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ARTICLE

Embedded flow-batch system with electrochemical detection for the determination of lead in propolis samples

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ABSTRACT

A lab-made prototype consisting of a potentiostat with a flow-batch system was designed and implemented under the commercial off the shelf paradigm. The prototype is light-weight and suitable for laboratory performance. Its performance was evaluated with linear sweep voltammetry (LSV) study of ferrocyanide/ferricyanide redox couple and the obtained results were comparable to that obtained with a commercial potentiostat. The system was applied in the determination of lead in propolis samples. For this purpose, bismuth film electrode by square wave anodic stripping voltammetry (SWASV) was employed. The obtained results were validated by atomic emission spectroscopy with inductively coupled plasma and very good agreement was obtained.

1. Introduction

In recent years, food and environment contamination has become a subject of great interest and social concern because of the adverse effects on the human health. The increase in industrial activity and the high vehicle population are primarily responsible for this pollution [1].

Bee products can constitute reliable indicators of environmental quality. These products can be contaminated through different sources such as air, water, plants and soil [2]. Additionally, the foraging activity of bees brings them into contact with a large number of pollutants that can be transferred to their products [3]. Among bee products, propolis is chemically complex resinous product collected by bees [4]. It is used in medicine and food industry due to its biological and pharmacological effects [5]. One of the main pollutants of propolis is lead, a toxic heavy metal, which can come from different sources.

Currently, laboratory assays are the most common way to determine the presence of contaminants in food samples. Unfortunately, these processes require highly trained laboratory analyst, sophisticated and expensive equipment, substantial amounts of time and sometimes consumption of toxic and expensive reagents.

Novel analytical processes require the use of light-weight and automated prototypes which imply a challenge for multidisciplinary research groups. The computational systems engineering play an important role on this process. Embedded computing systems (ECS)

have emerged as an important engineering discipline with principles rooted in electronics, real-time operating systems and software engineering. They are present in everyday life from industrial plants to home appliances. Most of them are implemented with system-on-a-chip technology (SoC) that integrates input/output interfaces, memory and CPU in a single chip. They usually interact with the environment measuring certain variables and controlling others. The use of ECS for portable instrumentation is becoming common. In particular, the introduction of flow-batch (FB) techniques has led to a completely new set of automated instruments that can be used in the field. Since its inception, flow-batch has transformed the way that sample analysis is handled. What used to be manual, slow and prone to error has become automated, rapid and precise. Through the use of an instantaneous stop-mixing chamber and computer-controlled flow-based sampling, it achieves extraordinarily high sampling rates and reliability [6]. In the literature, there are several manuscripts to determine metals in water samples by using FB methodology with electrochemical techniques. Bezerra dos Santos et al. determined zinc and lead using a boron-doped diamond electrode and a electrochemical flow cell [7] and lead and cadmium with an electrochemical analyser with on-line data transmission and a global positioning system [8]. The same authors employed a thermostated electrochemical flow cell with a bismuth film electrode to determine cadmium and lead [9]. Pierini et al. determine metals in raw propolis samples with a bismuth film electrode and a commercial potentiostat [10,11]. Several groups have designed and fabricated miniaturised single chip potentiostats from silicon that can be integrated [12,13]. The advantage of this approach is that potentiostats can be made very small making them ideal for applications such as *in vivo* neurotransmitter monitoring [14,15]. However, the setup and fabrication costs make this approach too expensive for the production of a few analysis devices. The choice of commercial-off-the-shelf (COTS) devices helps to reduce design time and costs while simplifying the implementation. With this paradigm, many researchers have developed their own devices for diverse applications [16–18]. Nevertheless, there is always present the problem of handling the samples and solutions manually.

The contribution of this work is the design and implementation of a light-weight prototype consisting of an embedded flow-batch system with electrochemical detection. For this purpose, low-cost microcontroller-based platform (ATMEL ATmega328 microcontroller in an Arduino open hardware kit) and a custom-built design with operational amplifiers are used to achieve instrumentation miniaturisation at low cost. The performance of the lab-made prototype was evaluated initially carrying out a linear sweep voltammetry (LSV) of potassium ferro/ferricyanide redox couple to verify its electrochemical sensing operation by comparing voltammograms with a commercial potentiostat. Furthermore, raw propolis samples with high concentration of lead commonly found in apiaries in the Province of Buenos Aires was analysed. For this proposed the developed prototype, with a bismuth electrode and a square wave anodic stripping voltammetry, was employed.

2. Experimental

2.1. *Hardware configuration*

In this section, we discuss the design and implementation of the measurement system hardware. The flow-batch methodology requires small amounts of reagents and samples and it generates small quantities of waste. All these aspects are aligned with the
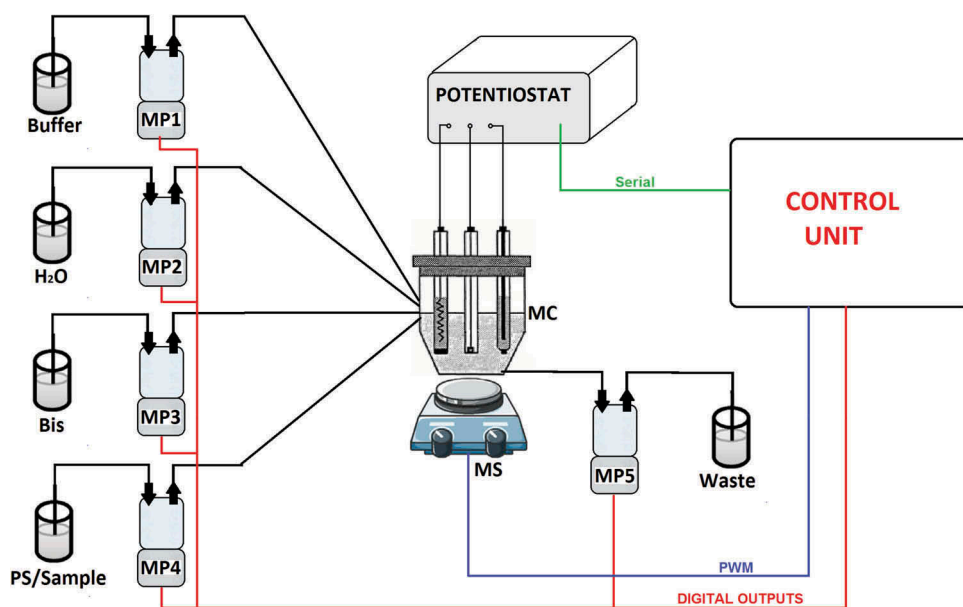
Figure 1. Embedded flow-batch system with electrochemical detection. MP: metering pump; MC: mixing chamber; MS: magnetic stirrer; Buffer: buffer solution of 0.1 M of sodium acetate; H_2O: ultra pure deionised water (>18MΩ); Bis: bismuth solution acidified with 5 mL of nitric acid; PS/Sample: lead standard solution or sample.

principles of the green chemistry. For a better description, the flow-batch system is divided into subsystems, which are described below. Figure 1 shows the system proposed and its different parts.

2.1.1. *Electrochemical cell*
The electrochemical reaction takes place in a laboratory-made glass mixing chamber (MC). This chamber offers the following features: 20 mL internal volume, four holes that allow the admission of the different fluids and at the bottom, a hole to remove the waste. Furthermore, contains a PTFE cap with three holes for connecting the electrodes.

2.1.2. *Distribution system*
The flow-batch methodology requires the injection of certain volumes of fluids into the mixing chamber (MC). For this reason, metering pumps (MP) are suitable for this kind of applications. A diaphragm metering pump MLP-200 with a displacement of 200 μL per cycle is chosen. The driving circuit for this pump is implemented with the integrated circuit ULN2803 that consists of an array of eight Darlington transistors specially designed to handle loads up to 500 mA per driver. For transporting fluids among the system parts, flexible tubes of 2.06 mm diameter are used. The addition of fluids into the MC is achieved by cycling the pumps at 1 Hz. A pump positioned at the outlet flushes the unused fluids to clean and to discharge the MC. The timing of the pumps is programmed on the microcontroller to deliver precise liquid volumes to the chamber. A magnetic stirrer (MS) is placed in the bottom of the MC to mix the solutions.

2.1.3. *Potentiostat*

In voltammetric techniques the potentiostat is responsible for setting a controlled potential between the working and reference electrodes to generate a redox reaction, and thus, to measure the obtained current. On this basis, an embedded system capable to establish controlled potential and record the current generated was implemented. This potentiostat contains three kinds of electrodes: working (WE), reference (RE) and counter electrodes (CE). The first one is the platform on which the electrochemical reaction takes place, The RE, is used as a point of reference in the electrochemical cell for the potential control and measurement. The CE is in charge of closing the current loop with the WE.

Figure 2 shows the schematic circuit of the topology chosen. To avoid variations in the potential of the reference electrode by the actual current flow, it is usually connected to the gate of a MOSFET transistor that has very high impedance or, like in the case of this work; it is connected to the input of an operational amplifier (Op-Amp). For the device denoted U2 in the figure, the operational amplifier LMP7721 was chosen. It has a very high input impedance and extremely low input current. With these characteristics it provides a good performance for the proposed application.

In this topology, the WE was clamped at a constant voltage by the potential applied to the positive terminal of op-amp denoted U3 in the figure. As the system has a single 5V voltage source, it was decided to clamp the potential voltage of WE in 2.048V through the reference voltage integrated circuit REF5020. Thus, the potential voltage
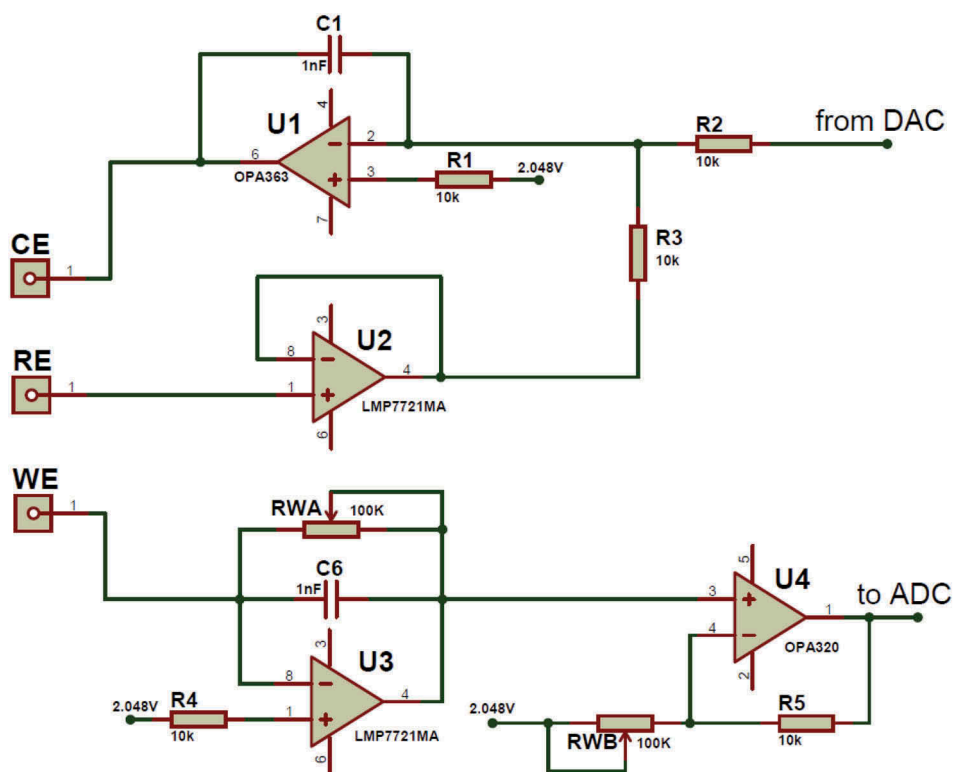

Figure 2. Schematic of the potentiostat topology for three electrodes cell configuration. WE: working electrode; RE: reference electrode; CE: counter electrode.

difference between the WE and the RE is considered negative or positive depending if the RE potential is greater than 2.048V or not respectively.

In the amplifier stage, the op-amp denoted U1 in the figure, the potential difference between WE and RE is compared to the value of Vbias (reference from DAC). The comparison is used to adjust the voltage of the CE, forcing a current through it which polarises the WE making the potential difference between the RE and WE equal to the desired Vbias. The capacitor C1 was placed in the feedback network to improve stability. For this stage, the operational amplifier OPA363 was chosen because it has a very high common mode rejection mode (90db) and a high open loop gain (100db). For the digital to analogue converter, the DAC7571 was selected. The requirements for this device are 12 bits precision, I2C communication interface, internal reference and low offset error.

In order to measure the current, a transimpedance amplifier was built. The system has to be able to measure different current intensities, thus it is necessary to have a variable gain knob to control the sensibility of the amplifier. As the sensor current was in the range of 1µA-1 mA, the amplifier was divided in two stages. The first stage a transimpedance amplifier with a variable gain up to 100.000 V/A, and the second one a voltage amplifier with a variable gain up to 100 V/V. In both stages, the feedback resistors, RWA and RWB, were implemented with a dual digital variable potentiometer integrated circuit TPL0102 connected to the I2C bus of the microcontroller. For the transimpedance amplifier, a LMP7721 op-amp was chosen because of its very high input impedance. The voltage amplifier was implemented with an OPA320 op-amp.

The output of the amplifier stage feeds an analogue to digital converter (ADC) ADS1014 with 12 bits precision, I2C communication interface and external reference. Figure 3 shows the circuit board developed.

Both converters and the digital potentiometer described above should be handled by a microcontroller. A measurement log history is saved in an SD memory or transmitted to a computer for later processing and analysis.
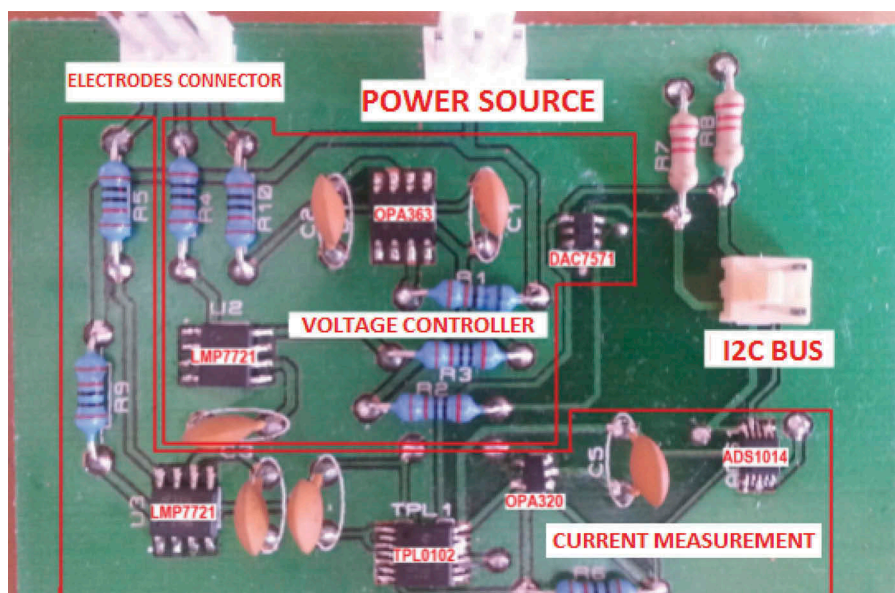

Figure 3. Printed circuit board of the potentiostat developed.

Table 1. Comparison between TI LaunchPad and Arduino UNO open-hardware kits.

	TI LaunchPad	Arduino UNO
MCU	TI M430G2553	ATMega328
Data bus	16 bits	8 bits
Clock speed	16 MHZ	16 Mhz
Storage	16 Kb	32 Kb
RAM	512 b	2 Kb
Digital I/O	8	14
Analogue inputs	8	6
Communication	USART, I2C, SPI	USART, I2C, SPI
Unit cost	$2.80/chip	$2.82/chip

The selection of the microcontroller hardware was done following the principle of the COTS paradigm. There were two possible solutions with very similar characteristics: the Atmel ATMega328 with the Arduino UNO development open hardware board and the Texas Instruments MSP430G2553 Launchpad platform. Table 1 shows the main characteristics of each microcontroller.

Both devices have features that meet the system requirements and both have similar costs, however, based on the popularity of the Arduino platform we finally selected this one.

A microSD TF Card Memory Shield Module with SPI interface was used to store the data. Arduino IDE comes with a SD library for card initialization and file management. This module has a level conversion circuit that can interface the TTL 5 V input/output ports of the Arduino board with the TTL 3.3 V of the memory card.

2.1.4. Control unit

Like in the case of the potentiostat, the automation of the system was done with an ATMega328 MCU (Arduino UNO open-hardware kit). This 8-bit MCU provides the necessary on-chip peripherals for this purpose at a low cost. Digital outputs were used for commanding the coils of the metering pumps. A Pulse Width Modulation (PWM) signal controls the DC motor of the magnetic stirrer. The UART unit was used to send the commands to the MCU of the potentiostat and to download the data stored in the SD memory card for subsequent processing.

2.2. Software description

The embedded system software is usually named firmware as it includes both the application and drivers of the different input/output devices. In the case of Arduino platform, there is an open Interface Development Environment that simplifies the software development.

2.2.1. Firmware of the potentiostat

The firmware of the potentiostat must be capable of performing the electroanalytical techniques required for the determination stage. The software has six main blocks that are listed below:

- Manage the serial port for the exchange of packets with other devices.
- Load the gain of the current measurement stage.
- Load the function to be performed (Pretreatment, linear sweep, square wave).
- Load the parameters of the function selected.

- Run the process, which automatically stops at the end of the scheduled task.
- Record the data to the SD memory card.

The system is configured to sample the current every 10 ms in the case of pretreatment or twice per period in the case of square wave in which the frequency is preconfigured by the user. Following the guidelines defined in the previous point, the firmware code was written using the following scheme:

2.2.2. Initialization
In this part, the communication parameters of the UART, SPI, I2C are configured. The variables which will then be used by the system are initialised and a potential difference of 0 V between the CE and the WE is established.

2.2.3. Main function
In this part, the periodic tasks of controlling, measuring and saving the data are performed. In what follows they are described with more details:

- Reading the serial port. If there are any data entering the serial port, they are read and interpreted.
- Implementation of functions. Once selected the function to perform and the parameters, it begins to run and only stops when the process is completed.

2.2.4. Communication
To control the potentiostat, a communication protocol to interact with it from other devices was developed. Communication is done via the serial port and is conducted by exchanging packets of information. The device that interact with the potentiostat sends a special character (command), which is interpreted by the Arduino MCU, and then sends the values of the variables involved in the process selected (parameters). Table 2 shows the available commands and detailed information of each one.

2.3. Reagents

All reagents were of analytical grade and ultra-pure water (>18 MΩ) was used. To remove the current migration, a buffer solution of 0.1 M sodium acetate pH 4.5 as

Table 2. Set of commands with detailed information.

Command:	**g**
Function:	Set the gain of the current amplifier stage
Parameters:	*Gain*: from 10 to 100,000,000
Command:	**$**
Function:	Set linear sweep function
Parameters:	E_i, E_f, *scanRate*
Command:	**%**
Function:	Set square wave function
Parameters:	E_i, E_f, ΔE_s, *f*, ΔE_{sw}
Command:	Set pretreatment function
Function:	*E*, *t*
Parameters:	
Command:	**&**
Function:	Enable cyclic mode
Parameters:	*none*

electrolytic support was used. This solution was prepared using sodium acetate trihydrate and acetic acid (Cicarelli).

Bismuth standard solution was prepared from the nitrate salt Bi $(NO_3)_3$ $5H_2O$ (Sigma-Aldrich 99.999%) in nitric acid (20% v/v). Lead stock solution 5.04 mg Pb L^{-1} was prepared from Pb $(NO_3)_2$ (Merck) acidified with nitric acid.

2.4. *Sample treatment*

Samples of raw propolis with high levels of lead were selected due to vehicular traffic and industries. The samples were obtained from different apiaries in the Province of Buenos Aires, Argentina, i.e. Tornquist, Bahia Blanca and Hilario Ascasubi. These samples were conditioned and a mineralization treatment was performed: raw propolis samples were weighted and calcined in furnace at 400°C to white ashes, taken up with 20% (v/v) nitric acid solution, filtered and making up to 25.0 mL with water [10].

2.5. *Procedure*

A three-electrode configuration consisting of a bismuth film prepared '*in situ*' on a glassy carbon electrode, Ag/AgCl (3 M NaCl), and a platinum foil as working, reference, and counter electrodes, respectively, were used. The glassy carbon electrode surface was polished manually with a different particle size (0.3 and 0.05 μm) of aluminas and sonicated in water during 30 s. This procedure was performed before start working and it is not necessary to repeat it. The authors have employed bismuth film electrodes in previous works and verified a good reproducibility electrochemical performance [10,11].

In order to determine lead in propolis samples square wave anodic stripping voltammetry (SWASV) measurements were carried out at pH 4.5 sodium acetate buffer solution containing 1000 μg L^{-1} of bismuth. Bismuth film has been deposited on glassy carbon electrode by applying a deposition potential of −1.4 V during 120 s under conditions of forced convection (stirring). Then, the stirring was stopped and after 10 s the measurements were carried out with the following parameters: potential range between −1.4 V and 0.2 V, potential step (ΔEs) 2 mV, frequency (f) 25 Hz and amplitude of the square wave (ΔEsw) 50 mV.

After each determination, a cleaning step was performed to remove the deposited bismuth or analyte onto the glassy carbon surface. Then, a potential of 0.2 V was applied for 30 s under conditions of mechanical agitation. All measurements were performed in the presence of oxygen.

3. Results and discussion

3.1. *Analytical performance of the potentiostat*

In order to study the performance of the lab-made light-weight prototype the response of 2.5 mmol L^{-1} potassium hexacyanoferrate (II) trihydrate (Sigma-Aldrich) in 0.1 mmol L^{-1} potassium chloride (Merck) as supporting electrolyte was analysed using the technique of linear sweep voltammetry (LSV). The electrochemical response of this prototype was compared against a commercial potentiostat (BASi-Bioanalytical System, Indiana, USA). In both cases, a three-electrode configuration was employed with a glassy carbon as
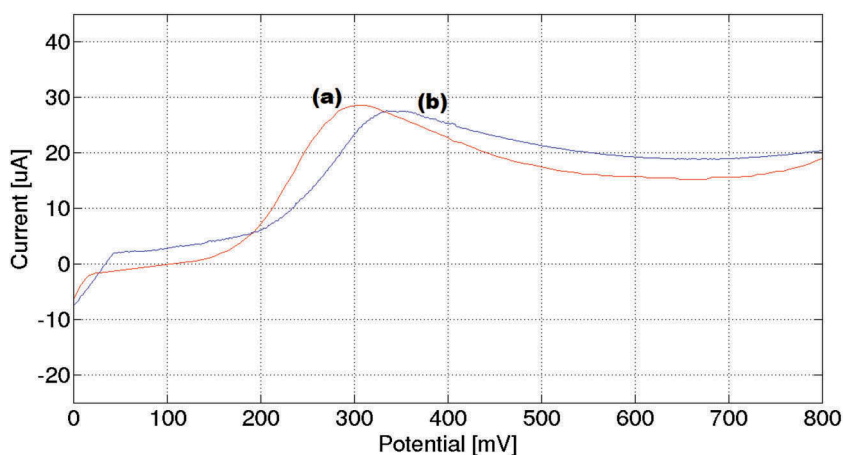
Figure 4. Comparison between the BASi-Bioanalytical System (a) and the proposed potentiostat (b) LSV were carried out in a solution of 2.5 mM potassium ferrocyanide in 0.1 M potassium chloride. Glassy carbon electrode, Ag/AgCl (3 M NaCl), and a platinum foil were used as working, reference, and counter electrodes, respectively. The LSV was carried out with the following parameters: Ei = 0 V, Ef = 0.8 V, scanRate = 100mV s^{-1}.
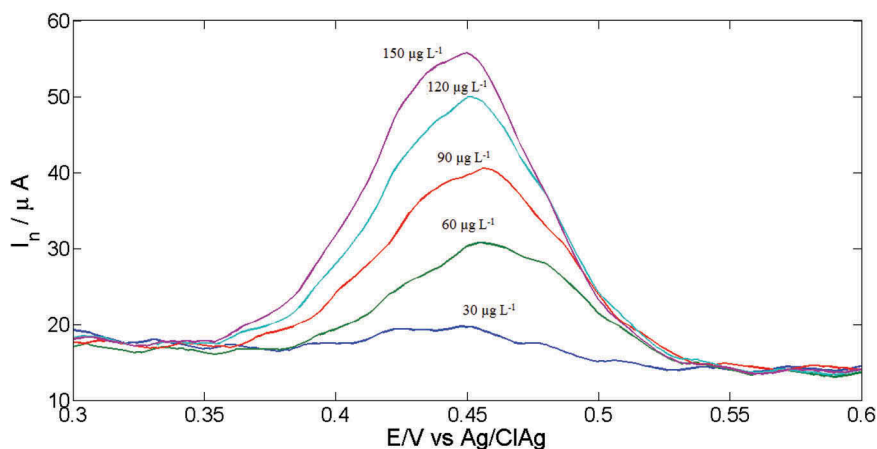
working electrode, Ag/AgCl (3M NaCl) as reference electrode and a platinum wire as counter electrode. All electrochemical experiments were performed at room temperature and without removing oxygen. The electrochemical response was carried out from 0 to 800 mV at different scan rates from 10 to 100 mV s^{-1}. Figure 4(a) shows the voltammogram obtained with the prototype developed. It can be seen the corresponding anodic peak current of the potassium ferro/ferricyanide redox couple between 300–320 mV and a peak current intensity of 27.5 µA. A similar anodic peak current in terms of shape, current intensity and potential was obtained by employing the commercial potentiostat (Figure 4(b)).

## 3.2. Analytical application: determination of lead in samples of propolis

One of the main pollutants present in raw propolis is lead, due mainly fuel and motor oil combustion, brake wear and enriched road dust resuspension [19,20]. The Argentine Food Code establishes the maximum lead concentration in raw propolis in 2 mg kg^{-1} [21]. The lab-made prototype was used to evaluate the concentration of lead in propolis samples [10]. For this purpose a bismuth film electrode and SWASV was employed.

3.2.1. Analytical parameters

To determine lead in propolis samples SWASV measurements were carried out at pH 4.5 sodium acetate buffer solution containing 1000 µg L^{-1} of bismuth. The range of lead concentrations was between 30 and 150 µg L^{-1}. Figure 5 shows the voltammograms obtained and very well defined peaks at a potential of 450 mV were observed. Figure 6 the shows the calibration curve. Considering that the lead content is different in the analysed samples, two volumes of calcinated solution were selected.

Figure 5. SWASV measurements for lead in the range of 30 and 150 μg L^{-1} were carried out at pH 4.5 sodium acetate buffer solution containing 1000 μgL^{-1} of bismuth. Glassy carbon electrode, Ag/AgCl (3M NaCl), and a platinum foil were used as working, reference, and counter, respectively. The potentiostat was configured with the following parameters: Ei = −1.4 V, Ef = 0.2 V, ΔEs = 2 mV, f = 25 Hz, ΔEsw = 50 mV.
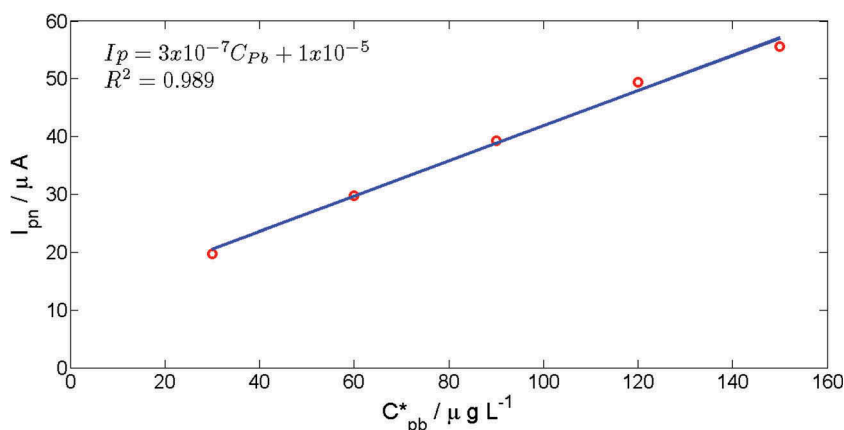
Figure 6. Calibration curve for the lead concentration in the range from 30 to 150 μgL$^{-1.}$

In the case of Tornquist and Bahía Blanca samples, the volume was 0.100 mL providing a concentration range of 7.50–37.50 mg Pb kg^{-1} raw propolis and a detection limit (LOD) of 3.33 mg Pb kg^{-1} raw propolis. Furthermore, 0.400 mL for Hilario Ascasubi sample was analysed and the linear range was 1.88–9.38 mg Pb kg^{-1} raw propolis with a LOD of 0.83 mg Pb kg^{-1} raw propolis. The detection limit was calculated in both cases, as three times Sy/x/slope [22]. Although in the literature there are papers with LOD values in raw propolis samples lower than those found in this manuscript, one of the objectives was to analyse samples with high concentration of lead commonly found in apiaries in the Province of Buenos Aires, Argentina. The repeatability and reproducibility were calculated as the percentage of the

relative standard deviation of 10 measurements using a 50 µg L^{-1} standard solution. The obtained values were 6.38 and 7.45% respectively.

3.2.2. Interferences

Raw propolis contains in its composition zinc and copper that may interfere in lead determination. In a previous paper, the authors showed that the zinc concentration do not influence in the quantitative determination of lead. Furthermore, copper content affect lead signal. Then, small amounts of hexacyanoferrate(II) solution (1 mmol L^{-1}) were used to mask completely the copper in these samples [10].

3.2.3. Analysis of samples

Table 3 shows the flow-batch parameters (time and pump setup) selected for the determination of lead in propolis samples. The lead content in the analysed samples are shown in Table 4. It can be observed that the lead content in the analysed samples was higher than the maximum established by Argentine Food Code in raw propolis (2 mg kg^{-1}). This fact may be due that the samples were collected in apiaries located in industrial and vehicular traffic zones: Bahía Blanca (industrial zone), Tornquist (near national route 33, RN33) and Hilario Ascasubi (near route 3 (RN3).

To validate the method, the lead concentrations obtained with the proposed method were compared with that obtained by atomic emission spectroscopy with inductively coupled plasma (ICP-AES, Shimadzu 9000). The results detailed in Table 4 show a good agreement between both techniques. Additionally, the application of a paired Student's t test confirmed that there is no statistical differences ($t_{estimated} = -0.49$, $t_{tabulated} = 4.30$, $n = 3$, and $\alpha = 0.05$) between the results obtained by both procedures [22].

Table 3. Flow-batch procedure.

Step	Event	Time [sec]	Pump setup [µL]@1Hz	Volume [mL]
1	Buffer (MP1)	75	200	15
2	Bismuth (MP3)	25	200	5
3	Sample (MP4)	5	20	0.1
4	Electroplating	120	0	0
5	Wait	10	0	0
6	Detection (SWASV)	32	0	0
7	Waste (MP5)[a]	150	200	0
8	H_2O (MP2)[a]	100	200	20
9	Stirring[a]	120	0	0
10	Waste (MP5)[a]	110	200	0

[a]Cleaning.

Table 4. Results of the determination of lead in raw propolis samples with the proposed method and the ICP-AES technique.

Sample	Pb [mgkg^{-1}]		Relative error
	Proposed method	ICP-EAS	
Tornquist	11.00 ± 0.08	9.94 ± 0.03	+10.6%
Bahía Blanca	7.15 ± 0.10	7.69 ± 0.02	−7.0%
H. Ascasubi	3.18 ± 0.05	2.94 ± 0.01	+8.1%

4. Conclusion

A lab-made light-weight prototype consisting of a potentiostat with an automated system was designed using the paradigm of COTS. This approach facilitates a quick implementation with already tested components that are commercially available with minimum customization; and allows rapid prototyping by concentrating the effort in the function of the device and not in hardware details.

The potentiostat allows electroanalytical measurements such as cyclic and square wave anodic stripping voltammetry. The automation step was performed by the flow-batch methodology by employing a glassy chamber with three electrodes. The measurement data are stored in the embedded memory of the device and can be transferred to a host PC for a subsequent processing. This is a rather important feature of the developed system. The information can be analysed and compared off-line with previous results or even data obtained with complementary devices. The embedded flow-batch system with electrochemical detection is simple, low cost, low consumption of reagents and provides results in considerably less time. Besides as it is automated, it can be repeated with identical setting as many times as necessary providing data obtained under the same strict conditions.

The designed prototype was evaluated by linear sweep voltammetry (LSV) of potassium ferro/ferricyanide redox couple. The results were compared with that obtained with the BASi-Bioanalytical System. Both instruments provide similar information showing the good performance of our low cost lab-made prototype with COTS. Besides, square wave anodic stripping voltammetry and a bismuth electrode were used to determine lead in propolis. The lead content was compared with those obtained with atomic emission spectroscopy with inductively coupled plasma (ICP-AES) and the results were satisfactory.

The proposed instrument can effectively substitute their more versatile and costly bench-type equivalents, especially for routine analytical applications.

Acknowledgments

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] R.E. Green and D.J. Pain, Food Chem. Toxicol. **50**, 4180 (2012). doi:10.1016/j.fct.2012.08.032.
[2] M.E. Conti and F. Botrè, Environ. Monit. Assess. **69**, 267 (2001). doi:10.1023/A:1010719107006.
[3] A. Badiou-Bénéteau, A. Benneveau, F. Géret, H. Delatte, N. Becker, J.L. Brunet, B. Reynaud and L.P. Belzunces, Environ. Int. **60**, 31 (2013). doi:10.1016/j.envint.2013.07.002.
[4] M.I. Nieva Moreno, M.I. Isla, A.R. Sampietro and M.A. Vattuone, J. Ethnopharmacol. **71**, 109 (2000). doi:10.1016/S0378-8741(99)00189-0.

[5] R.A. Laskar, I. Sk, N. Roy and N.A. Begum, Food Chem. **122**, 233 (2010). doi:10.1016/j.foodchem.2010.02.068.

[6] P.H.G. Dias Diniz, L.F. de Almeida, D.P. Harding and M.C.U. de Araújo, TrAC Trends Anal. Chem. **35**, 39 (2012). doi:10.1016/j.trac.2012.02.009.

[7] V.B. dos Santos, E.L. Fava, O.D. Pessoa-Neto, S.R. Bianchi, R.C. Faria and O. Fatibello-Filho, Anal. Methods **7**, 3105 (2014). doi:10.1039/C5AY00012B.

[8] V.B. dos Santos, E.L. Fava, N.S. de Miranda Curi, R.C. Faria, T.B. Guerreiro and O. Fatibello-Filho, Anal. Methods **6**, 8526 (2015). doi:10.1039/C4AY01811G.

[9] V.B. dos Santos, E.L. Fava, N.S. de Miranda Curi, R.C. Faria and O. Fatibello-Filho, Talanta **126**, 82 (2014). doi:10.1016/j.talanta.2014.03.015.

[10] G.D. Pierini, A.M. Granero, M.S. Di Nezio, M.E. Centurión, M.A. Zon and H. Fernández, Microchemical J. **106**, 102 (2013). doi:10.1016/j.microc.2012.05.015.

[11] G.D. Pierini, M.F. Pistonesi, M.S. Di Nezio and M.E. Centurión, Microchemical J. **125**, 266 (2016). doi:10.1016/j.microc.2015.11.038.

[12] W.S. Wang, W.T. Kuo, H.Y. Huang and C.H. Luo, Sensors **10**, 1782 (2010). doi:10.3390/s100301782.

[13] Y.T. Liu, M. Chen, Z.C. Li, Y. Wang and J. Chen, Microelectron. J. **46**, 716 (2015). doi:10.1016/j.mejo.2015.05.009.

[14] C. Mc Caffrey, K. Twomey and V.I. Ogurtsov, Sens. Actuat. B Chem. **218**, 8 (2015). doi:10.1016/j.snb.2015.04.063.

[15] S. Qin, M. Van der Zeyden, H.W. Oldenziel, I.T. Cremers and H.B. Westerink, Sensors **8**, 6860 (2008). doi:10.3390/s8116860.

[16] P. Avdikos and E. Efstathiou, Sens. Actuat. B Chem. **107**, 372 (2005). doi:10.1016/j.snb.2004.10.029.

[17] S. Kwakye and A. Baeumner, Sens. Actuat. B Chem. **123**, 336 (2007). doi:10.1016/j.snb.2006.08.032.

[18] Y. Shi, H. Dou, A. Zhou and Y. Chen, Sens. Actuat. B Chem. **131**, 516 (2008). doi:10.1016/j.snb.2007.12.053.

[19] O. Lambert, M. Piroux, S. Puyo, C. Thorin, M. Larhantec, F. Delbac and H. Pouliquen, Environ. Pollut. **170**, 254 (2012). doi:10.1016/j.envpol.2012.07.012.

[20] G.C. Lough, J.J. Schauer, J.S. Park, M.M. Shafer, J.T. DeMinter and J.P. Weinstein, Environ. Sci. Technol. **39**, 826 (2005). doi:10.1021/es048715f.

[21] J.J. De la Canal, *Código Alimentario Argentino* (Ed. De la Canal y Asoc. S.R.L., Buenos Aires, 2008).

[22] J.C. Miller and J.N. Miller, *Estadística y quimiometría para química analítica*, 4th ed. (Pearson Education, Madrid, 2002).