Batch injection electroanalysis with stainless-steel pins as electrodes in single and multiplexed configurations

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Abstract

In this work mass-fabricated stainless-steel pins are used as low-cost electrodes in a batch injection analysis (BIA) system with electrochemical detection (BIA-ED). The system consists of a polypropylene container including a potentiostatic three-electrode configuration cell designed using a pin coated with carbon ink as a working electrode and two bare pins as counter and reference electrodes. These pins are directly punched into the bottom of the container and connected to the potentiostat using a commercial female connection. The system shows good precision for measurements performed employing the same or different electrochemical cells (RSD 7.2 and 8.9 % respectively). The platform is applied to the determination of epinephrine in a pharmaceutical real sample showing accurate results. As a proof-of-concept, the feasibility of constructing a multiplexed BIA-ED system based on pins is evaluated by incorporating eight pin-based working electrodes in the container. The electroanalytical platform is completed with one reference and one auxiliary pin electrodes that is shared for all the eight working electrodes. It demonstrates the versatility that pins can provide to the construction of different electroanalytical systems. Moreover, the system is designed in such a way that an eight-channel micropipette can be employed for injections, making the system simpler and faster.

Keywords: batch injection analysis, electrochemical detection, pin-based electrode, multiplexed system, decentralized analysis, epinephrine.

1. Introduction

Batch injection analysis (BIA), introduced by Wang and Taha in 1991, is an injection analysis methodology used less commonly than flow injection analysis (FIA) or sequential injection analysis (SIA) [1] but with many unexplored possibilities. In BIA systems with integrated electrochemical detection (BIA-ED), a sample plug is injected from a micropipette tip directly on the surface of the working electrode, that is immersed in a large-volume background electrolyte solution [2]. A transitory signal proportional to the concentration of the analyte is obtained. The association of BIA with amperometric detection is a powerful tool for analysis in food, pharmaceutical and environmental fields [3–5]. BIA systems show similar advantages to those obtained with FIA systems (e.g., sampling rate, sample size, sensitivity, accuracy and reproducibility [6]). However, in BIA these advantages are obtained without using additional components commonly required in FIA: tubes, pumping systems and injection valves that can produce leaks or air bubbles [6] and increase the complexity and cost of the analytical system. They are usually replaced by an electronic micropipette, which is a material present in most analytical chemistry laboratories. Therefore, a BIA system with electrochemical detection can be considered a simple and portable analytical platform [7].

On the other hand, regarding electrochemical detection, at present there is a great interest in developing simple and low-cost electrochemical cells based on common and disposable materials [8,9]. The most extended method for the fabrication of low-cost electrodes relies on the deposition of metallic or carbon-based inks on flat substrates usually made of ceramic, glass or polymers using either thin- or thick-film technologies. In recent years, with the aim of using widespread available and easily disposable materials, paper [8,10–17] (even hydrophobic [18]), other cellulose-based materials such as cellophane [19], or plastic sheets commonly employed as transparency films [20] are being used as substrates. Apart

from the conventional use of screens or stencils, other methods of manufacturing electrodes have been developed, using deposition of the ink with a simple electrode configuration [17] or drawing on the surface of paper using a graphite-based pen [18] or pencil [15,16]. But, all these methods decrease the versatility of the electrochemical cell since they do not allow modifying the setting of the electrodes once they are fabricated. Furthermore, the mechanical stability of paper makes it not suitable for injection analysis.

Recently, and in collaboration with Whitesides group [21], the possibility of using mass-produced stainless-steel pins as electrodes (modified with carbon ink to be used as working electrodes) has been reported. The advantages of pins lie in their low-cost, disposability, widespread availability, high conductivity and easy storage. Moreover, the different parts of a pin allow easy handling and use as an electrode. For example, the head can be used as an electrode, while the sharp tip can be used to drill the substrate (e.g., hydrophobic paper [21] or transparency films [22]) and the shaft offers readily available connection points to the potentiostat. In recent works, we have demonstrated the use of pins as transducers of an enzymatic glucose sensor [22] as well as electrodes in a FIA system [23].

In most of the BIA systems reported, three independent electrodes (working, reference and counter electrodes) are used [7]. When conventional working electrodes are used, regeneration of their surface used to be necessary (e.g. glassy carbon, gold disk, …) and in the case of carbon paste, this is renewed. Moreover, the use of common reference electrodes can be cumbersome due to the special care they need. In order to avoid these inconveniences, during last years, the development of BIA systems based on disposable miniaturized electrodes and of adequate BIA cells for screen-printed electrodes have gathered importance as shown recent reported works [7,24–26]. This demonstrates that BIA is an underexploited methodology, which remains interesting and has been reinvented with the incorporation of miniaturized electrochemical cells. The research on integrating simple low-cost electroanalytical approaches is then a field of enormous interest and growing investigation.

In the present work, we develop the first electrochemical BIA system using prefabricated stainless-steel pins as electrodes. The three pins acting as working, reference and counter electrodes are directly inserted into the bottom of a polypropylene container and they can be easily replaced. The accuracy of this system was evaluated and, as a proof-of-concept, we tested its feasibility to determine epinephrine, a relevant analyte in the clinical field. Epinephrine, also known as adrenaline, is a hormone and chemical neurotransmitter that together with dopamine and norepinephrine belongs to the group of catecholamines. It is present in nervous tissues and body fluids and participates in biological reactions playing an important role in the control of central nervous, cardiovascular, hormonal and renal systems [27,28]. Furthermore, abnormal levels of epinephrine in body fluids are related to serious diseases including Alzheimer's and Parkinson's diseases as well as schizophrenia [29]. Moreover, epinephrine also can be utilized as drug, for example in anaphylactic shock treatment [30]. Therefore, its detection and quantification in physiological pH conditions is of great importance [31]. Since it is an electroactive species, electrochemical techniques are commonly used for their detection [28,29,31,32]. Pin-based electrodes can be considered disposable due to their low cost; however, they are able to produce precise measurements in a BIA platform over time. On the other hand, with the aim of saving time and money, developing multiplexed devices is an interesting challenge. Taking this into account and taking advantage of the versatility that pins offer, a multiplexed BIA system consisting of eight working electrodes is also developed here.

2. Experimental section

2.1. Chemicals

(±)-Epinephrine was purchased from Sigma-Aldrich. An injectable solution of epinephrine contained in a pre-filled 1 mL syringe (Adrenalina Level) was delivered by Laboratorios ERS. Isopropyl alcohol and N,N-dimethylformamide (DMF) were purchased from VWR International. Graphite ink (C10903P14) was

provided by Gwent Electronic Materials Ltd. Liquid glue SuperGOM was purchased from GomaGom®. Ultrapure water obtained from a Millipore Direct-QTM 5 purification system was used throughout this work. All other chemicals were of analytical reagent grade.

A 10 mM epinephrine stock solution was prepared daily in 10 mM HCl solution. Working solutions of epinephrine were prepared daily in 10 mM phosphate buffer saline (PBS), pH 7.0.

2.2. Materials and apparatus

Pins (AIN265925) were purchased from Metalúrgica Folch, S.L. These were 26-mm long stainless-steel pins with 0.59 and 1.5 mm of shaft and head diameters respectively. Polypropylene containers (16-cm long, 6.5-cm wide and 5.5-cm deep were obtained from the local market and used for constructing the BIA system. One-pin Dupont female cables were purchased from Amazon. Transparency film was purchased from Apli Paper S.A.U. The electronic pipettes used (1 and 8 channels) were Labnet Excel™ purchased from Labnet International, Inc.

In the case of the single BIA system, chronoamperometric and voltammetric measurements were performed with an ECO Chemie μAutolab type II potentiostat/galvanostat (Metrohm Autolab) interfaced to a Pentium 4 2.4 GHz computer system and controlled by Autolab GPES software version 4.9. Chronoamperometric measurements performed with the multiplex BIA platform were recorded using a μStat 8000 potentiostat (DropSens) interfaced to a Pentium 4 2.4 GHz computer system controlled by DropView 8400 2.0 software. All measurements were carried out at room temperature.

2.3. Pin-based BIA systems

As reference (RE) and counter (CE) electrodes, two stainless-steel pins were used without further modification. As working electrode (WE), a stainless-steel pin coated with freshly prepared carbon ink was used. The procedure to prepare the pins acting as WE, RE and CE, as well as the preparation of the carbon ink are detailed in the Supporting Information.

The single batch injection system (Fig. 1A-B) consisted of a polypropylene container where the three pins (WE, RE and CE) were inserted at the bottom. With the aim of avoiding cracking, the container was previously drilled with a heated pin. A pattern with a triangular disposition for the three pins, with 2 mm of distance in between, was prepared. Then, the pins serving as electrodes were inserted and sealed with liquid glue to avoid leaking. The head of the pins served as an electrode while the shaft was used as readily accessible point connection to the potentiostat. In order to achieve an easy handling interface between pins and potentiostat, 1-pin Dupont female cables were used as connectors (Fig. 1B).

The multiplexed BIA system (Fig. 1C-D) consisted of the polypropylene container where 8 WEs (carbon-coated pins) were placed. In this system, the RE and the CE were two 12-cm long stainless-steel wires. They were located as two parallel lines at both sides of the eight pins to ensure a better and easier control of the distances between each WE and the other two electrodes, RE and CE. The use of 1-pin Dupont female cables as interface between pins and potentiostat favored an easy connection not only for pins but also for the stainless-steel wires used as RE and CE.

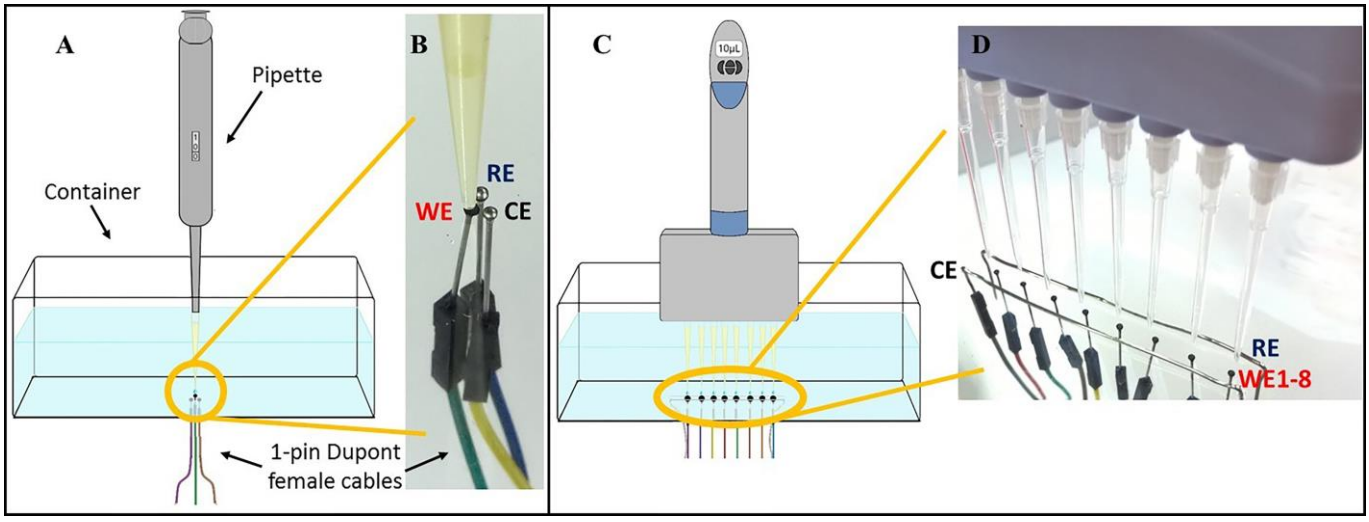

Fig. 1. (A) Scheme and (B) photograph of amplified detail of the electrode configuration of the single pin-based BIA system. (C) Scheme and (D) photograph of amplified detail of the electrode configuration of the multiplexed pin-based BIA system with eight WEs.

In both cases (single or multiplexed), a volume of 200 mL of 10 mM PBS pH 7.0 buffer was added to the container. Sample injections were performed using a conventional electronic micropipette (with one or eight channels for the single or multiplexed system respectively) held in a fixed position with a retort stand. The sample was injected ca. 2 mm away from the WE.

2.4. Epinephrine determination

The electroactive species, epinephrine, was measured directly by chronoamperometry. The epinephrine solution was injected into the head of the WE-pin (2 mm away) that was maintained at +0.5 V vs. a stainless-steel pseudo-reference electrode. Thus, the epinephrine was oxidized and the intensity of the current measured was proportional to the concentration.

The concentration of epinephrine in a real sample (injectable solution) was determined using the one-WE BIA system. The only sample treatment needed was dilution in 10 mM PBS pH 7.0.

3. Results and discussion

We have designed a very simple electrochemical BIA system using a polypropylene container from the local market and common stainless-steel pins as electrodes. Following our previous works where we designed electrochemical cells based on pins for thread microfluidics and analysis on flat hydrophobic surfaces [21], enzymatic glucose biosensing [22], and flow injection analysis [23], we develop here for the first time a cell for fast and simple batch injection analysis (BIA). In this BIA system all the pins used were made of stainless steel and the ones acting as WEs were coated with carbon ink. Due to the low cost of the pins (approx. 3.5 $ / 400 pins), they can be considered as disposable. However, as they are stable enough and precise measurements are obtained, they can be employed for a long period of time or a high number of measurements, as demonstrated in the FIA system [23].

The insertion of the pins in the container was very easy. Pins were heated and their thin sharp tip allowed drilling the polypropylene container at the bottom, allowing the production of patterns as desired without producing any cracking in the container. Very different designs are possible. For the evaluation of a single electrochemical cell, a triangular format was chosen since it allows achieving the lowest equal distance between all the three electrodes. However, in the case of the multiplexed format, working electrodes were aligned and two long wires were located at both sides, maintaining a constant distance between the WEs and RE / CE.

3.1. Optimization of the pin-based BIA system

In order to evaluate the performance of this pin-based BIA system, epinephrine was employed as an electroactive model species. Firstly, since there was not any previous related work about the behavior of this molecule on a pin-based electroanalytical system, a cyclic voltammogram was recorded in a 0.5 mM epinephrine solution in 10 mM PBS pH 7.0 (Fig. S1) using the same method we had previously described in our research group [22] (see Supplementary data). An oxidation process is observed at +0.32 V vs. a stainless-steel pseudo-reference pin, with a reduction process at +0.22 V. Although the difference between peak potentials is 100 mV for a process that involves two electrons [29,33,34], the intensity of the current of the cathodic process is very low (ipa/ipc = 4) and then an irreversible process occurs. In order to determine the best potential for epinephrine oxidation, a hydrodynamic curve (i vs. E curve) was then recorded by injecting 10 µL of a 0.5 mM epinephrine solution on the head of the WE, which was maintained at a fixed potential. This was varied between +0.1 and +0.7 V vs. a stainless-steel pseudo-reference electrode (Fig. 2). Although a lower potential could be applied, in order to ensure the oxidation of epinephrine and the precision of the measurements, +0.5 V was the potential chosen for further studies.

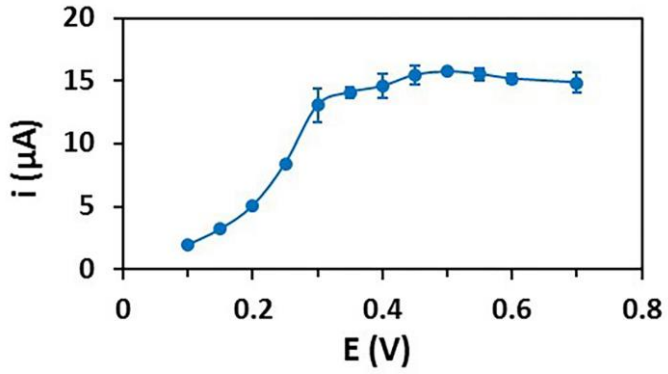

Fig.2. Hydrodynamic curve performed between +0.1 V and +0.7 V *vs*. a stainless-steel pseudo-reference electrode injecting 10 µL of a 0.5 mM epinephrine solution in 10 mM PBS pH 7.0. Error bars correspond to the standard deviation of 3 measurements. Experimental conditions: highest dispensing rate (5/5) and a volume of background electrolyte of 200 mL.

In a BIA system, a transitory signal (batch injection amperogram or biagram, that is a i-t curve) is obtained due to the dispersion of the analyte into the large volume of electrolyte. We evaluated the possibility of incorporating stirring in order to favor mass transport of the analyte. A magnet bar (5 mm diameter, 2 cm long) was introduced in the polypropylene container that was located on a stirring plate. The stirring rate chosen was low (100 rpm) so that the container solution is kept as quiet as possible to avoid noise that could distort the analytical signal. Moreover, we optimized the volume of sample injected. Fig. 3 depicts the peak current for different volumes of injection on the WE pinhead in both cases, with or without stirring.

As showed, higher solution volume provided higher analytical signal until ca. 10 µL, where it seems to stabilize. The precision in the current was similar for all the injection volumes tested (Fig. S2), with a value for the standard deviation corresponding to 10 µL of 0.9 µA. Therefore, 10 µL was the injection volume chosen for further studies. Regarding to the stirring, although there was a slight influence on the width of the peak at the baseline (Fig. S2B), which can produce some influence on the injection frequency, its effect in the analytical signal (height of the maximum of the peak), (Fig. 3) and precision

(Fig. S2A) was negligible. Then, for the sake of simplicity, the incorporation of stirring to this BIA system was discarded.

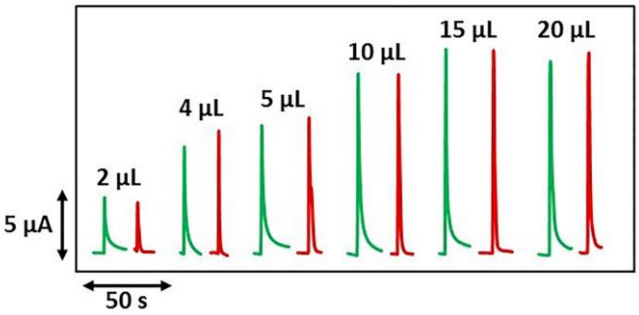

Fig. 3. Biagrams (i *vs*. t curves) recorded at a potential of + 0.5 V *vs*. a stainless-steel pseudo-reference electrode, performed without (green) and with (red) agitation injecting different volumes of a 0.5 mM epinephrine solution. Experimental conditions: highest dispensing rate (5/5) and volume of background electrolyte 200 mL.

We also studied the effect of the dispensing rate (injection speed). Although a manual micropipette could be used, in order to increase the precision, an electronic micropipette was employed due to the constant delivery rate. In our case, the electronic micropipette used has five different speeds to inject the solution. We tested these speeds injecting 10 µL of 0.5 mM epinephrine solution and evaluating the effect over the peak height and width obtained. As Fig. S3 shows, using higher dispensing rate, the peak current increased meanwhile peak width decreased. This fact is probably because of that at lower dispensing rates, a higher dispersion of the analyte solution in the electrolyte occurs and also lower mass transport factor takes place [35]. Since highest peak current (related to the limit of detection and quantitation) and lowest peak width (related to sample throughput) are aimed, the highest dispensing rate was chosen for further studies. Moreover, the precision of the width improved notoriously with the dispensing rate and is very adequate in the case of the peak current.

Once the sample had been injected, it arrived at the electrode surface and was oxidized. Then, it diffused rapidly into the background electrolyte. The effect of the volume of background electrolyte in the polypropylene container in the analytical signal was also studied (Fig. S4). As can be observed, the

volume of background electrolyte in the BIA cell did not affect in a significant way the peak current. Plus, the peak width decreases when higher background electrolyte volume was used. The main decrease is shown until 200 mL, with no important decrease observed for higher values. Thus, 200 mL was chosen as background electrolyte volume in the BIA cell. This study demonstrates that, even if slight evaporation of background electrolyte solution is produced because the container is open, it does not affect to the peak current. Therefore, the use of a closed container is not necessary in this case.

3.2. Precision of the BIA methodology

With the aim of knowing the precision and stability of the BIA system, several studies were performed. First, signals for several 10-µL injections of two epinephrine solutions with different concentrations were recorded (Fig. 4A). We have chosen solutions with concentrations quite different, 0.25 and 0.50 mM, and they were injected alternatively 10 times in order to determine if loss of analytical signal occurs. An intensity of current in the maximum of 5.7 ± 0.4 µA with a RSD of 7.9 % was obtained for a 0.25 mM epinephrine concentration, while for 0.50 mM, a peak current of 12.0 ± 0.6 µA with a RSD of 5.1 % was achieved. These results demonstrate this pin-based BIA system is robust and pin electrodes do not suffer saturation or passivation.

In order to evaluate the reproducibility of the system with time, injections of 10 µL of a 0.5 mM epinephrine solution were performed in 7 different days, changing the background solution every day (Fig. 4B). The intensity of current was 11.4 ± 0.8 µA with a RSD of 7.2 % (n = 49, 7 injections each day for 7 days; data in Table S1). Therefore, the system (same pins) was reproducible and, although the pins are low cost and disposable, they can be used at least for one week.

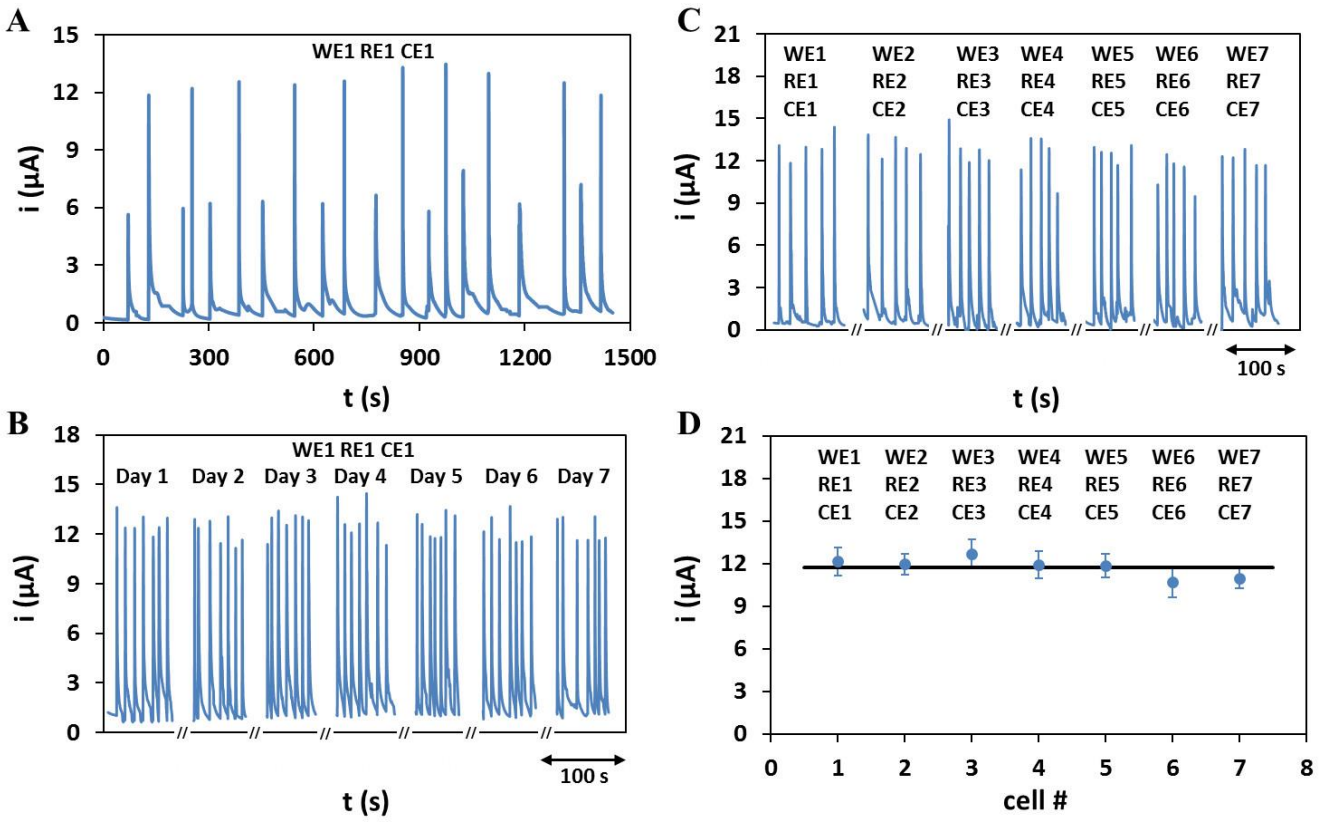

Fig. 4. Biagrams (i *vs*. t curves) recorded at a potential of + 0.5 V *vs*. a stainless-steel pseudo-reference electrode, performed by (A) injecting a 0.25 mM and a 0.50 mM epinephrine solution using the same WE, RE and CE, (B) injecting 10 µL of a 0.5 mM epinephrine solution using the same WE, RE and CE in 7 different days (7 injections each day), and (C) injecting 10 µL of a 0.5 mM epinephrine solution using 7 different WEs, REs and CEs (5 injections for each group of three electrodes). (D) Mean of the peak current of the biagrams (i *vs*. t curves) of Figure 4C (n = 5). Experimental conditions: highest dispensing rate (5/5) and a volume of background electrolyte of 200 mL.

Finally, we performed the evaluation of the reproducibility of the system when different pin-based electrodes were used. Using different WEs, REs and CEs, injections of a 0.5 mM epinephrine solution produced an intensity of current in the maximum of 12 ± 1 µA with a RSD of 8.9 % (n = 35, 5 injections for each one of the 7 different trios formed by one WE, one RE and one CE; Fig. 4C and 4D).

These RSD values obtained, always lower than 9 %, showed the robustness of this BIA system and the high usefulness of pins as electrodes for this kind of electroanalytical systems. Moreover, the same WE, RE and CE have been used to perform several measurements (optimizations and calibrations) without

loss of signal (after more than 300 injections as can be seen in Fig. S5). The width of BIA peaks at baseline was 8 ± 1 s (n = 3) so the sample throughput of the system is 450 h^{-1}.

3.3. Epinephrine determination: calibration and real sample analysis

The feasibility of this pin-based BIA system to determine the concentration of epinephrine was evaluated. In this way, the analytical signal was the intensity current due to the oxidation of the epinephrine at +0.5 V vs. stainless-steel pseudo-reference electrode. Fig. 5 shows the calibration curve obtained for measurements of epinephrine concentrations between 0.005 and 1 mM. The sensitivity obtained was 18.8 $\mu A \cdot mM^{-1}$ and the R^2 of the calibration curve was 0.995, showing an acceptable linearity in a very wide linear range. The limit of detection (LOD) was calculated according to the $3S_b/m$ criterium (m is the slope of the calibration plot in the significant range and S_b is the standard deviation of the intercept). Thus, the LOD was 1 µM. The analytical features of this pin-based BIA system are comparable with others before reported that required laborious modified electrodes (Table S2). For example, a wider concentration range is achieved in this work when compared with the use of a gold nanotube array electrode [29] or a screen-printed electrode modified with carbon nanofibers [36]. Besides the obvious low cost of the pins, this system passes over the need of modifying the electrodes with nanomaterials, SAMs, … reducing the complexity and cost of the assay. However, if lower LODs or higher selectivity were needed, the pin-based electrodes could be modified with the aim of improving the analytical characteristics.

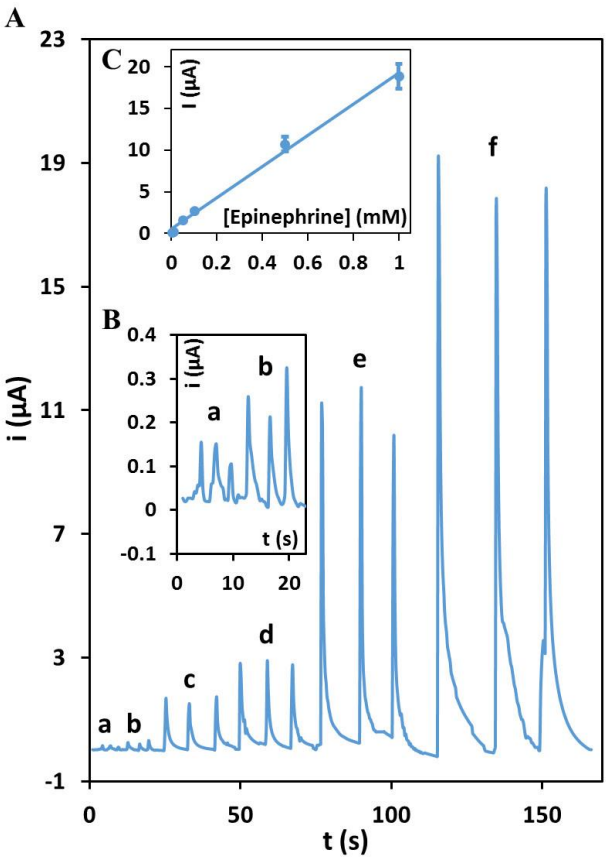

Fig. 5. (A) Biagrams (i *vs*. t curves), recorded for different concentrations of epinephrine (0.005 (a), 0.01 (b), 0.05 (c), 0.1 (d), 0.5 (e) and 1.0 mM (f)) applying a potential of +0.5 V *vs*. a stainless-steel pseudo-reference electrode. Inset: (B) Biagrams (i *vs*. t) amplified for 0.005 (a) and 0.01 mM (b) epinephrine solutions; (C) calibration plot in the epinephrine concentration range comprised between 0.005 and 1.0 mM (each point is the mean of 3 measurements corresponding to sequential injections). Experimental conditions: highest dispensing rate (5/5) and a volume of background electrolyte of 200 mL.

An injectable solution of epinephrine in a pre-filled 1 mL syringe was analyzed using the BIA system developed. The nominal value of this injectable solution was 1 mg·mL^{-1}. The sample was diluted 40 times in 10 mM PBS pH 7.0, and the epinephrine determination was performed by triplicate. Thus, a value of 1.1 ± 0.1 mg·mL^{-1} was obtained with the pin-based BIA system. This result, when compared with the labelled concentration of the sample, demonstrates a good accuracy and precision achieved by this BIA system using pins as electrodes.

3.4. Multiplexed BIA system

Nowadays, developing systems able to perform several measurements simultaneously still represents a major challenge. With this, analysis time, an increasingly valuable parameter, could considerably be reduced and measurements, which are made under identical external conditions, could be made precisely. With this aim, here we developed a multiplexed BIA system consisting on eight pins acting as WEs as shown in Fig. 1D. In order to evaluate the performance of this system epinephrine was also used as model analyte. In this case, RE and CE were two stainless-steel pieces employed with the aim of obtaining a simpler design and of assuring an area of the CE high enough considering that the multiplexed system has eight WEs.

Fig. 6A shows eight biagrams (i *vs*. t curves) recorded for each WE of the multiplexed BIA when 10 µL of a 0.5 mM epinephrine solution was injected. In Fig. 6B the mean intensity current of 3 peaks obtained for each WE are represented. It can be observed that the intensity currents were very similar for the eight WEs, with a RSD value of 9.2 % (for the 24 measurements). This is an acceptable value, even more considering that pin coating, device fabrication and injection were hand-made procedures. Therefore, pins allow constructing multiplexed BIA systems paving the way for using this system not only for simultaneous measurements, but also for multianalyte determination.

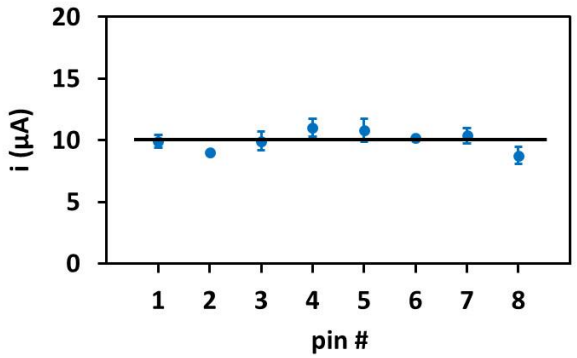

Fig. 6. Current intensity (at a potential of +0.5 V *vs*. stainless-steel pseudo-reference electrode) for injections of 10 µL of a 0.5 mM epinephrine solution in the multiplexed batch injection system consisting of eight pins coated with carbon ink acting as working electrodes (error bars correspond to the standard deviation of 3 measurements). Experimental conditions: highest dispensing rate (5/5) and a volume of background electrolyte of 200 mL.

4. Conclusions

Here we have developed the first BIA system with electrochemical detection using mass-produced stainless-steel pins as electrodes. The pin that acts as a working electrode is modified with carbon ink meanwhile the other two (reference and auxiliary) are washed but maintained as received. The methodology is very simple and user-friendly, since it is based on the injection of a sample solution to the head of the working electrode. No dilution occurs and a transitory signal is recorded. The precision between different cells and several days was adequate with values for RSD always lower than 9 %. The versatility of these low-cost electrodes allows designing multiplexed devices according to the needs of specific applications. Eight working electrodes have been aligned sharing reference and auxiliary electrodes. Precision was maintained, and in addition to that, if an electronic micropipette is employed, simultaneous measurements are involved decreasing analysis time notoriously. The BIA system based on pins here developed performed also an accurate analytical methodology, as demonstrated through the determination of epinephrine in pharmaceuticals. Moreover, the selectivity and sensitivity of the system could be improved modifying the pin-based electrodes either with recognition elements or with nanomaterials. Therefore, pins have shown that can be the basis for constructing promising simple, low-cost, portable and versatile electroanalytical injection systems.

Acknowledgements

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SUPPLEMENTARY DATA

Batch injection electroanalysis with stainless-steel pins as electrodes in single and multiplexed configurations

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PIN-BASED ELECTRODE PREPARATION

First of all, the stainless-steel pins that are going to be used were cleaned by sonication in isopropyl alcohol for 20 min. Two of these pins were used as reference (RE) and counter (CE) electrodes without further treatment. As working electrode (WE), one of these pins was used after coating with freshly prepared carbon ink. The carbon ink and the procedure to prepare it were optimized by us in a previous work [1]. This carbon ink consisted of a mixture 1 : 1 of graphite ink and DMF, prepared in an ultrasonic bath for 1 hour (37 kHz of frequency and 320 W of power) obtaining a homogeneous ink. The head of the pins was coated immersing it in the ink and leaving to dry for 15 min at 70 ºC. This process was repeated 3 times, but the drying time after the last immersion was 12 hours (instead of 15 min) in order to assure the complete evaporation of the solvent. After that, pins coated with carbon ink were ready to use as WEs.

CYCLIC VOLTAMMOGRAM OF EPINEPHRINE

In order to perform a cyclic voltammogram of epinephrine, the cell designed by us in a previous work was employed [1] (Figure S1 A). It was fabricated using a pin coated with carbon ink as working electrode and two bare pins as counter and reference electrodes. They were drilled on a transparency sheet where a 70-µL drop of solution was deposited covering the three heads of pins.

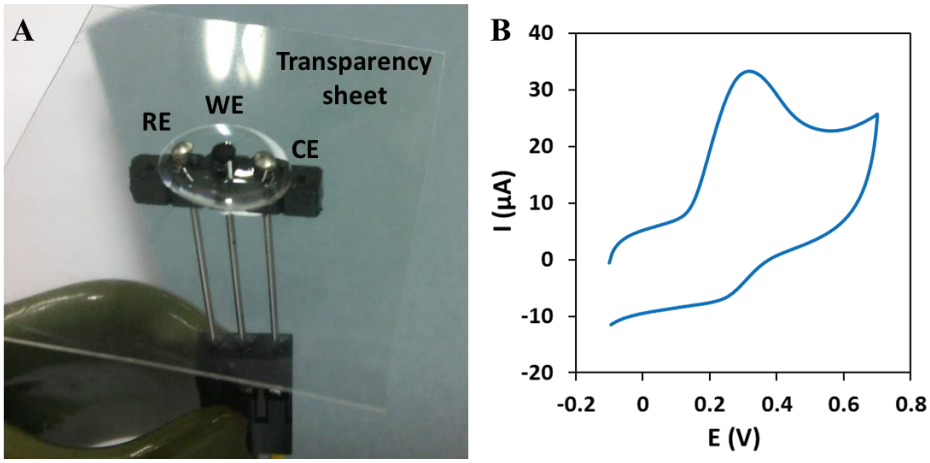

Figure S1. (A) Electrochemical cell employed for performing cyclic voltammetric studies. (B) Cyclic voltammogram recorded in a 0.5 mM epinephrine solution in 0.01 M PBS pH 7.0 at a scan rate of 50 $mV \cdot s^{-1}$ using the pin-based electrochemical cell.

EFFECT OF THE INJECTION VOLUME IN THE PRECISION OF THE ANALYTICAL SIGNAL

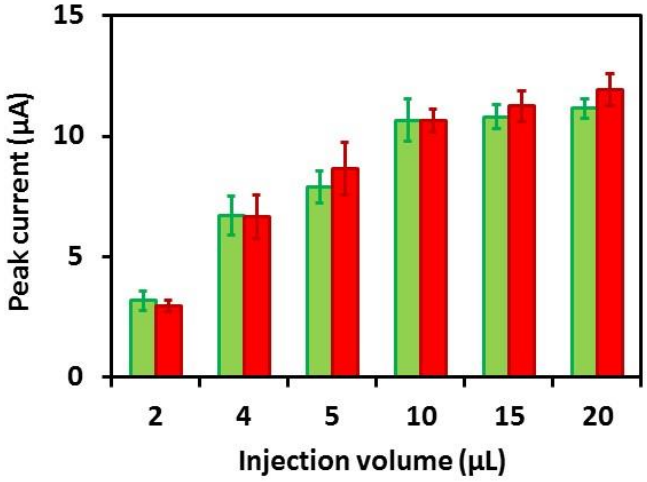

Figure S2. Peak current of the biagrams (i *vs*. t) recorded for different sample volumes injected, performed without (green) and with (red) stirring at a potential of + 0.5 V *vs*. stainless-steel pseudo-reference electrode, injecting 0.5 mM epinephrine solution (error bars correspond to the standard deviation of 3 measurements). Experimental conditions: highest dispensing rate (5/5) and volume of background electrolyte 200 mL.

EFFECT OF THE DISPENSING RATE ON THE ANALYTICAL SIGNAL

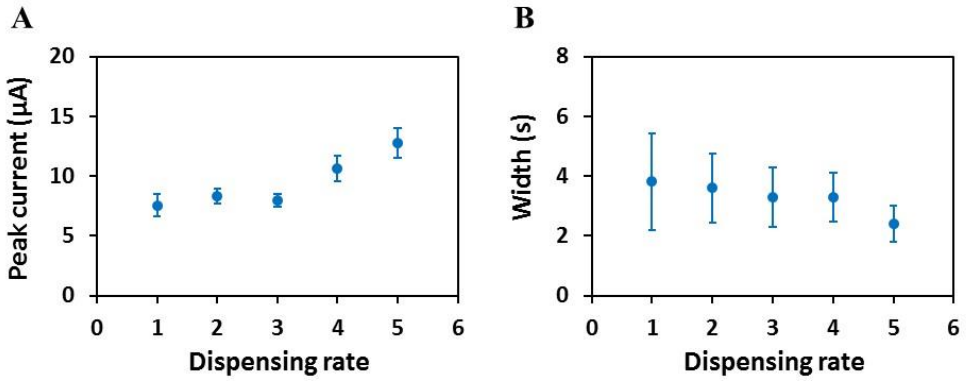

Figure S3. Variation of the intensity of the peak current (A) and the width of the peaks at the baseline (B) obtained in the biagrams (i *vs*. t curves) recorded applying a potential of +0.5 V *vs*. stainless-steel pseudo-reference electrode and injecting 10 µL of a 0.5 mM epinephrine solution at different speeds (error bars correspond to the standard deviation of 5 measurements). Experimental conditions: volume of background electrolyte 200 mL.

EFFECT OF THE VOLUME OF BACKGROUND ELECTROLYTE ON THE ANALYTICAL SIGNAL

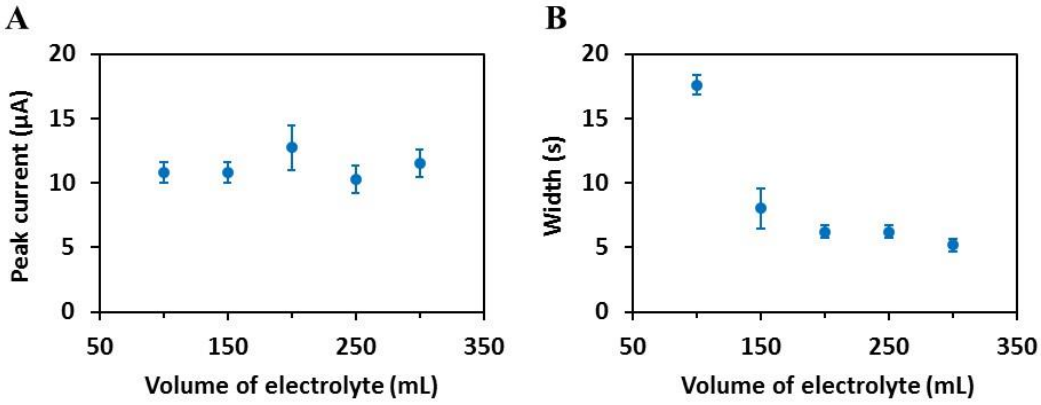

Figure S4. Variation of the intensity of the peak current (A) and the width of the peaks at the baseline (B) obtained in the biagrams (i *vs*. t curves) recorded applying a potential of +0.5 V *vs*. stainless-steel pseudo-reference electrode and injecting 10 µL of a 0.5 mM epinephrine solution using different volumes of electrolyte (error bars correspond to the standard deviation of 3 measurements). Experimental conditions: highest dispensing rate (5/5).

PRECISION OF THE SIGNALS OBTAINED WITH THE SAME WE, RE AND CE FOR 7 DAYS

Table S1. Current intensity of the peaks of Figure 4B.

	Peak current intensity (µA)						
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 6**	**Peak 7**
Day 1	12.47	11.43	11.57	12.07	10.97	11.72	11.95
Day 2	12.15	11.24	11.92	10.45	12.15	10.25	10.67
Day 3	10.17	12.00	12.36	11.28	11.91	12.11	11.48
Day 4	12.88	11.51	10.88	11.46	13.09	11.55	9.92
Day 5	12.09	11.29	10.48	10.67	10.80	12.11	12.03
Day 6	11.06	11.90	10.45	12.49	10.38	10.47	10.54
Day 7	11.77	11.79	10.09	10.31	11.84	10.29	10.43

MEASURMENTS USING THE SAME WE, RE AND CE IN DIFFERENT DAYS

The same WE, RE and CE have been used for more than 300 measurements without a marked trend demonstrating a good reproducibility. Fig. S5 shows random measurements for injections of 10 µL of 0.5 mM epinephrine solutions using the same WE, RE and CE (the last one injection is the number 304). These measurements are not continuous since several studies (optimizations and calibrations) were performed in between. As can be seen in the figure, the intensities of the peak current show no a decreasing trend and moreover, they show a deviation lower than 10 %.

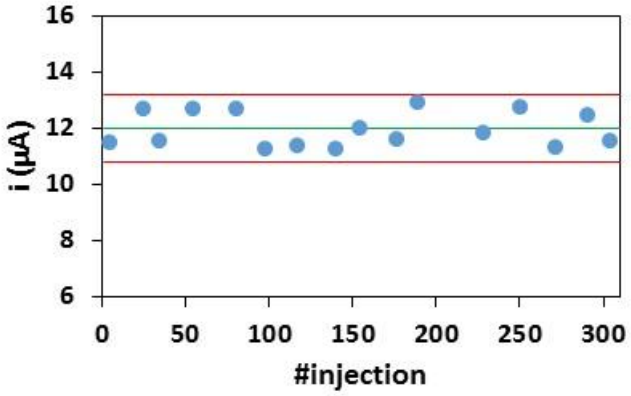

Figure S5. Intensities of peak current obtained in random biagrams (i *vs*. t curves) recorded applying a potential of +0.5 V *vs*. stainless-steel pseudo-reference electrode and injecting 10 µL of a 0.5 mM epinephrine solution using the same WE, RE and CE (the green bar corresponds to the average of the measurements and the red bars indicate the standard deviation corresponding to a of RSD of 10 %). Experimental conditions: highest dispensing rate (5/5).

COMPARISON OF CHARACTERISTICS OF ELECTROCHEMICAL SYSTEMS FOR EPINEPHRINE DETECTION

Table S2. Comparison of the main characteristics of different electrochemical systems for detecting epinephrine.

Electrode	**Technique**	**Concentration range (µM)**	**LOD (µM)**	**Sensitivity**	**Ref.**
Carbon ink modified stainless steel pin	Amperometry	5 - 1000	1	18.8 $\mu A \cdot mM^{-1}$	This work
Ordered mesoporous carbon modified GC electrode	DPV	0.5 - 200	0.2	0.6199 $\mu A \cdot \mu M^{-1}$	2
Au nanotube array electrodes	LSV / DPV	60 - 600 / 10 - 60	7.3 / 2.8	0.22 / 2.87 $mA \cdot cm^{-2} \cdot mM^{-1}$	3
L-cysteine SAMs modified Au electrode	DPV	0.1 - 2	0.01	0.7125 $\mu A \cdot \mu M^{-1}$	4
2-(2,3-dihydroxy phenyl)-1,3-dithiane SAMs modified Au electrode	DPV	0.7 - 500	0.51	0.01 $\mu A \cdot \mu M^{-1}$	5
Tyrosinase/Graphene modified SPCE	Amperometry	1 - 27.5	0.656	-	6
CNT-SPCE / CNF-SPCE / Graphene-SPCE	CV	1 - 60	1.06 / 1.98 / 3.52	-	7

GC: glassy carbon; SPCE: screen-printed carbon electrode; DPV: differential pulse voltammetry; LSV: linear sweep voltammetry; CV: cyclic voltammetry; SAMs: self-assembled monolayers; CNT: carbon nanotubes; CNF: carbon microfibers.

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