ELECTROCHEMICAL BIOSENSORS APPLICATION IN ENVIRONMENTAL, FOOD AND CLINICAL ANALYSIS

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Electrochemical biosensors are based on amperometric or potentiometric transducers coupled with immobilized enzymes placed in intimate contact with the sensor surface. Additional membranes cover the transducer and the enzyme to avoid electrochemical and enzyme interferences.

These probes are highly selective, stable and reproducible with response time of minutes or seconds.

Recently biosensors have found unique applications in envimonmental, food and clinical analysis.

Organophosphorus and carbamic pesticides have been determined using a choline biosensor. The reaction involves the folloving reactions:

1) choline esters → choline + organic acid
2) choline + 2 O_2 + H_2O → betaine + 2 H_2O_2

Reaction 1 is catalysed by cholinesterase enzymes; reaction 2 is catalysed by choline oxidase.

The current output due to the oxidation of H_2O_2 at a platinum electrode is correlated to the concentration of choline esters present in solution.

Since the cholinesterase enzymes are inhibited by organophosphorus and carbamic pesticides, measurements in presence and absence of these compounds result in a variation of cholinesterase activity which is correlated to the pesticide present in solution.

Fig. 1 shows the calibration curve of different organophosphorus insecticides determined with the above described procedure.

This procedure was used for the determination of anticholinesterase activity of some organophosphorus pesticides in spiked waters.

Table 1 shows some results obtained measuring the Total Anticholinesterase Activity (TAA) with the biosensor for pesticides on fresh water samples from several places in Central Italy compared with G.C. or HPLC analyses.

Electrochemical biosensors have been also used for analysis of glutamate, aspartate and lysine in food and pharmaceutical products.

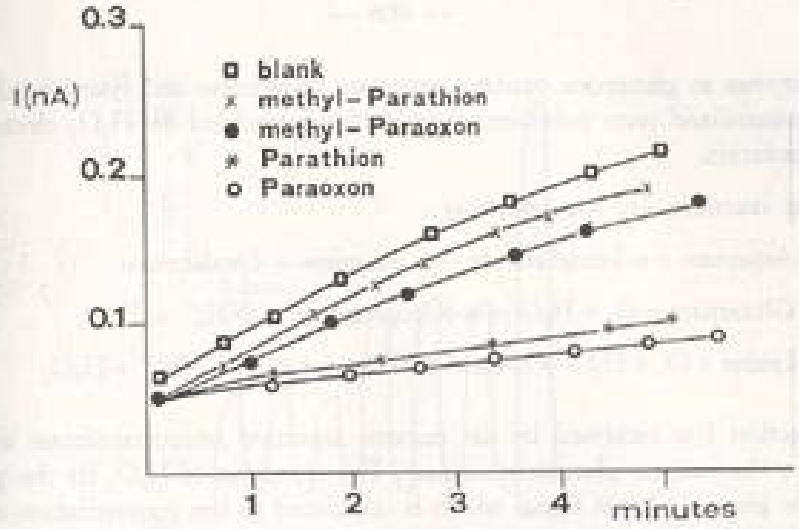

Fig. 1 - Choline-probe response to different organophosphorus insecticides 5 ppb concentration. Incubation time: 1 hour; BuCh 3×10^{-4}M, BuChE mU/ml, pH 7.5 (phosphate buffer).

Table 1 - *Analyses of water samples.*

Sample	Type	TAA as Paraoxon equiv.(biosensor) (ppb)	GC° and HPLC* analyses
Arrone (springs)	small stream	<0.5	no peaks
Arrone (urban area)	small stream	<0.5	no peaks
Arrone (agricultural area)	small stream	<0.5	no peaks
Arrone (agricultural area)	small stream	1.0	no peaks
Nemi	lake	1.5	1 un. peak*
Bracciano	lake	<0.5	no peaks
Albano	lake	<0.5	no peaks
Martignano	lake	<0.5	no peaks
Bolsena	lake	<0.5	no peaks
Alterno	small stream	<0.5	no peaks
S. Gregorio	spring	<0.5	no peaks
Monterosi	lake	<0.5	no peaks
Capodimonte	small stream	<0.5	no peaks
Castiglione	sea	<0.5	no peaks
Tiber (internal harbour)	river	<0.5	2 un. peaks°
Tiber (mouth)	river	1.0	3 un. peaks°

Enzymes as glutamate oxidase, aspartate transferase and lysine oxidase have been immobilised onto polymeric membranes assembled on H_2O_2 electrochemical transducers.

The reactions are the following:

1) Aspartate + α-ketoglutarate → Glutamate + Oxalacetate

2) Glutamate + O_2 + H_2O → α-Ketoglutarate + NH_4^+ + H_2O_2

3) Lysine + O_2 + H_2O → α-Keto-ε-Aminocaproate + NH_4^+ + H_2O_2

Reaction 1 is catalysed by the enzyme aspartate aminotransferase and reaction 2 by the enzyme glutamate oxidase. The detection of H_2O_2 by the platinum electrode gives a current signal which is correlated to the concentration of aspartate and glutamate present in samples.

Reaction 3 is catalysed by the enzyme lysine oxidase. Lysine in real matrix as foodstuff and feeds is determined following the procedure above described.

Analysis of these compounds have been carried out in batch, flow through and flow injection analysis.

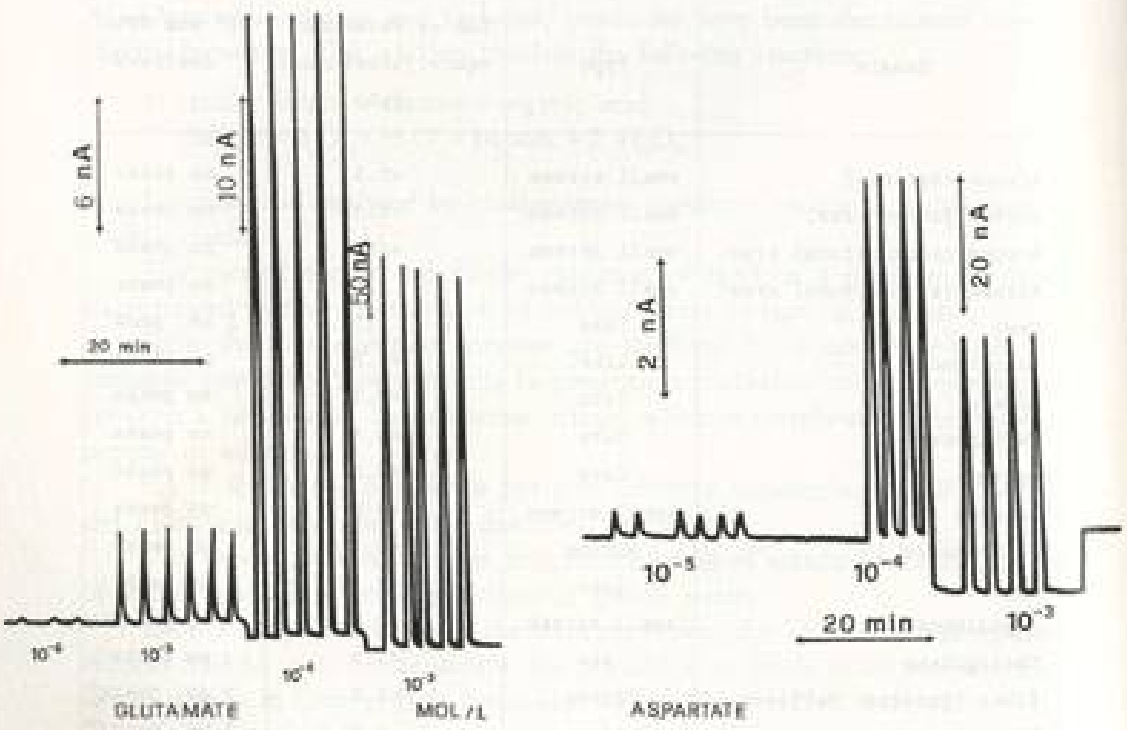

Fig. 2

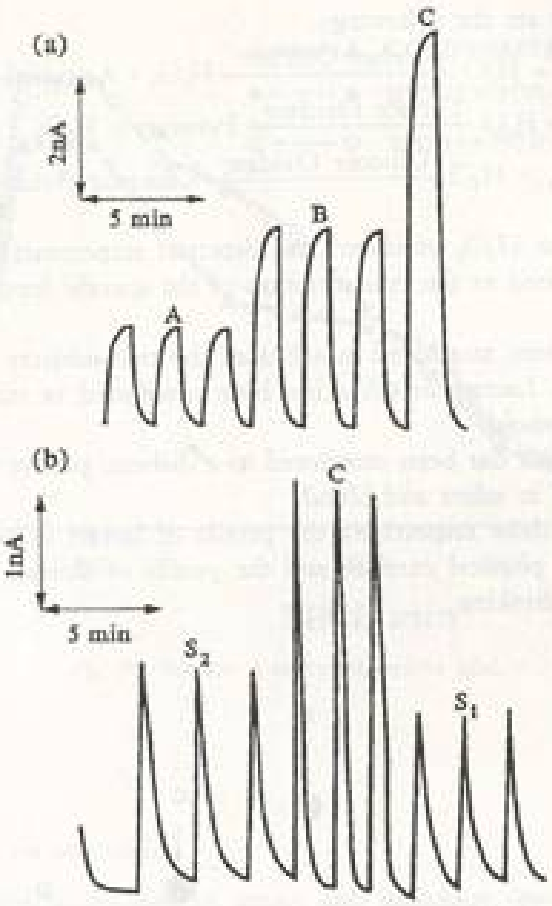

Fig. 3 - Reproducibility and response time of the bioprobe in flow through analysis and FIA a = flow through: lysine concentration in standard solution. A = 5×10^{-3}M; B = 10^{-4}M; C = 2×10^{-4}M; b = FIA; S_1 and S_2 foodstuff samples. C = lysine standard 5×10^{-4}M.

Fig. 2 shows some results obtained measuring glutamate and aspartate using a FIA procedure.

Fig. 3 shows some results obtained measuring lysine in flow through and FIA.

Analysis of glutamate, aspartate and lysine carried out using standard procedures correlated well.

Application of biosensor in clinical analysis led to the non invasive determination of alcohol, lactate and glucose in saliva and sweat. The enzymes used were alcohol oxidase, urease, glucose oxidase and lactate oxidase directly immobilised onto the surface of selected electrochemical transducers.

The reactions are the following:

$$\text{Alcohol} + O_2 + H_2O \xrightarrow{\text{Alcohol Oxidase}} H_2O_2 + \text{Acetaldehyde}$$

$$\text{Lactate} + O_2 + H_2O \xrightarrow{\text{Lactate Oxidase}} \text{Pyruvate} + H_2O_2$$

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + H_2O_2$$

In all cases the H_2O_2 produced was detected amperometrically at platinum electrodes and related to the concentration of the specific metabolite present in solution.

Alcohol has been monitored in saliva of the two subjects after injection of an alcoholic drink. Lactate in saliva has been monitored in runners to measure their anaerobic treshold.

Glucose in saliva has been monitored in a diabetic patient to study the correlation of glucose in saliva and blood.

Fig. 4 and 5 show respectively the profile of lactate in saliva in a subject before and after a physical exercise and the profile of alcohol content in saliva in a subject after drinking.

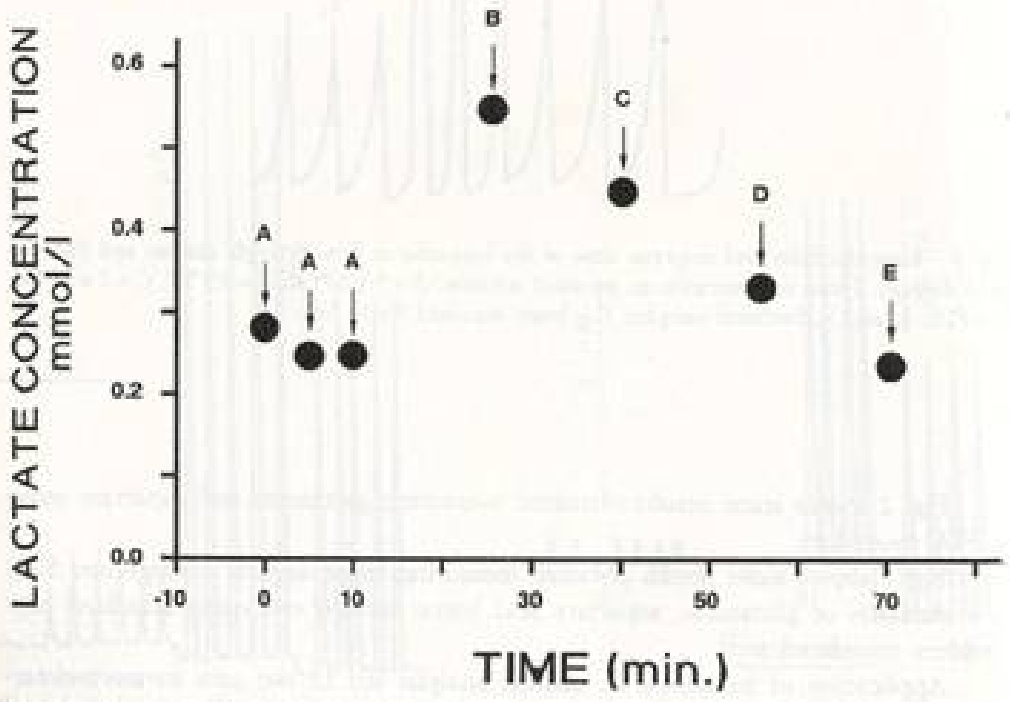

Fig. 4 - Lactate acidaemic curve. A, Saliva lactate measured while the subject was in the resting state; B, C, and E, saliva lactate measured immediately, 15, 30 and 45 min, respectively, after physical exercise.

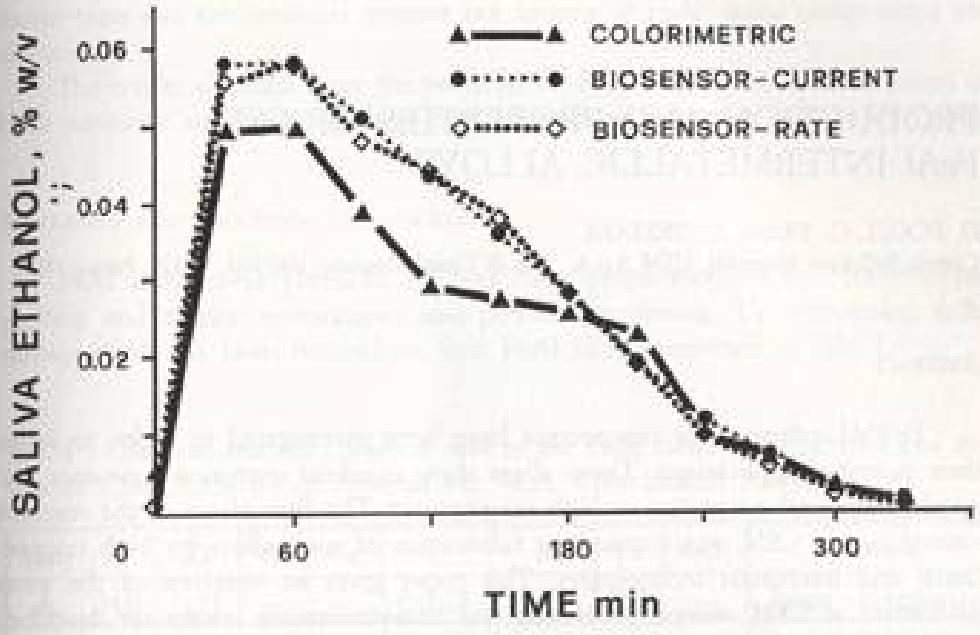

Fig. 5 - Subject A saliva ethanol vs time.

Research Activity on materials:

Our institution is involved in sensor and biosensor research since 1983, it has developed several techniques and a large number of electrochemical biosensors for clinical, food and environmental analysis.

Moreover there is a large group working on materials (ceramics, thick and thin film technology) for the realization of sensors for physical an chemical purposes.