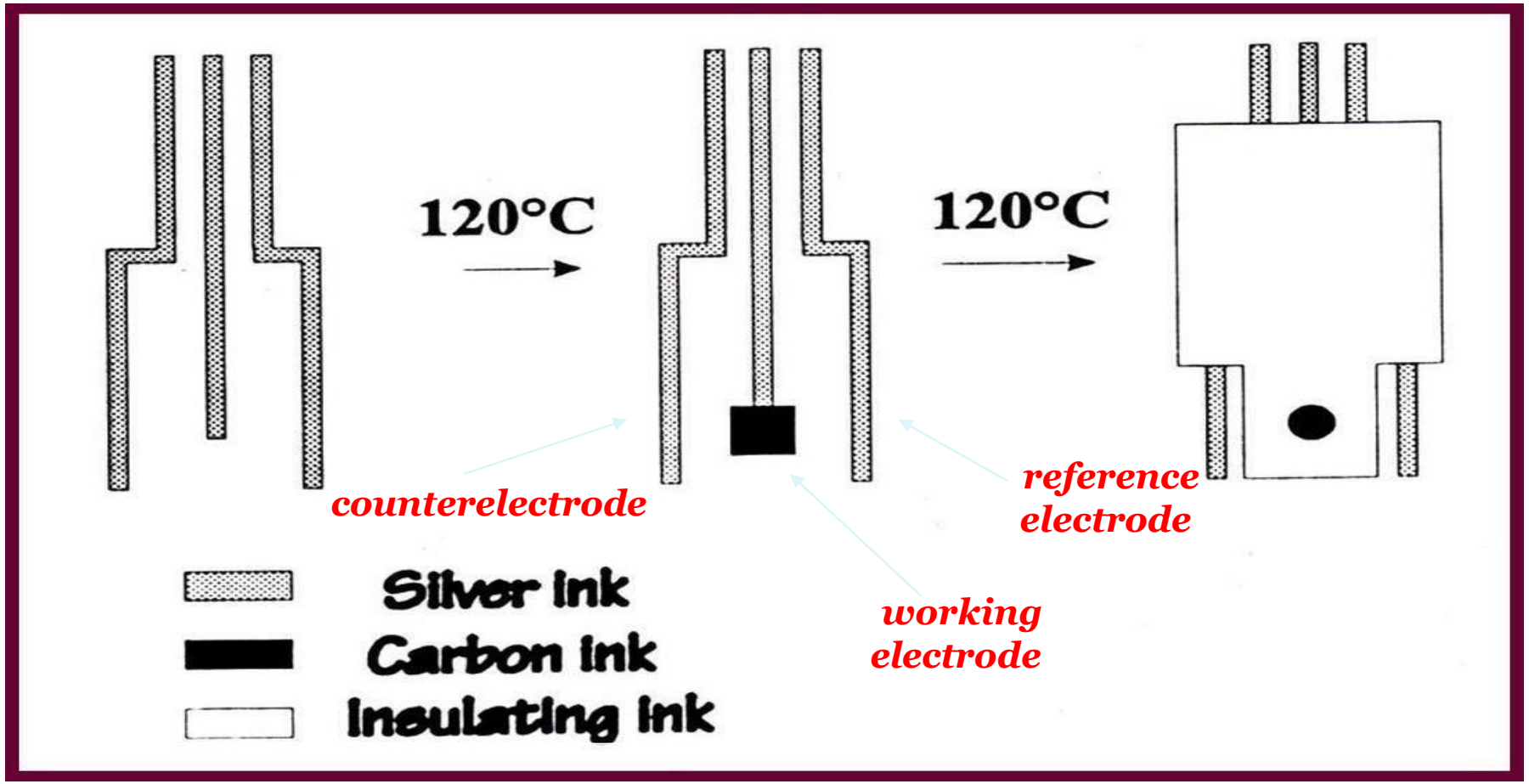
D. printing mesh;

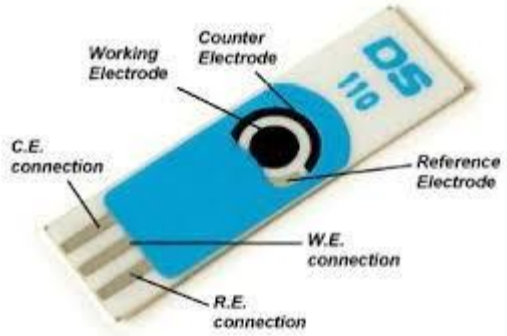
E. frame; F. printed ink



Advantages:
- Dimension
- Disposable
- Low-Cost

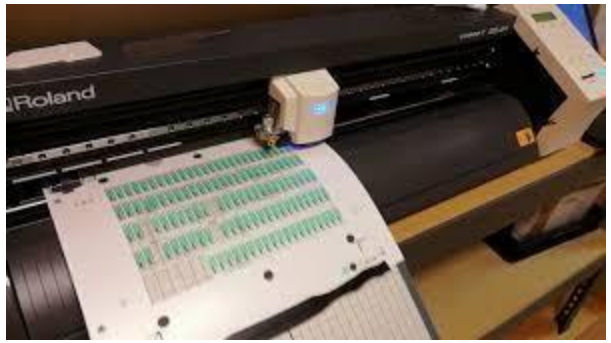
DISPOSABLE SCREEN-PRINTED CARBON ELECTRODES

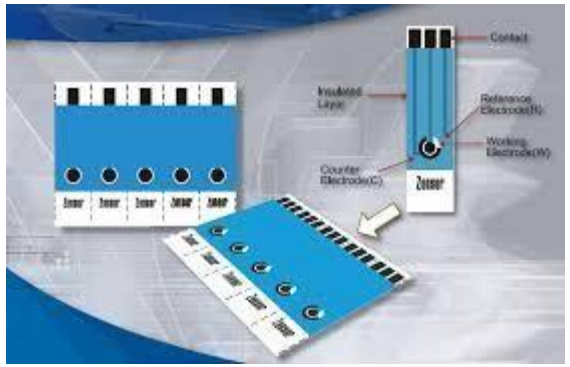


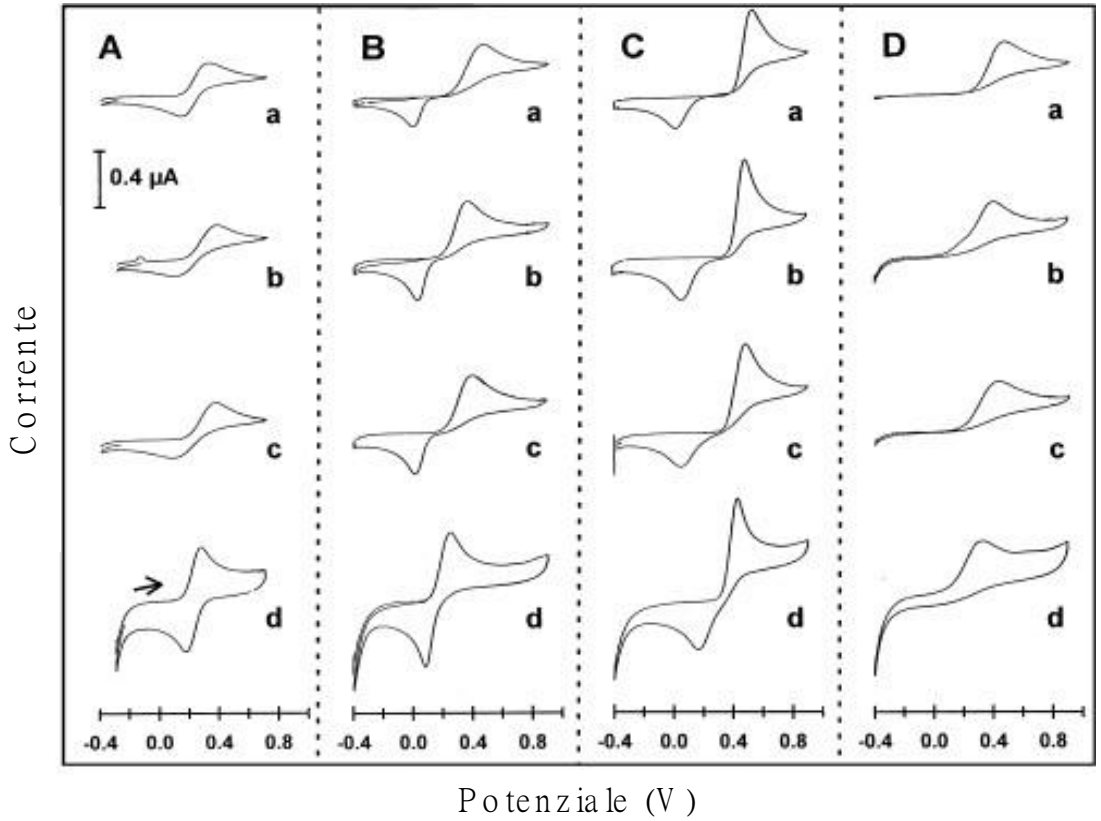

Working Electrode
Counter Electrode
C.E. connection
Reference Electrode
W.E. connection
R.E. connection
DS 110


DS 110
DS 550
DS 220BT
DS 220AT


Z&P

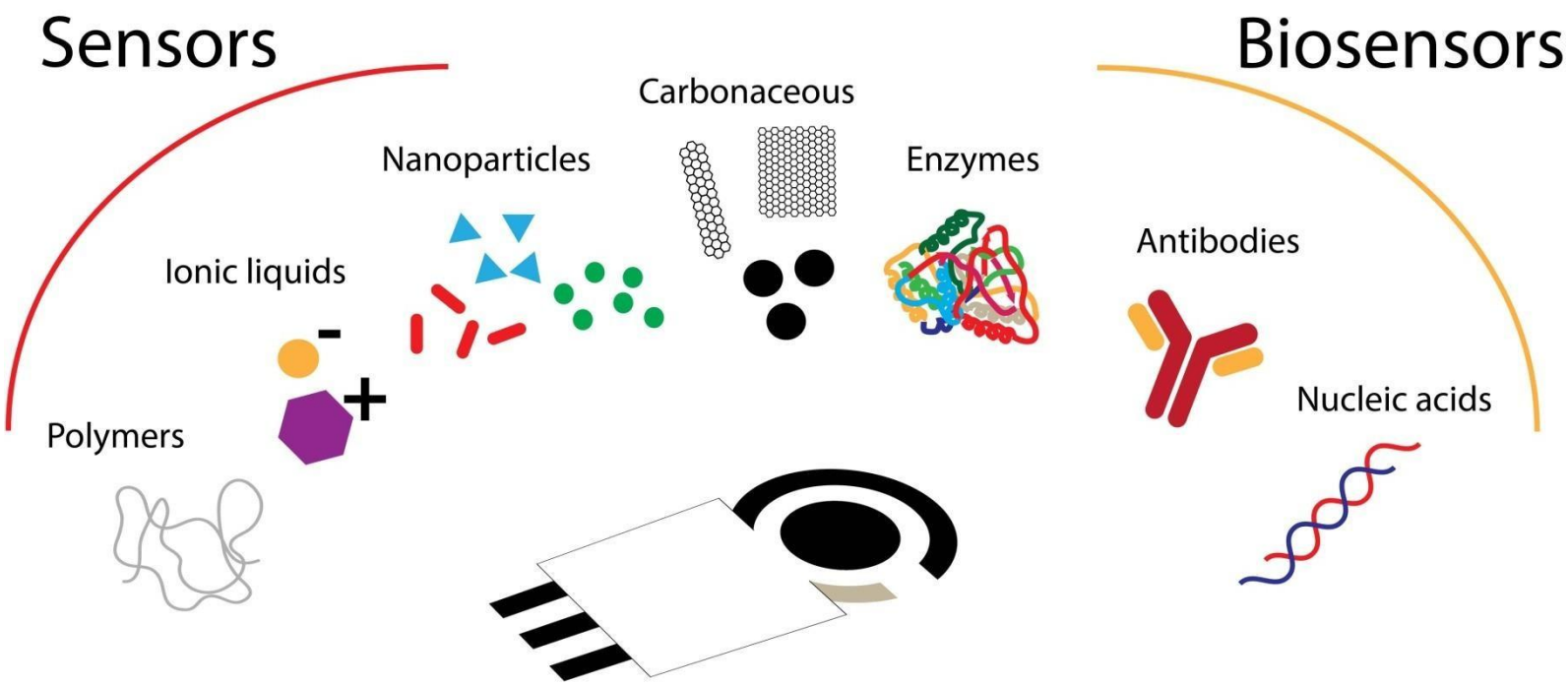

Roland


Contact
Insulated Layer
Reference Electrode(R)
Working Electrode(W)
Counter Electrode(C)
Zensor



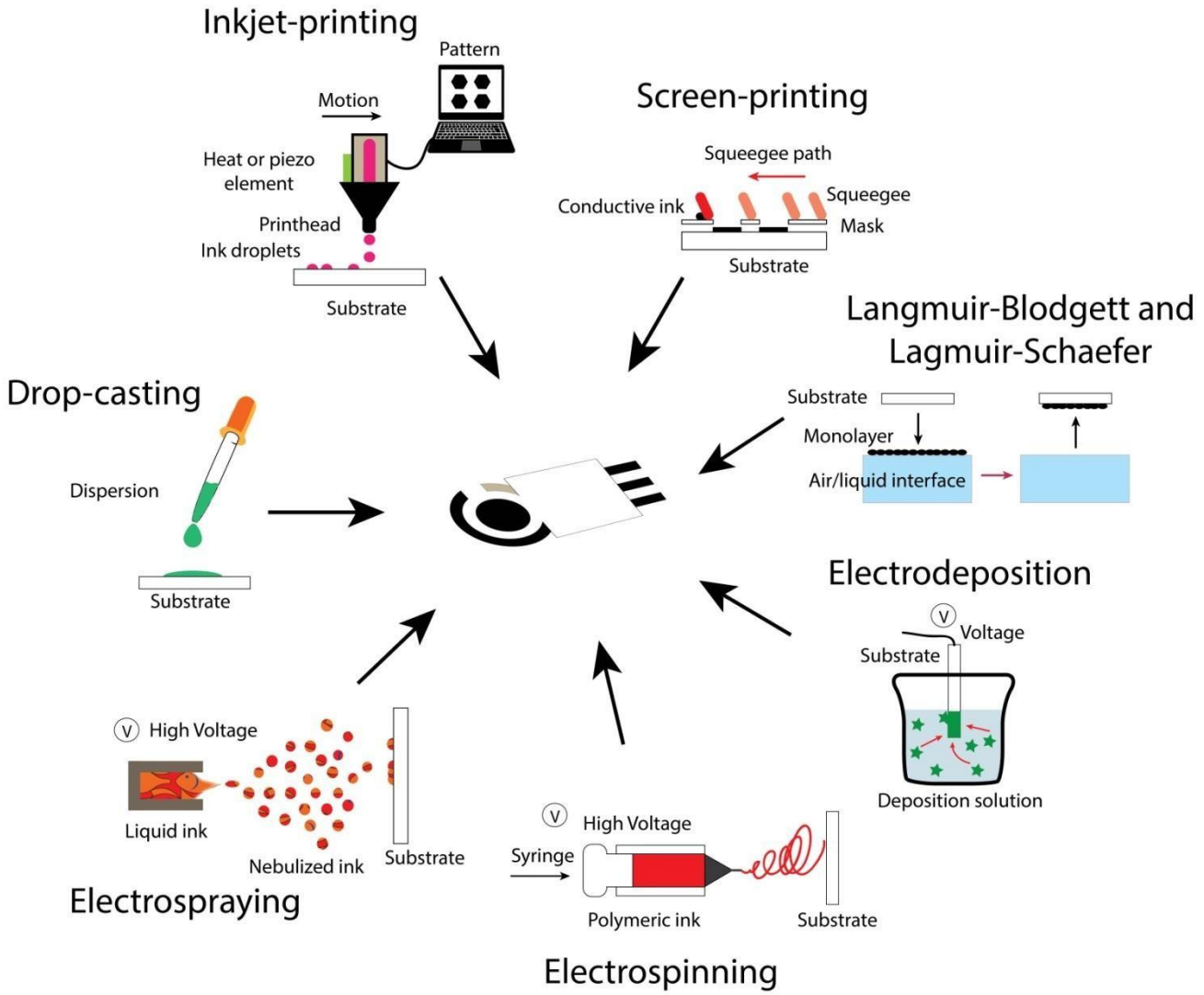
Cyclic voltammetries of ferricyanide (A), cathecol (B), acetamminophene (C), ascorbic acid (D) carried with dfferent screen-printed electrodes: Dupont (a), Ercon (b), Acheson (c) Gwent (d). Supporting electrolyte; KCl 0.1M (A), scan rate 20 mV/s.

Screen-Printed Electrodes



Screen-printed electrochemical (bio)sensors

How do we tune them?



TISSUE BASED BIOSENSORS

bovine liver (rich in catalase)

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

banana (rich in polyphenol oxidase)

$$\text{dopamine} + O_2 \rightarrow \text{dopamine quinone}$$

CELL BASED BIOSENSORS

measurement of ethanol using *acetobacter xylinum* (O_2 electrode)

Determination of the BOD (biological oxygen demand).

The BOD values indicate the amount of biochemicallly degradable organic material (carbonaceous demand) and the oxygen used to oxidise sulphides and ferrous ion.

Conventional methods include BOD_5 and BOD_7 which need 5 and 7 days.

BOD biosensors have been developed using *Trichosporon cutaneum, Bacillus subtilis, Hansenula anomala, etc.*

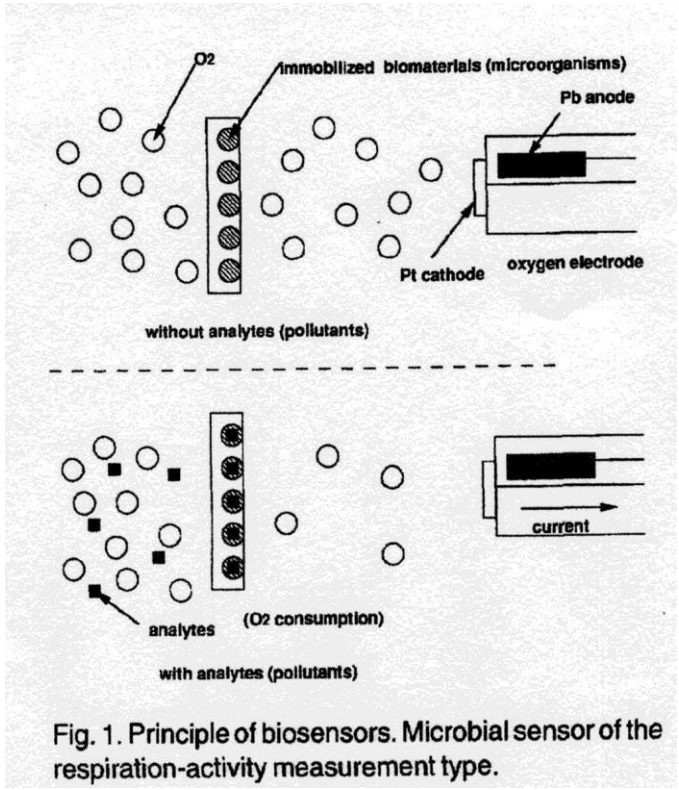
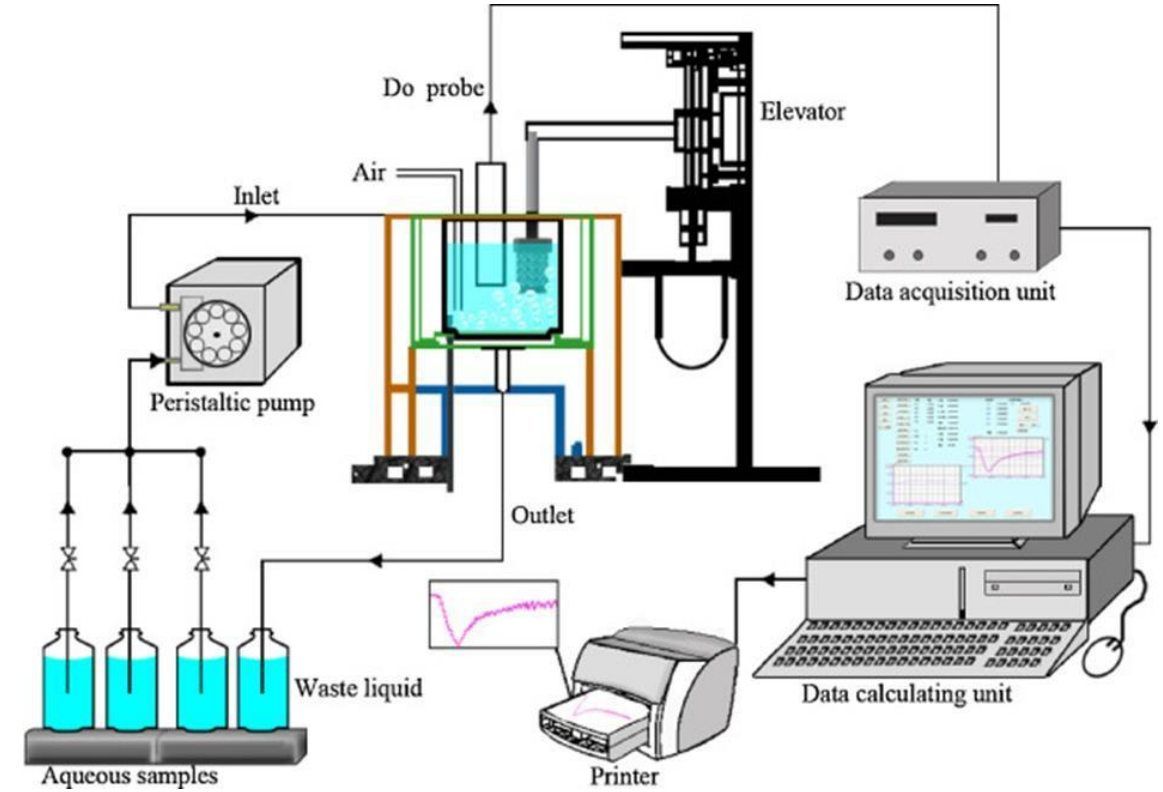
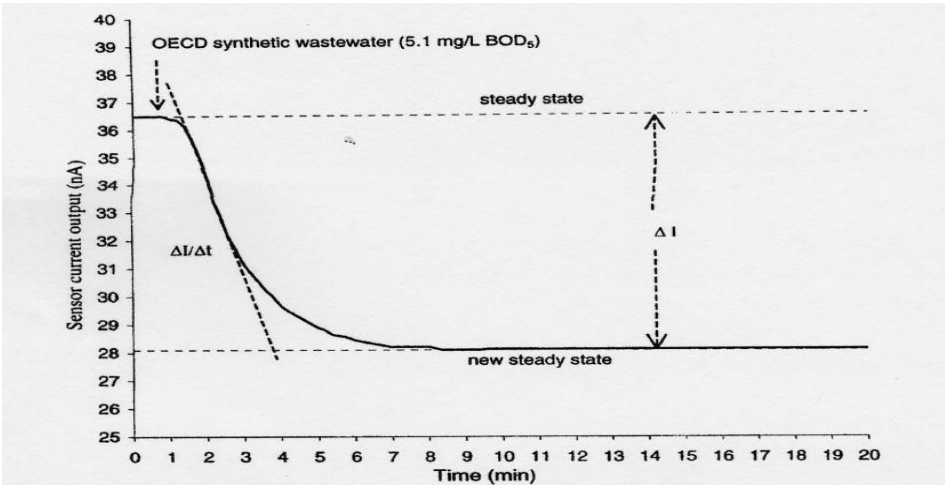


Fig. 1. Principle of biosensors. Microbial sensor of the respiration-activity measurement type.





Spectrophotometric enzymatic kits work as end-point reaction using enzymes in solution

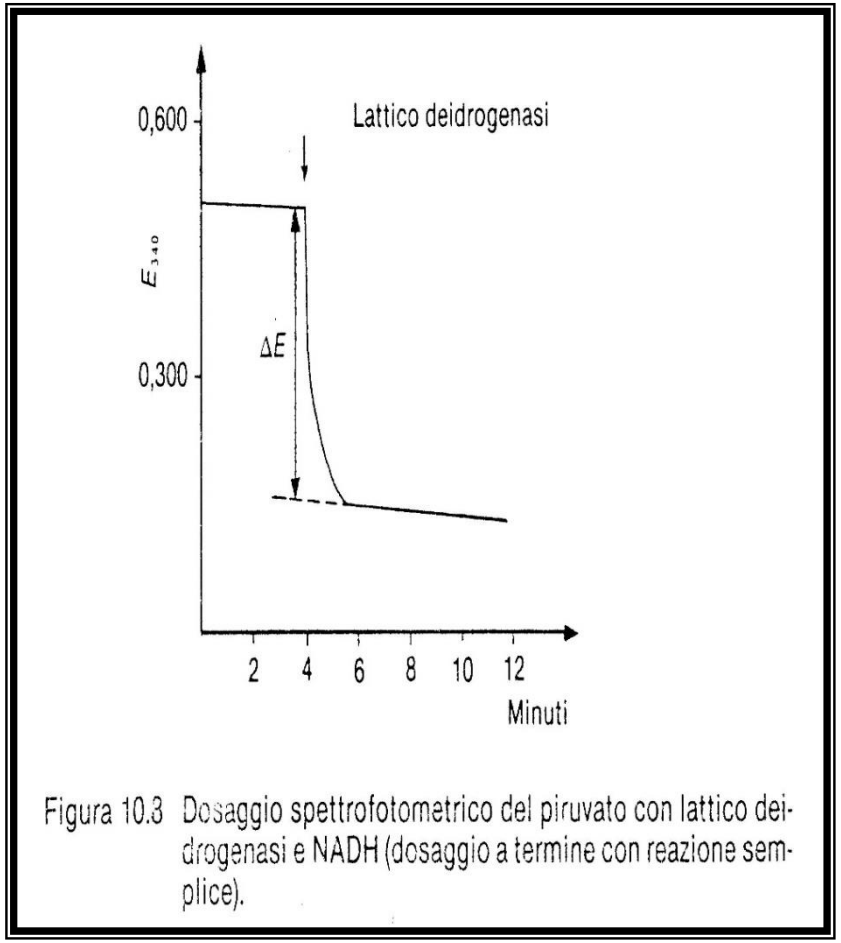


Figura 10.3 Dosaggio spettrofotometrico del piruvato con lattico deidrogenasi e NADH (dosaggio a termine con reazione semplice).

http://www.sigmaaldrich.com/life-science/metabolomics/enzymatic-kits.html

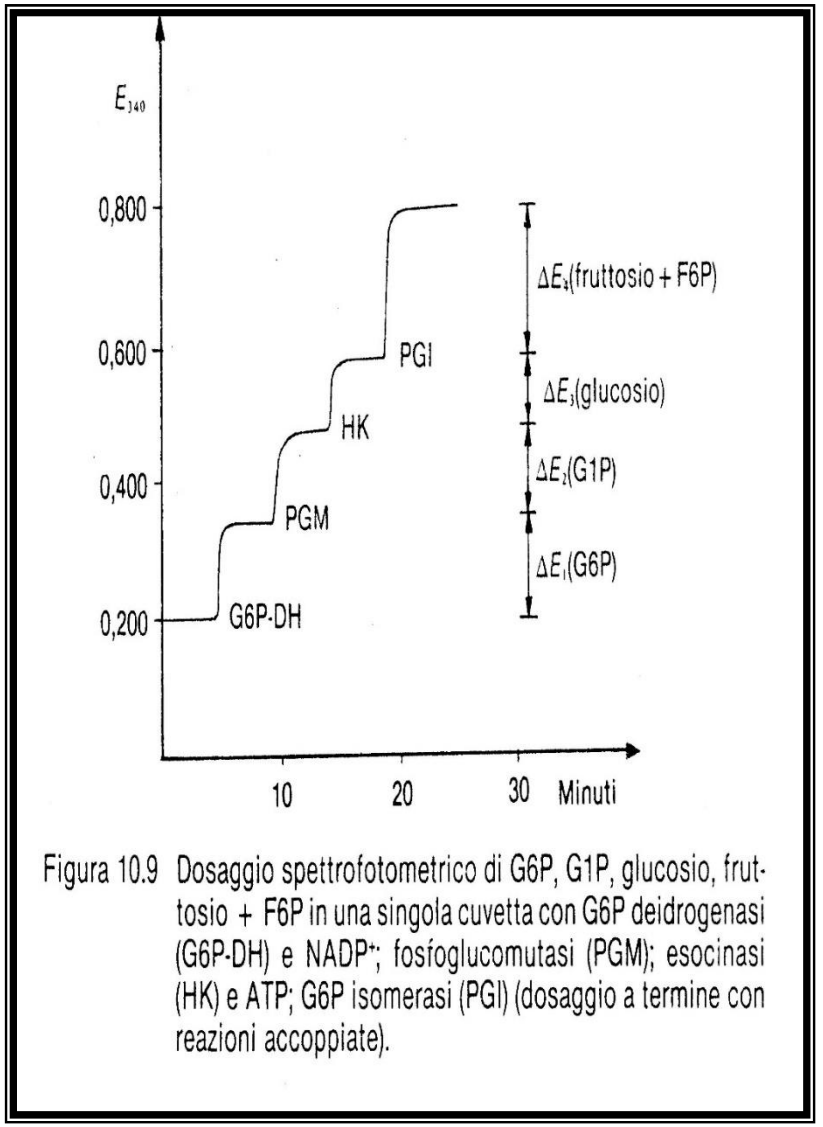
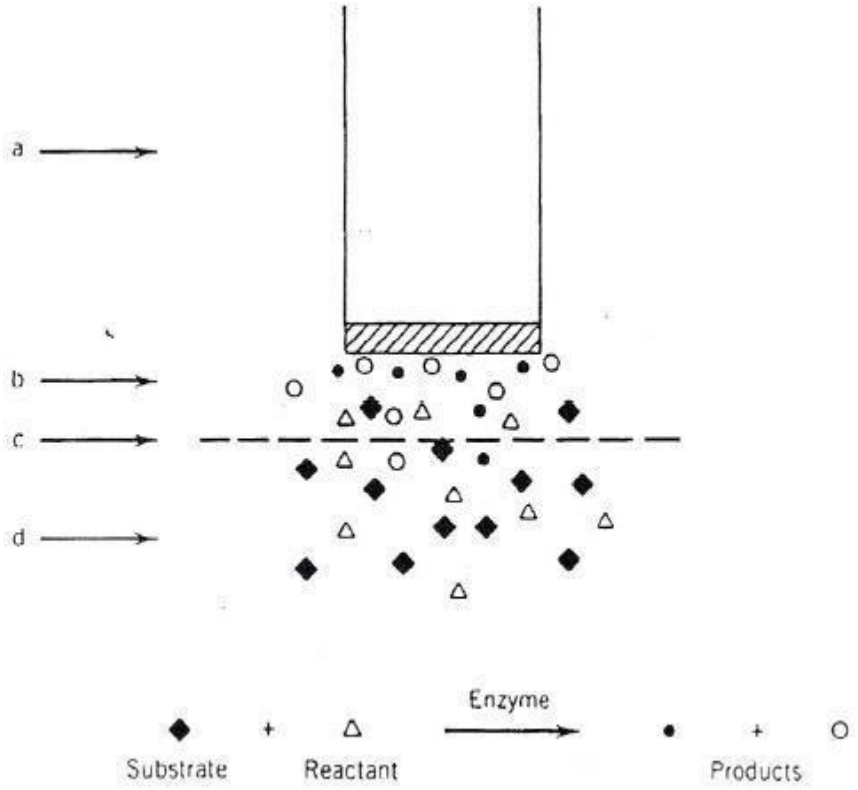


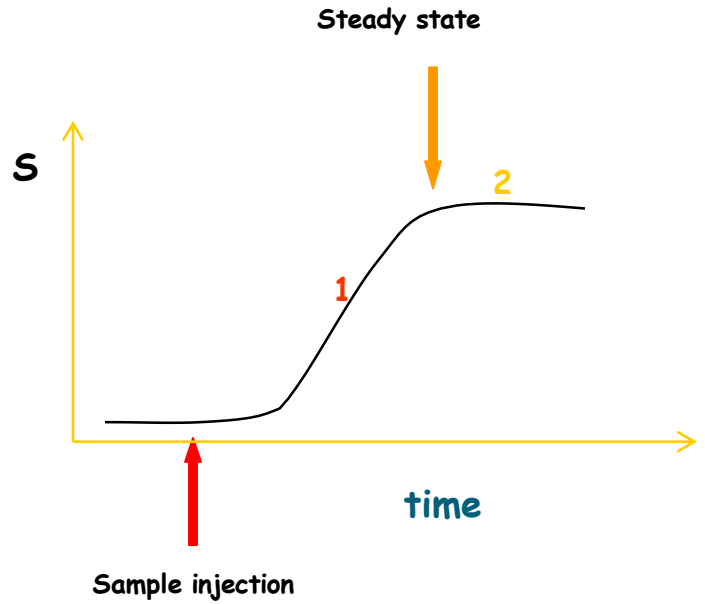
Figura 10.9 Dosaggio spettrofotometrico di G6P, G1P, glucosio, fruttosio + F6P in una singola cuvetta con G6P deidrogenasi (G6P-DH) e $NADP^+$; fosfoglucomutasi (PGM); esocinasi (HK) e ATP; G6P isomerasi (PGI) (dosaggio a termine con reazioni accoppiate).

Food Industry
Beer and Spirits
Milk and Dairy Products
Fruits, Vegetables and Nuts
Meat, Poultry, Pork and Fish (Animal Protein)
Wine
Drinking Water

Molecular Target
Carbohydrates and Sugars
Inorganic Ions
Lipids (Fats)
Amino Acids and Proteins

Enzyme electrode





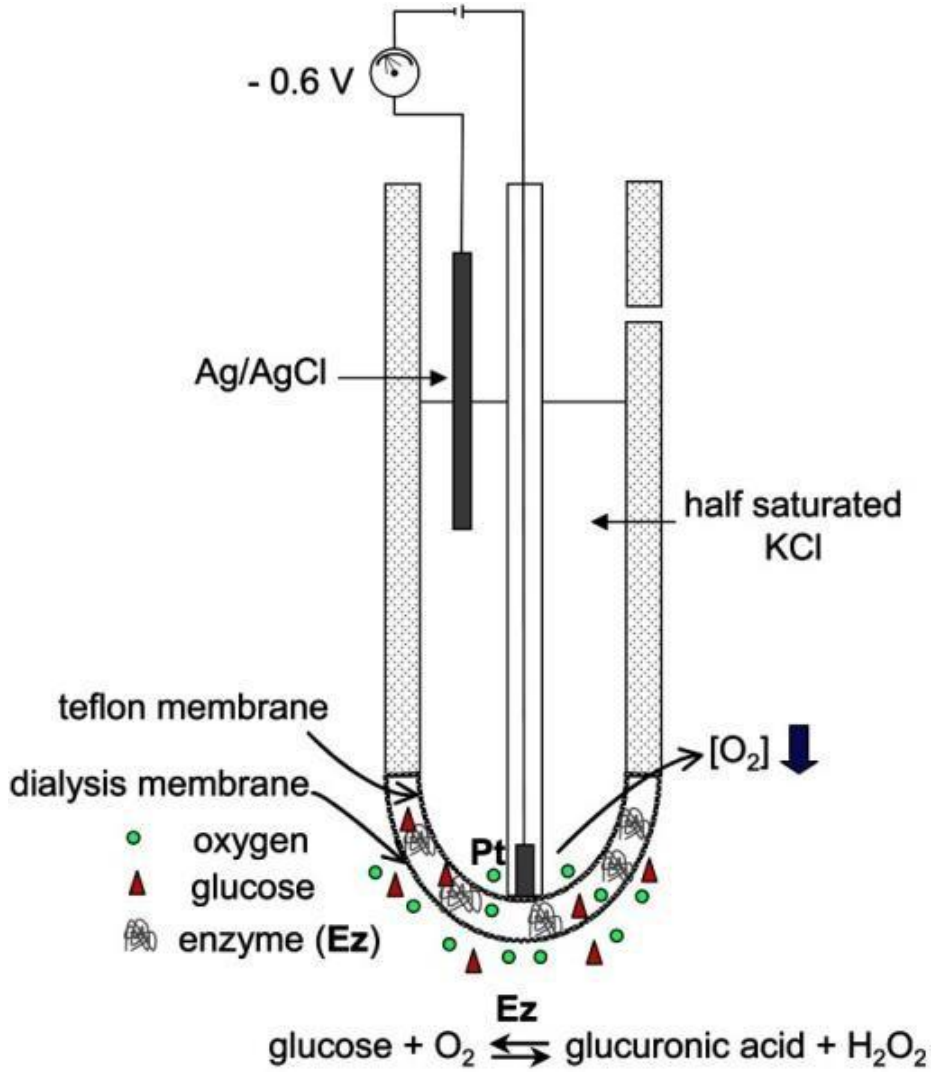
THE BIOLOGICAL ELEMENT SHOULD HAVE:

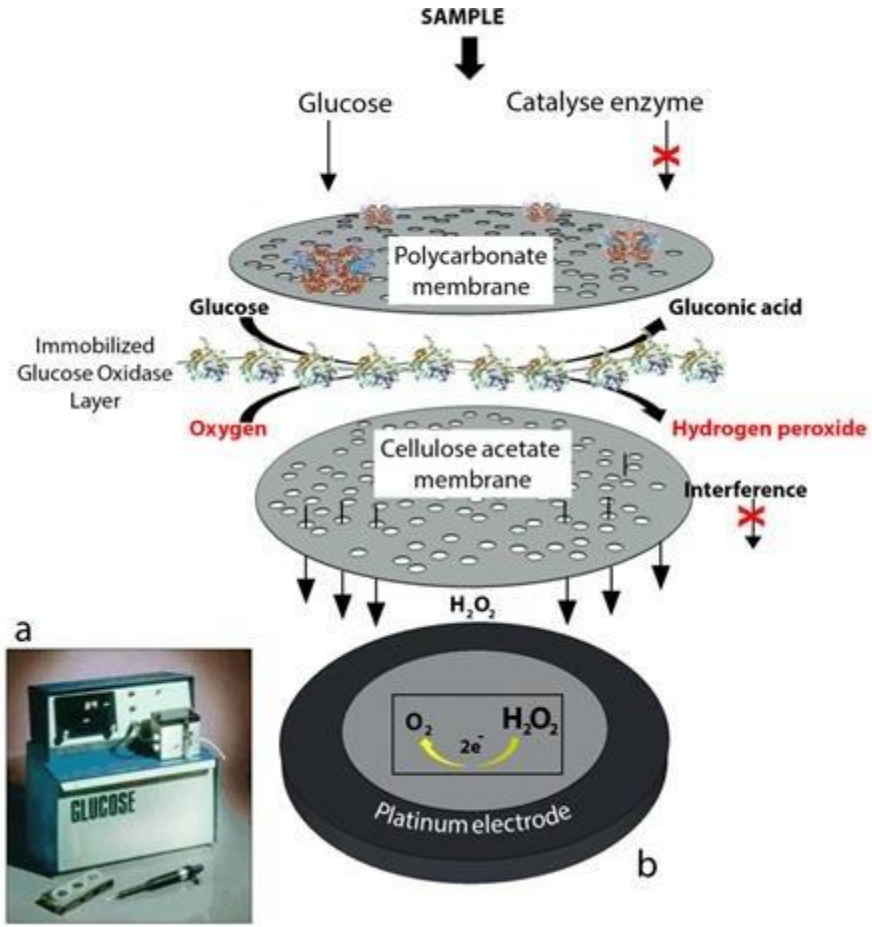
SPECIFICITY (HIGH SELECTIVITY) FOR THE ANALYTE(S)

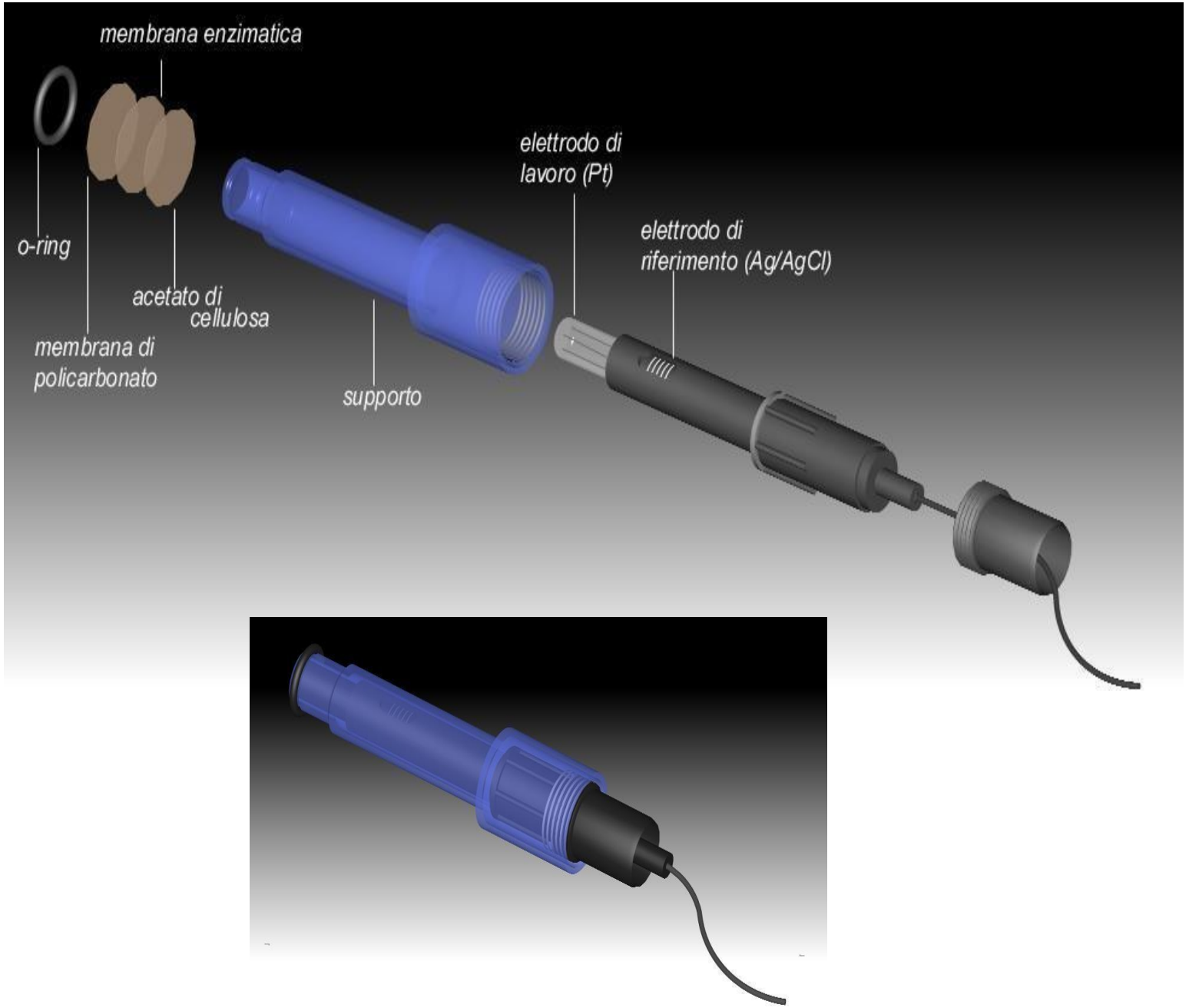
GOOD STABILITY IN OPERATING CONDITIONS (t, pH, μ)

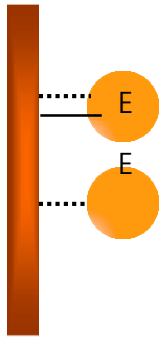
RETENTION OF SUFFICIENT BIOLOGICAL ACTIVITY WHEN IMMOBILISED

NO (VERY LOW) INHIBITION BY THE SAMPLE

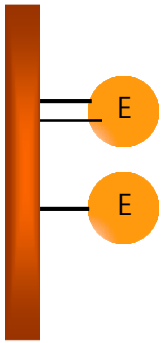

- 0.6 V
Ag/AgCl
half saturated
KCl
teflon membrane
dialysis membrane
oxygen
glucose
enzyme (Ez)
Pt
[O2]
Ez
glucose + O2 ⇆ glucuronic acid + H2O2


SAMPLE
Glucose
Catalyse enzyme
Polycarbonate membrane
Glucose
Gluconic acid
Immobilized Glucose Oxidase Layer
Oxygen
Hydrogen peroxide
Cellulose acetate membrane
Interference
H2O2
a
GLUCOSE
O2
H2O2
2e-
Platinum electrode
b

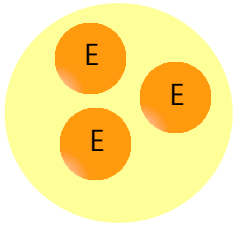

membrana enzimatica
o-ring
acetato di cellulosa
membrana di policarbonato
supporto
elettrodo di lavoro (Pt)
elettrodo di riferimento (Ag/AgCl)



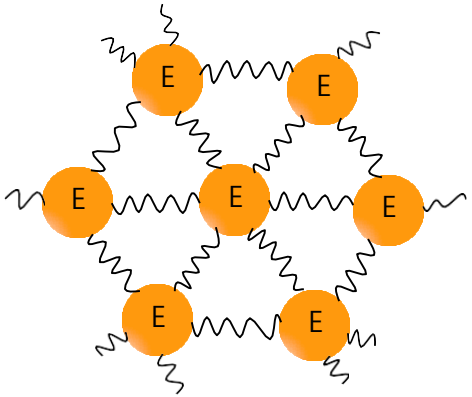
adsorption



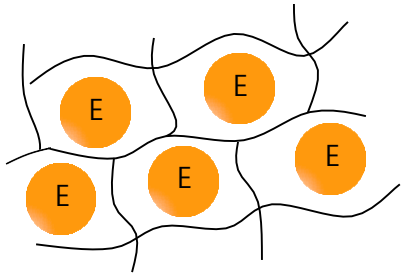
covalent binding

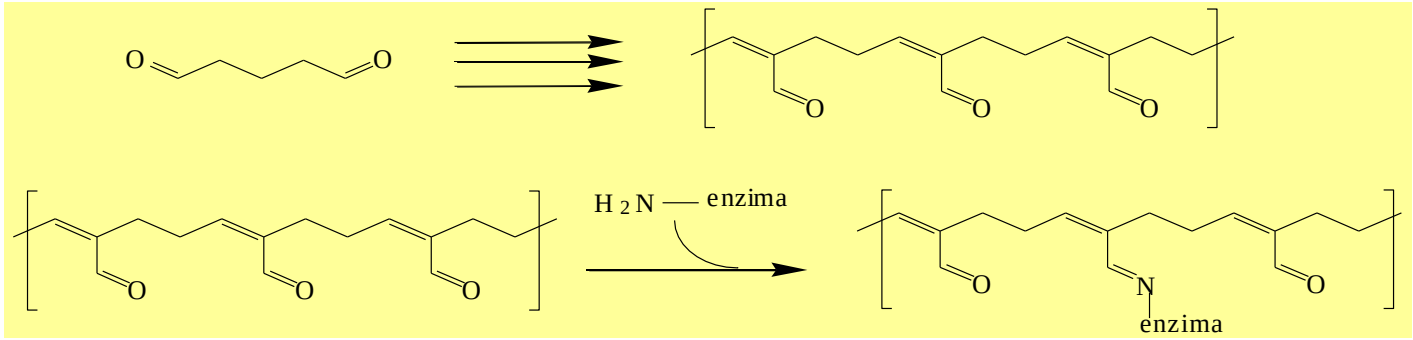


entrapment

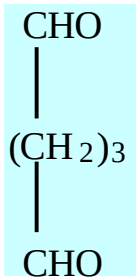


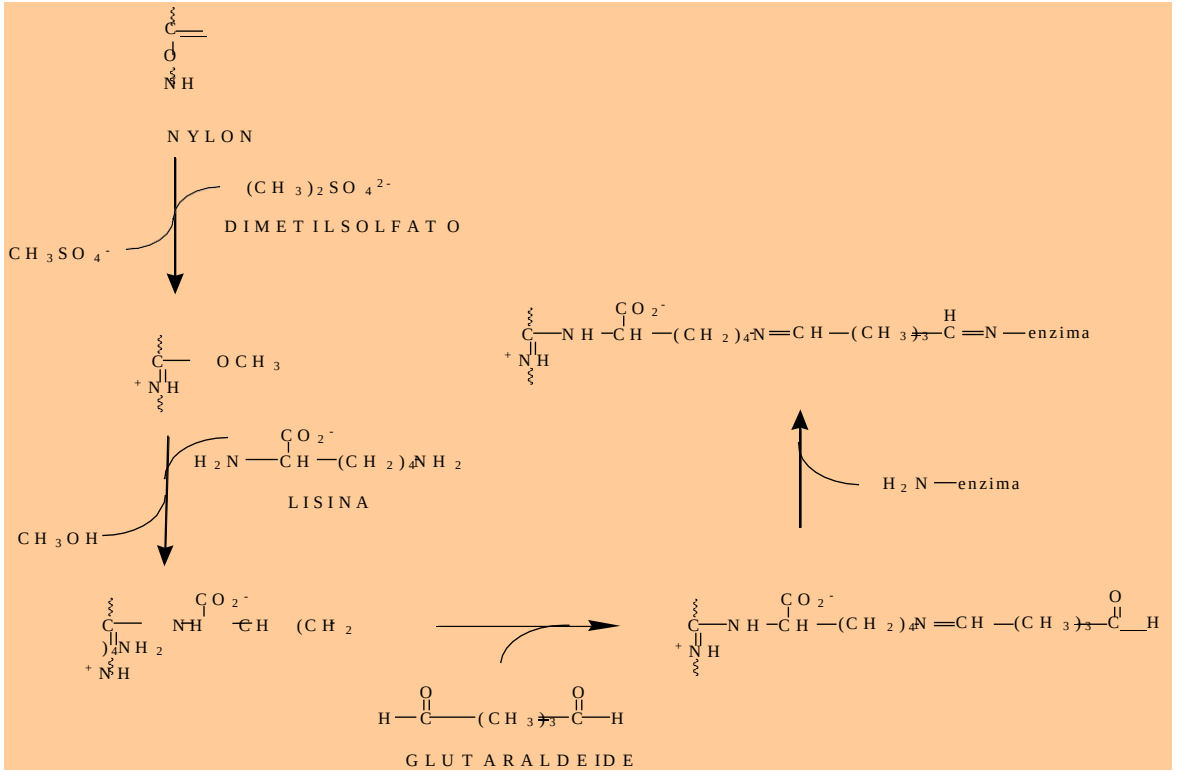
cross-linking



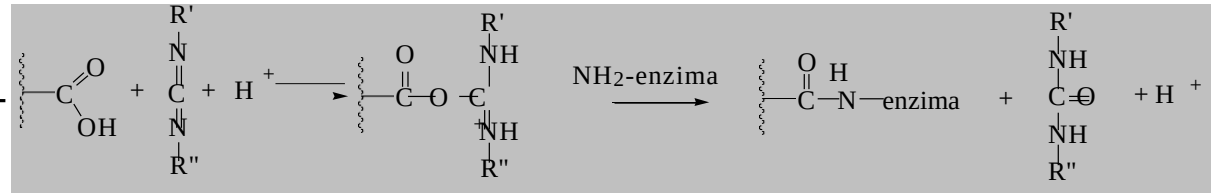


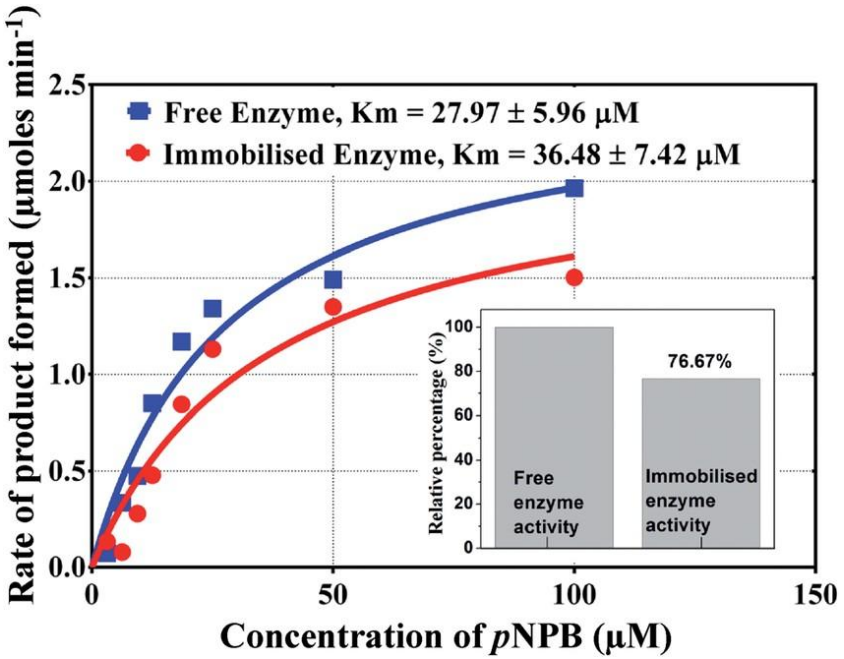
glutaraldehyde reactions: polimerization and lysine amino group

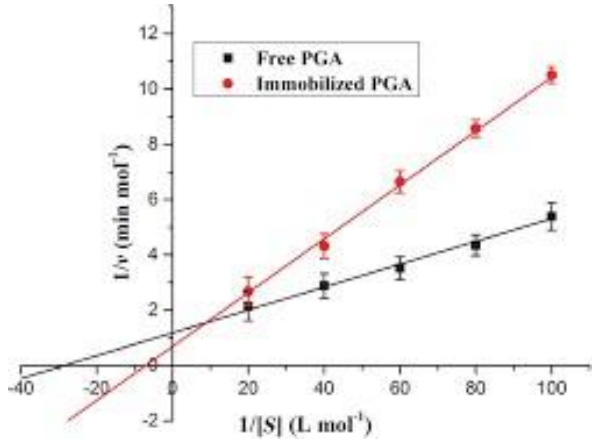


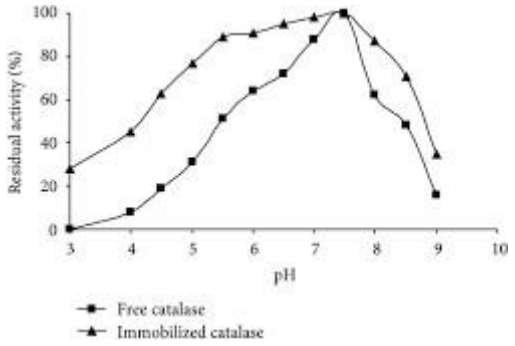


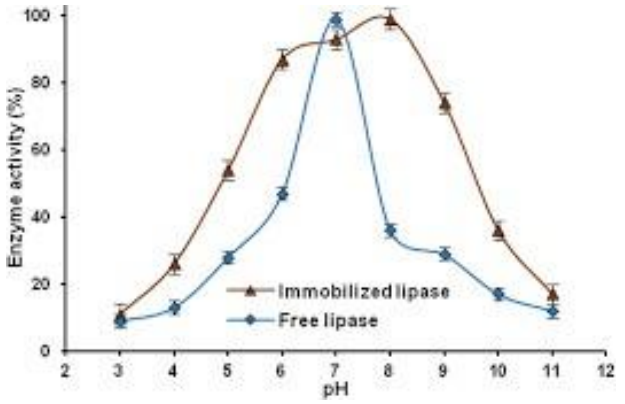
Immobilisation via carbodiimide











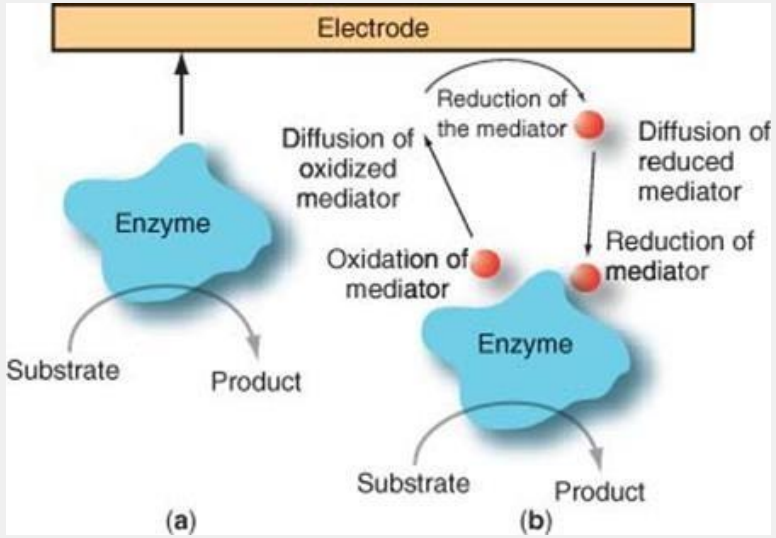
The immobilised enzyme has always a higher (apparent) K_m ,a shifted and larger optimum pH and lower enzymatic activity compared to the enzyme in solution

Enzyme electrodes are generally classified according to the mechanism of the electron transfer:

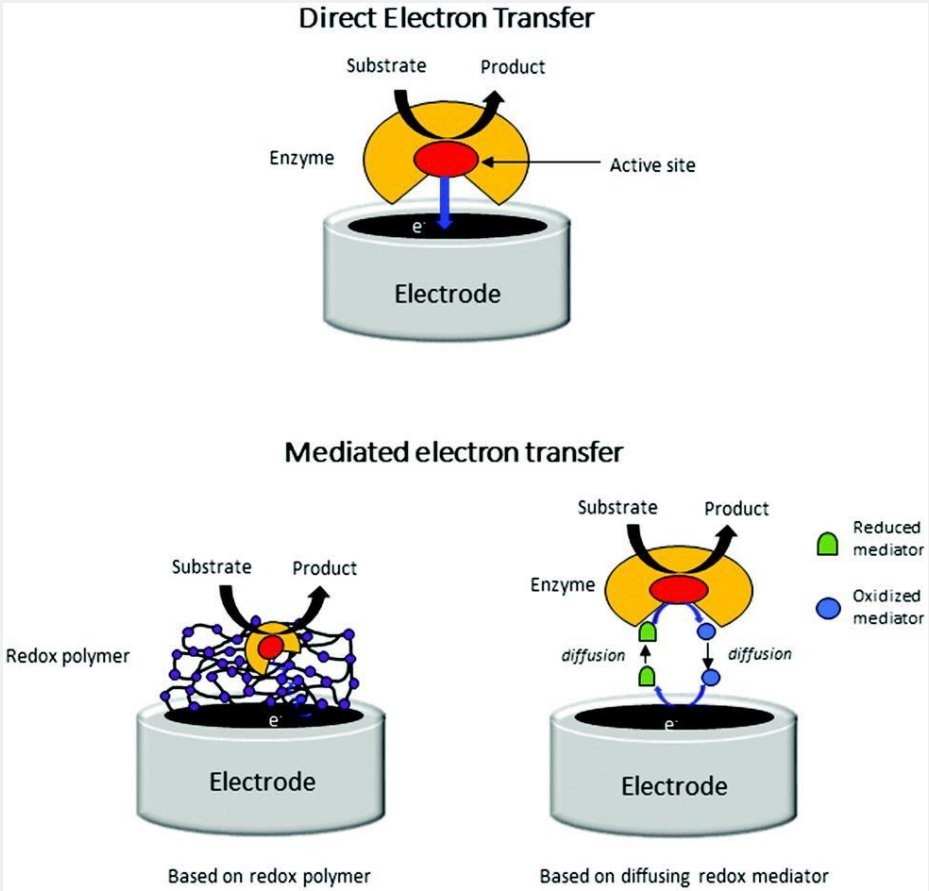
1.First generation: the enzyme is immobilised using a membrane that is in contact with the electrode surface. Usually there are other membranes to protect and regulate diffusion. Response time at the stady state is on the order of minutes

2.Second generation: electron transfer occurs via an electrochemical mediator in solution. The mediator shuttles electrons between the enzyme and the electrode.

3.Third generation: direct exchange of electrons between the electrode and the enzyme



Second and third generation



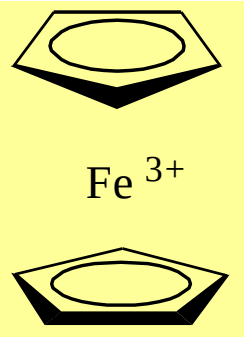
A good electrochemical mediator characteristics :

- rapid reaction with the enzyme
- rapid and reversible electron trasfer rate
- low overpotial for the redox reaction
- pH independent
- stability in the reaction medium in both redox forms
- Should not react with the dissolved oxygen in solution
- no toxicity

$Fe^{3+} + e^-$ Fe^{2+} $E° = +0,53\ V$

Ferrycianide $E° = +0,45\ V$

Ferrocene $E° = +\mathbf{0,165}\ V$

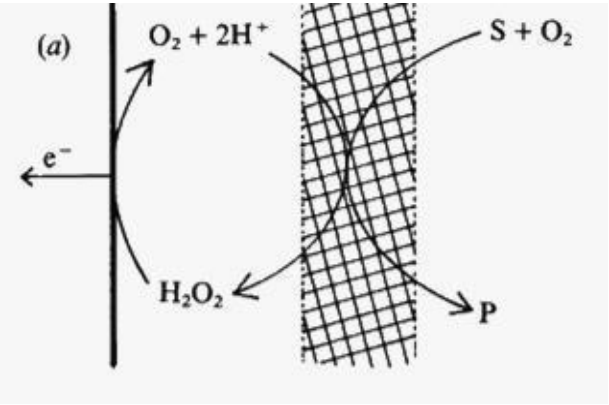


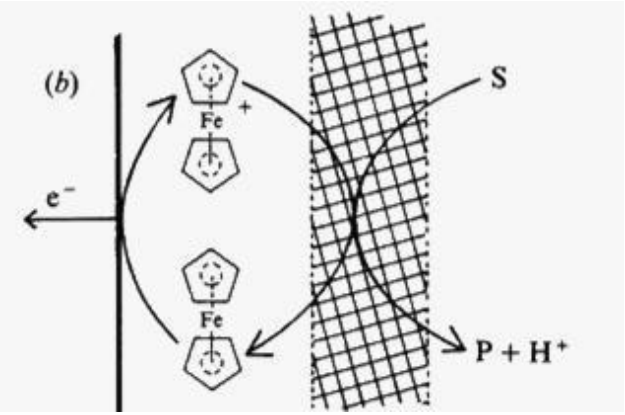
ferrocene is an excellent mediator for the oxidation of glucose catalysed by glucose oxidase

glucose + GOD_{ox} → gluconolactone (gluconic acid) + GOD_{rid} + $2H^+$

$GOD_{rid} + 2Fe^+ \rightarrow GOD_{ox} + 2Fe$

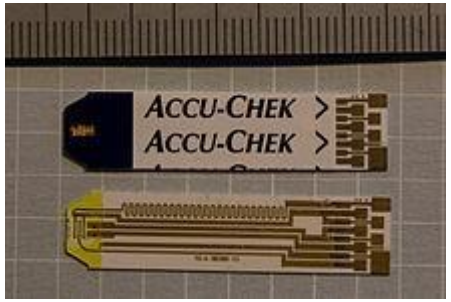
$2\ Fe - 2\ e^- \rightarrow 2\ Fe^+$



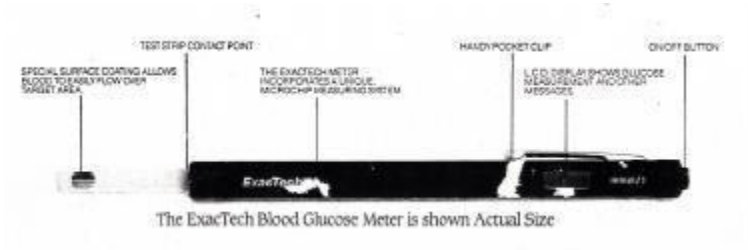



BAYER
3902M
GLUCOMETER ELITE®
Blood Glucose Meter/Lecteur de glycémie
Blutzuckermessgerät/Strumento per la
determinazione della glicemia/Bloedglucosemeter
Medidor de glucose no sangue
Medidor de glucosa en sangre
• Easy to use
• Very accurate
Now with memory/Maintenant avec mémoire
Jetzt mit speicher/Adesso con memoria
Nu met geheugen/Agora com memória
Ahora con memoria
GLUCOMETER ELITE


odeFree
OOD GLUCOSE TEST STRIP
GOLD ELECTRODE
50 x
SD BIOSENSOR

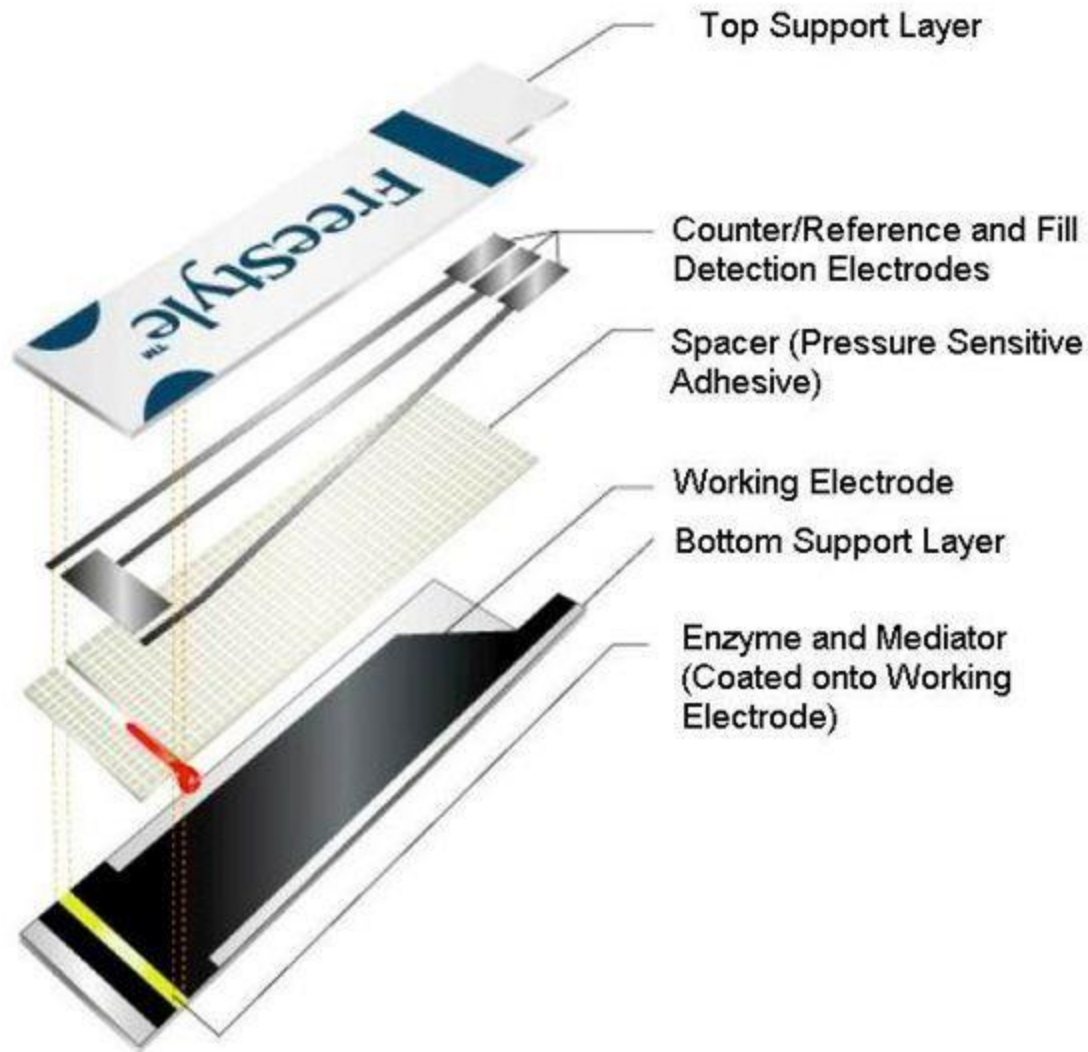

ACCU-CHEK
ACCU-CHEK


SIMPLE STEP BY STEP FUNCTION
1. Insert the ExacTech test strip in the meter and then place a blood sample on the target area.
2. Press the button immediately. The meter is now analysing the sample.
0 SECS
3. After a 30 second countdown, the result is displayed.
30 SECS


TEST STRIP CONTACT POINT
HANDY POCKET CLIP
ON/OFF BUTTON
THE EXACTECH METER INCORPORATES A UNIQUE MICROCHIP MEASURING SYSTEM
L.C.D. DISPLAY SHOWS GLUCOSE MEASUREMENT AND OTHER MESSAGES
ExacTech
The ExacTech Blood Glucose Meter is shown Actual Size

Test Strips

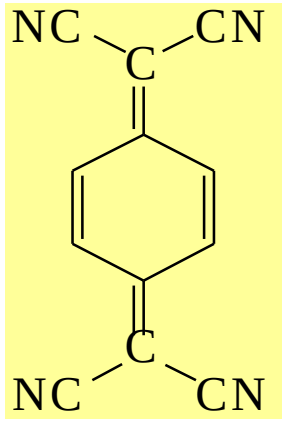
- When blood added, glucose is oxidized by enzyme coated on working electrode
- Voltage applied between working and reference electrode
- Measure current between working and reference electrode



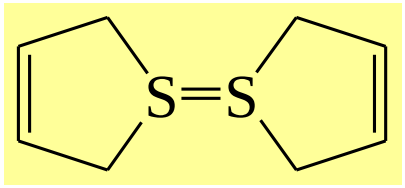
NATURAL	E(V) vs. SHC	SYNTHETIC	E (V) vs. SHC
Cytochrome a_3	+0,29	Esacyanoferrate(III)	+0,45
Cytochrome c_3	+0,24	2,6-dichlorophenol	+0,24
Ubiquinone	+0,10	Indophenolo	+0,24
Cytochrome b	+0,08	Ferrocene	+0,17
Vitamin K_2	-0,03	N-metilfenazium sulphate	+0,07
Rubredoxin	-0,05	Metilene blue	+0,4
Flavoproteins	da -0,4 a +0,2	Ftalocyanin	-0,02
FAD/$FADH_2$	-0,23	Fenosafranin	-0,23
FMN/$FMNH_2$	-0,23	Benzyl viologen	-0,36
NAD^+/NADH	-0,32	Methyl viologen	-0,46
$NADP^+$/NADPH	-0,32		
ferredossina	-0,43		

Third generation

Conduting salts



Tetracyanochinodimethane
(TCNQ)



Tetra tiafulvalene(TTF)

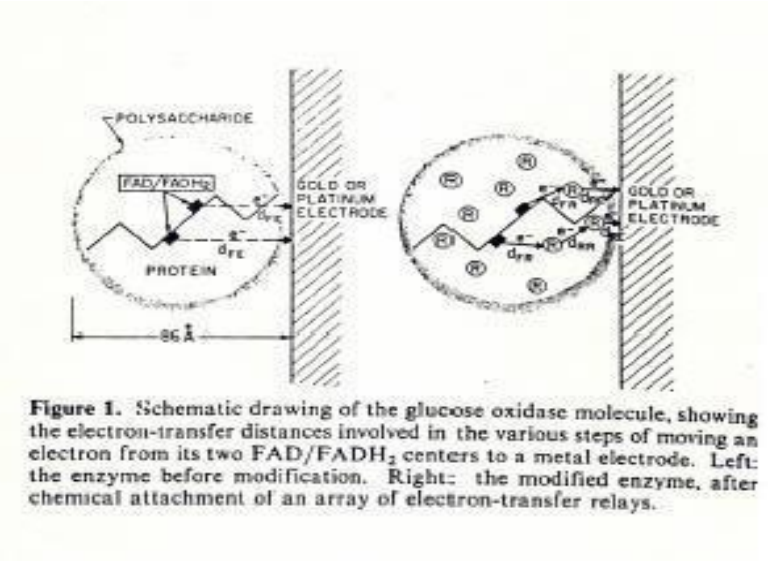
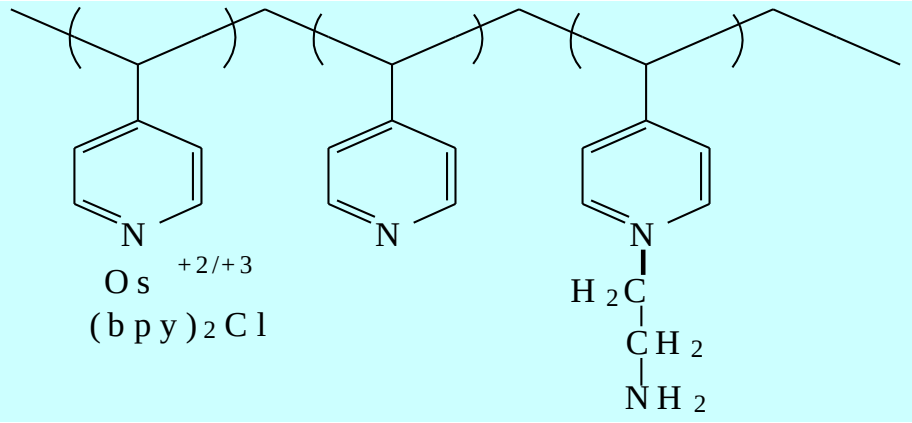


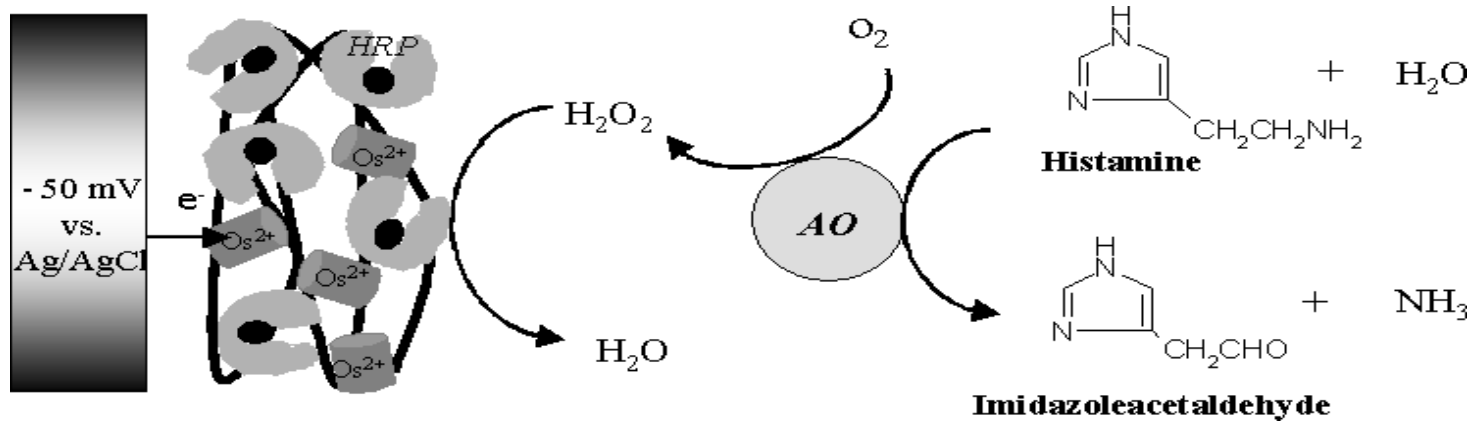
Figure 1. Schematic drawing of the glucose oxidase molecule, showing the electron-transfer distances involved in the various steps of moving an electron from its two $FAD/FADH_2$ centers to a metal electrode. Left: the enzyme before modification. Right: the modified enzyme, after chemical attachment of an array of electron-transfer relays.

Redox gel

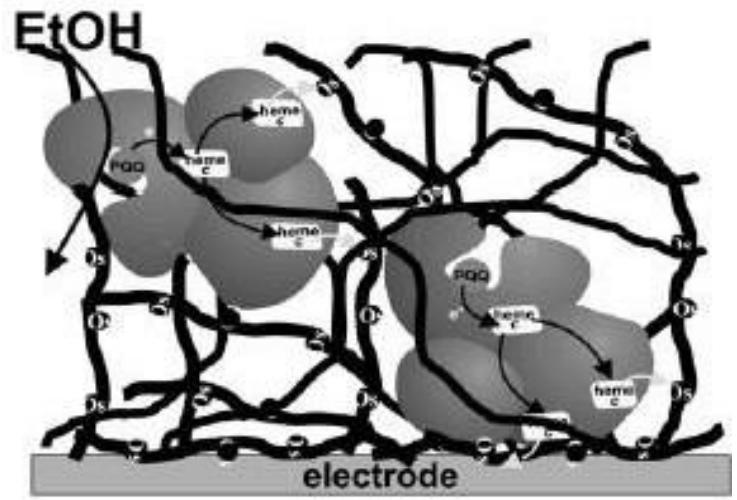


Struttura dell' l'osmio bipiridile legato a polivinilpiridina

Hystamine Biosensor



PQQ Alcohol Dehydrogenase entrapped in a Os hydrogel



NAD(P)H electrodes

The largest class of redox enzymes known is dehydrogenases which use the NAD(P)H / $NAD(P)^+$ couple as cofactor.

Oxidation of NADH at carbon and metal solid electrodes proceeds at high overvoltages (+400/ +700 mV vs. Ag/AgCl) via formation of the radical cation $NADH^{\cdot +}$. This can give side reactions (dimerisation) and adsorb onto the electrode (carbon).

A soluble mediator can be used to lower the overpotential and increase the electron transfer rate

$$NADH + Med_{ox} \Leftrightarrow NAD^+ + Med_{red}$$

at the electrode surface polarised at the appropriate E :

$$Med_{red} \rightarrow Med_{ox} + ne$$

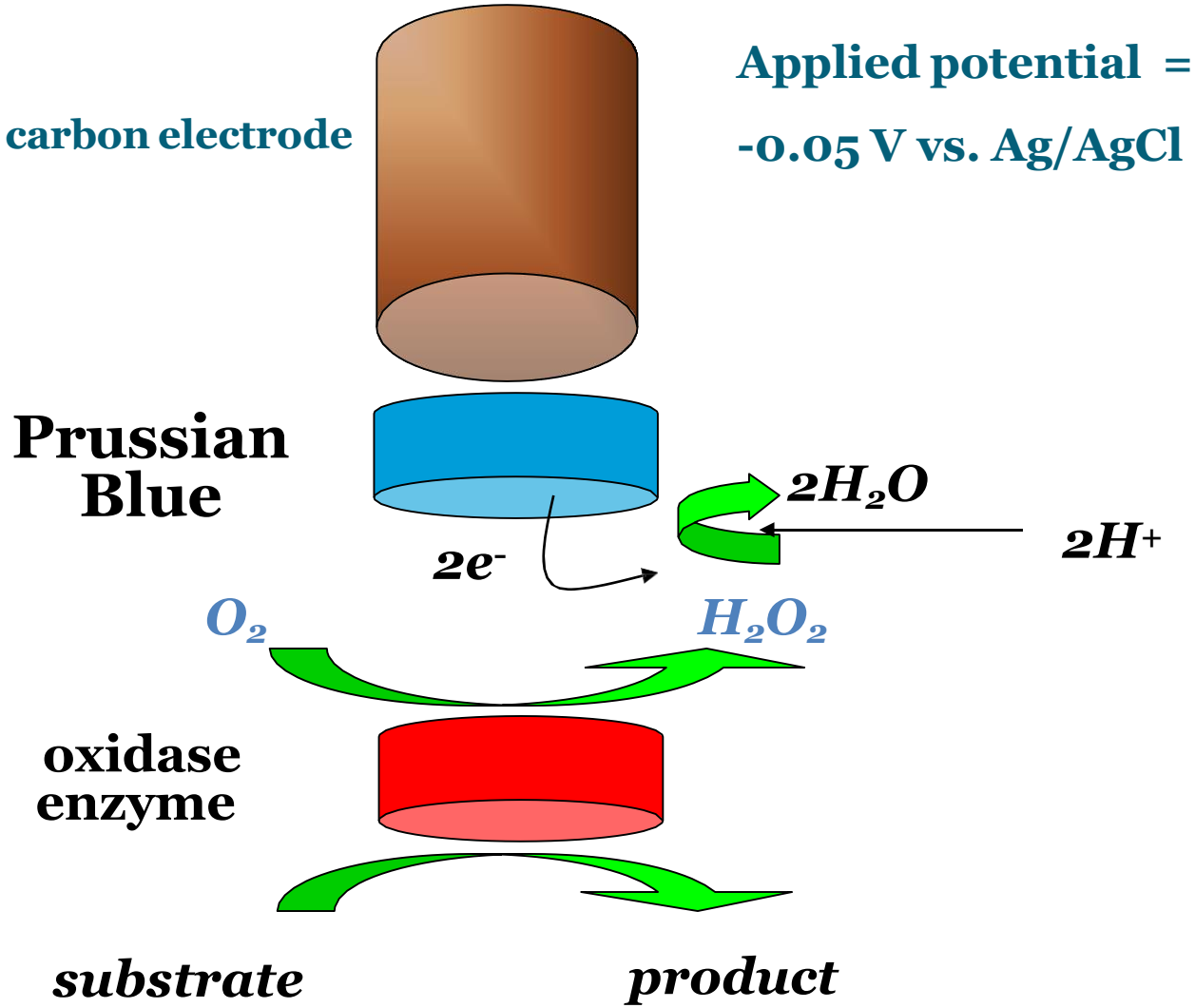
ortho- and para-quinones , quinone imines have been used and incorporated into larger molecules as indophenols, phenazines and phenoxazines

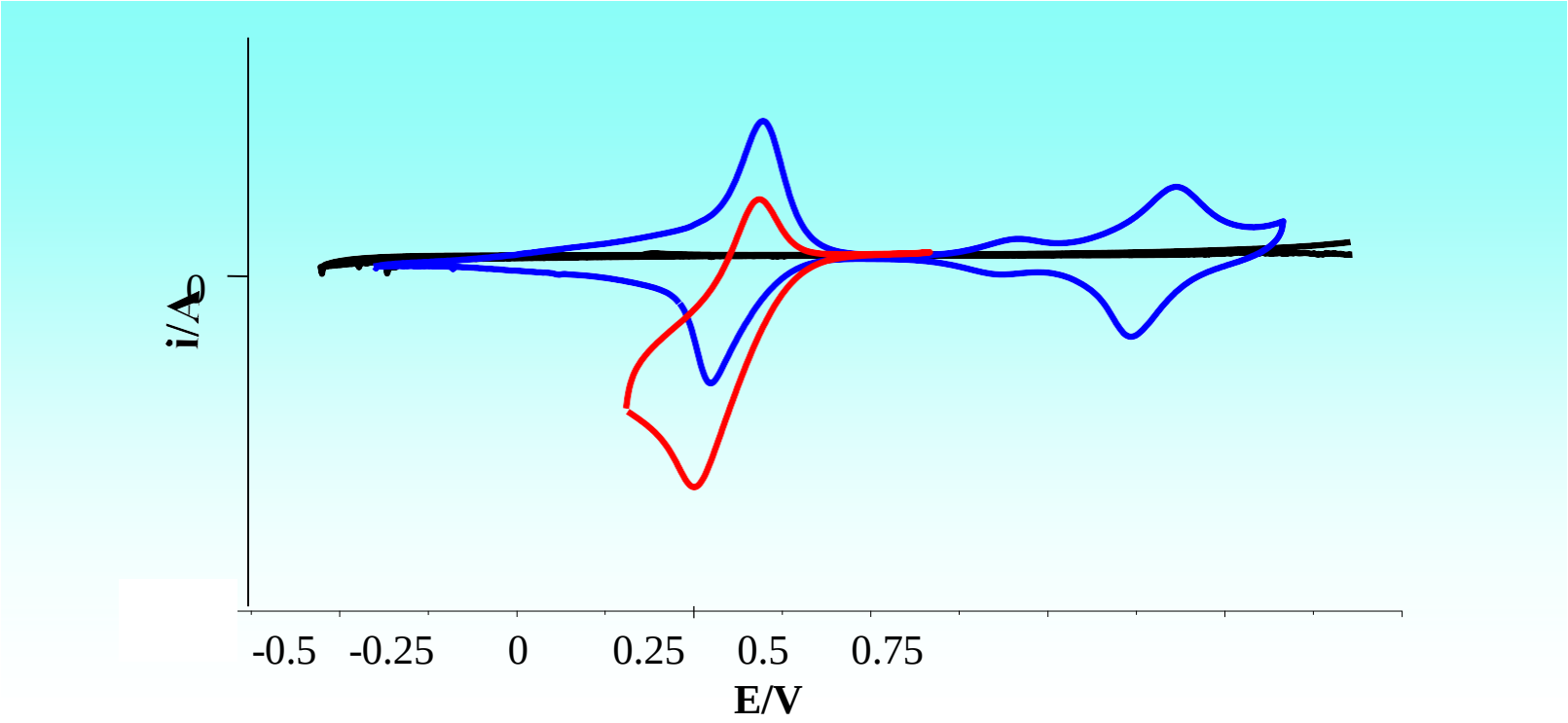
The mediator can be also immobilized at the electrode surface giving a **chemically modified electrode** for NADH

Sensitivity is excellent, major problems arise from selectivity and stability of the sensors

Reduced Prussian Blue is a selective catalyst for H_2O_2 reduction

$$K_2Fe^{II}[Fe^{II}(CN)_6] + H_2O_2 \rightleftharpoons KFe^{III}[Fe^{II}(CN)_6] + K^+ + OH^-$$



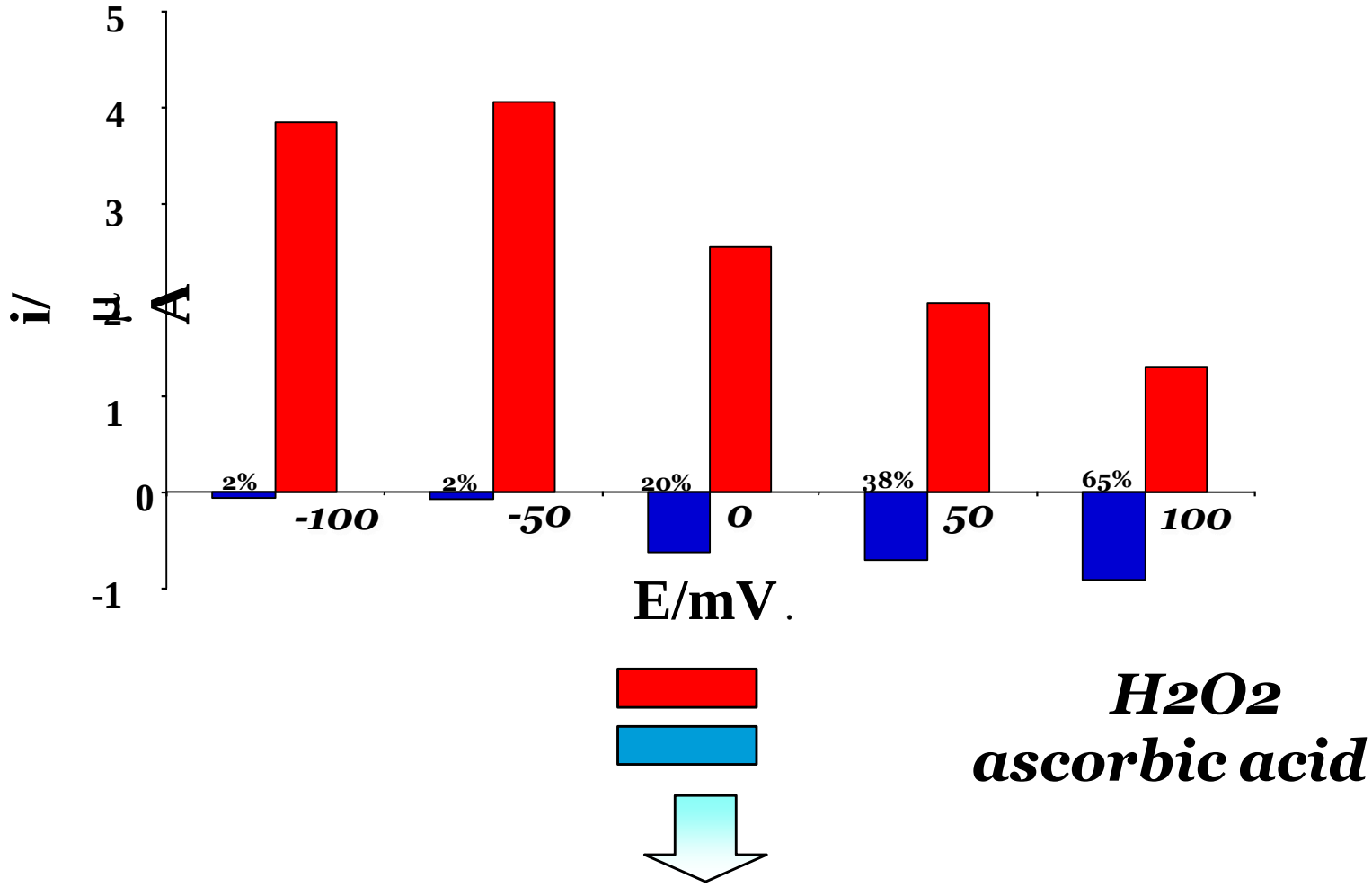


Bare electrode

Prussian Blue modified electrode

Prussian Blue modified electrode + H_2O_2

Selection of the applied potential



Potential selected - 50 mV vs. Ag/AgCl pseudo-ref.

Lysine biosensor, linear range and selectivity

$$\mathcal{L}\text{-lysine} + O_2 + H_2O \rightarrow \alpha\text{-keto-}\mathcal{E}\text{-aminocaproate} + NH_3 + H_2O_2$$

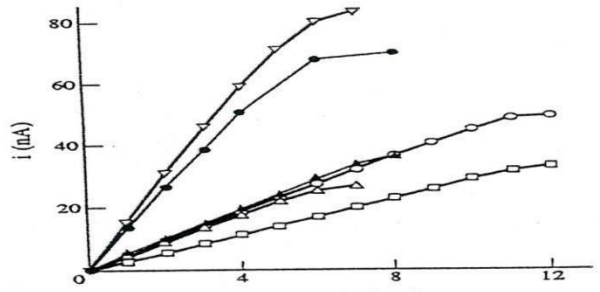


Fig. 1. Lysine calibration curves using two different immobilization procedures and protective polycarbonate membranes with different porosity. ▽ BSA/glutaraldehyde on Immobilon and 0.8-μm polycarbonate; ● Immobilon only and 0.8-μm polycarbonate; ○ BSA/glutaraldehyde on Immobilon and 0.03-μm polycarbonate; ▲ BSA/glutaraldehyde on Immobilon and 0.05-μm polycarbonate; △ Immobilon only and 0.05-μm polycarbonate; □ Immobilon only and 0.03-μm polycarbonate. Buffer phosphate 0.1M pH 7.0 $T = 25°$.

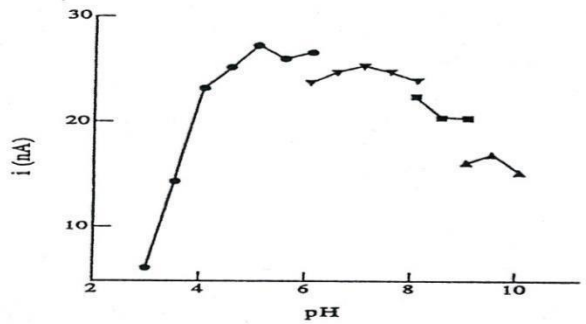


Fig. 2. Effect of pH on lysine oxidase activity. The enzyme activity was measured in the following buffers: ● citrate; ▼ phosphate; ■ tris; ▲ borax. $T = 25°$.

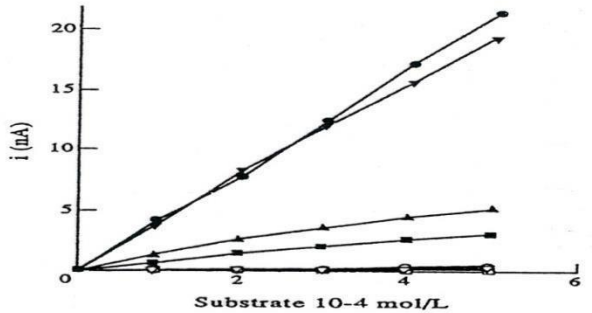
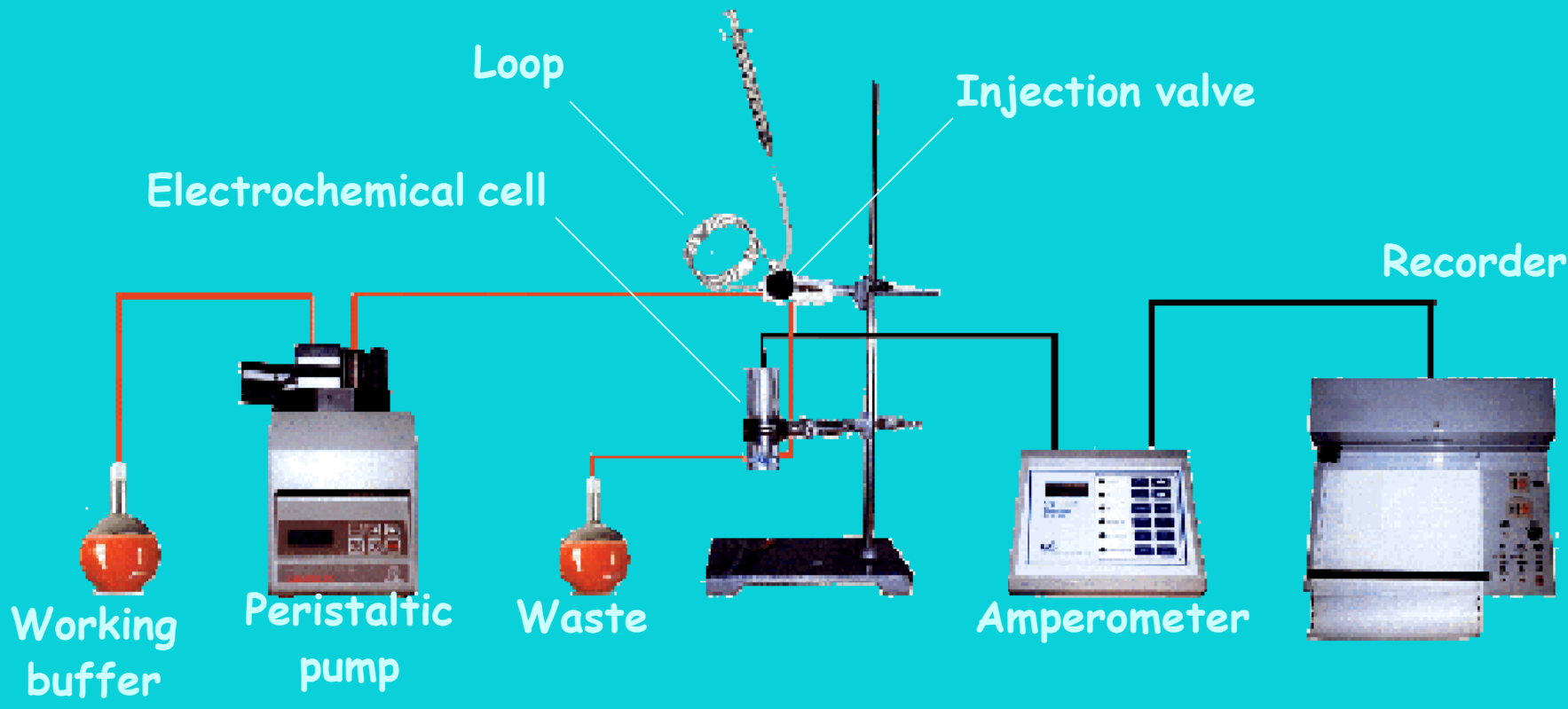
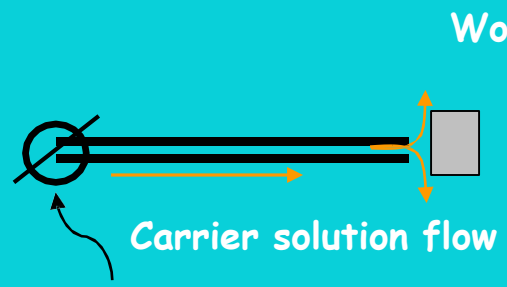
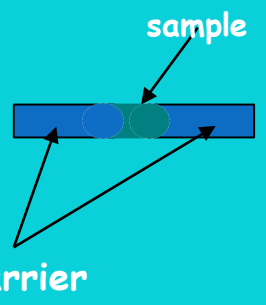
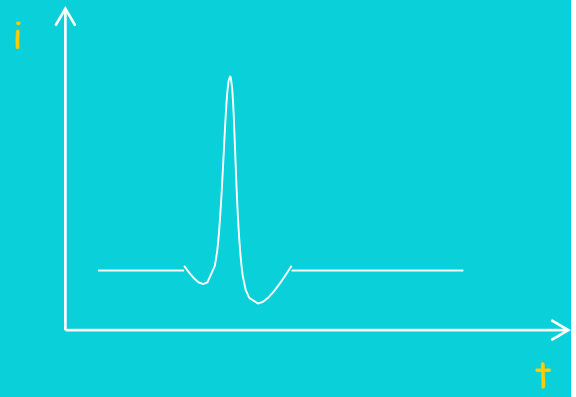


Fig. 4. Relative lysine oxidase activity toward some substrates pH 7.0 phosphate buffer $T = 25°$. ● lysine; ▼ lysine after the analysis of other aminoacids. ▲ ornithine; ■ arginine; ○ tyrosine; □ phenylalanine; ▽ histidine.


Loop
Injection valve
Electrochemical cell
Recorder
Working buffer
Peristaltic pump
Waste
Amperometer


Working electrode
Carrier solution flow
Sample injection through valve


sample
carrier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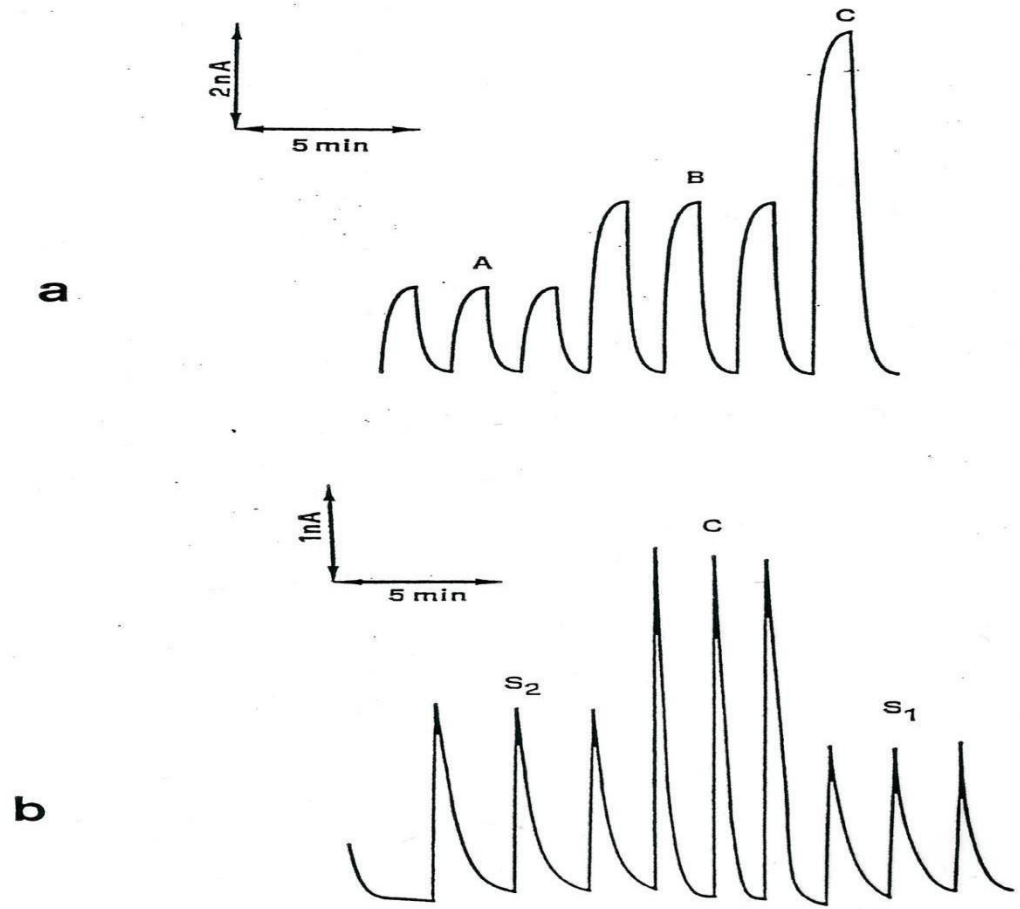


Figure 7 Reproducibility and response time of the bioprobe in flow through analysis and FIA.
a = flow through: Lysine concentration in standard solution.
A = $5 \cdot 10^{-5}$ mol/L; B = 10^{-4} mol/L; C = $2 \cdot 10^{-4}$ mol/L.
b = FIA; S_1 and S_2 foodstaff samples; C = Lysine standard $5 \cdot 10^{-4}$ mol/L.

Relative activity of 3 different purified lysine oxidase

compound	Yamasa	SIGMA	Univ. of Athens
Lysine	100	100	100
Phenylalanine	14	42	6
Arginine	2	17	0
Ornithine	3	14	0
Histidine	0	15	0
Furosine	0	0	0
Piridosine	0	0	0
Norleucine	3	17	3
AGPA*	0	0	0

Microwave hydrolysis + biosensor , analysis time 30 min

Sample	**Amino-acid analysis (mM)**	**L-lysine biosensor (mM)**	**Recovery (%)**
Milk	1.684	1.493 ± 0.014	88.66
pasta	0.299	0.308 ± 0.012	103.01

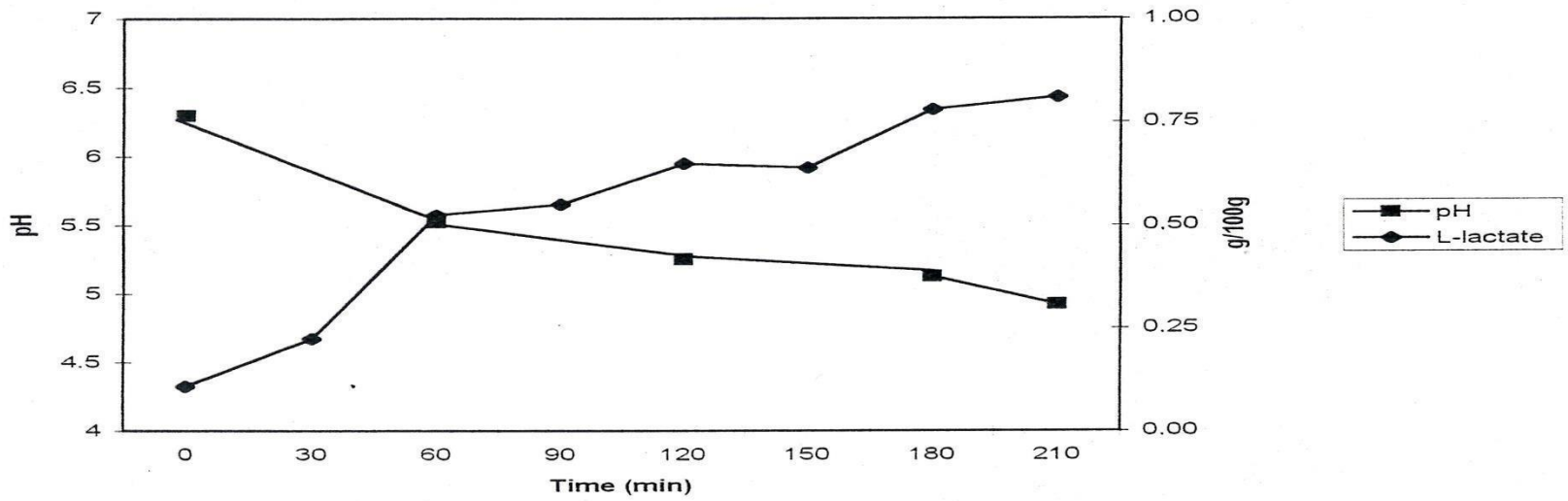
Lactic acid monitoring during mozzarella cheese manufacturing

In mozzarella cheese manufacture L-Lactic acid is the main product of lactose fermentation generated by selected cultures of lactic acid bacteria. A progressive acidification of the curd md the whey occurs and had to be carefully controlled.

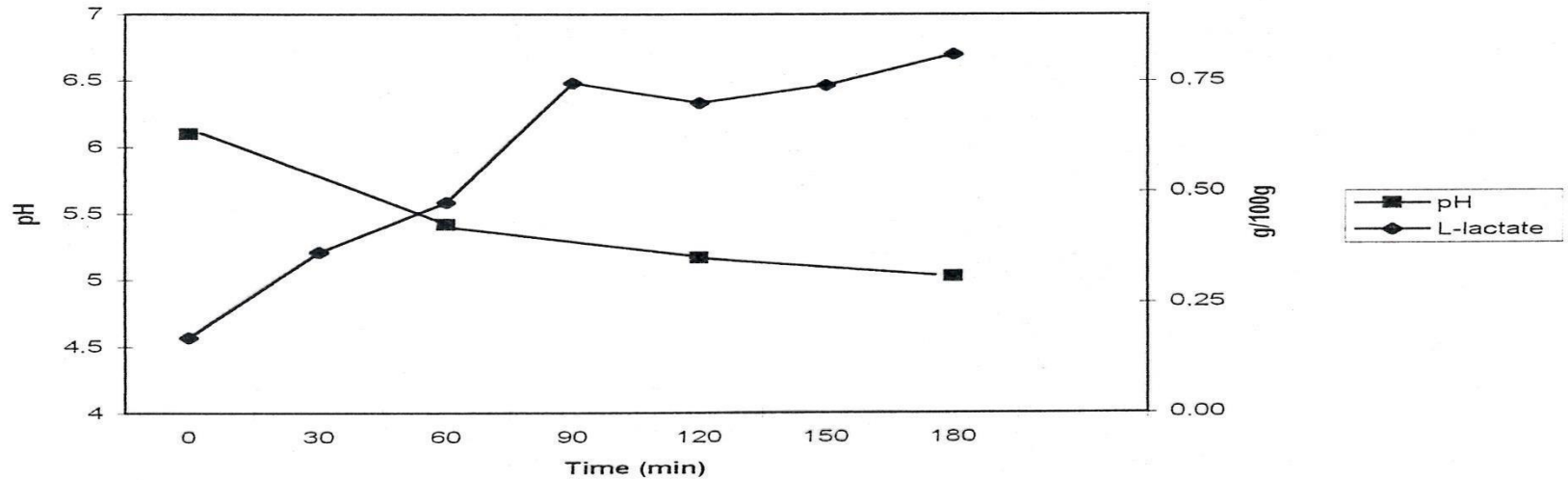
Particularly, the pH of the "stretching point" is important in order to avoid loss of fats, a decrease in yield and low reproducibility of themanufacturing.

Optimum pH is 4.9 for water-buffalo milk and 5.1 for cow milk. At these pH values there is a great increase in the buffer capacity due to casein (isoelectric point pH ~ 5) and low molecular weight acids

A sensitive measurement of lactic acid in real time can be useful in the optimization of the mozzarella cheese manufacture

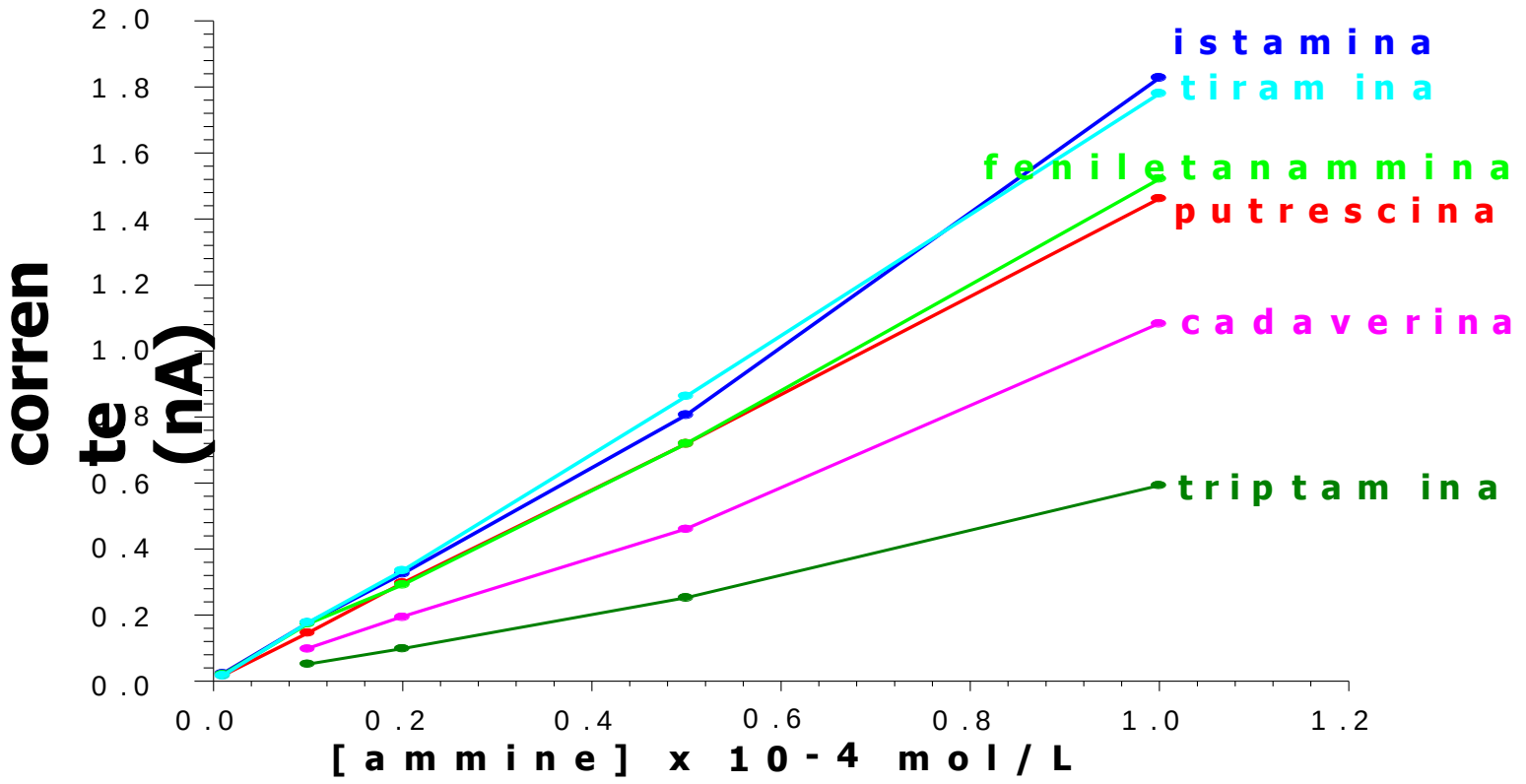


B



Biogenic amines

$$R\text{-}CH_2\text{-}NH_2 + O_2 + H_2O \longrightarrow R\text{-}CHO + NH_3 + H_2O_2$$



Total amines (mg/mL), expressed as equivalents of Put, measured in apricot samples using DAO biosensor[a].

		Storage time 20 days, temp. 0 ± 1°C		
variety	at harvest	LDPE	Super L	air
Pellecchiella				
ripening time I	5.6 ± 0.5	3.0 ± 0.2	2.9 ± 0.6	3.4 ± 0.6
ripening time II	5.5 ± 0.2	3.1 ± 0.4	3.0 ± 0.7	3.6 ± 0.0.2
Boccuccia				
ripening time I	5.2 ± 0.2	2.2 ± 0.1	3.7 ± 0.7	4.9 ± 0.2
ripening time II	5.1 ± 0.2	4.6 ± 0.8	4.6 ± 0.4	5.1 ± 0.1

$$\text{alcohol} + O_2 \xrightarrow{AOx} \text{aldehyde} + H_2O_2$$

Immobilization: PEI on Pall Immunodyne
Storage: 1% sucrose
Optimised operative conditions: 0.1 M phosphate buffer pH 7.0 + 0.02% Tween. Flow rate 1 mL/min; injection loop 500 µL.
Analitycal performances:
detection limit 10^{-6} mol/L
linearity 2 x 10^{-6} / 10^{-3} mol/L
stability: 20% decrease after 200 samples

$$\text{glycerol} + \text{ATP}(Mg^{2+}) \xrightarrow{GK} \text{glycerol-3-P} + \text{ADP}$$

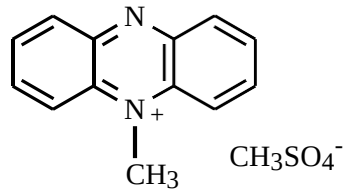
$$\text{glycerol-3-P} + O_2 + H_2O \xrightarrow{GPO} \text{glycerone-3-P} + H_2O_2$$

Immobilization: GK on aminopropyl glass beads (via glutaraldehyde), GPO on Immunodyne
Storage: DEAE-dextran/lactitol (1/5%)
Optimised operative conditions: 0.1 M borate buffer pH 8.5 + 3 mM ATP(Mg^{2+}) + 0.02% Tween. Flow rate 0.5 mL/min; injection loop 250 µL.
Analitycal performances:
detection limit 10^{-6} mol/L
linearity 2.5 x 10^{-6} / 5 x 10^{-4} mol/L
stability: 40% decrease after 200 samples

Fructose

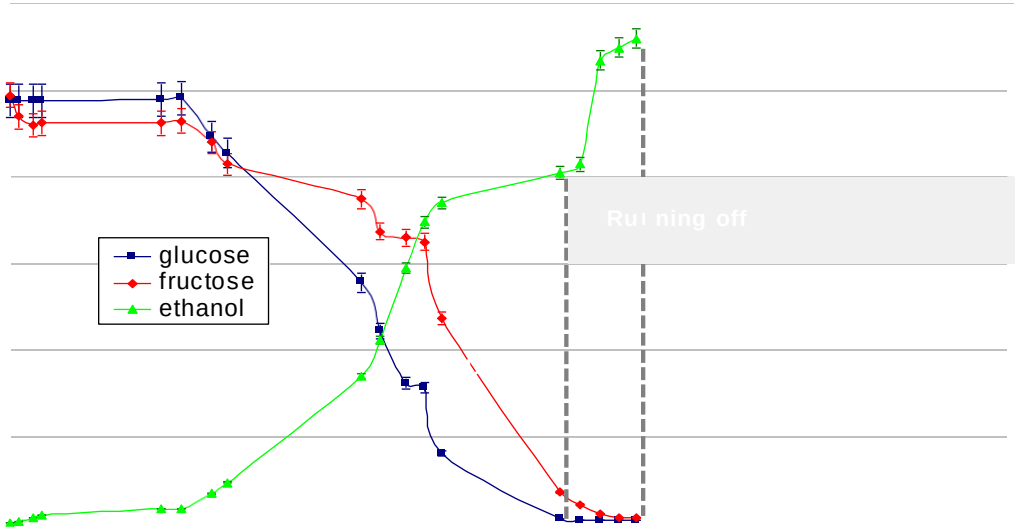
fructose + 2 PMS^+ —***FDH***→ ketofructose + 2 PMSH

PMSH —***+100 mV (Pt)***→ $PMS^+ + e^-$

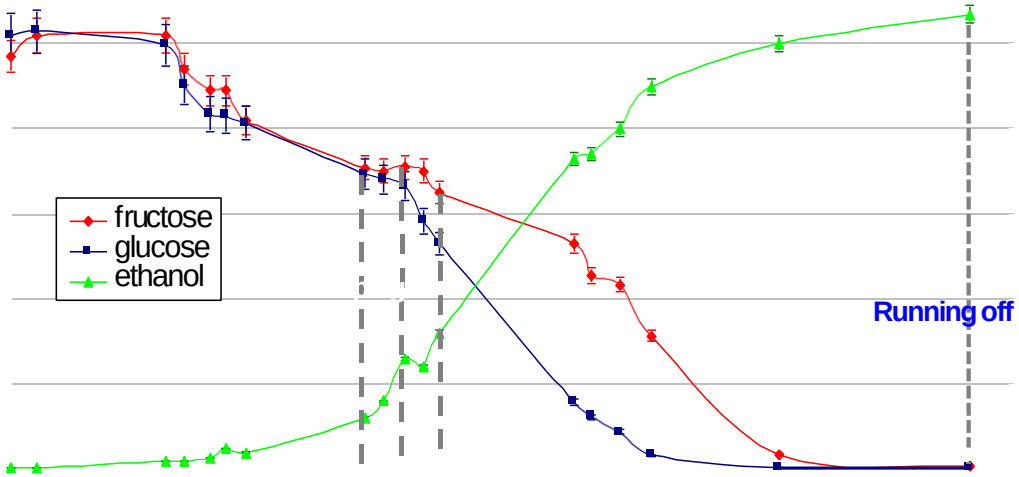


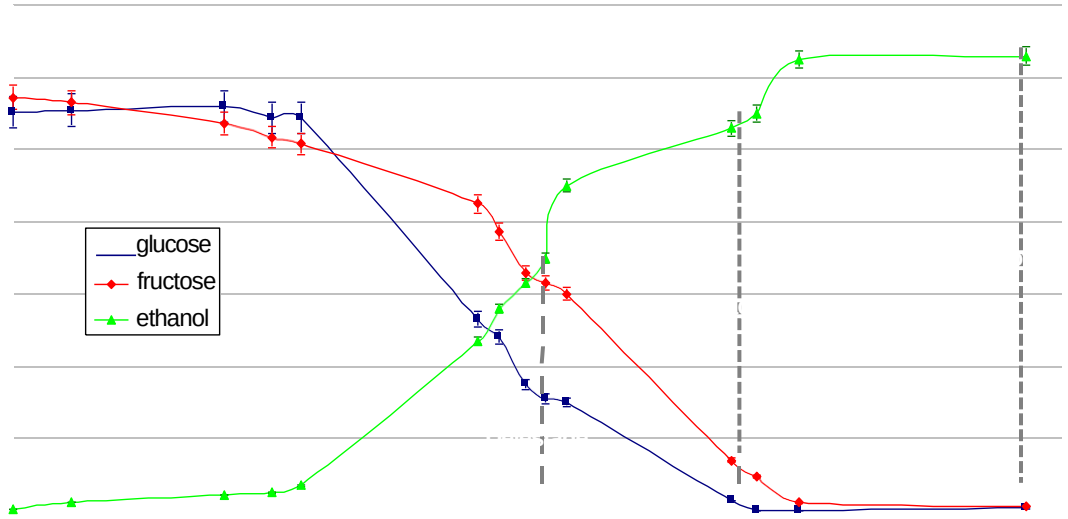
Immobilization: BSA-glutaraldehyde on Immobilon AV
Storage: DEAE-dextran/lactitol (1/5%)
Optimised operative conditions: 0.1 M citrate/phosphate buffer pH 4.5 + 0.02% Tween. Flow rate 0.5 mL/min; injection loop 100 µL.
Analitycal performances:
detection limit 5 x 10^{-7} mol/L
linearity 10^{-6} / 8 x10^{-4} mol/L
stability: 30% decrease after 200 samples

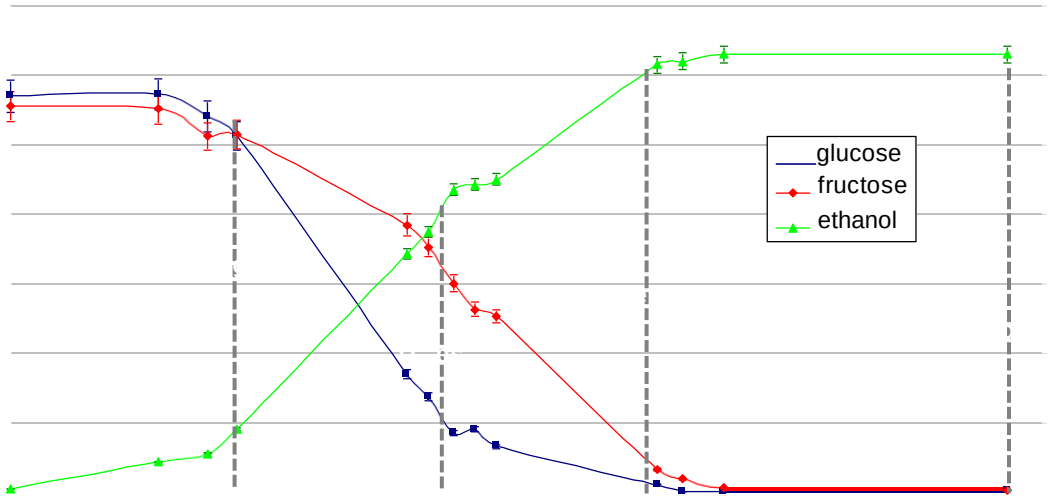
One delestage



Pumping-over




glucose
fructose
ethanol


glucose
fructose
ethanol

Fructose:Glucose ratio during alcoholic fermentation