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Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 400–404
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
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Short communication

Fast BIA-amperometric determination of isoniazid in tablets

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Received 7 March 2006; received in revised form 12 April 2006; accepted 14 April 2006
Available online 24 May 2006

Abstract

This paper proposes a new, fast and precise method to analyze isoniazid based on the electrochemical oxidation of the analyte at a glassy carbon electrode in 0.1 M NaOH. The quantification was performed utilizing amperometry associated with batch injection analysis (BIA) technique. Fast sequential analysis (60 determinations h^{-1}) in an unusually wide linear dynamic range (from 2.5×10^{-8} to 1.0×10^{-3} M), with high sensitivity and low limits of detection (4.1×10^{-9} M) and quantification (1.4×10^{-8} M), was achieved. Such characteristics allied to a good repeatability of the current responses (relative standard deviation of 0.79% for 30 measurements), were explored for the specific determination of isoniazid in isoniazid–rifampin tablet.

Keywords: Amperometry; Batch injection analysis; Glassy carbon electrode; Isoniazid; Pharmaceutical products

1. Introduction

Isoniazid (pyridine-4-carboxilic acid hydrazide or isonicotinic acid hydrazide) is a widely used drug alone in the prophylaxis and in combination with other antituberculars in the treatment of all forms of tuberculosis [1].

Several methods for the analysis of isoniazid are available in the literature such as titrimetry [2,3], spectrophotometry [4–6], chemiluminescence [7–9], fluorimetry [10], high performance liquid chromatography (HPLC) [11–16], capillary electrophoresis [17–19], and electroanalytical [20–22].

Electroanalytical techniques can be advantageous because they provide good sensitivity, precision and accuracy, simplicity and rapidity. In spite of this, there are only a few papers that report the analysis of isoniazid employing electroanalytical methods. Differential pulse and square-wave adsorptive (cathodic or anodic) stripping voltammetry have been the most employed techniques with results showing low limits of detection. On the other hand, the use of mercury electrodes is not recommendable due to the toxicity of this metal. In addition, the voltammetric techniques require previous accumulation steps, precluding a high sampling frequency. Therefore, the utilization of a system with high speed and good precision for fast electrochemical analysis of isoniazid is desirable.

An interesting alternative can be the batch injection analysis (BIA) technique coupled with amperometry. It consists in the injection of a sample plug (from a micropipette tip) directly onto an electrode surface that is immersed in a relatively large volume of electrolyte. Transient response peaks are observed by reflecting the passage of the sample zone over the detector. Such dynamic batch operation yields many attractive features similar to that observed in flow injection analysis (FIA) systems such as speed, sample size, sensitivity, and reproducibility. In addition, as an advantage over FIA, it eliminates some problems common to flow systems like the use of pump or valves, expense of manifold components and disposal of reagent/carrier solutions [23]. BIA technique has been successfully implemented in our laboratory by combining it with electrochemical single [24,25] or modified sensors [26–29] for the quantification of several organic compounds.

In this work, a simple and rapid method for the isoniazid quantification is described and applied to its determination in isoniazid–rifampin tablet. It was based on the electrochemical oxidation of isoniazid just after the injection of the analyte directly onto the glassy carbon electrode surface, which was held at 150 mV (versus $Ag/AgCl_{KCl\,sat.}$) in alkaline medium (0.1 M NaOH) during the operation.

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doi:10.1016/j.jpba.2006.04.006

2. Experimental

2.1. Instruments and apparatus

All electrochemical measurements were performed utilizing an EG&G potentiostat/galvanostat (PAR, model 263A) interfaced to a Pentium microcomputer. For voltammetric experiments, a conventional three-electrodes cell consisting of a glassy carbon-working electrode (Model TL-5, Bioanalytical System, 0.071 cm^2 apparent area), a platinum wire auxiliary electrode and a miniaturized $Ag/AgCl_{KCl\,sat.}$ reference electrode were employed. The same electrodes were also used in the batch injection analysis (BIA) system for amperometric measurements, as previously described [28]. The electrolyte volume inside the BIA cell was only 40 ml. The injection of the sample or standard solutions was carried out utilizing a motorized electronic micropipette EDP Plus EP-100, from Rainin Instruments. The frequency was 60 injections h^{-1}.

2.2. Reagents and materials

All reagents were of analytical grade (purchased from Sigma or Merck). The stock solutions were prepared with Millipore® Milli-Q ultra pure water. The solutions of isoniazid (standard and sample) were prepared every day and further diluted with supporting electrolyte (0.1 M NaOH) just before the amperometric measurements. The investigated tablet formulation (containing 200 mg isoniazid and 300 mg rifampin) was kindly donated.

2.3. Procedure

Before the electrochemical experiments, the glassy carbon electrode was mechanically polished with 0.02 μm alumina slurry on a felt cloth, rinsed with deionized water and then dried at room temperature.

The experimental conditions for the amperometric batch injection analysis were optimized varying all possible parameters that could influence the results. In this way, the most suitable conditions were found to be as follows: analysis potential = 150 mV, sampling volume = 100 μl, injection speed = 75.3 μl s^{-1}, and electrode-pipette tip distance = 2 mm. In all experiments the standard or sample solutions were injected directly onto the electrode surface, immersed in the supporting electrolyte. Before amperometric measurements, tablets were dispersed in water and the resultant solution was filtered. The filtrate was made up to 100 ml in a calibrated flask. From this solution (nominally containing 1.46×10^{-2} M isoniazid) were done the subsequent dilutions. In these last solutions, was included also the adequate amount of electrolyte (NaOH) sufficient to attain exactly the same concentration as in the BIA cell (=0.1 M).

3. Results and discussion

3.1. Cyclic voltammetry

Fig. 1 illustrates the electrochemical oxidation of isoniazid at a glassy carbon electrode immersed in 0.1 M NaOH. The cyclic voltammograms presented in this figure exhibit a single well-defined irreversible oxidation peak, which is probably due to the oxidation of the hydrazide moiety.

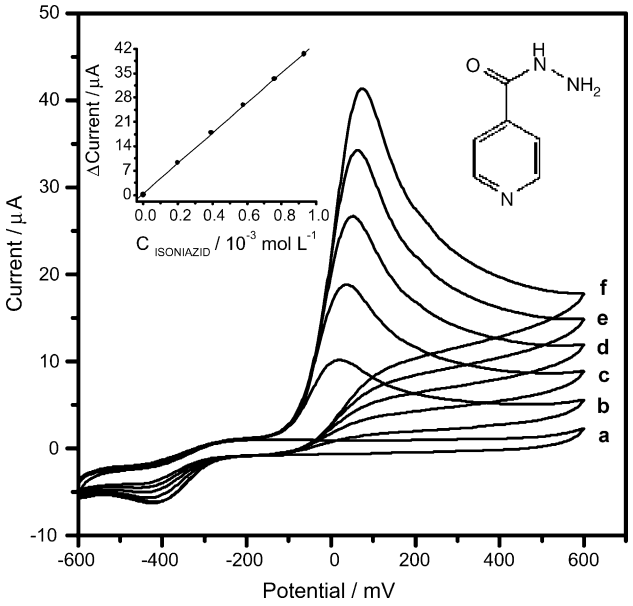

Fig. 1. Cyclic voltammograms obtained for a glassy carbon electrode in 0.1 M NaOH before (a) and after (b–f) additions of 0.20 mM (b), 0.39 mM (c), 0.58 mM (d), 0.76 mM (e), and 0.93 mM (f) isoniazid (final concentrations). Scan rate: 100 mV s^{-1}; electrode area: 0.071 cm^2. Inset: correspondent calibration curve and schematic representation of isoniazid structure.

At lower concentrations, the peak potential is close to zero and then shifts to more positive values with the concentration increasing. This suggests that the electrode surface is partially blockaded by the adsorption of the electrochemical reaction products. However, the effect of fouling seems to be more pronounced in acid media.

In the present work, it was possible to perform fast, simple and reproducible analysis of isoniazid utilizing a glassy carbon electrode (coupled with a BIA system) in 0.1 M NaOH without significant problems associated to electrode surface fouling. The loss of activity was minimized owing to use of alkaline solutions as supporting electrolyte and also to the narrow time period in which the analyte remained in contact with the electrode during the injection of small sample volumes in the BIA operation. Moreover, the method supplied wide linear working range and low limits of detection and quantification.

3.2. Batch injection analysis

Linear relationship between current magnitude and isoniazid concentration was observed, as it can be seen in the inset of Fig. 1. This demonstrates the usefulness of the glassy carbon electrode for the isoniazid quantification in 0.1 M NaOH, making possible its use for electroanalytical purposes using the batch injection analysis technique. First of all, experiments were performed looking for the best potential for the analysis of this analyte, now in lower concentrations and in flowing condition. Therefore, 100 μl samples of a 5.0×10^{-5} M isoniazid solu-

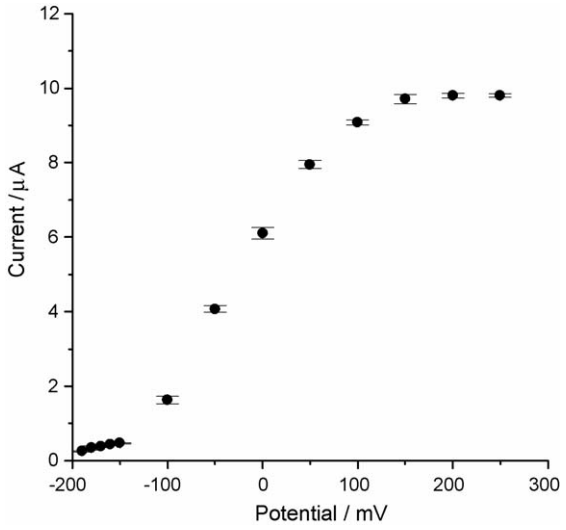

Fig. 2. Plot of the average currents (and errors bars) obtained after sequential injections (in triplicate) of 100 μl of a 5.0×10^{-5} M isoniazid solution onto a glassy carbon electrode as a function of the applied potential. Supporting electrolyte: 0.1 M NaOH; injection speed: 75.3 μl s^{-1}; electrode-pipette tip distance: 2 mm; electrode area: 0.071 cm^2.

tion were injected while holding the electrode at fixed potentials (from −200 to +250 mV). The result of this experiment is displayed in Fig. 2. The injections were done in triplicate for each potential and the average data of the current peaks and the standard deviations (represented as error bars) were calculated for each series. The current signals showed a continuous increase as a function of the applied potential until 150 mV and then leveled off. Hence, the potential of 150 mV was chosen as the best for the amperometric quantification of isoniazid.

The effect of other parameters was also investigated in order to get the optimized conditions for isoniazid determination. The best distance between the electrode and the pipette tip was found to be 2 mm, whereas 100 μl was the most suitable sample volume. The fastest dispensing rate allowed in the programmable pipette (75.3 μl s^{-1}) was found to be the most appropriate injection speed.

In order to establish the linear working range, a series of experiments was performed with standard solutions of isoniazid, which were conveniently diluted to reach different final concentrations. The results obtained showed an unusually vast linear region. In Fig. 3(A) are showed the calibration plots obtained for five decades of concentration.

Practically the same result was obtained, with minimum variations of the slopes, indicating that the sensitivity of the electrode was maintained in spite of the increasing current demand. Omitting the first experimental point (1.0×10^{-8} M), the calibration plot showed a large response region, between 2.5×10^{-8} and 1.0×10^{-3} M, with linear regression represented by the equation $I = 5.31 \times 10^{-7}$ A + 0.17 A l mol^{-1} (correlation coefficient > 0.999). For concentrations higher than 1.0×10^{-3} M, the slope decreased significantly (not shown), suggesting a saturation behavior. The electrolyte volume inside the BIA cell was only 40 ml. However, as was demonstrated before [30], the contribution of the analyte concentration inside the cell (which

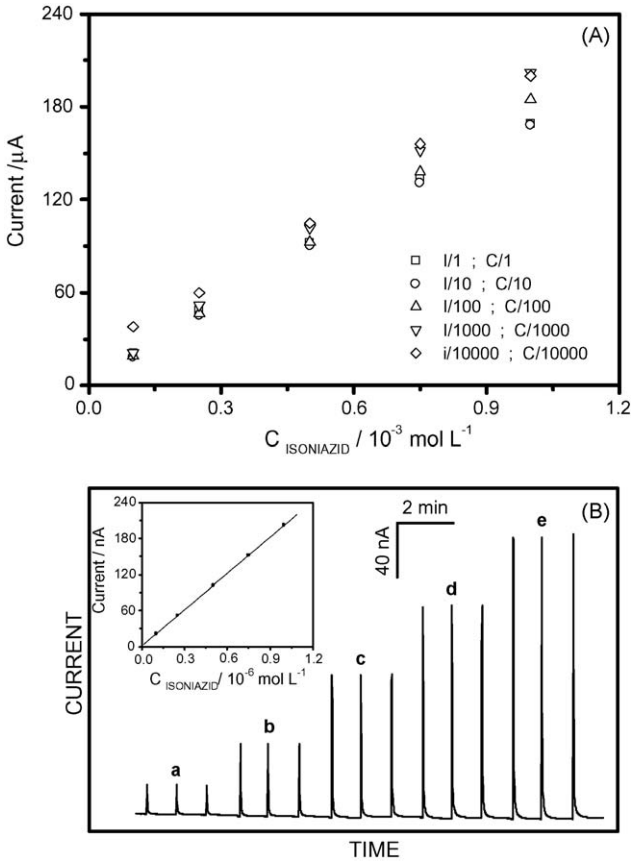

Fig. 3. (A) Calibration plots for BIA amperometric responses utilizing standard solutions of isoniazid with concentrations changing between: (◊) 1.0×10^{-8} and 10.0×10^{-8} M, (▽) 1.0×10^{-7} and 10.0×10^{-7} M, (△) 1.0×10^{-6} and 10.0×10^{-6} M, (◯) 1.0×10^{-5} and 10.0×10^{-5} M, (□) 1.0×10^{-4} and 10.0×10^{-4} M. (B) BIA amperometric responses after injections of (a) 1.0, (b) 2.5, (c) 5.0, (d) 7.5 and (e) 10.0×10^{-7} M isoniazid solutions. Inset: correspondent calibration plot. For measuring conditions see Fig. 2.

increases when successive injections are done) is not very significant. In our conditions, for 5×10^{-5} M isoniazid injections, the base line initially was 25 nA, and after additions of analyte correspondent to 100 and 200 injections, the base line grown to 70 and 112 nA. On this condition, variation of the base line corresponds to ∼0.5 and 1% of the signal measured for isoniazid during the injection series.

The system had singular behavior exhibiting BIA current values with very favorable signal-noise ratio, demonstrated by a particularly stable base line even at very low concentrations (Fig. 3(B)). For the smaller peaks of this series, corresponding to injections of 1×10^{-7} M and 100 μl of solution, there is only 1×10^{-11} mole of analyte. Even so, there is a well-defined peak, thanks to the high mass transport generated during each injection. Oppositely, when the injection ends, the current decreases exponentially. The analyte suffers a huge dilution in the electrolyte and in addition, on the electrode surface is consumed and so its concentration in this region is even lower. The proportion of analyte oxidized during each injection can be evaluated by integration of the peak currents recorded during these experiments. The software supplied with the PAR 263A provides the charge involved in each experimental peak. Considering a reac-

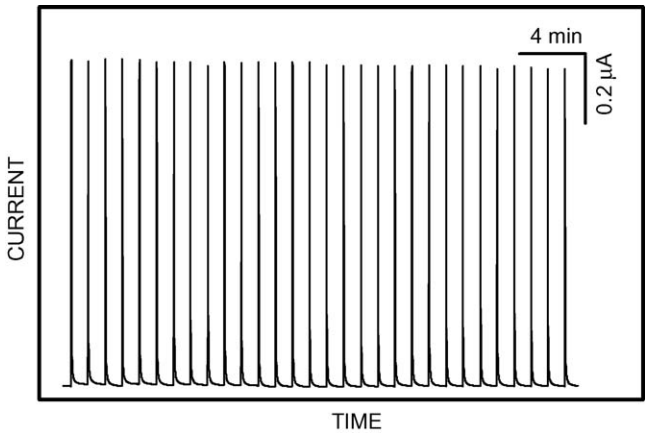

Fig. 4. BIA amperometric responses of a glassy carbon electrode after 30 consecutive injections of 100 μl of a 5.0×10^{-6} M isoniazid solution. For measuring conditions see Fig. 2.

tion involving two electrons [20–22] was verified that less than 1.2% of the analyte is oxidized during the BIA-injection process.

A detection limit of 4.1×10^{-9} M (three times the standard deviation of the blank [31]) was calculated, utilizing a calibration plot in the range of 2.5×10^{-8} to 1.0×10^{-7} M. In this same way, the limit of quantification was determined as 1.4×10^{-8} M (10 times the standard deviation of the blank [31]).

The repeatability of the measurements was also studied. The response of 30 successive injections of a 5.0×10^{-6} M isoniazid solution, with a sampling frequency of 60 determinations h^{-1}, is illustrated in Fig. 4. The relative standard deviation found for the complete series of injections was 0.79%, corroborating the ability of the electrode for multiple sequential analysis of isoniazid in alkaline media. When the isoniazid concentration was increased of 10 times (for 5.0×10^{-5} M), the relative standard deviation calculated for 30 measurements was 2.1% (not shown), revealing also a good stability in spite of the electrode to be subjected to conditions more favorable to fouling, since higher concentration of the analyte was employed.

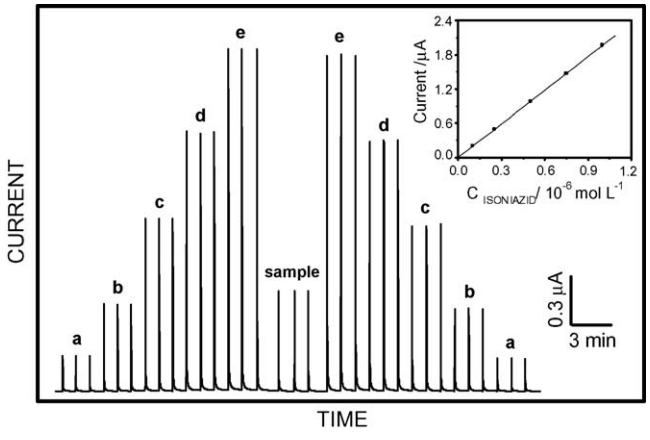

Fig. 5. Results for analysis of isoniazid in isoniazid–rifampin tablet utilizing a glassy carbon electrode. Inset: calibration plot obtained after injections of (a) 1.0, (b) 2.5, (c) 5.0, (d) 7.5 and (e) 10.0×10^{-6} M isoniazid standard solutions. For measuring conditions see Fig. 2.

3.3. Analysis of isoniazid in isoniazid–rifampin tablets

The proposed method was applied for the determination of isoniazid in a tablet combination with rifampin. A typical set of BIA signals, recorded for standard solutions of isoniazid (from 1.0×10^{-6} to 10.0×10^{-6} M) and of the sample is presented in Fig. 5. The proportionality between the current and the concentration of the standard solutions was confirmed using the calibration plot shown in the inset. Excellent accuracy and precision were obtained: 197.7 ± 0.3 mg/isoniazid/tablet (labeled value = 200 mg).

4. Conclusions

The present work demonstrated the feasibility of determining isoniazid by a simple and very rapid way. Using amperometry associated with the batch injection analysis technique the analysis could be carried out with high speed and good precision and accuracy. The utilization of alkaline medium (0.1 M NaOH) minimized the fouling effect of isoniazid on the glassy carbon electrode. Wide linear working range, low limits of detection and quantification and high sampling frequency were easily achieved. The substitution of the toxic mercury electrodes by the more environmentally friendly solid (unmodified or modified) electrodes is also a worldwide tendency.

Acknowledgements

The authors gratefully acknowledge the fellowships from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP-Process 03/07225-6) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Processes 304031/85-2 and 504654/2004-7). This work received financial support from SAMUTI, IM^{2}C and RENAMI projects.

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