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Development of a new integrated easy to use micro-electrochemical platform for food analysis and staphylococcal enterotoxin B detection

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Abstract

A new electrochemical platform was established during this work for food diagnosis and carrying on tests in situ for the detection of different toxin species. It has the advantage to be easy to use, portable and can help to conduct such tests in a very specific way, instantly and doesn't require expensive equipments or laborious experiments. This entire platform is based on combining an electronic portable system with home manufactured screen printed sensors, and running both cyclic voltammetry and chronoamperometry techniques for electro analytical experiments. The detection of staphylococcal enterotoxin type B will be carried as an application in order to validate the well performance of this electrochemical workstation.

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Keywords: nanotechnology, screen printing, miniaturization, system integration, electrochemical detection, toxin

Introduction

Since a long time, Toxins present in food have gained a major interest in worldwide as they threaten the existence of the human race since they cause over 76 million cases annually resulting in 325,000 hospitalizations, 5,000 deaths

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and medical cost had reached almost 37.1 billion [1]. Among the 200 known diseases, we can distinguish some viral health problems caused by different types of bacteria such as *Campylobacter jejuni*, *Listeria monocytogenes*, *Salmonella enterica*, *Escherichia coli*, *Staphylococcus Aureus* and Mycotoxins [1].

Consequently, to address these problems, many culture-based techniques and rapid methods such as ELISA and PCR have been developed. Although, they served to solve many health problems, they still remain slow and time consuming.

By the time, and with the intervention of the new technologies (ICTs) and their integration in various fields, scientists tried to develop the domain of biochemistry especially with the need of simultaneous tests in-vivo and rapid responses, where does the idea of biosensors. Since the 60's, biosensors have shown a stunning behavioral performance since they are rapid, easy-to-use, help to increase turnover in the world of economy by saving products from being defected in the domain of food industry and help to solve many health problems and save lives in the case of some fatal diseases.

The aim of this work is to develop a micro-electrochemical platform that responds to food industrial needs and has many advantages as the opportunity of miniaturization and portability of the system that can help to achieve analysis in situ and implement microchips in green fields where we can lend a hand to detect the degree of toxicity of biological aliments. In fact, a graphical interface was developed to help to generate an electronic signal relative to the reference electrode for the simulation of the electro-analytical compounds and then the acquisition and the treatment of the concerned signal. This electronic part was coupled with a new screen printed electrochemical chip that contains two active detection surfaces developed by means of Au with mutual electrodes of Ag/AgCl and carbon. This selection was based on the interaction that may happen between the specific target and the toxicity level of the chip. The fonctionalization of the surface for the detection of staphylococcal enterotoxin type-B will be based on self assembled monolayers (SAM) using physical adsorption of protein A on Au-cysteamin surfaces and then the characterization of different coating steps were assured by electrochemical techniques.

A. Screen Printing Technology: fabrication of microchips

Screen printing technology has been widely used since the two last decades in the electrochemical biosensing field [2]. Comparing to other techniques of micro-fabrication such as lithography and evaporation process, screen printing has the opportunity to be easy to handle, doesn't require laborious experiments and offers an important amount of mass production in a very short delay. In addition, SPEs are used as an economical solution for point-to-care tests since they are disposable, not expensive, have the opportunity to be miniaturized depending on the application, and can be used in many environmental applications as in our case [2, 3].

Gold, Ag/AgCl and carbon pastes were used for this approach. The employment of gold as an active surface is based on the strong S bonds that Au creates while the generation of self assembled monolayers (SAMs) for the detection of SEB, that's why it tends to be used in the SPEs. Carbon was used basically for its stable and inert chemical properties and for its low cost [4].

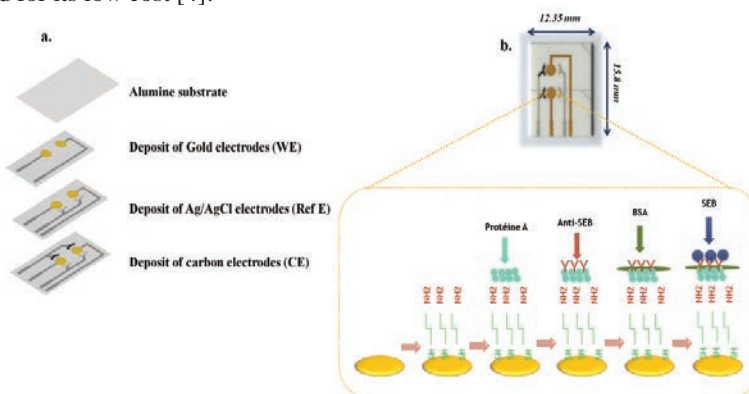


Figure 1: Different steps of fabrication of the micro-sensor: (a) stages of the micro-sensor formation; (b) Presentation of the final product of the SPEs

After the preparation of the design of the microchip using modeling software, we passed through the steps of pretreatments and printing process. First of all, we start by emulating the different designed masks on the stainless steel screens. Then, we proceed with the screen printing process by depositing the gold, Ag/AgCl and carbon pastes, respectively. The different steps of deposition and formation of the micro-sensors are presented in figure 1.a.

Finally, after the drying and the curing of each applied paste with precise thermal treatment, the sensor is left for cooling and the entire product is shown in figure 1.b.

The validation of this immunosensor will be carried using staphylococcal enterotoxin type-B as a target molecule. Both specific and non specific detection of SEB will be described. The functionalization of the surface will be based on the protocol described in figure 1.b. It is based on the formation of self assembled monolayers (SAM) using physical adsorption of protein-A on Au-cysteamin surfaces. Then, deposition of BSA, Anti-SEB and the target molecule will be held. The characterization of different coating steps will be assured using CV and SWV as electrochemical techniques.

B. Electronic system for data acquisition

Electrochemical methods such as cyclic voltammetry, chronoamperometry and square wave voltammetry have been used to help investigations in biomedical and industrial analysis and applications. According to this theme, many types of potentiostats have been developed by different companies and studies in order to facilitate electrochemical measurements and develop new instruments of detection and characterization which are compatible with each application [5]. Electrochemical detection has the advantage to generate a sensitive response compared to other sensors with, rapid, selective and require simple instrumentation [6]. Whereas, many factories require a license agreement for the use of their instruments and they can be controlled only by experts from the company itself. Also, many instruments are not portable or need a larger current-potential range. Facing these problems and with the need of miniaturization and the portability of the electrochemical platform, we managed to design, construct and validate a LabView-PC based system in order to control both cyclic voltammetry (CV) and chronoamperometry (CA).

As we can see in figure 2, the whole system is composed of both software and hardware parts. The interfacing between the computer and the micro-sensor is assured by the DAQ NI-USB 6009 and an external electric control board. Concerning the software part, it presents a graphical interface that has the advantage to be considered as a virtual instrument that controls the NI-DAQ. It is divided into two blocks: The first one, its purpose is to generate the necessary signal either a ramp (CV) or a DC potential (CA) depending on the desired application. The second block is mainly used for the signal acquisition and display.

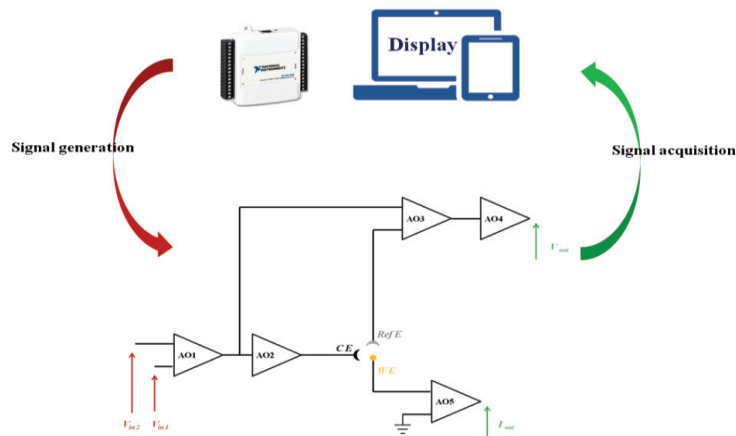


Figure 2: Schematic presentation of the electronic system

For the external board, it was basically described in the “Bulletin of the Union of Physicists” in 2002. It’s composed of an assembly of FET-input amplifiers to control the signal applied on the electrodes. In fact, in order to maintain a stable potential with an inconsiderable current over all the circuitry and especially the reference

electrode, we applied a high impedance amplifier on the reference. Also, NI-USB 6009 has no current entry, so, using a simple resistance can not solve the problem since it will disrupt the signal for the acquisition of the current between counter and work electrodes and will change the potential over the reference electrode. That's why using a trans-impedance amplifier will solve many problems and helps to display the exact current in the electrochemical cell. It has also the advantage to put the work electrode into a virtual ground.

The validation of the electronic system was approved using both board circuitry and without it. Classic electrochemical cell was used in the experiments with 10mM of sulfuric acid solution containing $K_3[Fe(CN)_6]$.

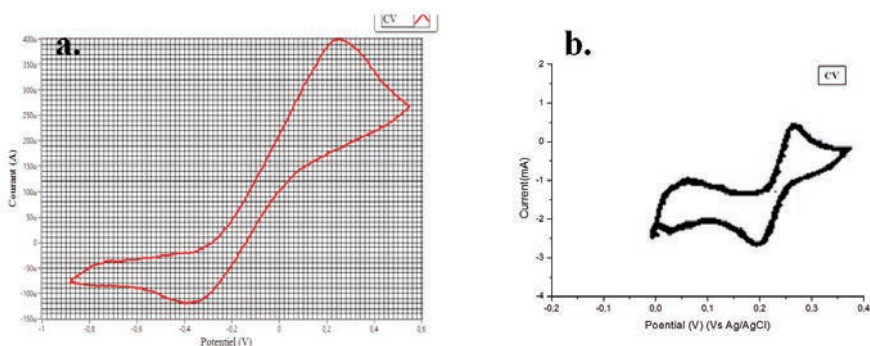


Figure 3: Diagrams of CV using 10 mM of acid sulfuric solution containing redox couple: (a) CV without external card and smoothed with savitsky-Golay filter; (b) CV using the external board and an oscilloscope for the display of results.

Diagrams showed in figure 3 show us cyclic voltammograms with oxydation and reduction pics. Without the external card, the acquired signal is affected by noise and have to be smoothed using a numeric filter named “Savitsky-Golay” filter.

C. Conclusion and future works

Results presented in this work, although primary and preliminary, show the possibility to make electrochemical measurements using this new developed electronic system. Further work will be carried to combine both home-manufactured SPEs and the electronic system to detect SEB as target molecule. Also, organizational and morphological study will be carried by AFM for the different steps of functionalization

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