MULTIPARAMETRIC SYSTEM FOR BIOSENSING TECHNOLOGIES APPLICATIONS BASED ON MICROARRAY ELECTRODES

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ABSTRACT

The biosensor platform here described has been designed for the use of new, versatile, electric devices, such as MEA (Micro Electrodes Array). The system has been developed applying the principles of the amperometric transduction on each specific device. This paper explains the main techniques of measurement of this platform, and is focused on MEA devices with four wells, which allow the use of up to four different types of biomediators. The Design and construction of the platform are also presented. The paper has been structured with the following sections: hardware, mechanical system, software and biochemical application. The described platform can find application in two different fields: biomedical, for the determination of possible anomalies in the human body (e.g. through the detection of cholesterol, catecholamines, glutamate, and bilirubin in blood samples), and environmental, for the detection of pesticides or heavy metals in water or foods (through the use of special biomediators such as photosystem II (PSII), or thylakoid membranes, extracted from plants or green algae). The signals coming from the biological material are correlated to the concentration levels that detect each biomedidadors according to the calibration line and the sensitivity of the material.

Keywords: Amperometry, MEA, Biomediator, Biomedical Biosensors.

SISTEMA MULTIPARAMÉTRICO PARA APLICACIONES DE TECNOLOGÍA DE BIOSENSORES BASADAS EN MICROMATRICES DE ELECTRODOS.

RESUMEN

La plataforma biosensoristica aquí descrita ha sido diseñada para el uso de nuevos dispositivos eléctricos y versátiles como el MEA (Matriz de Microelectrodos), el sistema ha sido desarrollado aplicando los principios de la transducción amperometrica en cada dispositivo especifico. Este documento explica las principales técnicas de medición de esta plataforma y se centra en los dispositivos MEA de 4 pozos, que permiten el uso de hasta 4 tipos diferentes de biomediadores. El diseño y la construcción también son presentados. El documento se ha estructurado con las siguientes secciones: Hardware, sistema mecánico, software y la aplicación bioquímica. La plataforma descrita puede encontrar aplicación en dos campos diferentes: biomédico para la determinación de posibles anomalías en la sangre humana (por ejemplo: A través de la detección de colesterol, las catecolaminas, el glutamato, y de la bilirrubina en muestras de sangre), y en el campo ambiental para la detección de pesticidas o materiales pesados en agua o alimentos (mediante el uso de biomediadores especiales, tales como el fotosistema II (PSII) o en las membranas tilacoidales extraídas de algas verdes o de plantas). Las señales procedentes del material están asociadas a los niveles de concentración de cada biomedidador acorde a la linea de caibracion y la sensibilidad de cada material.

Palabras clave: Amperometria, MEA, Biomediador, Biosensores biomédicos.

1.   INTRODUCTION

The platform is based on a biosensoristic application [1] where MEA are used to canalize the acquisition of many electrical signals coming, at the same time, from a different biomediator located in a different well. The new measurement technology is therefore utilized for the data acquisition and is integrated in the platform.

The main advantage of 4-wells MEAs is the versatility that is the possibility of using the same sample in different conditions, or many samples in the same conditions. As an example, from a blood sample one may determine four different analytes by depositing in the 4 wells four different specific enzymes. In this way, it could be possible, for instance, to measure cholesterol levels in the first well, bilirubin in the second well, catecholamines in the third well, and glutamate in the last well. This procedure would accelerate the process of diagnosis, in the way that the system can detect 4 different analytes using one single blood sample.

The same application can be used for the determination of lactose and fructose sugars in foods [2] like yogurt, juice or milk with different transduction systems.

2.   PLATFORM DESCRIPTION

The platform main stream consists in providing the small-medium enterprises (SMEs) applicants with a multidisciplinary, efficient methodology to design and produce compact miniaturized biosensors for large application lines; both for environmental and biomedical applications. With an efficient methodology to design and produce compact miniaturized biosensors for large application lines (environmental and biomedical analyses). The new multi/parameters sensor for biosensor applications is based on electrochemical-electrical-optical transduction mechanisms, MEA (Micro Electrode Array), and advanced modular techniques.

The platform has been integrated with four systems: chemical-biological, mechanical, hardware and software that are described in the following block diagram (Fig. 1.).
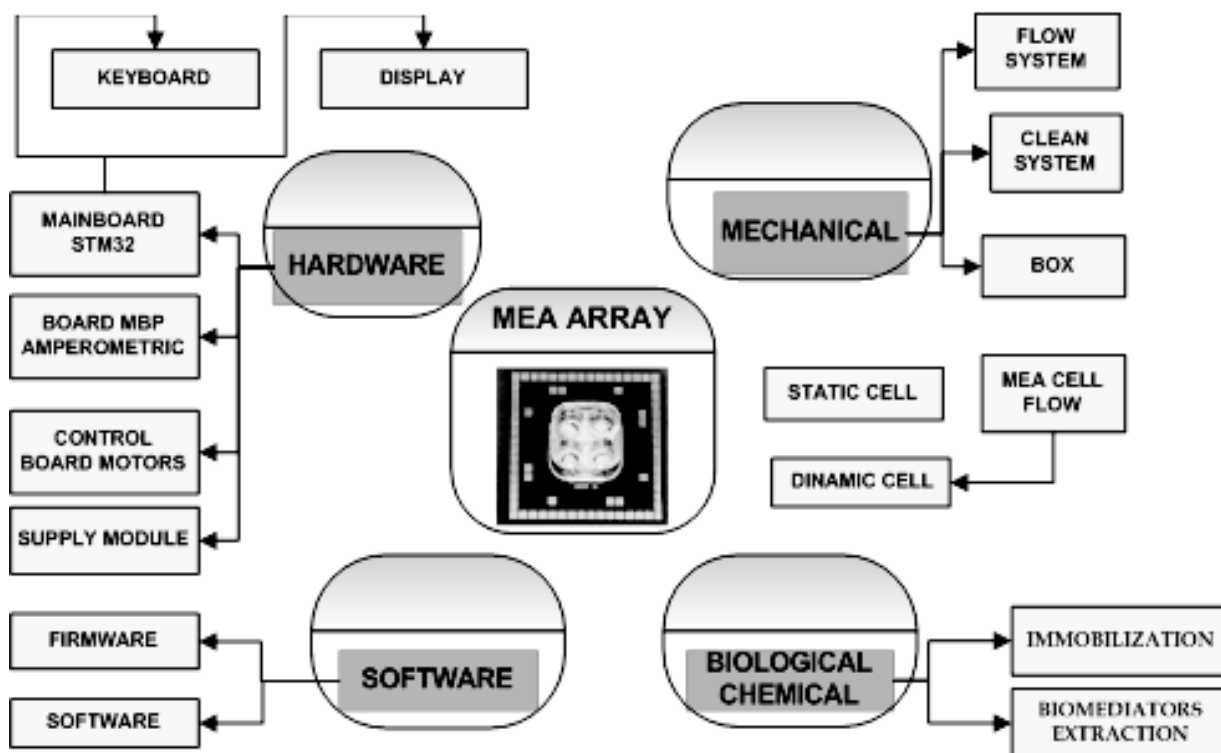

Fig. 1 – block diagram of the platform

3.   DESIGN AND FABRICATION

3.1   Microelectrode array (MEA) chip
The electrochemical signals generated by the bioreceptors and their interaction with target analytes and physical-chemical conditions are small and depend on the distance of the signal source to the electrode. Due to the low electrical conductivity of the extra biological space (buffer, sample solution, etc...), signal amplitudes decrease with increasing distance of the signal source to the electrode [3]. Therefore, a high spatial resolution of the electrode array and/or a close interface between electrode and biomediator is very important for a high signal-to-noise ratio [4].

These are the reasons why MEAs are extensively used for biomedical applications with electrogenic cell cultures, which is the first and main commercial application now, and why their technology has been selected to be used in this biosensor platform with double application such as environmental monitoring and biomedical analysis. The figure 2 is described: a. Micrograph of the round microelectrode tip (Metal=Au, Diameter=30µm); and in the figure 2b MEA with SU8 pattern with 4 different culture areas. Images figure 2a and figure 2b from L. Lorenzelli, FBK-Irst, Trento, Italy.
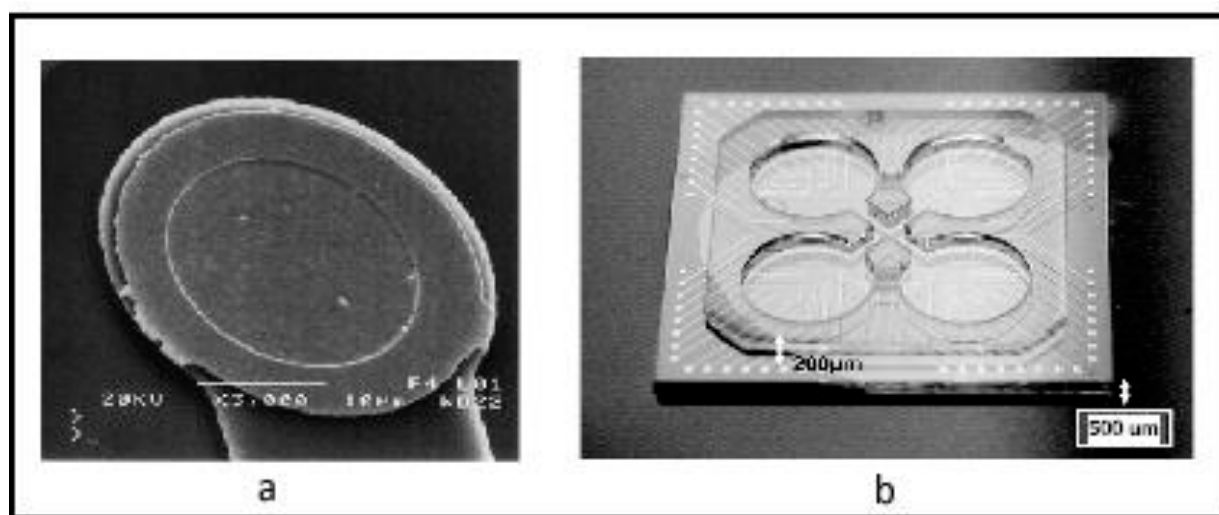

Fig. 2 – MEA: a. SEM particular size of the active zone, b. configuration with 4 wells areas

3.2   Mechanical integration
The integration of the sensor has been conducted from two points of view, mechanical and electronic respectively, according to the structural and geometrical requirements and to the output signal and processing requirements.

This scheme can be easily implemented thanks to the specification on the chip substrate that is made of transparent quartz. This material complies with all electronic and optical requirements and with the microelectronic fabrication process.

3.3   Electronic integration
The electronic integration started from the design of the electronic control board that contains the read-out and processing modules of both electrical and optical sensors. The electronic read-out system is

designed to deliver an efficient and precise measurement and to enable a semiautomatic process with minimum operator's intervention. The electronic system must allow the selection of the channel to be read-out (among all signal output channels coming related to the MEA sensor-24 channels) and of the variable to measure; it must control data acquisition and the important environmental parameters to be kept constant, such as temperature or CO_2 pressure for human cell cultures measurement systems.

3.3.1   Microprocessor STM32
The electronic board is based on the use of the microprocessor STM32 that was the first component to be selected and procured, which with its flexibility, high number of I/O ports, high data handling capacity and computational power allows

designing an electronic control system for a wide range of applications. The design of the electronic board is focused on the MEA cell which is demanding in terms of signal read-out due to the high number of electrodes operating at the same time and requiring a real time recording for this reason the electronic architecture has been defined after a study of the different commercially available MEA chips in order to design a board compliant with most of them.

3.3.2   Pin out of the MEA amperometric sensor

The pin out of the bonded and packaged MEA has been defined and shown in Figure 3, composed by (3 channels) / (9 microelectrodes) with workings by well and (2 channels) / (2 microelectrodes) counters by well can be read-out in total 12 channels workings and 8 channels counters. The others channels/microelectrodes has been defined with (2 channels)/ (4) microelectrodes by well for applications of resistance and conductance, in total 8-channels resistance/ conductance. The Figure 3 represents the connections between the PCB pads (Figure 3a) and the connector pins on the lateral side of the PCB (Figure 3b) to take out the signals.
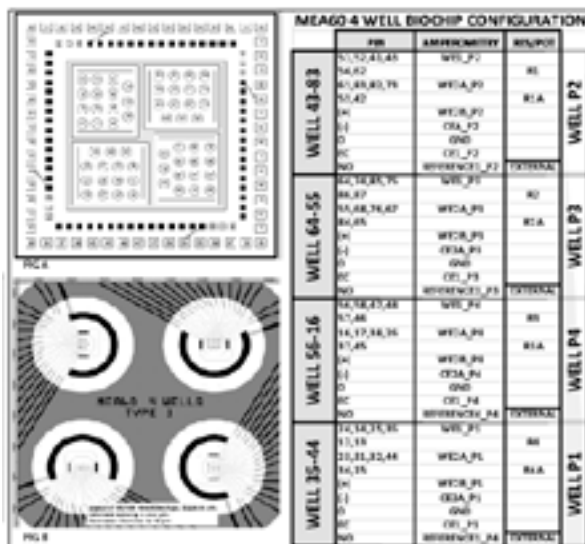

Fig. 3 Microelectrodes distribution and pins and functions connection.
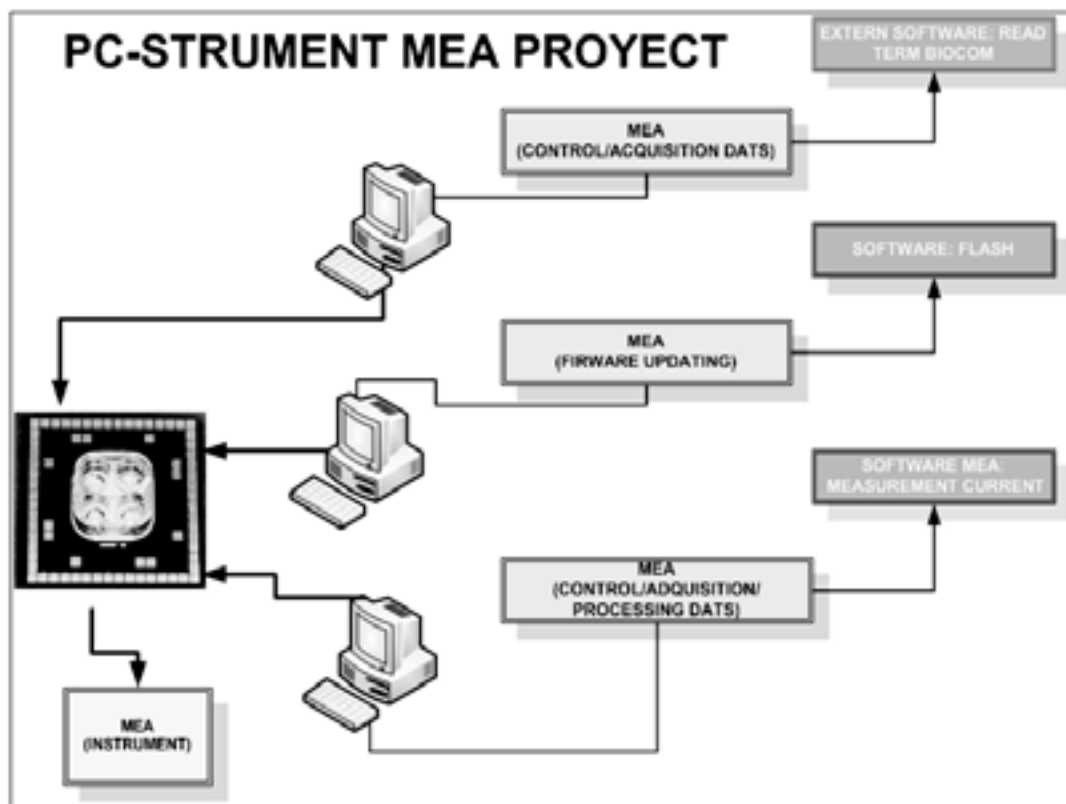

Fig. 4 – Connection between Hardware and Software

3.4 Software development

This document provides a global overview of the software development allowing the normal run of several processes related to the biosensoristic platform designed. In the figure 4 several ways of connection between Software and Hardware of the instrument are described the physical connections through the peripherals, the interface with the instrument, the acquisition and real-time processing systems of input and output data.

Before describing software details of the MEA system, composed by several menus and submenus, it is necessary to illustrate the process which led to the design and realization of a system integrating different technologies and exploiting results from years of experience in research and development of novel biosensors. The essential requirement of the software must be an efficient management of the direct communication between the data processing computer and the instrument and between the instrument and the user: thus a correct acquisition and statistical/graphical elaboration of measurement data, easy user/instrument interface for the control of main measurement parameters.
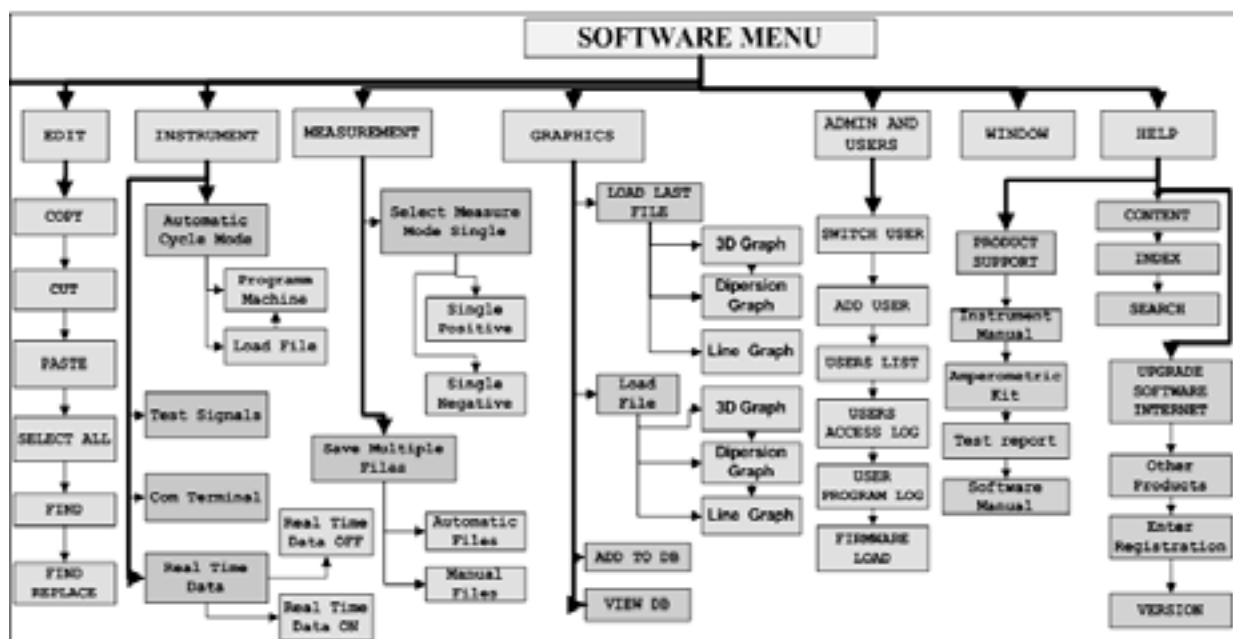

Fig. 5 –Software Menu

The figure 5 describes the basic requirements chosen for the MEA proposal, it is focused on the following aspects: Instrument control, data storage, data elaboration, presentation of data. We are going to the fundamental aspects to keep into account while designing the instrument software is illustrated.

3.5 Firmware development

The firmware has been developed based in an architecture consisting in the use of functions (type of subprograms), these are called for execution of the main program, in function of the hardware design. The Figure 6 describes the diagram of amperometry using a microprocessor STM32 ARM with an ADC Chip, the diagram describes the selection of 4 possible potential values with a range from +700 mV to -700 mV by each well on the MEA. These values depend on the desired application through external reference electrode. The application can work 3 amperometry ranges: 5 uA, 1 uA to 500 nA, the readout system of amperometry are intended to be used in copies or 4 wells at the same time, the connections at other pheriphericals as display, keyboard and the buzzer to microcontroller are explain too. Additionally, it is show the TLC5923 chip is a driver that lets you control the light sources at different time periods and with different intensities of light for applications related to of Photosystem I, II (PSII).
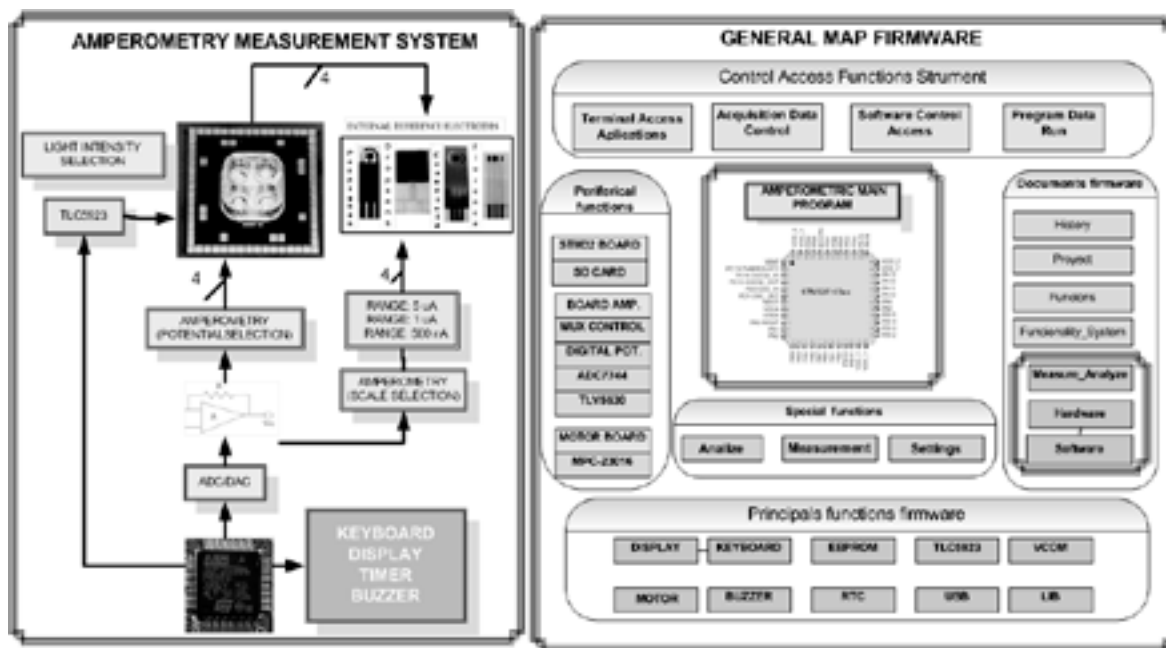
Fig. 6–Firmware Map

In the figure 7 is described the tool menu which allows the operator to select the measurement mode, the amperometric mode, settings and examples developed for each application. From the first menu it is possible to select the basic parameters for setting a standard measurement: flow, range, polarity (positive or negative), potential, light intensity level (useful for photosensitive biomediators), time (divided into darkness, light or relax times) and cycle (which can be set from 0 to 16 minutes, from 0 to 60 seconds, or from 0 to 20 minutes, in order to allow the photosynthetic material to return at its initial conditions).
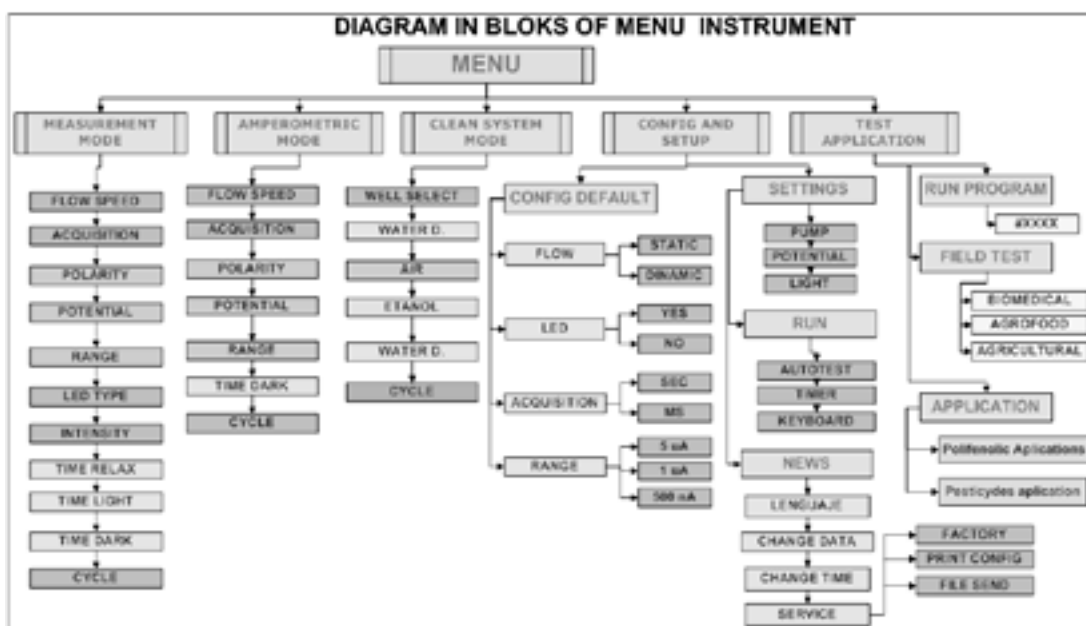

Fig. 7 –Instrument Menu

4. Immobilization procedure with Nafion®

Nafion® is a sulfonated tetrafluoroethylene based fluoropolymer-copolymer and it has received a considerable amount of attention as a proton conductor because of its excellent thermal and mechanical stability. The MEA electrode is cleaned in a mixture solvent of 40% of water, 60% alcohol and 1% KOH for 30 seconds, then rinsed with deionized water for 20 seconds and immersed in a buffer containing HNO_3, HCl, water (1:3:4, v/v/v) for 30 seconds, and finally rinsed again with deionized water. Biological material is dropped on the MEA; after the suspension dried, 1% Nafion® is spotted on the biological material. Another method used for biomediators immobilization is the Laser-Induced Forward Transfer (LIFT) process, which is based on the irradiation of the biomediator, using a pulsed laser [5].

5. Experimental results

5.1 Measurement tests on the external reference electrodes

Amperometric measurements were achieved on MEA in order to test different external reference electrodes and finally chose the best set up to obtain a good signal with high amplification and low noise [6]. The electrodes for the reference tests were chosen among several electrodes manufactured by companies or institutions, as schematized (figure 8).
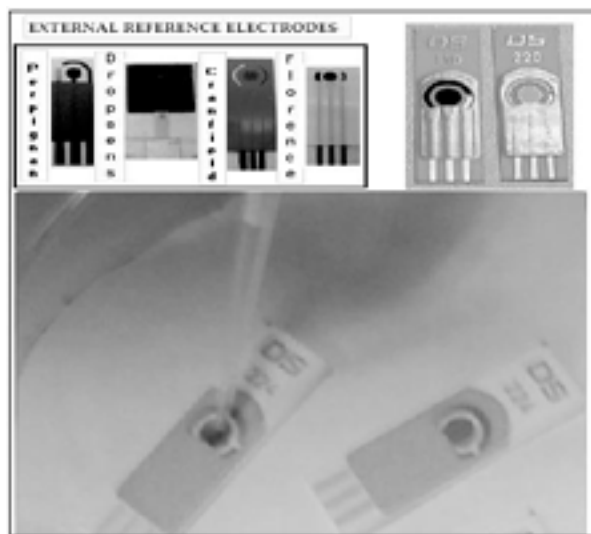

Fig. 8- Screen printed electrodes from different companies employed as reference electrodes.

All the measurement tests were compared to the tests which employ the gold screen printed electrodes from DropSens (see figure 9).

The measurement was conducted in static mode by adding to the buffer solution the appropriate amount of the enzyme and of the target analyte and registering the current development in solution.
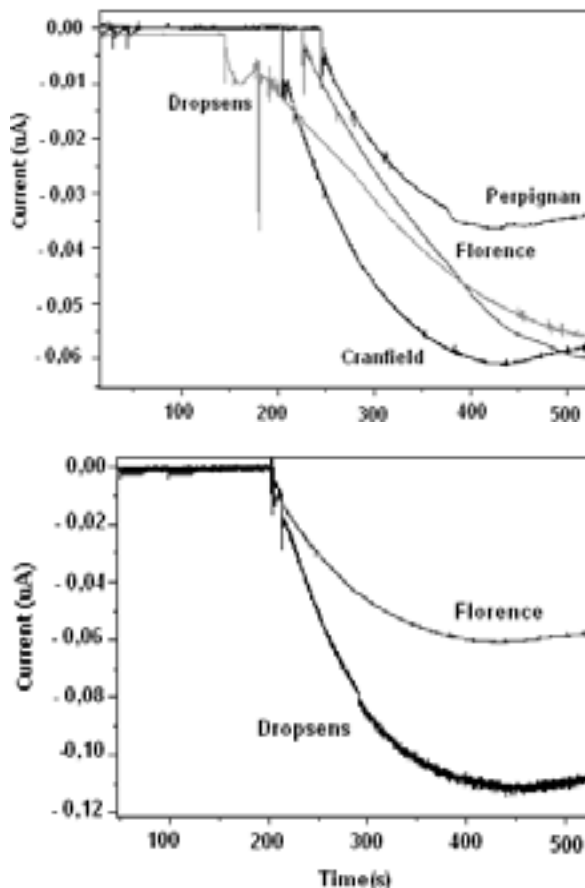

Fig. 9- responses of the laccase activity on catechol substrate with different reference electrodes are reported.

5.2 Measure and test with enzyme

The biosensor was developed taking into account a new advanced biotechnological technique which employ a physical support based micro electrodes array (MEA). This array of gold electrodes [7] allow to accommodate with different methodologies different kind of biological recognition elements able to detect a wide range of target analyte and appropriate to several application field.

The biosensor was configured in order to use 4 biomediators, and was tested by using the enzyme Laccase from Trametes versicolor, the Tyrosinase enzyme from *Agaricus bisporus* and the green photosynthetic algae *Chlamydomonas reinhardtii* [8], for the detection of different classes of pesticide, such as phenolic, triazinic and ureas pesticides, and the dehydrogenase enzymes for the detection of sugars. The MEAs permit to sense the recognition mechanisms [9], between the biomediator and the target analyte by means of several transduction systems, such as potentiometric, amperometric, resistometric and optical systems, due to its physical configuration. Multi electrode arrays (MEAs) or microelectrode arrays are devices that contain multiple plates or shanks through which neural signals are obtained or delivered, essentially serving as neural interfaces that connect neurons to electronic circuitry. The use of MEAs (Multi-electrode arrays) has been growing in popularity over the last several years. The physical configuration of the microelectrode enables recording from diverse biological preparations including macromolecules as well as cells and tissues. It allows easily acquiring clean, high quality signals and performing sophisticated experiments. This technique shows several advantages including low-noise and stable recording, superior signal-to-noise ratio, excellent electrical stimulation and no need for pre-amplifier.

Table 1- Lists of biological recognition elements and the analyte detected.

Agrofood components-contaminants subclasses	Agrofood components-contaminants classes	Biological recognition elements	Transduction system
Glucose	Sugars	Glucose dehydrogenase	Amperometric
Lactose	Sugars	b-Galactosidase	Amperometric
Lactate		Lactate dehydrogenase	Amperometric
Catechol	Phenolic compounds	Laccase Tyrosinase	Amperometric
Short chain fatty acid	Lipids	Lipase	Potentiometric Optical Conductometric
Bisphenol A	Phenolic compounds	Tyrosinase	Amperometric
Atrazine Prometryn	Triazinic herbicides	Photosynthetic microorganisms	Optical
Diuron Linuron	Ureic herbicides	Photosynthetic microorganisms	Optical

5.2.1 Amperometry on tyrosinase enzyme for phenols detection

In the figure 10 are represented the measurement curves employed to perform the amperometric tests of the Tyrosinase activity on catechol substrate as control test for the MEA biosensor: SPE_AU vs MEA (4well_AU).
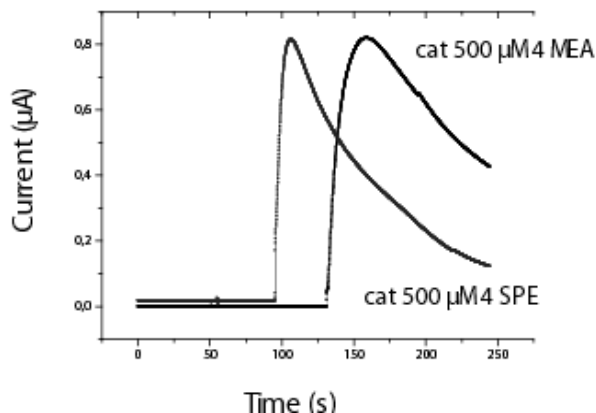

Fig. 10- Amperometry Tyrosinase electrode inmobilized on SPE VS MEA4 wells.

5.2.2 Amperometry on laccase enzyme for phenols detection

In the figure 11 are represented the measurement curves were employed to perform the amperometric tests of the Laccase activity on catechol substrate as control test for the MEA biosensor: SPE_AU vs MEA (4well_AU).
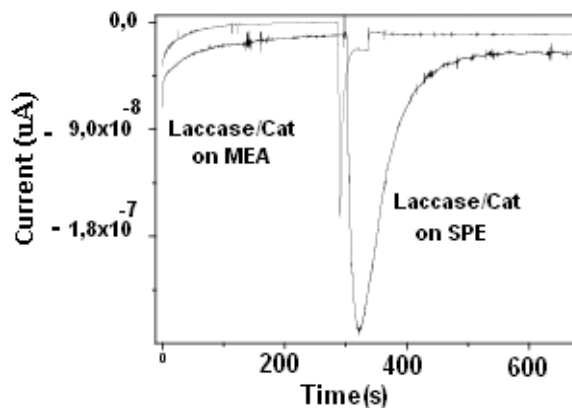

Fig. 11 Amperometry Laccase electrode inmobilized on SPE VS MEA4 wells.

5.3 Measure and test with thylakoids material

In plants and algae, photosynthesis takes place in organelles called chloroplasts (size 2x5µm). A typical plant cell contains about 10 to 100 chloroplasts. The chloroplast is enclosed by a membrane. Within the membrane is an aqueous fluid called the stoma. The stoma contains stacks (grana) of thylakoids, which are the site of photosynthesis. The thylakoids are flattened disks, bounded by a membrane with a lumen or thylakoid space within it (see figure 12).
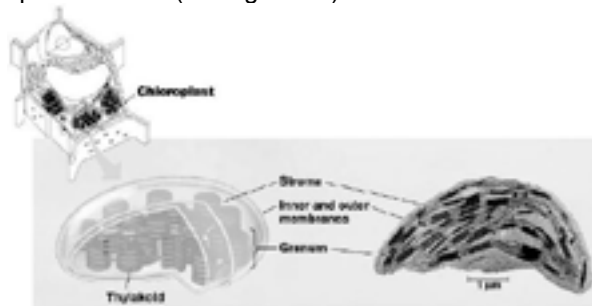

Fig. 12 – Schematic representation of thylakoid membranes.

The site of photosynthesis is the thylakoid membrane, which contains integral and peripheral membrane protein complexes, including the pigments that absorb light energy, which form the photosystems. Photosynthesis of higher plants starts with the charge separation process in the photosynthetic reaction centre of Photosystem II (PSII). PSII is a light-driven, water–plastoquinone oxidoreductase which catalyses the most thermodynamically demanding reaction in biology. This multi-enzymatic complex is embedded in the thylakoid membranes of plants, algae and cyanobacteria [8].
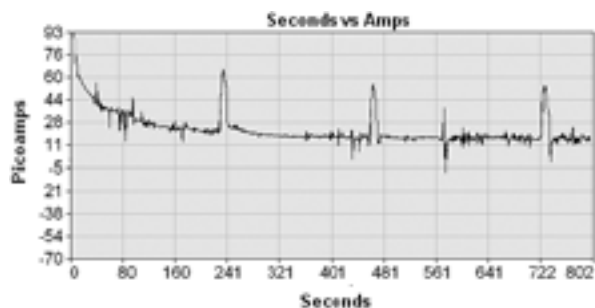

Fig. 13 – Amperometric measurements of electron transfer of spinach thylakoid membranes immobilized on MEA _Au_4WELL.

5.3.1 Amperometry on thylakoids

The amperometric measurements [9] of electron transfer of spinach thylakoid membranes immobilized on MEA _Au_4WELL are represented in the figure (13).

6. CONCLUSION

We can assert that the best response of the enzyme activity on the target analyte was obtained by using the screen printed electrode from Cranfield's Laboratory as reference electrode.
The voltametric and amperometric data obtained by the MEA 4 wells and 1 well instruments, on tyrosinase and laccase enzymes on catechol as substrate, can be compared to the data obtained by using standard laboratory potentiostat and to the data from the literature. In particular, the voltametric tests showed that the best potential for the enzyme transformation of the catechol substrate was -30 mV for laccase enzyme and +200 mV for tyrosinase enzyme.

In several measurement tests interfering current signals were observed, probably due to the noise and the spikes of the basal current. These interfering signals could be due to the following reasons: the instrument set-up is not isolated and the current values detected by the systems were registered in the range of pico-ampers and nano-ampers, where the ratio signal-to-noise is too low; in this context it could be essential to add a Faraday cell in order to obtain a well isolated configuration of the system. the biological component were added by pipetting enzymes and/or substrate to the chamber buffer solution, allowing to give rise false positive current signals; furthermore, biological residues may be absorbed by the gold support since there is not a cleaning system to avoid this phenomenon; in this context it could be essential to employ immobilised biomediators and flux substrate, real samples and cleaning solutions by microfluidic systems.

7. ACKNOWLEDGMENT

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