

## Desarrollo de nuevos instrumentos biosensores ambientales para el análisis de herbicidas

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## Resumen

Se presenta el desarrollo de nuevos biosensores, especialmente sensores a electrodos serigrafados de carbono, oro o sensores impresos (screen printed sensors- SPE). Estos instrumentos permite realizar análisis multiparamétrico.

## Palabras clave

Biosensor, sensores serigrafados, biomediodor, fluorímetro, PSII

## Abstract

Development of new biosensors is presented, especially sensors screen printed carbon electrodes, gold or printed sensors (screen-printed sensors SPE). These instruments allow for multi parametric analysis.

## Key Words

Biosensor, screen printed sensor, biomediodor, fluorímetro, PSII

# Development of New Environmental Biosensors Instruments for Herbicides Analysis

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## 1. Introduction

Biosensors are analytical devices composed of a recognition element of biological origin and a physical-chemical transducer where the interaction between the analyte and the recognition element produces a physicochemical change (see fig.1). This change is detected and mea-

sured by the transducer converting the signals into analytical information. The design and construction of these devices requires an imaginative combination of biological, chemical, physical and engineering disciplines. Biosensors will find application in a variety of analytical fields.

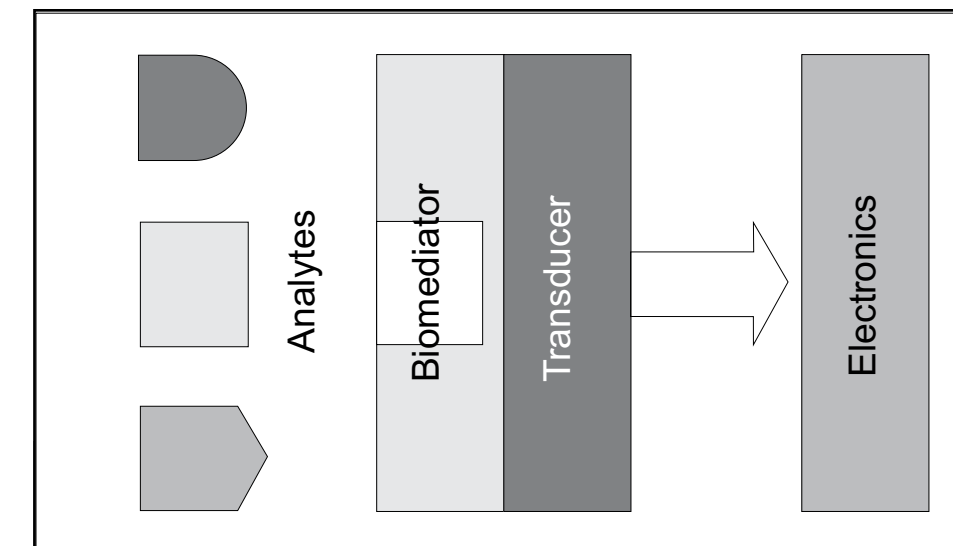


Fig. 1. Description of the biosensor: the principal characteristic are sensibility and selectivity

The last 10 years have witnessed a constant growth of interest in the field of large-scale analysis, using proteins or enzymes to perform biochips development. Thus, miniaturizing, parallelizing and reducing the analysis time and cost were required and became a crucial challenge.

Biosensors represent a rapidly expanding field, at the present time, with an estimated 60% annual growth rate; the major impetus coming from the health-care industry, food quality appraisal and environmental monitoring. The estimated world analytical market is about 13,6 K millions Euro per year of which 30% is in the health care area. There is clearly a vast market expansion potential as less than 0.1% of this market is currently using biosensors. Research and development in this field is wide and multidisciplinary, spanning biochemistry, bioreactor science, physical chemistry, electrochemistry, electronics and software engineering. Most of

this current endeavor concerns potentiometric and amperometric biosensors and colorimetric paper enzyme strips.

## 2. Biosensor based in screen printed electrodes

Screen printed electrodes (SPE) technologies appears to be an interesting tool to permit the achievement of marketable biosensors taking advantage of the low cost and mass production possibilities. The SPE is made with three electrodes called working (central electrode), counter and reference; the two first are in carbon, while the electrode reference is in gold or silver; the types of SPE depending of the number of electrodes. In the figure 2 describes the characteristic and types of the SPEs

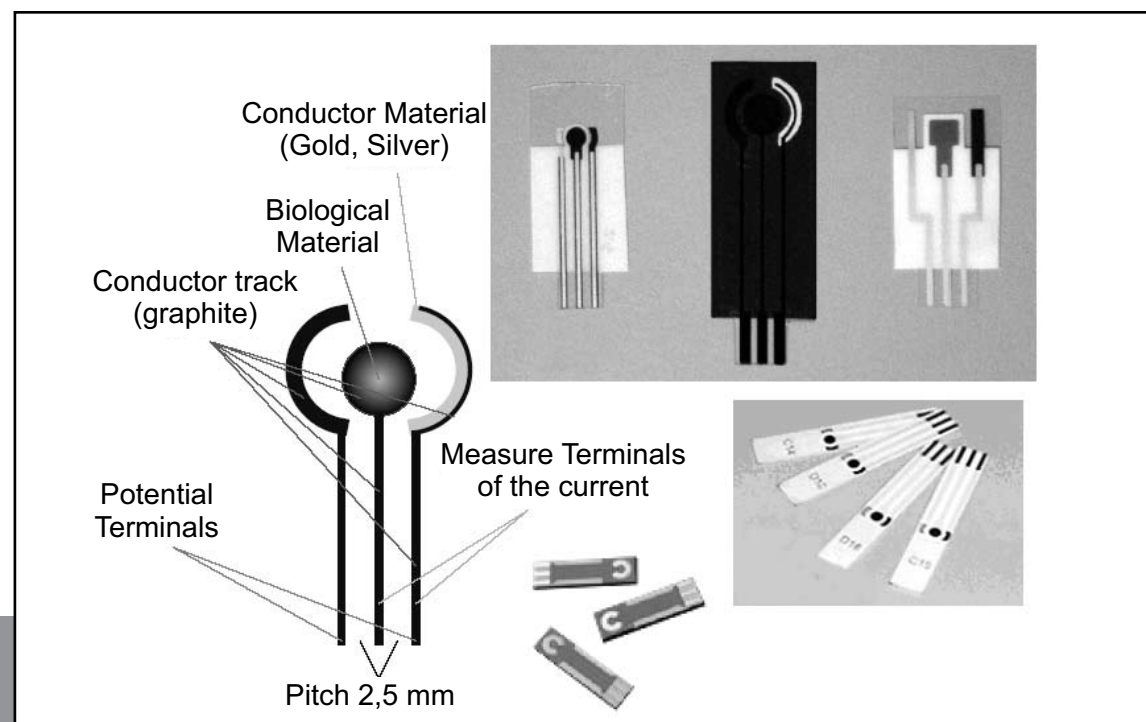


Fig. 2. Characteristic and types Screen Printed Electrodes

SPE biosensor performances and reliability depend mainly on the detection-transduction system and on the immobilization procedure involved in.

Indeed, on one hand, the SPE sensors have to be able to generate an interference-free and sensitive signal, and on the other hand, the sensing layer design has to maintain the biological molecules activity and stability.

## 3. Biosensors based in the photosystem ii (psii)

Traditionally, photosynthesis is regarded as the light-dependent production of oxygen and biomass from water and carbon dioxide. With an increasing knowledge of photosynthetic reaction mechanisms and structural details of reaction centers, culminating recently with 3-D structures of Photosystem II (PSII), new technological applications become visible which utilize isolated photosynthetic reaction center proteins for the construction of biosensors [1]. These proteins are natural nanostructured complexes which behave as sophisticated molecular devices.

The advantage of using PSII-based devices is given by the fact that this enzyme complex specifically recognizes certain analytes, some of which are widely used commercially as herbicides. Chemicals such as triazines, phenylurea, diazines and phenolic compounds, used for crop control, are able to bind specifically and reversibly to the D1 subunit of PSII within its QB-binding pocket, also called herbicide binding niche. Upon binding, these compounds alter or inhibit electron transfer by displacing QB, thus blocking electron flow, oxygen evolution and changing the fluorescence properties of PSII (see Fig.

3). These changes can be easily detected by electrochemical or optical systems.

Most frequently used biosensing systems for monitoring herbicides utilize intact cells or PSII particles isolated from plants, algae or cyanobacteria to measure either changes in fluorescence either in photocurrent due to the inhibition of electron transport by means of artificial mediators [2][3].

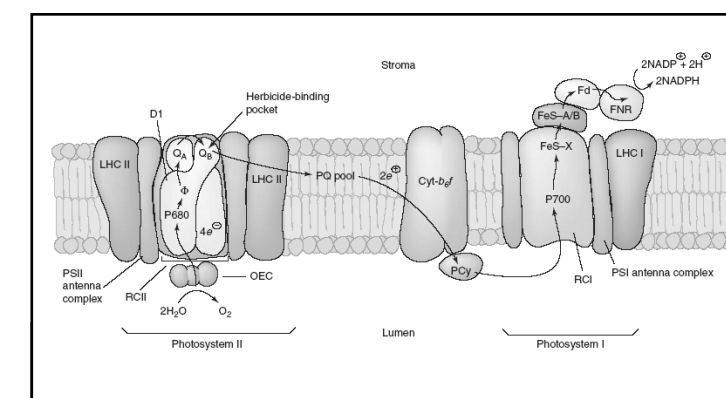


Fig. 3. Thylakoids membrane : characteristic of the Photosystem I and II

Recent advances in PSII molecular biology have produced a number of site-directed mutants characterized by alterations in the amino acid composition of the reaction center protein D1 [4]. Notably, modifications in only one amino acid within the QB-binding pocket can change photosynthetic activity and herbicide binding considerably. The herbicide binding site consists of about 65 amino acids. Depending on position and type of amino acid substitution, chemically different inhibitors show differential affinity for their binding niche. It has been shown that a mutation which causes resistance towards one inhibitor class can lead to hypersensitivity towards other classes of inhibitors. Apparently, within the large space of the herbicide binding niche chemically different inhibitors only in part overlap with amino acids lining the pocket cavity.



Fig. 4: Picture of the optical biosensor instruments at the end of fabrication, showing the electronic control and measure boards and the optical modules with the LEDs on (right side). The biological containers arrays to be settled over the optical modules are still unmounted.

Three different photosynthetic activities can be monitored to detect the chemical/physical interactions: electron transport, oxygen evolution and fluorescence emission [7], [8]. Two detection mechanisms have been implemented for monitoring water pollution: optical and electrochemical (see Fig. 4) [9], [10], [11]. In the first system, a Photosystem II biomediator is excited by a blue LED and the remitted light by fluorescence is measured by a photodiode. Its photogenerated current is amplified and converted into a voltage signal (see Fig. 5) [12], [13], [14]. A timer controls the fluorescence process by turning the LED on at fixed intervals. In the second system the PSII biomediator is attached to a screen-printed electrode which measures the amount of current inhibited by the pollutant interaction.

Both the systems are completely modular for easy maintenance and replacement and equipped with electronic control boards, while each cell hosting the biological material allows a flow of liquid (e.g. pollutant water) with the employment of pumps and valves.



Fig. 5: Picture of the amperometric biosensor is shown in a photograph with two flow cells for biomediators in series.

In this study the D1 protein-herbicide interactions have been investigated by the combination of homology based protein modelling, virtual mutagenesis and engineering techniques in algal PSII. Ideally, the D1 protein modified for sensing applications should show a higher herbicide binding affinity, maintain its electron transport capacity and exhibit enhanced stability compared to the wild-type protein. For this purposes the unicellular green alga *Chlamydomonas reinhardtii* has been selected because its D1-encoding *psbA* gene can be manipulated very easily using PCR fragments and a tailor-made deletion mutant.

Since a major drawback of using photosynthetic material is its short life-time, various immobilization techniques have been developed to improve its stability. A largely tested method is the immobilization of photosynthetic material in an albumin-glutaraldehyde crosslinked matrix [5]. Other systems comprise physical immobilization on organic matrix followed by lyophilization. We tested a particular new promising technique which utilize light emitting polymers (LEP) with the double function of cross linkers and exciting source instead of LEDs.

The new biosensors has the unique feature of utilizing algae mutants proposed by a computer model and implemented by site-directed mutagenesis. The sensors are able to distinguish subclasses of photosynthetic herbicides as reported in [6]. These setups resulted in reusable, portable biosensors for the detection of herbicide subclasses with a life-time of about  $60 \pm 6$  hours for isolated PSII and of days for intact cells; detection limits between  $4.44E-08$  and  $9.21E-10$  depending on the transducer and the tested herbicide.

## Conclusion

The biosensor instrumentation presented here is based on an innovative measurement cell where biomediator, excitation light source, electrodes and flow are integrated in a compact miniaturized sensor.

In this and previous works already reported with success, the fabrication of biosensors employing mutants of Photosystem II extracted from higher plants, deposited on screen-printed electrodes for pesticide detection in agriculture and environmental applications

The aim of the present study throught of biosensor was to assess the effects of space environment on the photosynthetic activity of various microorganisms and to select space stress tolerant strains.

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