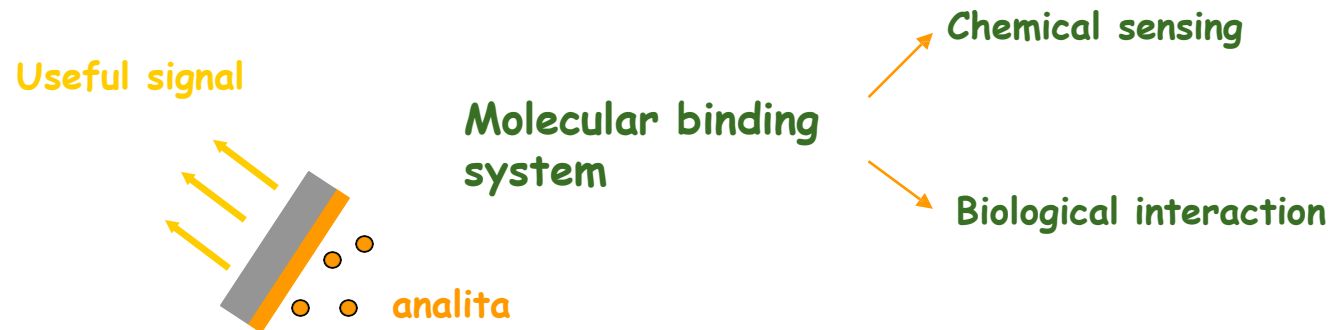


# What are sensors and biosensors?

"...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 "Recommended 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



Biosensors are chemical sensors nwhich the binding element is a biological molecule

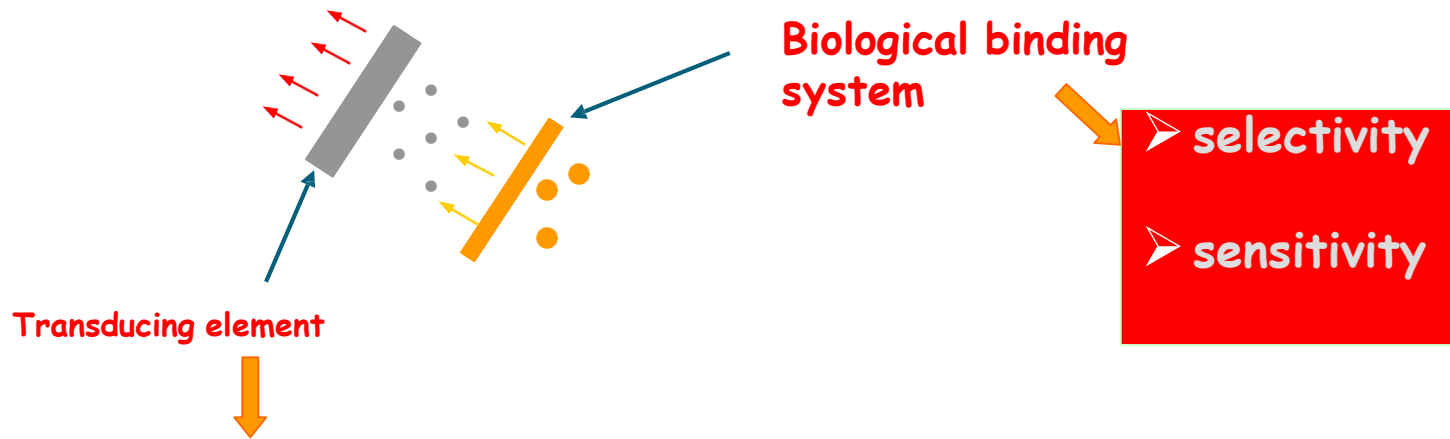
# Self-testing



## **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



➤ selectivity  
➤ sensitivity

## Tranducers

- electrochemical
- optical
- piezoelectric
- thermal

- potentiometric
- amperometric
- voltammetric
- conducttimetric,  
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 synthetic receptor

es: peptides, aptamers, molularly imprinted polymers

## Ion selective electrodes for biosensors

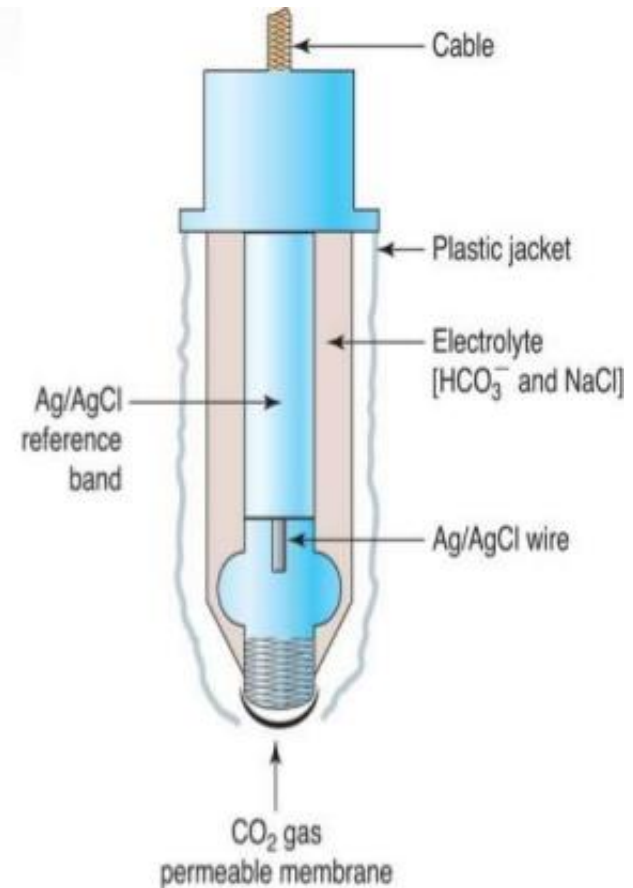
The most used ISEs for the development of biosensors have been  $\text{CO}_2$  and  $\text{NH}_3$  probes.

This potentiometric gas-sensors are realised using a pH glass electrode and a reference behind a gas permeable membrane (polytetrafluoroethylene, 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  $\text{NH}_3$  or  $\text{CO}_2$  (diffused from the sample) in the electrolyte causes a change in  $\text{H}^+$  that is measured at the electrode.

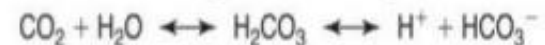
This is related to the partial pressure of the gas in the sample solution. Immobilisation of a suitable enzyme onto the surface leads to the detection of metabolites in the  $10^{-5}$  -  $10^{-2}$  mol/L range.

## PCO<sub>2</sub> ELECTRODE

- Measurement of PCO<sub>2</sub> in routine blood gases
- A modified pH electrode with a CO<sub>2</sub> permeable membrane covering the glass membrane surface
- A bicarbonate buffer separates the membranes
- Change in pH is proportional to the concentration of dissolved CO<sub>2</sub> in the blood

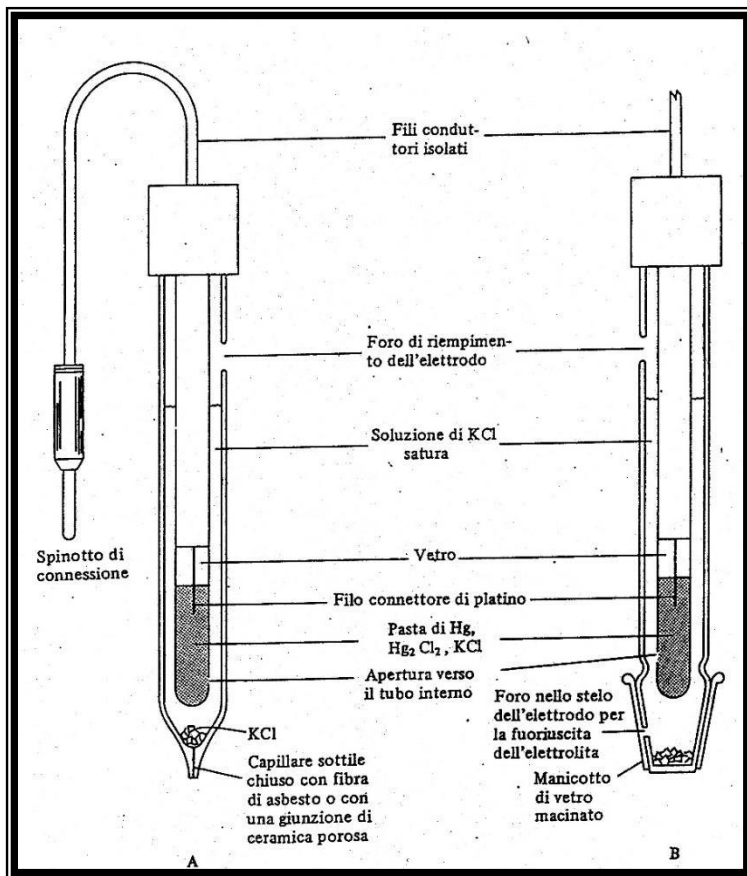


Reaction occurring in the electrolyte solution:

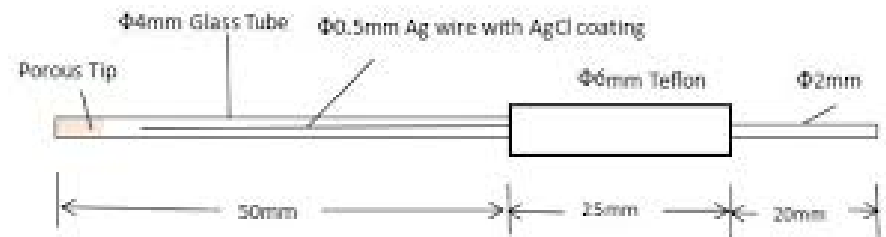


# Reference electrodes

calomel



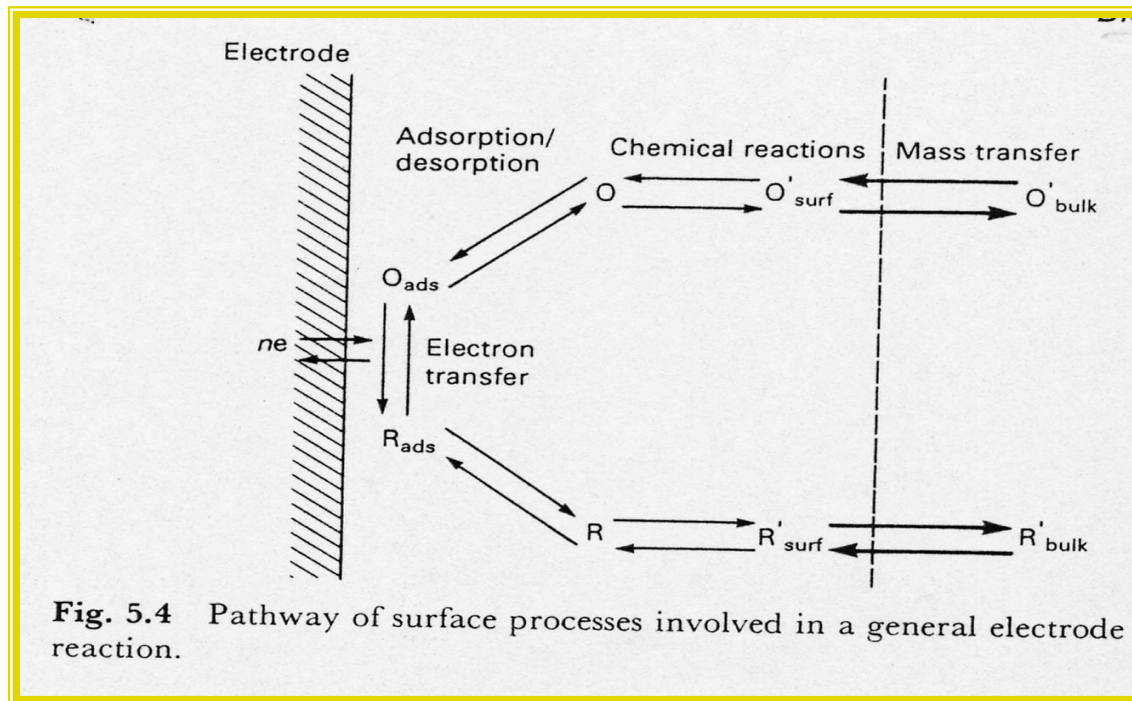
Ag/AgCl



CS901 Ag/AgCl Reference Electrode

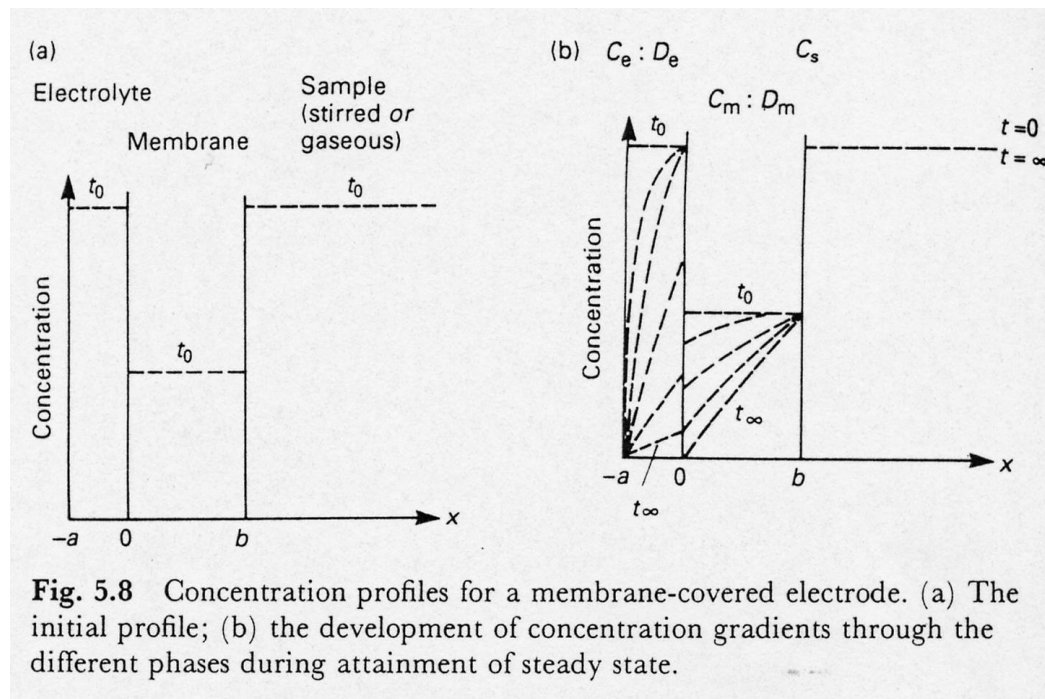
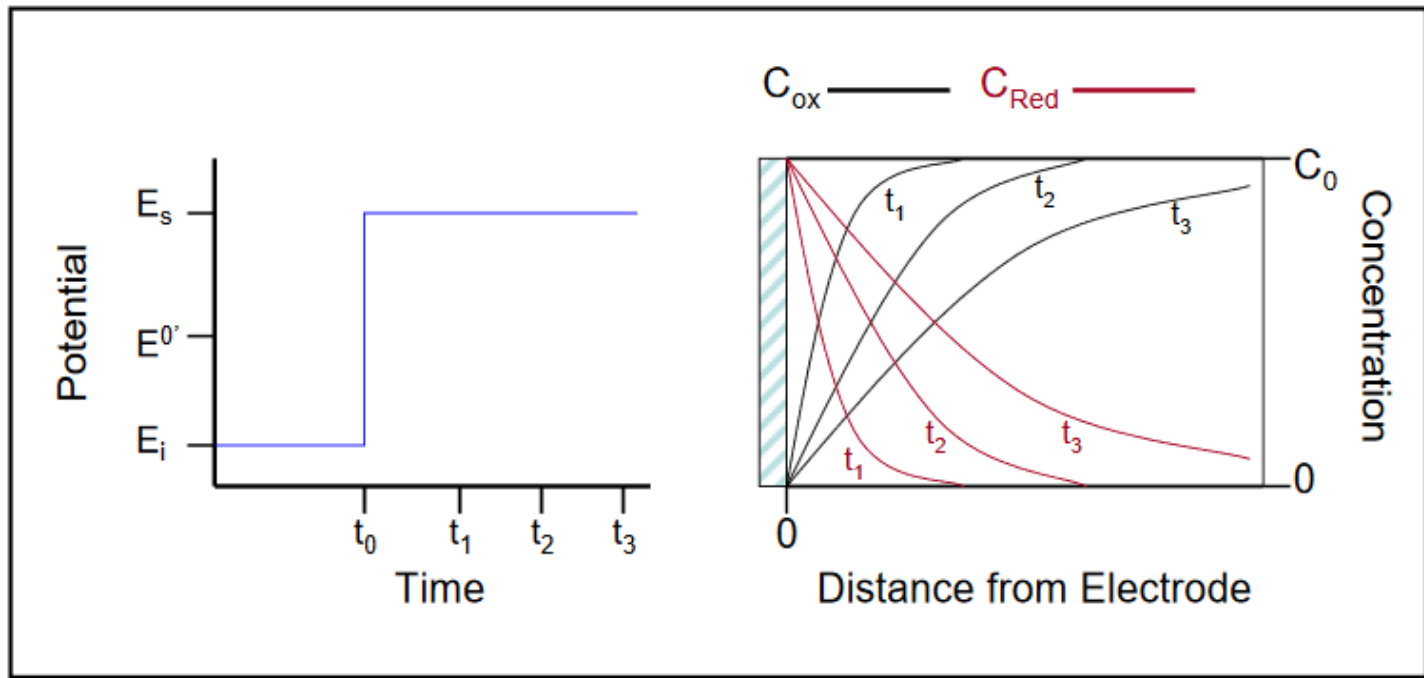
Amperometric sensors monitor the current flow when a selected fixed potential is applied at a working electrode with respect to a reference electrode. The current generated by the oxidation (or reduction) of a compound is dependent by:

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

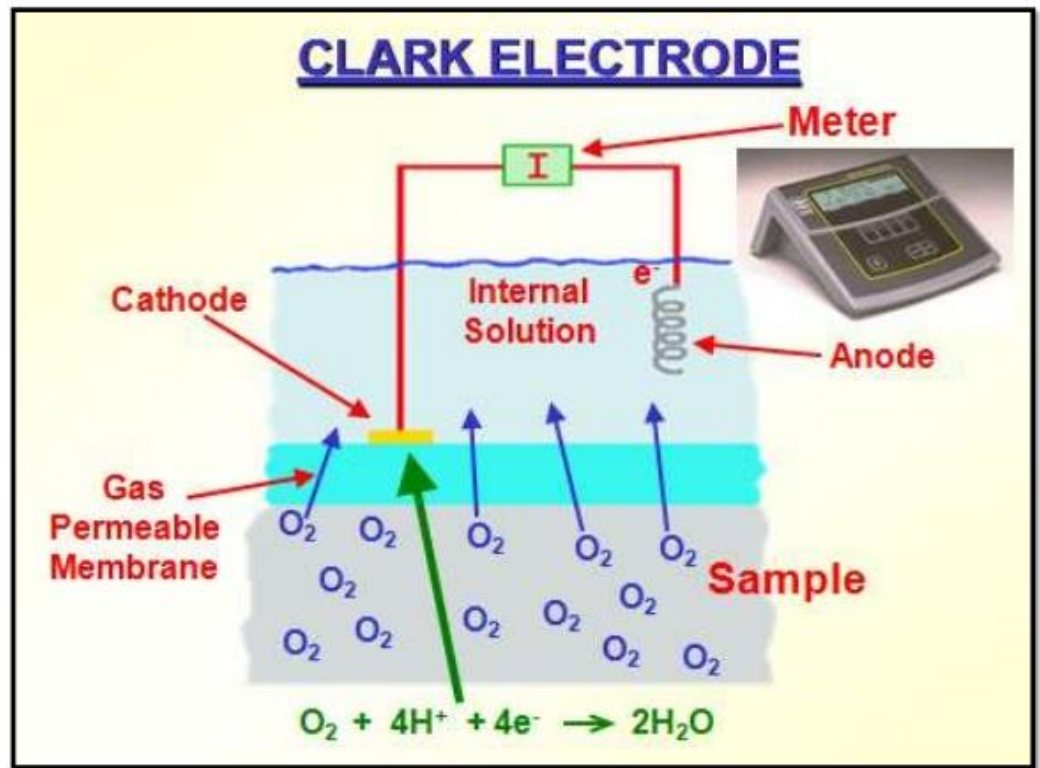
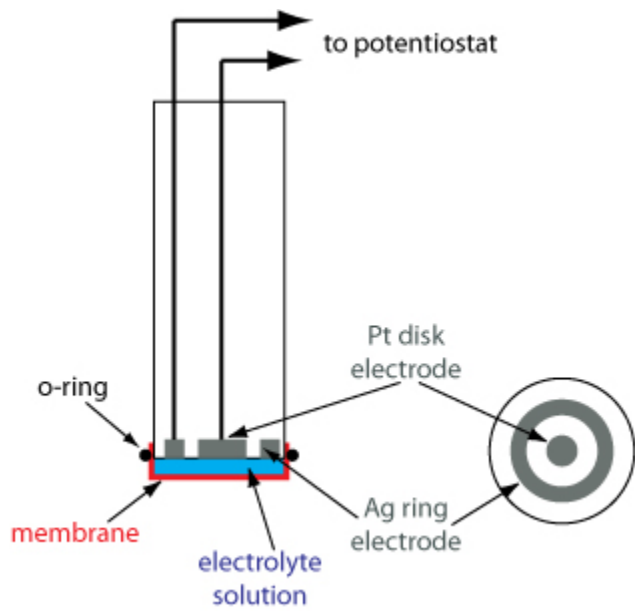


**Fig. 5.4** Pathway of surface processes involved in a general electrode reaction.





**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

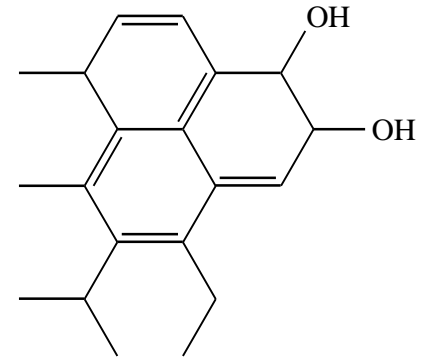
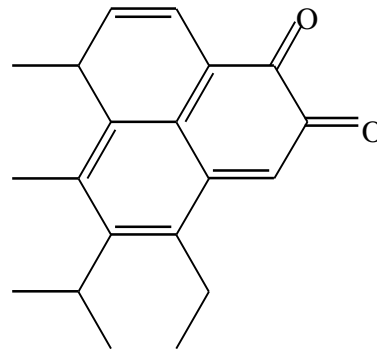
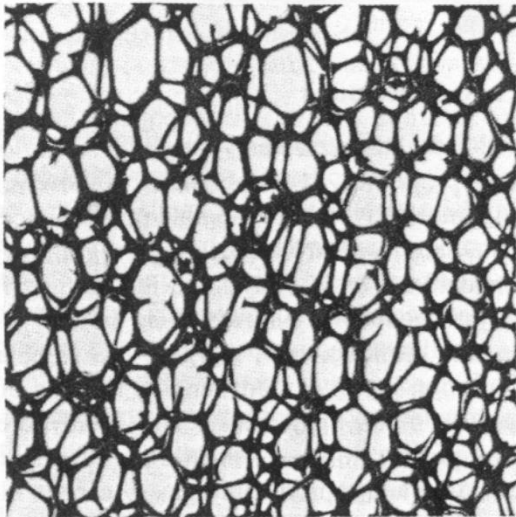
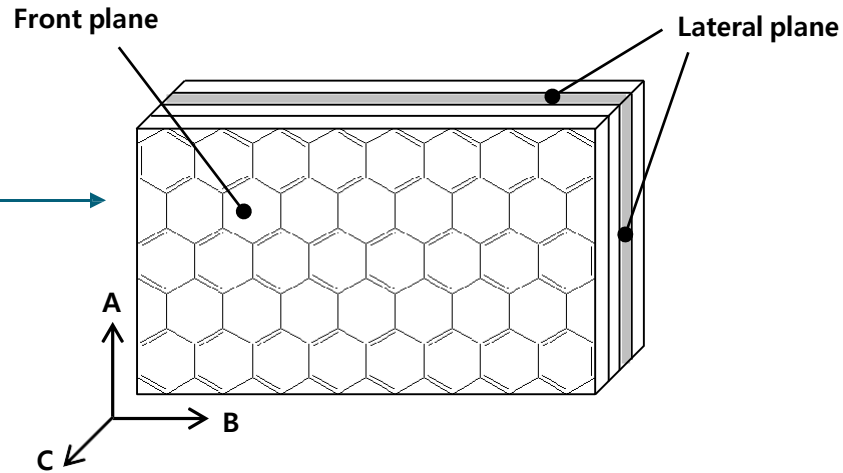
The gas permeable membrane protects the electrodes from contamination, provides for reproducible conditions of oxygen transport and minimize undesirable changes in electrolyte composition. Ideally, it should be with low permeability and high diffusivity for oxygen.

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.

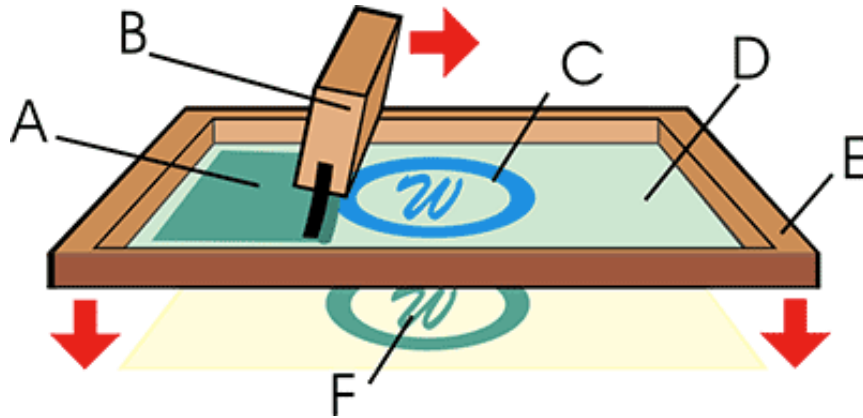
The anode is Ag/AgCl and a neutral or alkaline electrolyte is used in the final assembling.

# Conventional carbon based probes:

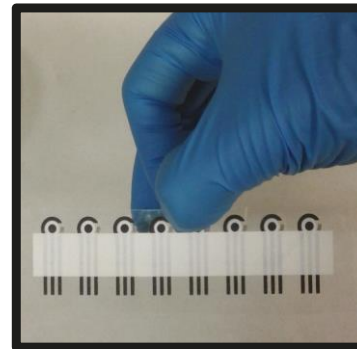
- graphite
- carbon paste
- pyrolytic graphite
- glassy carbon



# Printing electrodes: serigraphy

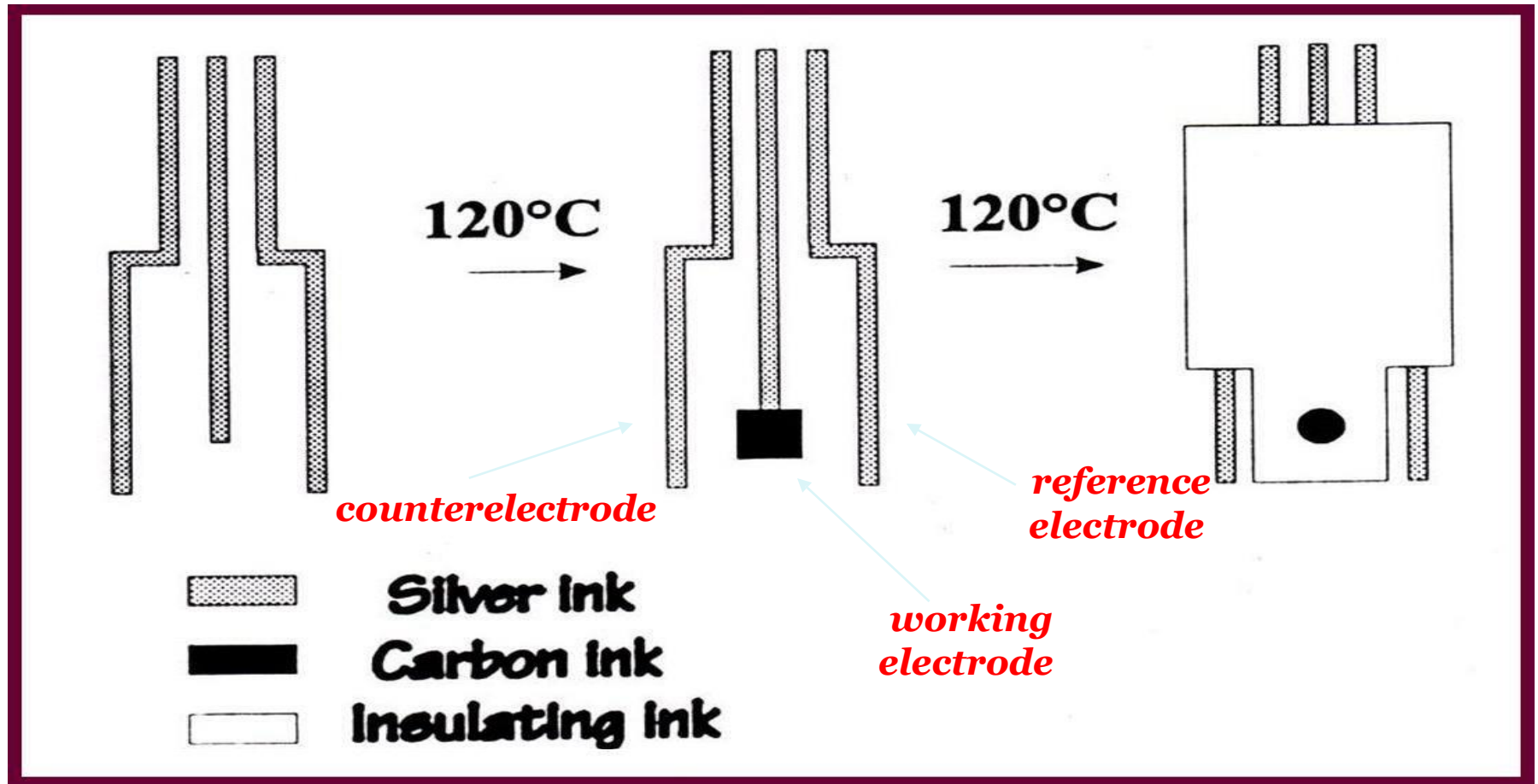


- A. ink; B. squeegee;
- C. printing mask;
- D. printing mesh;
- E. frame; F. printed ink

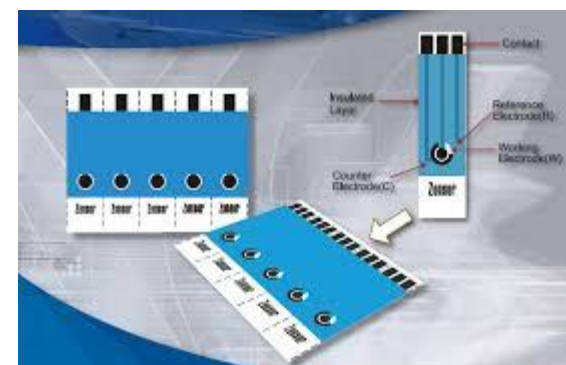
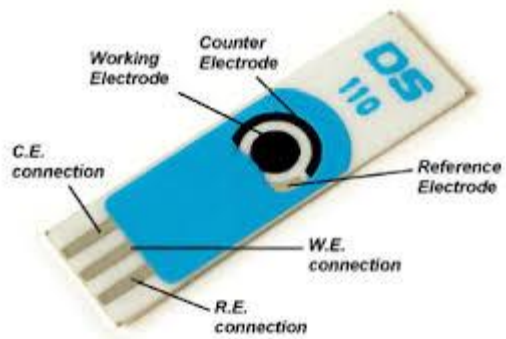


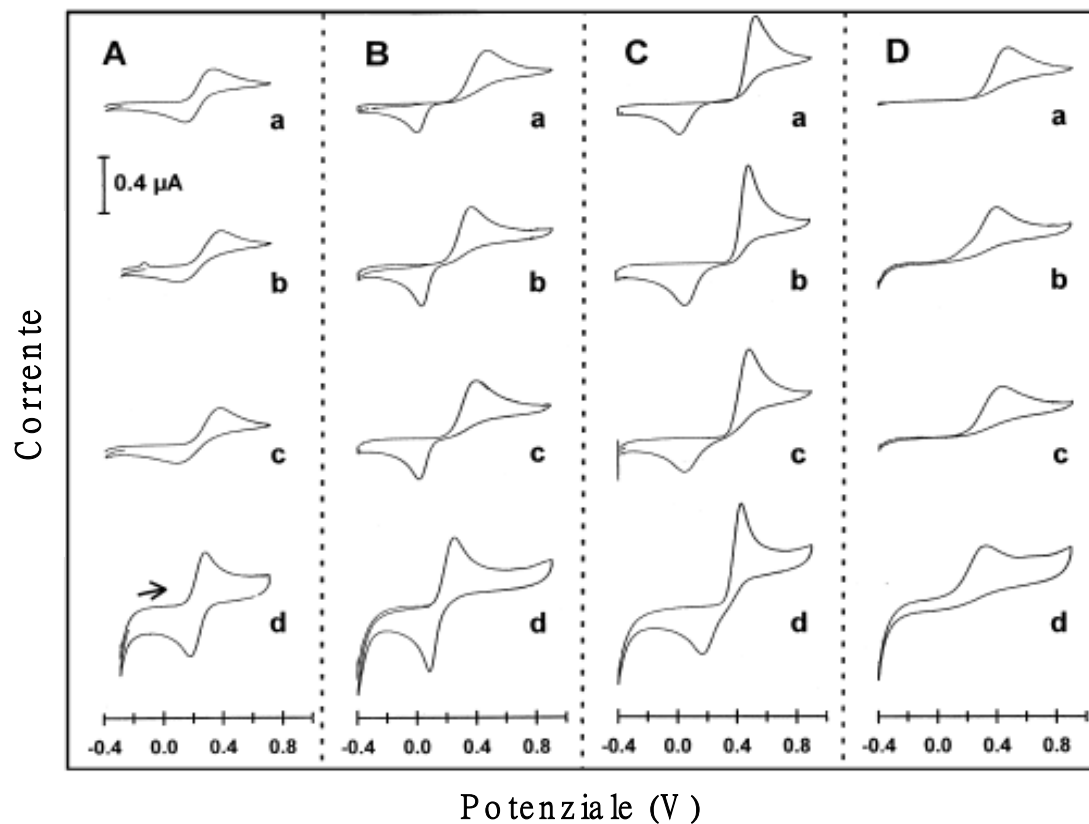
- Advantages:**
- Dimension
  - Disposable
  - Low-Cost

# DISPOSABLE SCREEN-PRINTED CARBON ELECTRODES









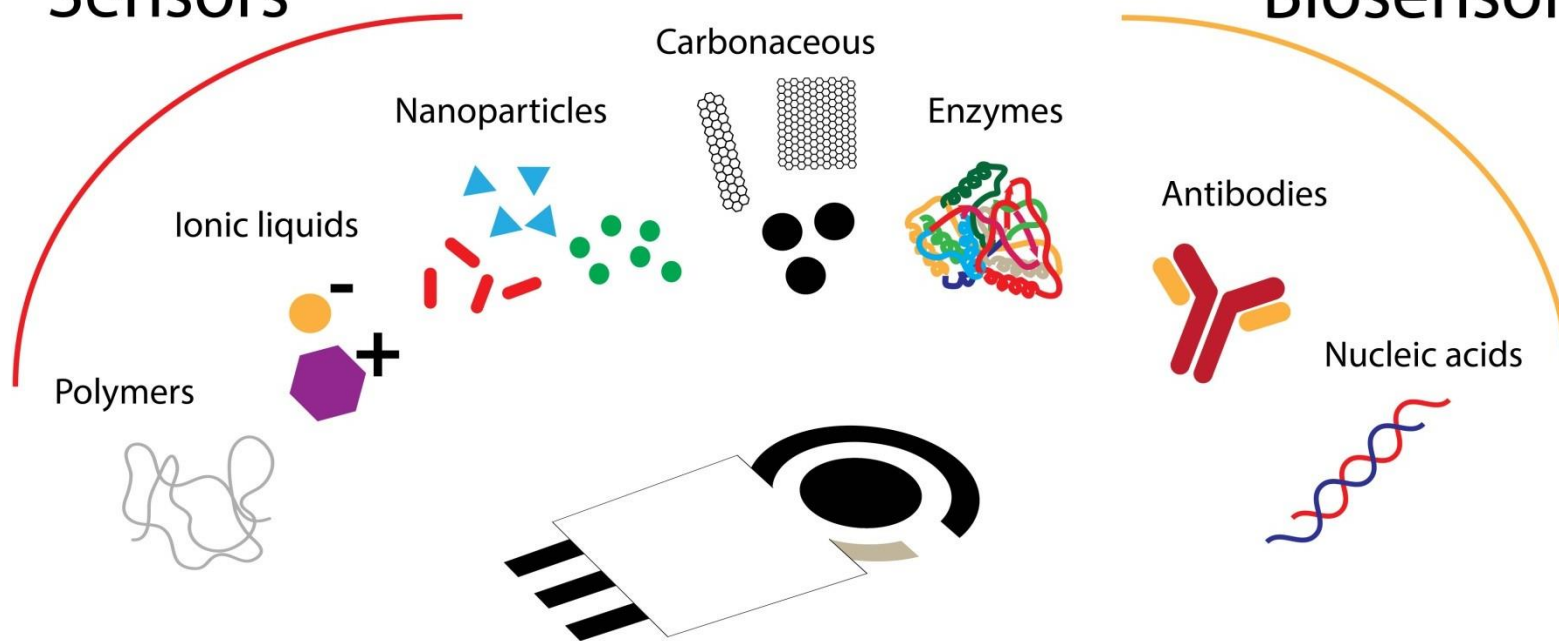
Cyclic voltammeteries of ferricyanide (A), catechol (B), acetaminophene (C), ascorbic acid (D) carried with different screen-printed electrodes: Dupont (a), Ercon (b), Acheson (c) Gwent (d). Supporting electrolyte; KCl 0.1M (A), scan rate 20 mV/s.



# Screen-Printed Electrodes

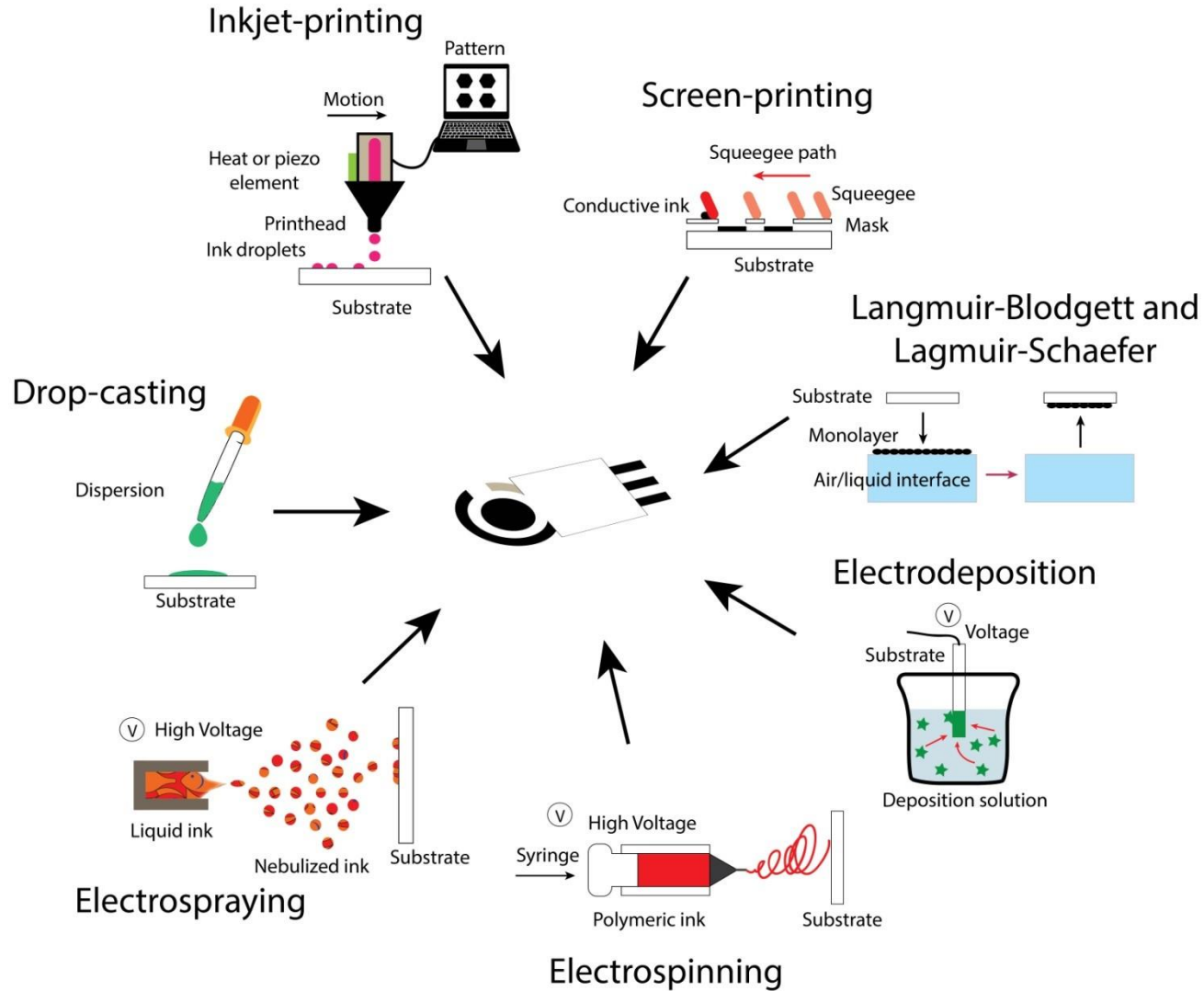
## Sensors

## Biosensors



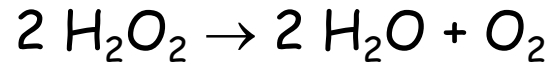
Screen-printed electrochemical (bio)sensors

# How do we tune them?

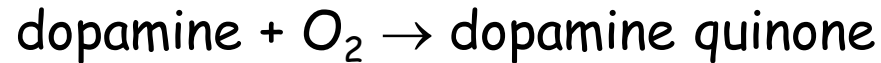


## TISSUE BASED BIOSENSORS

bovine liver (rich in catalase)



banana (rich in polyphenol oxidase)



## CELL BASED BIOSENSORS

measurement of ethanol using *acetobacter xylinum* ( $\text{O}_2$  electrode)

Determination of the BOD (biological oxygen demand).

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

Conventional methods include  $\text{BOD}_5$  and  $\text{BOD}_7$  which need 5 and 7 days.

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

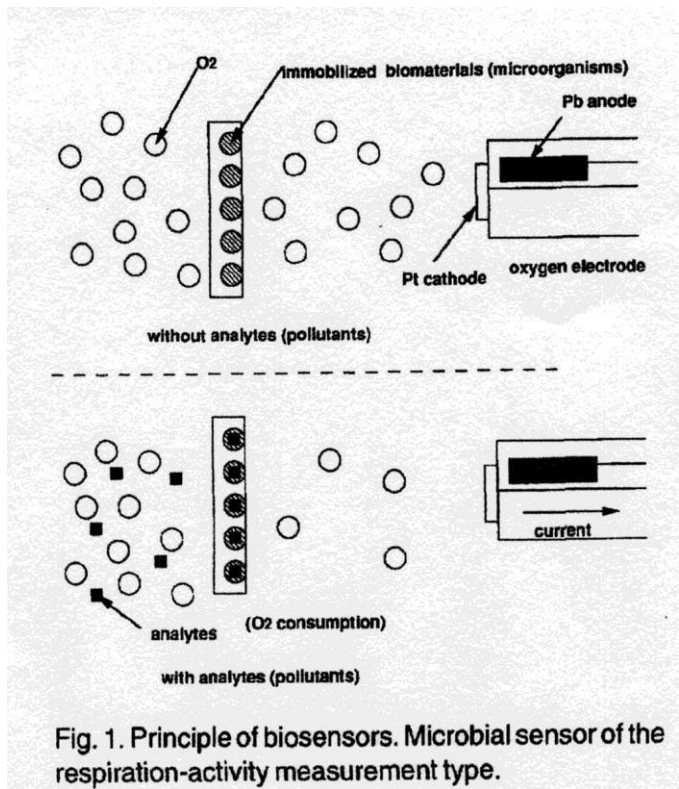
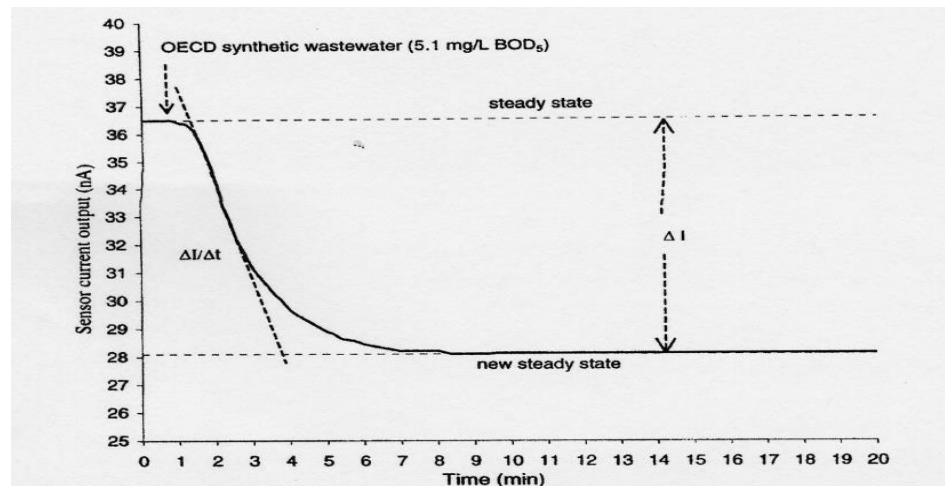
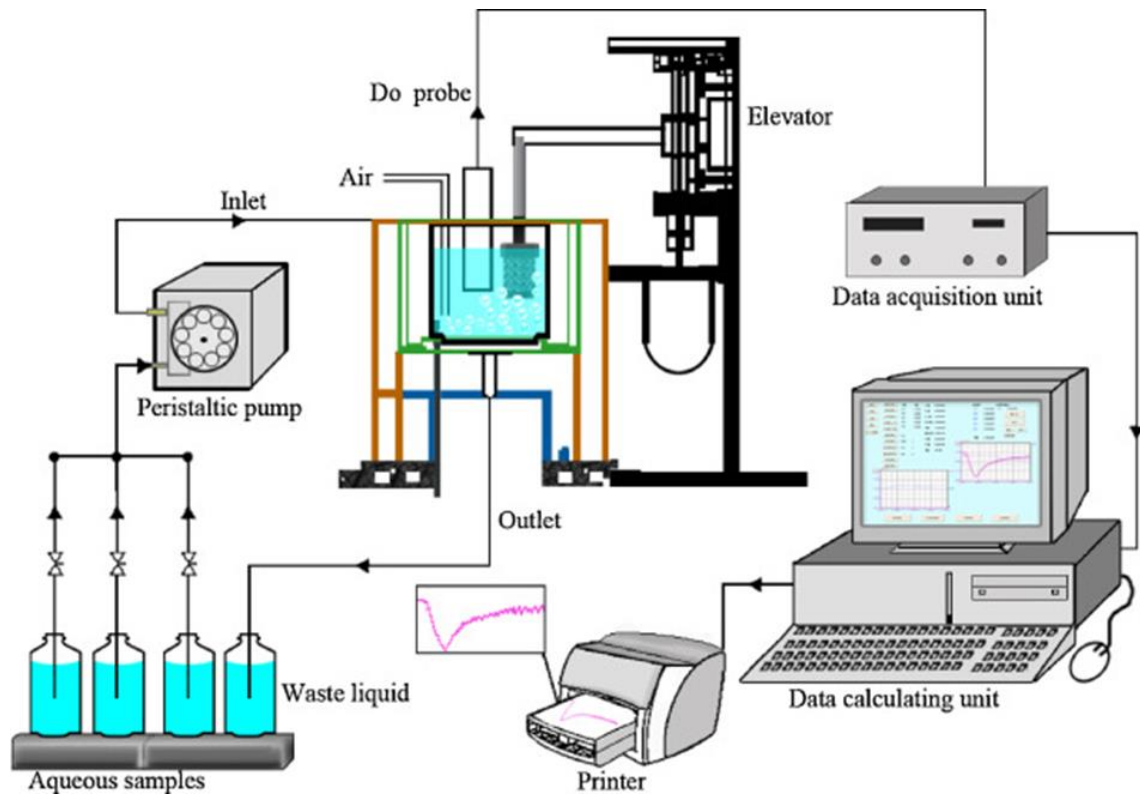
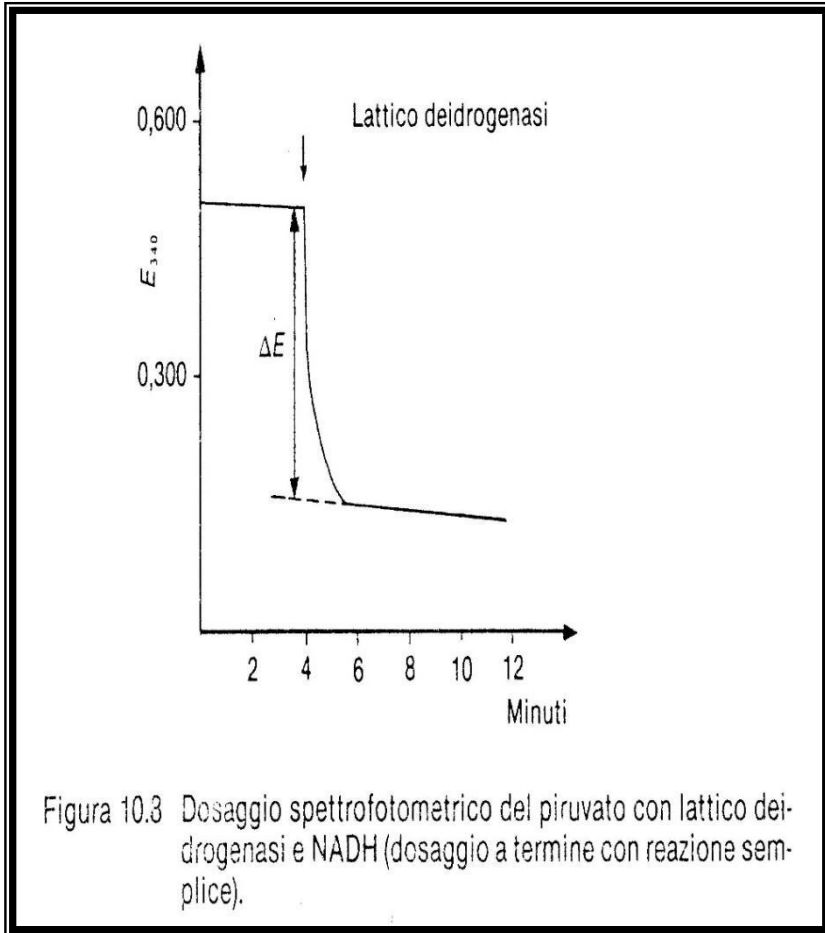


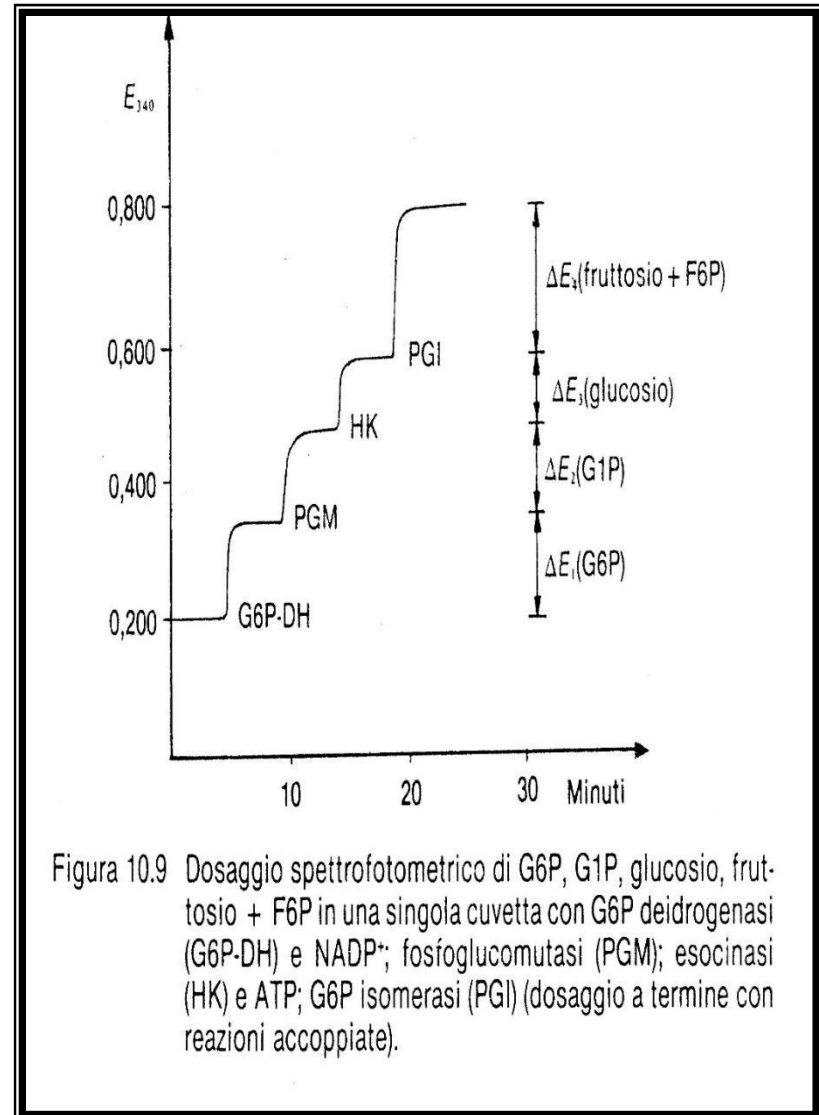
Fig. 1. Principle of biosensors. Microbial sensor of the respiration-activity measurement type.



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



<http://www.sigmaldrich.com/life-science/metabolomics/enzymatic-kits.html>





## 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

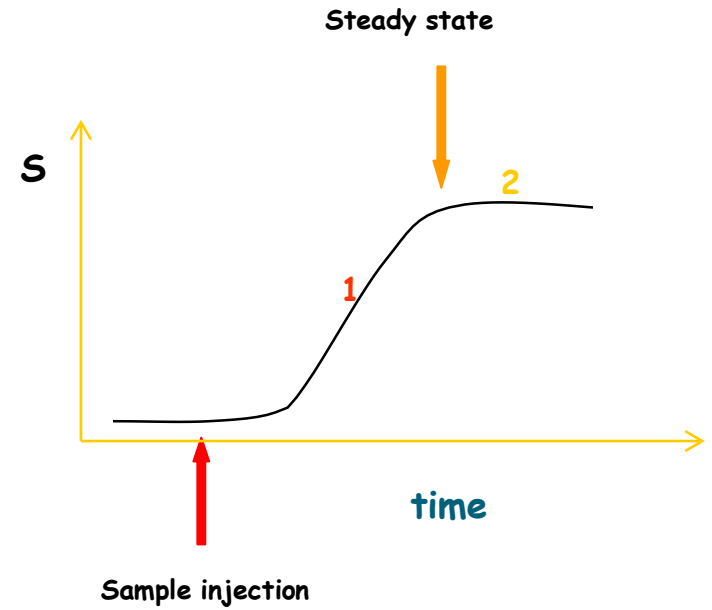
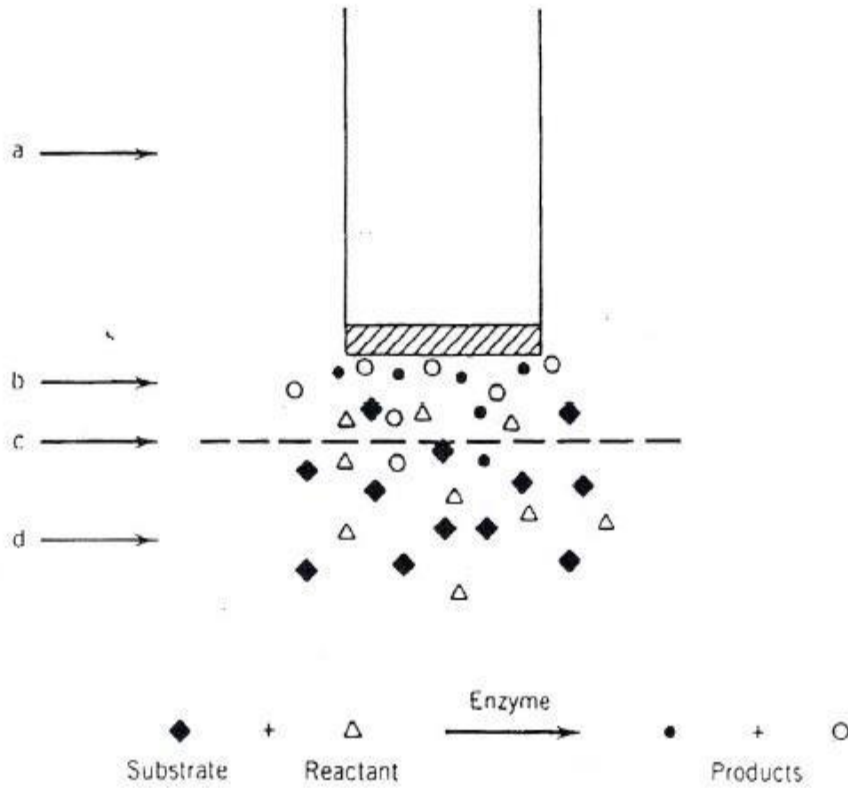
Carbohydrates and Sugars

Inorganic Ions

Lipids (Fats)

Amino Acids and Proteins

# Enzyme electrode



**THE BIOLOGICAL ELEMENT SHOULD HAVE:**

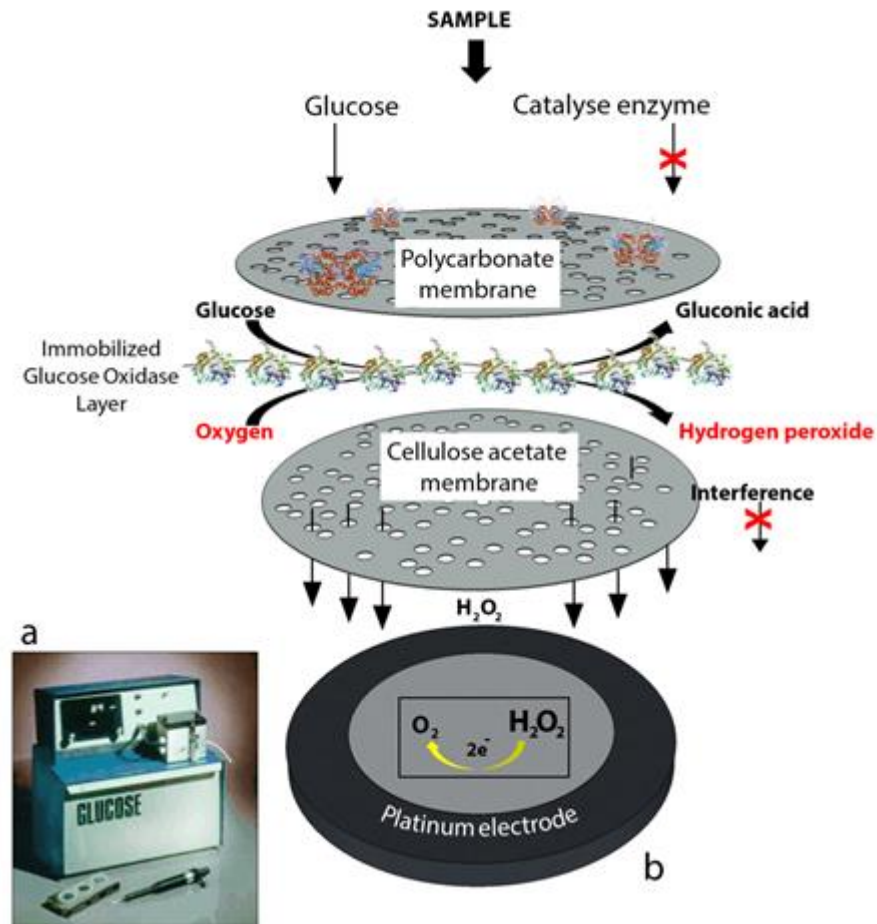
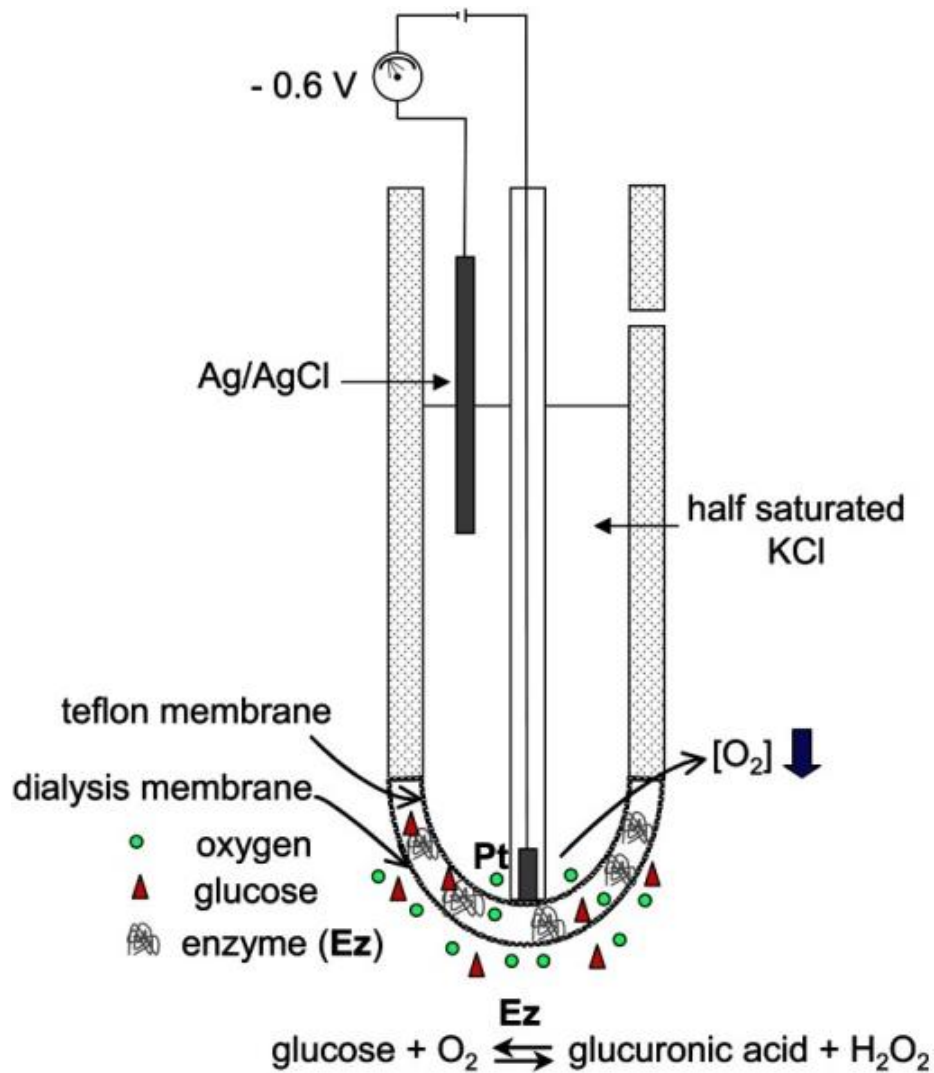
**SPECIFICITY (HIGH SELECTIVITY) FOR THE ANALYTE(S)**

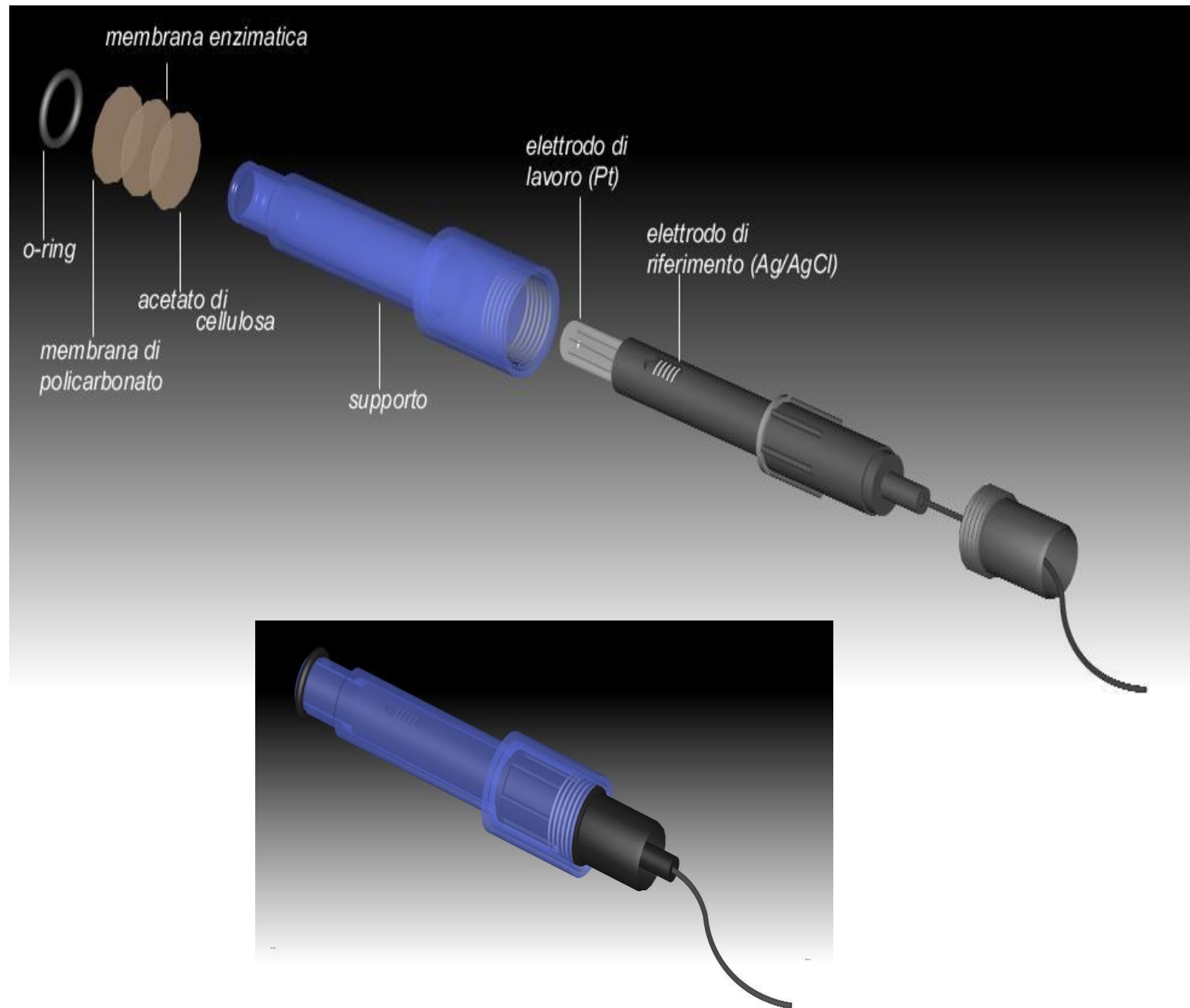
**GOOD STABILITY IN OPERATING CONDITIONS (t, pH,  $\mu$  )**

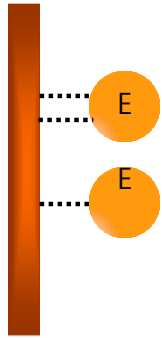
**RETENTION OF SUFFICIENT BIOLOGICAL ACTIVITY WHEN  
IMMOBILISED**

**NO (VERY LOW) INHIBITION BY THE SAMPLE**

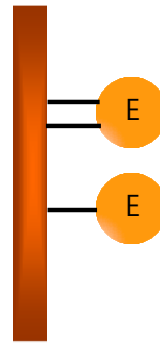




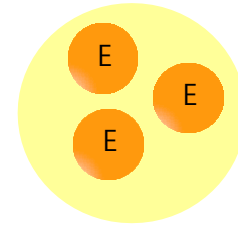




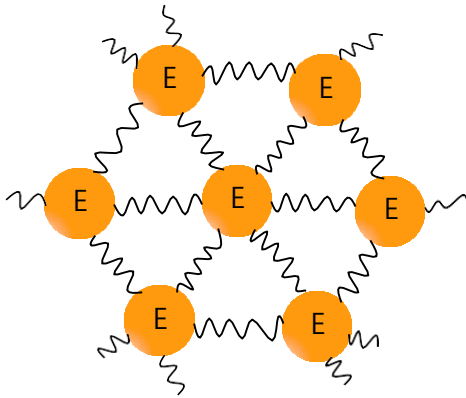
adsorption



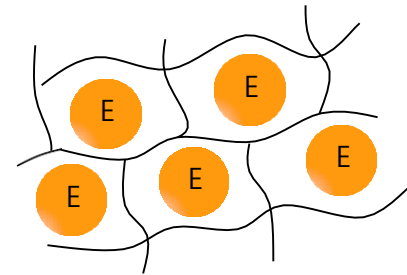
covalent  
binding

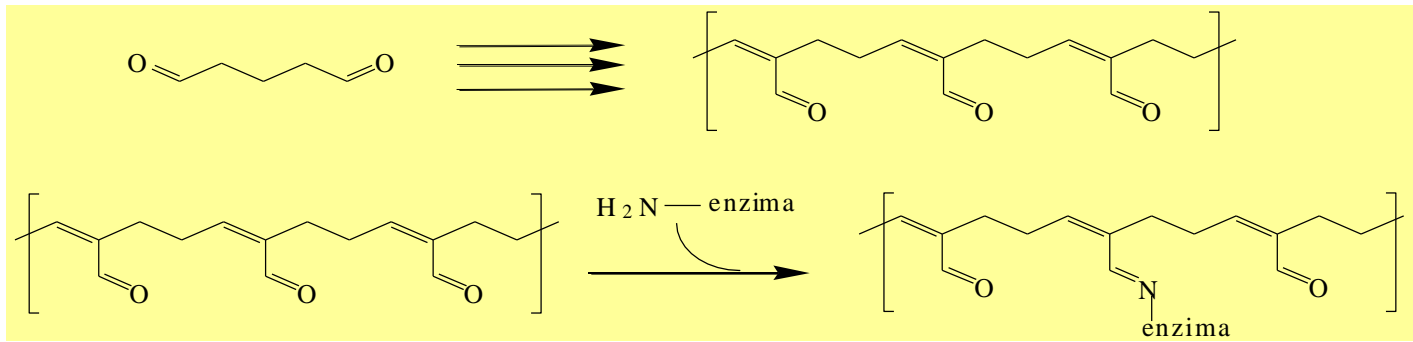


entrapment

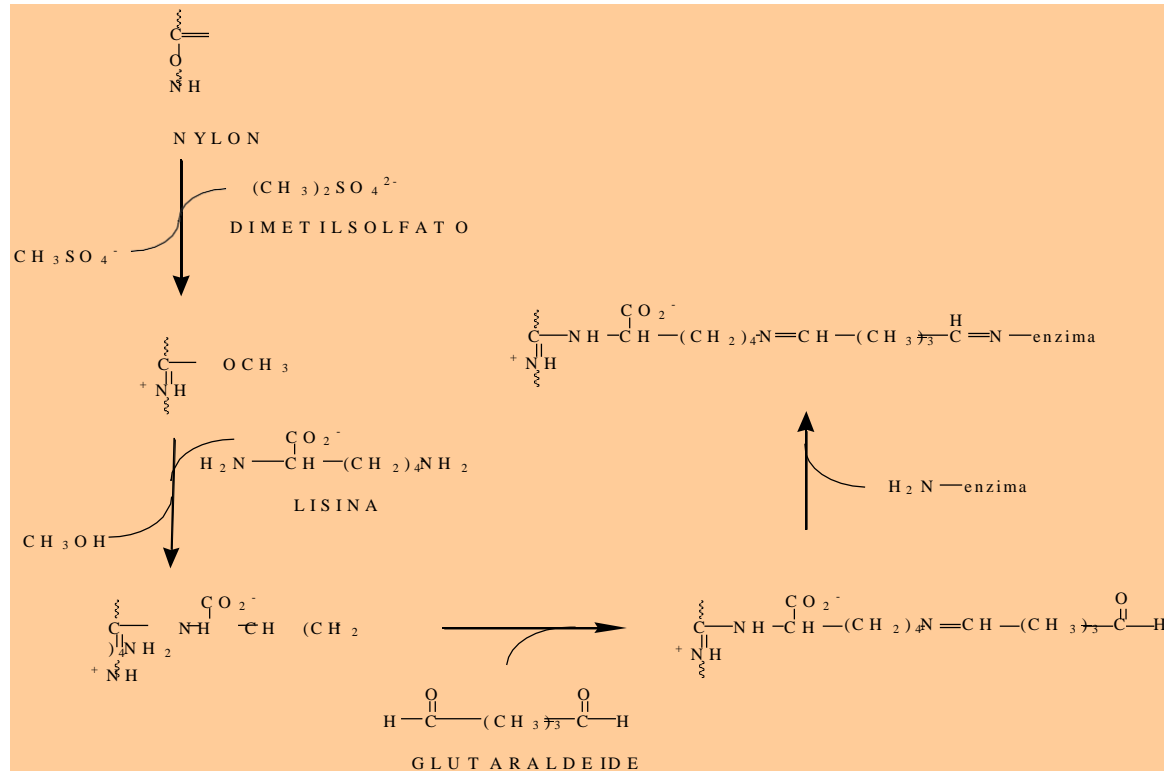
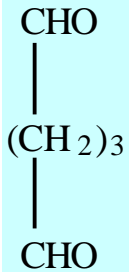


cross-linking

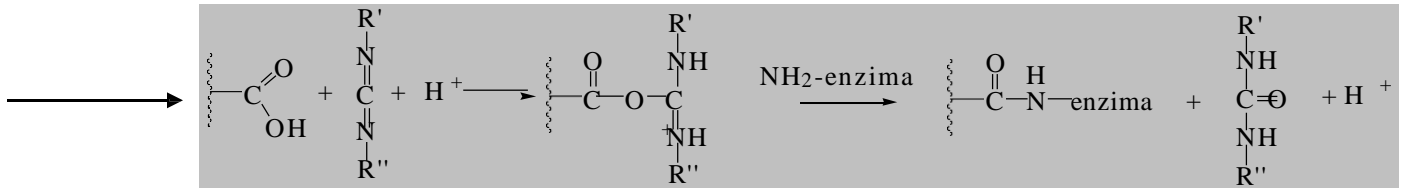


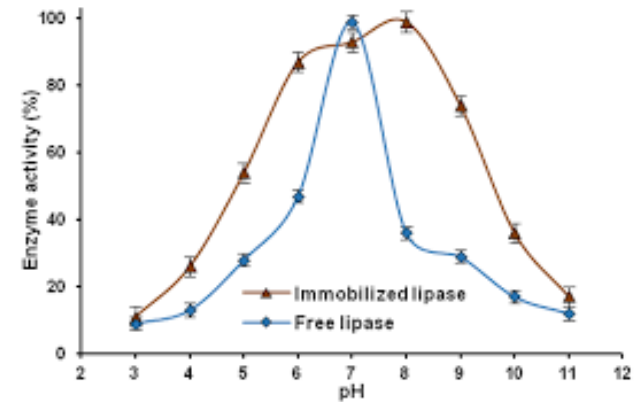
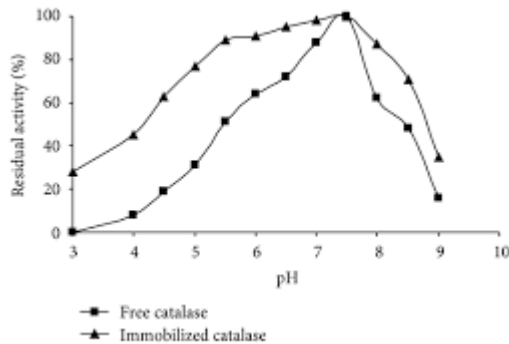
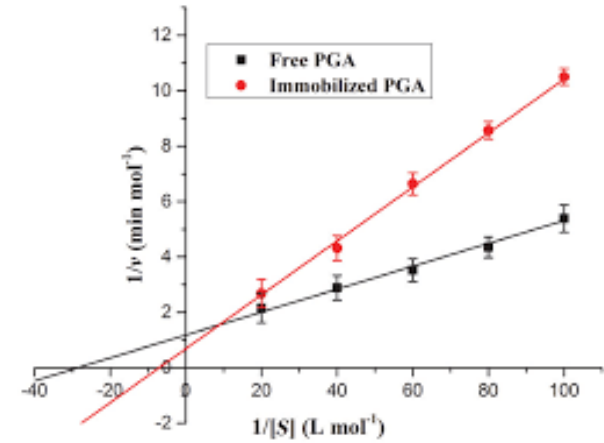
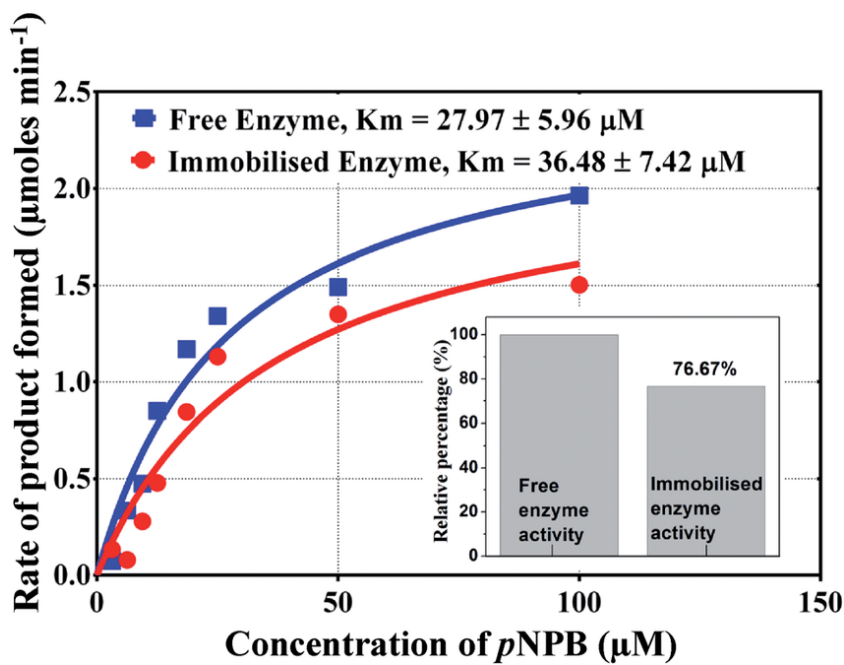


glutaraldehyde reactions: polymerization and lysine amino group



Immobilisation via carbodiimide





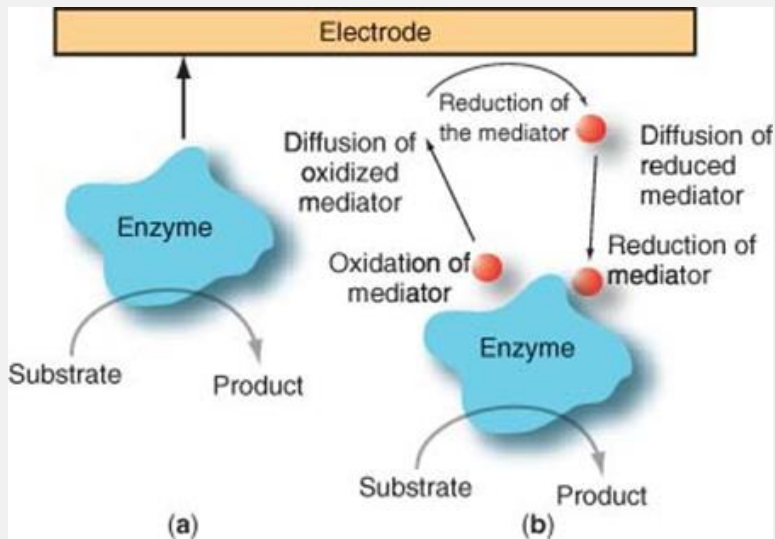
The immobilised enzyme has always a higher (apparent)  $K_m$ , a shifted and larger optimum pH and lower enzymatic activity compared to the enzyme in solution

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 steady state is on the order of minutes

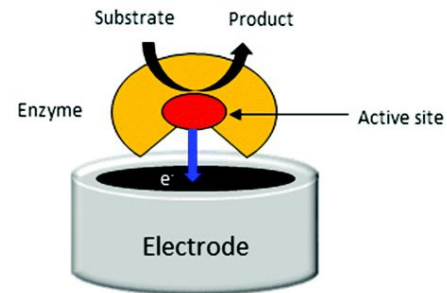
2. Second generation: electron transfer occurs via an electrochemical mediator in solution. The mediator shuttles electrons between the enzyme and the electrode. Faster response times.

3. Third generation: direct exchange of electrons between the electrode and the enzyme. Very fast response time.

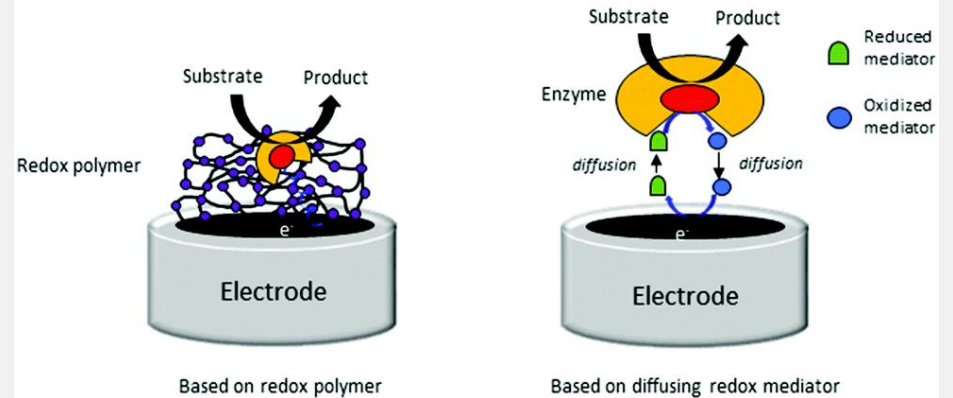


## Second and third generation

### Direct Electron Transfer



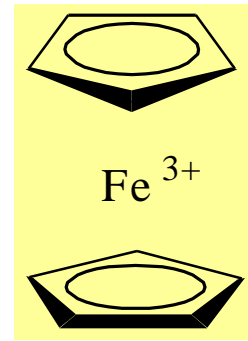
### Mediated electron transfer



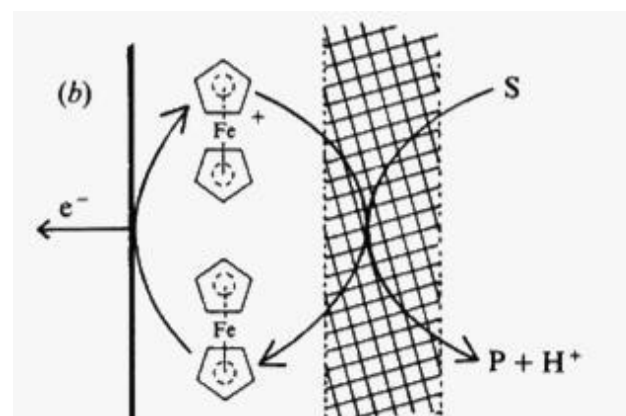
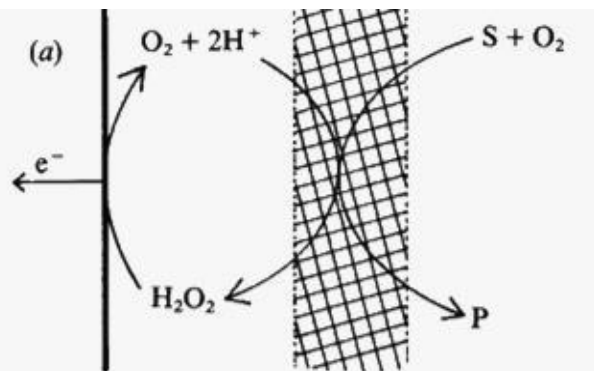
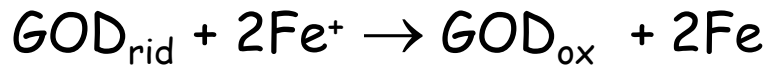
A good electrochemical mediator characteristics :

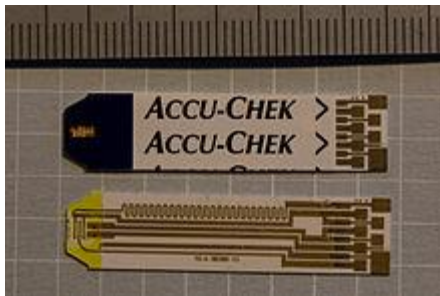
- rapid reaction with the enzyme
- rapid and reversible electron transfer rate
- low overpotential for the redox reaction
- pH independent
- stability in the reaction medium in both redox forms
- Should not react with the dissolved oxygen in solution
- no toxicity





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





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**



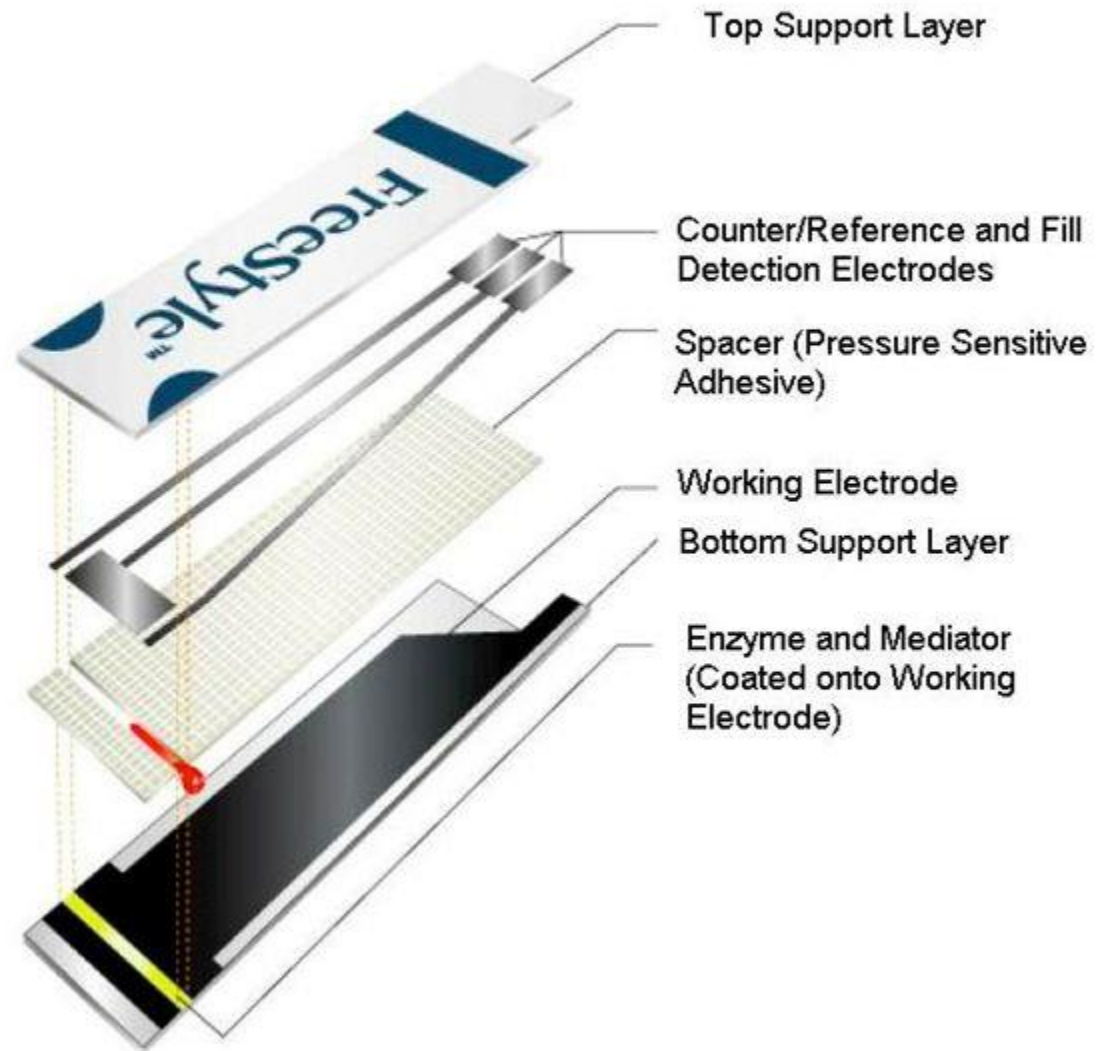
3. After a 30 second countdown, the result is displayed. **30 SECS**



The ExacTech Blood Glucose Meter is shown Actual Size

# Test Strips

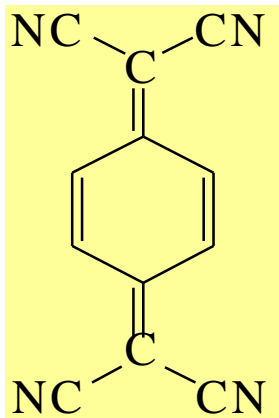
- When blood added, glucose is oxidized by enzyme coated on working electrode
- Voltage applied between working and reference electrode
- Measure current between working and reference electrode



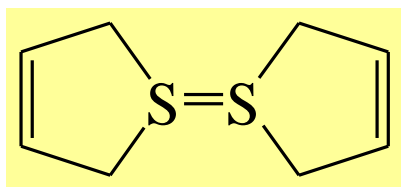
NATURAL	E(V) vs. SHC	SYNTHETIC	E (V) vs. SHC
Cytochrome a <sub>3</sub>	+0,29	Esacyanoferrate(III)	+0,45
Cytochrome c <sub>3</sub>	+0,24	2,6-dichlorophenol	+0,24
Ubiquinone	+0,10	Indophenolo	+0,24
Cytochrome b	+0,08	Ferrocene	+0,17
Vitamin K <sub>2</sub>	-0,03	N-metilfenazium sulphate	+0,07
Rubredoxin	-0,05	Metilene blue	+0,4
Flavoproteins	da -0,4 a +0,2	Ftalocyanin	-0,02
FAD/FADH <sub>2</sub>	-0,23	Fenosafuranin	-0,23
FMN/FMNH <sub>2</sub>	-0,23	Benzyl viologen	-0,36
NAD <sup>+</sup> /NADH	-0,32	Methyl viologen	-0,46
NADP <sup>+</sup> /NADPH	-0,32		
ferredossina	-0,43		

# Third generation

## Conducing salts



Tetracyanoquinodimethane  
(TCNQ)



Tetra thiafulvalene(TTF)

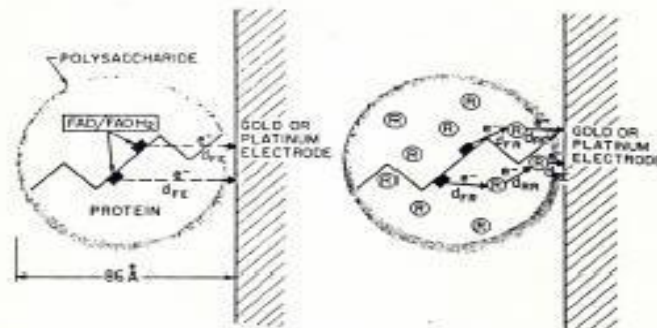
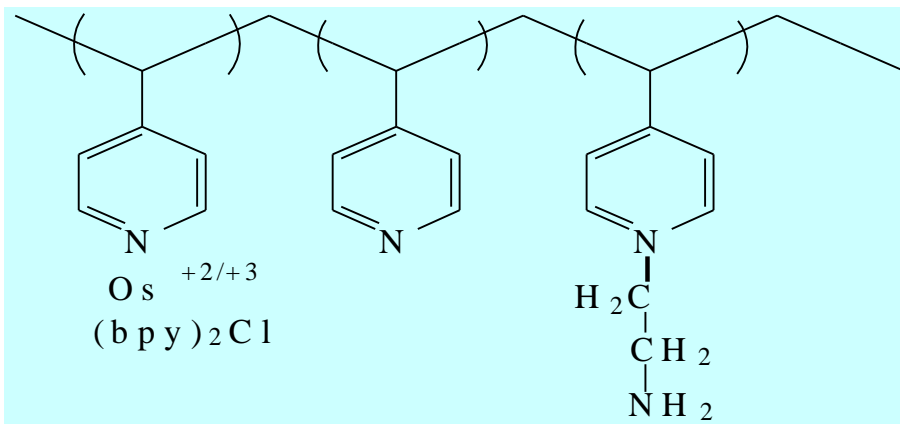


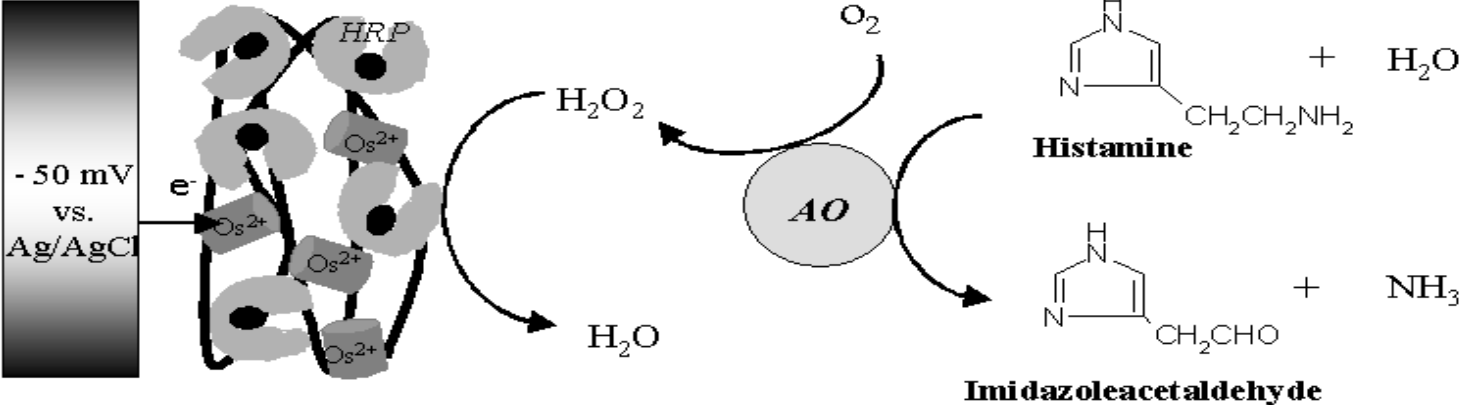
Figure 1. Schematic drawing of the glucose oxidase molecule, showing the electron-transfer distances involved in the various steps of moving an electron from its two FAD/FADH<sub>2</sub> centers to a metal electrode. Left: the enzyme before modification. Right: the modified enzyme, after chemical attachment of an array of electron-transfer relays.

## Redox gel

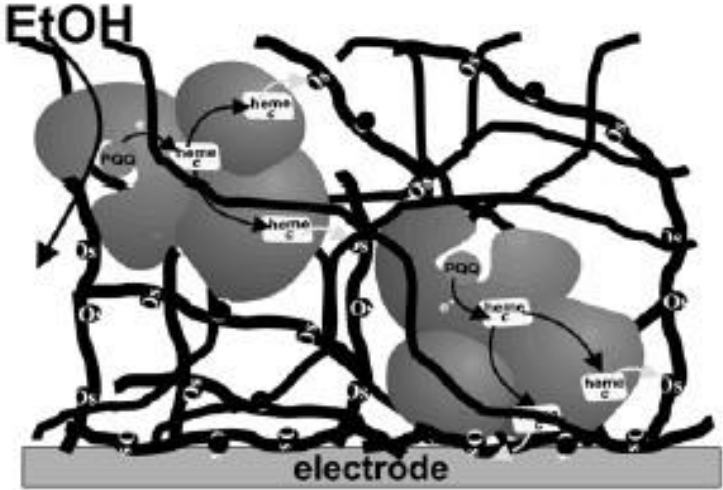


Struttura dell' osmio biperidile legato a polivinilpiridina

# 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)<sup>+</sup> 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<sup>•+</sup>. 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



at the electrode surface polarised at the appropriate E :

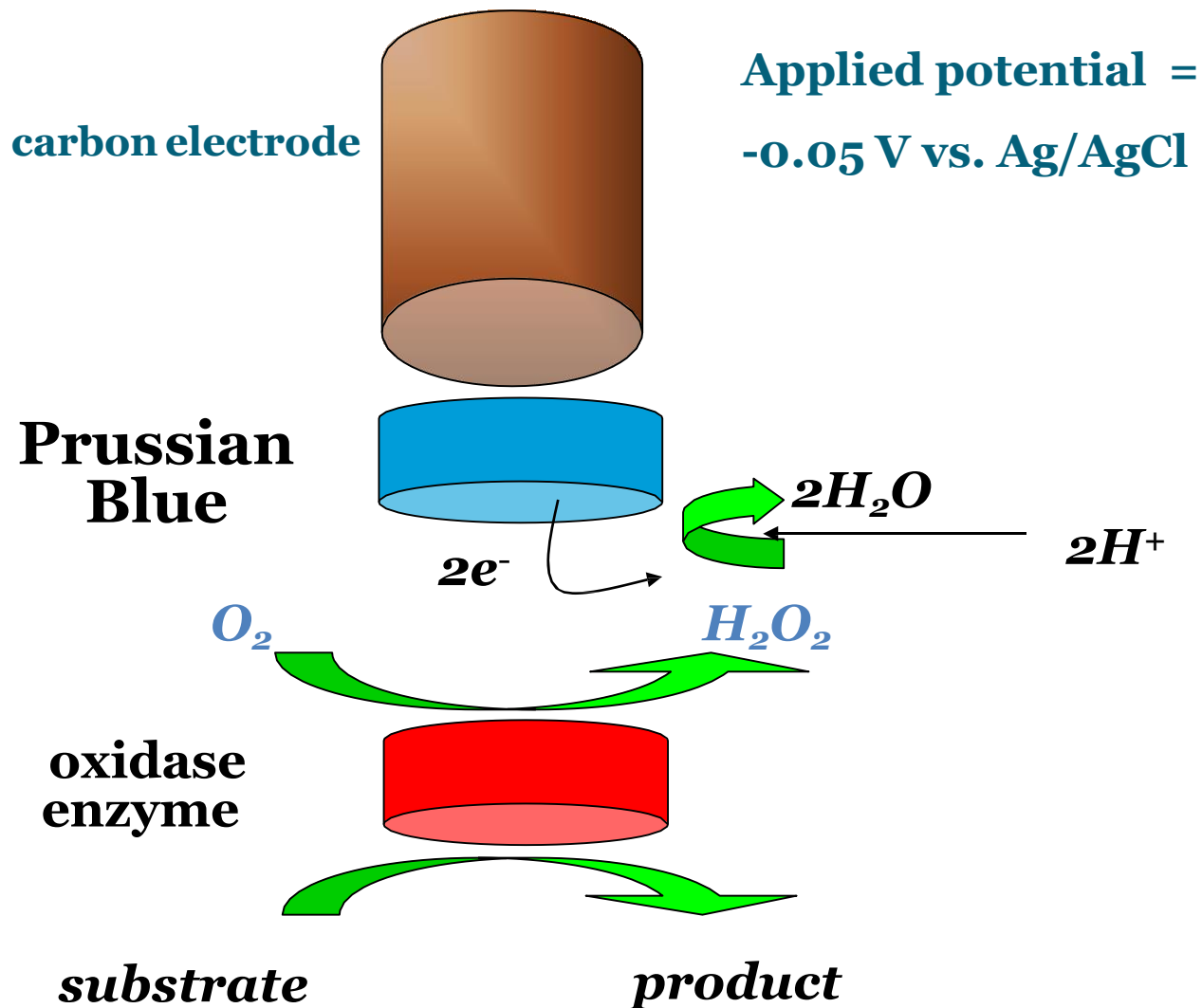


ortho- and para-quinones , quinone imines have been used and incorporated into larger molecules as indophenols, phenazines and phenoxazines

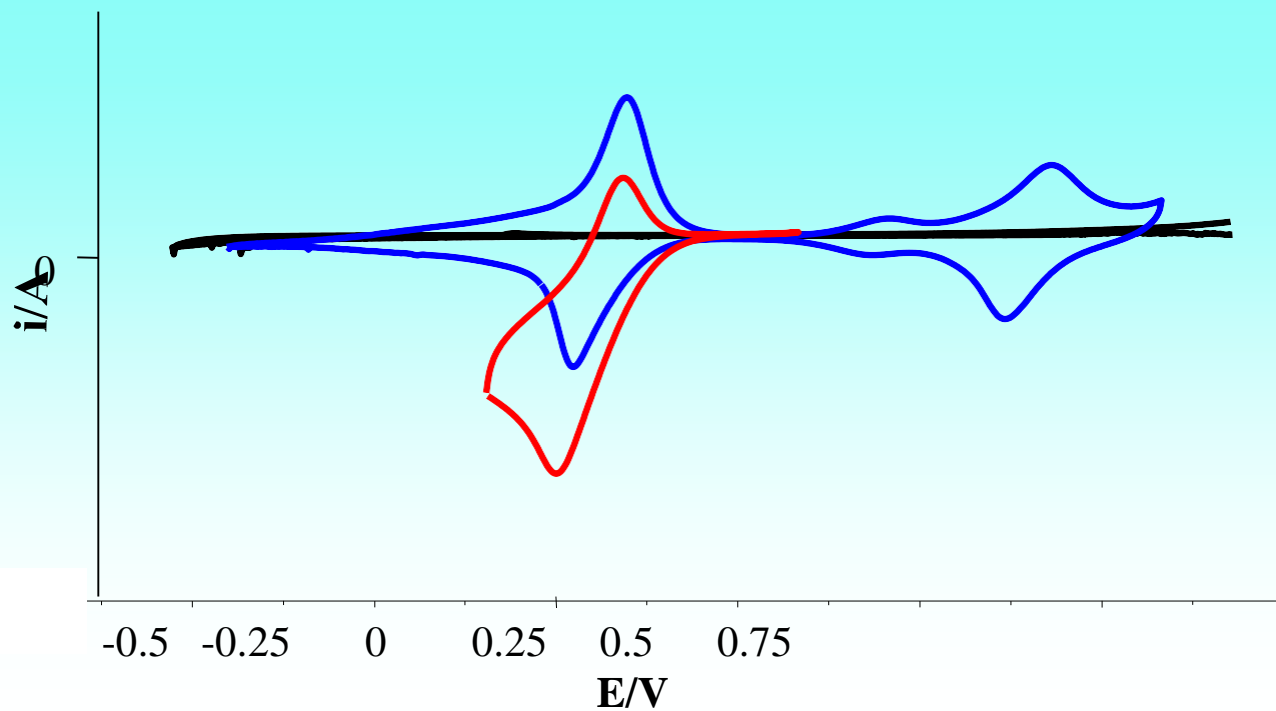
The mediator can be also immobilized at the electrode surface giving a chemically modified electrode for NADH

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***





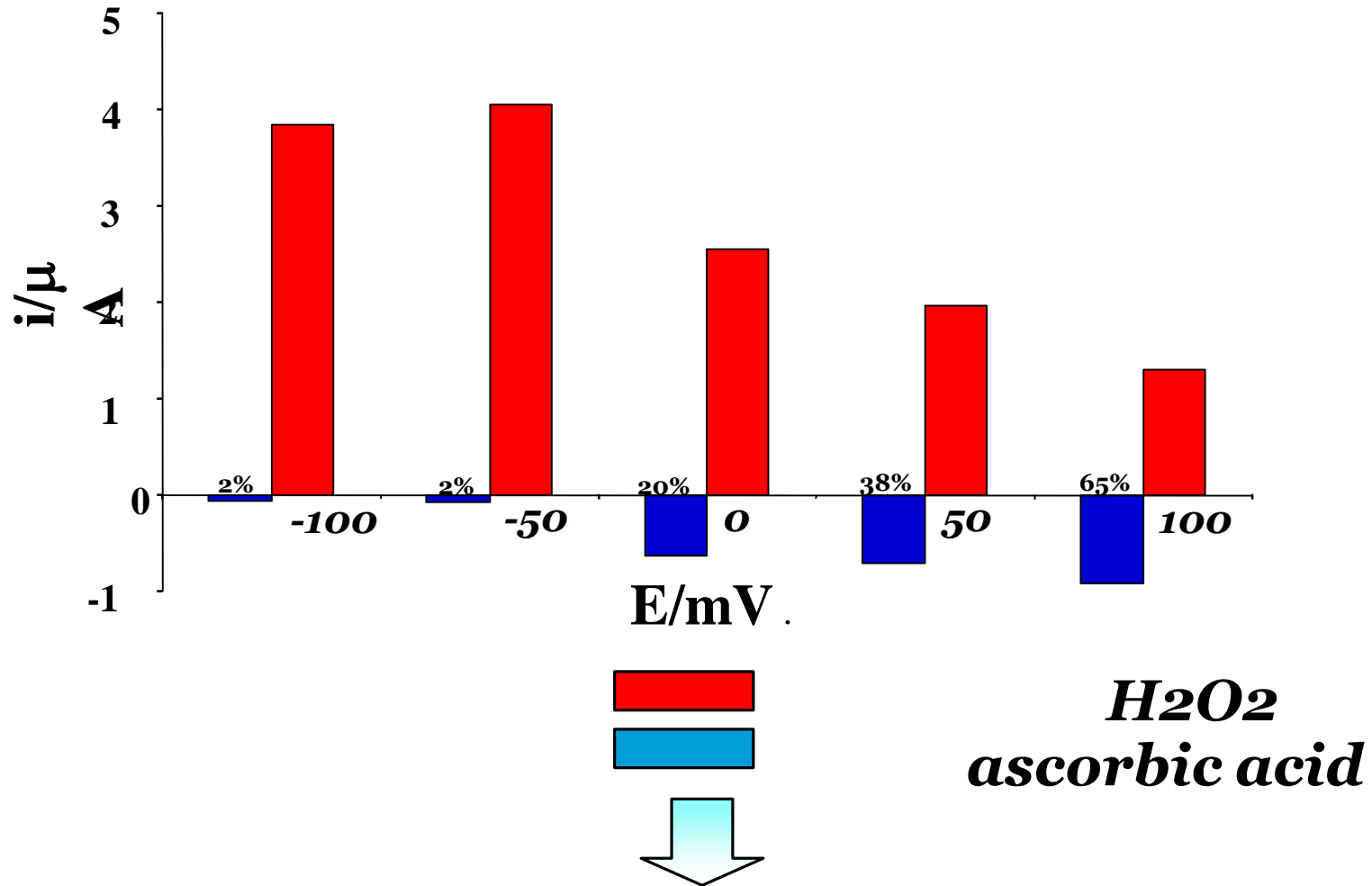


**Bare electrode**

**Prussian Blue modified electrode**

**Prussian Blue modified electrode + H<sub>2</sub>O<sub>2</sub>**

# *Selection of the applied potential*



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

# Lysine biosensor, linear range and selectivity

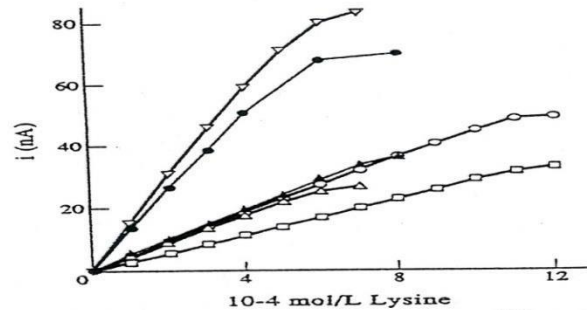
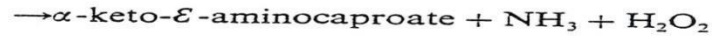
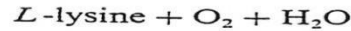


Fig. 1. Lysine calibration curves using two different immobilization procedures and protective polycarbonate membranes with different porosity.  $\nabla$  BSA/glutaraldehyde on Immobilon and  $0.8\text{-}\mu\text{m}$  polycarbonate;  $\bullet$  Immobilon only and  $0.8\text{-}\mu\text{m}$  polycarbonate;  $\circ$  BSA/glutaraldehyde on Immobilon and  $0.03\text{-}\mu\text{m}$  polycarbonate;  $\blacktriangle$  BSA/glutaraldehyde on Immobilon and  $0.05\text{-}\mu\text{m}$  polycarbonate;  $\triangle$  Immobilon only and  $0.05\text{-}\mu\text{m}$  polycarbonate;  $\square$  Immobilon only and  $0.03\text{-}\mu\text{m}$  polycarbonate. Buffer phosphate  $0.1\text{M}$  pH 7.0  $T = 25^\circ$ .

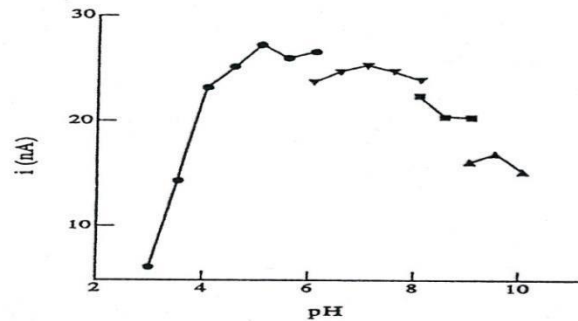


Fig. 2. Effect of pH on lysine oxidase activity. The enzyme activity was measured in the following buffers:  $\bullet$  citrate;  $\nabla$  phosphate;  $\blacksquare$  tris;  $\blacktriangle$  borax,  $T = 25^\circ$ .

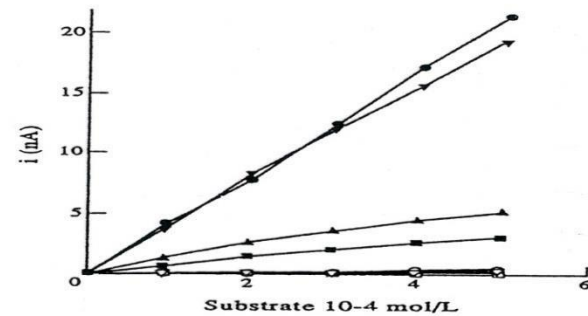
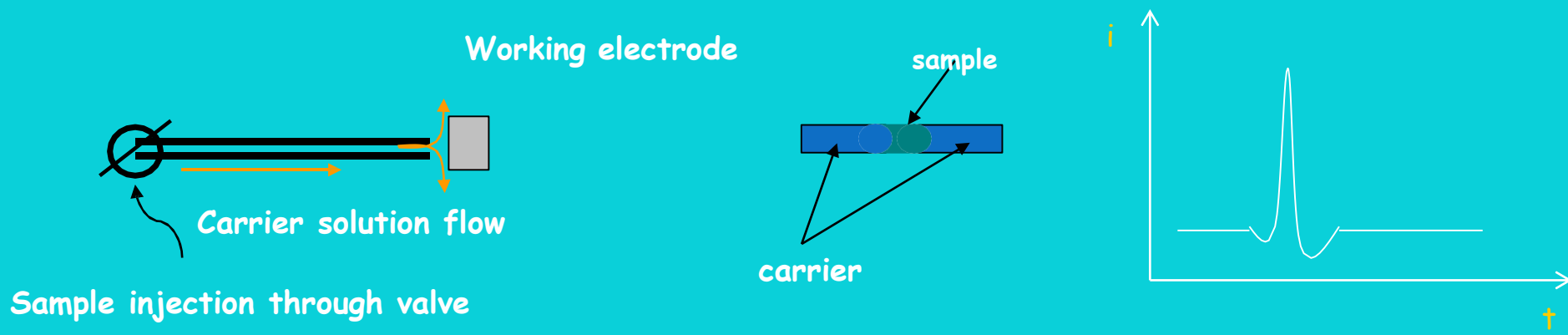
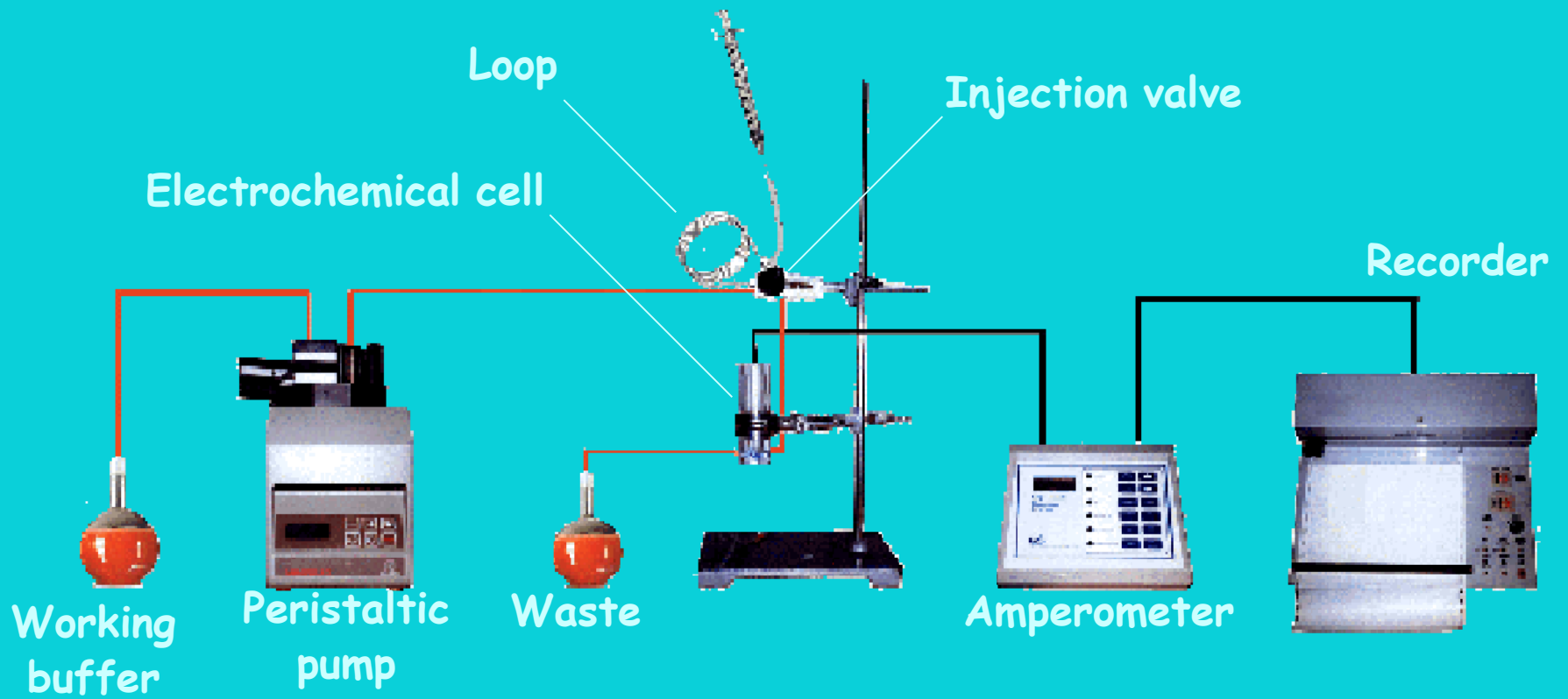
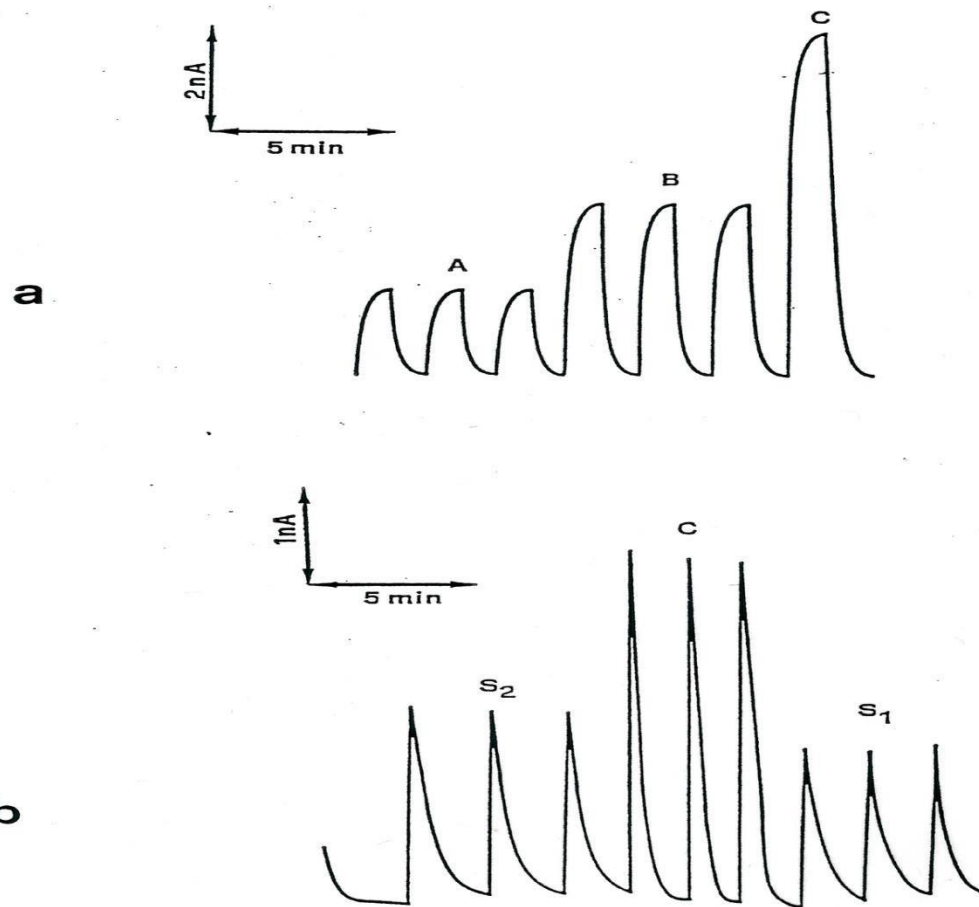


Fig. 4. Relative lysine oxidase activity toward some substrates pH 7.0 phosphate buffer  $T = 25^\circ$ .  $\bullet$  lysine;  $\nabla$  lysine after the analysis of other aminoacids.  $\blacktriangle$  ornithine;  $\blacksquare$  arginine;  $\circ$  tyrosine;  $\square$  phenylalanine;  $\nabla$  histidine.





**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<sub>1</sub> and S<sub>2</sub> foodstuff 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 (%)
<b>Milk</b>	1.684	1.493 ± 0.014	88.66
<b>pasta</b>	0.299	0.308 ± 0.012	103.01

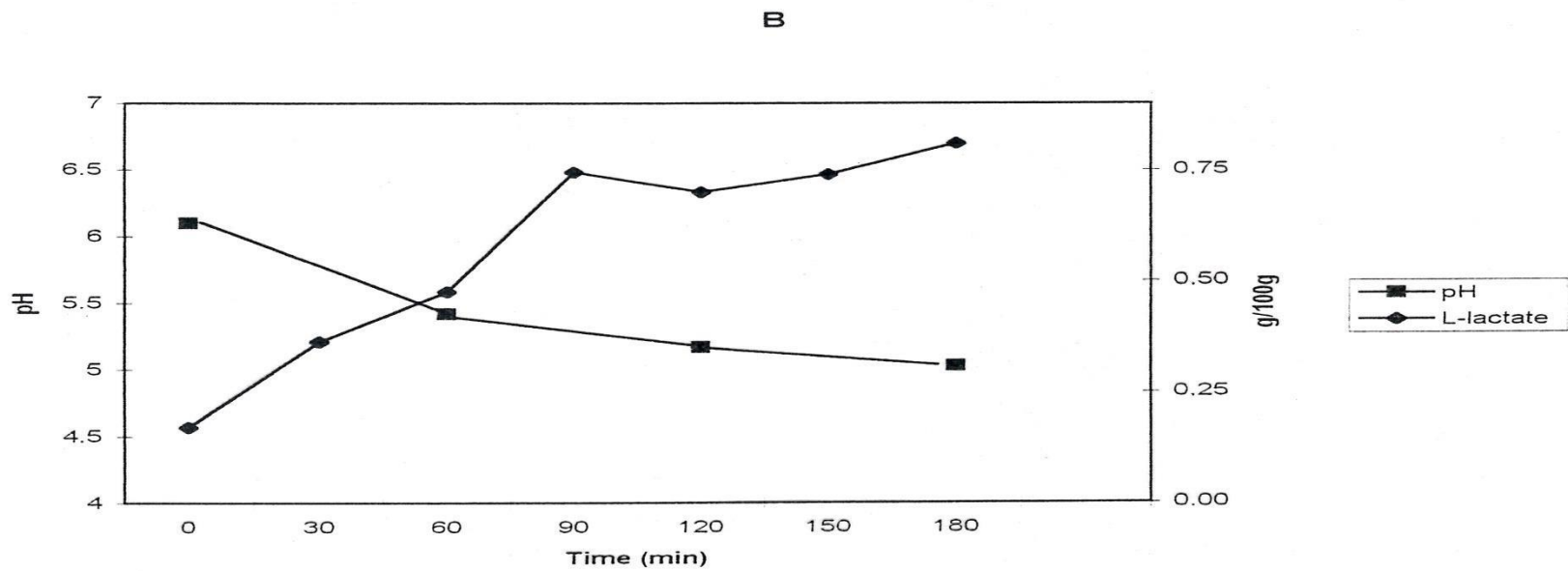
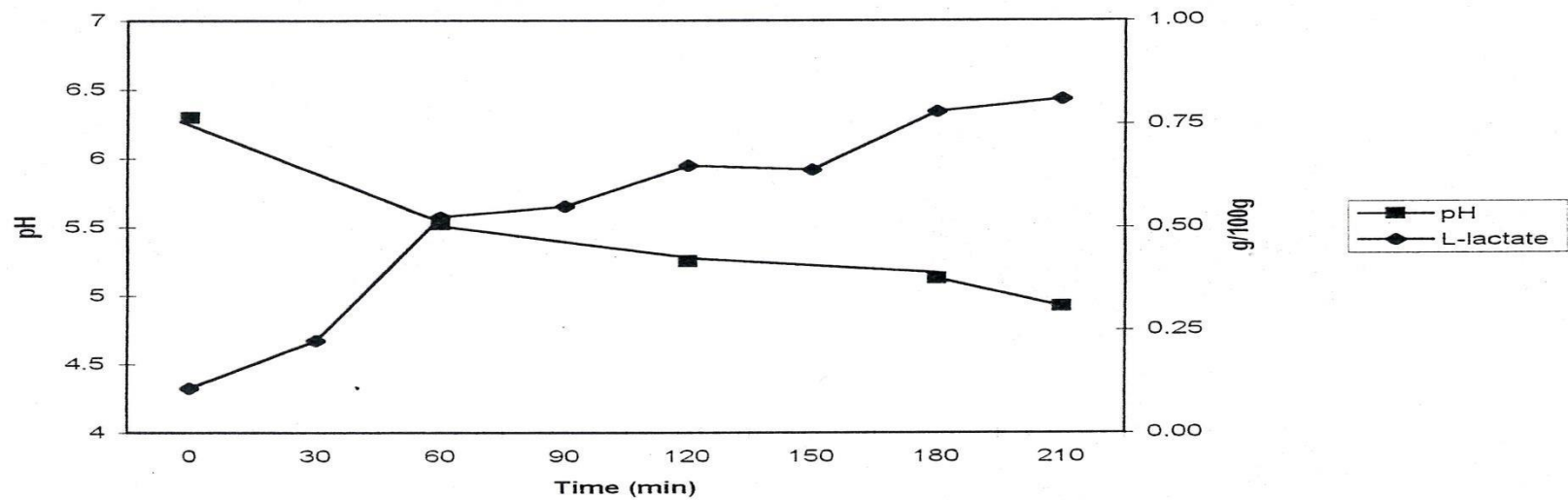
## Lactic acid monitoring during mozzarella cheese manufacturing

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 and 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 the manufacturing.

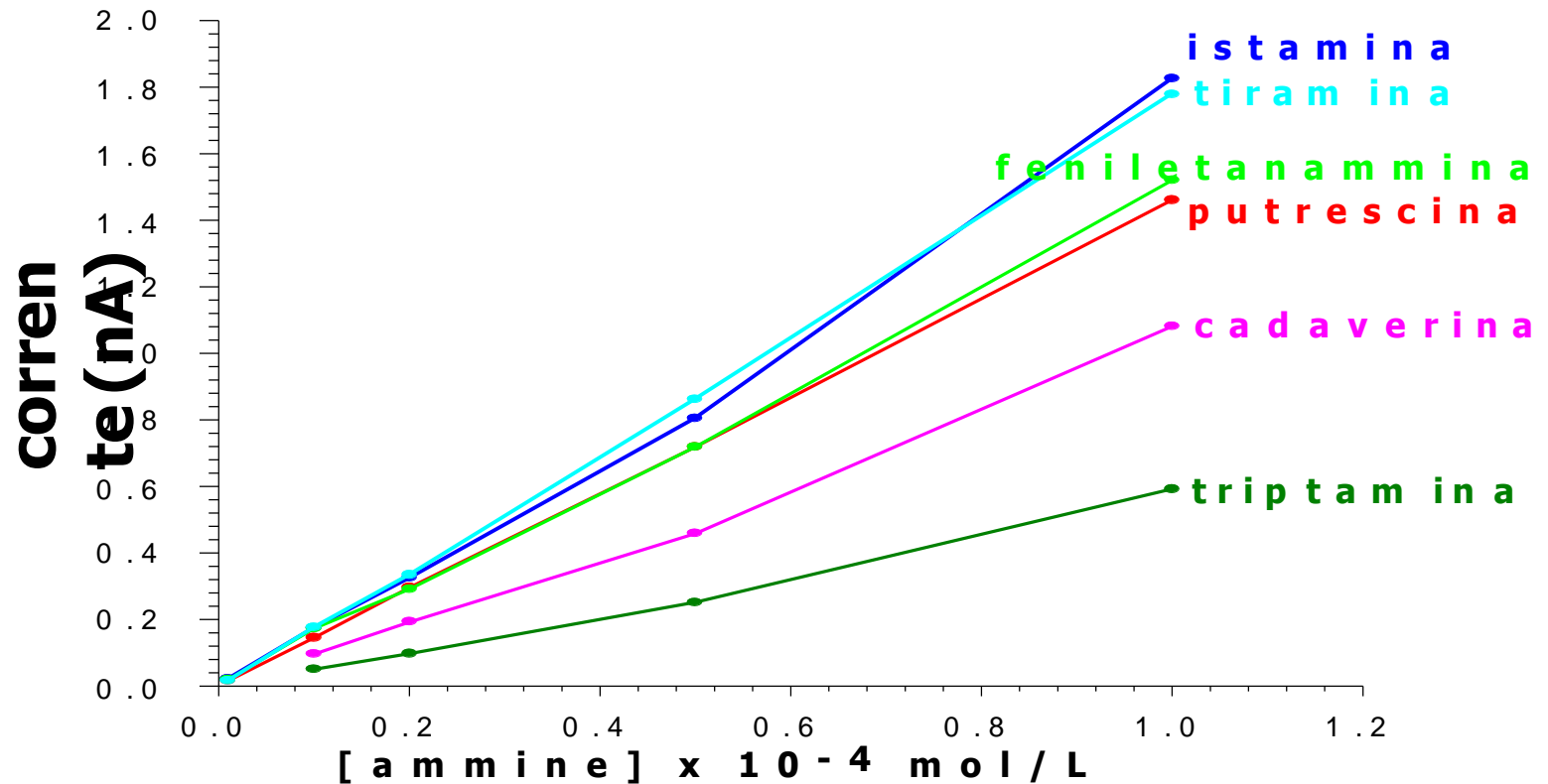
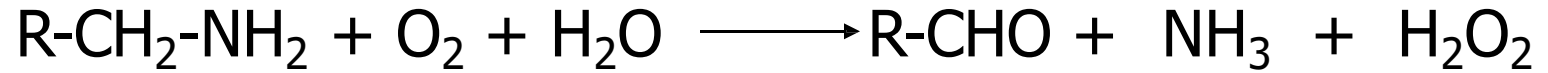
Optimum pH is 4.9 for water-buffalo milk and 5.1 for cow milk. At these pH values there is a great increase in the buffer capacity due to casein (isoelectric point pH ~ 5) and low molecular weight acids

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



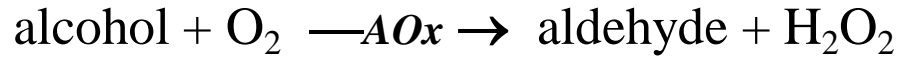


# Biogenic amines



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

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



**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  $\mu\text{L}$ .

**Analytical performances:**

detection limit  $10^{-6}$  mol/L

linearity  $2 \times 10^{-6} / 10^{-3}$  mol/L

stability: 20% decrease after 200 samples



**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<sup>2+</sup>) + 0.02% Tween. Flow rate 0.5 mL/min; injection loop 250  $\mu\text{L}$ .

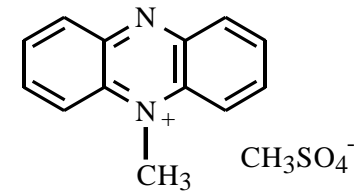
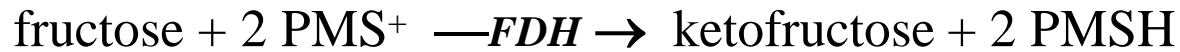
**Analytical performances:**

detection limit  $10^{-6}$  mol/L

linearity  $2.5 \times 10^{-6} / 5 \times 10^{-4}$  mol/L

stability: 40% decrease after 200 samples

# Fructose



**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  $\mu$ L.

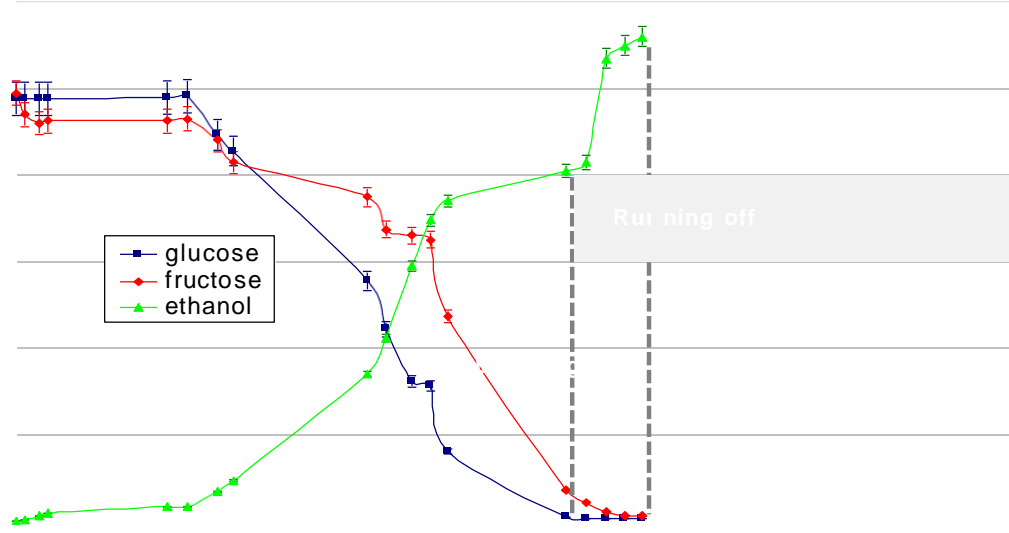
**Analytical performances:**

detection limit  $5 \times 10^{-7}$  mol/L

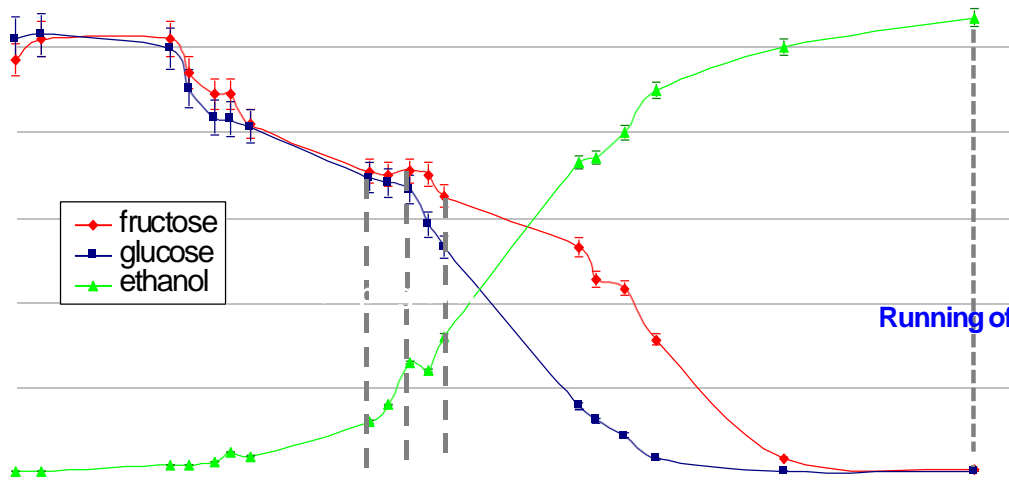
linearity  $10^{-6}$  /  $8 \times 10^{-4}$  mol/L

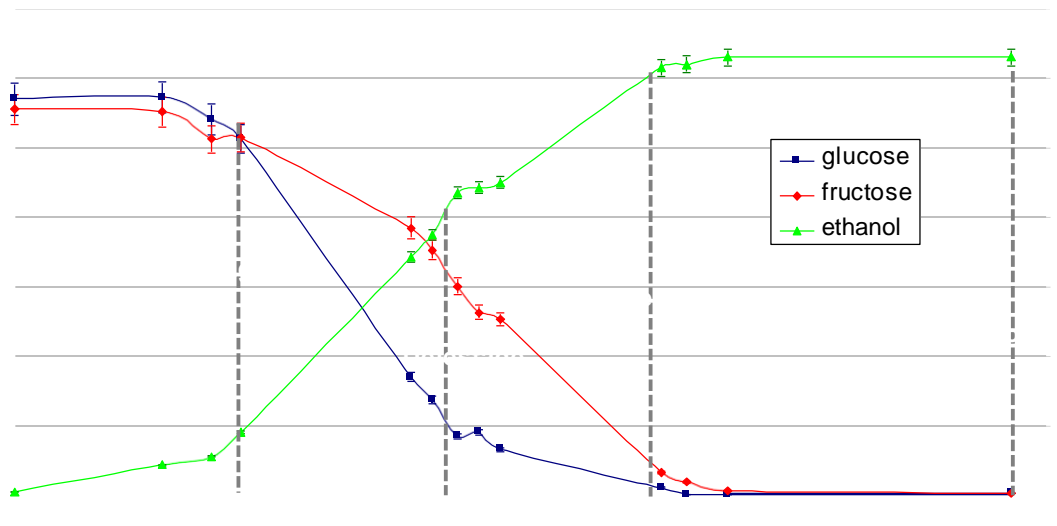
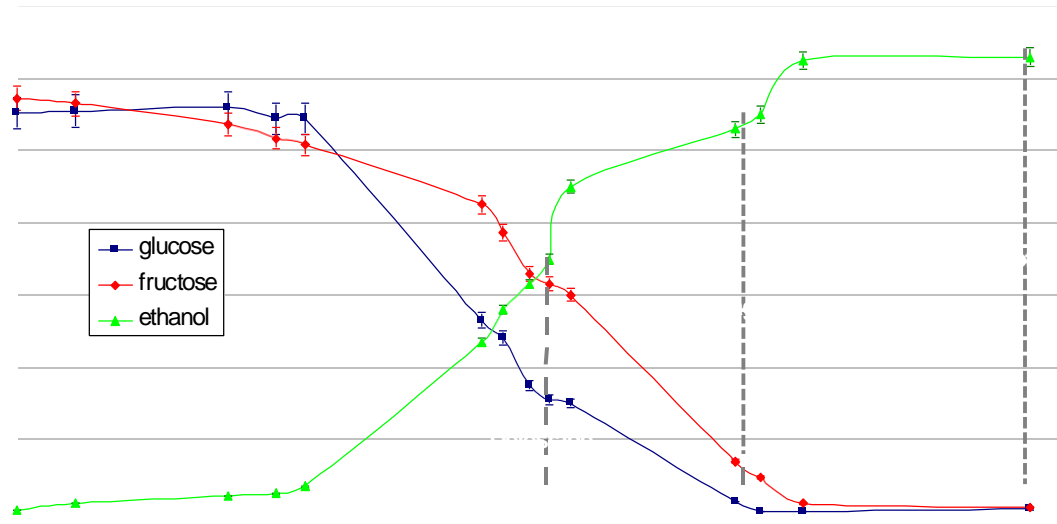
stability: 30% decrease after 200 samples

# One delestage

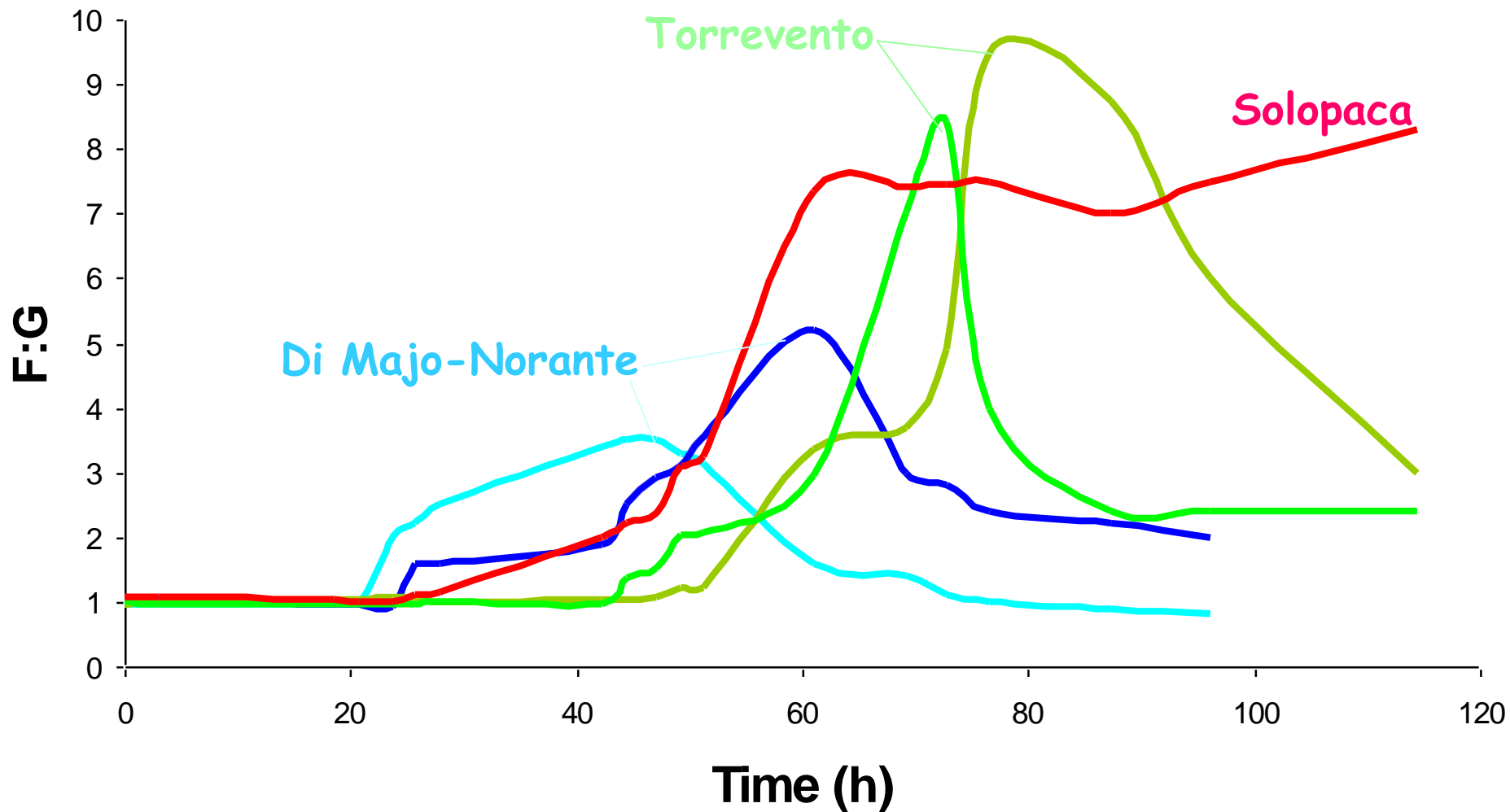


# Pumping-over

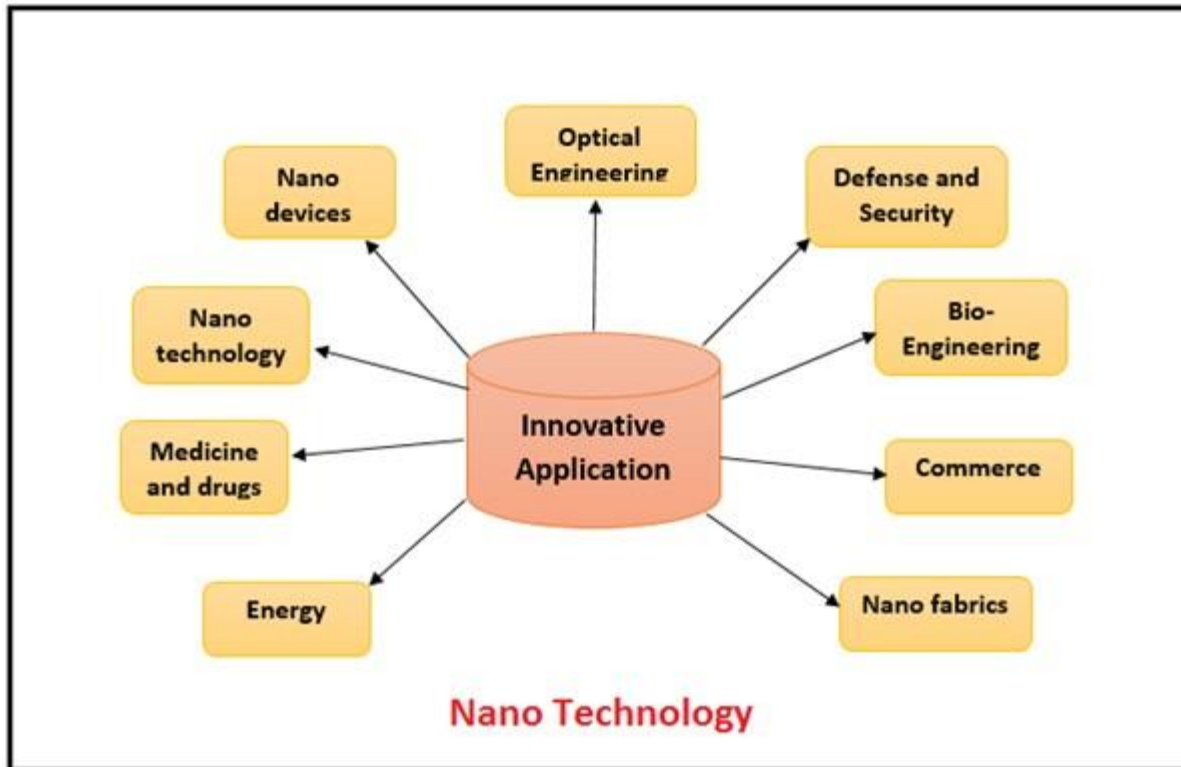




# Fructose:Glucose ratio during alcoholic fermentation



**Nanotechnology** is science, engineering, and technology conducted at the nanoscale, which is about 1 to 100 nanometer





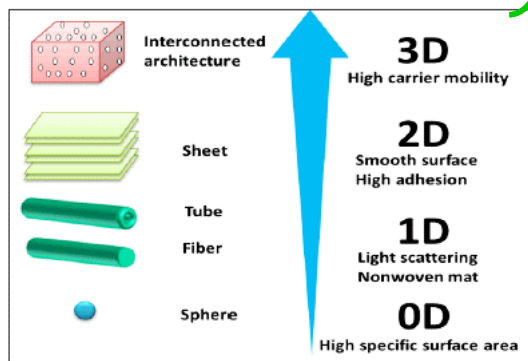
# Nanomaterials

## Pros



- ✓ Different chemical nature
- ✓ Different morphologies
- ✓ High surface/volume ratio
- ✓ High functionalizability
- ✓ Easy interfaceability
- ✓ Size/morphology dependent properties → **tunability**

Sensor  
sensitivity & selectivity  
improvement



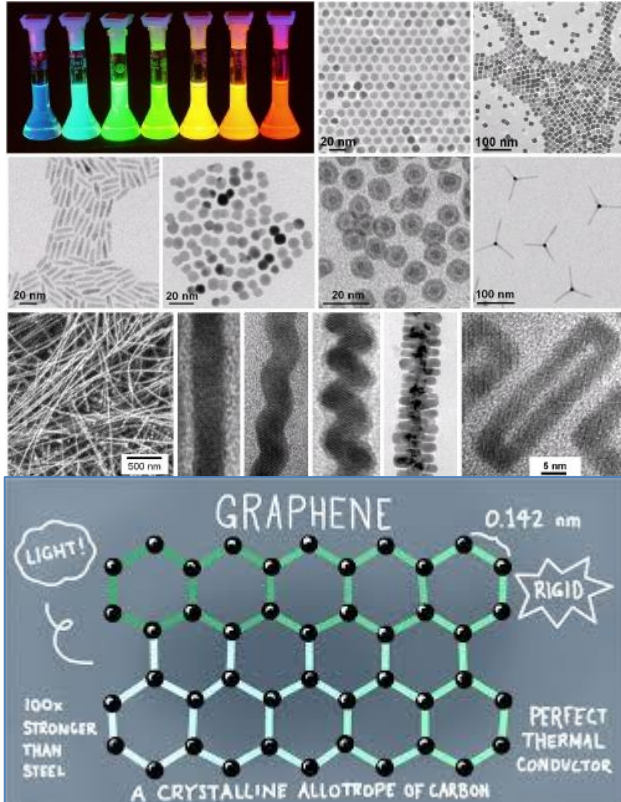
## Potential drawbacks



- ? Waste disposal
- ? Potential toxicity

Figure 1: Schematic illustration of structural dimensionality of nanomaterials with expected properties.

# Nanomaterials employed in electrochemical sensor



## Carbon based nanomaterials:

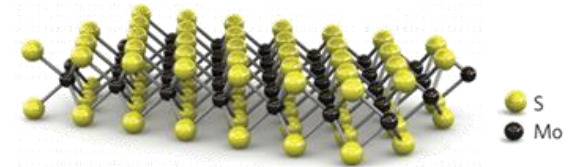
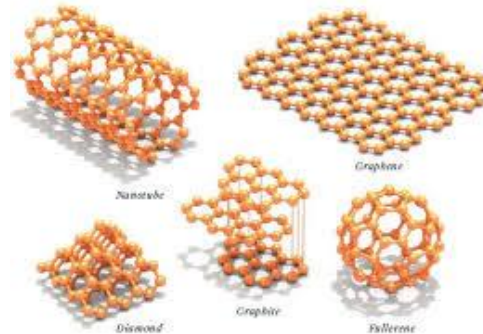
- Nanotubes
- Fullerenes
- Graphene
- Etc...

## Nanoparticles:

- Metal nanoparticles
- Metal Oxide nanoparticles

## Graphene-like nanomaterials:

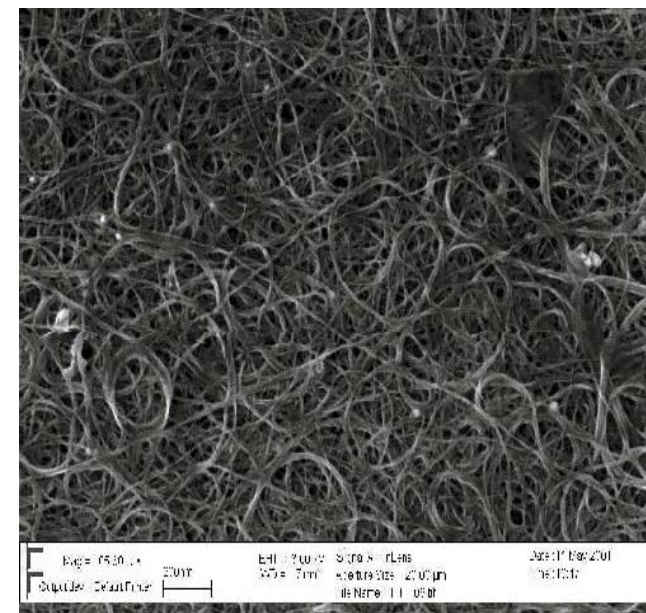
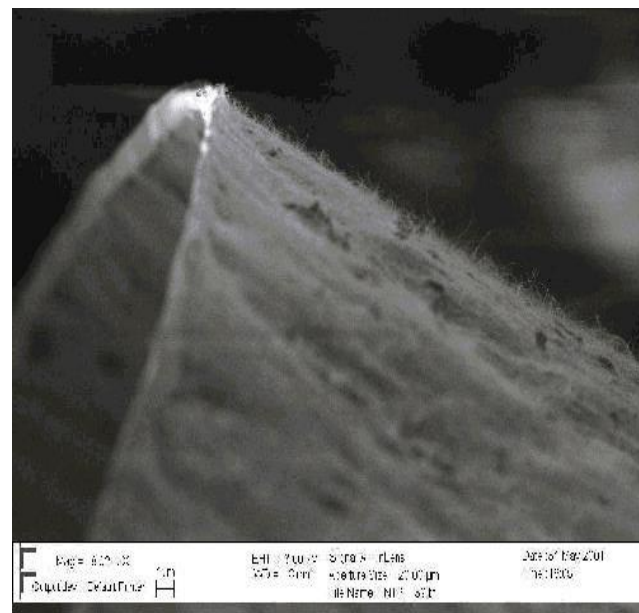
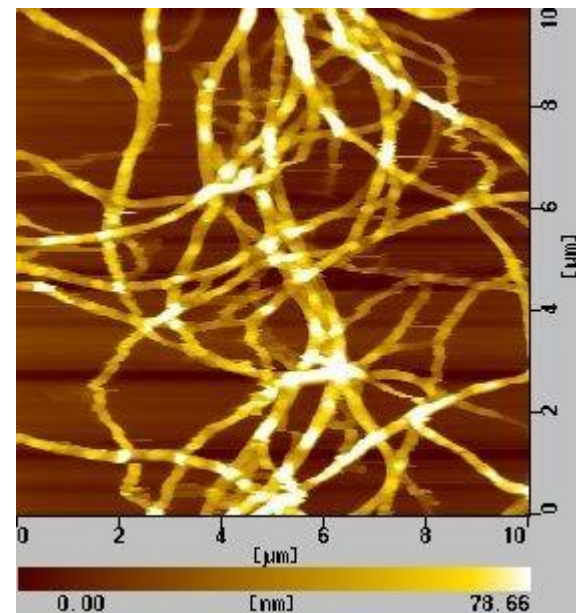
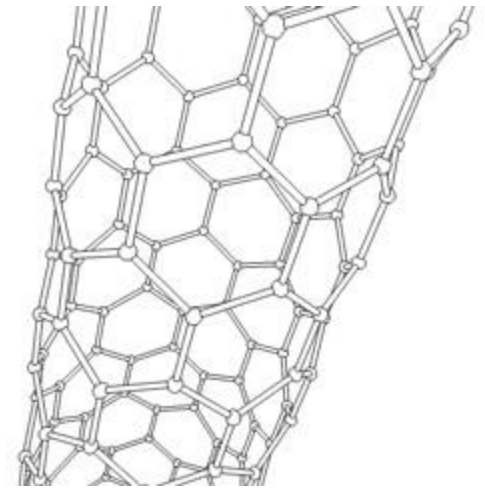
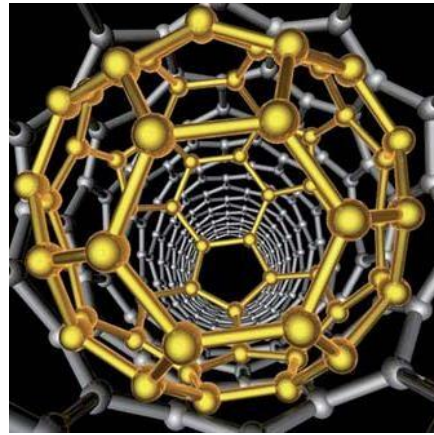
- e.g. Transition Metal Dicalchogenised (TMD)



# CARBON NANOTUBES

## CHARACTERISTICS

- porous structure;
- high mechanical strength;
- easy to be modified;

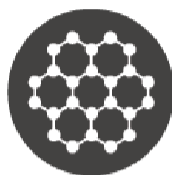




# GRAPHENE: THE CARBON-BASED 'WONDER MATERIAL'

Since its discovery in 2003, graphene has been a hot topic in chemistry and materials science research. It's been linked with water purification, electronics, and biomedical applications. However, how close are we really to using graphene in our day-to-day lives? This graphic looks at its properties, uses, and future.

## WHAT IS GRAPHENE?



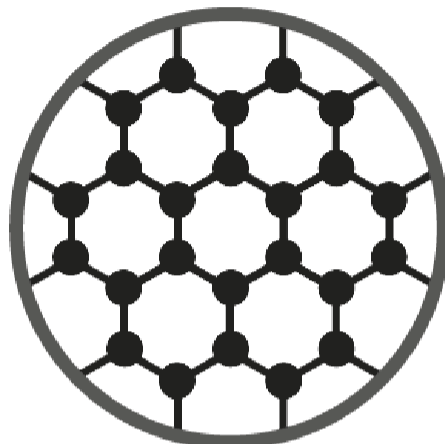
SINGLE LAYER OF CARBON ATOMS

HONEYCOMB-LIKE STRUCTURE

GRAPHITE IS LAYERS OF GRAPHENE

ISOLATED IN 2003 IN MANCHESTER

Graphene is a single layer of graphite, the carbon-based material found in pencil leads. Graphite has been known for centuries, but graphene was only isolated in 2003, by shearing layers off of graphite using sellotape. It's a single atom-thick layer of carbon atoms, that are arranged in a flat, hexagonal lattice structure.



## POTENTIAL USES OF GRAPHENE



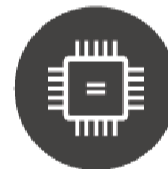
### TOUCH SCREENS IN DEVICES

Graphene's transparency and conductivity means that it can be used in displays and touchscreens. However, currently these are more expensive to produce than the currently used material, indium tin oxide.



### WATER FILTRATION SYSTEMS

Graphene allows water to pass through it, but not other liquids and gases, so it can be used in water purification. Researchers are working on a device that could be capable of filtering salt from sea water.



### IN ELECTRONIC DEVICES

Graphene has been touted as silicon's successor, and has been used to make very fast transistors. However, its conductivity cannot be 'switched off' as silicon's can. Other 2D materials seem more promising.



### MEDICAL SENSORS & DRUG DELIVERY

Several biomedical applications are being explored for graphene, including drug delivery, cancer therapy, and its use as a sensor. However, its toxicity profile must be investigated before any clinical uses.



### ENERGY STORAGE & COMPOSITES

Graphene-based energy storage devices are possible. It can also substitute for graphite in normal batteries, improving efficiency. Additionally, it can be added to materials to make them stronger and more lightweight.

## THE PROPERTIES OF GRAPHENE



HIGH ELECTRICAL CONDUCTIVITY



200X STRONGER THAN STEEL



THIN AND LIGHTWEIGHT



HIGH THERMAL CONDUCTIVITY



VERY HIGH TRANSPARENCY

Graphene's 'wonder material' reputation stems from its superlative properties. It is a million times thinner than a piece of paper, yet stronger than diamond, and 200 times stronger than steel, due to the strong carbon-carbon bonds. It's also a flexible material, and conducts heat and electricity better than copper. Being only one atom thick, almost 98% of visible light passes through graphene, making it transparent.



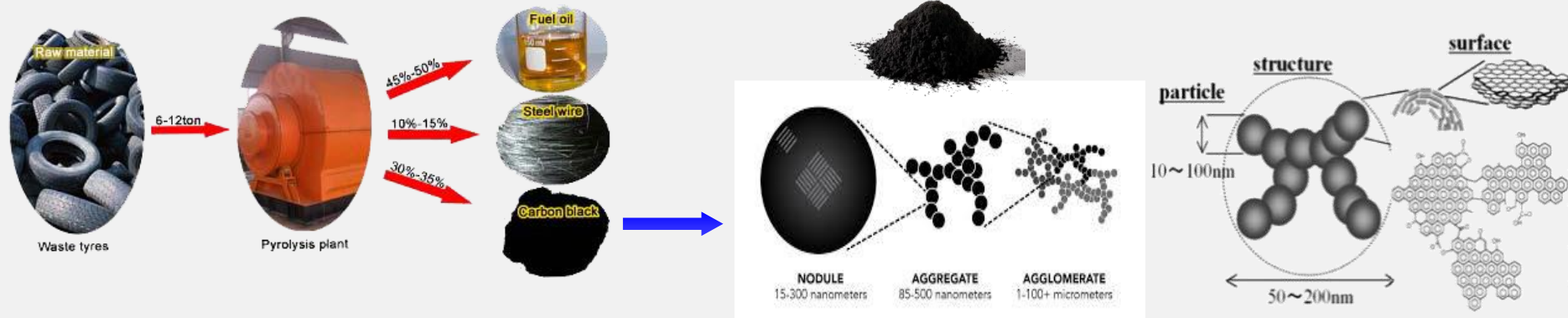
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# Carbon based nanomaterials (Carbon Black, CB)

## Nano Carbon Black

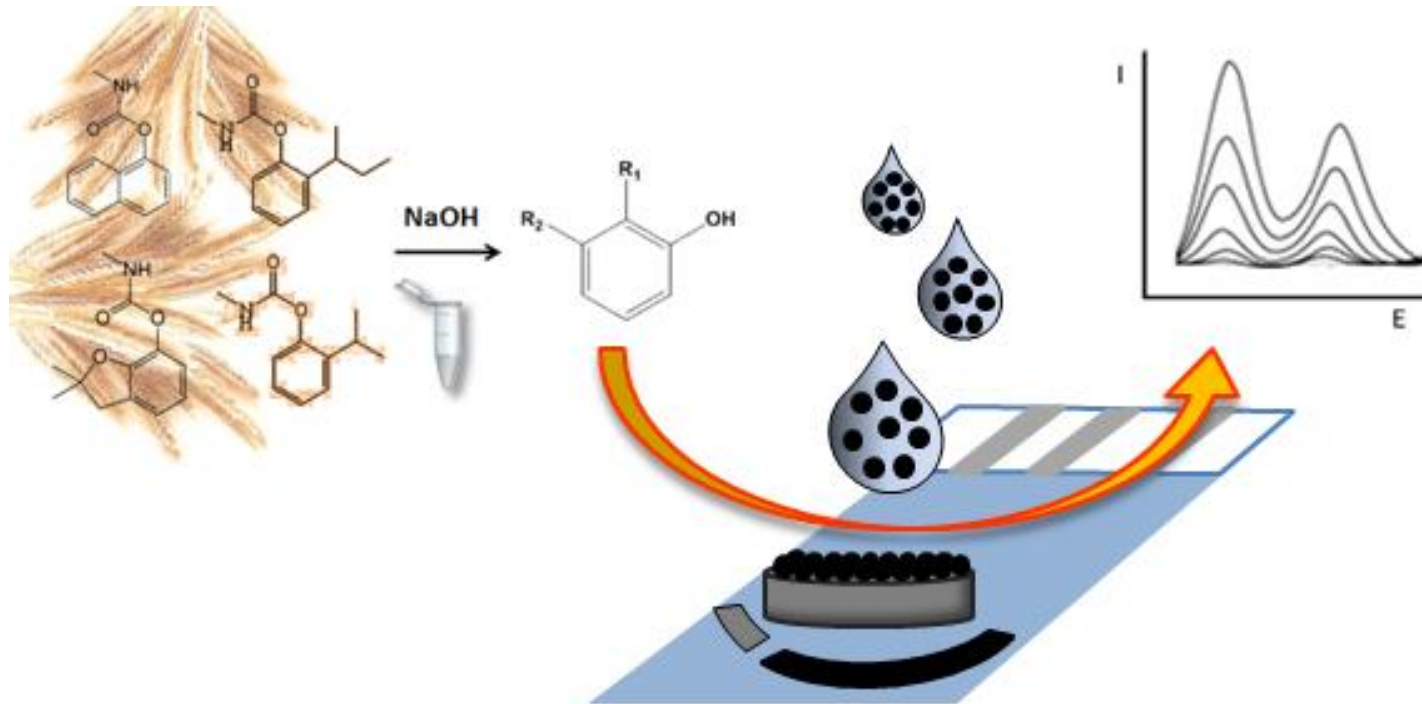


- Electrocatalysis → Selectivity
- High surface → Sensitivity
- Resistance to fouling → Reproducibility
- Faster electron transfer → Improving separation performance

### CB compared with other nanomaterials:

- Very low cost
- No synthesis
- No impurities due to synthesis
- Easily dispersible
- Large number of defect sites

# SPE CBNPs for direct analysis of carbamates in grain samples



Contents lists available at ScienceDirect

Talanta

journal homepage: [www.elsevier.com/locate/talanta](http://www.elsevier.com/locate/talanta)

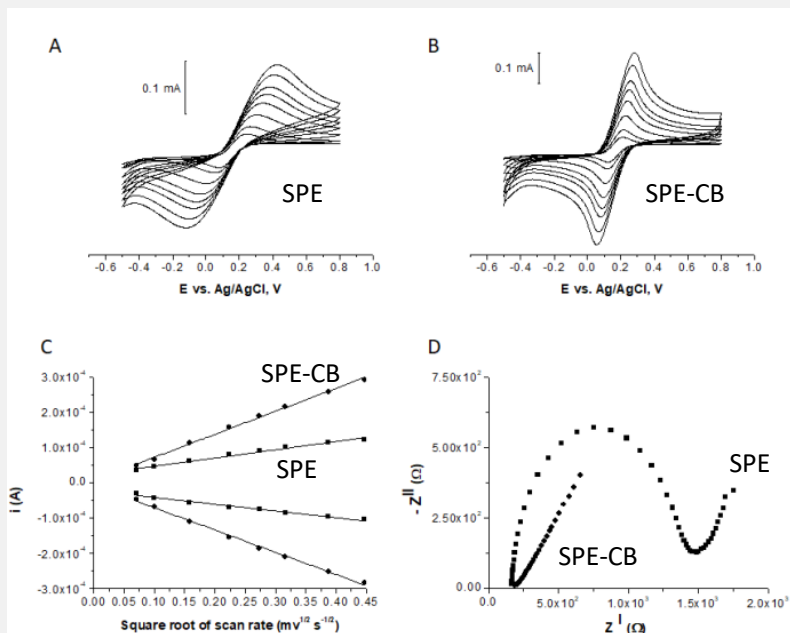


Nano carbon black-based screen printed sensor for carbofuran, isoprocarb, carbaryl and fenobucarb detection: application to grain samples

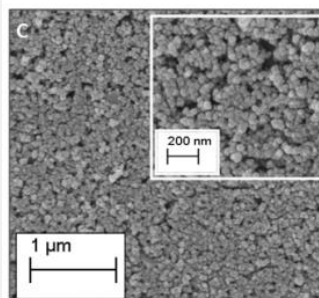
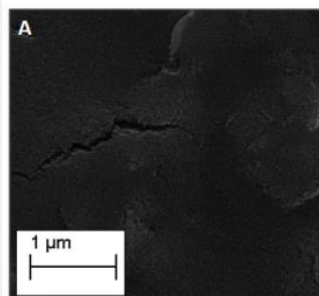
Flavio Della Pelle, Claudia Angelini, Manuel Sergi, Michele Del Carlo, Alessia Pepe, Dario Compagnone\*

# Nano carbon black-based screen printed sensor for carbofuran, isoprocarb, carbaryl and fenobucarb detection: application to grain samples

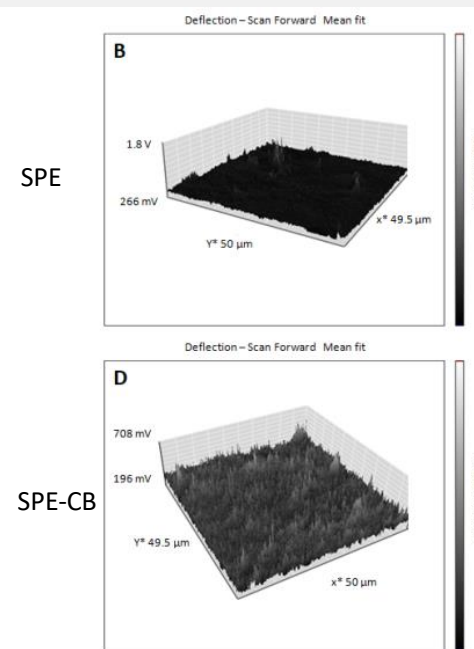
## SPE vs. SPE-CB electrochemical performance



## SEM

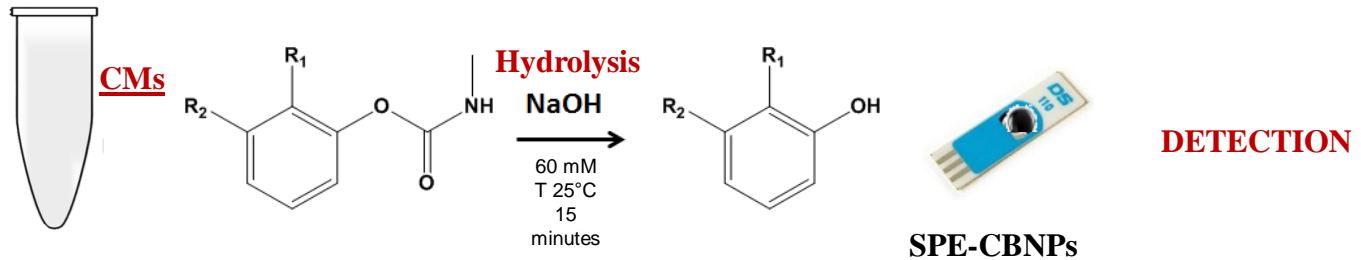


## AFM



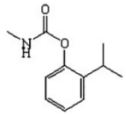
1  $\text{mmol L}^{-1}$  ferricyanide solution in 0.1  $\text{mol L}^{-1}$  KCl of SPE CV performed at 5, 10, 25, 50, 75, 100, 150 and 200  $\text{mV s}^{-1}$

# SPE CBNPs for direct analysis of carbamates in grain samples

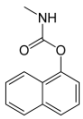


## SPE-CBNPs vs. CMs

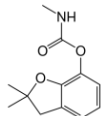
**Isoproc carb**



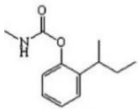
**Carbofuran**



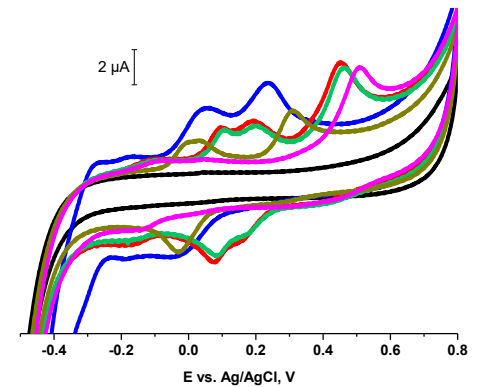
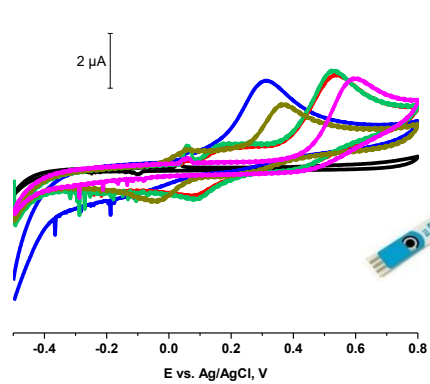
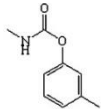
**Carbaryl**



**Fenobucarb**



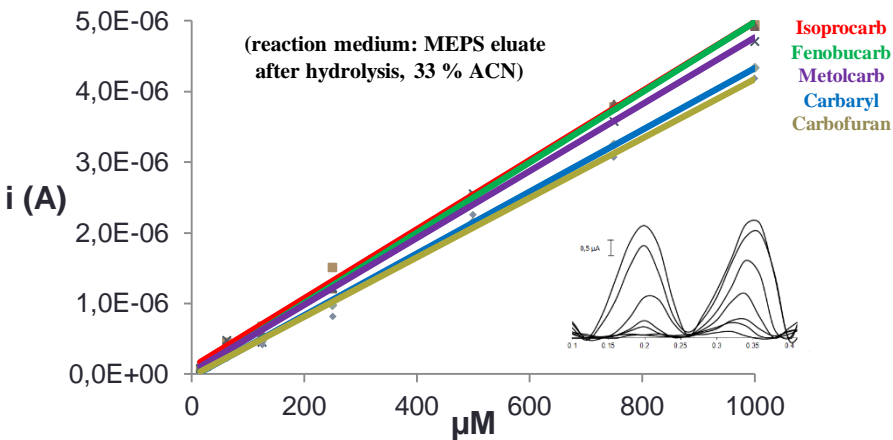
**Metolcarb**





# SPE CBNPs for direct analysis of carbamates in grain samples

## SPE-CBNPs CMs Calibration, Reproducibility and Fouling resistance



**Peak intensity** (RSD, n=7): < 0.9 %

**Peak potential** (RSD, n =7): < 4,8 %

**Inter electrode reproducibility** (RSD, n=10): < 6.6 % p.i and < 3,4 % p.E.

**Fouling** (peaks RSD):

DPV (n = 30, 250 μM) 96 % v.s.32 %

CV (n = 20, 500 μM) 94 % v.s 15 %

Analyte	Linear range (μmol L <sup>-1</sup> )	Regression equation (Y=am + b)	Coefficient of determination n (r <sup>2</sup> )	Detection limit (μmol L <sup>-1</sup> )	Quantification limit (μmol L <sup>-1</sup> )
Isoprocarb	0.1-100	y = 3E-08x + 5E-09	0.9971	0.6	0.7
Carbofuran	0.1-100	y = 6E-08x - 1E-08	0.9999	0.4	0.5
Carbaryl	0.1-100	y = 6E-08x + 2E-08	0.9983	0.4	0.5
Fenobucarb	0.1-100	y = 3E-08x - 8E-09	0.9996	0.6	0.7
Metolcarb	0.1-100	y = 6E-08x + 4E-08	0.9980	0.3	0.4

# Nano carbon black-based screen printed sensor for carbofuran, isoprocarb, carbaryl and fenobucarb detection: application to grain samples

## Pesticide recoveries in grain samples

Recoveries : 78–102%

Correlation:  $r = 0.952$

Accuracy: relative error between 9.0% and -7.8%

Analyte Spiked (mg Kg <sup>-1</sup> )	UHPL C-MS/MS recovery (%) <sup>a</sup>					CB-SPE recovery (%) <sup>a</sup>					Relative error (%)				
	HW	HWO	SW	SWO	MZ	HW	HWO	SW	SWO	MZ	HW	HWO	SW	SWO	MZ
CA															
0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD					
0.25	82 ± 6	87 ± 3	83 ± 9	89 ± 12	82 ± 15	88 ± 1	84 ± 4	76 ± 7	93 ± 8	85 ± 12	-6.8	3.1	9.0	-4.1	-3.8
0.50	85 ± 2	84 ± 13	83 ± 2	81 ± 8	93 ± 7	88 ± 3	88 ± 11	80 ± 5	80 ± 6	90 ± 4	-3.4	-4.7	3.4	0.6	3.1
0.75	82 ± 10	78 ± 7	84 ± 1	82 ± 7	80 ± 4	85 ± 5	80 ± 9	87 ± 8	81 ± 9	84 ± 2	-3.7	-2.3	-3.0	1.2	-5.6
CF															
0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD					
0.25	80 ± 14	102 ± 10	83 ± 10	82 ± 14	81 ± 8	86 ± 9	97 ± 7	86 ± 6	79 ± 10	80 ± 9	-7.8	4.8	-3.7	3.4	1.9
0.50	78 ± 7	96 ± 6	78 ± 5	78 ± 5	79 ± 13	81 ± 4	100 ± 2	81 ± 3	80 ± 5	83 ± 11	-3.3	-4.8	-3.6	-2.9	-5.6
0.75	79 ± 9	100 ± 9	84 ± 11	99 ± 131	79 ± 16	82 ± 5	100 ± 5	87 ± 7	100 ± 8	84 ± 9	-3.4	-0.6	-3.1	-0.7	-6.4
IC															
0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD					
0.25	82 ± 5	80 ± 8	78 ± 8	84 ± 5	79 ± 8	82 ± 5	81 ± 6	82 ± 2	86 ± 9	80 ± 5	-1.0	-0.5	-5.3	-2.4	-1.2
0.50	82 ± 7	85 ± 4	79 ± 2	78 ± 8	79 ± 5	81 ± 8	85 ± 4	81 ± 4	82 ± 6	78 ± 8	1.3	-0.2	-3.0	-4.5	1.6
0.75	96 ± 2	96 ± 6	80 ± 3	95 ± 2	92 ± 14	96 ± 4	98 ± 9	80 ± 3	96 ± 4	97 ± 11	0.0	-2.0	-4.9	-1.8	-5.6
FB															
0	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD					
0.25	83 ± 9	87 ± 7	83 ± 15	80 ± 4	87 ± 9	84 ± 5	92 ± 4	80 ± 13	79 ± 4	88 ± 3	-0.9	-5.3	8.0	4.5	-0.9
0.50	79 ± 12	78 ± 9	97 ± 2	78 ± 5	80 ± 9	78 ± 8	82 ± 11	102 ± 0	78 ± 2	78 ± 6	7.7	-5.7	-5.2	4.7	5.1
0.75	89 ± 11	78 ± 8	102 ± 7	94 ± 13	79 ± 13	84 ± 11	81 ± 10	99 ± 3	96 ± 9	84 ± 11	5.6	-4.1	3.3	-2.5	-6.8

<sup>a</sup> Mean value (n = 3) of three different extracts were employed for the recovery and relative error calculation for both CB-SPE and UHPLC-MS/MS methods.

# Transition Metal Dichalcogenides and their hybrid nanomaterials

Objective

MoS<sub>2</sub> hybrids

WS<sub>2</sub> hybrids

Group VI Transition Metal Dichalcogenides

## Transition Metal Dichalcogenides (TMDs)

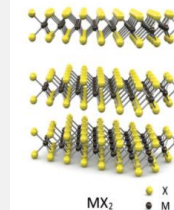
TMDs are a family of 2D nanomaterials with general formula MX<sub>2</sub> (M: Group 4-10 and X: S, Se and Te)

Arranged in a multilayered structure held by weak van der Waals forces

TMDs nanosheets easily prepared by exfoliation

Periodic table highlighting transition metals (M) and chalcogens (X) for MX<sub>2</sub> formation. The legend indicates M = Transition Metal and X = Chalcogen. The chalcogen group (S, Se, Te) is highlighted in red, and the transition metal groups (Groups 4-10) are highlighted in blue.

TMDs general structure



## Polyphenols electrochemical sensing

Limitations using common electrodes

- Tendency to passivation (so called Fouling)

Nanomaterials

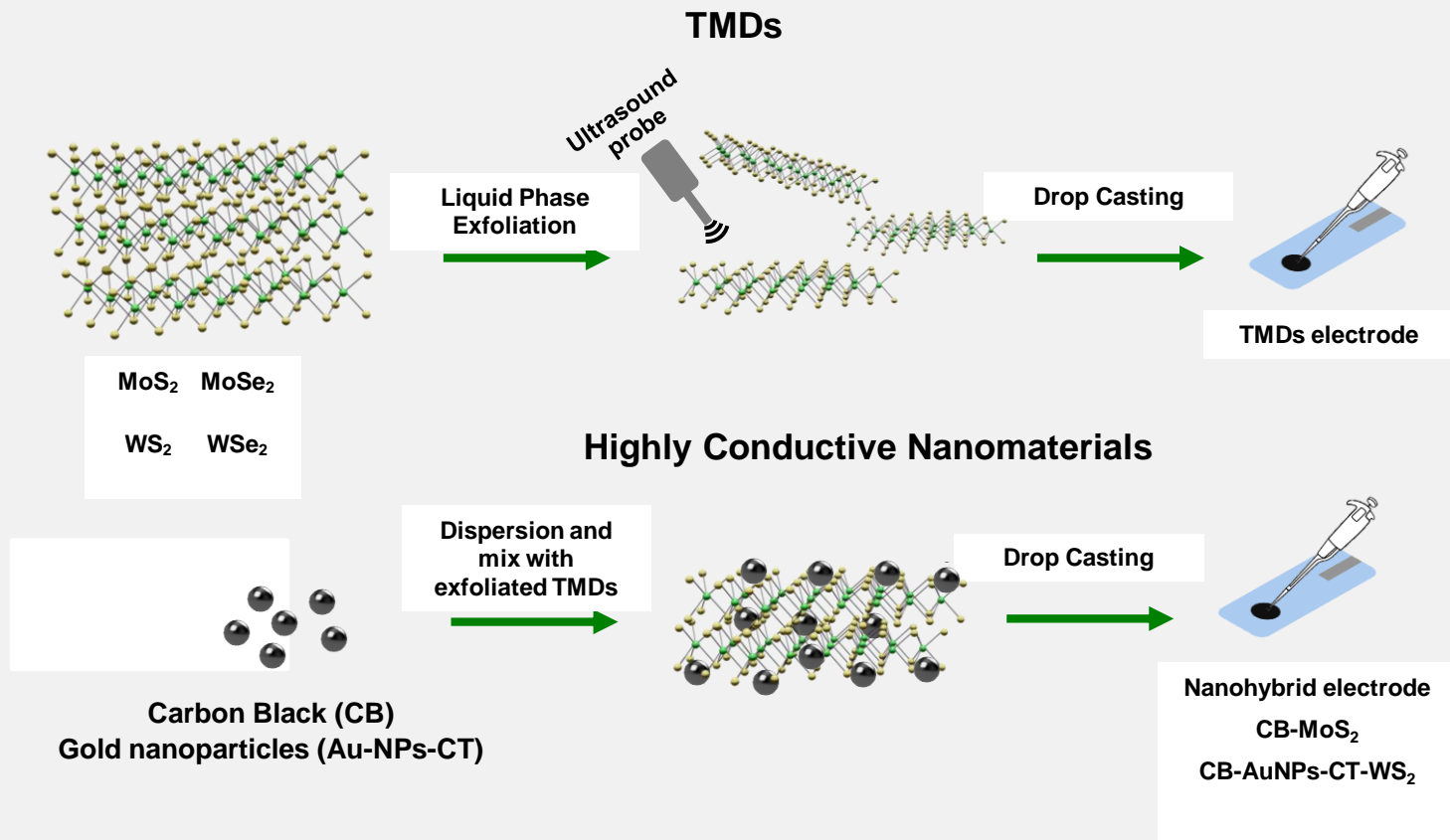
✓TMDs are poor conductive materials

✓Hinder its catalytic capabilities

Nanohybrids materials

# Transition Metal Dichalcogenides and their hybrid nanomaterials

## Electrode preparation methods



# Transition Metal Dichalcogenides and their hybrid nanomaterials

Objective

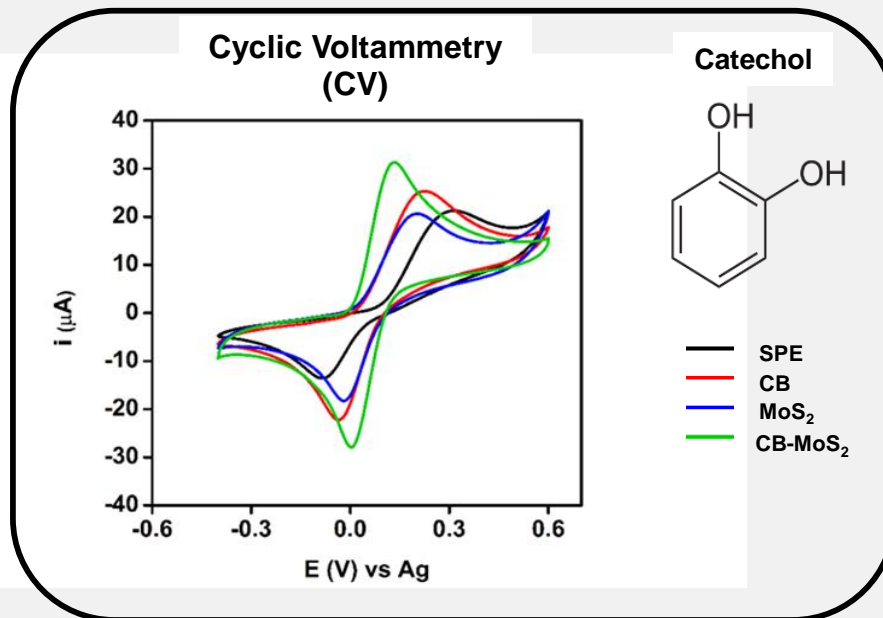
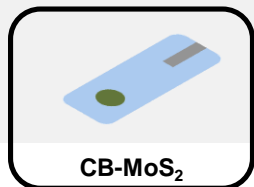
MoS<sub>2</sub> hybrids

WS<sub>2</sub> hybrids

Group VI Transition Metal Dichalcogenides

## Electrochemical characterization

Catechol as representative redox moiety in food polyphenols



Catechol moiety is present in highly antioxidant polyphenols

CB-MoS<sub>2</sub> nanohybrids shows an enhanced and synergistic electrocatalytic effect towards catechol

D. Rojas, F. Della Pelle, M. Del Carlo, E. Fratini, A. Escarpa, D. Compagnone.  
Microchim. Acta. 186 (2019) 363.

# Transition Metal Dichalcogenides and their hybrid nanomaterials

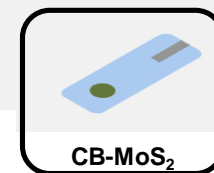
Objective

MoS<sub>2</sub> Nanohybrids

WS<sub>2</sub> Nanohybrids

Group VI Transition Metal Dichalcogenides

Application of CB-MoS<sub>2</sub> electrochemical sensors to different food matrices

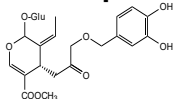


✓ Catalytic effect extensive to complex structures

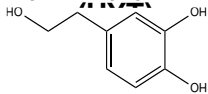
Correlation matrix: electrochemical sensor and well established analysis

	CB-MoS <sub>2</sub>	HPLC-UV	ABTS	AuNPs	FC
Olive Oil		0.995	-	-	-
Cocoa		-	0.966	0.949	0.972

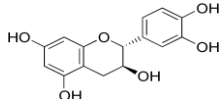
Oleuropein



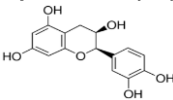
Hydroxytyrosol



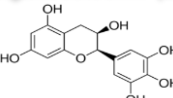
Catechin (CT)



Epicatechin (EP)



Epigallocatechin (EG)



Olive oil



Cocoa



✓ Antifouling effect: nanohybrid n=50 measurements vs n=1 carbon

D. Rojas, F. Della Pelle, M. Del Carlo, E. Fratini, A. Escarpa, D. Compagnone. Microchim. Acta. 186 (2019) 363.

F. Della Pelle, D. Rojas, A. Scroccarello, M. Del Carlo, G. Ferraro, C. Di Mattia, M. Martuscelli, A. Escarpa, D. Compagnone, Sensors Actuators B Chem. 296 (2019) 126651.



Co-funded by:



# Transition Metal Dichalcogenides and their hybrid nanomaterials

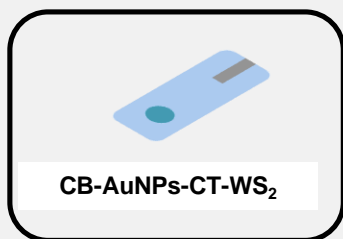
Objective

MoS<sub>2</sub> NanoHybrids

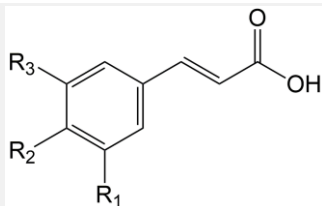
WS<sub>2</sub> NanoHybrids

Group VI Transition Metal Dichalcogenides

## WS<sub>2</sub> nanoHybrids electrochemical behavior towards different hydroxycinnamic acids (hCNs)



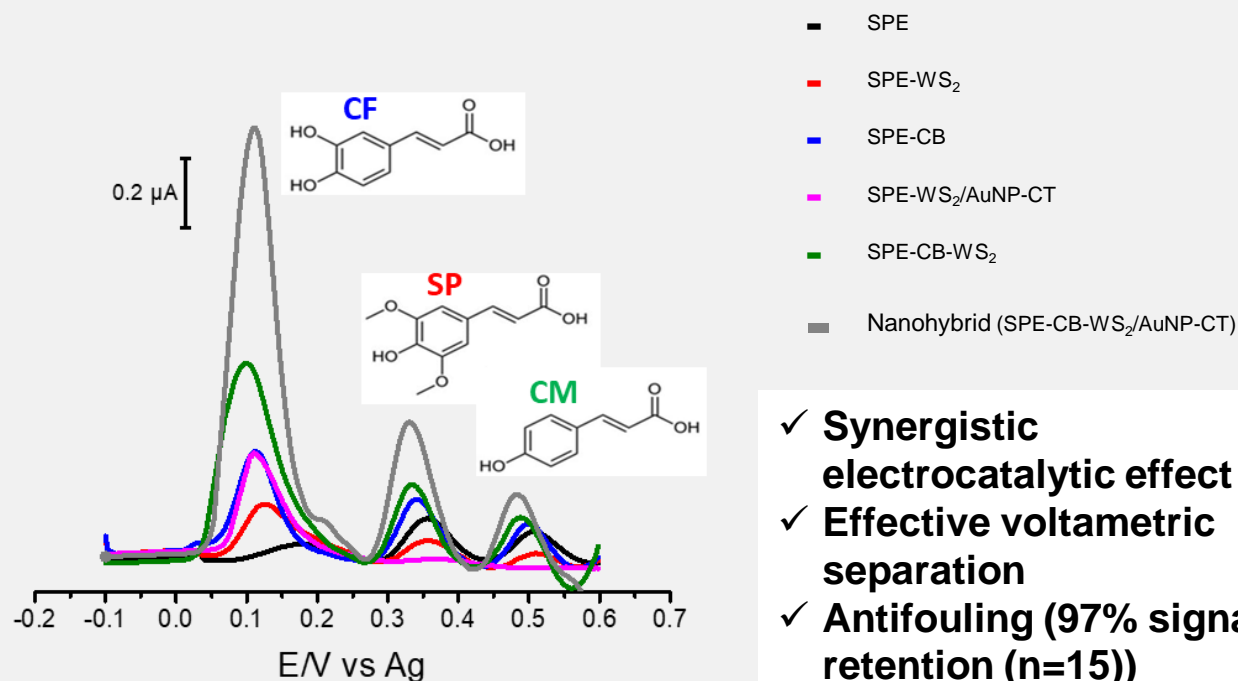
### hCNs general structure



R<sub>1</sub>=R<sub>2</sub>=OH Caffeic Acid

R<sub>1</sub>=R<sub>3</sub>=OCH<sub>3</sub> R<sub>2</sub>=OH Sinapic Acid

R<sub>2</sub>=OH Coumaric Acid



- ✓ Synergistic electrocatalytic effect
- ✓ Effective voltametric separation
- ✓ Antifouling (97% signal retention (n=15))

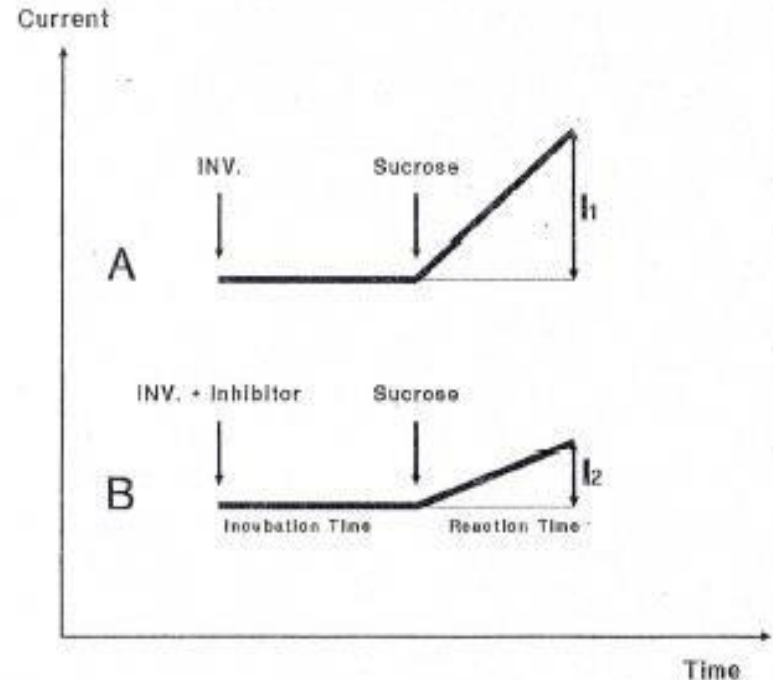
F. Della Pelle, **D. Rojas**, F. Silveri, G. Ferraro, E. Fratini, A. Scroccarello, A. Escarpa, D. Compagnone. *Microchim. Acta.* 187 (2020) 296.

# Biosensors based on enzyme inhibition

There is a great demand for rapid and sensitive analytical methods for the determination of mercury and related compounds in environmental samples. The environmental risk and toxicological concern of mercury and its compounds, especially methylmercury, have stimulated the research into various new methods of trace analysis.

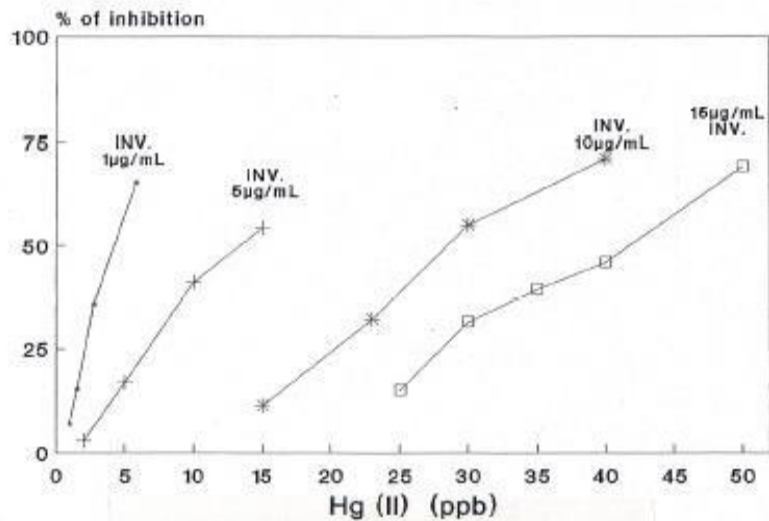
Many enzymes are inhibited specifically by low concentrations of certain chemical substances.

Toxicity of mercury depends on its chemical form. For example methyl mercury is more toxic than HgII (1).

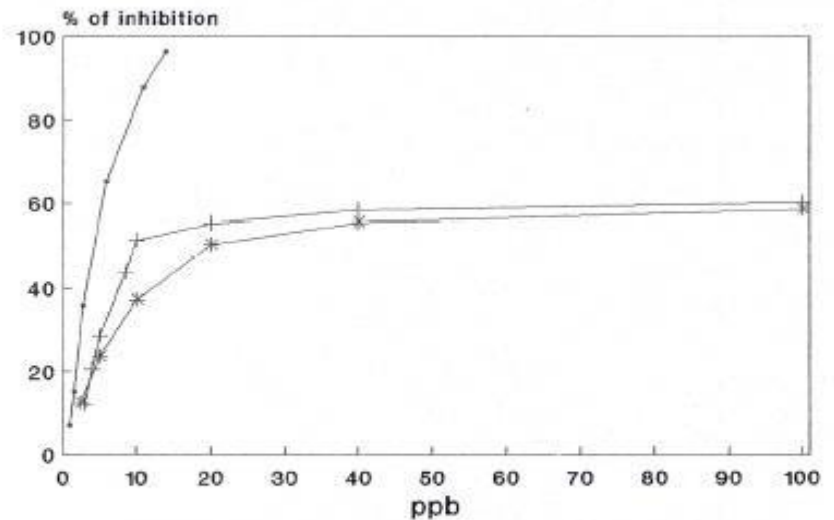


Typical current-time curves obtained in the absence (A) and in the presence (B) of inhibitor.





Calibration curves of Hg(II) with different enzyme concentrations. 5mM sucrose, phosphate buffer pH 6.0. 10 minutes incubation time.



Calibration curves of Hg(II) (.), MetHg (+) and EtHg (\*). 1 µg/mL invertase; 5 mM sucrose; phosphate buffer pH 6.0; 10 min. incubation time.

## **Biosensor for organophosphate and carbamates pesticides (phytochemicals)**

**High acute toxicity (200.000 deaths/year in the 80s)**

**High chronic toxicity**

**Moderate persistence**

**Mechanism of action:**

**inhibition of acetylcholinesterase (AChE)**

**Classical methods:**

**GC-NPD o GC-MS, LC-MS**

# Scheme of the measurement

## step 1

0.125 U/ml AChE

1 mL buffer +  
standard or sample

**10' incubation**

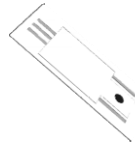
## step 2

Substrate  
(0.3 mM)

**120''**

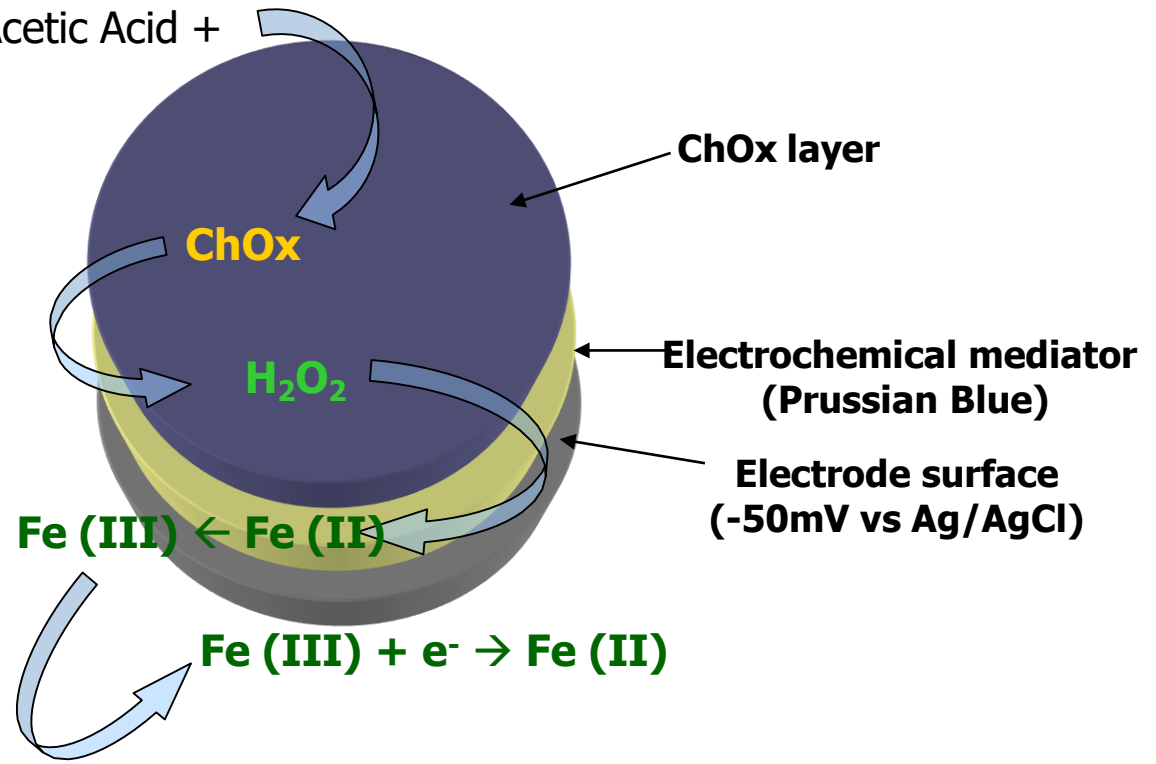
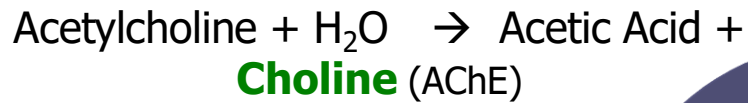
**-50 mV vs Ag/AgCl**

$$RA \% = 100 * \frac{(I_0 - I_s)}{I_0}$$



## Screen printed electrode

### Working electrode reactions pathway



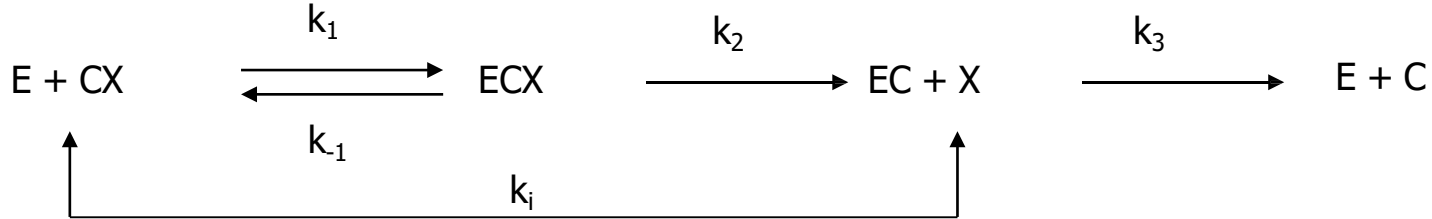
Ability to detect at ng/mL;  
Precision = 10%;  
Total analysis time = 20 min

## Project PON target molecules:

Diclorvos (organophosphate )  
Pirimifos-metile  
(thiophosphate)

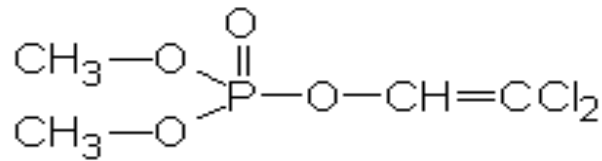
**anticholinesterasic**

### Mechanism of AChE inhibition



**E = enzyme; CX = carbamate or organophosphate; X = leaving group;  $K_d = k_{-1} / k_1k_2$  carbamylation or phosphorylation rate constant;  $k_3 =$  decarbamylation or dephosphorylation rate constant;  $k_i =$  bimolecular rate constant**

# Dichlorvos



**Solubility in water:**

**16 g/l**

2,2-Dichlorovinyl dimethyl phosphate

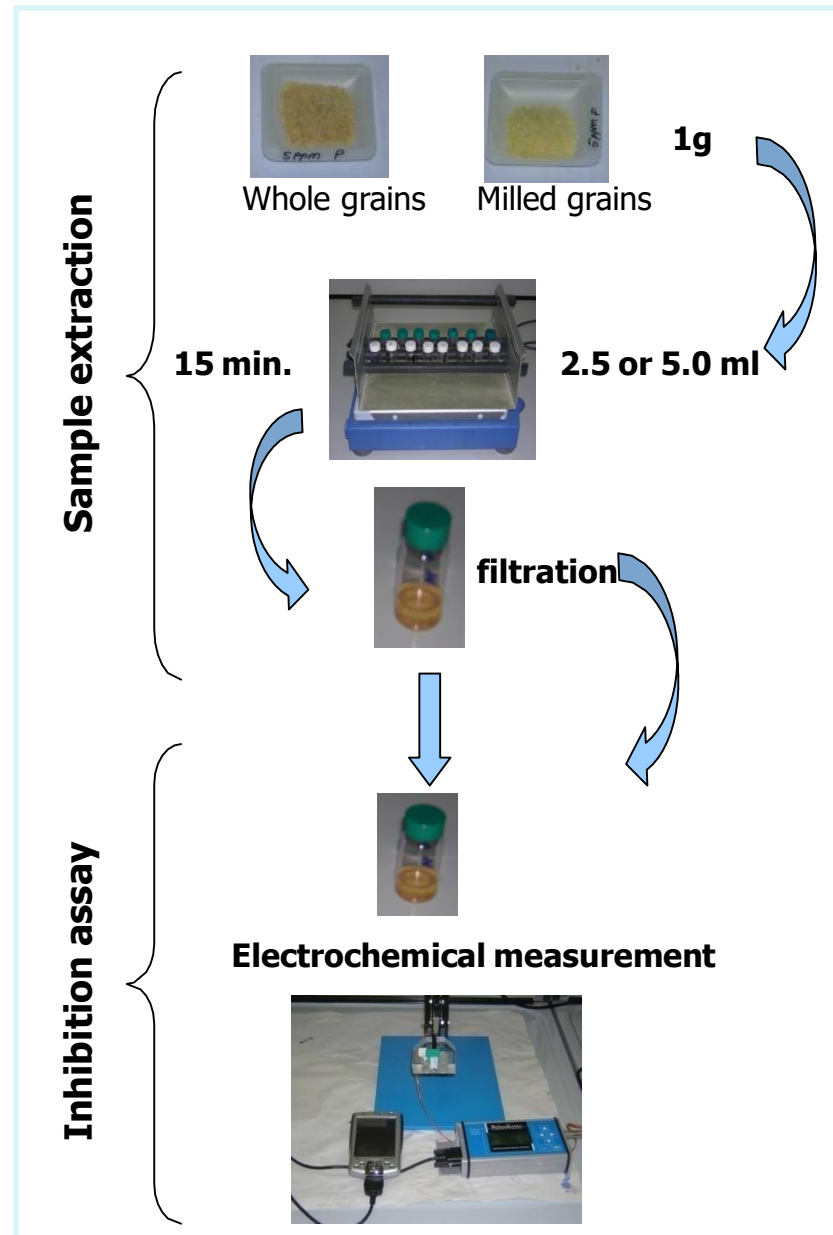
- Commercial formulation **Didivane**® is used as broad spectrum grain protectant insecticide.
- Effective in controlling a wide range of insects that attack stored grain products.
- It provides long-term protection against re-infestation from insects.

The European Union regulates the maximum admissible level in **durum wheat** at **2 mg/Kg** (European Directive 2001/57/CE)

## Quantitative Usage for dichlorvos

The annual agricultural use of dichlorvos was estimated as 248,000/year during 90' (ATSDR, 1997). Estimates done in late 1990s indicate that 60% of dichlorvos used worldwide was for plant protection, 30% was for public hygiene and vector control, and **10% to protect stored crops (WHO, 1999)**.

# Extraction + assay protocol



# Matrix effect on the RA% and the I%

## Experimental conditions:

Extraction in **measuring buffer** (1g/10ml)

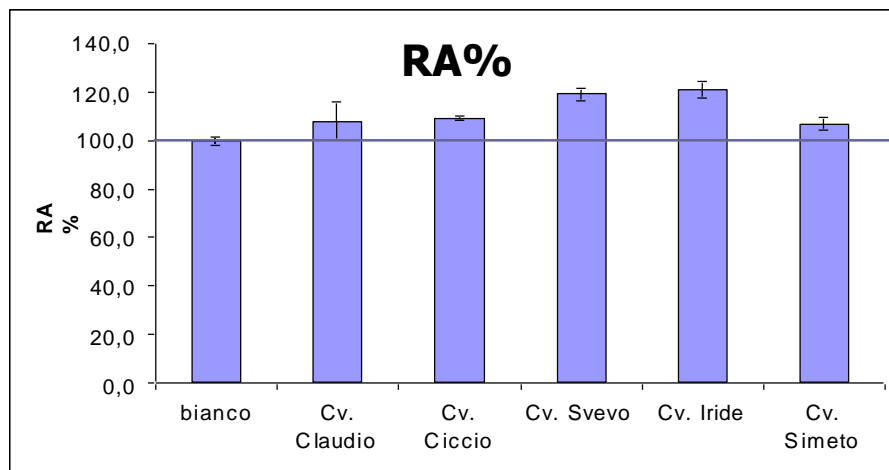
Phosphate buffer pH 7.4, KCl 100 mM

10% matrix

AChE 0.125 U/ml, Ach 0.3 mM

Incubation time: 10 min.

$$RA\% = 100 - (I_0 - I_s / I_0) * 100$$



## Experimental conditions:

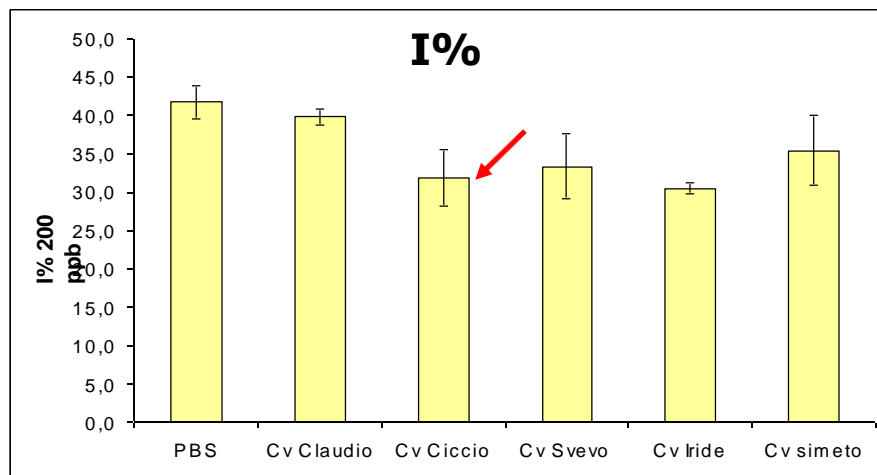
Phosphate buffer pH 7.4, KCl 100 mM

10% matrix

AChE 0.125 U/ml, Ach 0.3 mM

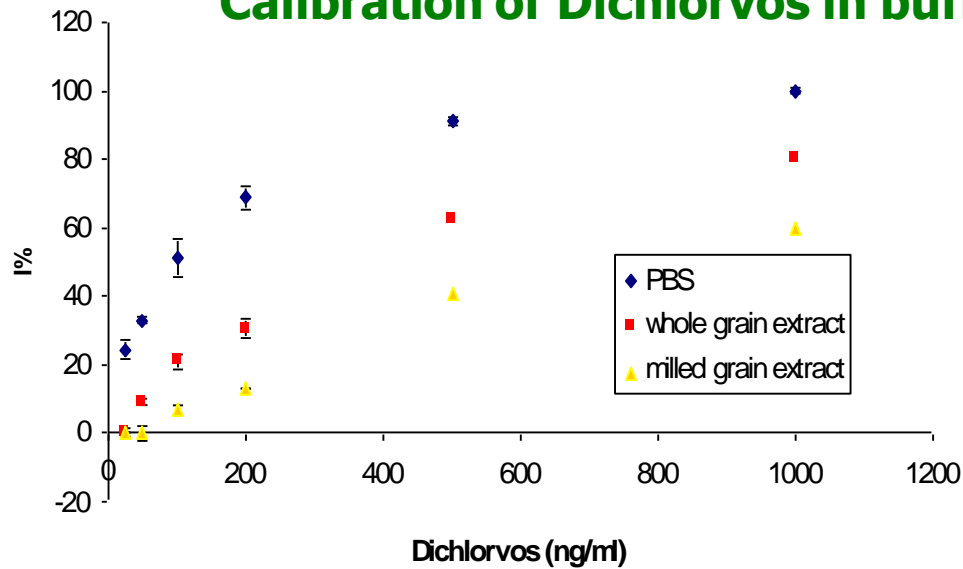
200 ng/ml dichlorvos

Incubation time: 10 min.





# Calibration of Dichlorvos in buffer and 10% matrix



**Buffer**

$$y = -1035.26 + \frac{1124.83}{\left\{1 + e^{-\left[\frac{(x + 776.54)}{303.60}\right]}\right\}}$$

**R=0.998**  
**LOD= 8 ng/ml**  
**I<sub>50%</sub>=230 ng/ml**

**Whole grains extract (10%)**

$$y = -463.20 + \frac{566.30}{\left\{1 + e^{-\left[\frac{(x + 755.36)}{489.98}\right]}\right\}}$$

**R=0.992**  
**LOD= 45 ng/ml**  
**I<sub>50%</sub>=360 ng/ml**

**LOD = 0.45 mg/Kg**

**milled grains extract (10%)**

$$y = -20.86 + \frac{83.82}{\left\{1 + e^{-\left[\frac{(x - 276.15)}{218.04}\right]}\right\}}$$

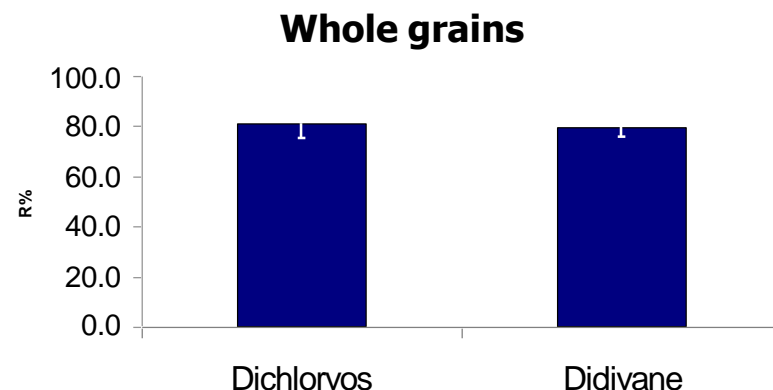
**R=0.998**  
**LOD= 130 ng/ml**  
**I<sub>50%</sub>=650 ng/ml**

**LOD = 1.3 mg/Kg**

# Recovery from spiked samples (2 mg/Kg)

## Experimental conditions:

Spiked samples  
Extraction: 1g **whole grains sample** in 10ml buffer  
AChE 0.125 U/ml, Ach 0.3 mM  
Incubation time: 10 min.  
**n=5**



Mean I%: 21.0 ± 1.3

Mean I%: 19.2 ± 1.2

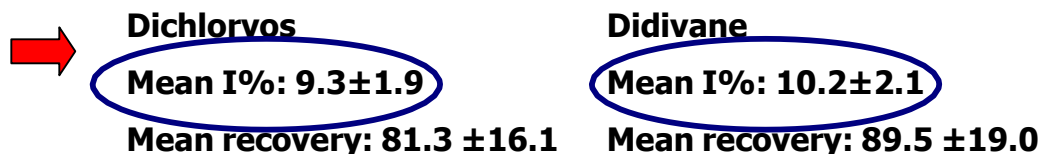
Mean recovery: 81.3 ± 5.8

Mean recovery: 79.5 ± 3.5

## Experimental conditions:

Spiked samples  
Extraction: 1g **milled grains sample** in 10ml buffer  
AChE 0.125 U/ml, Ach 0.3 mM  
Incubation time: 10 min.  
**n=5**

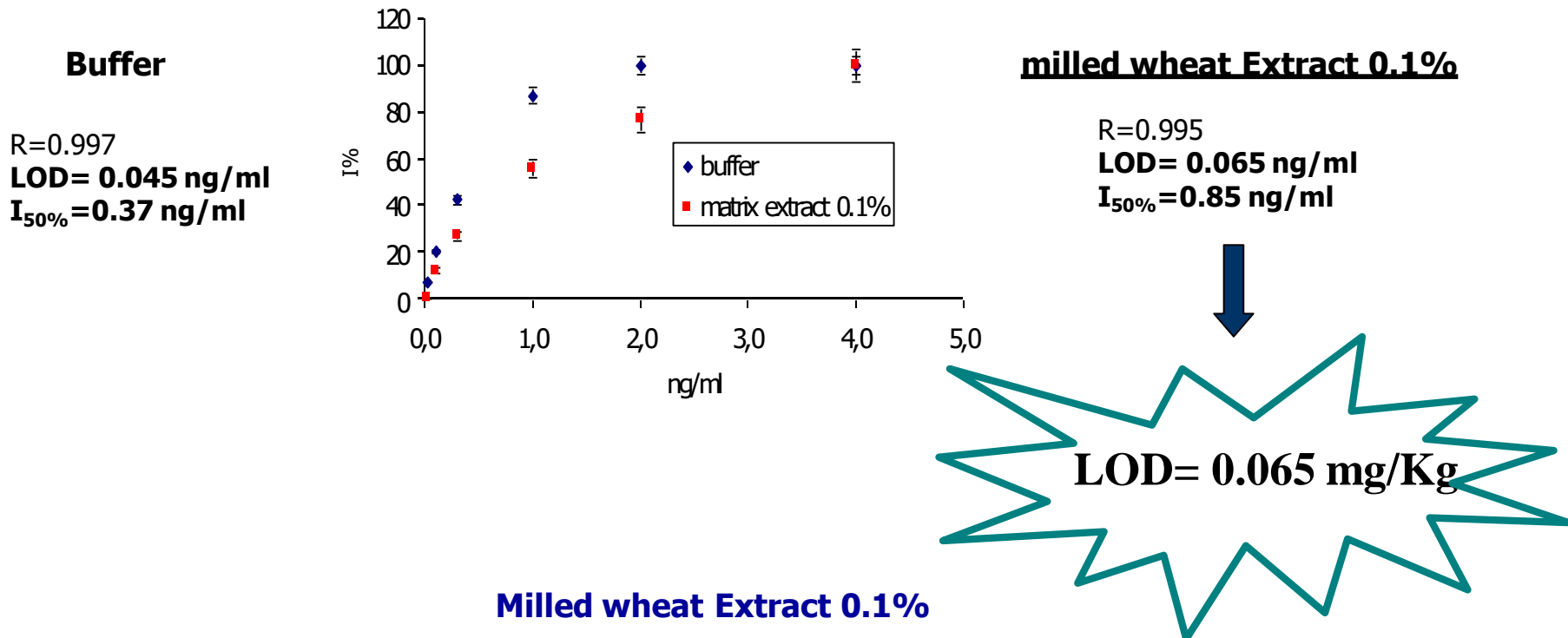
## Milled grains



**I%<sub>LOD</sub> = 8% !!**

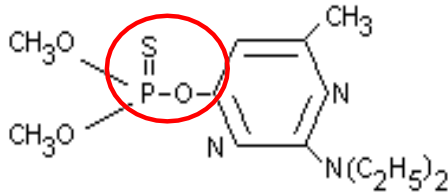
# Use of recombinant acetylcholinesterase (rAChE)

rAChE: Mutant AChE from *Drosophila melanogaster* Clone B3 specific for dichlorvos  
 Fournier D et al. Protein Engineering, Vol. 15, No. 1, 43-50, January 2002



	Mean I% ±SD	Calc. Conc. measuring soln. (ng/ml)	Mean recovery ±SD (mg/Kg)
<b>Dichlorvos (n=3)</b>	70.2±4.5	1.5	75.0±4.8
<b>Didivane (n=3)</b>	69.2±7.3	1.45	72.5±7.6

# Pirimiphos methyl



**Solubility in water:**

**5 mg/l**

**S. methanol, ethanol, acetone: all proportions**

(O-[2-(diethylamino)-6-methyl-4-pyrimidinyl]O,O-dimethylphosphorothioate)

- Commercial formulation Actellic® is used as broad spectrum grain protectant insecticide.
- Effective in controlling a wide range of insects that attack stored grain products.
- It provides long-term protection against re-infestation from insects.

The European Union regulates the maximum admissible level in durum wheat at **5 mg/Kg** (European Directive 2001/57/CE)

## Quantitative Usage Analysis for Pirimiphos-Methyl (EPA 1989-1997)

Total annual USA usage was 12,000 pounds active ingredient (a.i.). Total usage is allocated mainly to **stored corn grain** (39%), **stored sorghum grain** (15%), **corn seed** (5%) and **sorghum seed** (5%).

In **Europe** it is mainly used for wheat, corn, barley (45% in Scotland and Ireland) storage.

In **Italy** the major durum wheat transforming brands indicate pirimiphos methyl as a pest control chemical for post-harvest storage.

# Effect of methanol and acetone on the Residual Activity of AChE

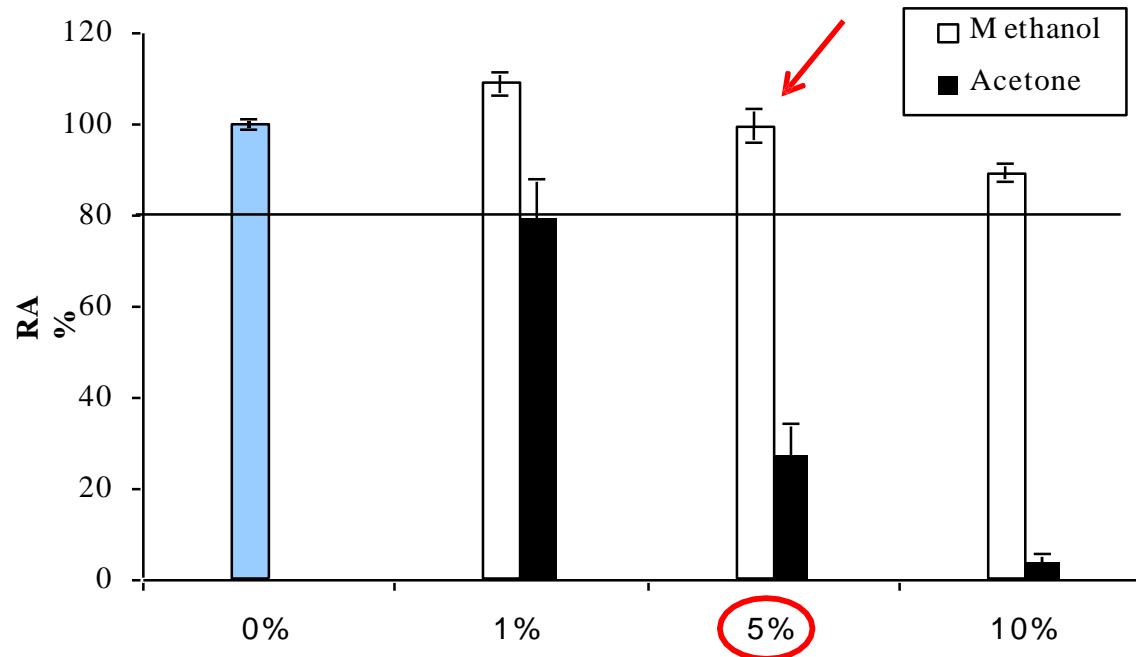
## Experimental conditions:

Phosphate buffer pH 7.4, KCl 100 mM

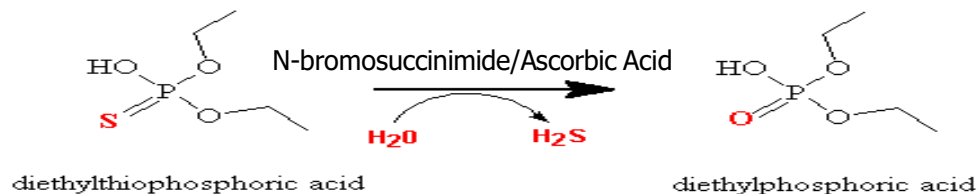
AChE 0.125 U/ml, Ach 0.3 mM

Incubation time: 10 min.

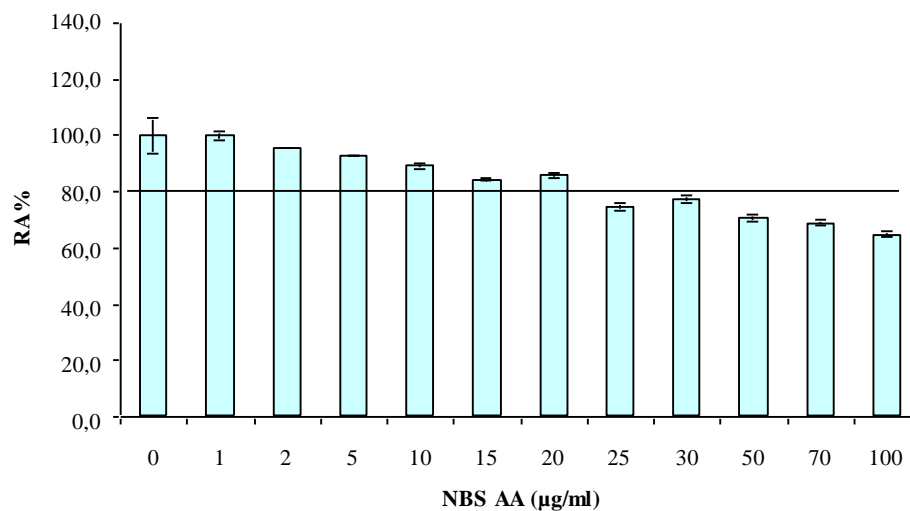
$$RA\% = 100 - (I_0 - I_s / I_0) * 100$$



# Oxidation of pirimiphos methyl



**Influence of NBS and AA concentration on the blank response evaluated as RA%.**



**Inhibition obtained with 250 ng/ml of pirimiphos methyl using different concentration of NBS and AA.**

NBS-AA µg/ml	I% (n=3)	SD	CV%
5	31.5	2.2	6.9
10	28.6	2.4	8.3
25	28.2	3.1	10.9

# Paper as substrate



Paper can...

Store

Filter

React

Drawbacks...

Reagents diffusion...

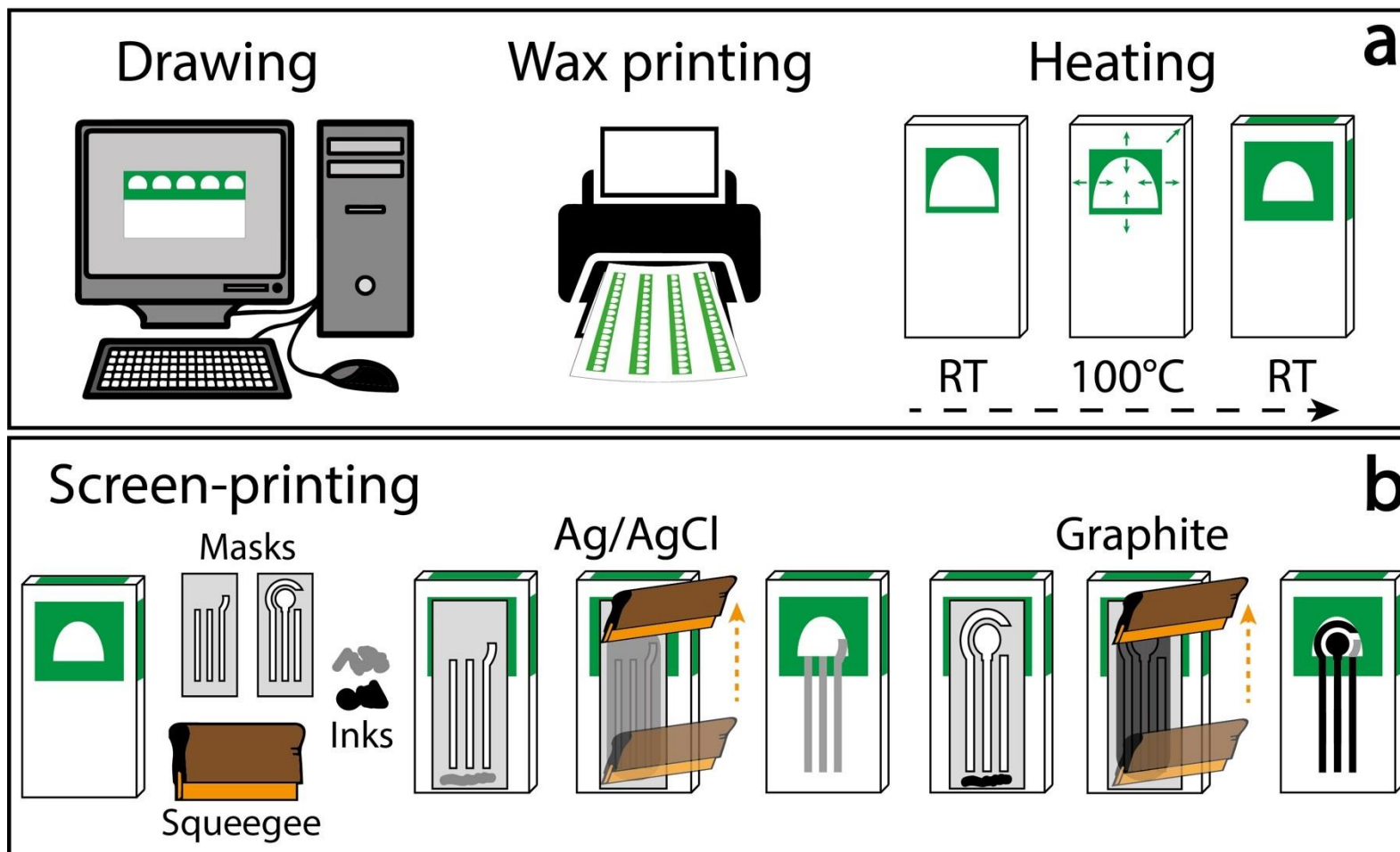
Electrical noise! ☹️

An hydrophobic  
barrier

is needed...

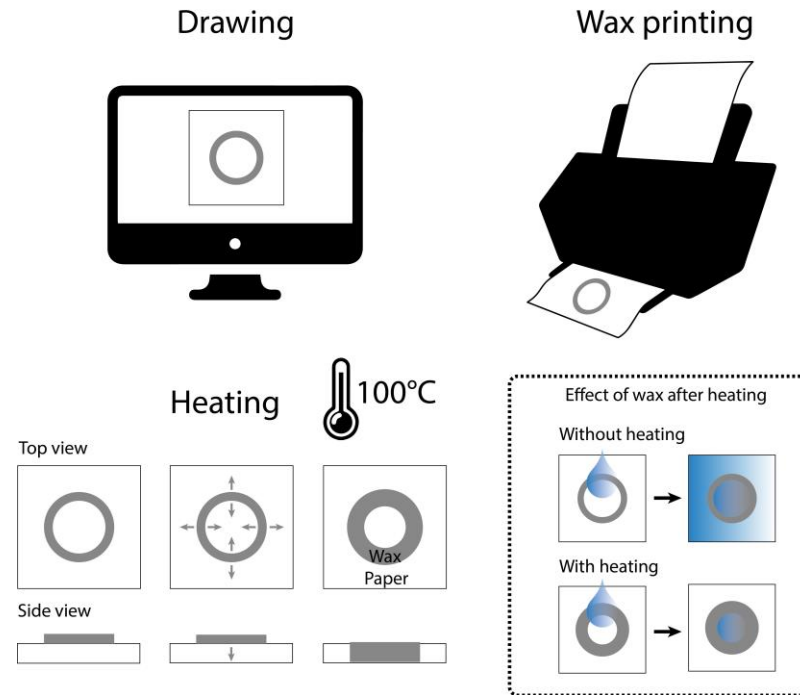
# From Paper to E-Paper

Few and easy steps





# Hydrophilicity matters



... also the cost!

Costs of the components for producing one device (all the costs have to be intended in Euro).

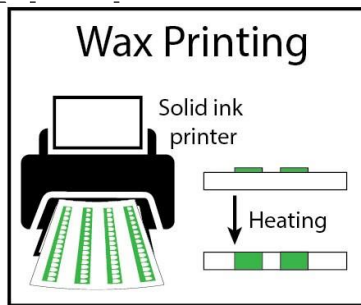
Substrate	Ag/AgCl ink	Carbon ink	Insulator	Substrate	Total cost	Saving <sup>c</sup>
Polyester	0.010	0.007	0.003 <sup>a</sup>	0.013	0.033	45%
Whatman #1			0.001 <sup>b</sup>	0.007	0.025	30%
Office paper			0.001 <sup>b</sup>	0.0001	0.018	/

<sup>a</sup> Insulator ink.

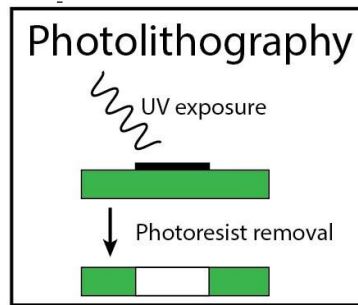
<sup>b</sup> Wax.

<sup>c</sup> Calculated as  $1 - [\text{Office paper}/\text{Other}] \times 100$ .

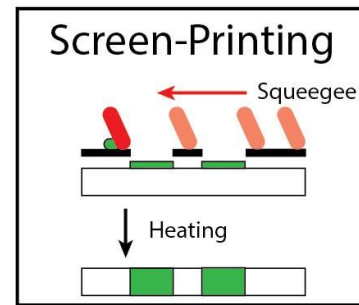
# It depends on what you need and you have!



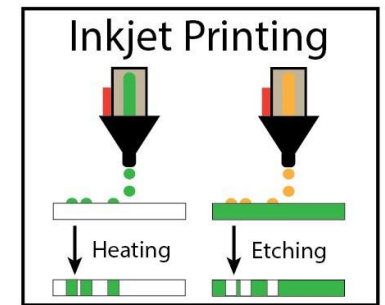
Sustainable  
Low resolution



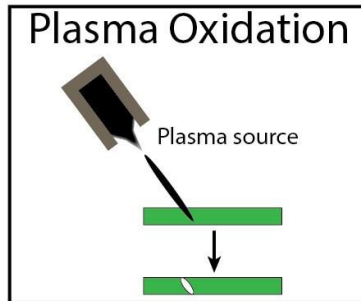
High resolution  
Expensive



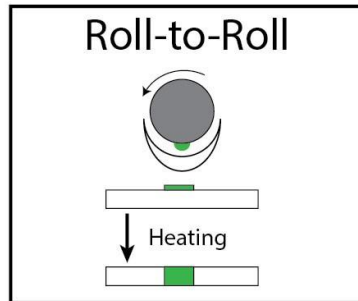
Easiness  
Ad hoc masks



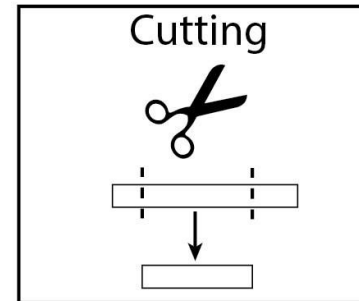
Reduced waste  
Expensive printer



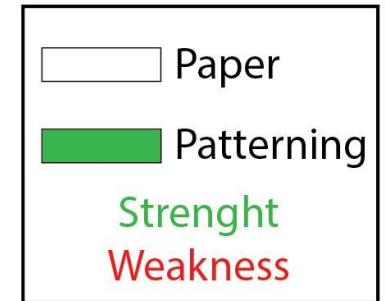
Cheap patterning  
Hydrophobized paper



Mass scalable  
Too many steps



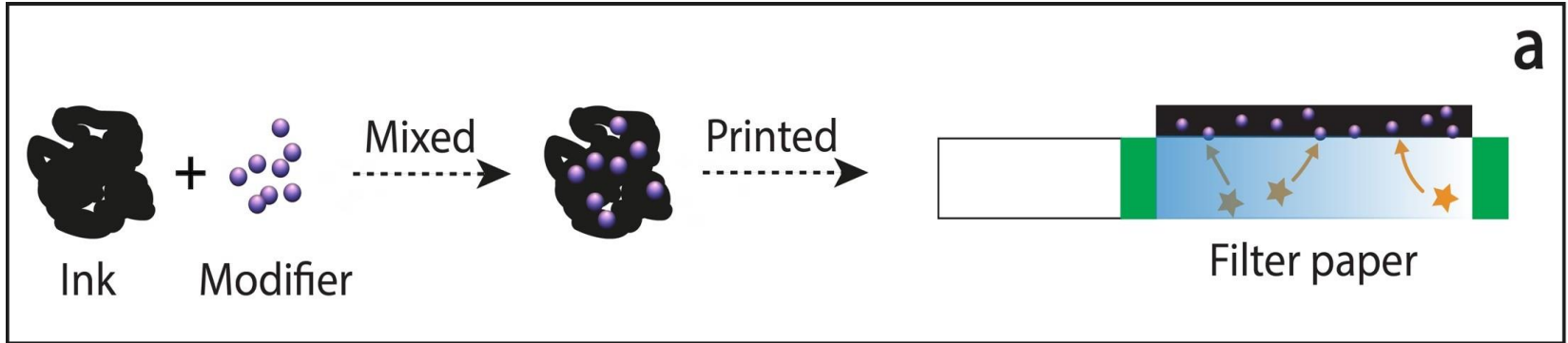
Low-cost  
No channels



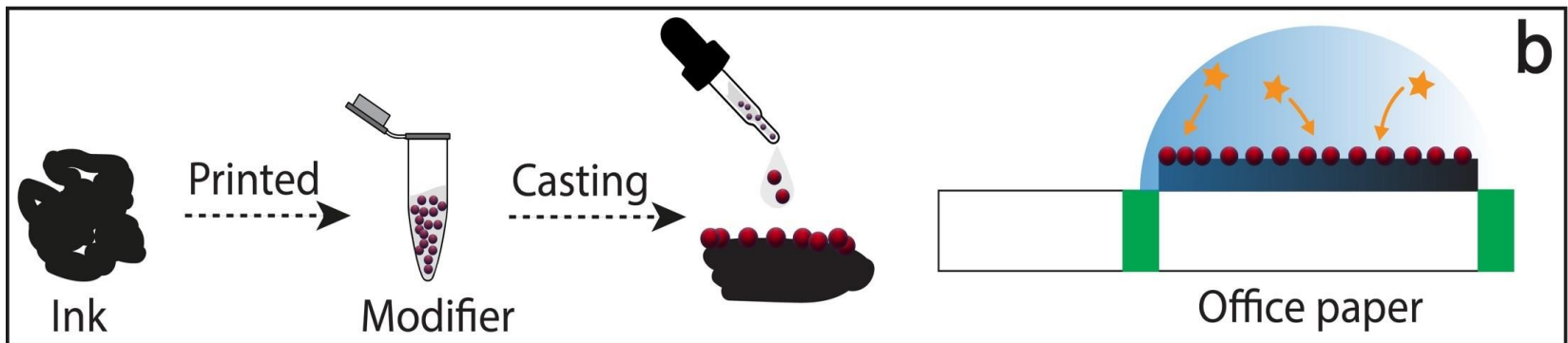
Strenght  
Weakness

# Which E-Paper?

## Porous

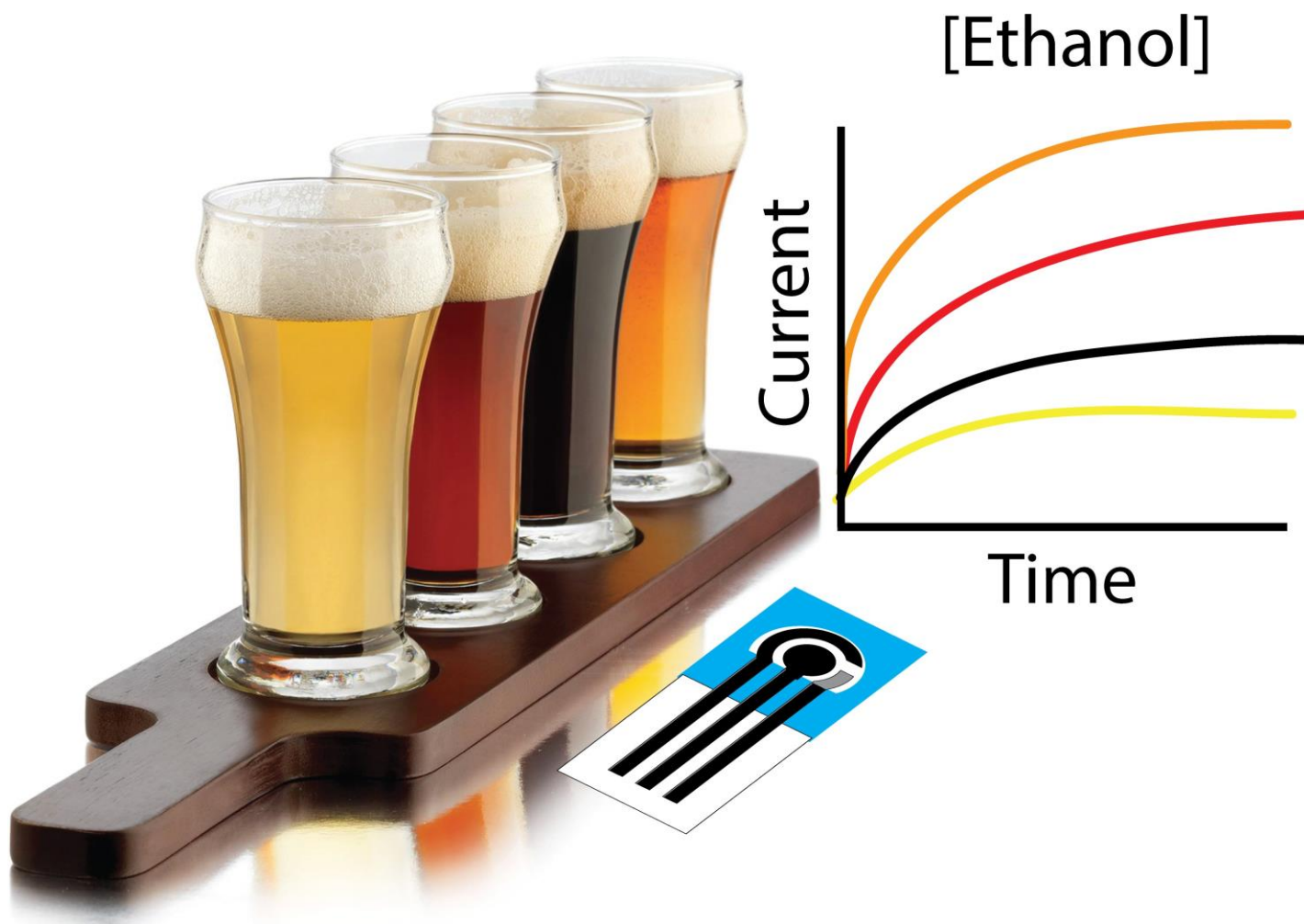


## Non porous

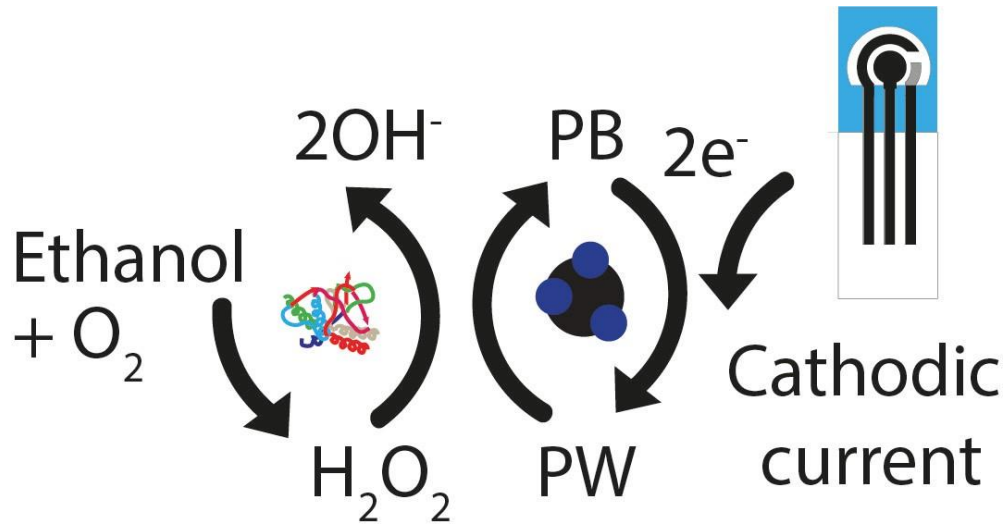


Anyway, paper is the substrate... we need to make these strips ad-hoc

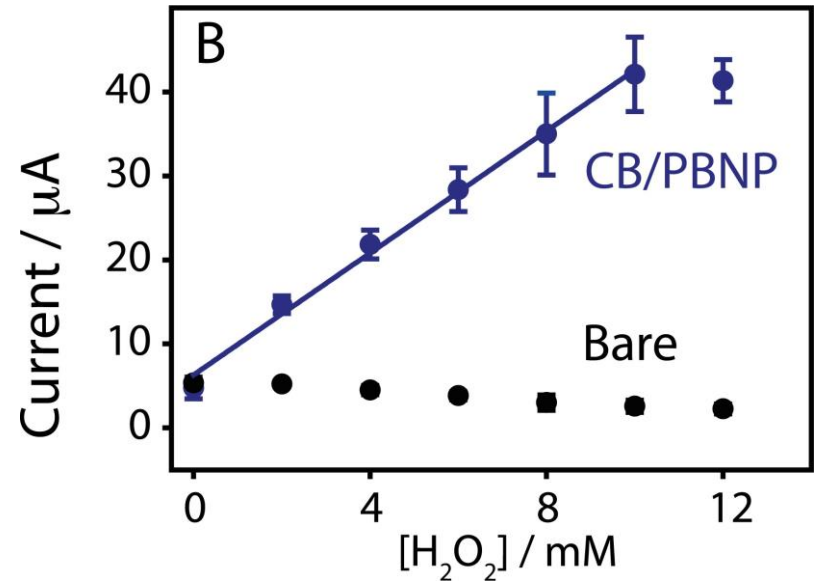
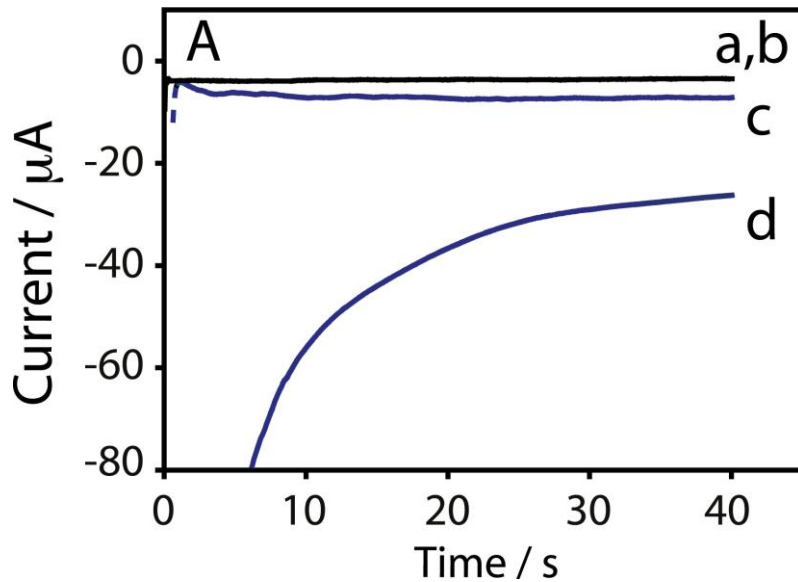
# Office paper for ethanol



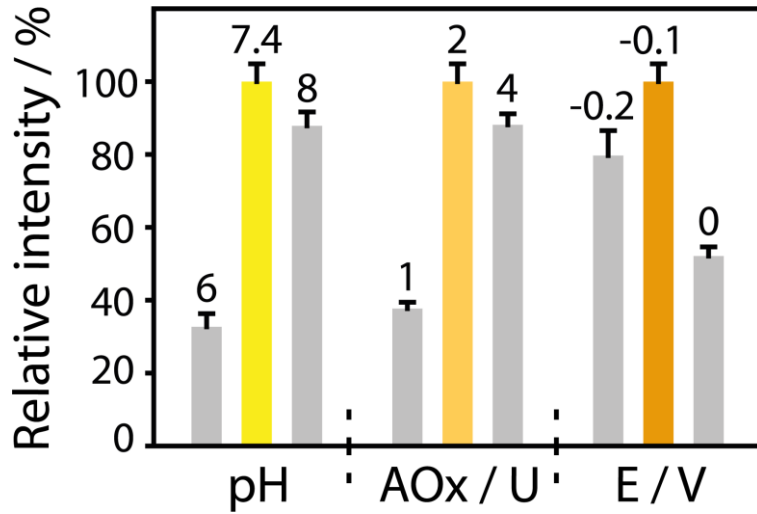
# Detection mechanism



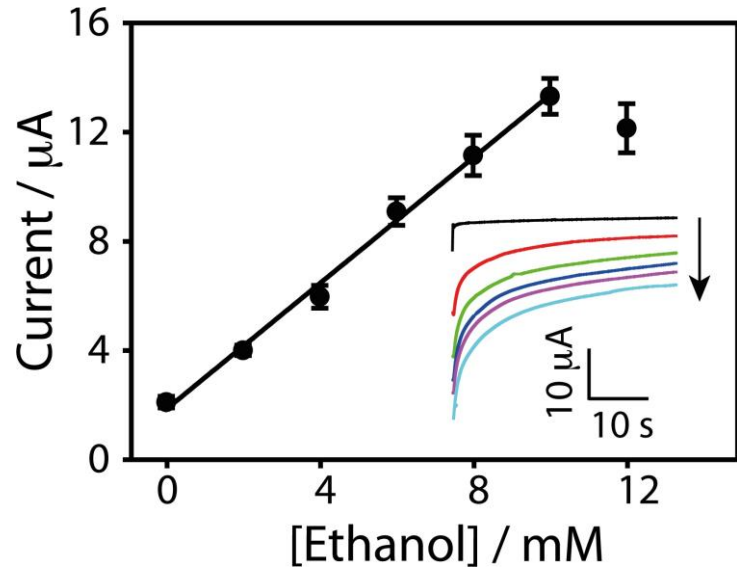
- Office paper
- Carbon Black
- Prussian Blue
- Alcohol oxidase



## Optimization



## Calibration curve



**LOD = 0.5 mM**

**Linear range up to 10 mM**

**RSD = 8 %**

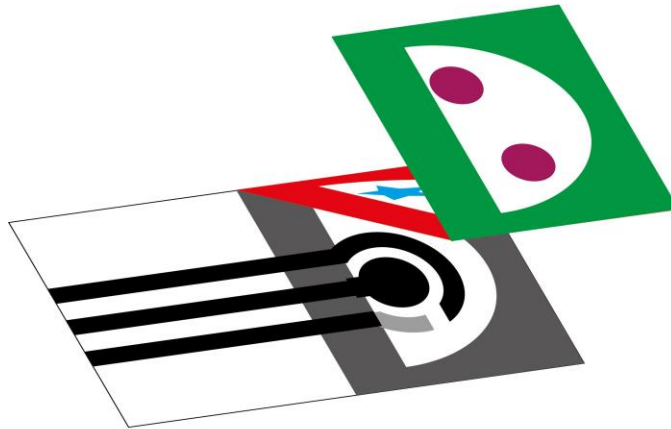
## Accordance with label

Detection of ethanol in commercial beers.

Beer	Lager Best Bräu, Poland	Weiss Franziskaner, Germany	Pilsner Ceres, Denmark	Alcohol free Tourtel, Italy
Label [ethanol]/%vol (M)	4.7% (0.805 M)	5% (0.856 M)	4.6% (0.787 M)	<0.5% (0.086 M)
Found [ethanol]/%vol (M)	4.7 ± 0.4 (0.805 ± 0.075)	5.0 ± 0.4 (0.86 ± 0.07)	4.4 ± 0.2 (0.75 ± 0.04)	0.34 ± 0.03 (0.059 ± 0.004)
RSD/%	9.3	8.1	5.3	6.8

# 3-D paper origami for pesticides

## Filter paper + office paper



Substrate pad

Enzyme pad

Paper-based SPE



Waxed filter paper



Enzyme + buffer



Waxed office paper

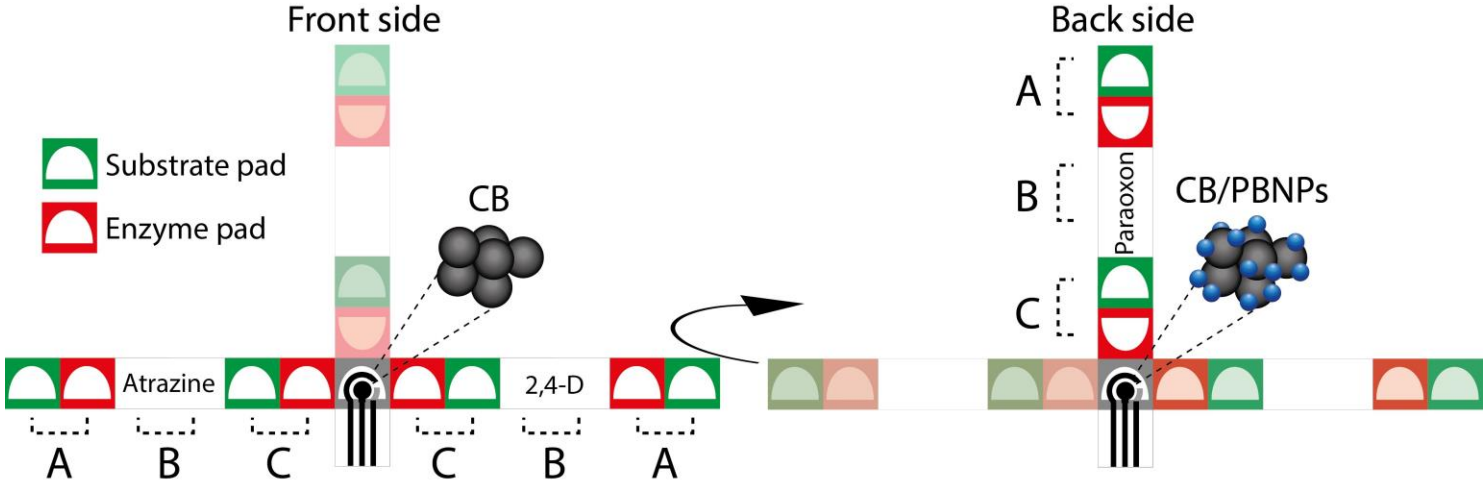


Substrate + electrolyte

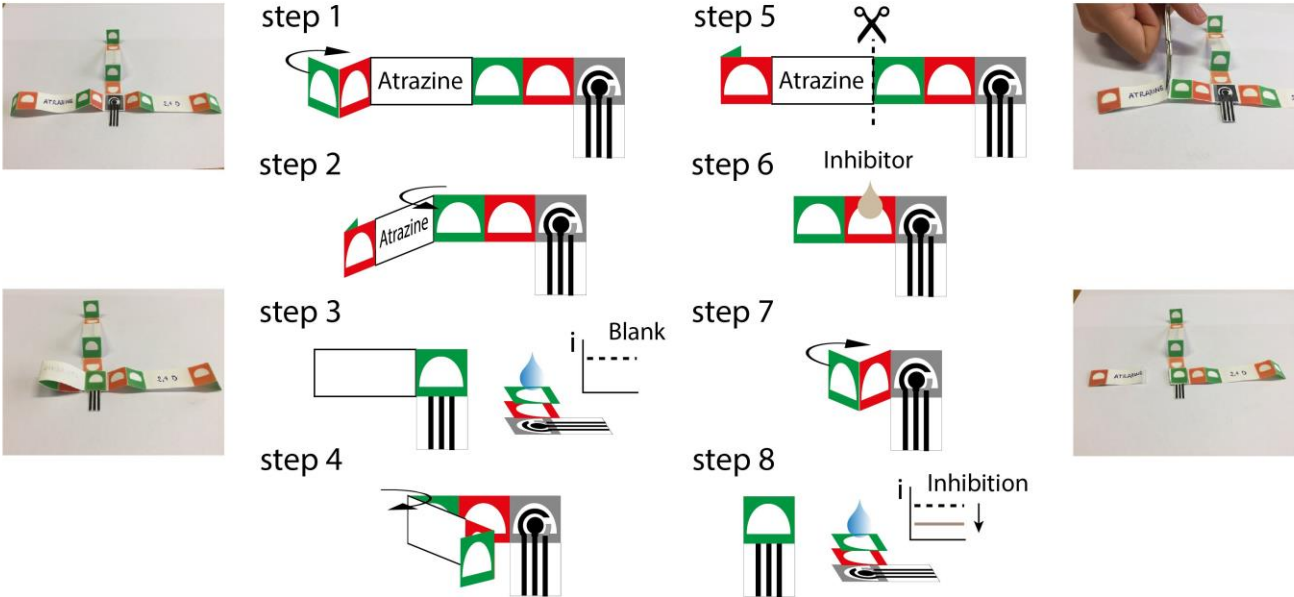
**Paraoxon, 2,4-dichlorophenoxyacetic acid, and atrazine by inhibition of butyrylcholinesterase, alkaline phosphatase, and tyrosinase**



# Configuration

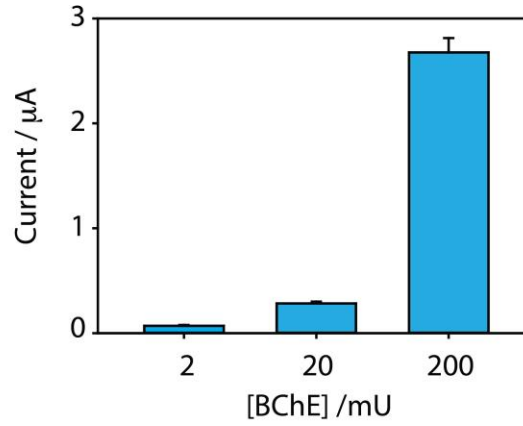
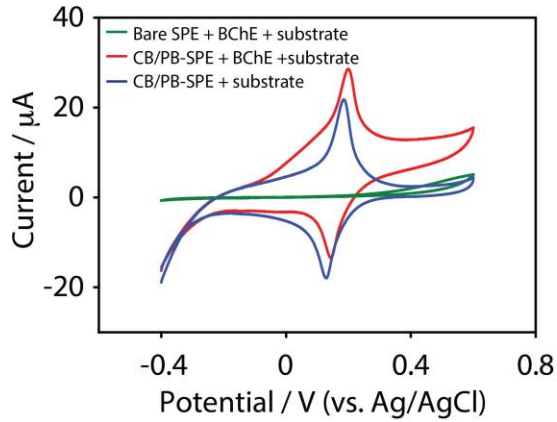


# Measurements, e.g. Atrazine





# E.g. paraoxon detection



**LOD = 2 ppb**

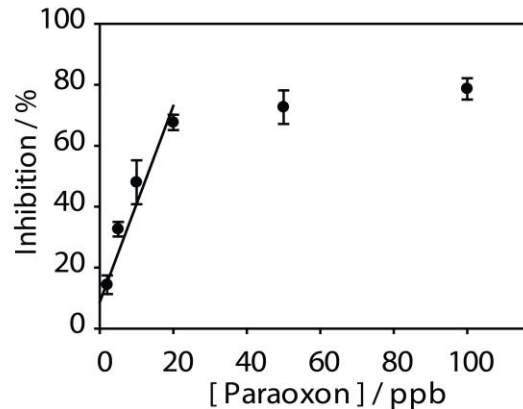
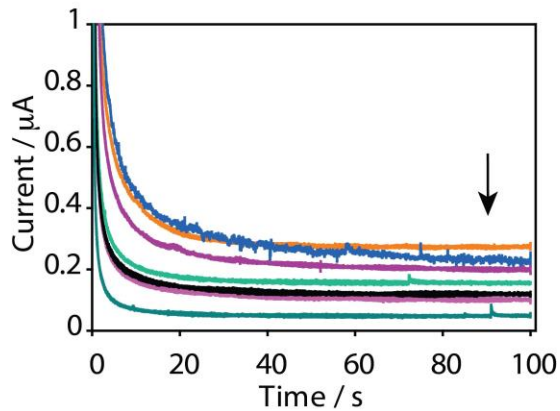
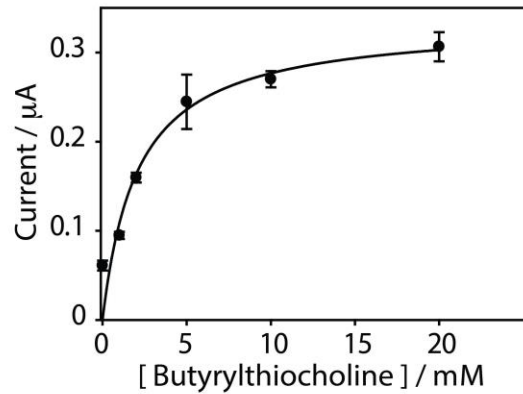
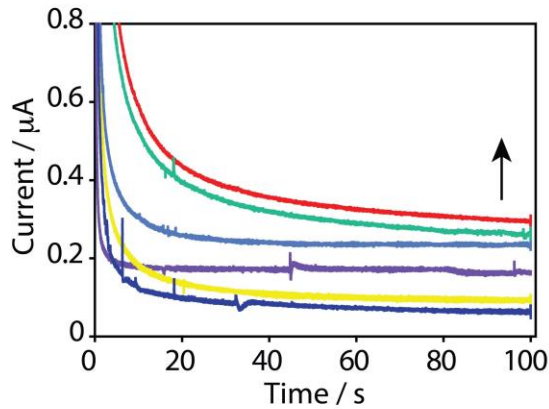
**Linear range up to 30 ppb**

**RSD = 11%**

**Real sample: River water**

**Linear up to 30 ppb**

**Recoveries: 90 and 88%  
(10 and 20 ppb)**

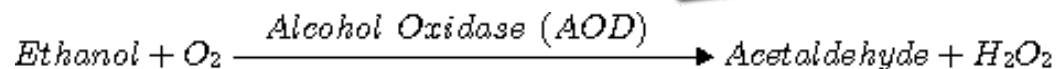


# Multianalita (zuccheri, alcol, acidi organici) Universal Sensor

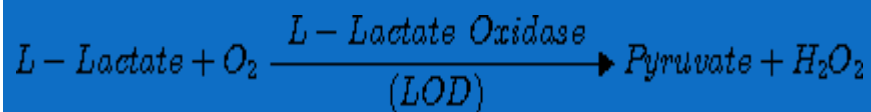


# Etanolo

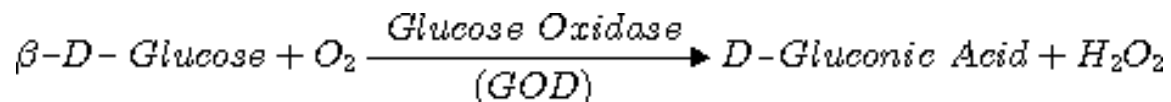
Analex  
instruments



# Acido lattico, monouso (Sens-Lab)



Glucose, Lactate, Ethanol,  
Methanol, Ammonia, Glycerol,  
Sucrose, Lactose and Glutamine






# SENZYTEC 1 portable laboratory

## Biosensori per l'Industria Alimentare

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