

Corso di Biosensori

Programma

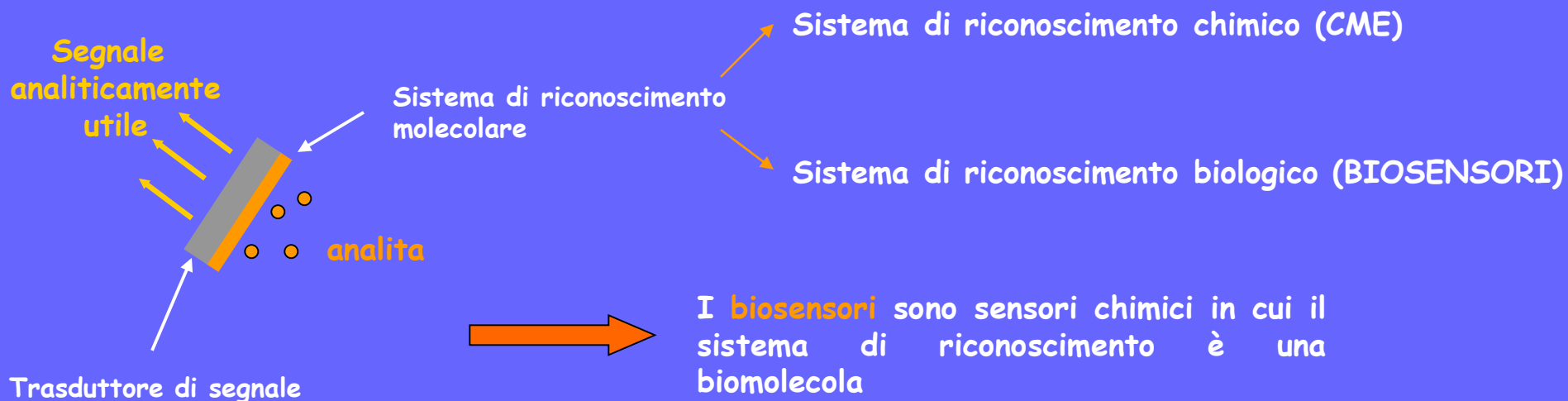
Definizione e classificazione, Biosensori elettrochimici basati su tessuti e cellule, misura di BOD, enzimi ossidasi e deidrogenasi per la realizzazione di biosensori, trasduttori di platino e grafite, elettrodi monouso, sensori per la misura di NADH. Tecniche di immobilizzazione. L'elettrodo ad enzima: selettività, sensibilità, tempo di vita e di risposta. Strategie per l'eliminazione delle interferenze. Elettrodi di seconda e terza generazione. Sensori elettrochimici e nanomateriali. Misura in flusso e ad iniezione in flusso. Applicazioni in campo clinico; i biosensori a glucosio per il monitoraggio del diabete. Elettrodi ad enzima per il controllo di qualità degli alimenti. Elettrodi ad inibizione per la determinazione di fitofarmaci. Biosensori di affinità. Confronto tra tecniche ELISA e immunosensori: realizzazione e caratteristiche analitiche. Immunosensori elettrochimici per micotossine. Sensori ottici e loro caratteristiche, sensori ottici a risonanza plasmonica di superficie e piezoelettrici. Caratteristiche analitiche e potenziali applicazioni. Sensori ottici e nanomateriali. Sensori a DNA, monitoraggio dell'ibridazione. Recettori biomimetici per la realizzazione di biosensori. Lab-on-chip.

Cosa è un biosensore?

"...a biosensor is a self-contained intergated 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."

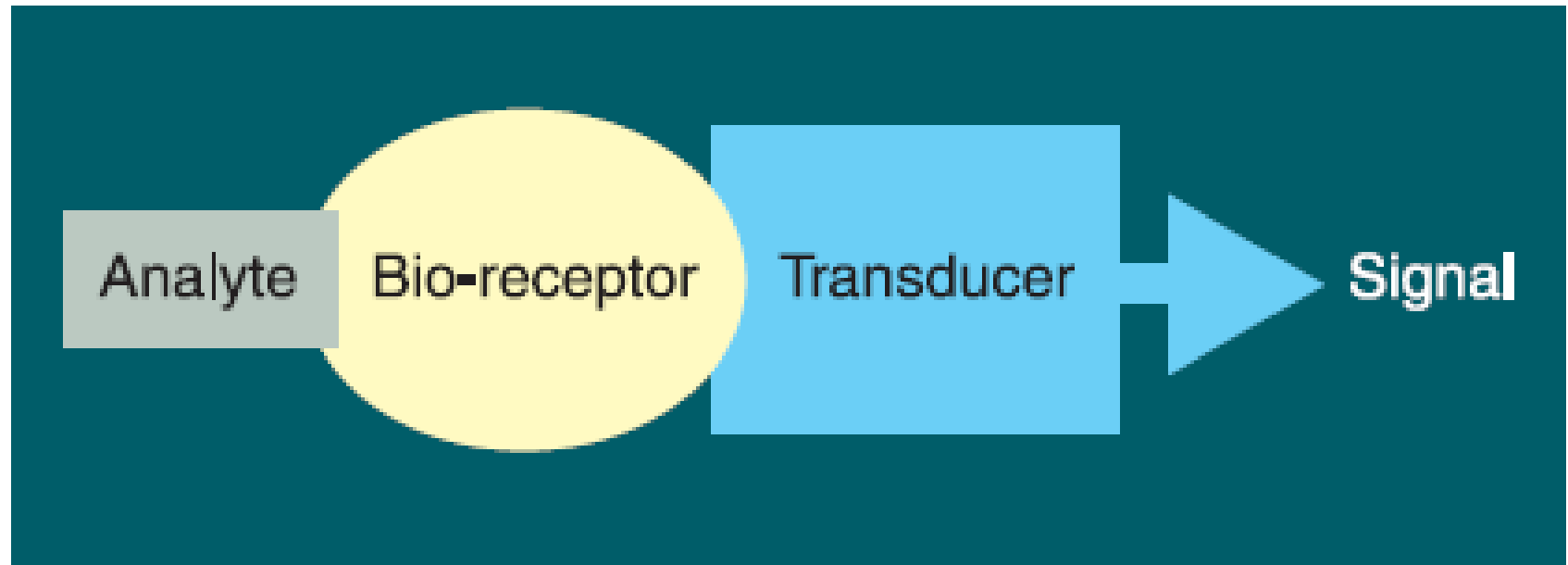
Technical report "Reccomended definition and classification"
IUPAC (Physical Chemistry and Analytical Chemistry Divisions) 2001

Un **sensore** è un dispositivo in grado di trasformare informazioni fisiche o chimiche, come la concentrazione di uno o piu' componenti di una soluzione, in un segnale analiticamente utile.

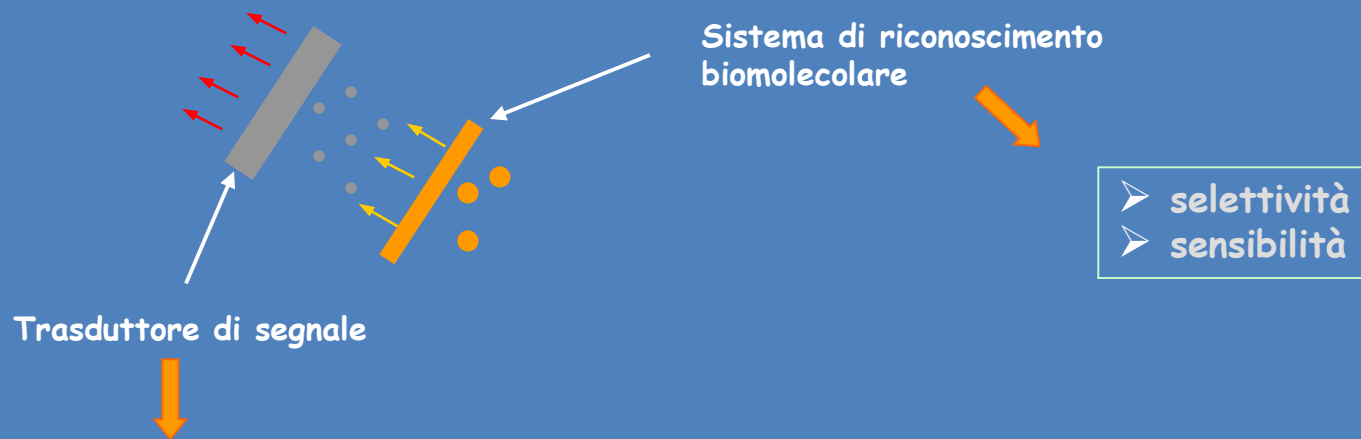




Schematic Diagram of a Biosensor



Il sistema di riconoscimento biologico traduce informazioni del 'dominio biochimico' (solitamente la concentrazione) in un segnale chimico o fisico con una determinata sensibilità.



➤ selettività
➤ sensibilità

➤ trasferimento del segnale generato dalla bio-molecola al dominio elettrico
➤ sensibilità
➤ selettività

Principali tipi di trasduttori

- elettrochimico
- ottico
- piezoelettrico
- termico



- potenziometrici
- amperometrici
- voltammetrici
- conduttometrici

Classificazione in base all'elemento di riconoscimento molecolare

Biosensori catalitici

la reazione di riconoscimento catalizza una reazione chimica
es: enzimi, cellule, tessuti

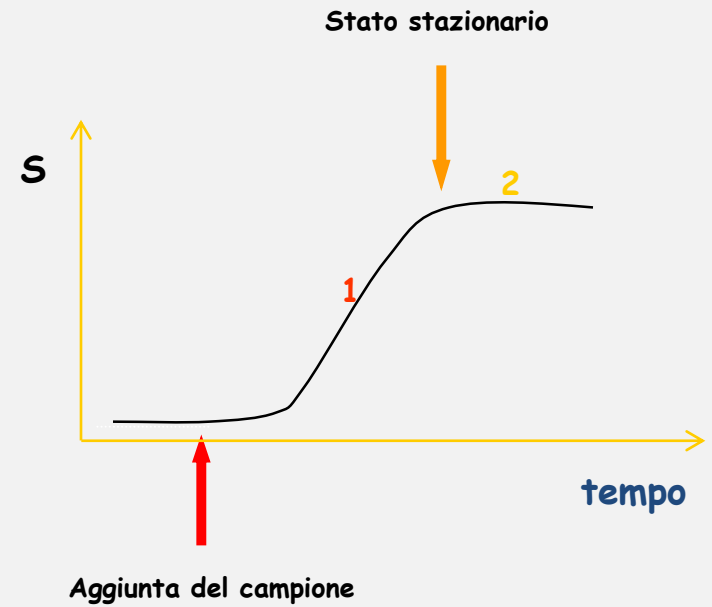
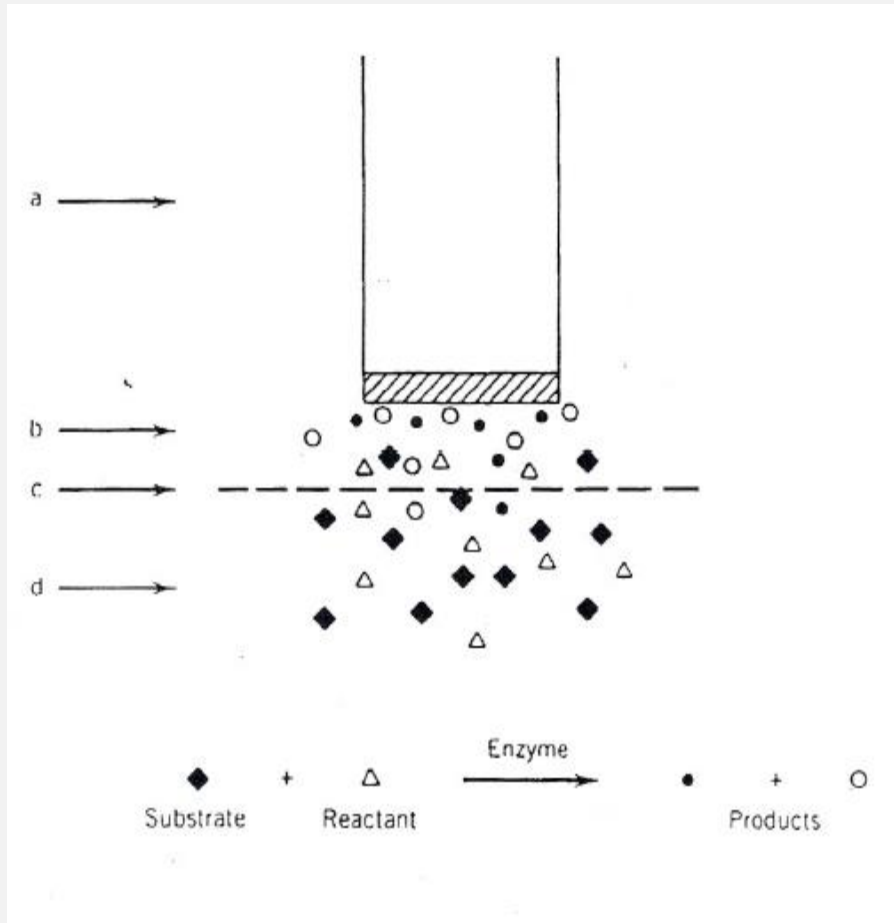
Biosensori di affinità

la reazione di riconoscimento determina la formazione di un complesso
es: anticorpi, filamenti di oligonucleotidi, recettori biologici

Biosensori biomimetici

la reazione di riconoscimento avviene tra un recettore sintetico e l'analita con formazione di un complesso
es: peptidi sintetici, MIP, aptameri

Elettrodi ad enzima

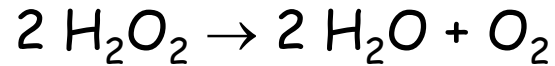


L'elemento biologico deve possedere:

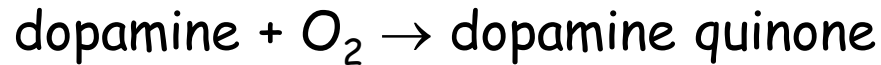
- Elevata selettività per l'analita
-
- Stabilità nelle condizioni di misura (t, pH, μ)
- Deve ritenere sufficiente attività biologica dopo l'immobilizzazione
- Non deve essere inibito dal campione

TISSUE BASED BIOSENSORS

bovine liver (rich in catalase)



banana (rich in polyphenol oxidase)



CELL BASED BIOSENSORS

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

Determination of the BOD (biological oxygen demand).

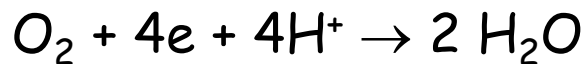
The BOD values indicate the amount of biochemically degradable organic material (carbonaceous demand) and COD the oxygen used to oxidise sulphides and ferrous ion.

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

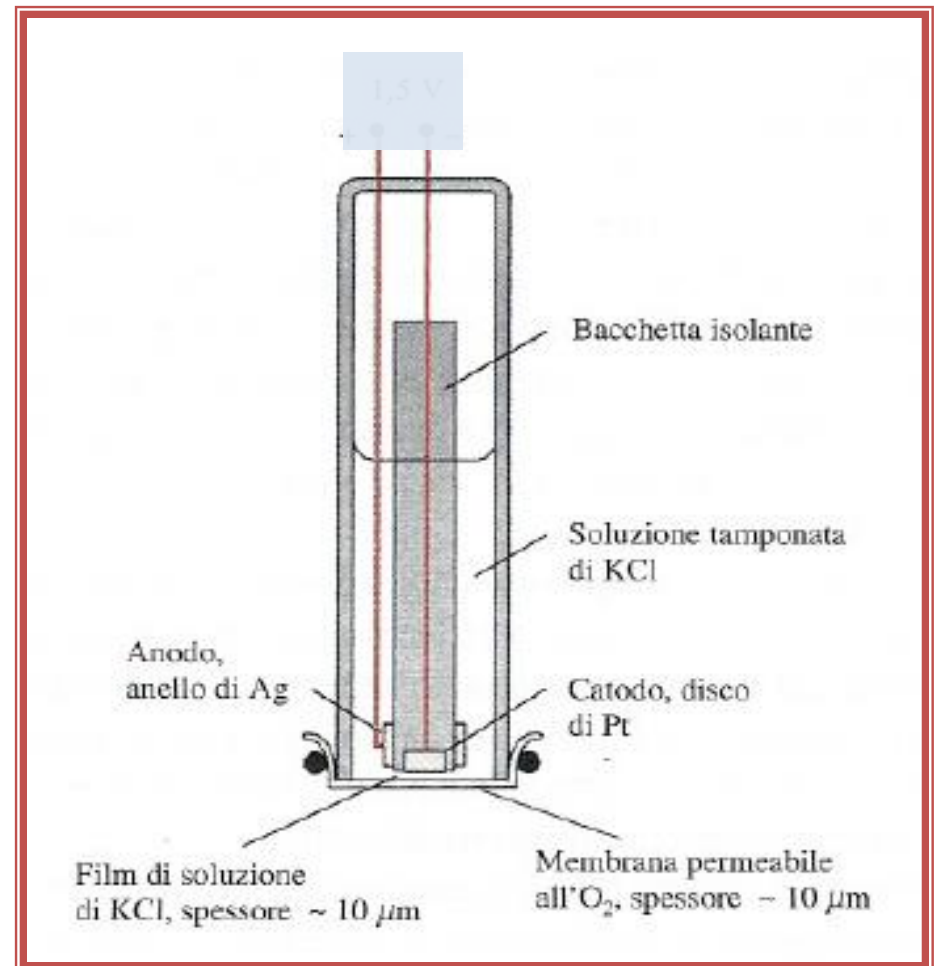
BOD biosensors have been developed using *Trichosporon cutaneum*, *Bacillus subtilis*, *Hansenula anomala*, etc.

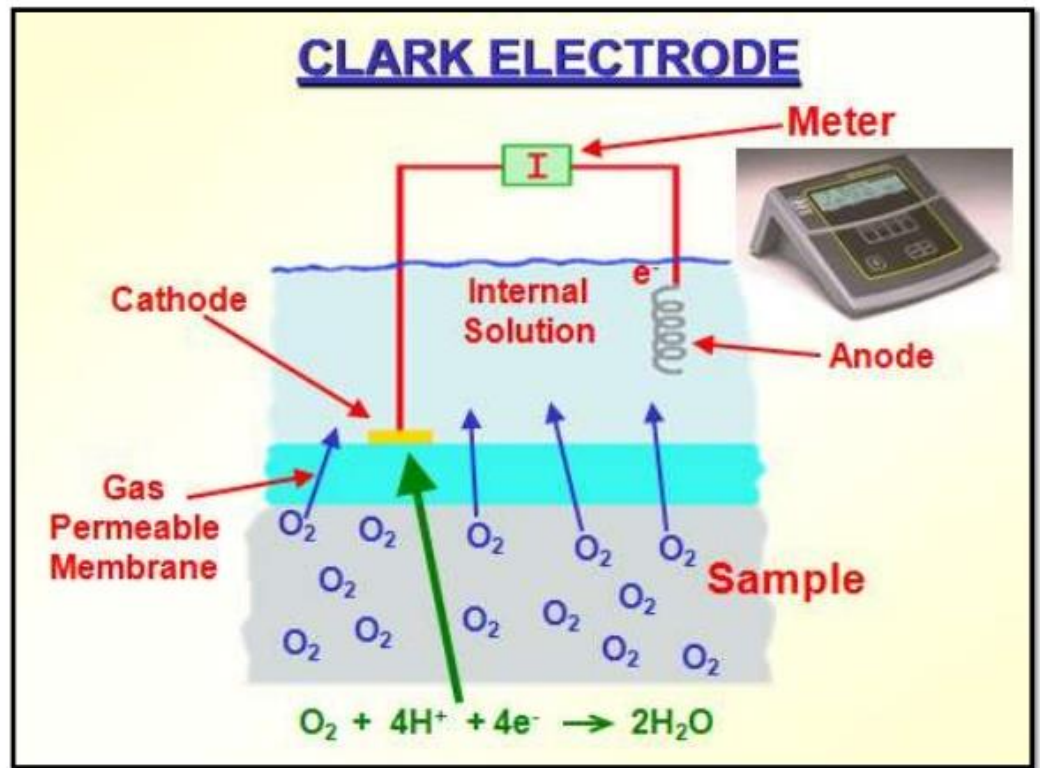
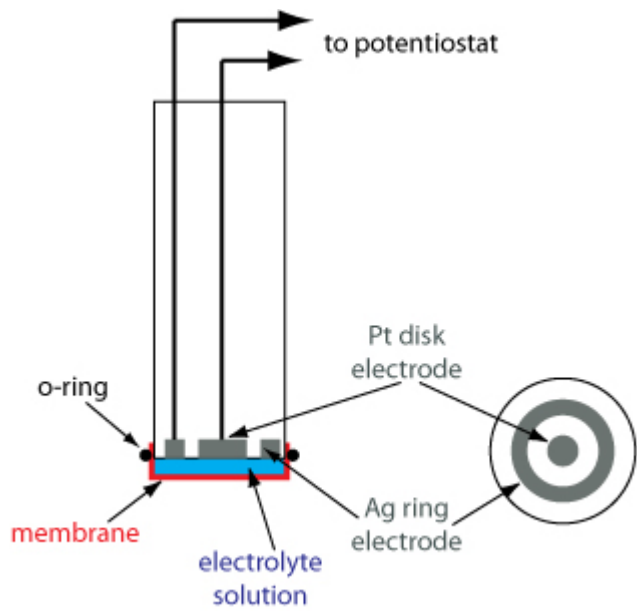
In amperometria si applica un potenziale fisso all'elettrodo (nella zona della corrente di diffusione) di lavoro e si misura la variazione di corrente in presenza dell'analita. E' necessario utilizzare delle strategie appropriate per la selettività della misura.

Nell'elettrodo ad ossigeno di Clark si applica un potenziale di $-0.7\text{ V vs. Ag/AgCl}$ ad un elettrodo di platino (od oro). La reazione all'elettrodo di lavoro è



La selettività è garantita dalla membrana gas-permeabile (e.g. polipropilene) posta tra la cella elettrochimica e la soluzione campione.





Applied potential
-0.7 V vs.
Ag/AgCl



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

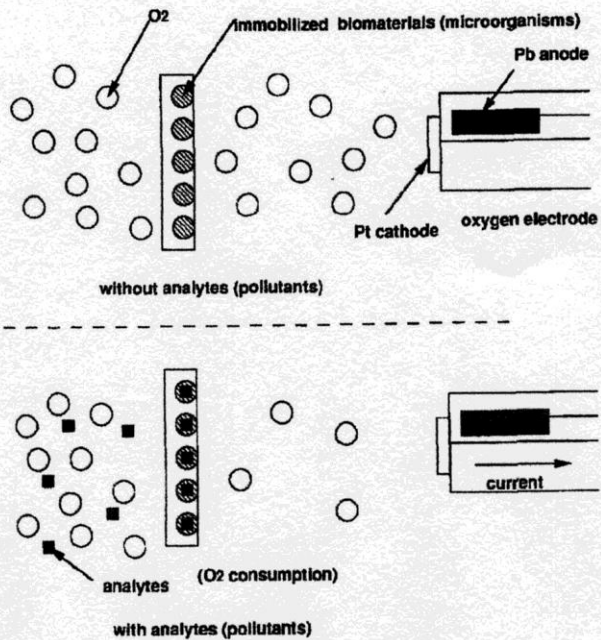


Fig. 1. Principle of biosensors. Microbial sensor of the respiration-activity measurement type.

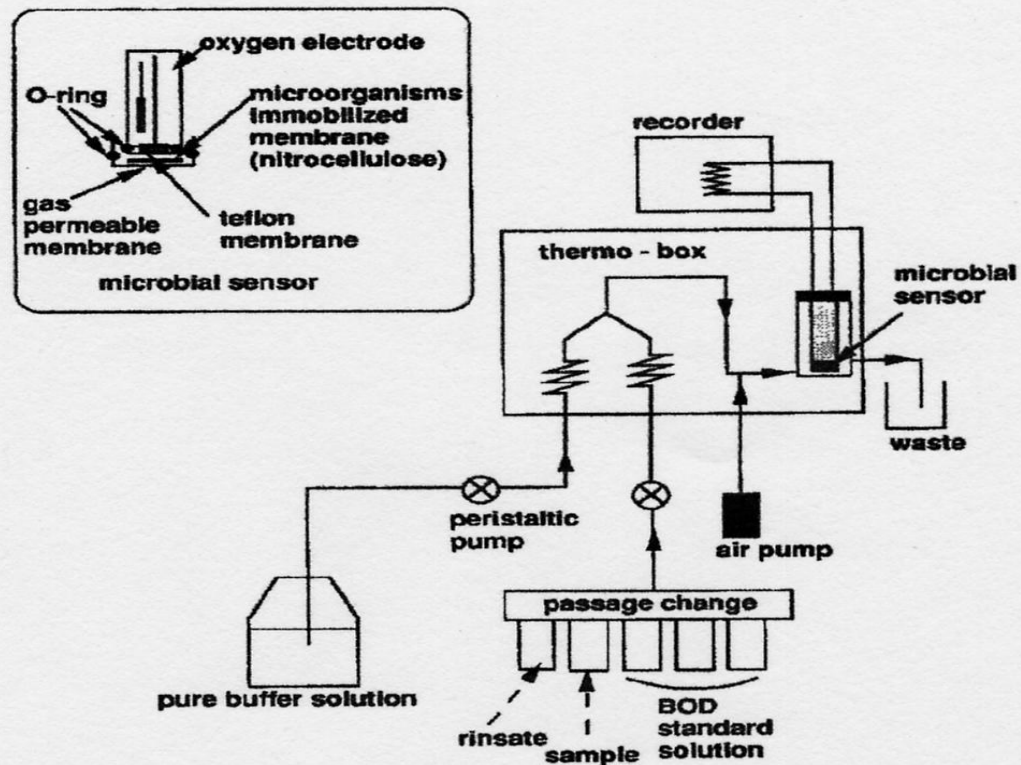
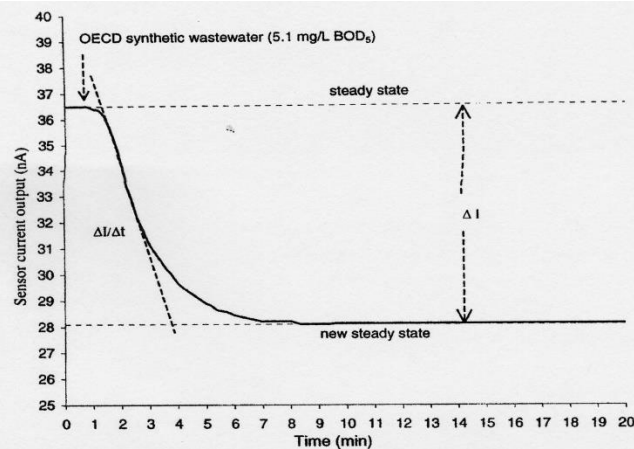
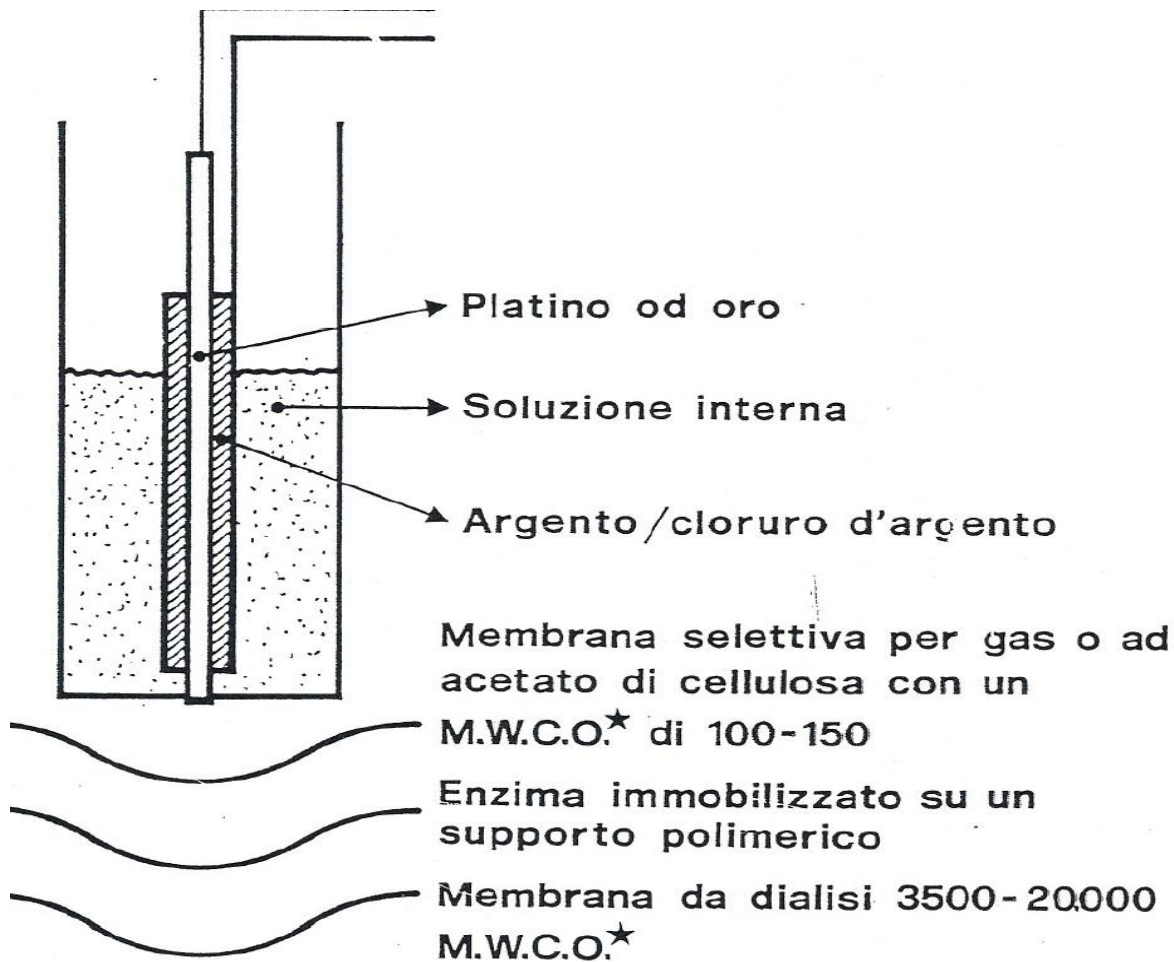


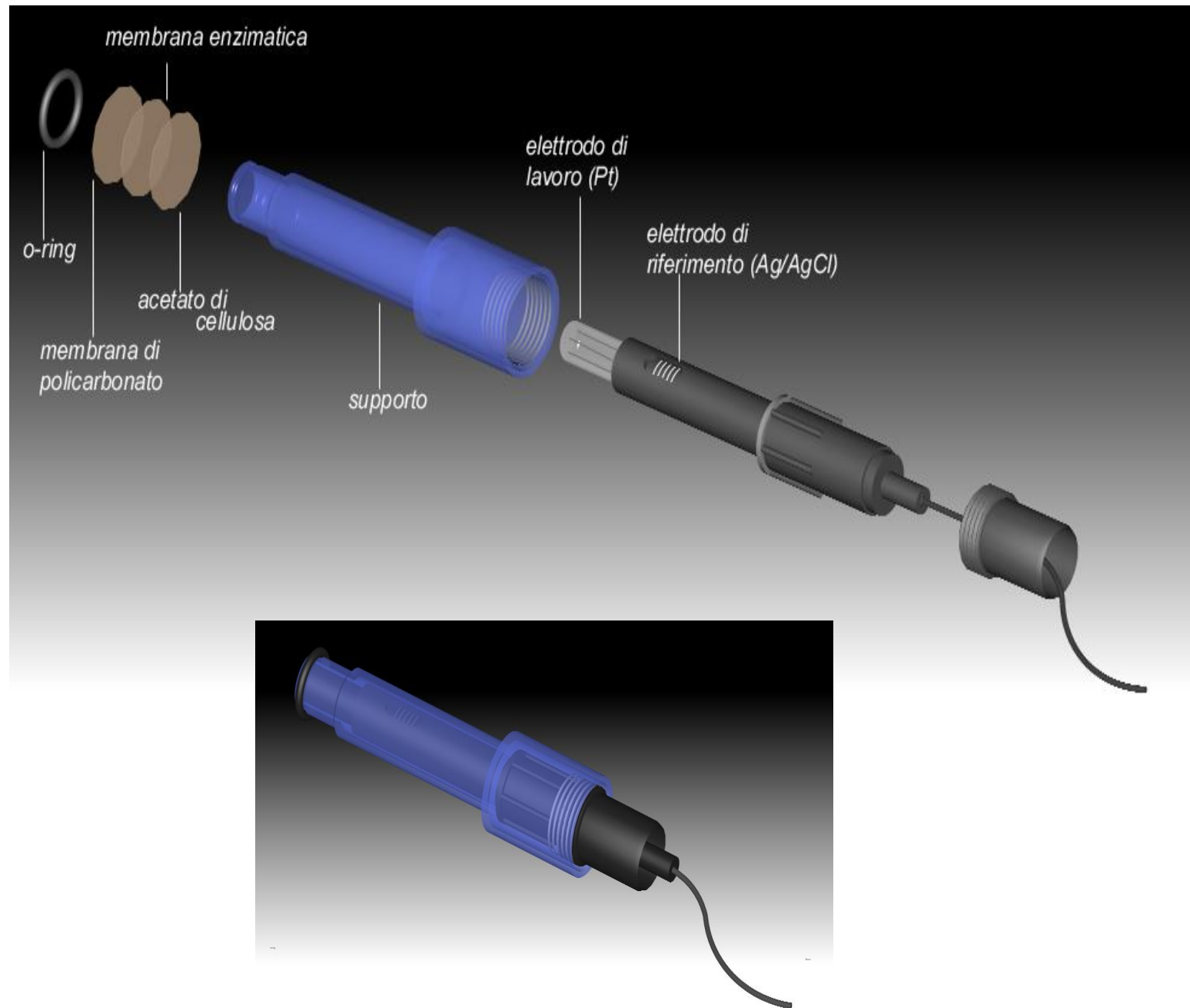
Fig. 2. Example of commercial BOD sensor.



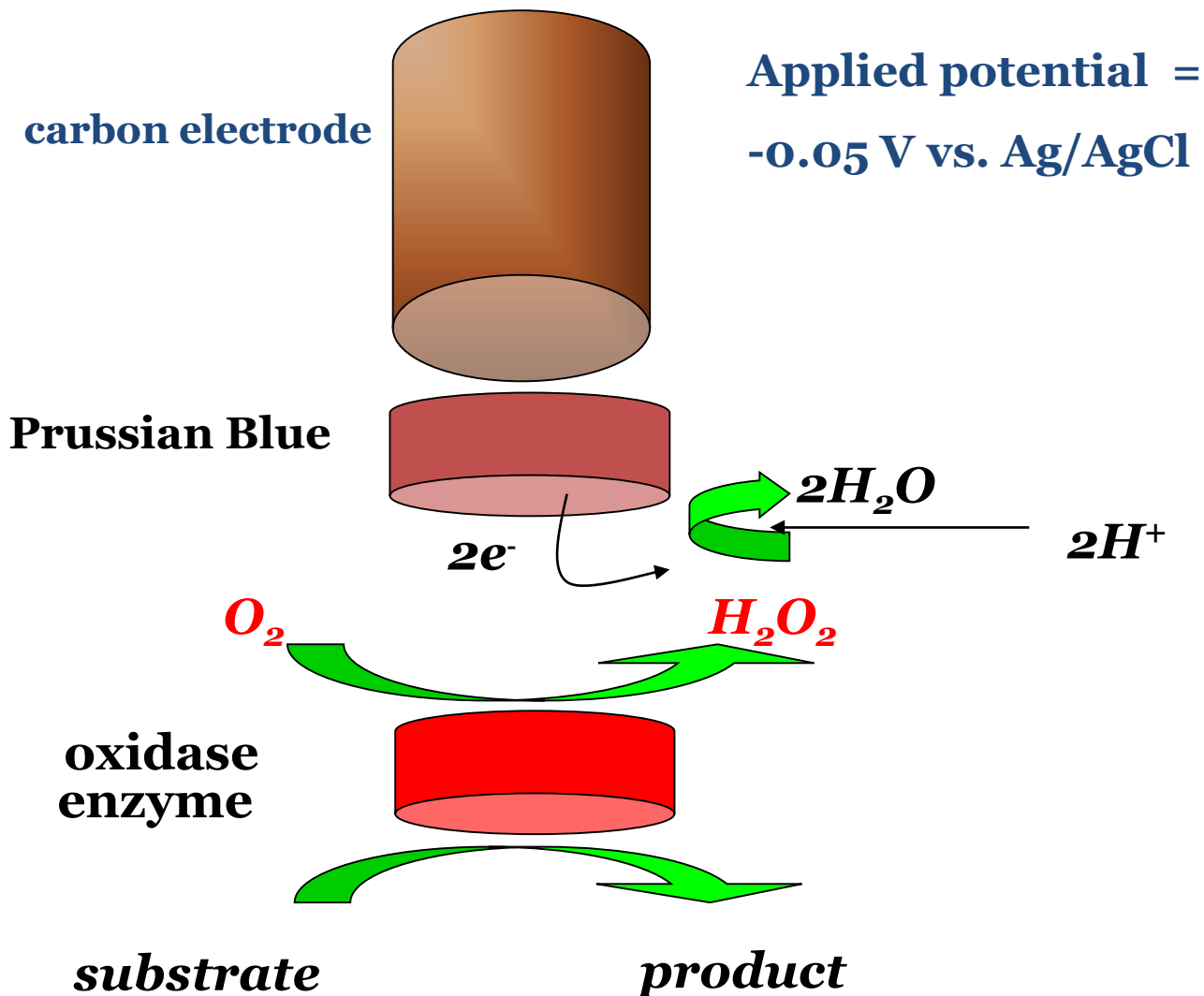


METHOD	SENSOR	CONC. RANGE mol/l
Potentiometry	H ⁺ , Na ⁺ , CN ⁻ NH ₄ ⁺ , I ⁻	10 ⁻⁵ -10 ⁻²
Amperometry	Pt/H ₂ O ₂	10 ⁻⁷ -10 ⁻³
GAS Sensor Potentiometry	NH ₃ , CO ₂	10 ⁻⁴ -10 ⁻²
GAS Sensor Amperometry	Oxygen	10 ⁻⁵ -10 ⁻⁴

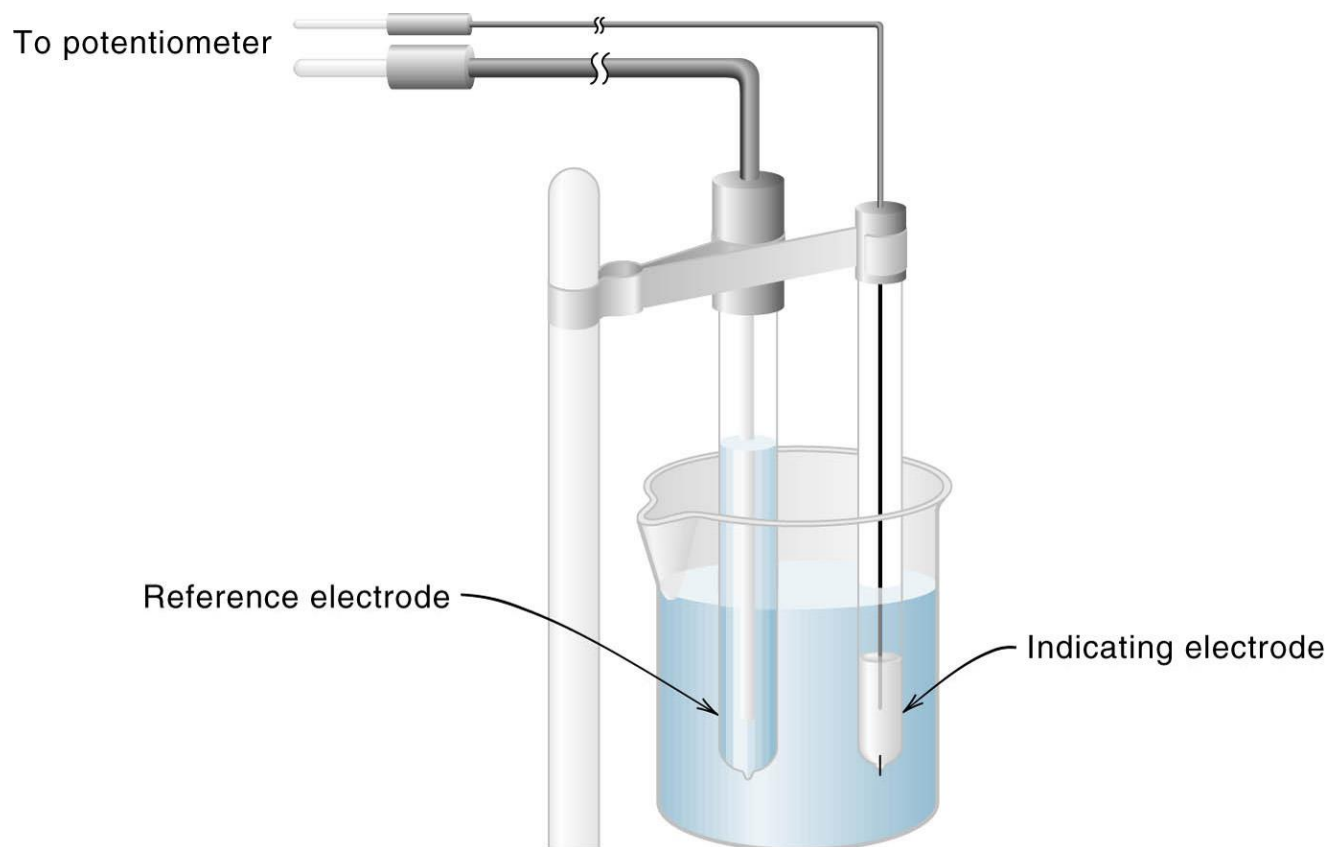
★ M.W.C.O. (molecular weight cut-off) è il peso molecolare massimo che le sostanze possono avere per poter attraversare la membrana



Reduced Prussian Blue is a selective catalyst for H_2O_2 reduction



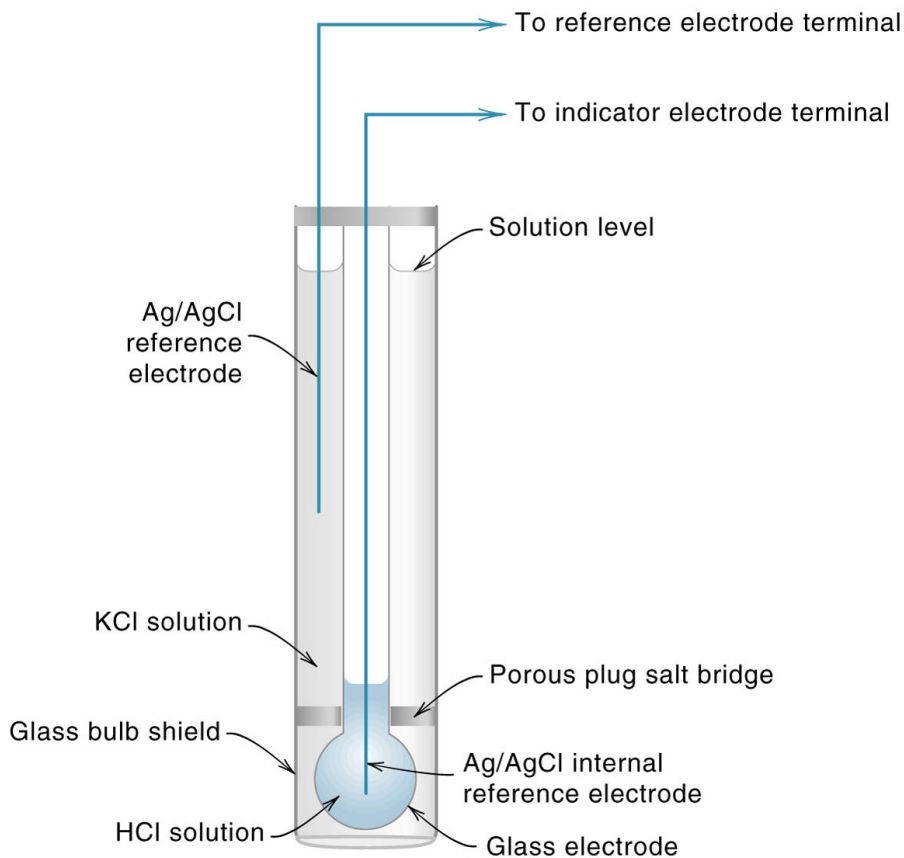
In potenziometria si usa un elettrodo di riferimento (a potenziale costante) ed un elettrodo indicatore



Cella per misure potenziometriche.

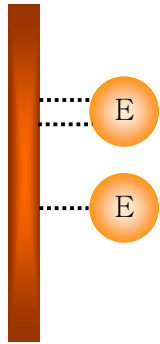
Elettrodo di riferimento+ elettrodo a pH

Limitato uso in elettrodi ad enzima perchè si lavora in soluzioni tamponate

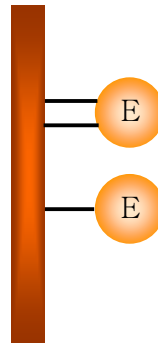


Elettrodo a pH combinato e pHmetro

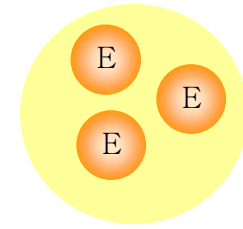
Immobilizzazione degli enzimi



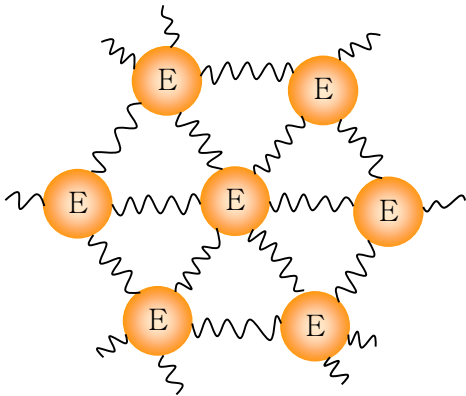
adsorbimento



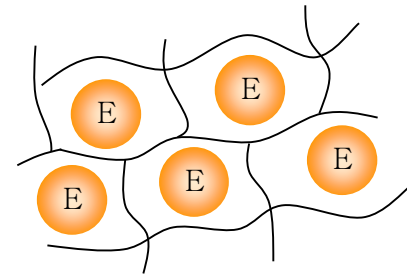
Legame
covalente



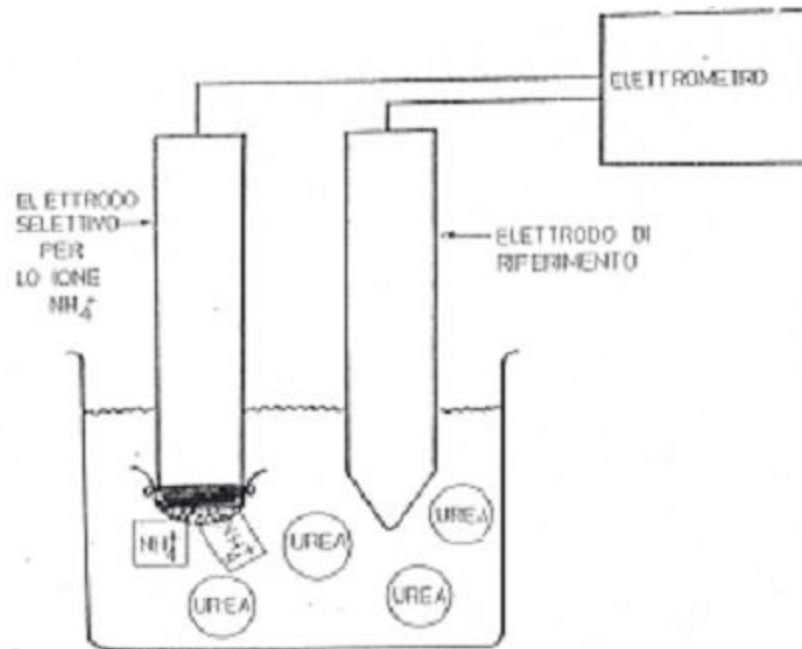
incapsulamento



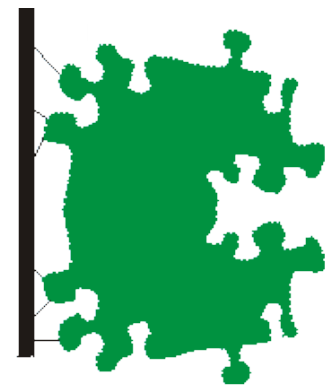
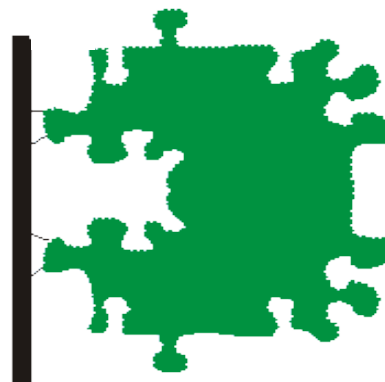
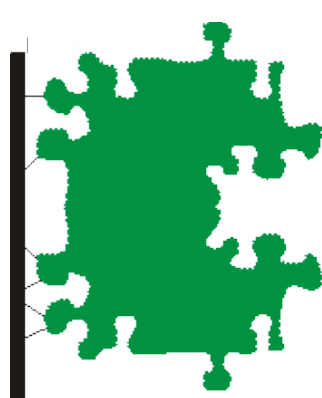
cross-linking



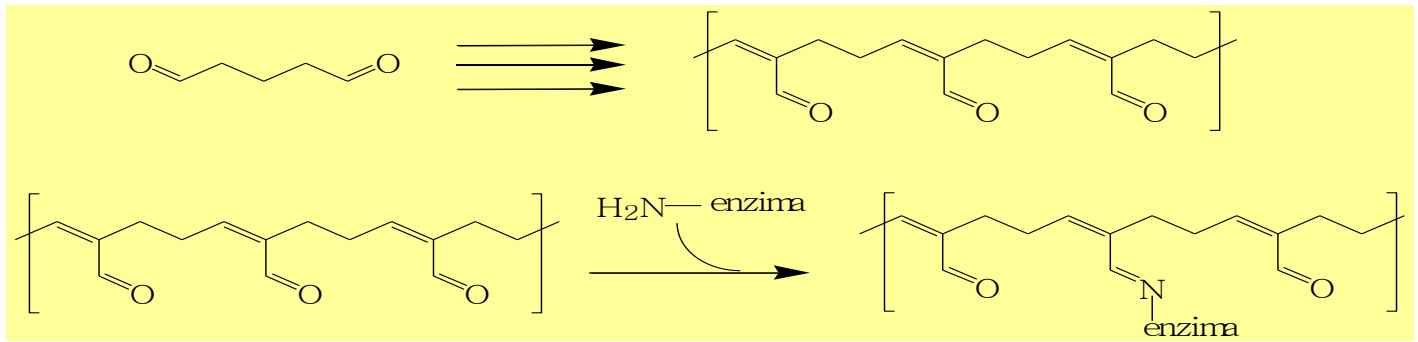
intrappolamento



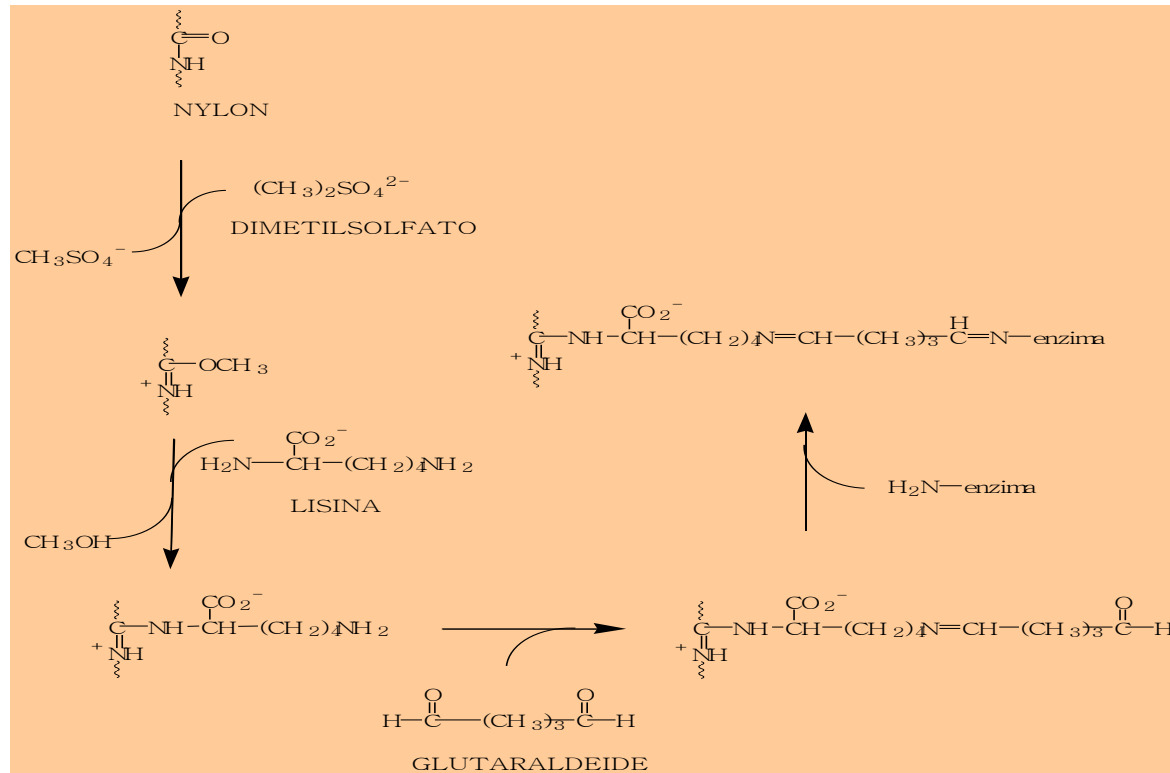
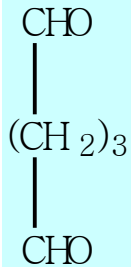
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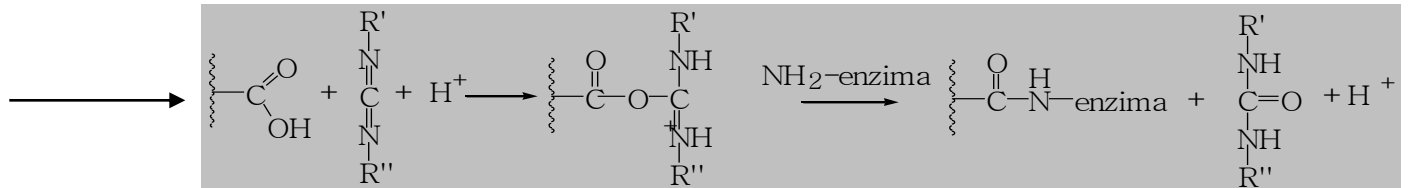
legame covalente



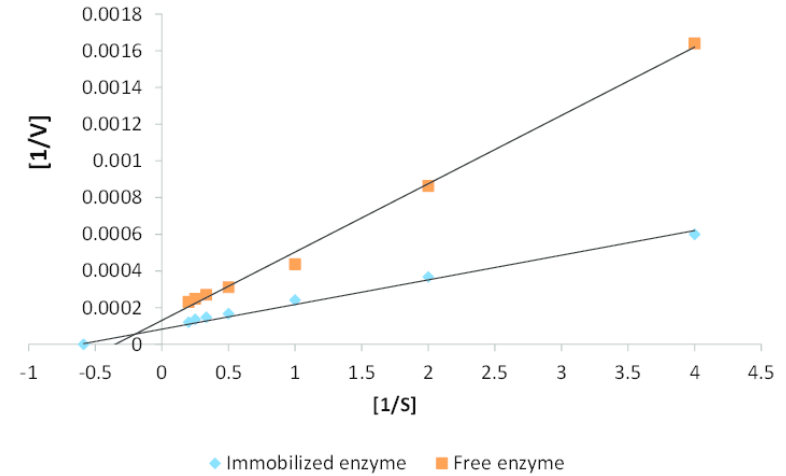
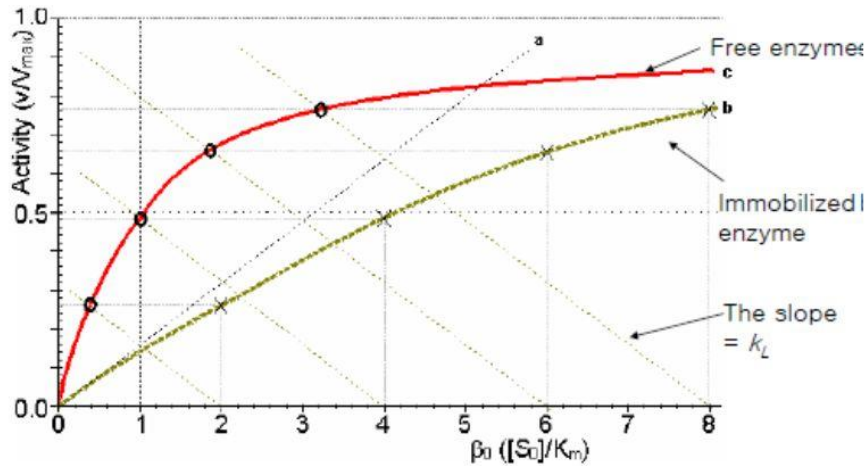
Reazioni della glutaraldeide: polimerizzazione e reazione con la catena laterale dei residui di lisina



Immobilizzazione
via carbodiimide

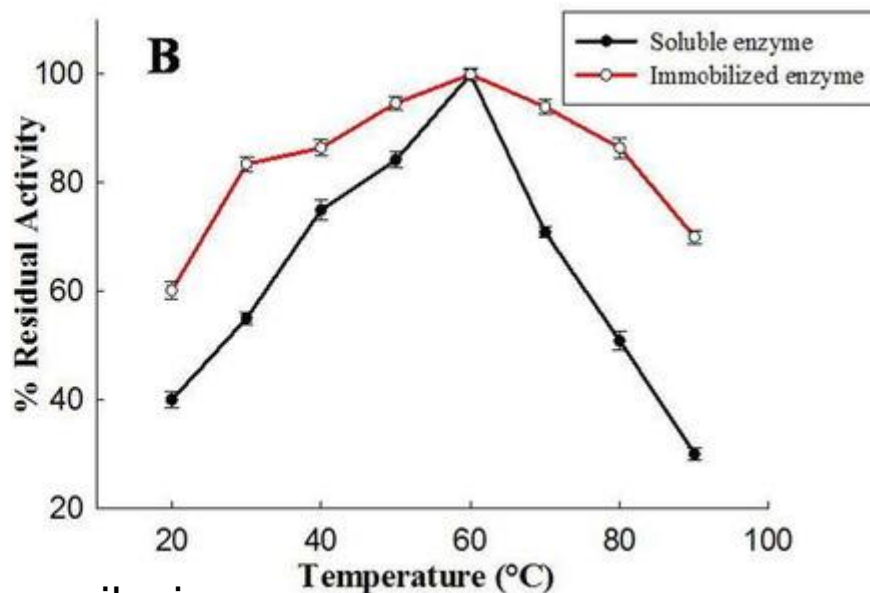
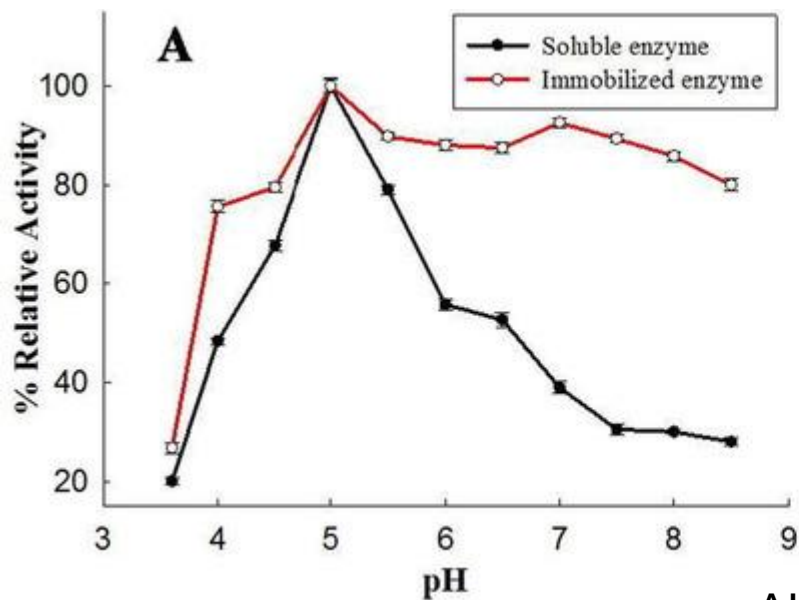
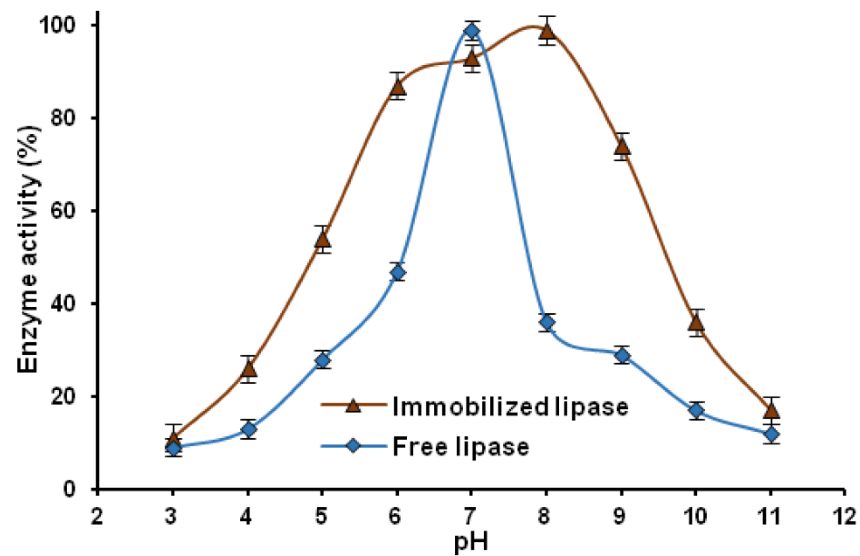


Kinetics of immobilized enzyme in nonporous solid support



Un enzima immobilizzato presenta sempre una K_m più elevata e una attività inferiore rispetto all'enzima in soluzione

L'optimum di pH è spostato ed allargato, l'enzima è più stabile alla temperatura



Alfa-amilasi

Analita	Enzima	Tempo di risposta (min)	Stabilità (giorni)
Glucosio	Glucosio ossidasi	2	>30
Colesterolo	Colesterolo ossidasi	3	7
Ammine	Monoammina ossidasi	4	14
Ossalato	Ossalato ossidasi	4	60

La stabilità in condizioni di lavoro è forse il parametro + importante !

(n campioni prima di cambiare il sensore o parte di esso)

Dipende dal campione che sto misurando oltre che dal biosensore!

Altri enzimi redox usati per gli elettrodi ad enzima

Deidrogenasi NAD(P)H dipendenti (piu' di 600 tipi diversi - difficili da immobilizzare e non semplice la misura del NADH)

Biosensore a tirosinasi per composti fenolici

FDH

D-fruttosio \rightarrow 5-Keto-D-fruttosio + $2e^-$

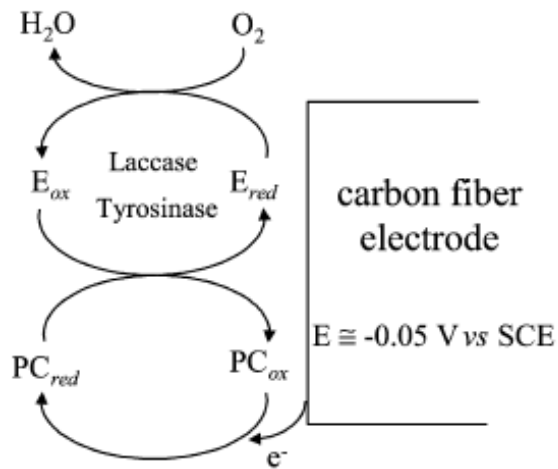
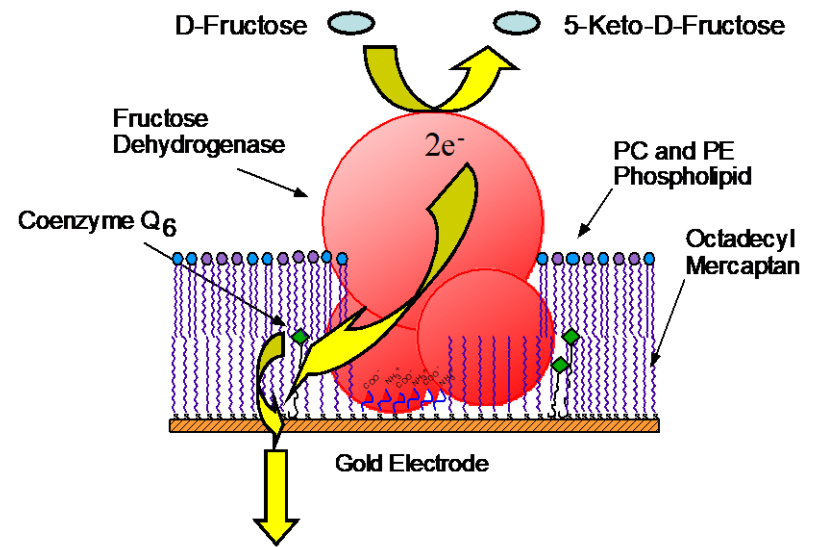


Fig. 1. Reactions mechanism on the laccase- and tyrosinase-based biosensor. PC: phenolic compound; E: enzyme; *red* and *ox* are the reduced and oxidized forms.



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^{•+}. 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

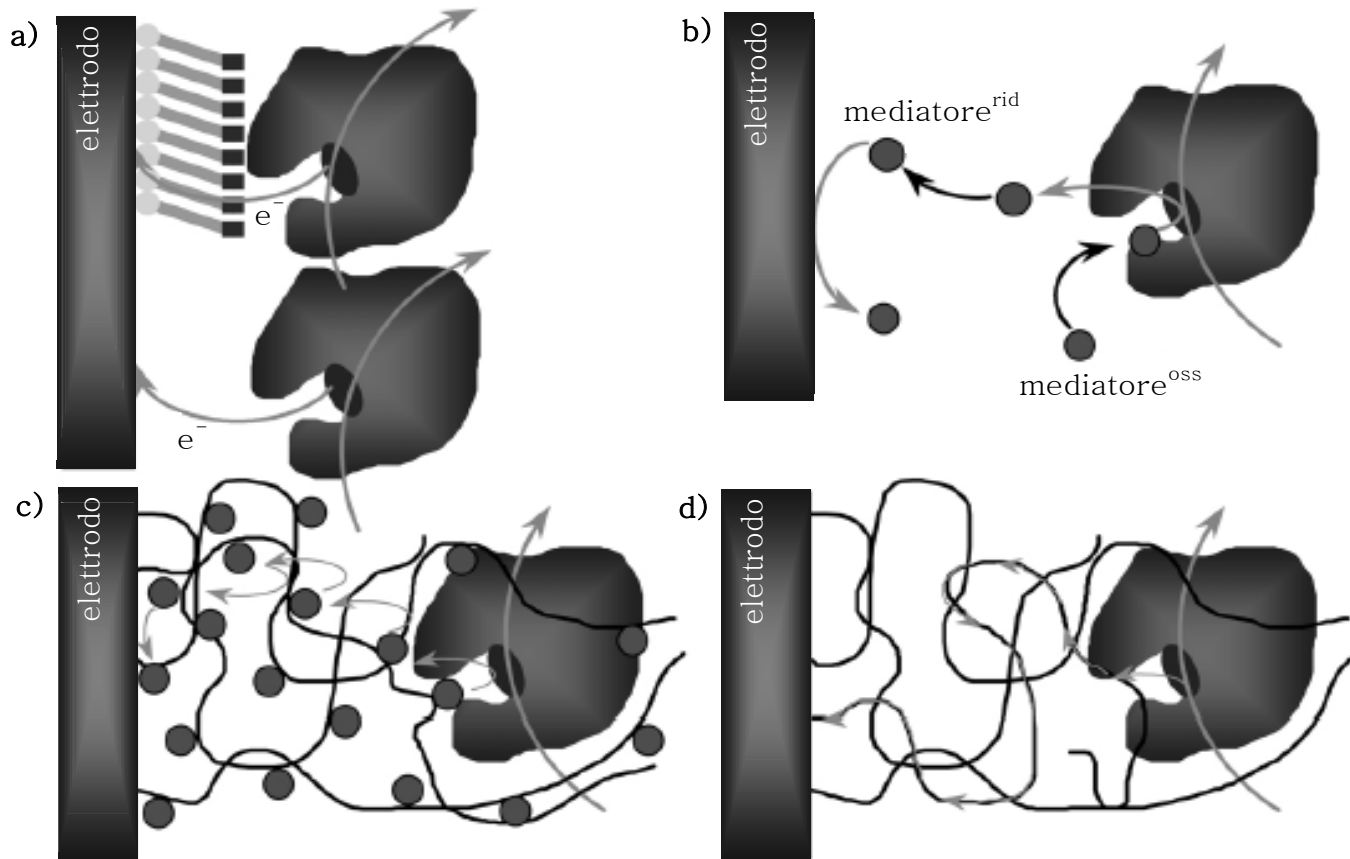
Sensitivity is excellent, major problems arise from selectivity and stability of the sensors

I sensori enzimatici vengono generalmente classificati in tre classi, distinte in base al meccanismo con cui avviene il trasporto elettronico.

1. Prima generazione: l'enzima è strettamente connesso all'elettrodo con l'ausilio di una membrana, che lo protegge da specie che potrebbero danneggiarlo o interferire nella misura. Il tempo di risposta del sistema è piuttosto lungo, dal momento che l'analita deve diffondere attraverso la membrana per raggiungere l'enzima

2. Seconda generazione: il trasferimento elettronico avviene tramite un mediatore elettrochimico posto in soluzione, che funziona da sistema navetta per gli elettroni tra l'enzima e l'elettrodo, velocizzando notevolmente la risposta del sensore.

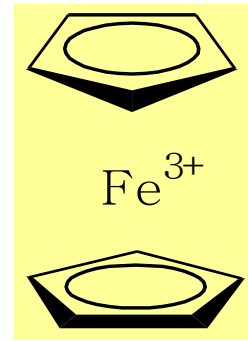
3. Terza generazione: si tratta di sensori in cui vi è accoppiamento diretto tra elettrodo ed enzima. La risposta è praticamente immediata e questo permette l'eliminazione di molte interferenze.



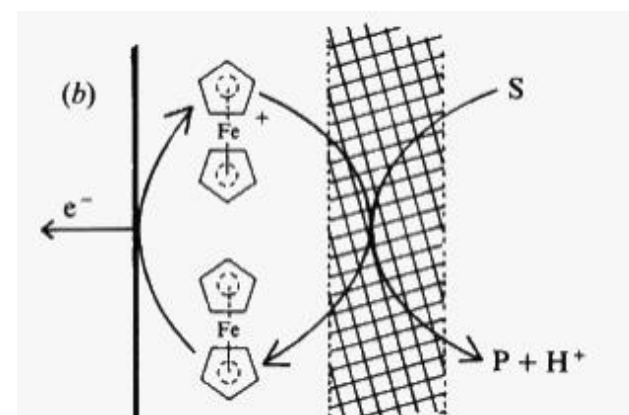
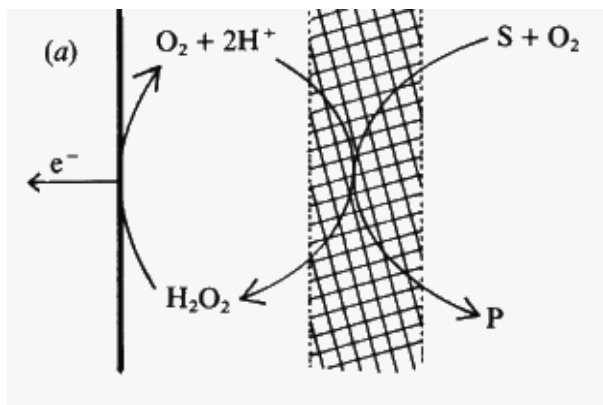
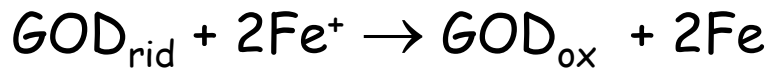
Rappresentazione schematica dei diversi meccanismi con cui può avvenire il trasferimento elettronico tra enzima e superficie dell'elettrodo. (a) Trasferimento elettronico diretto tra l'elettrodo nudo o modificato con un monostrato. (b) Meccanismo shuttle da parte di un mediatore redox in soluzione. (c) Gli elettroni saltano tra i diversi centri redox di un idrogel polimerico modificato. (d) Trasferimento elettronico attraverso cavi molecolari conduttori.

Un buon mediatore deve possedere le seguenti caratteristiche:

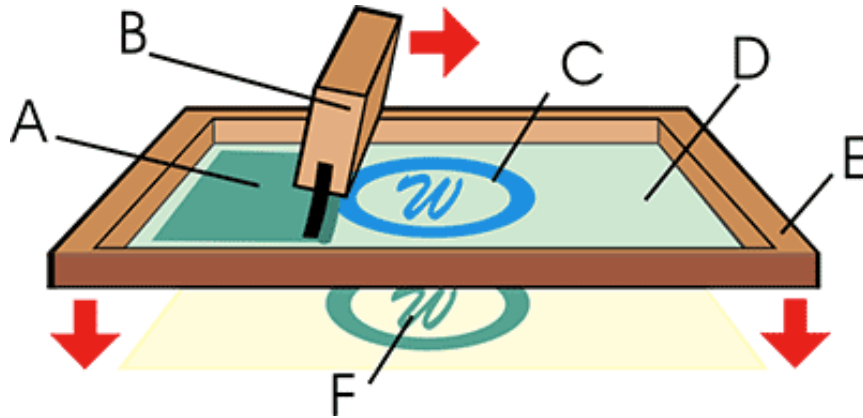
- Deve reagire rapidamente con l'enzima
- Deve avere cinetiche di trasferimento elettronico rapide, ovvero reversibili
- Il sovrapotenziale per la rigenerazione deve essere basso
- Non deve essere influenzato da variazioni di pH
- Deve essere stabile nel mezzo utilizzato, sia nella forma ossidata che in quella ridotta
- Non deve reagire con l'ossigeno disciolto in soluzione
- Non deve essere tossico



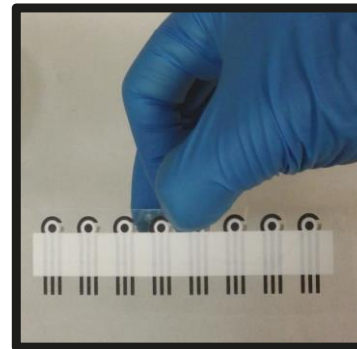
Il ferrocene è un ottimo mediatore per l'ossidazione del glucosio da parte della glucosio ossidasi:



Printing electrodes: serigraphy

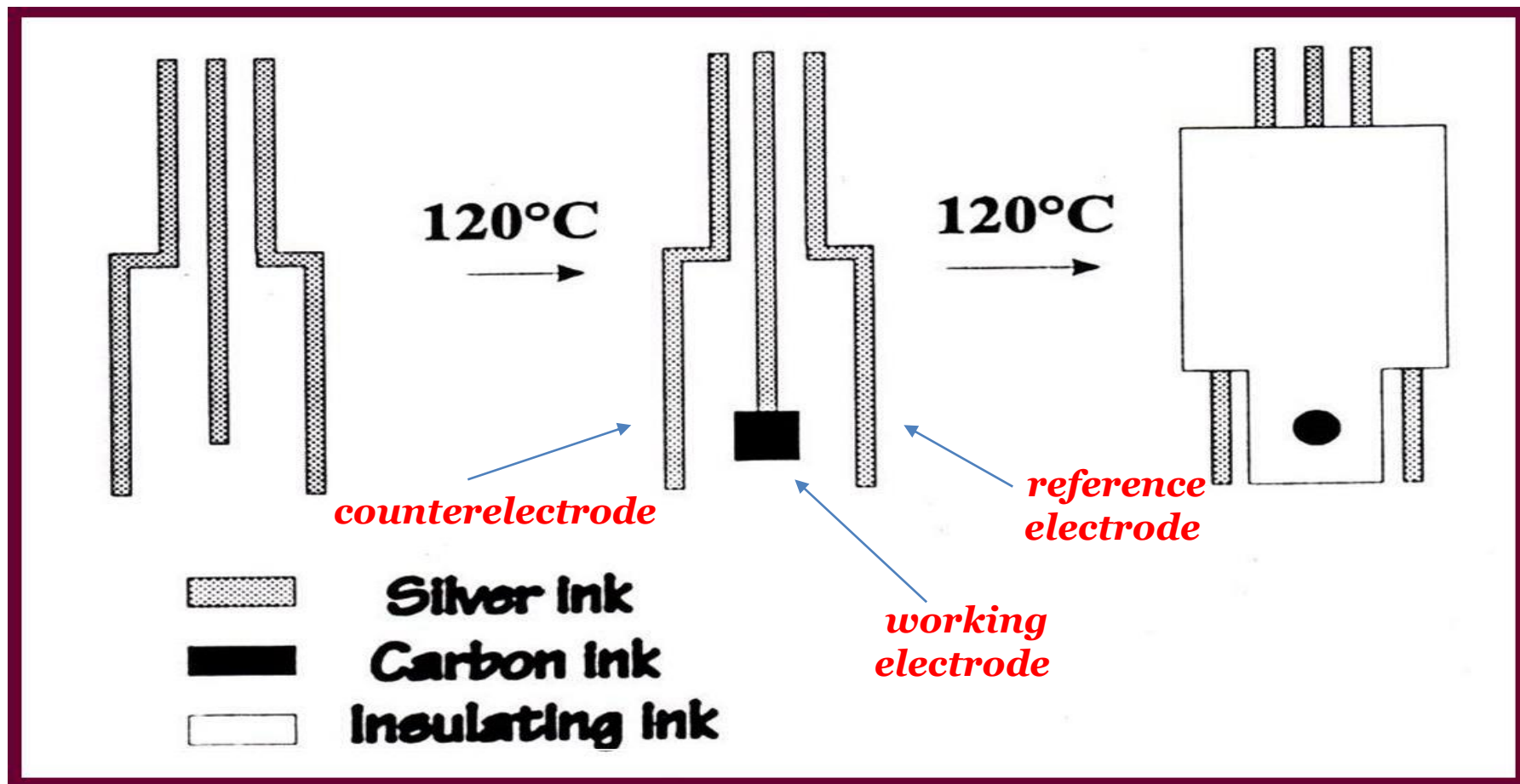


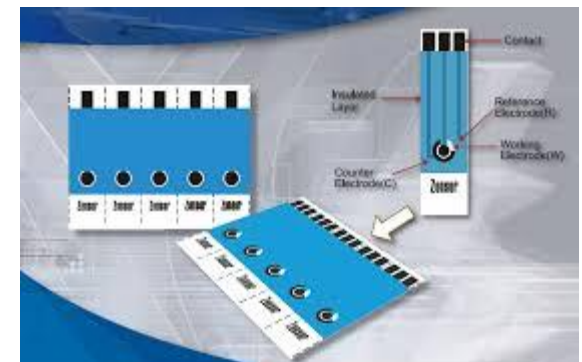
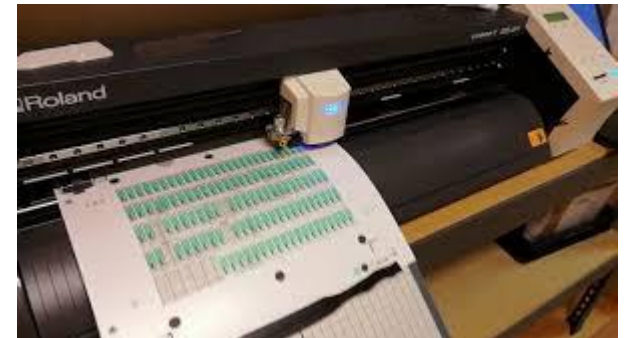
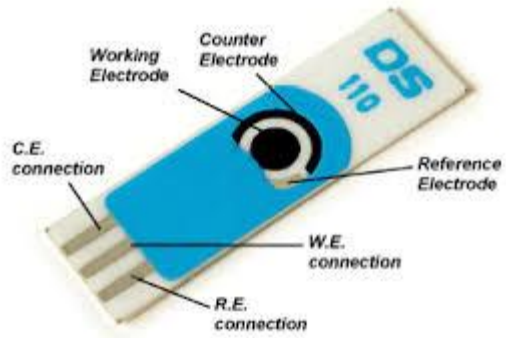
- A. ink; B. squeegee;
- C. printing mask;
- D. printing mesh;
- E. frame; F. printed ink



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

DISPOSABLE SCREEN-PRINTED CARBON ELECTRODES





BAYER 3902M
GLUCOMETER ELITE®

Blood Glucose Meter/Lecteur de glycémie
 Blutzuckermessgerät/Strumento per la
 determinazione della glicemia/Bloedglucosemeter
 Medidor de glucosa no sangue
 Medidor de glucosa en sangre

- Easy to use
- Utilisation facile
- Einfache Bedienung
- Facile de usare
- Fácil de utilizar
- Fácil manejo
- Very accurate
- Grande précision
- Hohe Präzision
- Molto accurato
- Ultra-precisione
- Muito preciso

Now with memory/Maintenant avec mémoire
 Jetzt mit Speicher/Adesso con memoria
 Nu met geheugen/Ahora con memoria

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**



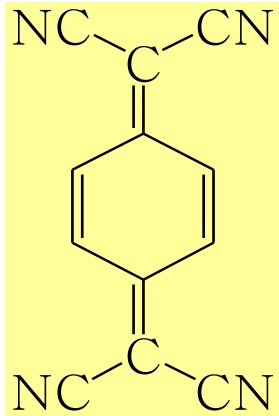
- Lo sviluppo della ricerca sui biosensori è stato trainato dai vantaggi economici derivanti dall'elevato numero di diabetici e dal conseguente enorme numero di analisi richieste

MEDIATORI REDOX

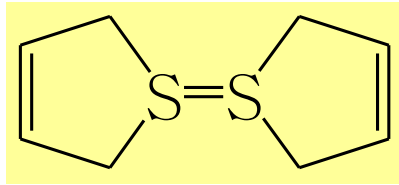
NATURALI	E(V) vs. SHC	ARTIFICIALI	E (V) vs.SHC
Citocromo a ₃	+0,29	Esacianoferrato(III)	+0,45
Citocromo c ₃	+0,24	2,6-diclorofenolo	+0,24
Ubichinone	+0,10	Indofenolo	+0,24
Citocromo b	+0,08	Ferrocene	+0,17
Vitamina K ₂	-0,03	Idrogenosolfato di N- metilfenazonio	+0,07
Rubredossina	-0,05	Blu di metilene	+0,4
Flavoproteine	da -0,4 a +0,2	Ftalocianina	-0,02
FAD/FADH ₂	-0,23	Fenosafranina	-0,23
FMN/FMNH ₂	-0,23	Viologeno di benzile	-0,36
NAD ⁺ /NADH	-0,32	Viologeno di metile	-0,46
NADP ⁺ /NADPH	-0,32		
ferredossina	-0,43		

Terza generazione

Sali conduttori



Tetra tiafulvalene (TTF)



Tetracyanoquinodimetano (TCNQ)

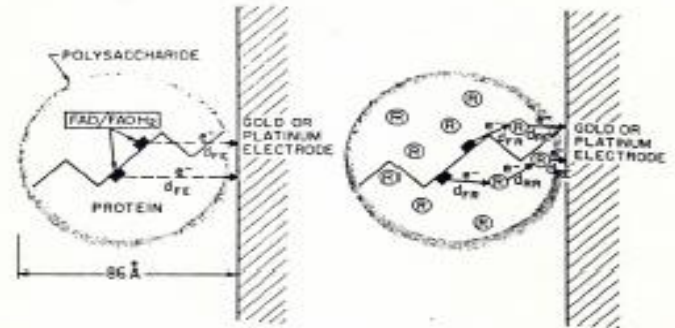
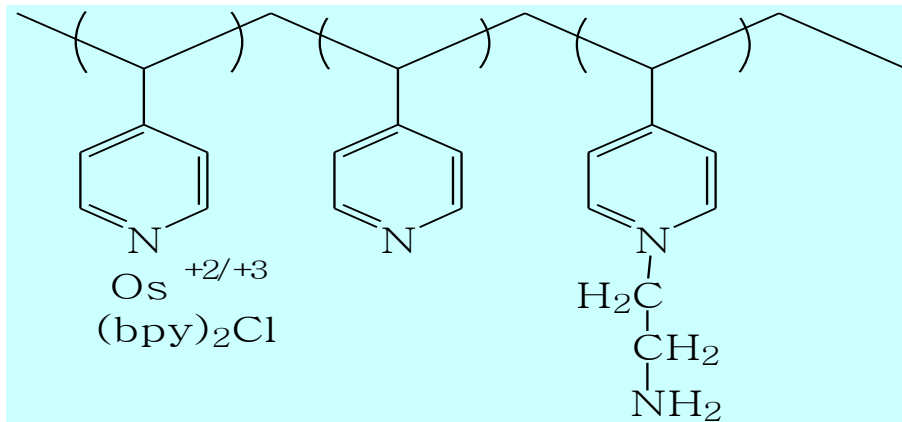


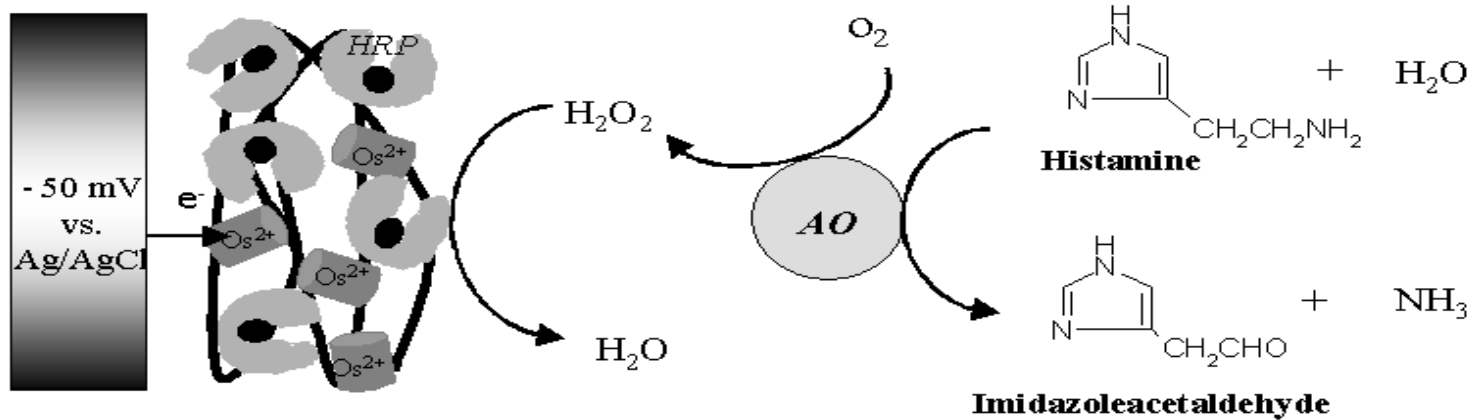
Figure 1. Schematic drawing of the glucose oxidase molecule, showing the electron-transfer distances involved in the various steps of moving an electron from its two FAD/FADH₂ centers to a metal electrode. Left: the enzyme before modification. Right: the modified enzyme, after chemical attachment of an array of electron-transfer relays.

gel redox

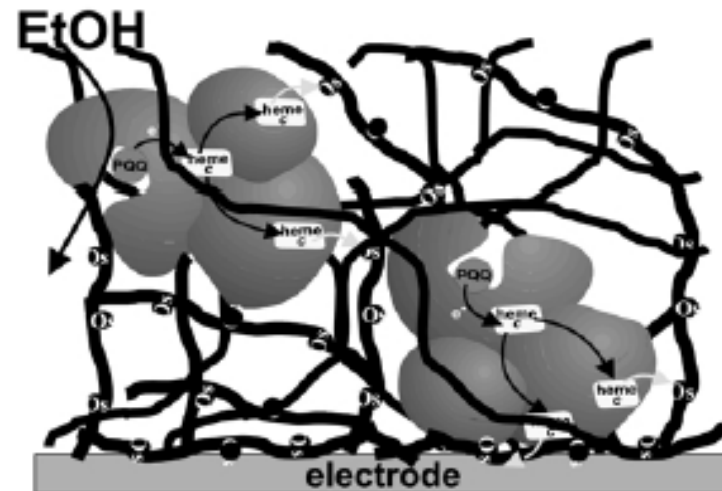


Struttura dell' osmio biperidile legato a polivinilpiridina

Biosensore Bienzimatico a Istamina



Rappresentazione schematica del complesso QH-ADH intrappolato all'interno di un idrogel modificato con osmio



Alcohol biosensor

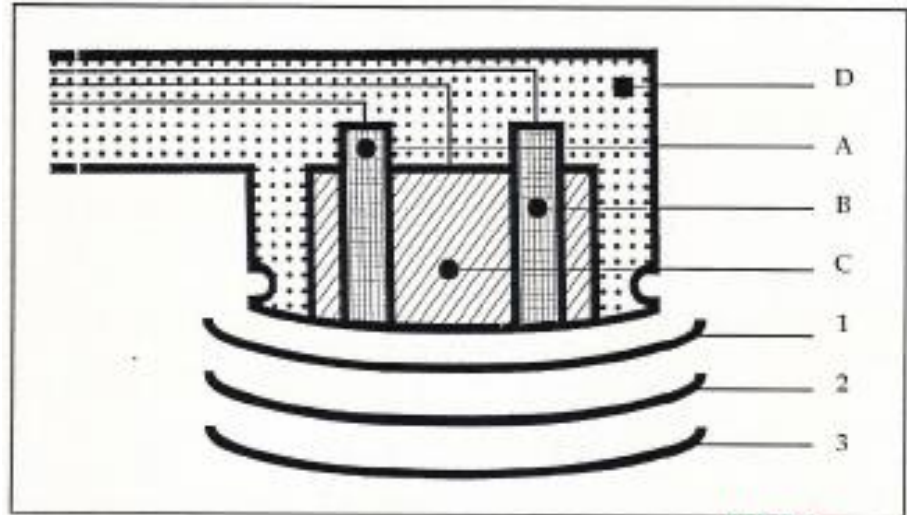
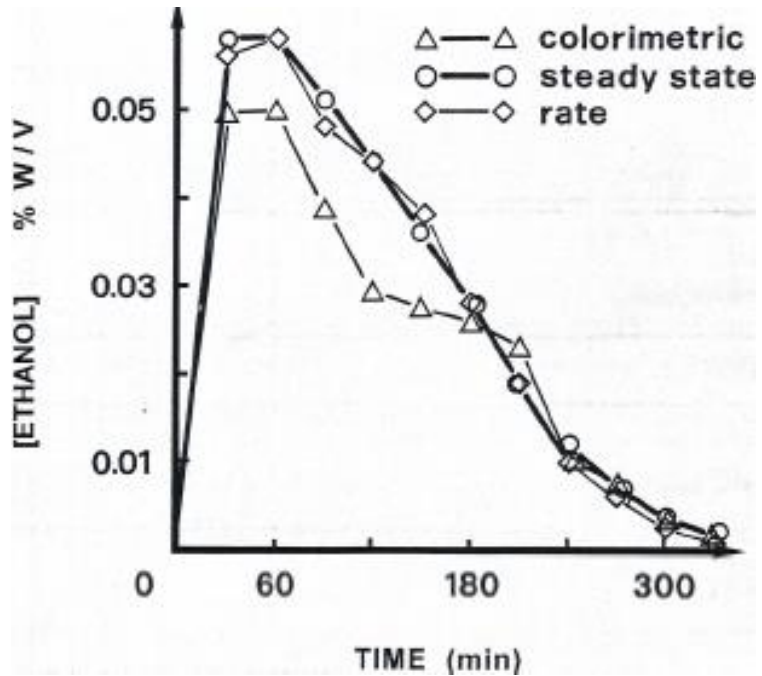


Figura 25. Schema ed assemblaggio di un doppio elettrodo a ^{ETANOL} ~~testante~~ A e B = elettrodi di platino; C = elettrodo ad Ag/AgCl; D = Plexiglass; 1 = membrana di acetato di cellulosa (100 m.w.c.a.) 2= membrana con l'enzima immobilizzato, 3 = membrana di policarbonato.

Biosensore a lisina

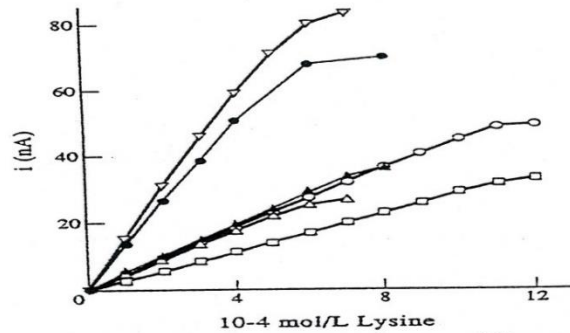
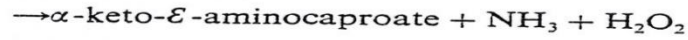
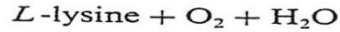


Fig. 1. Lysine calibration curves using two different immobilization procedures and protective polycarbonate membranes with different porosity. ∇ BSA/glutaraldehyde on Immobilon and $0.8\text{-}\mu\text{m}$ polycarbonate; \bullet Immobilon only and $0.8\text{-}\mu\text{m}$ polycarbonate; \circ BSA/glutaraldehyde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

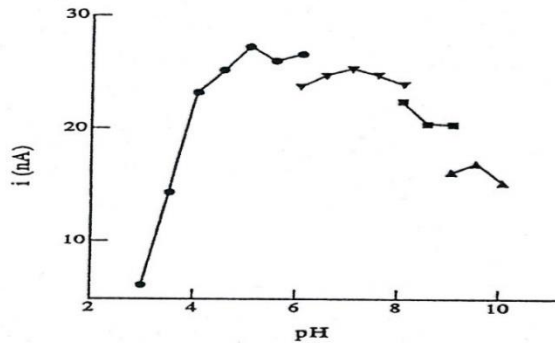


Fig. 2. Effect of pH on lysine oxidase activity. The enzyme activity was measured in the following buffers: \bullet citrate; \blacktriangledown phosphate; \blacksquare tris; \blacktriangle borax, $T = 25^\circ$.

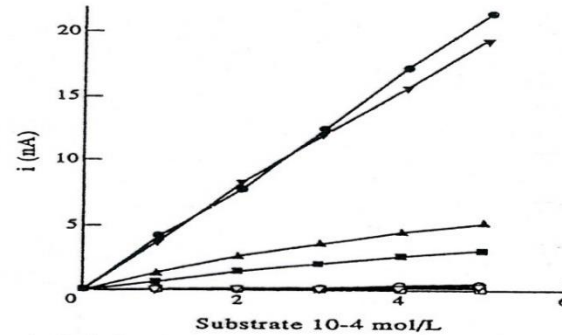
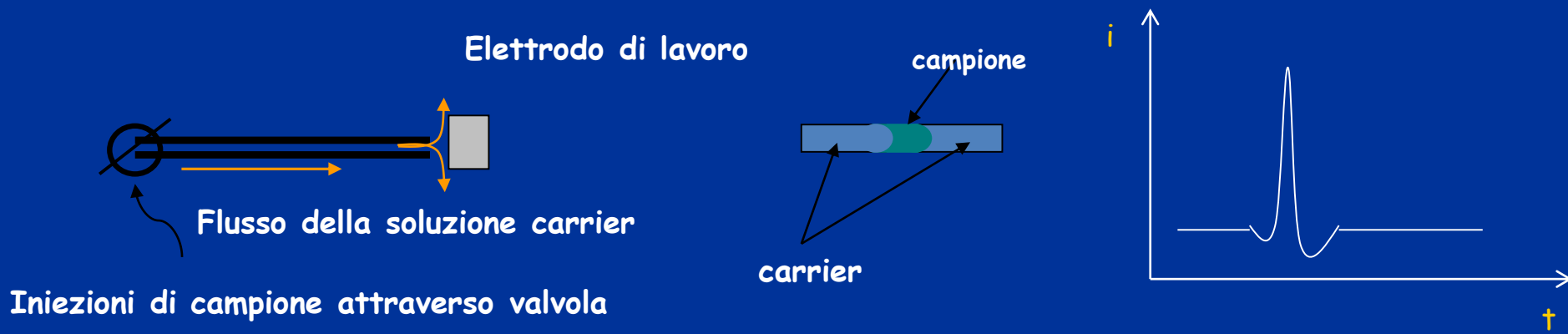
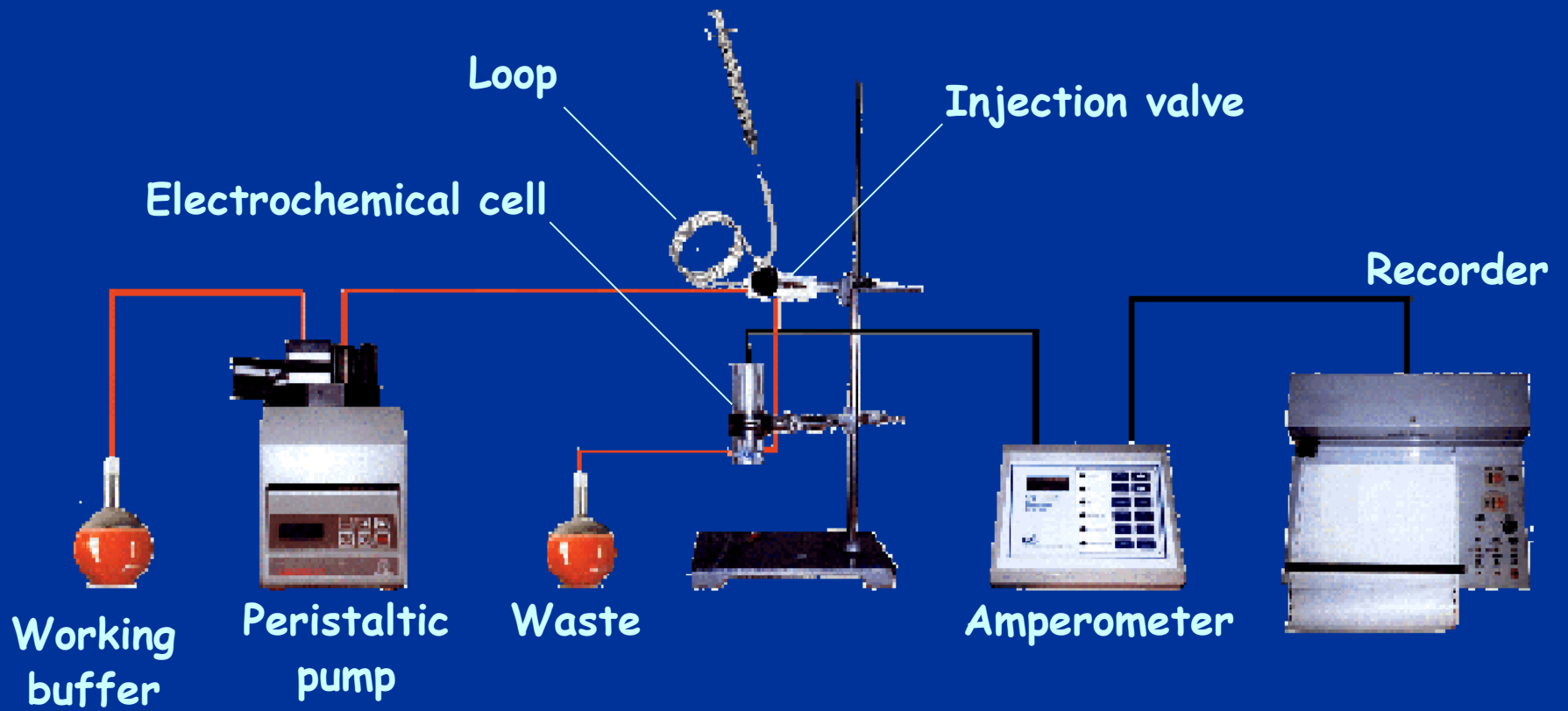


Fig. 4. Relative lysine oxidase activity toward some substrates pH 7.0 phosphate buffer $T = 25^\circ$. \bullet lysine; \blacktriangledown lysine after the analysis of other aminoacids. \blacktriangle ornithine; \blacksquare arginine; \circ tyrosine; \square phenylalanine; ∇ histidine.



ELECTROCHEMICAL BIOCELLS IN FOOD

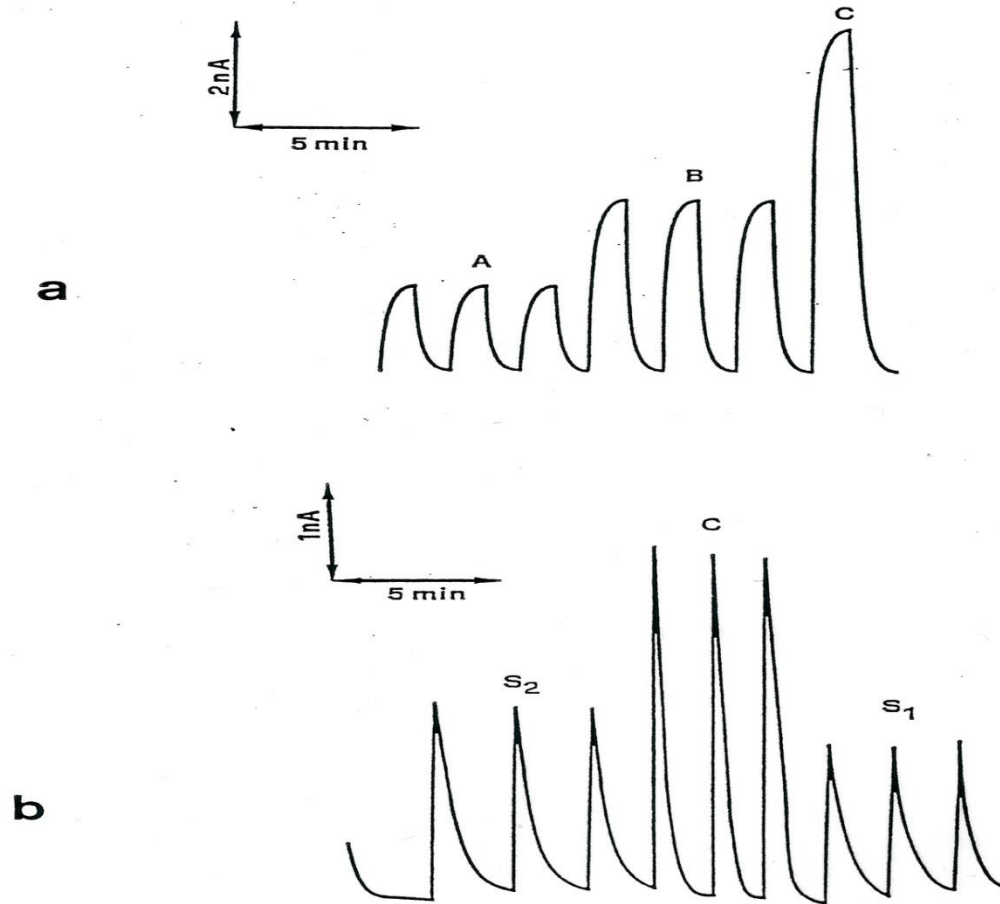


Figure 7 Reproducibility and response time of the bioprobe in flow through analysis and FIA.
a = flow through: Lysine concentration in standard solution.
A = $5 \cdot 10^{-5}$ mol/L; B = 10^{-4} mol/L; C = $2 \cdot 10^{-4}$ mol/L.
b = FIA; S_1 and S_2 foodstuff samples; C = Lysine standard $5 \cdot 10^{-4}$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

Attività relativa di 3 preparazioni di lisina ossidasi

compound	Yamasa	SIGMA	From partner 3
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

Sample	Amino-acid analysis (mM)	L-lysine biosensor (mM)	Recovery (%)
L (milk)	1.684		
		1.493 ± 0.014	88.66
M (pasta)	0.307		
		0.537 ± 0.039	177.23
N (pasta)	0.299		
		0.308 ± 0.012	103.01

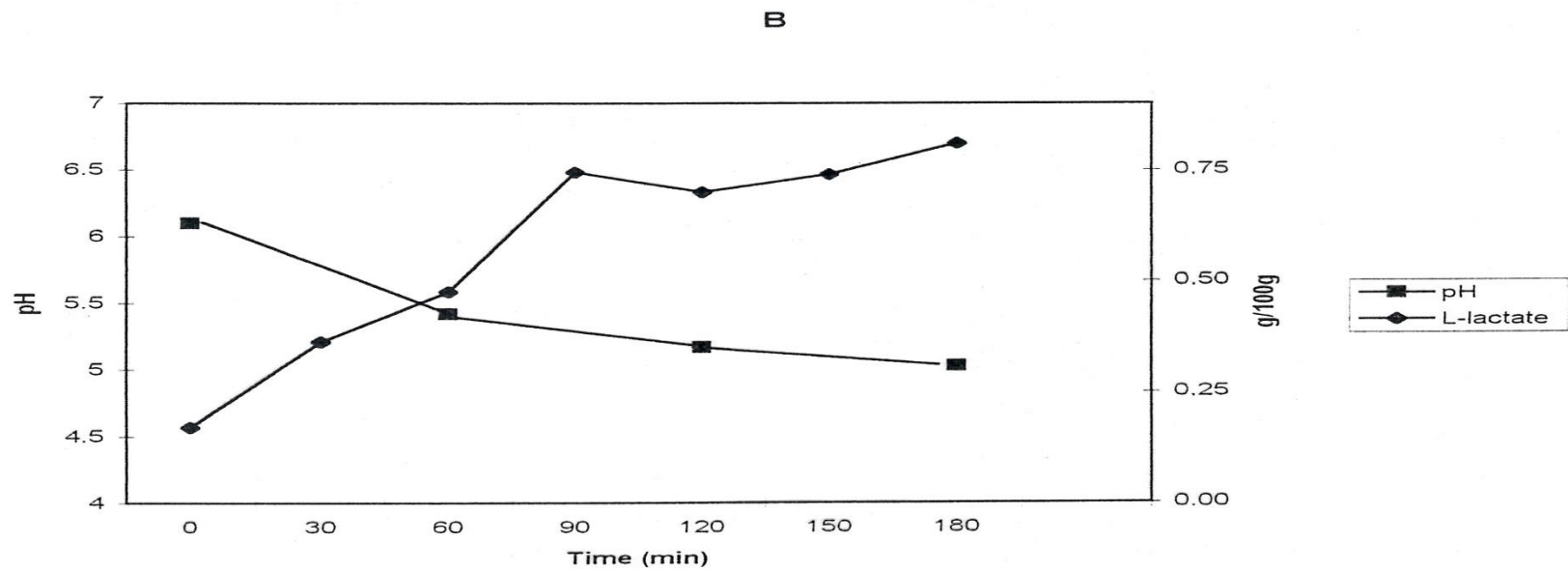
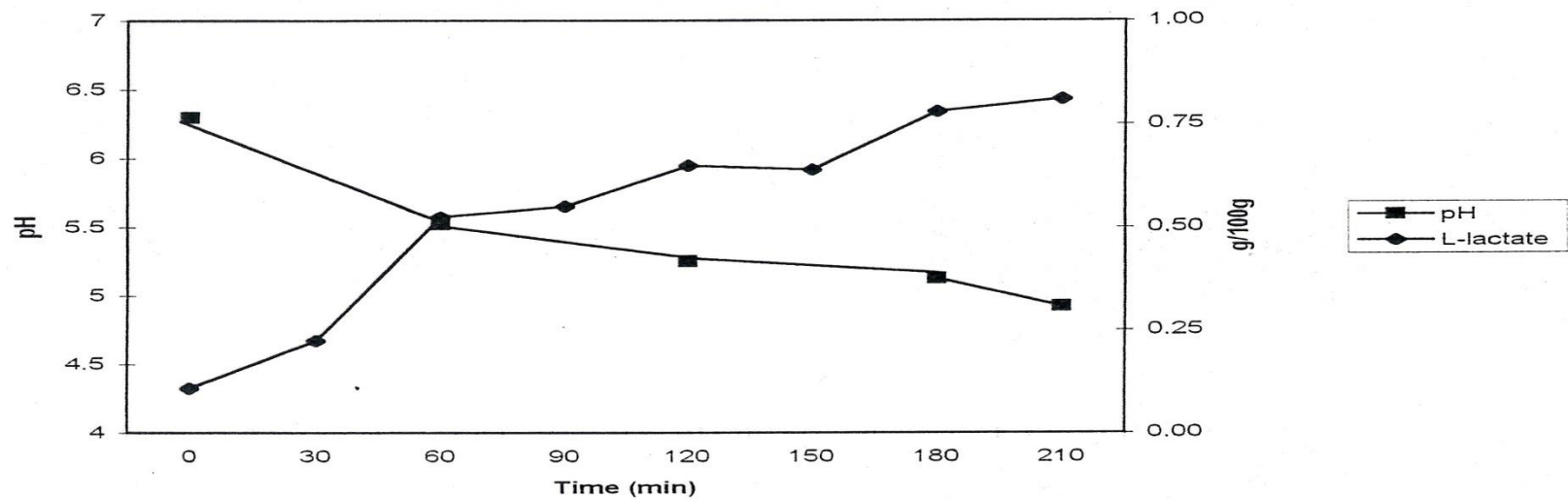
Lactic acid monitoring during mozzarella cheese manufacturing

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 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



Enzyme electrodes for fish freshness



$$\mathbf{K (\%)} = \frac{\text{inosine} + \text{hypoxanthine}}{\text{ATP} + \text{ADP} + \text{IMP} + \text{I} + \text{HxR} + \text{U}} \times 100$$

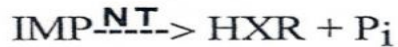
$$\mathbf{K_I (\%)} = \frac{\text{inosine} + \text{hypoxanthine}}{\text{IMP} + \text{inosine} + \text{hypoxanthine}} \times 100$$

$\mathbf{K} < 20 \rightarrow$ suitable for “sashimi” (sliced raw fish)

$20 < \mathbf{K} < 40 \rightarrow$ fish has to be cooked

$\mathbf{K} > 40 \rightarrow$ not suitable for human consumption

Le reazioni enzimatiche coinvolte nella degradazione dell' IMP ad acido urico sono le seguenti :

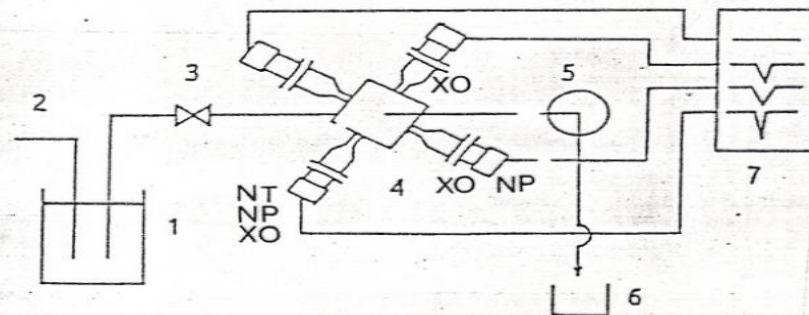


IMP, HXR ed HX possono essere determinati utilizzando elettrodi ad ossigeno o ad acqua ossigenata accoppiati ai suddetti enzimi.

Il consumo di ossigeno o la produzione di H₂O₂ in soluzione, determinano una variazione del segnale di corrente che e' direttamente proporzionale alla concentrazione dei metaboliti in esame.

Un gruppo di ricerca giapponese (Watanabe ed altri) ha realizzato un sistema basato su elettrodi ad ossigeno, per la determinazione del valore di K.

Tre elettrodi, con gli enzimi immobilizzati su opportune membrane, venivano inseriti in una cella a flusso continuo:



Is this Nano?

Nanotechnology is a “*system of innovative methods to control and manipulate matter at near-atomic scale to produce new materials, structures, and devices*”.



Nanomaterials (NMs)

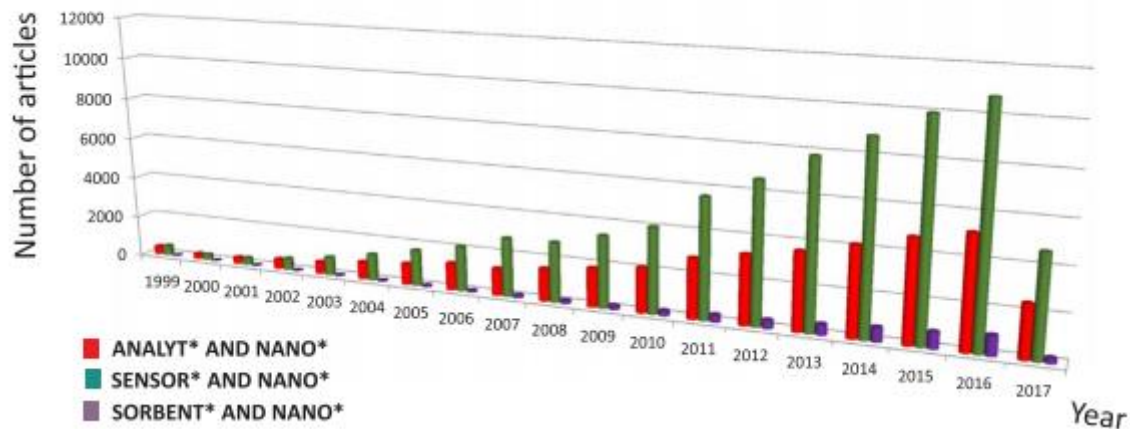
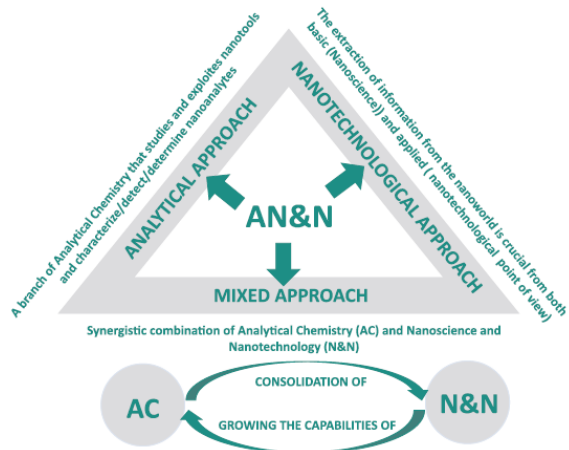
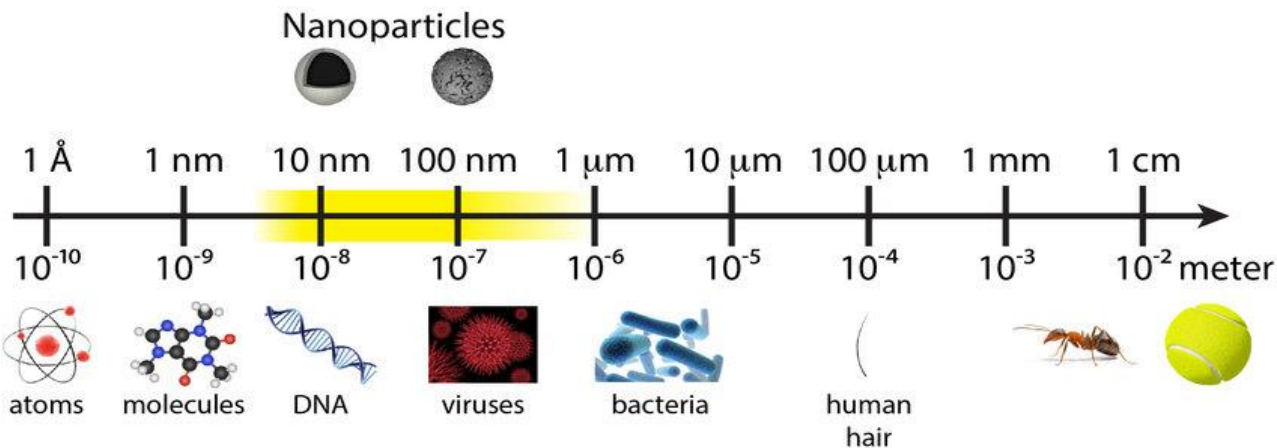
Materials in the range of 100 nm are considered to be nanoparticles. They exhibit a wide range of properties, including optical, electrical, catalytic, magnetic, and



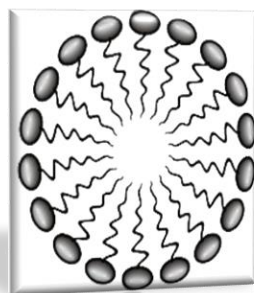
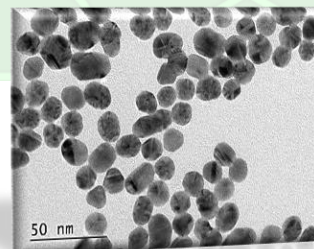
Considerations on the EU definition of a nanomaterial: Science to support policy making

Eric A.J. Bleeker*, Wim H. de Jong, Robert E. Geertsma, Monique Groenewold, Evelyn H.W. Heugens, Marjorie Koers-Jacquemijns, Dik van de Meent, Jan R. Popma, Anton G. Rietveld, Susan W.P. Wijnhoven, Flemming R. Cassee, Agnes G. Oomen

Is this Nano?

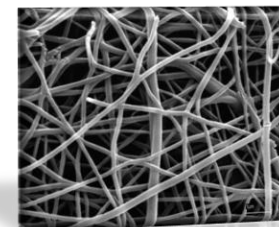


Nano-objects
Nano-
particles



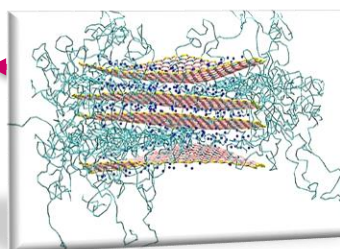
Micell
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Nanofib
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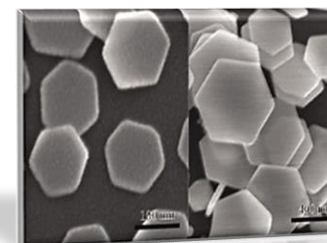


Nanomaterials (NMs)

Nanoformulation
s



Nanopla
te



Nanocomposite

Nanotecnologie e nanomateriali nel mondo

INAIL



Entro il **2020** il **20%** circa di tutti i prodotti fabbricati nel mondo impiegheranno una certa quota di nanotecnologie (stima ILO, 2010)

I LAVORATORI NEL SETTORE

= 100.000

2008

400.000

Il tasso di crescita mondiale è stimato pari al **25% annuo**

2020 (stima ROCO M)

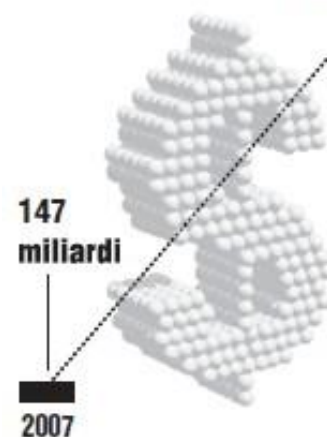
6 milioni



IL VALORE DEI NANOPRODOTTI

Dati in dollari americani (stima ROCO M)

3.000 miliardi



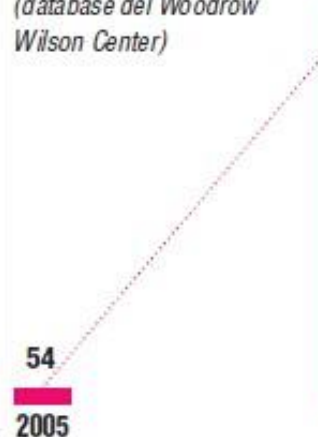
LA LORO DIFFUSIONE

Numero di prodotti nei quali si trovano nanomateriali (database del Woodrow Wilson Center)

1.317

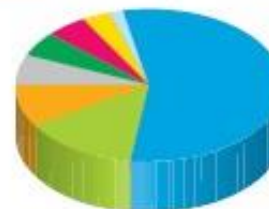
54
2005

2011

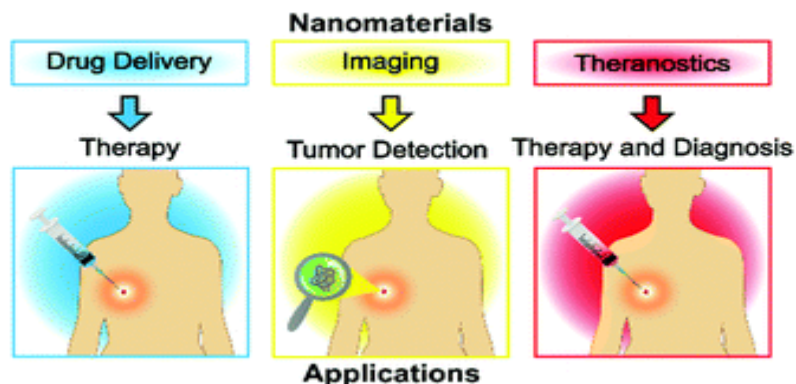


LE AREE DI UTILIZZO

- Salute e benessere
- Casa e giardino
- Cibo e bevande
- Automobile
- Elettronica e informatica
- Varie
- Elettrodomestici
- Prodotti per bambini



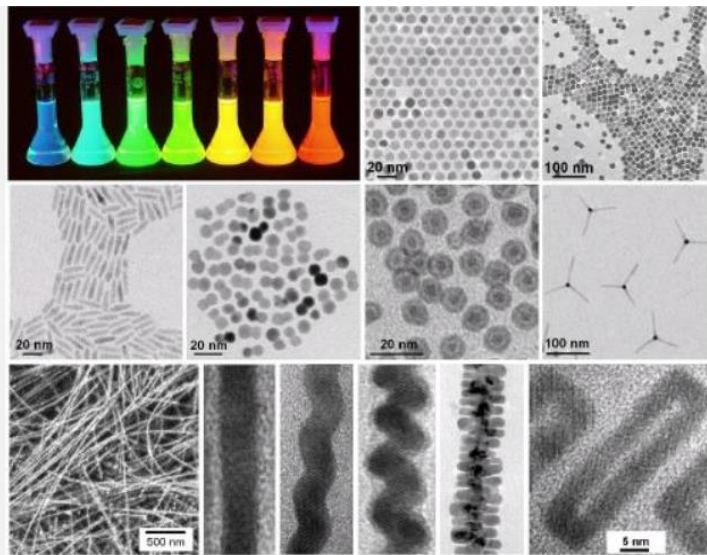
centimstri.it



Nanomaterials for electrochemical sensors

- Metal Nanoparticles;
- carbon nanotubes
- graphene;

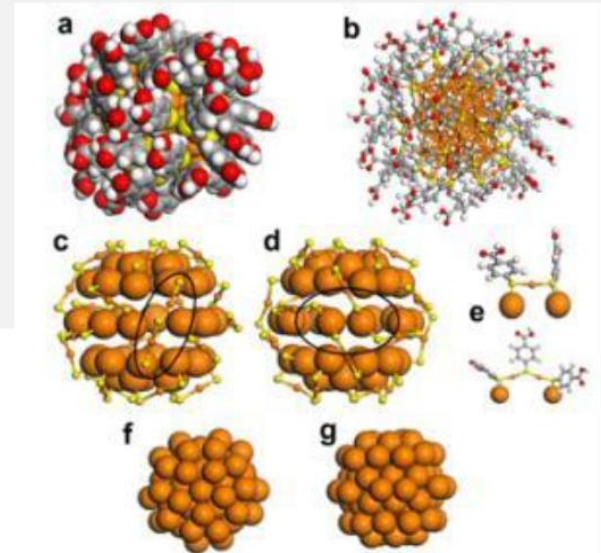
- Different properties with respect to micromaterial;
- catalytic
- High Surface/Volume;



SWNT



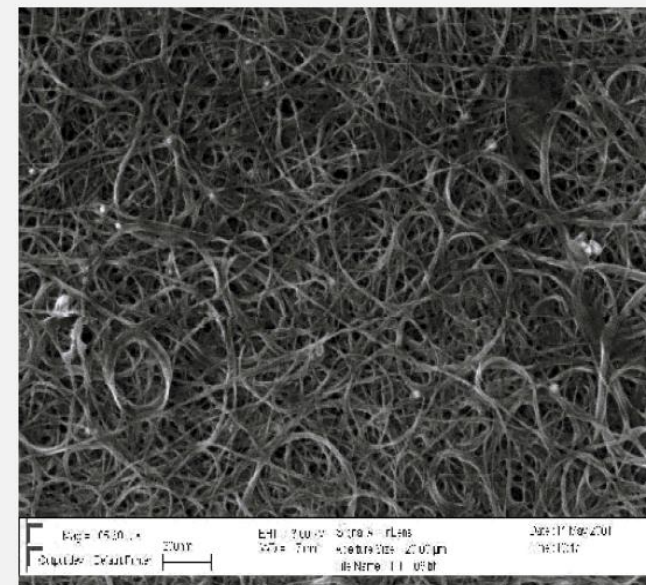
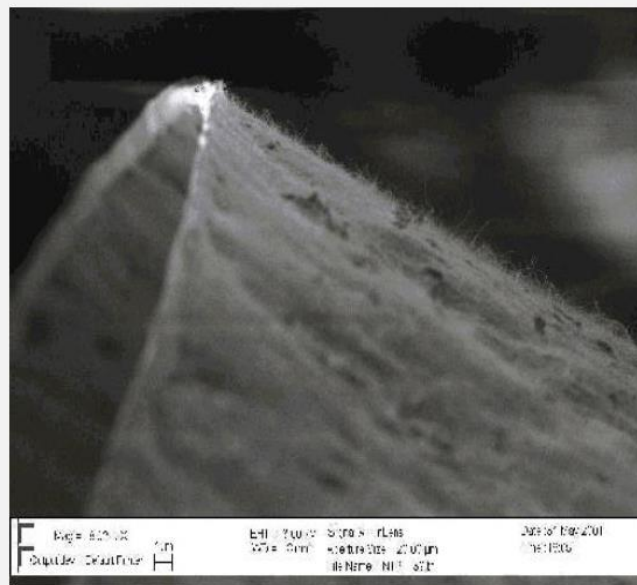
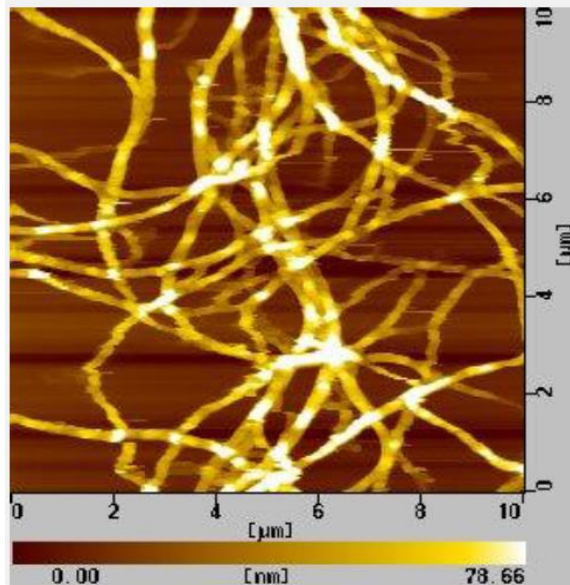
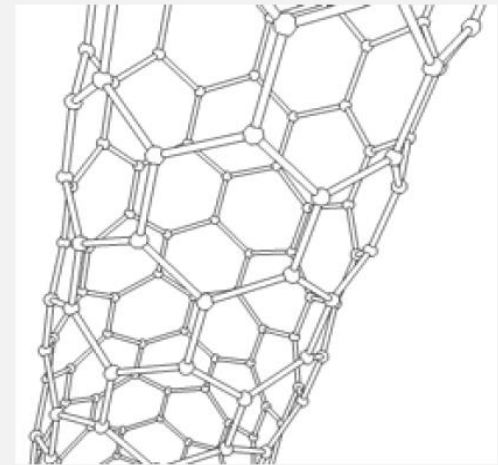
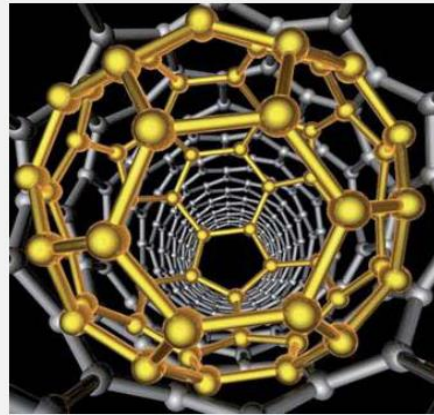
MWNT



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?

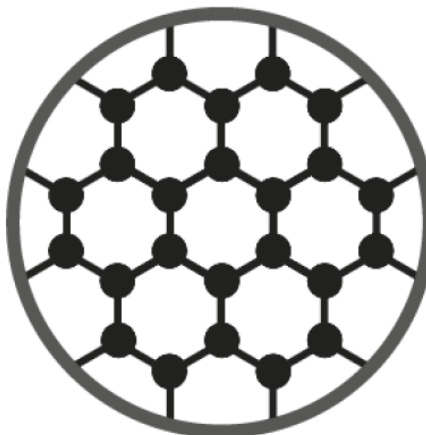


SINGLE LAYER OF CARBON ATOMS

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.

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.

POTENTIAL USES OF GRAPHENE



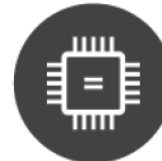
TOUCH SCREENS IN DEVICES

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.

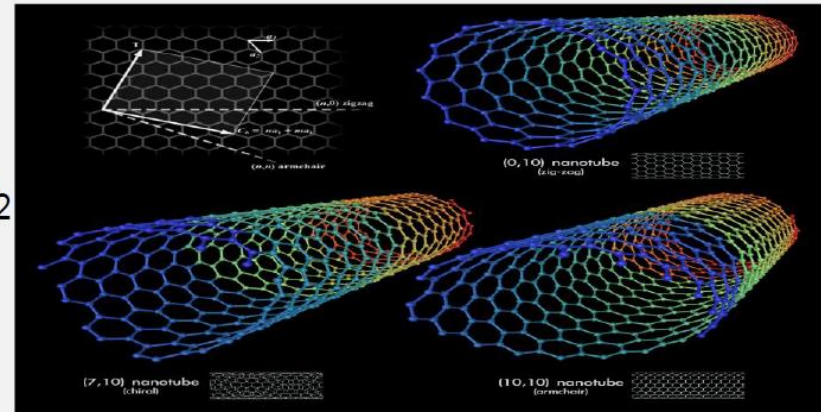
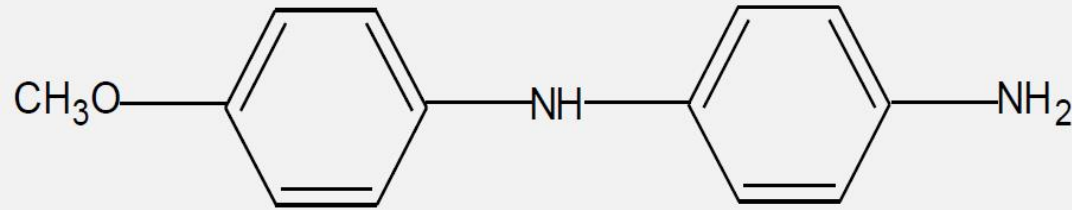


ENERGY STORAGE & COMPOSITES

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.



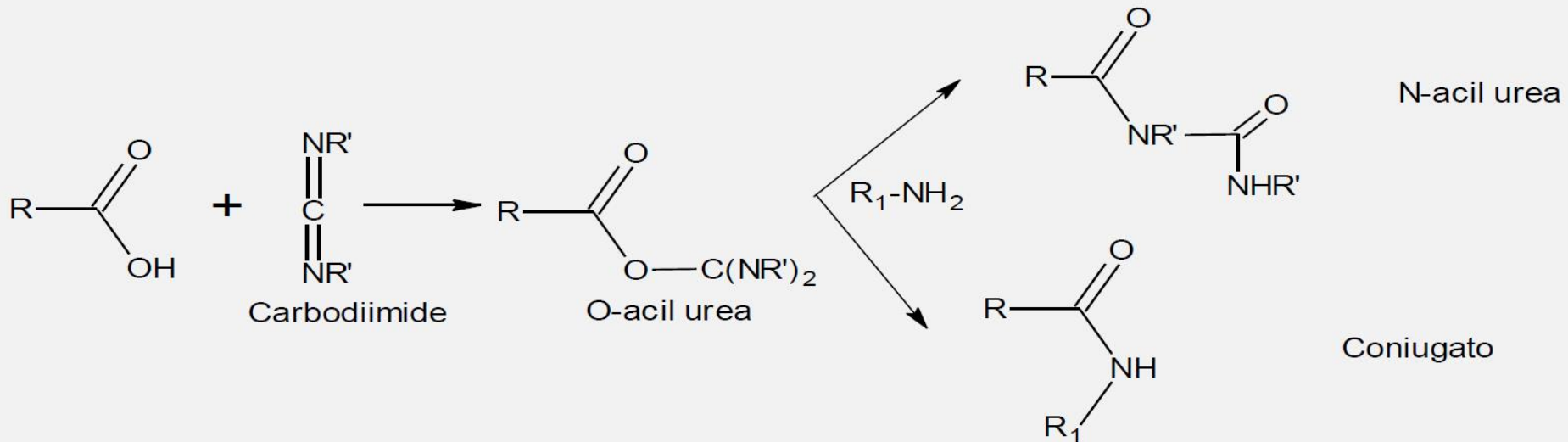
NADH sensor based on Variamine Blue on single wall carbon nanotubes onto Screen printed electrodes

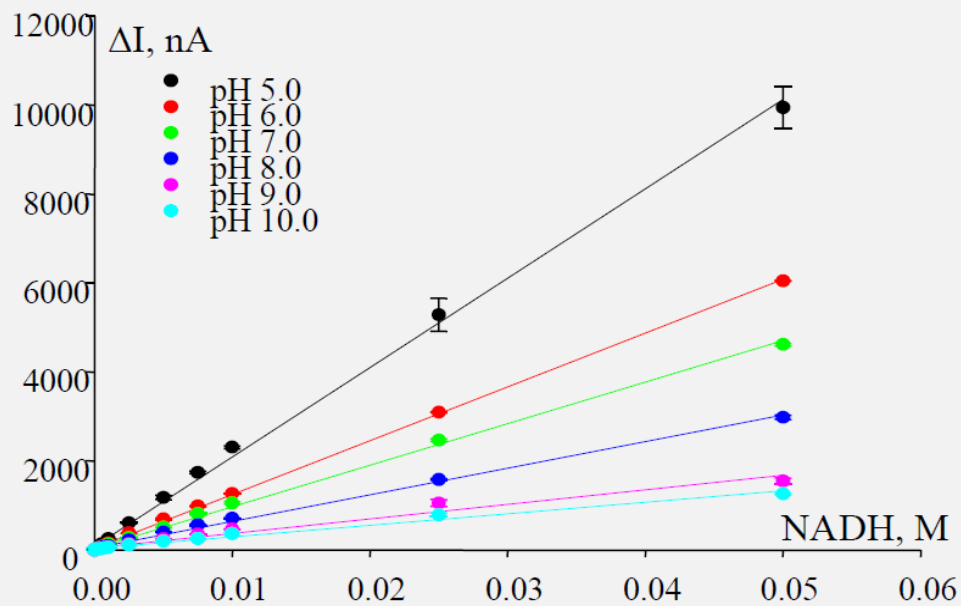
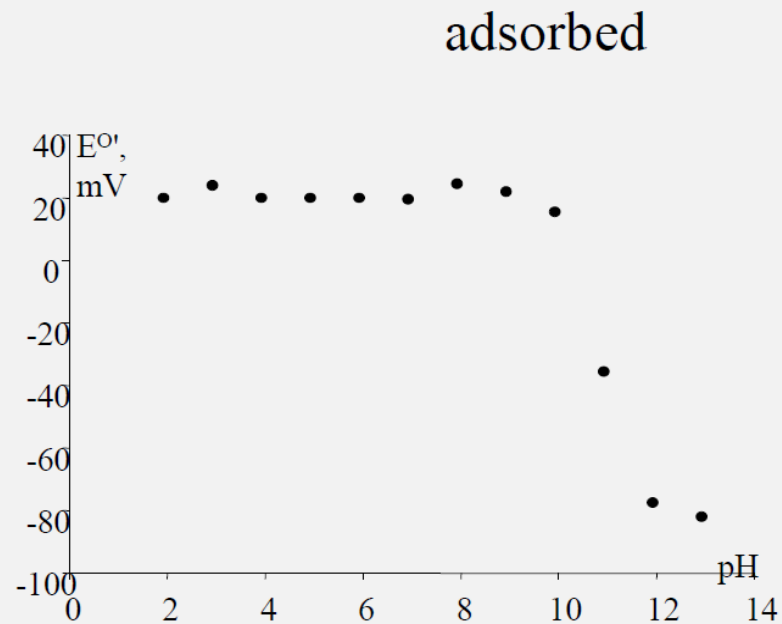
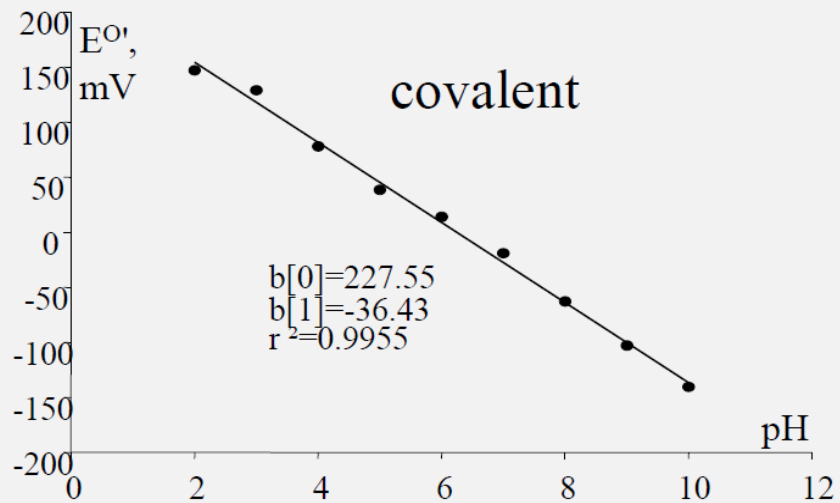


METODO EDC

(1-ethyl-3-(3-dimethylamminopropyl)carbodiimide)

SWCNTs-COOH







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Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb



Reduced graphene oxide-carboxymethylcellulose layered with platinum nanoparticles/PAMAM dendrimer/magnetic nanoparticles hybrids. Application to the preparation of enzyme electrochemical biosensors



Boryana Borisova^a, Alfredo Sánchez^a, Sandra Jiménez-Falcao^a, Miriam Martín^b,
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^d IMDEA Nanoscience, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain

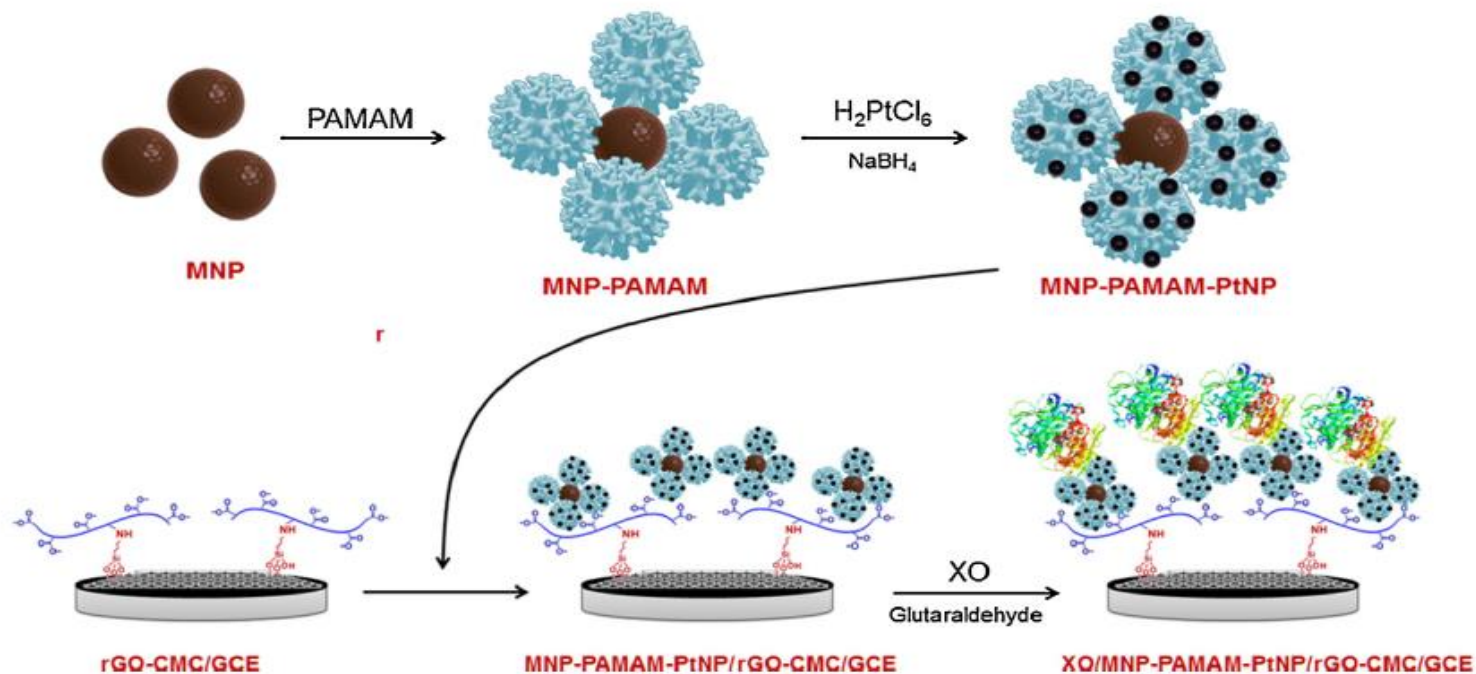


Fig. 1. Schematic display of the preparation of the MNP-PAMAM-PtNP and the XO/MNP-PAMAM-PtNP/rGO-CMC/GCE enzyme electrode.

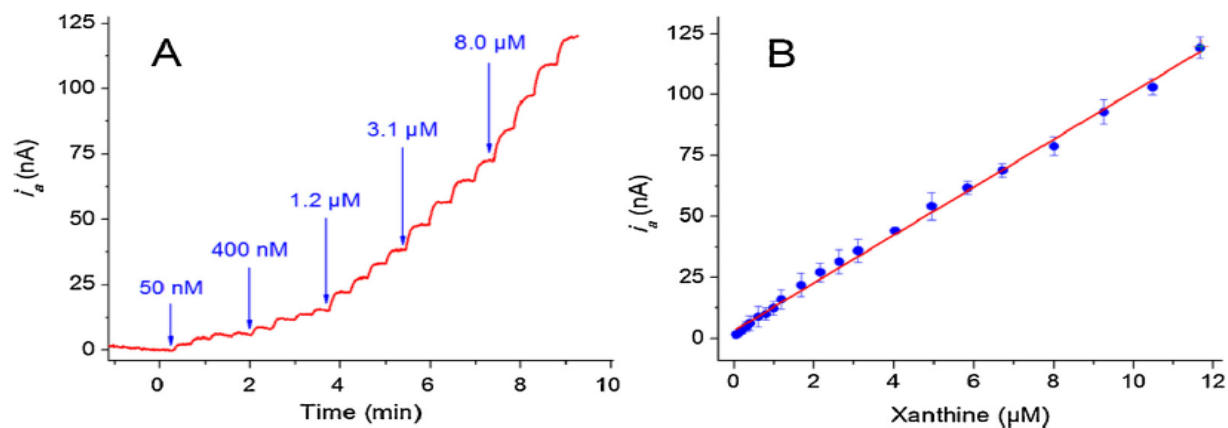


Fig. 5. Amperometric responses (A) and calibration curve (B) recorded with the XO/MNP-PAMAM-PtNP/GO-CMC/GCE biosensor. $E_{\text{app}} = +600$ mV, stirring condition: 300 rpm.



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Original Article

Novel electrochemical xanthine biosensor based on chitosan–polypyrrole–gold nanoparticles hybrid bio-nanocomposite platform



CrossMark

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bio-nanocomposite

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gold nanoparticles

xanthine oxidase

ABSTRACT

The aim of this study was the electrochemical detection of the adenosine-3-phosphate degradation product, xanthine, using a new xanthine biosensor based on a hybrid bio-nanocomposite platform which has been successfully employed in the evaluation of meat freshness. In the design of the amperometric xanthine biosensor, chitosan–polypyrrole–gold nanoparticles fabricated by an *in situ* chemical synthesis method on a glassy carbon electrode surface was used to enhance electron transfer and to provide good enzyme affinity. Electrochemical studies were carried out by the modified electrode with immobilized xanthine oxidase on it, after which the biosensor was tested to ascertain the optimization parameters. The Biosensor exhibited a very good linear range of 1–200 μM , low detection limit of 0.25 μM , average response time of 8 seconds, and was not prone to significant interference from uric acid, ascorbic acid, glucose, and sodium benzoate. The resulting bio-nanocomposite xanthine biosensor was tested with fish, beef, and chicken real-sample measurements.

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creativecommons.org/licenses/by-nc-nd/4.0/

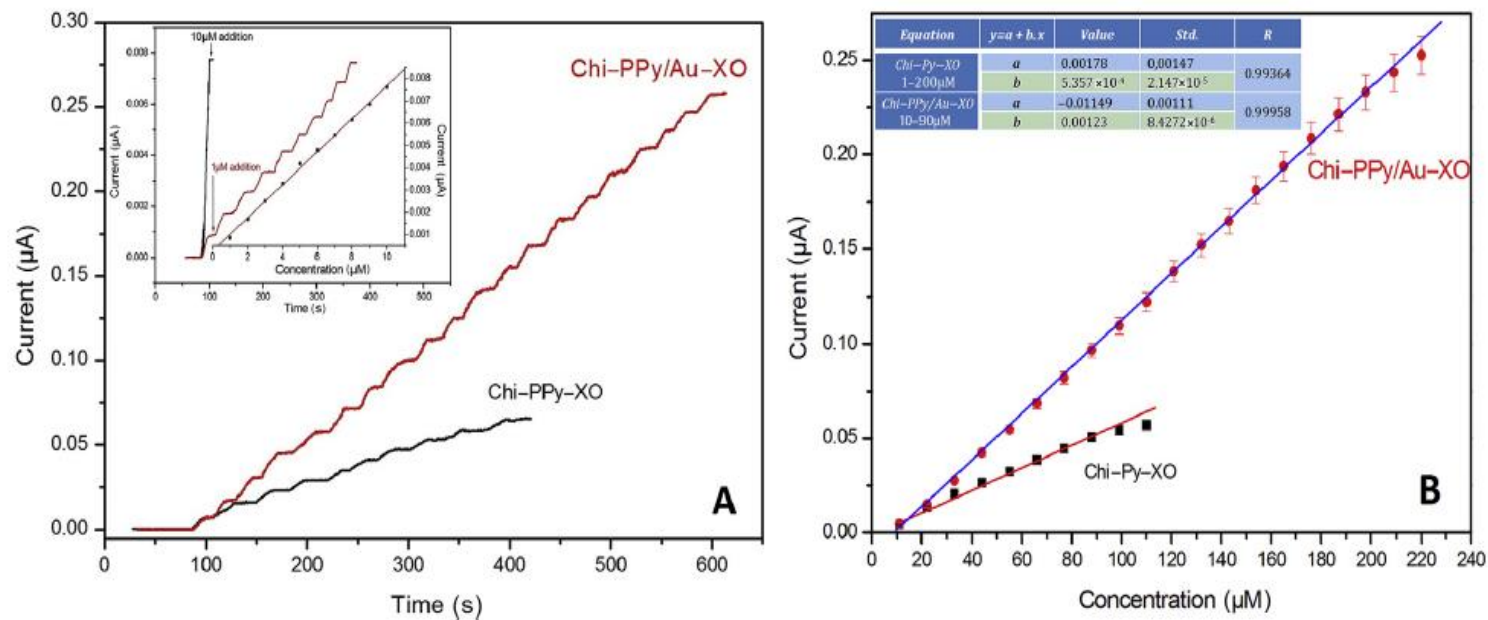


Figure 3 – (A) Amperometric response of biosensing electrodes to successive addition of 10 μM xanthine at an applied potential + 0.70 V in stirred 10 mM PBS (pH 7.0) (Inset: amperometric response of the biosensor to successive addition of 1 μM xanthine). (B) Calibration curves for the amperometric response of the biosensing electrodes.

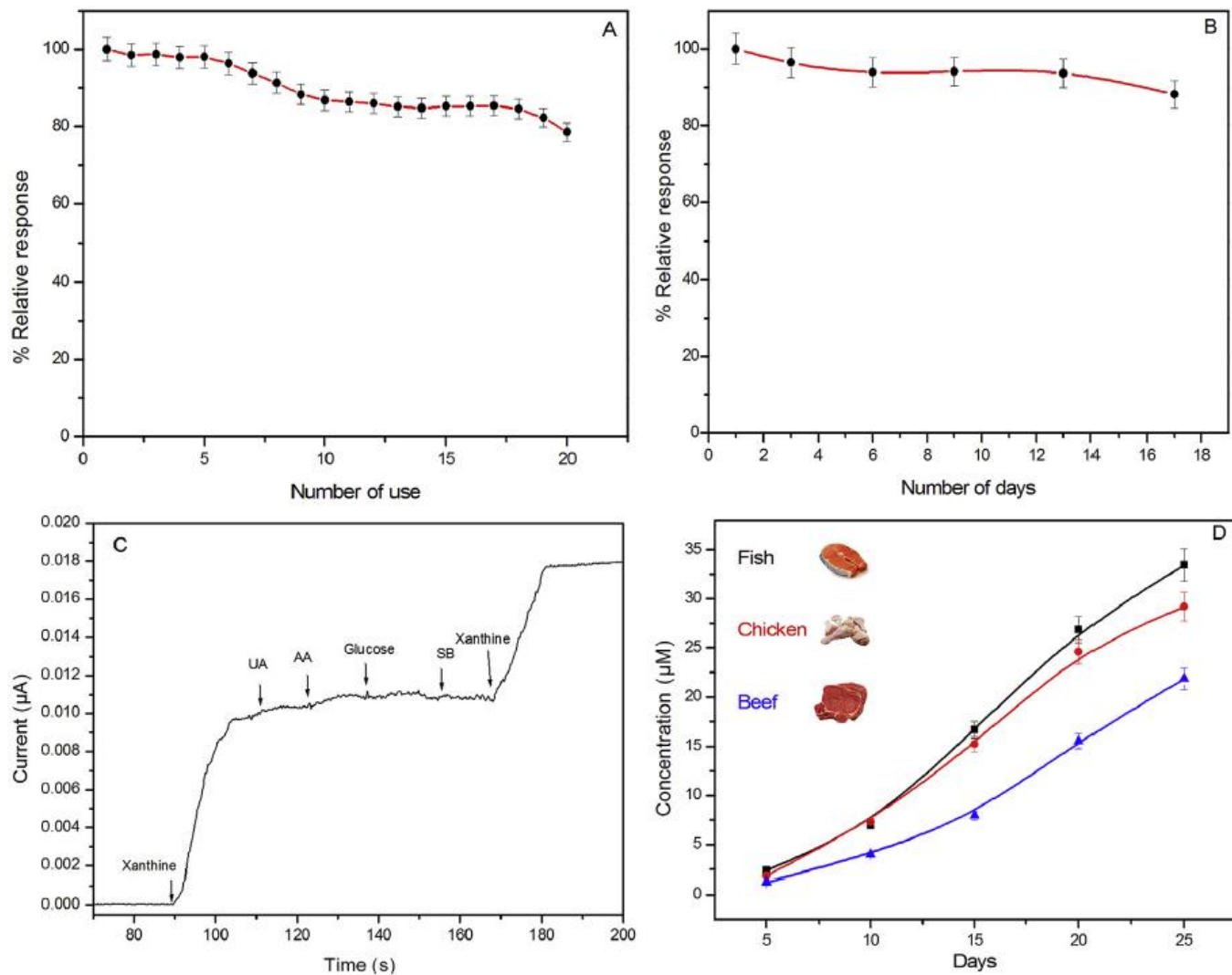
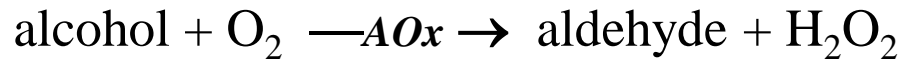


Figure 4 – (A) Operational stability of the biosensing electrode to xanthine additions (10 mM PBS pH 7.0). (B) Long-time stability of biosensing electrode by tracking the amperometric responses of enzyme electrodes in intervals of 18 days (10 mM PBS pH 7.0). (C) Interference effect of potential interferants (uric acid, ascorbic acid, glucose, and sodium benzoate) to the amperometric response of the biosensing electrode (10 mM PBS pH 7.0). (D) Determination of xanthine concentrations in fish, chicken, and beef samples during 25 days (10 mM PBS pH 7.0). AA = ascorbic acid; SB = sodium benzoate; UA = uric acid.



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 .

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 $\text{ATP}(\text{Mg}^{2+})$ + 0.02% Tween. Flow rate 0.5 mL/min; injection loop 250 μL .

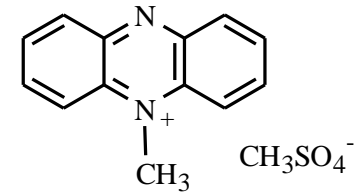
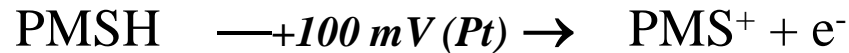
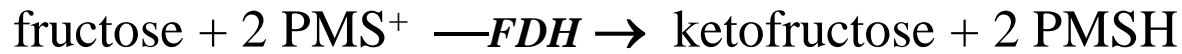
Analytical performances:

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 μ L.

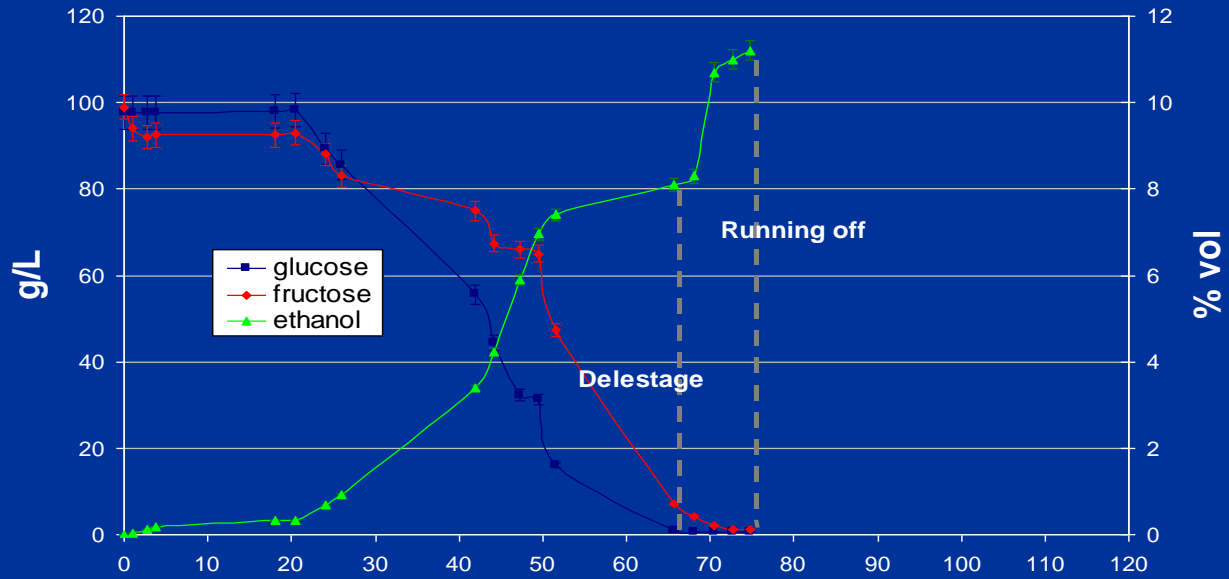
Analytical performances:

detection limit 5×10^{-7} mol/L

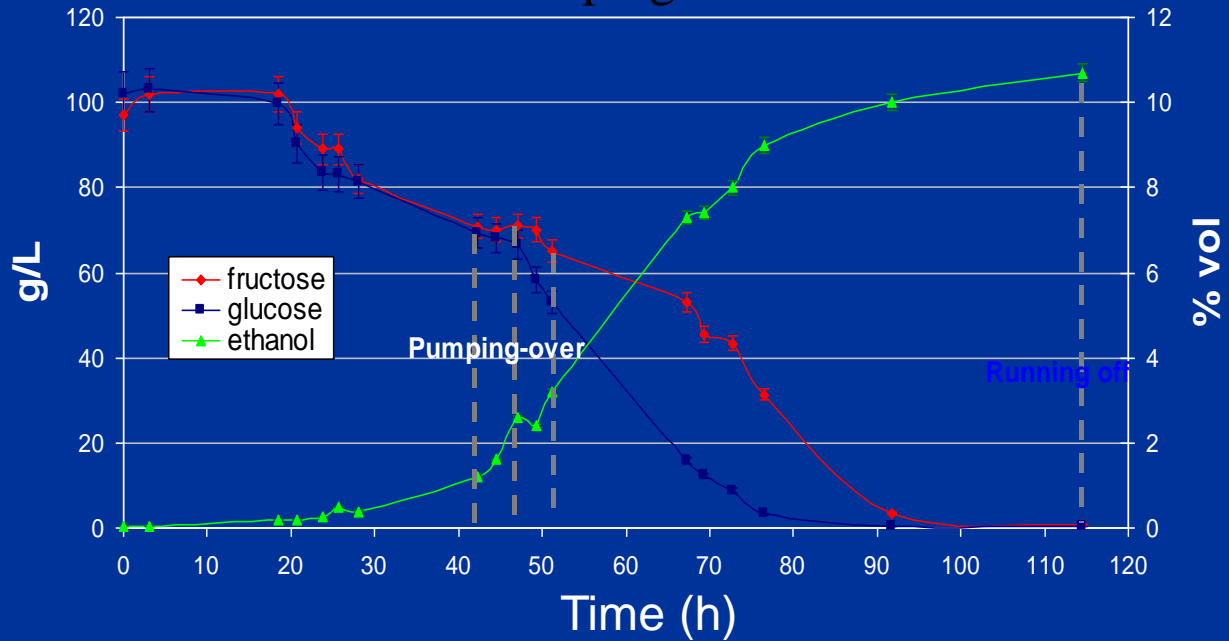
linearity 10^{-6} / 8×10^{-4} mol/L

stability: 30% decrease after 200 samples

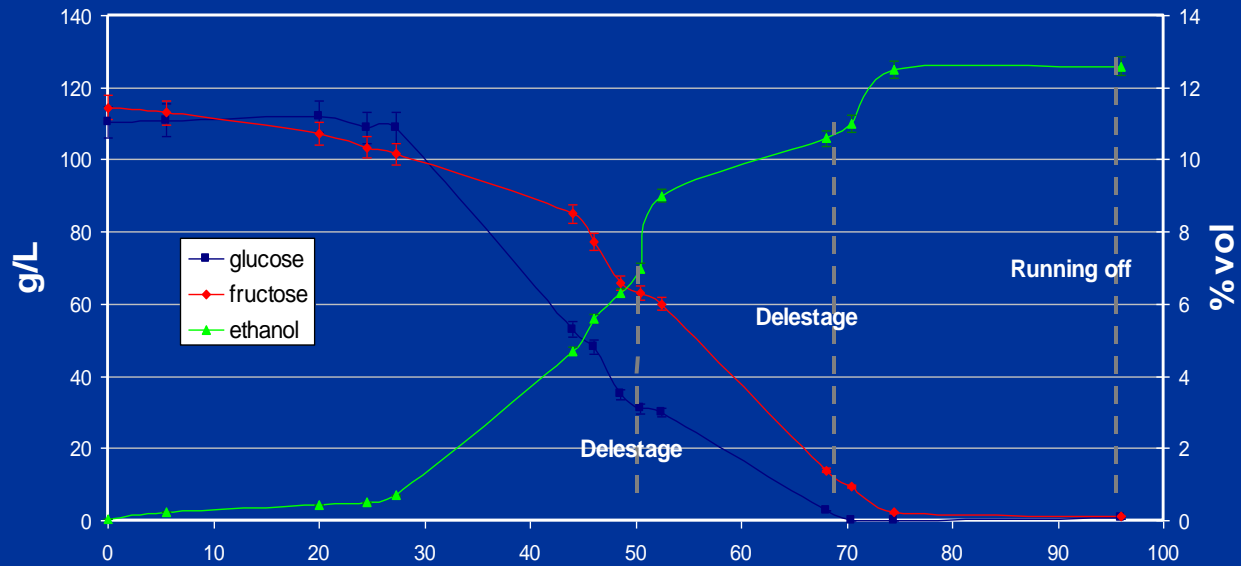
One delestage



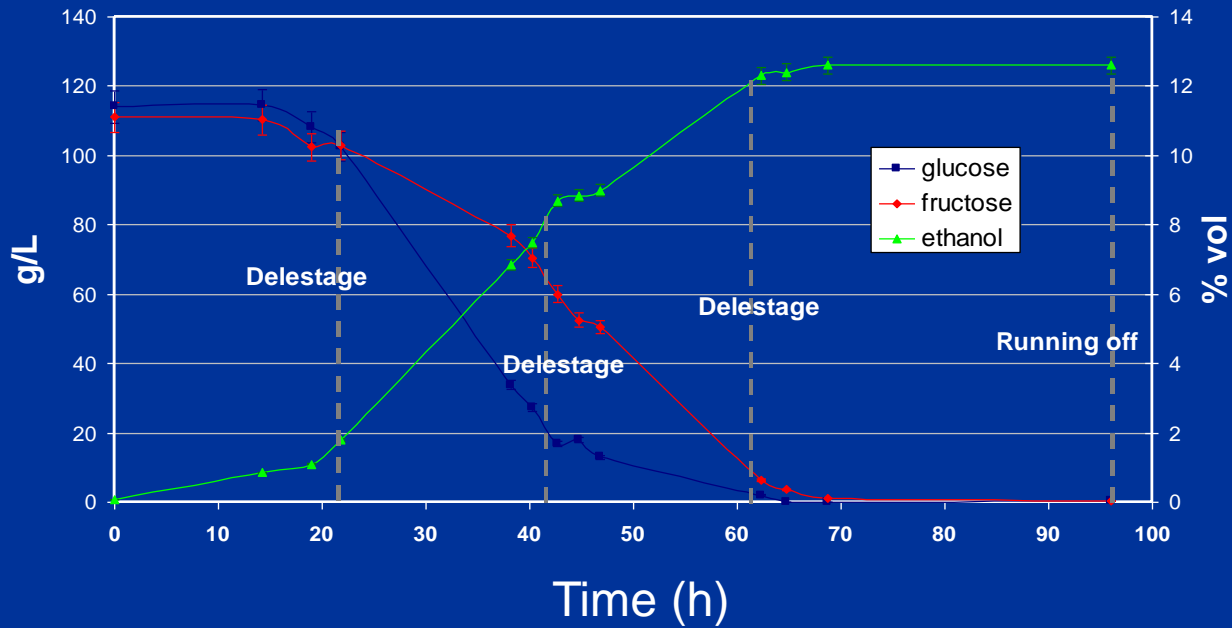
Pumping-over



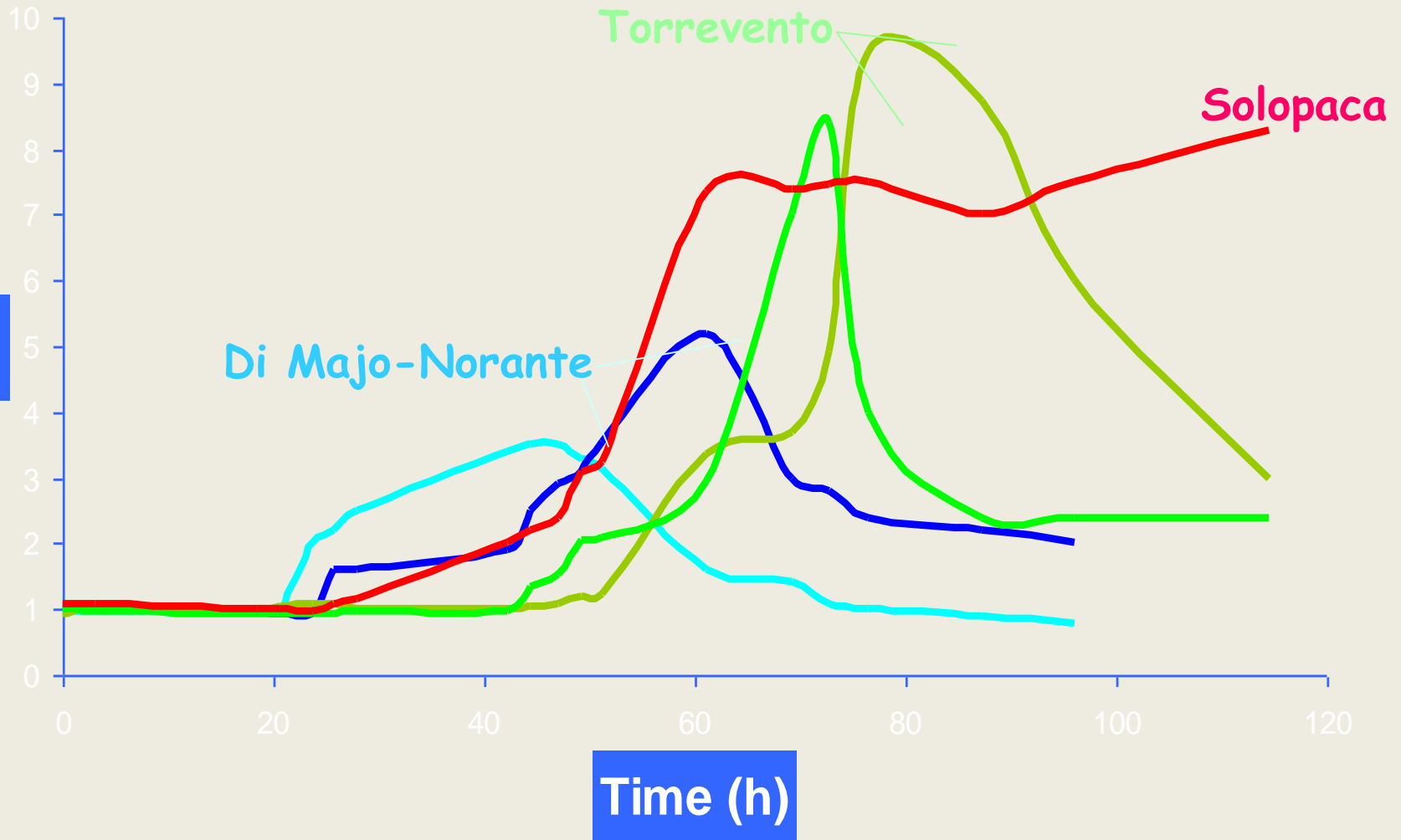
Delayed anthocyan extraction



More Delestage

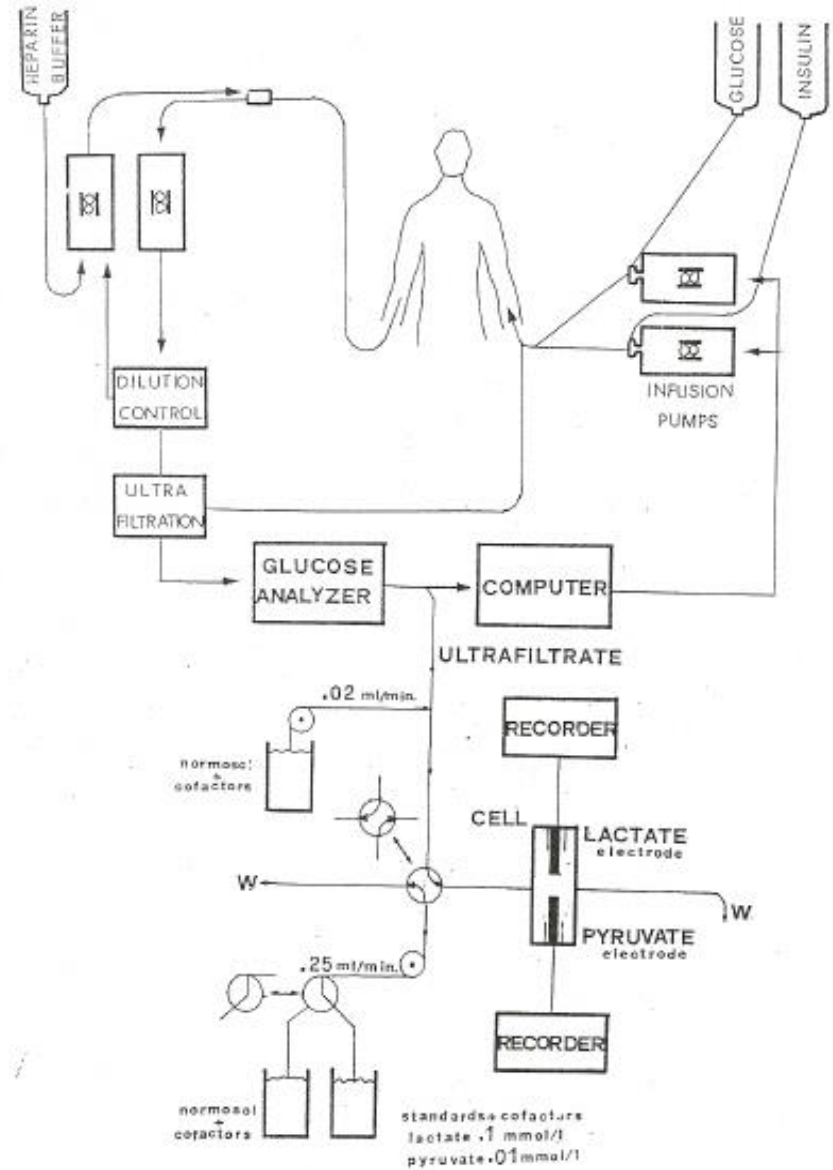


Fructose:Glucose ratio during alcoholic fermentation



Betalike

Sistema automatico per il controllo glicemico

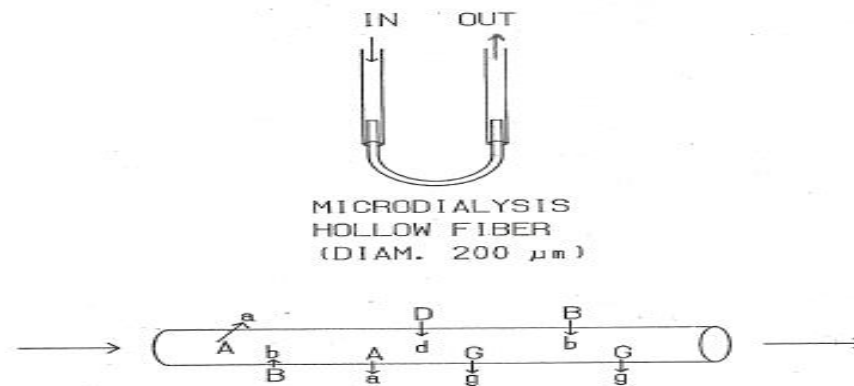


FEATURE OF MICRODIALYSIS

The extracellular space is the compartment in the body where substances travel between cells. It is the crossroad of nutrients, metabolites, waste products, transmitters, hormones, drugs and toxins. The ability to study this compartment has so far been very limited as it is not possible to draw liquid from it. Instead, scientists have been using blood samples which reflect the collected chemistry of many different organs and tissues of the body.

With microdialysis there is now a way to remove chemical substances from the extracellular fluid of the body without removing liquid and a way to introduce substances without injecting fluid. It is an attempt to copy the function of blood vessels by introducing an artificial blood vessel into the tissue - a microdialysis probe.

- The perfusion system is simple: due to the presence of a membrane, the probe is a closed liquid system and the flow is unidirectional.
- It makes it possible to sample continuously for hours or days.
- It can be used to recover and/or introduce substances in the medium.
- It collects a representative sample of all substances in the analysed medium.
- The size of the perfused area can be regulated by varying the length of the membrane.
- The membrane excludes large molecules such as proteins and enzymes, i.e. purifies the sample so that it is possible to introduce directly into the analytical instrument.
- It is possible to monitor multiple analytes by coupling other analytical techniques to the microdialysis system.



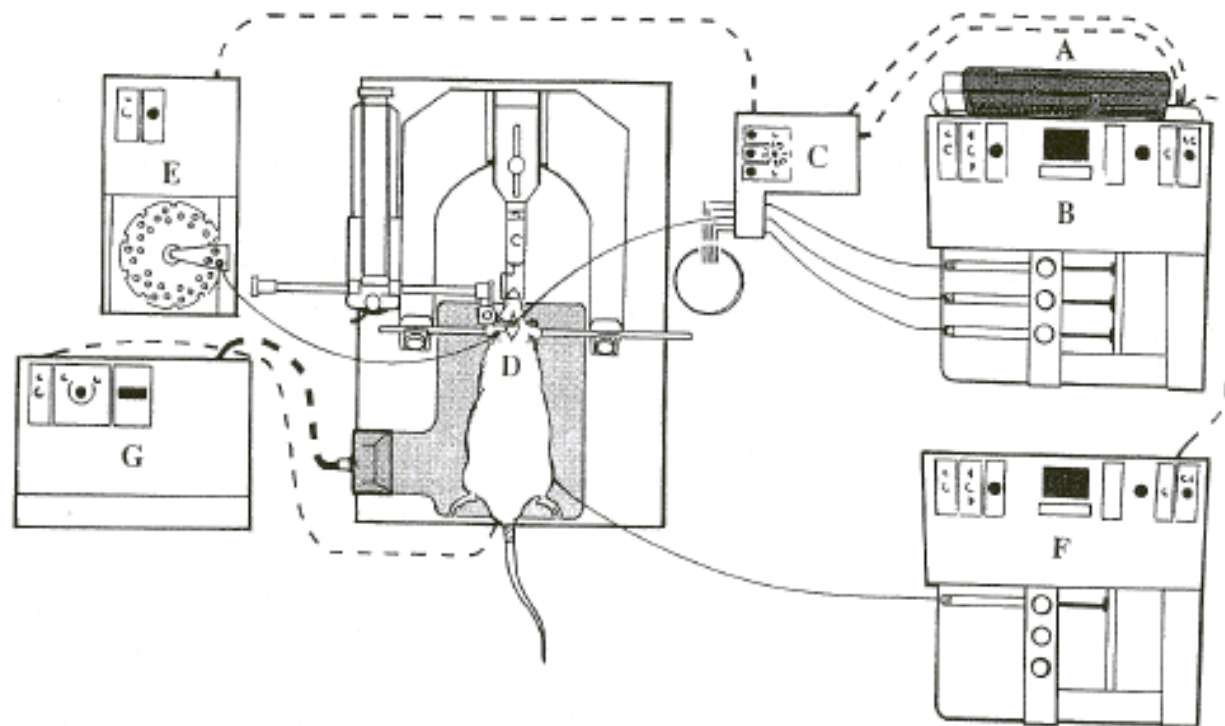
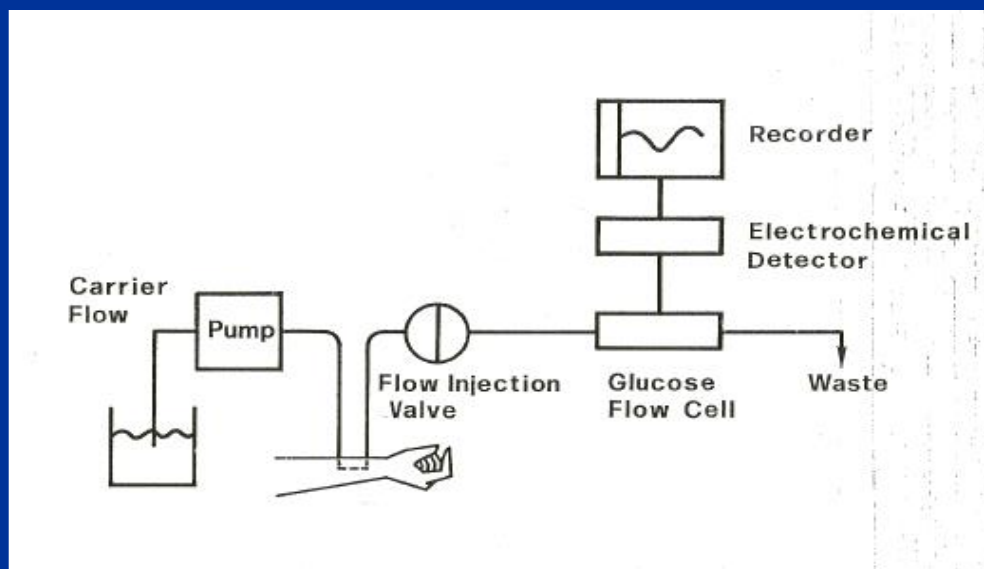


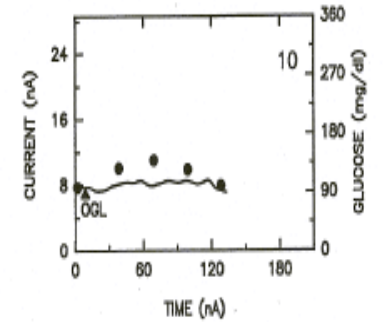
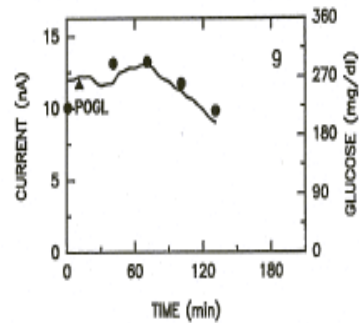
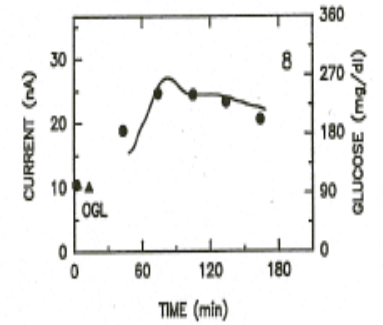
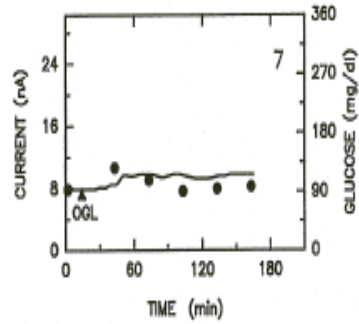
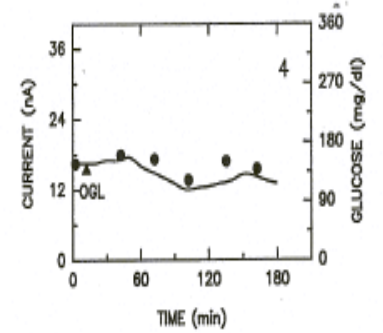
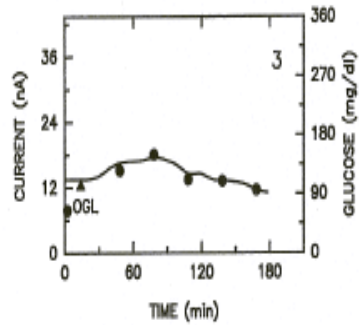
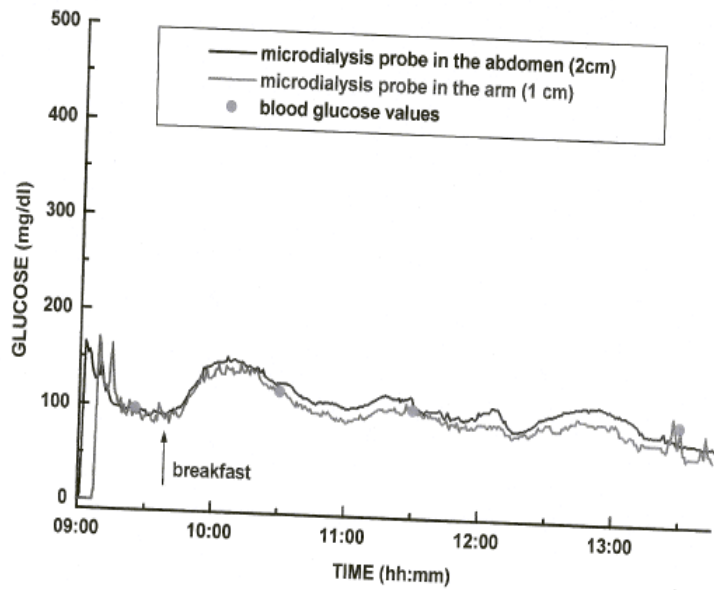
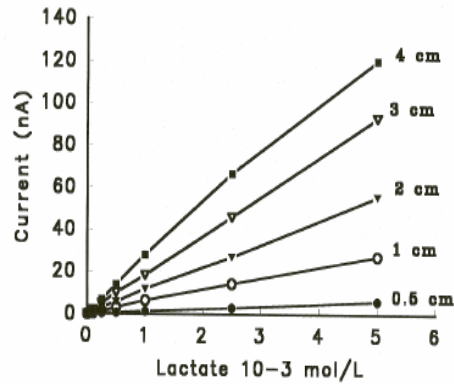
Fig. 2. Schematic drawing of the instruments used during a microdialysis experiment on a rat. A = computer controlling the experiment, B = perfusion pump with three syringes containing different perfusion fluids, C = syringe selector which selects one of the syringes of the pump (B) under computer control (A), D = anaesthetized rat in a stereotaxic instrument. The microdialysis probe is held in the instrument and placed in the brain, E = microfraction collector, which collects fractions under the control of the computer (A) or the pump (B), F = second pump used to inject a drug systemically under the control of the computer (A), G = temperature controller to maintain the anaesthetized animal at the correct temperature.

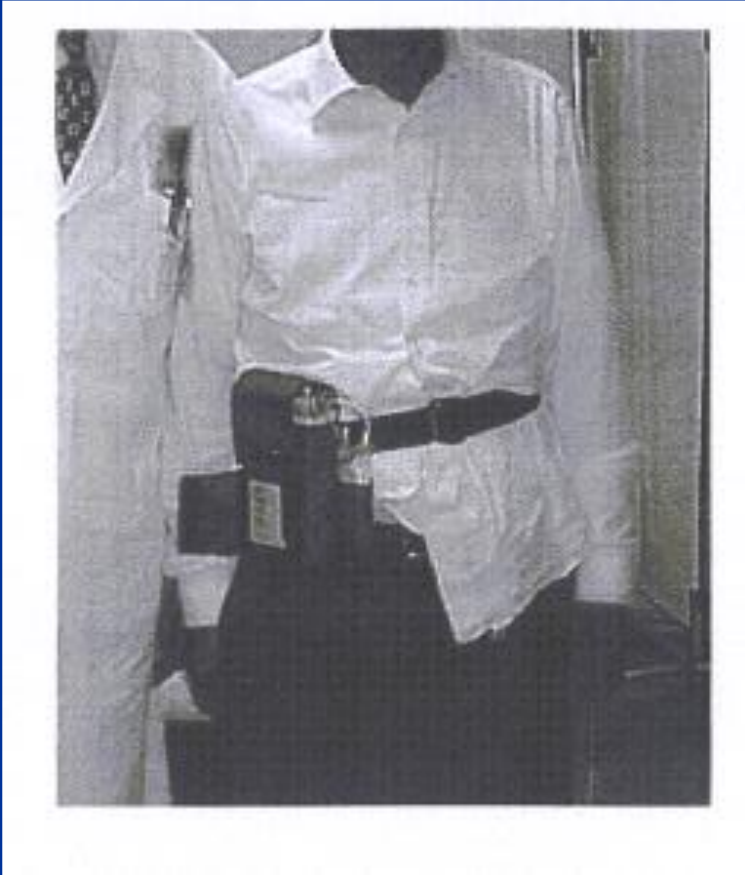
CARATTERISTICHE DI UN SENSORE PER LA MISURA "IN VIVO"

- essere biocompatibile;
- mostrare stabilità di risposta per tutto il tempo della misura (che può essere anche 24 ore o più);
- misurare in un range di concentrazione pari al range fisiologico sia in condizioni normali che soprattutto patologiche;
- essere miniaturizzabile, in modo che la sua inserzione "in vivo" e la sua permanenza non siano dolorose;
- non risentire della presenza di sostanze interferenti presenti nei fluidi biologici, che non possono essere eliminate.



Calibration curves of lactate using different microdialysis fibers
Flow rate 30 μ l /min

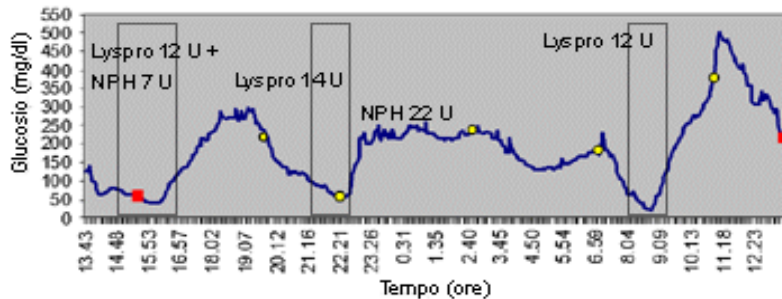




GlucoDay® S è uno strumento innovativo, sviluppato dalla ricerca Menarini, per il monitoraggio continuo sottocutaneo del glucosio, per un periodo di 48 ore, nei pazienti umani.

GlucoDay® S nel mondo è il primo strumento basato sulla tecnica della "microdialisi" ad avere ottenuto la marcatura CE ai sensi della Direttiva sui Dispositivi Medici, la quale è indispensabile per la commercializzazione di un prodotto nell'ambito dell'Unione Europea.

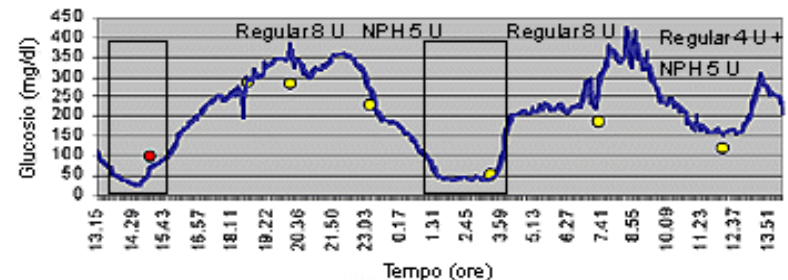
GlucoDay® S è un dispositivo professionale che deve essere utilizzato solo da personale medico qualificato e addestrato e che è stato specificamente progettato per indagini cliniche o diagnostiche sui pazienti. **GlucoDay® S** è classificato come Dispositivo Medico, Classe IIA.



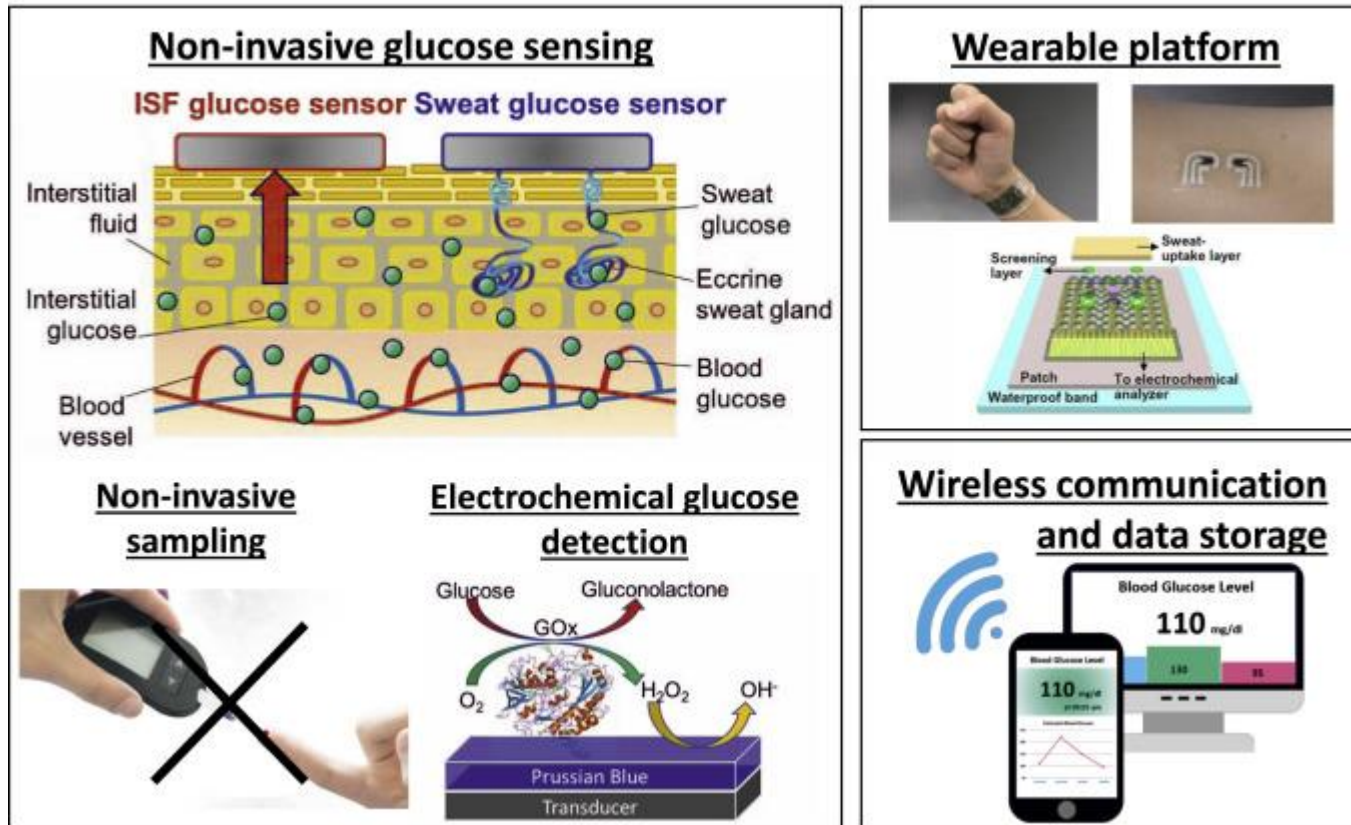
In questo giovane paziente diabetico sono stati rilevati 3 episodi di ipoglicemia post-prandiale (dopo pranzo, cena e colazione), come potete vedere in questo profilo. Le misure della glicemia con sangue venoso sono rappresentate in rosso, quelle con sangue capillare sono rappresentate in giallo.



In questo paziente con DM1 **GlucoDay® S** ha rivelato un episodio di ipoglicemia prolungata durante la notte.



Wearable epidermal glucose sensors



Da : **Jayoung Kim, Alan S.Campbell, Joseph Wang**
Talanta Volume 177, 15 January 2018, Pages 163-170
Wearable non-invasive epidermal glucose sensors: A review

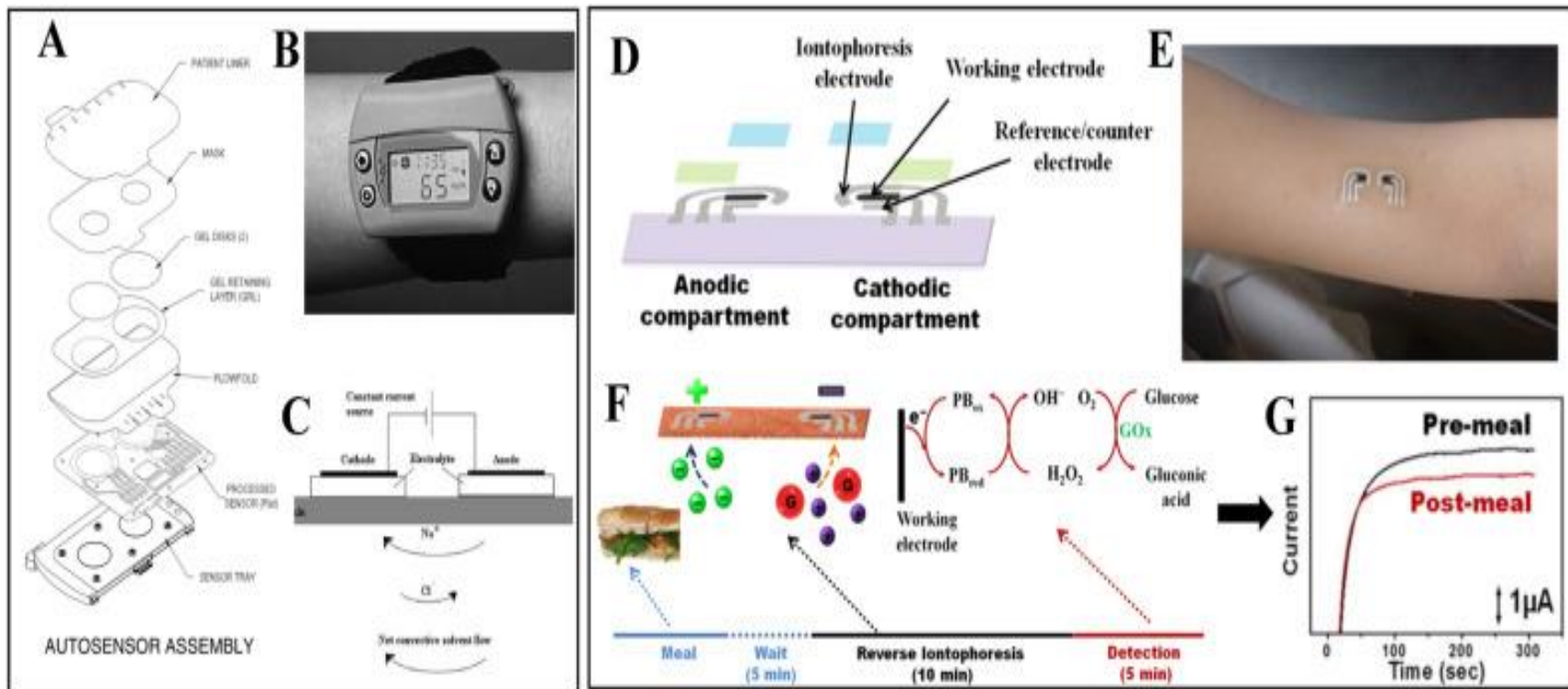


Fig. 2. Epidermal [glucose](#) sensing in skin ISF through reverse [iontophoresis](#). A) Exploded view of GlucoWatch® biographer assembly. B) Depiction of GlucoWatch® biographer display. Reproduced with permission [50]. Copyright 2001, Elsevier. C) ‘Reverse iontophoresis’ process for glucose extraction. Reproduced with permission [48]. Copyright 2000, Taylor & Francis Group. D) Tattoo-based printable iontophoretic sensing configuration. E) Photograph depiction of tattoo based printable iontophoretic sensing system applied to human subject. F) Schematic representation of tattoo-based printable glucose sensing system operation. G) Tattoo-based printable glucose sensing system output on human subject before and after meal. Reproduced with permission [25]. Copyright 2014, American Chemical Society.

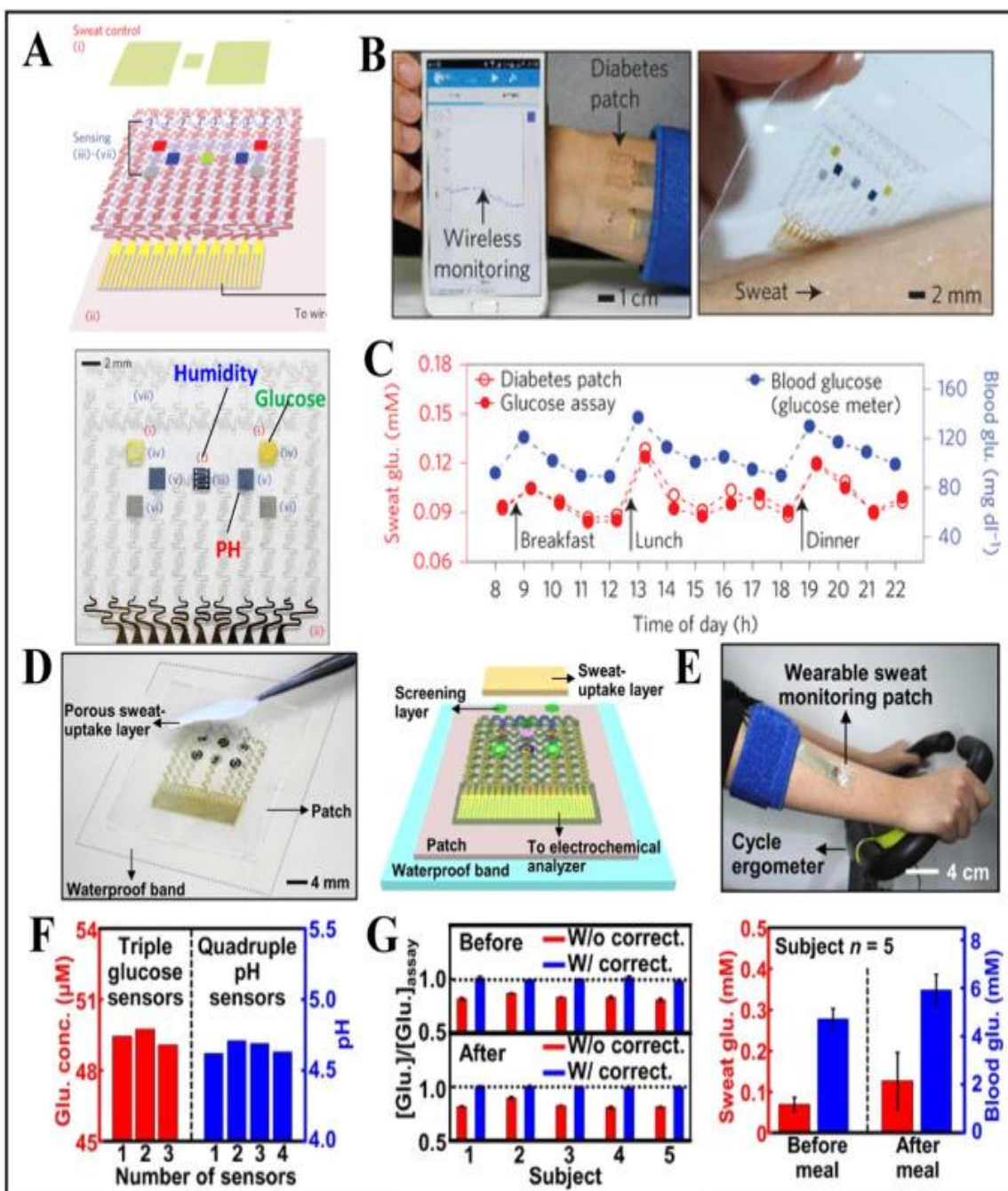


Fig. 3. Epidermal [glucose](#) monitoring with flexible patch. A) Schematic representation and photograph depiction of flexible diabetes patch composed of sweat control, multiplexed sensing and therapeutic components. B) Photograph depiction of integrated diabetes monitoring and therapy system applied to human subject. C) On-body glucose monitoring on human subject over the course of three meals with comparison to *ex vivo* and blood glucose analysis. Reproduced with permission [\[40\]](#). Copyright 2016, Macmillan Publishers. D) Photograph depiction and schematic representation of wearable sweat analyte monitoring patch. E) Photograph of the subject using a cycle ergometer for sweat generation with the wearable patch on the subject's arm. F) Multimodal glucose and pH sensing to improve detection accuracy. G) Comparison of sweat glucose concentration measured using on-body sweat glucose monitoring patch and blood glucose concentration before and after meal. Reproduce with permission [\[41\]](#). Copyright 2017, American Association for the Advancement of Science.

Table 1

Summary of epidermal wearable glucose sensors.

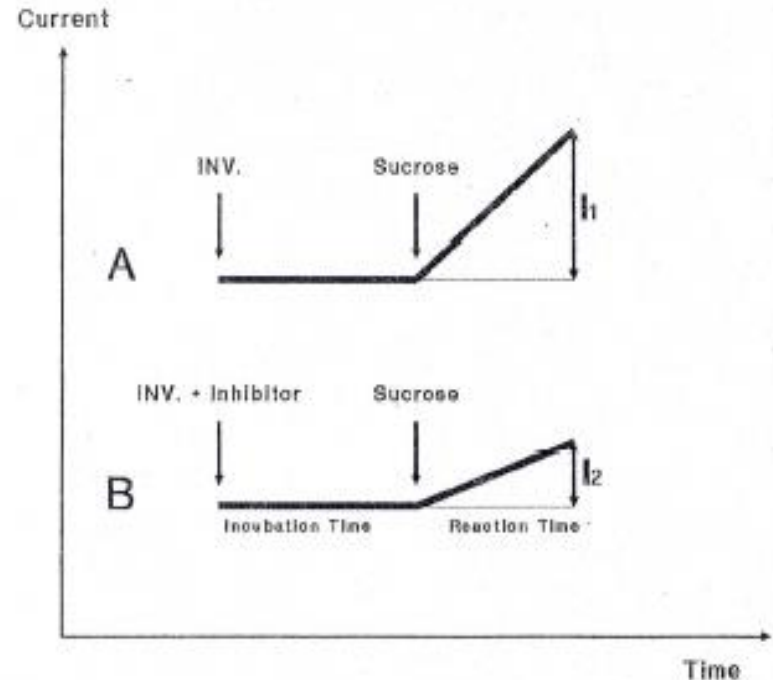
Wearable glucose sensor	Biofluid	Sampling method	Advantages	Next Steps	Refs
GlucoWatch® biographer	ISF	Reverse iontophoresis	<ul style="list-style-type: none"> ● FDA approved ● First commercialized product of this type ● Continuous monitoring ● Electronics for measurement and data storage were combined 	<ul style="list-style-type: none"> ● Minimize skin irritation ● Shorter warming up period ● Interference by sweat generation ● Time lag compared to blood glucose 	[50]
Temporary tattoo	ISF	Reverse iontophoresis	<ul style="list-style-type: none"> ● Cost effective ● Easy to wear ● No skin irritation 	<ul style="list-style-type: none"> ● Single use ● Study stability and reproducibility towards continuous use 	[25]
Multiplexed wearable, flexible array patch	Sweat	Exercise	<ul style="list-style-type: none"> ● Simultaneous multiplexed sweat sensing ● Integration of customized wireless electronics ● Extensive characterization of sweat 	<ul style="list-style-type: none"> ● Establish correlation to blood glucose ● Large-scale validation 	[38]
Sensor array patch coupled with induced sweating	Sweat	Iontophoresis (Stimulated)	<ul style="list-style-type: none"> ● Integration of iontophoretic sweat generation with glucose sensing ● Integration of customized wireless electronics 	<ul style="list-style-type: none"> ● Extension to on-body monitoring 	[60]
Graphene- based stretchable patch	Sweat	Exercise	<ul style="list-style-type: none"> ● Accurate monitoring by combination of pH, temperature, and humidity ● Nanomaterials-based sensitive glucose sensor ● Soft materials incorporated for stretchable/flexible device 	<ul style="list-style-type: none"> ● Increase the sampling frequency ● Replacement of the commercial analyzer with conformal interface ● Large-scale validation 	[40]
Wearable patch, multimodal glucose sensor	Sweat	Exercise	<ul style="list-style-type: none"> ● Controlled sweat uptake ● Improved accuracy of glucose sensing (multimodal sensing array) and correction with sweat pH value 	<ul style="list-style-type: none"> ● Demonstrate continuous monitoring ● Replacement of the commercial analyzer with conformal interface 	[41]
Eyeglasses sensor	Sweat	Exercise	<ul style="list-style-type: none"> ● Real-time continuous monitoring of sweat glucose ● Integration with customized wireless electronics 	<ul style="list-style-type: none"> ● Detailed study and validation ● Temperature/pH compensation 	[42]

Biosensori ad inibizione enzimatica

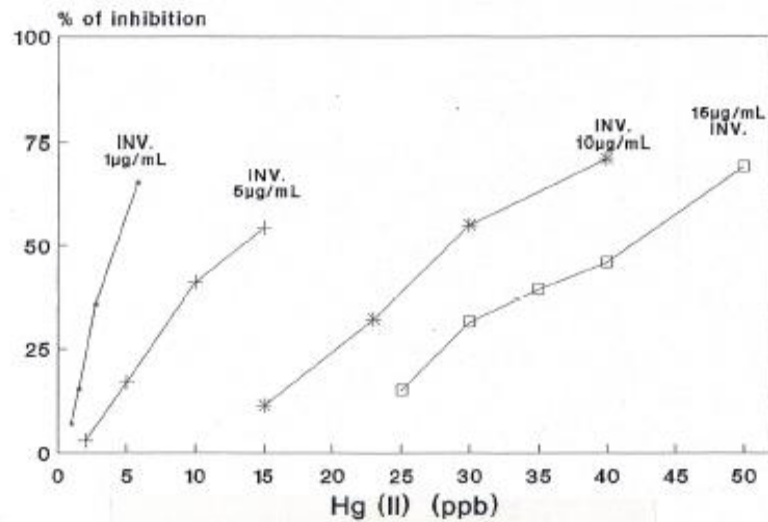
There is a great demand for rapid and sensitive analytical methods for the determination of mercury and related compounds in environmental samples. The environmental risk and toxicological concern of mercury and its compounds, especially methylmercury, have stimulated the research into various new methods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

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

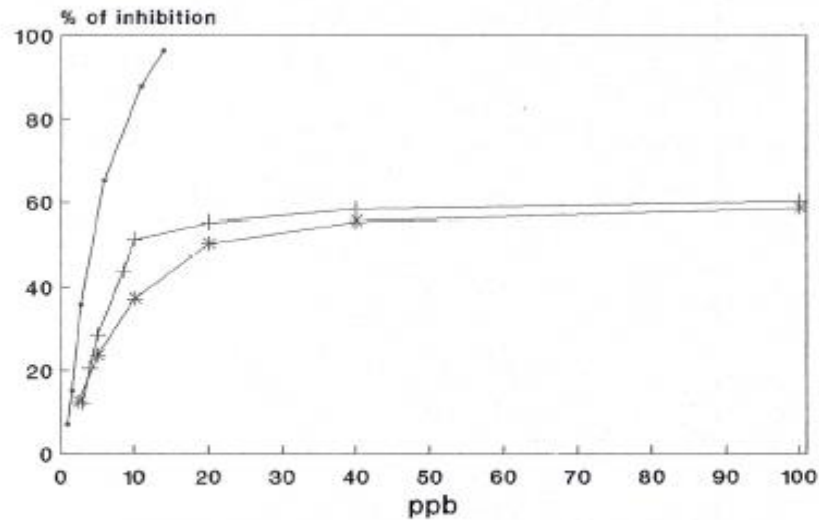
Toxicity of mercury depends on its chemical form. For example methyl mercury is more toxic than HgII (1).



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.

Biosensore per pesticidi organofosforici e carbammici

Elevata tossicità acuta (200.000 decessi/anno negli anni 80*)

Alta tossicità cronica

Moderata persistenza

Meccanismo di azione:

inibizione dell'enzima acetilcolinesterasi (AChE)

Tecniche analitiche strumentali:

Organofosforici → GC-NPD o GC-MS

Carbammati → LC-MS

*J. Jeyratnam World's Health Quarterly, 1990, 43, 139-152

Scopo del lavoro: fornire un sistema di misura semiquantitativo dei residui OP o C in alimenti

Caratteristiche del metodo:

semplicità (operatori non esperti)

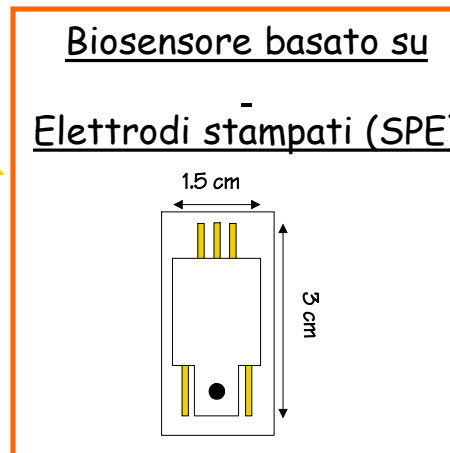
rapidità (15' analisi)

robustezza (utilizzabile in campo)

compatibile con matrici non purificate (tecniche di estrazione semplificate)

Dispositivo proposto:

• strumentazione portatile



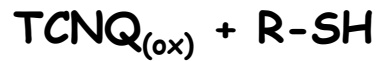
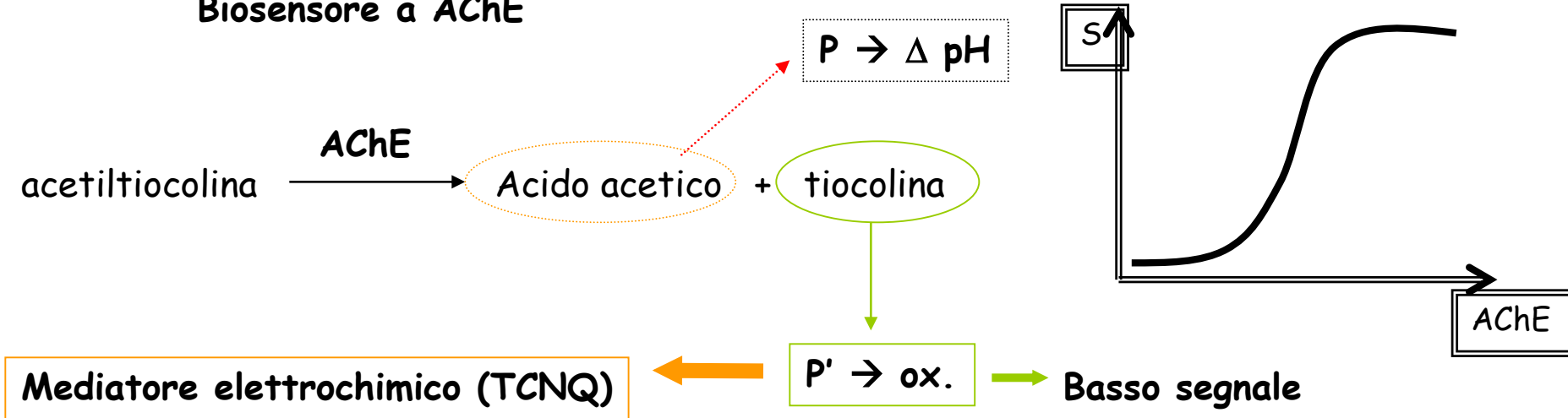
• minimo trattamento del campione

Principio del metodo

- Dosaggio dell'inibizione dell'attività di AChE



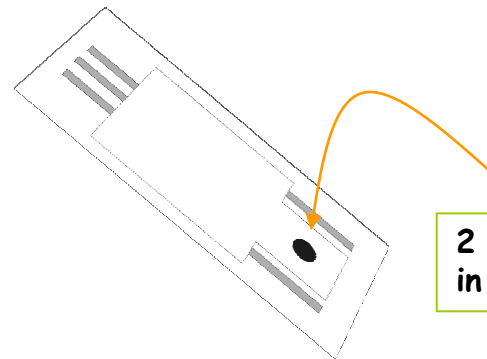
Biosensore a AChE



DPV (150-700 mV) $\text{TCNQ}_{(\text{ox})}$

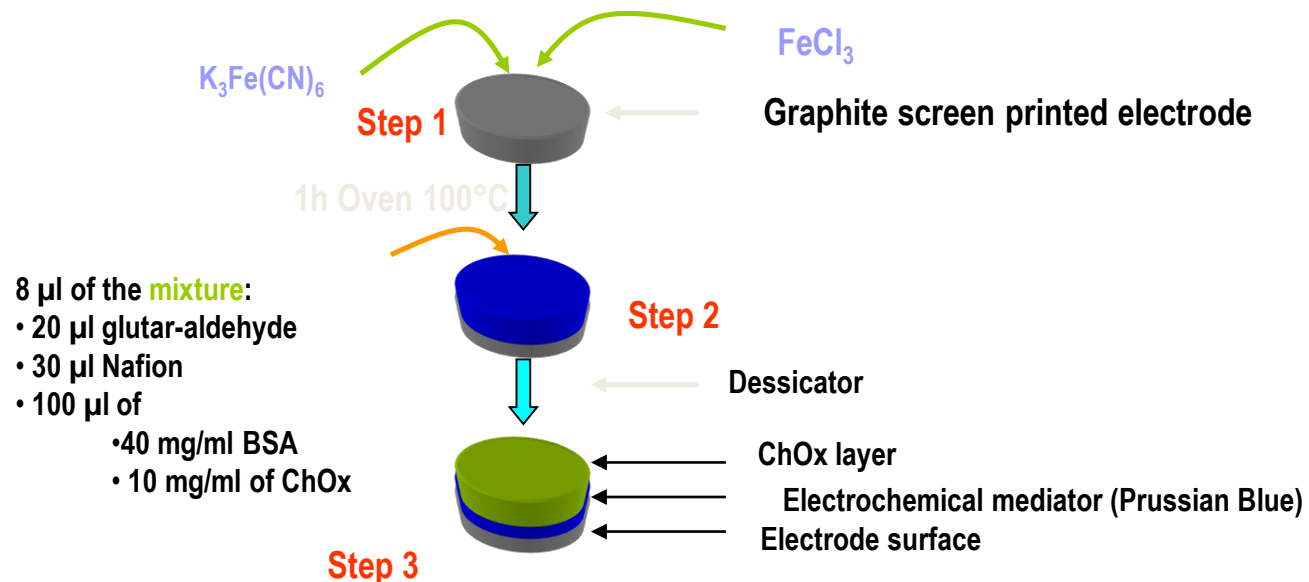
Modificazione degli elettrodi (CME): immobilizzazione del TCNQ

Intrappolamento in matrice polimerica

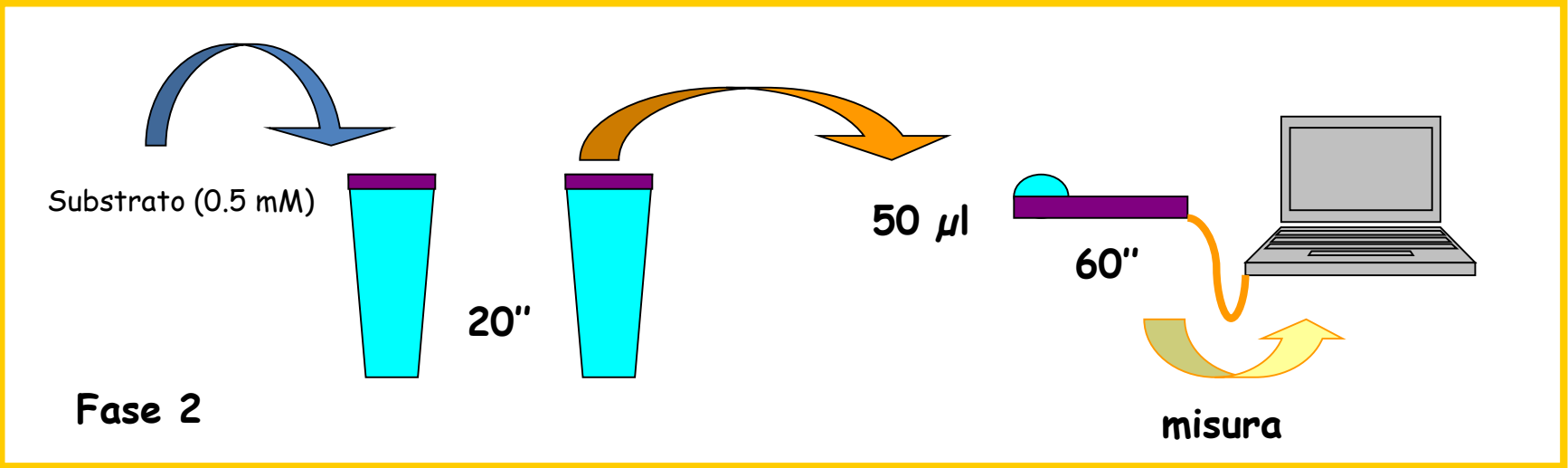
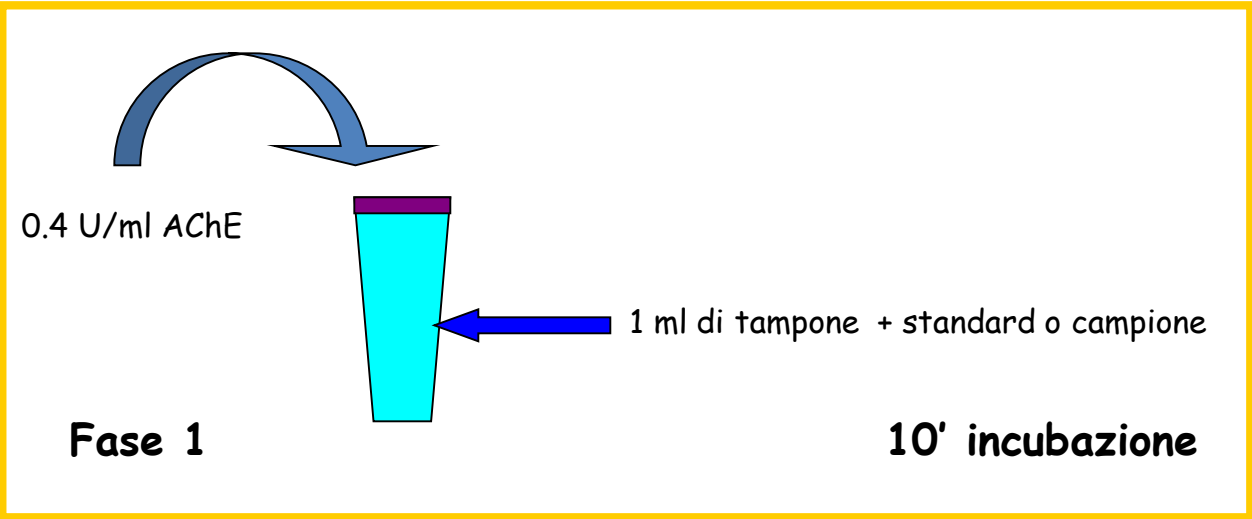


2 μl di 0.5 mM TCNQ
in Nafion 5%

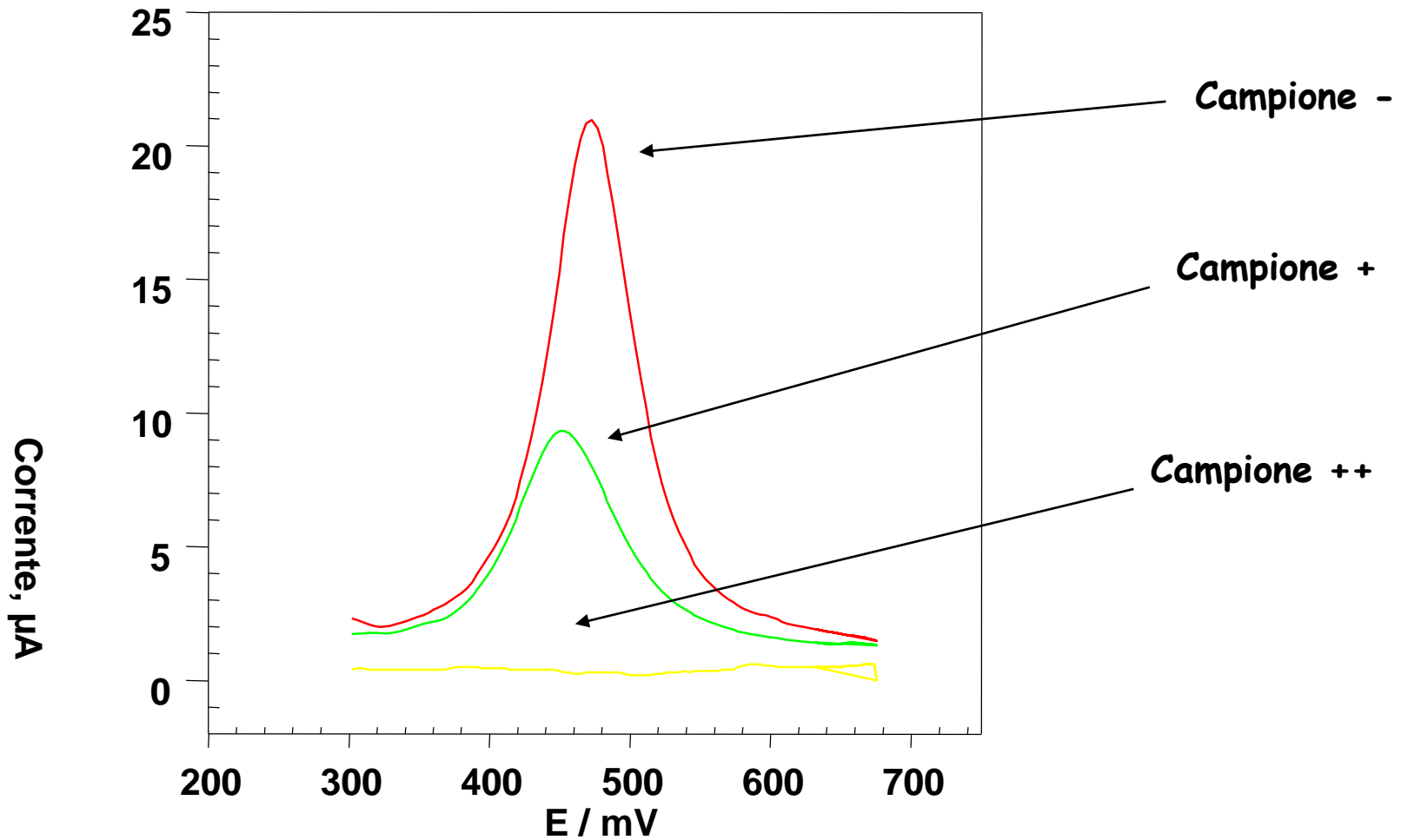
Modificazione degli elettrodi: immobilizzazione di colina ossidasi/Prussian Blue



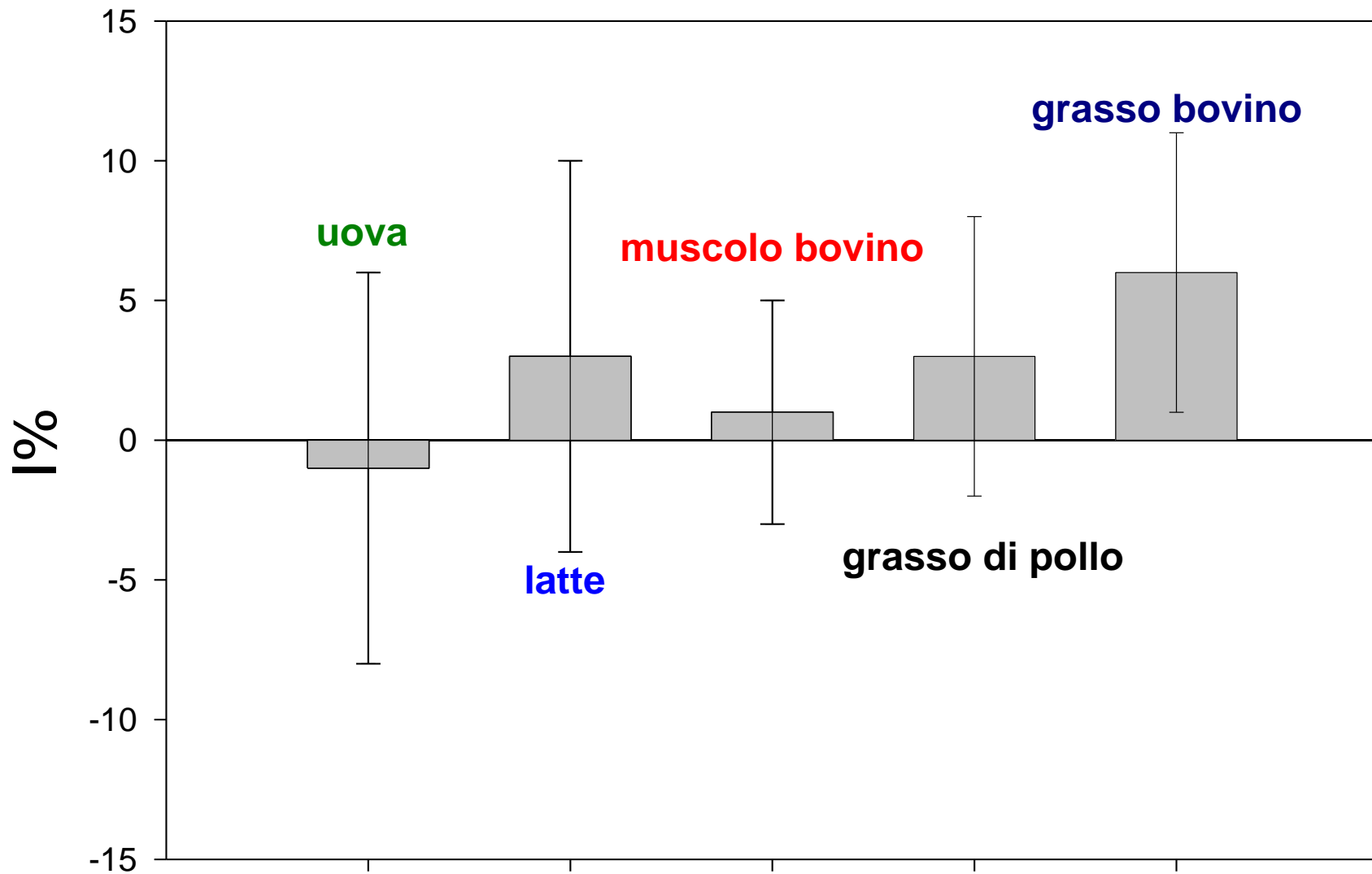
Fasi della misura



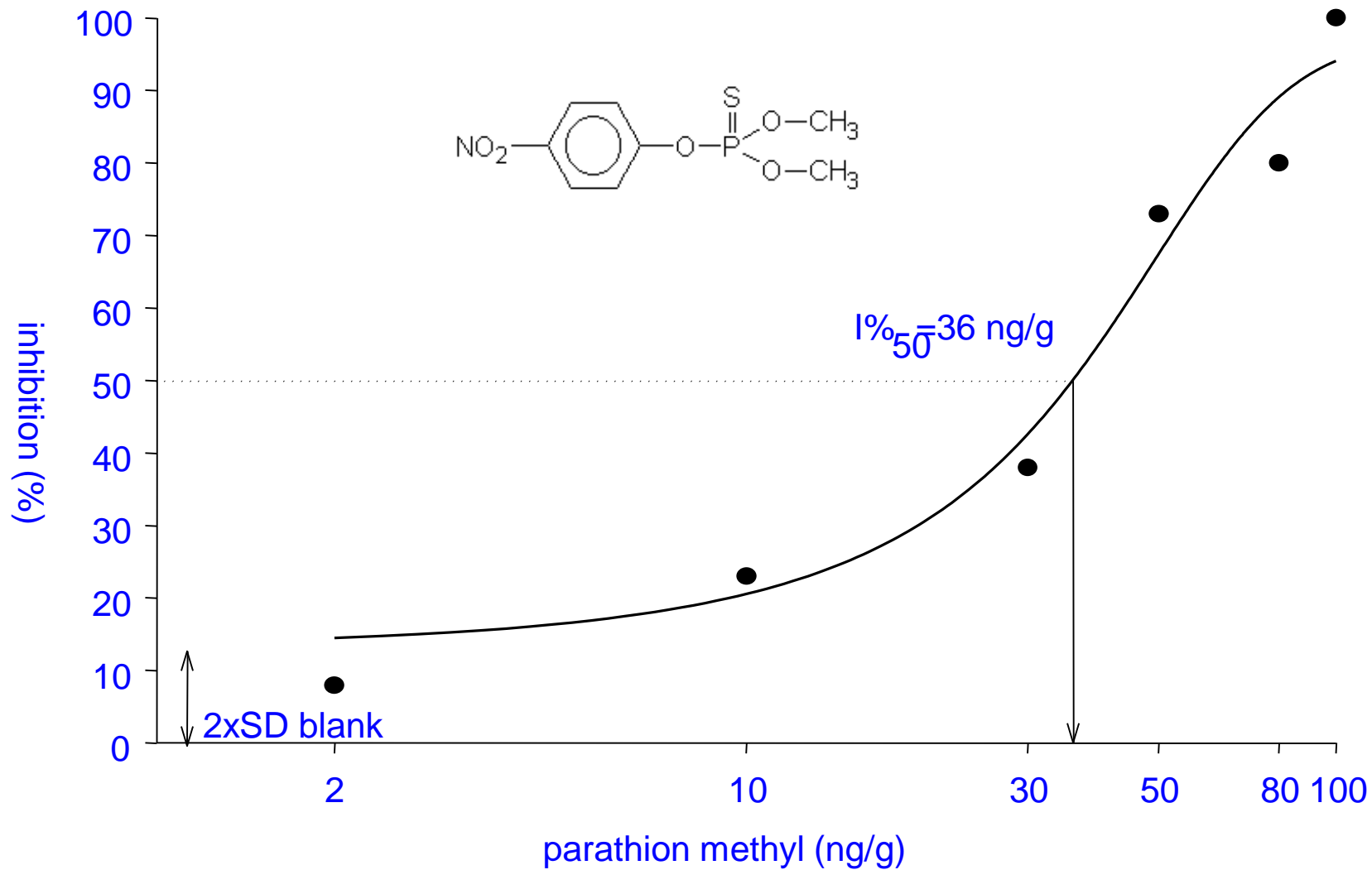
Segnali elettrochimici e interpretazione qualitativa



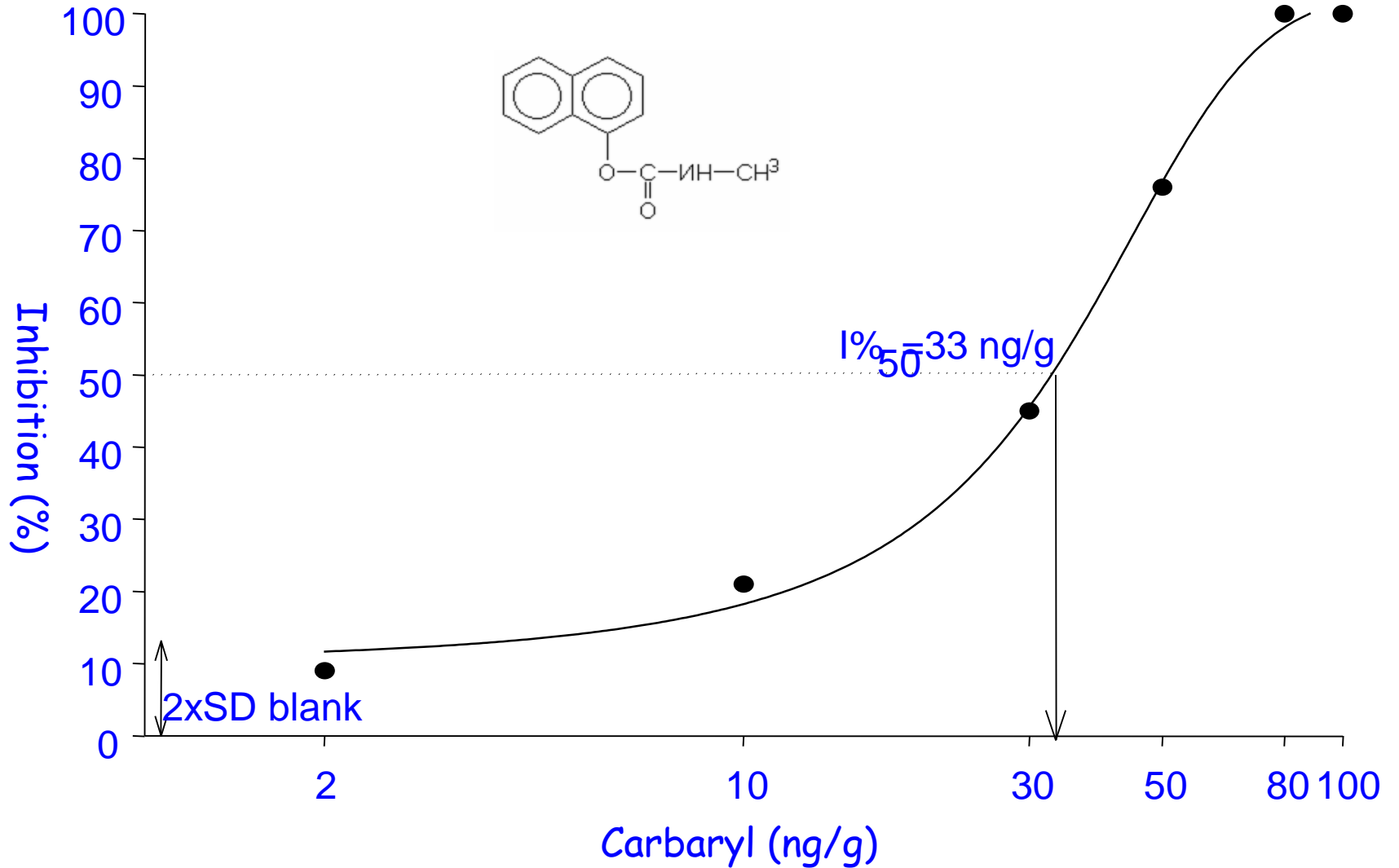
Valutazione dell'effetto matrice



Parathion methyl - curva di calibrazione



Carbaryl - curva di calibrazione



Analisi di campioni di origine animale contaminati

Matrici non contaminate:

Latte
Uova
Carne
miele

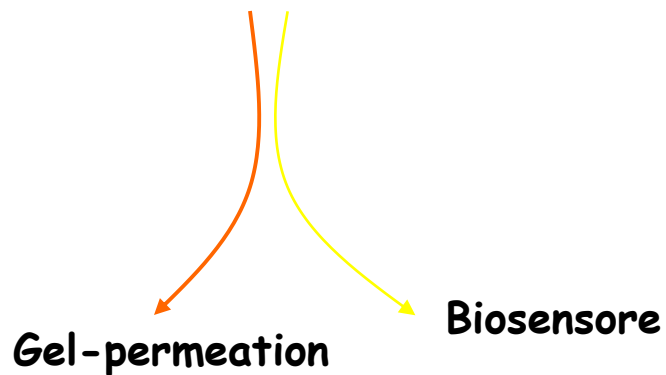
+

Pesticidi 10 ng/g:

Carbaryl
Methyl parathion
Diclorvos
Mevinphos

Estrazione con acetone/esano

← Matrici contaminate

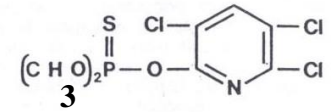


GC-NPD
LC-MS

→ Recupero 80 - 100%

	Falsi negativi	Recupero medio (%)
Latte	NR	60
Uova	NR	80
Carne	NR	60
miele	NR	90

Applicazione del metodo al monitoraggio di Clorpirifos-metile in vigna



Clorpirifos metile

Garantire l'adeguato livello di protezione alla vigna verificando il reale livello di insetticida presente:

- ➔ Ridurre il numero di trattamenti**
- ➔ Ridurre il quantitativo di P.A applicato**
- ➔ Monitorare il livello di residuo in fase di raccolta**

**Introdurre un nuovo approccio per l'estrazione:
estrazione con tampone acquoso**

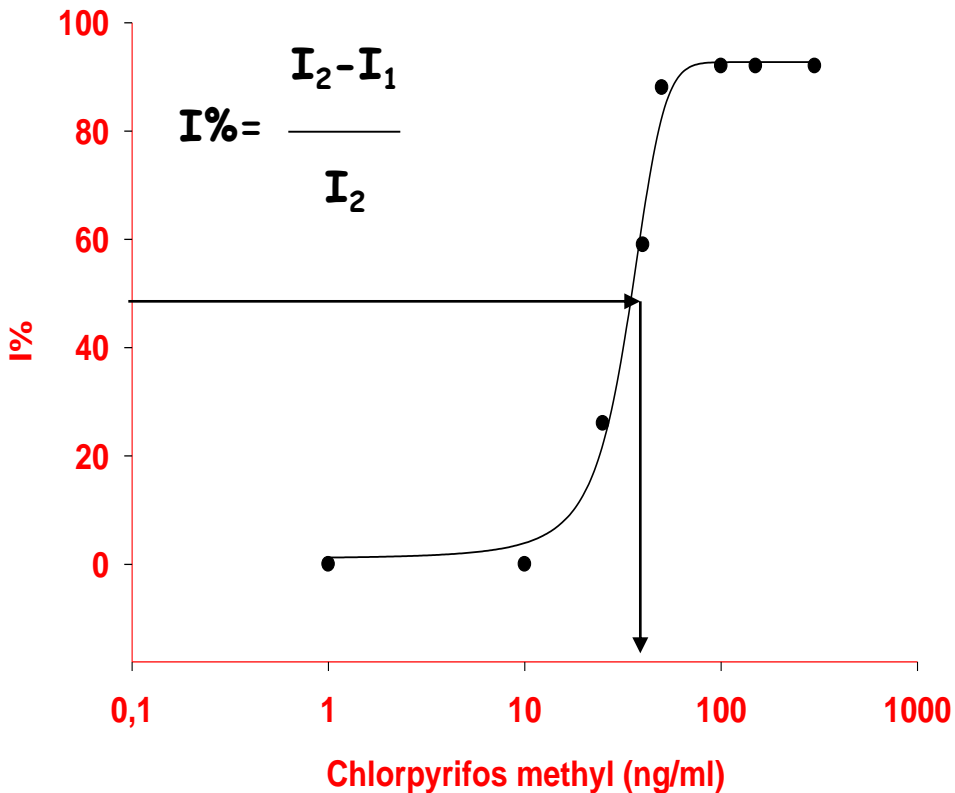
FASI DI LAVORO

Ottimizzazione del biosensore

Confronto P.A. vs Preparato commerciale (Reldan)

Confronto estrazione con solvente vs estrazione con tampone

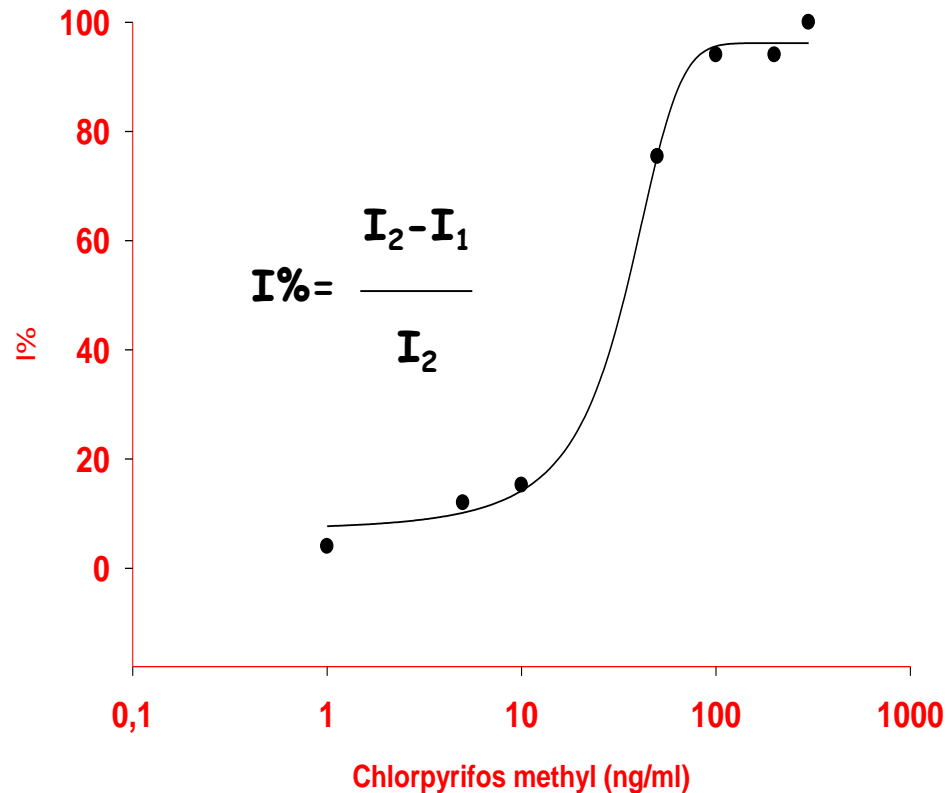
Reldan 22



DL = 25 ng/ml

Range di misura 25-100 ng/ml

Standard puro



Correlazione reldan - standard puro

$$Y = 0.85 x + 15.40$$
$$r^2 = 0.992$$

Analisi su campioni di vite trattate con Reldan (clorpirifos metile)

Matrici: acini e foglie

Estrazione:

Etilacetato,
Mix, sonicatore
centrifuga

Tampone fosfato
Agitazione manuale 1'
10' T.A.

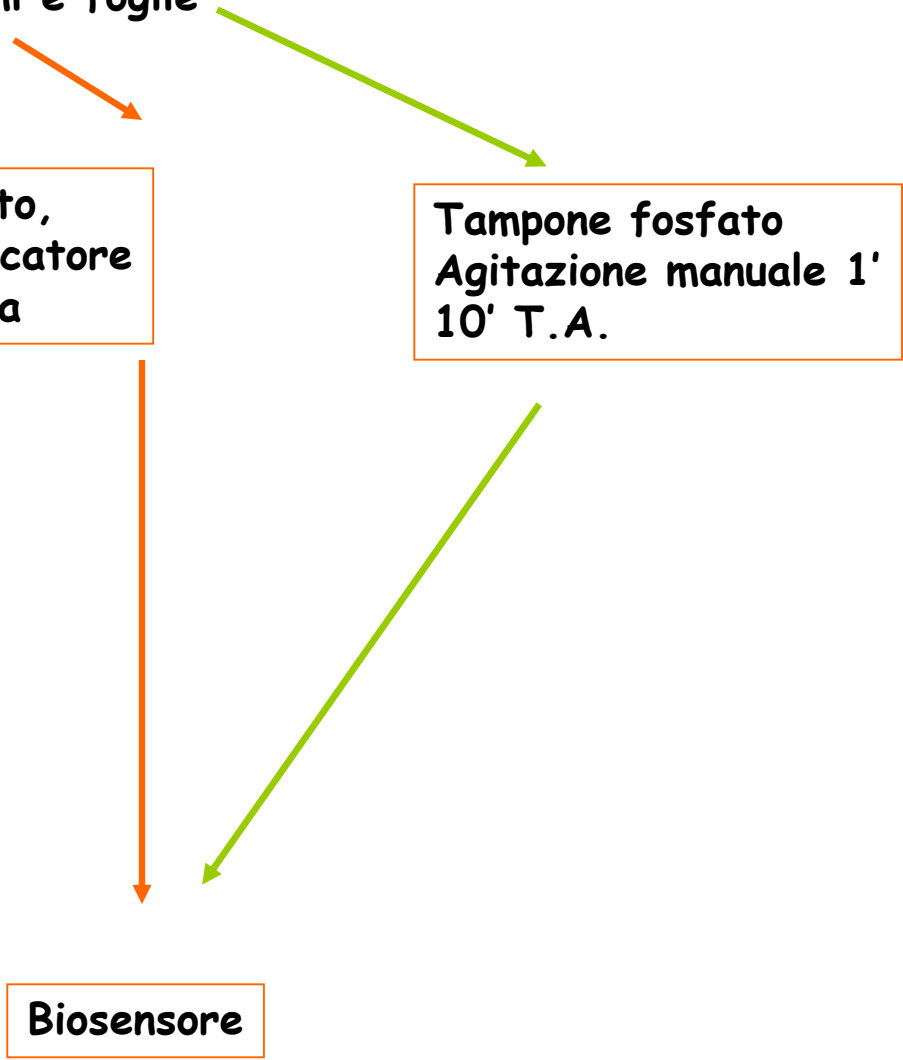
Purificazione:

SPE-C18
+
Liquido liquido

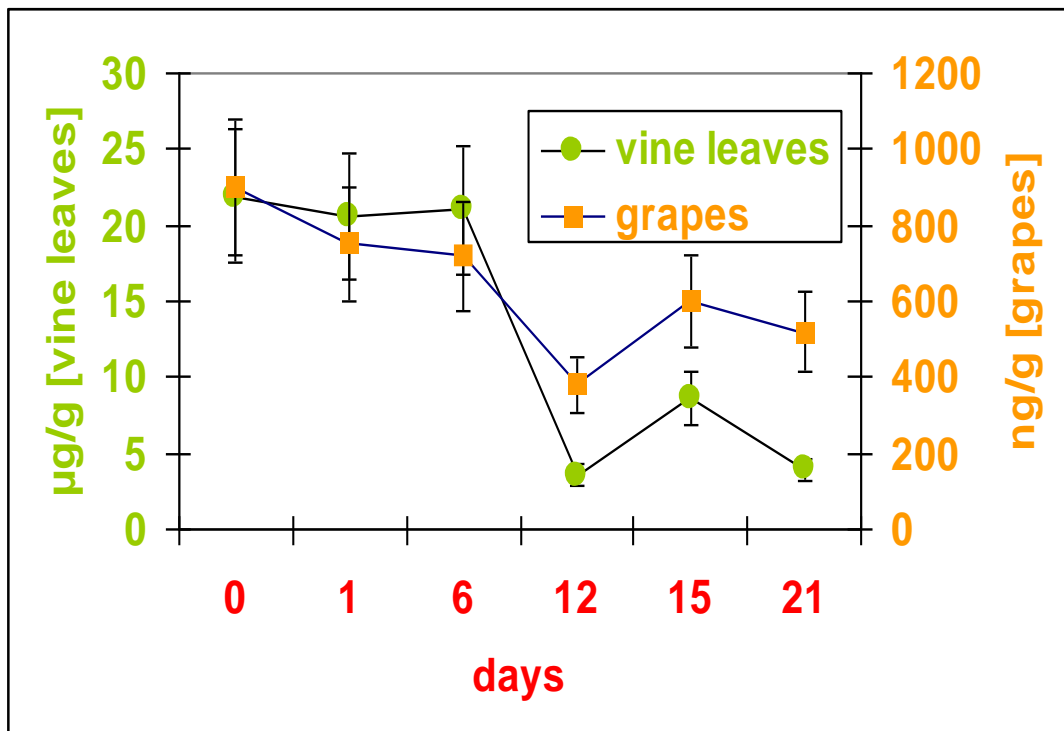
Analisi:

GC-NPD

Biosensore



Risultati biosensore



Risultati GC (acini):

Campione	ng/ml
0	700
1	800
5	650
11	350
14	450
21	400

Rapporto superficie/massa

Rapporto uva/foglie

Foglia >> acino

1-6 giorno 1:25

12-21 giorno 1:12

Rapporto superficie esposta/superficie

Foglia > acino