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Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 315–319
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Flow injection amperometric determination of procaine in pharmaceutical formulation using a screen-printed carbon electrode

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Received 16 December 2005; received in revised form 5 June 2006; accepted 6 June 2006
Available online 17 July 2006

Abstract

A rapid and simple method for procaine determination was developed by flow injection analysis (FIA) using a screen-printed carbon electrode (SPCE) as amperometric detector. The present method is based on the amine/hydroxylamine oxidation from procaine monitored at 0.80 V on SPCE in sodium acetate solution pH 6.0. Using the best experimental conditions assigned as: pH 6.0, flow rate of 3.8 mL min^{-1}, sample volume of 100 μL and analytical path of 30 cm it is possible to construct a linear calibration curve from 9.0×10^{-6} to 1.0×10^{-4} mol L^{-1}. The relative standard deviation for 5.0×10^{-5} mol L^{-1} procaine (15 repetitions using the same electrode) is 3.2% and detection limit calculated is 6.0×10^{-6} mol L^{-1}. Recoveries obtained for procaine gave a mean values from 94.8 to 102.3% and an analytical frequency of 36 injections per hour was achieved. The method was successfully applied for the determination of procaine in pharmaceutical formulation without any pre-treatment, which are in good accordance with the declared values of manufacturer and an official method based on spectrophotometric analysis.

Keywords: Procaine; FIA amperometric; Screen-printed carbon electrode

1. Introduction

Procaine (2-dietylaminoethyl *p*-aminobenzoate) [1], also named as novocaine or neocaine was first synthesized in 1905, and was the first injectable man-made local anesthetic. This drug acts by blocking pain sensation from specific areas of the body [2] by reversible blocks of the impulse conduction along nerve axons and other excitable membranes that utilize sodium channels as the primary means of action potential generation. It is strongly used until nowadays due to its quick action and lower toxicity than other anesthetics.

There are many analytical methods indicated for the determination of procaine in pharmaceutical formulations and biological matrices. The present United States Pharmacopoeia [1] recommends the employ of an extraction-spectrophotometric method based on its maximum absorbance signal at 280 nm. The utilization of other spectrophotometric techniques methods are also described in the literature [3–5] and alternative methods is reported such as fluorimetry [6], chemiluminescence [7] and analytical methods involving separation techniques such as high-performance liquid chromatography [8,9] and gas chromatography [10]. Several electrochemical procedures for determination of procaine have been investigated. Taking into consideration, that electroanalytical methods can offer many advantages in relation to other methods such as high sensitivity, fast response and extreme simplicity. The possibility to use ion selective electrodes (ISEs) for the procaine determination was studied by Kataky and Palmer [11], which reported detection limits close to 10^{-5} mol L^{-1}. Zhou et al. [12] described the use of metal-oxide dispersed glassy carbon electrode as chromatographic amperometric detector for the procaine determination. A wide linear range between 7.3×10^{-9} and 8.1×10^{-7} mol L^{-1} of procaine was reported. Voltammetric techniques using chemically modified electrodes for procaine determination have also reported. Wang et al. [13] have proposed a differential pulse voltammetric method by using a pumice modified carbon paste electrode, which 30 s of pre-concentration leads to a detection limit of 5.0×10^{-8} mol L^{-1}. A detection limit of 2.0×10^{-7} mol L^{-1} procaine was obtained by Wu et al. [14] employing a carbon nanotube film coated electrode, using accumulation time of 4 min, under adsorptive stripping anodic voltammetric conditions.

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doi:10.1016/j.jpba.2006.06.001

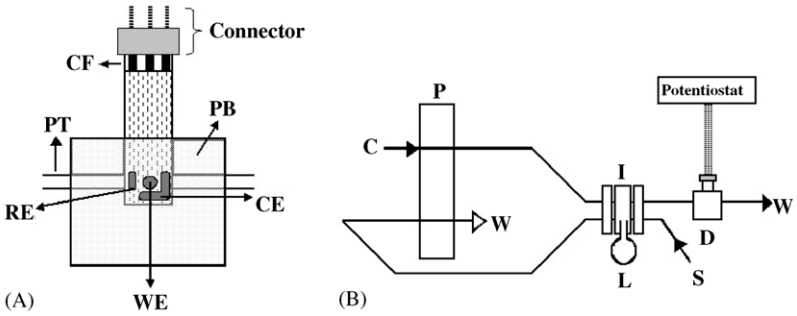

Fig. 1. Schematic diagram of the electrochemical flow cell (A) used in the amperometric measurements in flow injection system. (A) PB, polyurethane resin block; RE, reference electrode; CE, counter electrode; WE, working electrode; CF, contact field; PT, polyethylene tubing (flow). Schematic diagram of the flow system (B) used for evaluation of the SPCE for procaine determination. P, peristaltic pump; I, manual injector; S, sample or reference solutions; L, sample volume; C, carrier solution; D, electrochemical flow cell; W, waste.

In the recent years there is clearly a growing demand for rapid, reliable, inexpensive sensors for the determination of different kind of analytes in biomedical, environmental, and industrial samples [15]. Screen-printing is capable of producing a wide range of geometries (either as arrays or single electrodes) and can be used to print whole electrode systems, reference working and counter electrode, each with their own tailored characteristics; most importantly, these can be made both cheaply and with a high degree of precision. The main advantage of screen-printed carbon electrodes (SPCE) over conventional electrodes is that the problems of carry over and surface fouling is alleviated, as they can be used only once and then discarded [15].

In addition, it is important to mention that alternative automatic procedures based on flow injection technique with amperometric and voltammetric detectors have been widely valorized in pharmaceutical, food, forensic and clinical sciences [16,17]. The main factors that it contributes are low consumption of reagents and samples, better repeatability, high sample throughput, easier medium exchange after analyte accumulation, reduction of the risk of contamination during the analysis step, combined with good precision and high sensitivity, good selectivity, as well as relative low cost of the instrumentation.

The present work reports the electrooxidation of procaine on a screen-printed carbon electrode, and its application as an amperometric detector for procaine determination using a flow injection analysis system. The method was optimized evaluating the influence of several parameters (applied potential, pH, flow rate, sample volume and analytical path) on the amperometric response for procaine determination in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

Cyclic voltammetric and amperometric measurements were carried out with a μAUTOLAB (Ecochemie) controlled by a personal computer using the GPES 4.9 software. The screen-printed carbon electrodes were purchased from Oxley Developments & Company, UK, where all the electrodes are made by carbon conducting ink. The measurements were performed in a flow cell specially developed to adapt the screen-printed carbon electrode, which design is shown in Fig. 1A. The body of the electrochemical flow cell was fabricated with polyurethane resin from vegetable oil (20 mm × 40 mm × 50 mm) kindly supplied by Dr. E.T. Cavalheiro (IQSC, USP, Brazil). The effective volume of the flow cell is 95 μL. The design of the screen-printed carbon electrode used in all the electrochemical experiments is also shown in Fig. 1A. The system is based on an alumina ceramic base with 50 mm long, 10 mm wide and 0.85 mm of thick, where on to this surface is lined up the working (W), the reference (R) and the auxiliary (CE) electrodes, printed with carbon ink. The contacting field on end of the sensor was connected with the active part by ink carbon channel (C), covered by a dielectric protection layer. The sensor was connected with a cable to the potentiostat. For cyclic voltammetry the potential was ranged from 0.5 to 1.4 V versus Ag/AgCl and from 0.2 to 1.2 V versus carbon printed at a scan rate of 50 mV s^{-1}, stationary solutions were used in such cases. The amperometric measurements were performed by chronoamperometry in a flow injection analysis system.

The electrochemical flow cell was inserted in a one-channel flow injection analysis system schematically represented in Fig. 1B. The system was assembled with a peristaltic pump (Ismatec, model 78001-00, Switzerland) and a manual injector made of acrylic with two fixed sidebars and a sliding central bar. The manifold connections were made with polyethylene tubing (0.8 mm i.d.). Sodium acetate solution 0.10 mol L^{-1} (pH 6) was used as the carrier solution. The analytical path was 30 cm and the entire flow injection system was kept at room temperature.

2.2. Reagents and solutions

All solutions were prepared using water from a Millipore Milli-Q system. All chemicals were of analytical reagent grade and were used without further purification. The supporting electrolyte used for all experiments was a 0.10 mol L^{-1} sodium acetate solution adjusted in the desired pH value. A 1.0×10^{-3} mol L^{-1} procaine stock solution was prepared daily by dissolving procaine (Sigma–Aldrich) in 10 mL of the same sodium acetate solution and used to prepare procaine reference solutions. The proposed FIA procedure was carried out to deter-

mine procaine in the pharmaceutical formulation Timpanol® (Hexal) by diluting the sample with appropriate volumes in 0.1 mol L^{-1} sodium acetate solution, in order to obtain a final solution of 3.0×10^{-5} mol L^{-1} procaine. No additional sample pretreatment was required. The content of procaine in the pharmaceutical formulation was determined by standard addition method and the results were compared with official method reported in the literature [1].

3. Results and discussion

3.1. Electrochemical behavior

The oxidation of procaine was studied by cyclic voltammetry in order to elucidate its electrochemical behavior. Cyclic voltammograms indicate that the electrochemical oxidation of procaine is represented by an anodic peak at +1.05 V versus Ag/AgCl in 0.10 mol L^{-1} sodium acetate solution (pH 6.0) as shown in Fig. 2 (curve A), this peak can be attributed to oxidation of amino group to hydroxylamine, in a process involving two protons and two electrons, as demonstrated previously [13]. There is no peak on the reverse scan indicating that the process present characteristic of an irreversible global process. The magnitude of the anodic peak currents decreased after successive cycling, suggesting that the product of electrode reaction is adsorbed on the electrode surface, which can be removed by washing with electrolyte solution that promotes the complete reestablishment of the voltammetric currents.

When the carbon printed reference electrode is used instead the Ag/AgCl reference electrode (Fig. 2B) the cyclic voltammogram presents essentially the same form but there is a shift potential of around 300 mV to less positive potential attributed to the use of pseudo reference electrode, which peak potential was constant in all the voltammograms and screen-printed carbon electrodes tested.

The effect of potential scan rate on the voltammetric response for oxidation of 1.0×10^{-4} mol L^{-1} procaine on the carbon screen-printed electrode was investigated from 5 to 100 mV s^{-1}. The anodic peak current varied linearly with the square root of the scan rate according to the equation: $I/nA = 5.2 + 120.3\nu^{1/2}$ (mV s^{-1})$^{1/2}$, with a linear correlation coefficient of 0.9993 ($n = 5$), suggesting that procaine oxidation follows a diffusion-controlled mechanism.

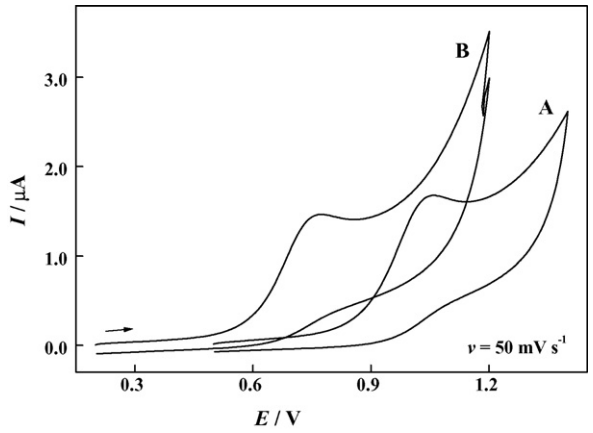

Fig. 2. Cyclic voltammograms obtained for the oxidation of a solution of 1.0×10^{-3} mol L^{-1} procaine in 0.1 mol L^{-1} sodium acetate solutions at pH 6.0 on a SPCE using a conventional reference electrode (curve A) and a carbon printed electrode (curve B). Scan rate = 50 mV s^{-1}.

The effect of pH on the oxidation of procaine (1.0×10^{-4} mol L^{-1}) at screen-printed electrodes was investigated over a pH range between 4.0 and 7.0 in static conditions. It was observed that procaine presented a well-defined peak at less acidic conditions than pH 5.0. In addition, pH values lower than 4.0 were avoided since the oxidation of procaine occurs very closely to the electrolyte/electrode discharge. The anodic peak potential obtained for procaine in sodium acetate solutions at pH values ranging from 4.5 to 7.0 has presented a shift of 60 mV by pH unit to less positive values, indicating that the electrode process is influenced by protonation reactions, as observed on conventional electrodes [18]. The optimum pH for procaine detection was 6.0.

3.2. Flow injection analysis

The effect of several parameters on the amperometric response of procaine in the flow injection conditions using a screen-printed carbon electrode was investigated in order to optimize the FIA parameters. These include the effects of applied potential, flow rate, sample volume and analytical path.

The effect of the applied potential was studied from 0.65 to 0.90 V versus carbon printed electrode in 0.10 mol L^{-1} sodium acetate solution at pH 6.0 using sample volume of 75 μL and procaine concentration of 5.0×10^{-5} mol L^{-1}. The amperometric response increased with increasing working potential from 0.65 to 0.80 V (versus carbon printed), above this value the obtained signal became almost constant. Therefore a working potential of 0.80 V was chosen for FIA measurements, this value is in agreement with the potential where maximum current is observed for procaine oxidation, observed in voltammetric curves recorded in static conditions.

The effect of the flow rate on the magnitude of amperometric response was also investigated, testing values from 1.1 to 6.5 mL min^{-1}, under applied potential of 0.80 V and sample volume of 75 μL of procaine 5.0×10^{-5} mol L^{-1} solution. The transient signals obtained in different flow rates are shown in Fig. 3. The results showed that the flow injection current response is markedly dependent of the flow rate form 1.1 to 3.8 mL min^{-1}. Maximum value was obtained above flow rate of 3.8 mL min^{-1}. The increase in the flow rates usually leads to a change in the diffusion profile at the electrode surface and therefore promotes increases the efficiency of the mass transport [19]. The residence time (R_t) is defined as the change in current between initial and maximum signal. The washing time (W_t) was evaluated by the time necessary to the maximum signal return to baseline. The evaluation of these parameters studied, indicates that the R_t and W_t decrease from 90 to 15 s and from 200 to 50 s, respectively. Therefore, the flow rate of 3.8 mL min^{-1} ($R_t = 40$ s and $W_t = 70$ s) was selected for further experiments where better engagement between reagent consumption and analytical frequency was obtained.

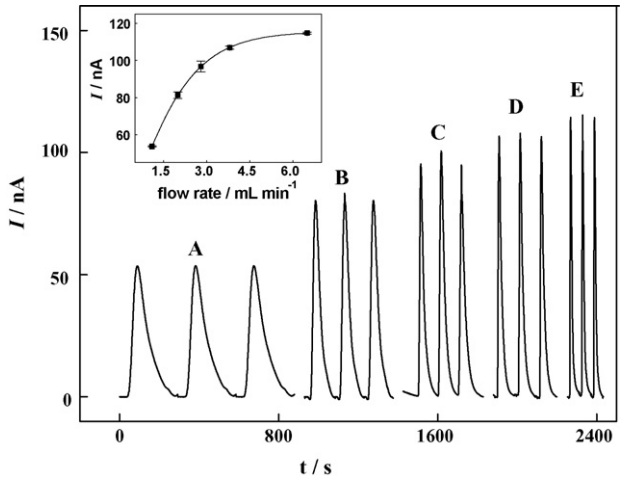

Fig. 3. Transient current signal obtained in triplicate for the SPCE for a sample volume of 75 μL of 5.0×10^{-5} mol L^{-1} procaine under different flow rates: (A) 1.1 mL min^{-1}; (B) 2.0 mL min^{-1}; (C) 2.8 mL min^{-1}; (D) 3.8 mL min^{-1}; (E) 6.5 mL min^{-1}. The inset shows the influence of flow rate on the amperometric response.

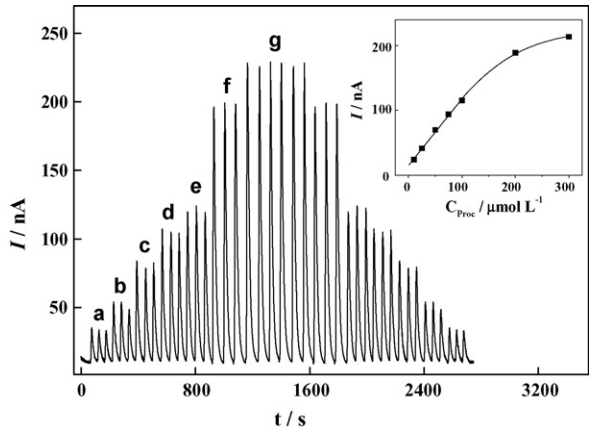

Fig. 4. Transient current signals obtained in triplicate for procaine solutions: (a) 9 μmol L^{-1}; (b) 25 μmol L^{-1}; (c) 50 μmol L^{-1}; (d) 75 μmol L^{-1}; (e) 100 μmol L^{-1}; (f) 200 μmol L^{-1}; (g) 300 μmol L^{-1} procaine. The inset shows the analytical curve for procaine. Applied working potential +0.80 V vs. carbon printed; sample volume 100 μL of procaine; flow rate 3.8 mL min^{-1}.

The effect of the sample volume from 25 to 200 μL, on the analytical signal was also investigated by changing the length of the sample loop (5–40 cm) for a 5.0×10^{-5} mol L^{-1} procaine in 0.10 mol L^{-1} sodium acetate pH 6.0. The amperometric response increased with the increase of sample volume from 25 to 100 μL reaching a plateau for sample volumes higher than 100 μL. Therefore, a sample volume of 100 μL was used in the further experiments.

The analytical path represented by the distance from the manual injector to electrochemical flow cell was also studied in the range 25–55 cm under the same experimental conditions as selected before. The results obtained showed that the amperometric response of the screen-printed carbon electrode is practically constant in the entire investigated interval, and an analytical path of 30 cm was chosen for optimum amperometric response.

In order to obtain the analytical curve for procaine, amperometric measurements were carried out in sodium acetate solution (pH 6.0) containing different procaine concentrations using the optimized operating conditions for the FIA procedure.

Fig. 4 illustrates the transient current signals for different procaine concentrations. The current values (at 0.80 V) obtained gave a linear relationship with procaine concentrations from 9.0×10^{-6} to 1.0×10^{-4} mol L^{-1} (see inside Fig. 4). This plot could be represented by the equation: $I/nA = 9.0 + 1.3 \times 10^{6} C_{\text{procaine}}$ (mol L^{-1}), with a linear correlation coefficient of 0.9997 ($n = 6$). From 1.0×10^{-4} mol L^{-1} a deviation from linearity occurred due to saturation of the electrode surface. The detection limit [20] was 6.0×10^{-6} mol L^{-1} (three times the standard deviation of the intercept/slope). The relative standard deviation (R.S.D.) for 15 replicates of 5.0×10^{-5} mol L^{-1} procaine was 3.2% and the analytical frequency was 36 injections per hour. These experimental results show that the current response are very reproducible and unless the screen-printed carbon electrode is disposable, in this work possesses long-term stability. It was observed that a same screen-printed carbon electrode can be used at least for 100 measurements.

3.3. Analytical applications

In order to investigate the analytical application of this method, the matrix effect was evaluated by addition-recovery experiments carried out in a commercial pharmaceutical formulation containing procaine. The results obtained by standard addition method are shown in Table 1. Recoveries ranging from 94.8 to 102.3% of procaine were obtained using the proposed FIA amperometric procedure. This is a good evidence of the accuracy of the proposed method.

The proposed FIA amperometric procedure was applied for the determination of procaine in the pharmaceutical formulation Timpanol®. The procaine content was determined by the standard addition method and compared with an official method proposed in the literature [1]. The content of procaine calculated by both methods is reported in Table 2. The results are in agreement with the results obtained by extraction-spectrophotometric method, as well as the label values described by the industrial laboratory.

Table 1
Results of addition-recovery experiments using FIA amperometric procedure for three different standard concentrations of procaine in pharmaceutical formulations

Solution reference (mol L^{-1})/sample (mol L^{-1})	Recovery[a] (%)
5.0×10^{-5}/0.0	100.0 ± 2.5
4.0×10^{-5}/1.0×10^{-5}	97.8 ± 3.0
3.0×10^{-5}/2.0×10^{-5}	100.1 ± 2.2
2.0×10^{-5}/3.0×10^{-5}	99.4 ± 0.9
1.0×10^{-5}/4.0×10^{-5}	98.7 ± 1.7
0.0/5.0×10^{-5}	100.2 ± 1.3

[a] $n = 3$.

Table 2
Mean values obtained for the determination of procaine in pharmaceutical formulation by FI amperometric procedure in comparison with the official method

Sample	Timpanol®
Label value	50 mg mL^{-1}
Official method[a]	52 ± 1 mg mL^{-1}
FIA method[a]	53 ± 2 mg mL^{-1}

[a] $n = 3$.

The statistical calculations for the assay results showed good precision of the FIA amperometric method. The results obtained were also compared by applying the *F*-test and *t*-test at 95% confidence level [21]. In either case the calculated *F* or *t* values not exceed the theoretical values ($F_{3,3} = 9.28$, $t_6 = 2.45$), confirming that there are no significant differences between the results obtained by both procedures.

4. Conclusions

This work demonstrates that the screen-printed carbon electrode is a promising analytical tool, which can be used as simple sensors able to detect procaine in simple and rapid way than other electroanalytical methods proposed in the literature. The screen-printed carbon electrode offers the advantage to be easily coupled with FIA systems. The developed system offers a reliable method for procaine determination in pharmaceutical formulations without prior chemical or electrochemical surface treatment. There is also no need of sample pre-treatment. In the flow analysis condition the adsorption effect was minimized, demonstrating by the good reproducibility between the measurements, suggesting that FIA amperometric procedure offers a good possibility for extending the technique in routine analysis of procaine in the pharmaceutical formulations. In addition, some advantages of the screen-printed electrode can be also taken into consideration such as: low cost, easy construction and storage, potential for miniaturization, facility of automation and construction of simple equipment.

Acknowledgements

The scholarships granted by FAPESP (contract no. 03/05567-7 (ALS) and no. 04/00111-8 (MFB)), CAPES and CNPq are acknowledged.

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