Implementation of electrochemical elements for an alternative detection of ochratoxin A

D H Aristizabal[1,2], D A Giraldo[1,2], S Sanchez[1], G Taborda[2] and A Baeza[3]

[1] Servicio Nacional de Aprendizaje (SENA), Manizales, Colombia
[2] Universidad de Caldas, Manizales, Colombia
[3] Universidad Nacional Autónoma de México, Ciudad de México, México

E-mail: dhernab@sena.edu.co

Abstract. Ochratoxin A (OTA) is a nephrotoxic metabolite, hepatotoxic and carcinogenic produced mainly by Aspergillus and Penicillium fungi. Usually, the mycotoxin analysed through the technique of high performance liquid chromatography. This method is expensive and takes a lot of time. Therefore, expected to automate a device of low cost, minimal instrumentation micropolarographic (MIMP) for the analysis. For this purpose, the right programming environment and the manufacturing of the software for the graphical user interface selected automation of the MIMP through the design and simulation of the circuit, imprint and assembled in a container with connection ports. Lastly, validation and analysis of Ochratoxin A by cyclic voltammetry from analogous MIMP, automatic MIMP and a potentiostat, in order to corroborate the registered data. This way, voltammetry analysis of coumarin is obtained and the simulation of the MIMP electric circuit. Therefore, this are the key data during the investigation, because the molecule of OTA can be monitored through an acid hydrolysis of amides, composed by a phenylalanine and coumaric acid group, generating the significant decrease in the costs and time of analysis.

1. Introduction

Ochratoxin A is a secondary metabolite with some toxicity level, produced by fungi genus Aspergillus and Penicillium, which causes immune and hepatic diseases [1-5]. Therefore, the OTA considered worldwide as a contaminant in food products. Found in some cereals, spices, fruits, wine, beer and coffee [6]. The food analysis of the metabolite used in immune-affinity tests with chromatographic techniques, which represents raised costs [7]. Thus, the automation of a minimum micropolarographic instrumentation device in electrochemistry designed, to evaluate the consequences in food of this metabolite, using chemical analysis [8]. For this purpose, the use of electronic circuits programmed by means of Arduino microcontrollers is required and analyse the data captured through graphical user interfaces (GUI) [9,10]. In this regard, some of the principal development environment programs to design specific graphical user interfaces in the electroanalytical chemistry field used, according to the need. This could be associated with the data acquisition speed, analysis techniques or comfort for the design of the GUI. Matlab has developed some applications for these interfaces and has become one of the most used software for design GUI, applied in voltammetric monitors of geochemical level in the analysis of marine waters, to follow the levels of sulphur, gases, metals and dissolved gases. The data process and registration should be done as soon as possible by the constant change of the water column in the ocean, therefore the implementation of this type of software allow us to know in depth the natural phenomena 'in vivo', since there could not access a raw team [11]. Other programming

environment as KDevolv used in the research work [12], which proposes to improve significantly the chronoamperometry technique used in electrochemical analysis using programming code. With the construction of the software, the objective is to pause the experiments or reverse the sign of the applied current, in order to minimize the undesirable effects of the burden of double layer capacitance and to offset uncompensated ohmic loss. Finally, the programming environment employed during the process of automation of the MIMP, due to it is free of use and pleasant to work. The proposed methodology is a contribution to process sample collection for specific laboratories. The instruments currently used for the analysis of samples are highly priced units and required trained personnel to carry out the analysis and to understand the data. These analytical systems are bulky to be used 'in-situ'. In many cases, the longtime delays involved in this process are unacceptable, and online processing is required. Most electrochemical sensor systems researches focus in analog systems. However, the development of this unit is get a graph of the Ochratoxin type A detection [13].

2. Materials and methods

2.1. Electronic Application

The MIMP system is composed of: electrical supply 12V Stern transformer; resistance of 5kΩ; rheostat of 10kΩ guarantees the resistance variability; voltmeter, allows the measurement of the imposed potential; three electrode electrochemical cell (also an auxiliary electrode, one for reference and one for indicator) and an ammeter, allows the measurement of the current flowing through the electrochemical cell. Two multimeters STERN used as a voltmeter and ammeter. The entire circuit assembled on a disposable plastic box [8]. With the description of the MIMP, it begins the automation process that implements two operational amplifiers with adder-subtracter configuration, thanks to an array of 1KΩ elements (see Figure 1). This is powered by two 9V batteries, controlled using a digital potentiometer X9C103P in a range of +/-2V using an Arduino nano board. Thanks to the arrangement of resistances, the system generates controlled potential sweeps to 100mVs^{-1}, which processed by reading on Arduino pin configuration-controller and sent to the free processing software, which finally generates the voltammetry and presents the information recorded in both the wave of oxidation, and the reduction of the analytic. (see Figure 1).
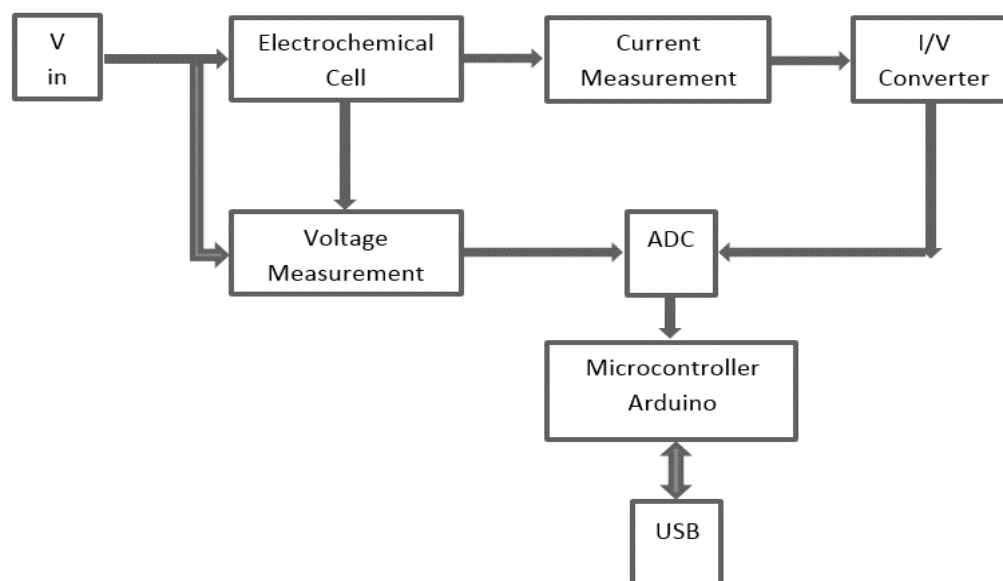
Figure 1. Block diagram of the control/acquisition data module.

2.2. Chemical Analysis
The voltammetry analysis with the MIMP performed in a glass cell of 4mL with a set of three electrodes: reference electrode Ag°/(Ag$^+$Cl$^-$), graphite electrode and vitreous carbon working electrode counter and starts with the electroactivity domain. For this purpose, adding 1mL of PBS 0.1mol L^{-1} buffer at pH 8.0, 0.5mL of electrolyte support 0.1mol L^{-1} of hexadecyltrimethylammonium bromide (CTAB) dissolved in methanol, 0.5mL of Acetonitrile-methanol mixture in proportion 60:40 reagent grade and nitrogen bubbling for 2 minutes. Then, new solutions are prepared for the analysis in triplicate in concentrations of 0,001molL^{-1}, 0,002molL^{-1} and 0,004molL^{-1}, coumarin dissolved in the same form in a mixture of Acetonitrile-Methanol in proportion 60:40 and a sweep rate used of 50mVs^{-1}. After each measurement, the work electrode polished with alumina, to guarantee that the surface area of interaction remains constant. Similarly, it comes with Gamry potentiostat interface of a 1000, with a slight change in the concentration of the solutions of analysis, diluting the concentration 1000 times to avoid overruns on the readings, because the equipment has greater sensitivity compared to the MIMP.

3. Results and discussion
The voltage generated by a dual source of 9V, was restricted to 2V and this voltage then measured between an electrode of reference and the voltage on the working electrode, using an operational amplifier. To do this, it was used a subtracter configuration which determines the difference between these two voltages and thus acquire the measurement required for the construction of voltammograms. In parallel form, it studies the participation of the current in the circuit, determined as an electro counter. Is in here that occurs a conversion from current to voltage through Ohm's law, a turning point during the investigation, because the Arduino microcontroller only receives voltage through its input pins. Additionally a new restriction appears. The values of the potential must be positive for this purpose. An offset implemented, which consisted in applying 2V both of the depleted potential and in the converted from the current. With these ensured that the Arduino board will only conduct readings between 0V and 4V, since the operational amplifier offset settings comes -2V the add 2V, reading will be 0V, otherwise when is received 2V in addition to 2V, at the end the result will be 4V, read by the microcontroller. These data in cyclic voltamperometric should treated with a simple programming Arduino code, since for the cycle there is also a need for negative values. Therefore, the data received by using Arduino analogue pins through the analog-digital conversion, multiplied by -2 to secure the cycle voltamperometry of +/-2V generated by the sweep of potential that gives the lines used [14].

Using the MIMP and applying the cyclic voltamperometry technique achieved to determine coumarins (see Figure 2). In this way, potential of reduction of the compound obtained in -1.5V as those reported in the work carried out by [15] (see Table 1). This results due to the transfer of two electrons and two protons in the carbonyl group to generate the hydroxyl group as a reduced species. Thus, the radical intermediate species may be more stable thanks to the alkaline medium. Regarding the corroboration of the preliminary analysis of the analyte with the 1000 Gamry interface reference, similar potential reductions with linear correlations from R^2=0.9998 are observed with regard to the increment of the cathodic peak current and increasing of the concentration. (see Figure 3 and Table 2).

Table 1. Cathodic peak current and potential half-wave to each concentration by the MIMP.

| C (mol/L)	i_{pc} (μA)	$E_{1/2}$ (mV)
0.001	205	-1.573
0.002	230	-1.567
0.004	274	-1.570
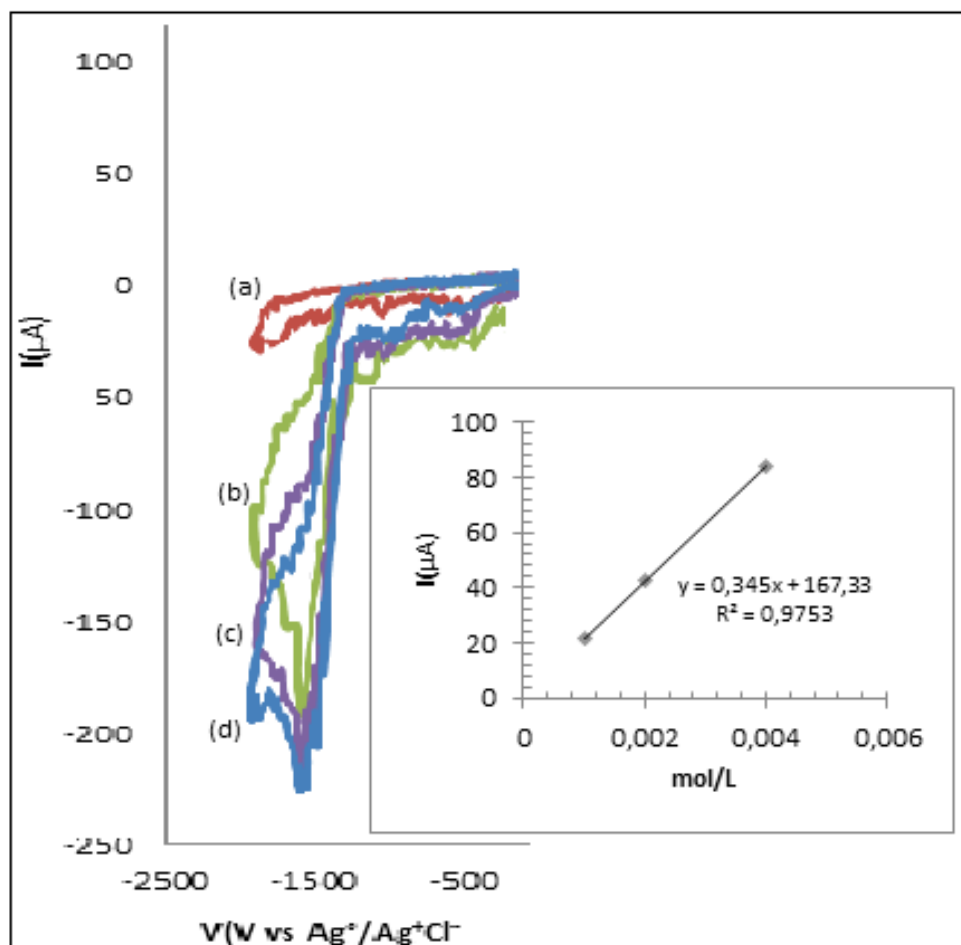
Figure 2. Cyclical voltammograms of (a) electroactivity domain, (b) 0.001mol L^{-1}, (c) 0.002mol L^{-1}, (d) 0.004mol L^{-1} of coumarin at a sweep rate of 50mVs^{-1} on a carbon electrode vitreous. Support electrolyte 0.1mol L^{-1} bromide hexadimethrine (CTAB), buffered solution of phosphates 0.5mol L^{-1} at pH: 8.0 and Acetonitrile-Methanol in proportion 60:40 reagent grade with the analog MIMP.

These findings are useful for the research because of the molecule of Ochratoxin A has a group of phenylalanine and a group coumaric, therefore recognize the potentials of reduction, which the metabolite found useful for the analysis of the compound.

Table 2. Cathodic peak current and potential of half wave to each concentration with the Gamry interface 1000.

C (mol/L)	i$_{pc}$ (μA)	E$_{1/2}$ (mV)
0.0001	21.5	-1.515
0.0002	42.4	-1.496
0.0004	84.3	-1.505
0.0006	121.9	-1.510
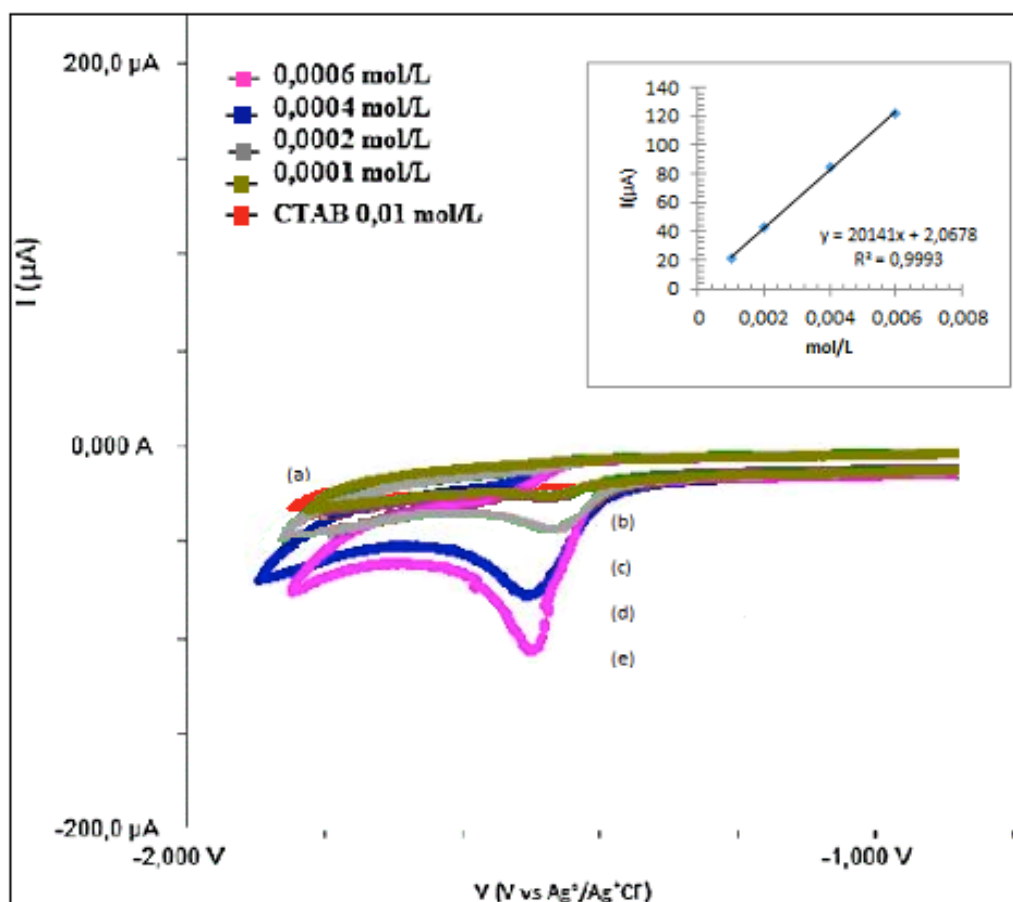
Figure 3. Cyclical voltammograms of (a) electroactivity domain, (b) 0.0001mol L^{-1}, (c) 0.0002mol L^{-1}, (d) 0.0004mol L^{-1}, (e) 0.0006mol L^{-1} of coumarin, at a sweep rate of 50mVs^{-1} on a carbon electrode vitreous. Support electrolyte 0.1mol L^{-1} hexadimethrine bromide (CTAB), buffered solution of phosphates 0.5mol L^{-1} at pH: 8.0 and Acetonitrile-Methanol in proportion 60:40 reagent grade with the potentiostat Gamry interface 1000.

4. Conclusions and expected results

Until now, the partial automatizing of MIMP is in progress, which turns out to be an important tool of chemical analysis for monitoring mycotoxins present in foods, in relation of traditional commercial equipment, both for chromatography analysis and electrochemestry. This way, appears new contributions and alternatives for electrochemical analysis to low-cost and less time, from analog electronics and programming applied to microcontrollers and software design of graphical interfaces free of use. In relation to the expected results, the data is plot from current and voltage through the free software application 'Processing' through code lines graph, obtaining curves from the analytical signal. In this way, the implementation of electroanalytical techniques will be fast and economic, concerning the conventional design until the time of the MIMP.

In appreciation to

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