

VOLTAMMETRIC DETERMINATION OF NITROIMIDAZOPYRAN DRUG CANDIDATE FOR THE TREATMENT OF TUBERCULOSIS

C. Yáñez,² S. Bollo,² L. J. Núñez-Vergara,² and
J. A. Squella^{1,*}

¹Bioelectrochemistry Laboratory

²Chemical and Pharmaceutical Sciences Faculty,
University of Chile, P.O. Box 33, Santiago, Chile

ABSTRACT

PA-824 (2-nitro-6-(4-trifluoromethoxy-benzyloxy)-6,7-dihydro-5H-imidazo [2,1-b][1,3]oxazine) a nitroimidazopyran drug candidate for the treatment of tuberculosis was electrochemically studied with the aims of to propose a new electroanalytical method and to investigate the potential of an electrochemical system to mimic phase I reduction metabolism in PA-824. The electrochemical behavior was studied using fast polarography, differential pulse polarography, differential pulse voltammetry and cyclic voltammetry. PA-824 was electrochemically reducible showing one reduction peak due to the four-electron reduction of the nitro group to form the hydroxylamine derivative. A method for the determination of PA-824 by differential pulse polarography

*Corresponding author. E-mail: asquella@ciq.uchile.cl

and differential pulse voltammetry at pH 7 were proposed. The differential pulse polarography method has adequate accuracy and precise with a mean recovery of 101.11% and a standard relative deviation of 2.27%. The detection limit was 2.23×10^{-7} M. Furthermore, for comparative purposes an alternative differential pulse voltammetry and UV spectrophotometric methods were developed. On the other hand, from the electrochemical point of view, PA-824 shows similar reduction capabilities that metronidazole supporting the hypothesis that both drugs could share a similar reductive metabolism.

Key Words: Nitroimidazopyran; Voltammetric; Drug analysis

INTRODUCTION

Mycobacterium tuberculosis, which causes tuberculosis, is the greatest single infectious cause of mortality worldwide, killing roughly two million people annually (1). Estimates indicate that one-third of the world population is infected with latent *M. Tuberculosis* (2). The synergy between tuberculosis and the AIDS epidemic (3–5), and the surge of multidrug-resistant clinical isolates of *M. Tuberculosis* have reaffirmed tuberculosis as a primary public health thread. In spite of the above, new antitubercular drugs with new mechanisms of action have not been developed in over thirty years. However, recently, Stover et al. (6) reported a series of compounds containing a nitroimidazopyran moiety that posses antitubercular activity. Lead compound PA-824 (Figure 1) (2-nitro-6-(4-trifluoromethoxy-benzyloxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3] oxazine) showed potent bactericidal activity against multidrug-resistant *M. Tuberculosis* and promising oral activity in animal infection models. The exact action mechanism of PA-824 is unknown but there are some evidences (6) indicating that the metabolic activation of PA-824 by *M. Tuberculosis* may involve a nitro-reduction step analogous to that required for metronidazole activation (7).

A survey of the literature reveals that no attempt has been made to study the electrochemical behavior of nitroimidazopyran compounds. However, several reports pertaining to the electrochemical behavior of different nitroimidazole compounds are available (8–11). Generally both the biological and the electrochemical activity of nitroimidazoles is dependent upon the nitro group reduction process. In this work, we studied by TAST, differential pulse polarography (DPP), differential pulse

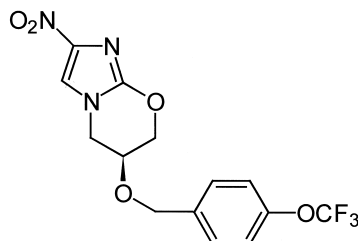


Figure 1. Chemical structure of PA-824.

voltammetry (DPV) and cyclic voltammetry (CV) the electrochemical behavior of the nitroimidazopyran PA-824, in buffered aqueous/alcohol protic media, mainly focused to develop an electroanalytical method to determine this compound.

On the other hand, drug metabolism is normally divided into phase I, II and III metabolism (12). Phase I metabolism involves primarily functionalization, including oxidation, reduction, hydrolysis and isomerization. In the present case, probably, the most important phase I metabolism pathways are enzyme-catalyzed reductions similar to the metabolism of metronidazole (7). Consequently other aim of our studies was to investigate the potential of an electrochemical system to mimic phase I reduction metabolism in PA-824.

EXPERIMENTAL

Reagents and Solutions

PA-824 was obtained from PathoGenesis Corporation, Seattle, Washington, USA. All reagents employed were of analytical grade.

Stock solutions of PA-824 were prepared at a constant concentration of 1×10^{-3} M in ethanol. An aliquot of stock solution was taken and diluted with ethanol/0.4 M Britton-Robinson buffer mixture (20/80), to obtain a final working solution concentration between 1×10^{-6} and 1×10^{-4} M.

Apparatus

Electrochemical experiments, differential pulse polarography (DPP), fast polarography, differential pulse voltammetry (DPV) and cyclic

voltammetry (CV) were performed with a totally automated BAS 100 voltammetric analyzer, coupled with a GCME stand. A dropping mercury electrode (DME) as working electrode, a platinum wire counter electrode, and an Ag/AgCl as reference electrode were used for the measurements. For DPV and cyclic voltammetry a hanging mercury drop electrode (HMDE) as a working electrode was used. All potential values in this paper have been referred to the Ag/AgCl reference electrode.

Spectrophotometric measurements were carried out with an UV-Vis spectrophotometer ATI Unicam Model UV3, using 1 cm quartz cell.

Analytical Studies

For calibration plots, a series of ten solutions containing PA-824 concentrations between 1×10^{-6} and 1×10^{-5} M in ethanol/0.4 M Britton-Robinson buffer mixture (20/80) at pH 7 were prepared.

For polarographic and voltammetric measurements, each sample was transferred to a polarographic cell, degassed with nitrogen for 10 minutes and recorded at least twice.

For UV spectrophotometric calibrations plot, ten solutions containing PA-824 concentrations between 1×10^{-5} and 1×10^{-4} M in ethanol/0.4 M Britton-Robinson buffer mixture (20/80) at pH 7 were prepared.

For recovery study, a series of twelve solutions were independently prepared by weighing approximately the same mg amount of PA-824 and then dissolving in ethanol/0.4 M Britton-Robinson buffer mixture (20/80) at pH 7. The mg amount of PA-824 in the sample solution was calculated from prepared standard calibration plot.

RESULTS AND DISCUSSION

Electrochemical Behavior

PA-824 in hydroalcoholic solution (0.4 M Britton-Robinson buffer/ethanol, 80/20), shows a cathodic response in a wide range of pH (2–12). Our first experiment was to check the stability of the PA-824 solutions by recording differential pulse polarograms over chosen time intervals between 0–180 min. The results show that there is no significant change in the intensity of cathodic peak current with time delay when the experiments are performed at room temperature and in presence of light at pHs 3, 7 and 10. The stability test results for a pH 7 solution are showed in Figure 2, indicating that this compound is highly stable below these conditions.

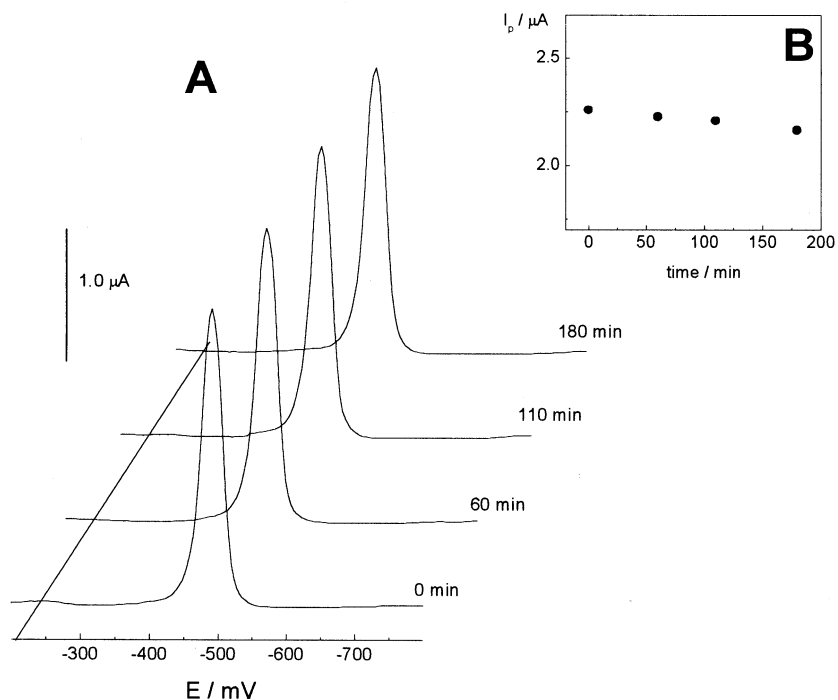


Figure 2. (A) Differential pulse polarograms of 0.1 mM PA-824 solution at different times (0.4 M Britton-Robinson/ethanol, 80/20, pH 7). (B) Variation of I_p with the time.

The obtained results both by differential pulse and tast polarography techniques (Figure 3A, 3B, respectively) show only one well-defined cathodic signal in all the pH range studied. This signal was shifted toward more cathodic potential upon increasing the pH, indicating that this compound would be more easily reducible in acidic media. A polarographic maxima can be observed in the tast polarographic waves (Figure 3B), indicating a probable adsorption phenomena or other related phenomena that acts affecting the streaming of the solution in the vicinity of the electrode. Analyzing the evolution of peak potential (E_p) with pH (Figure 4A), it is possible to observe that there is a linear dependence between pH 2–10, and up to pH 10 the peak potential was independent to the H^+ concentration. The same results was obtained from tast polarographic experiments (Figure 4B) confirming that in spite of the observed polarographic maxima the polarographic parameters obtained from tast waves are reliable.

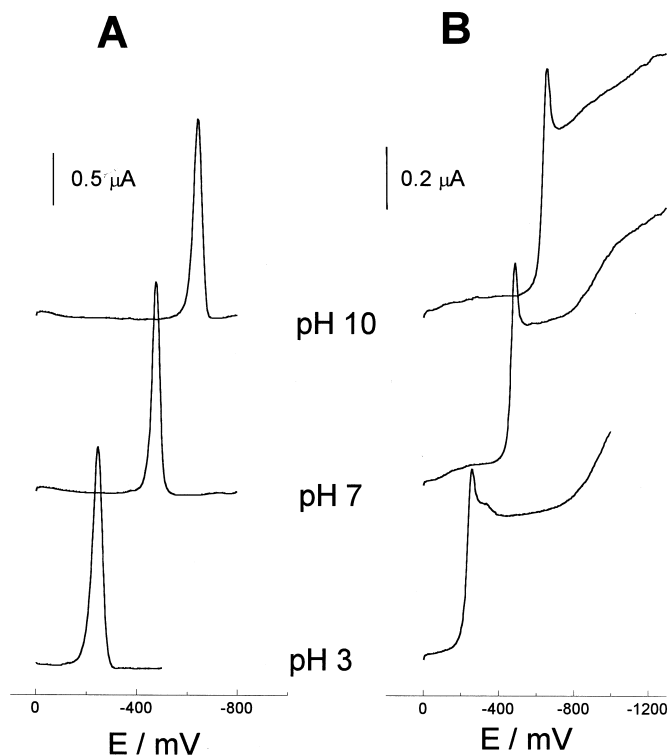


Figure 3. Differential pulse (A) and fast polarograms (B) of 0.1 mM PA-824 solution at different pH (0.4 M Britton-Robinson/ethanol, 80/20).

Furthermore, from the plot of the limiting current vs pH (Figure 4C), a pH-independent zone between pH 2–10 was observed. This behavior is indicative of diffusion controlled limiting currents. Then, up to pH 11, a strong decrease of the limiting current was observed. This decrease in the limiting current was also observed in the peak current by DPP mode with a 4.2 times lower peak current at pH 12 when compared with pH 10.

Cyclic voltammetric experiments performed at pH 3, 7 and 10 showed only one irreversible peak. We have studied the evolution of the peak current with the sweep rate finding a diffusion-controlled behavior of the electroreduction of PA-824. In Figure 5 we can observe the linear relation between $\log I_p$ versus $\log \nu$ with a slope near to 0.5 confirming the above assumption. From this result, we can conclude that the observed polarographic maxima was due to problems in the streaming of the solution

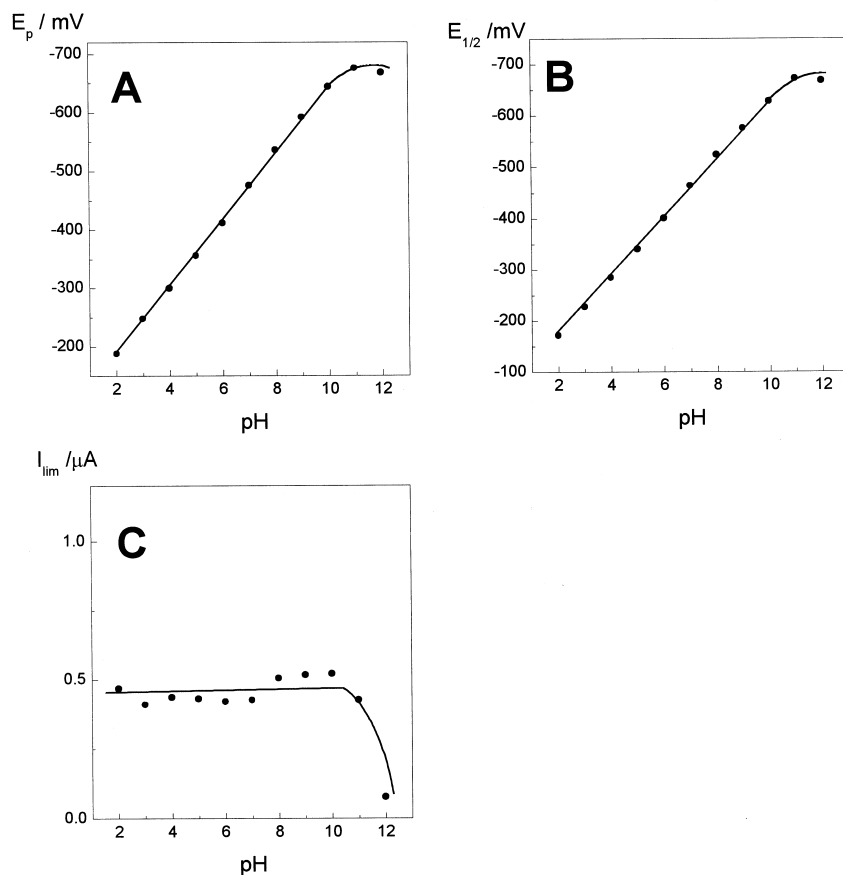


Figure 4. pH dependence of the differential pulse potential peak (A), half-wave potential (B) and limiting current (C) of 0.1 mM PA-824 solution (0.4 M Britton-Robinson/ethanol, 80/20).

to the electrode surface more than strong adsorption of reactants or products.

In order to elucidate the number of electrons transferred in the reduction process we compared the limiting currents of the PA-824 with limiting currents of equimolar solutions of metronidazole, at the same experimental conditions. We found that both, metronidazole and PA-824, showed a similar value of the limiting current measured by fast polarography (Figure 6), which could indicate that the same number of electrons are transferred. According to the well-known mechanism of metronidazole (13), which correspond to the

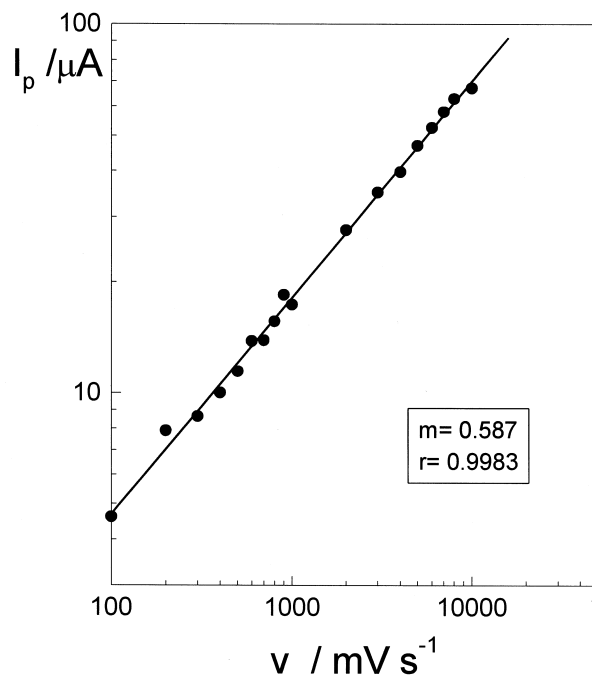
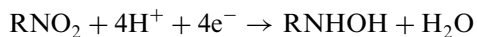


Figure 5. Plots of $\log I_p$ against $\log v$ of 0.1 mM PA-824 solution (0.4 M Britton-Robinson/ethanol, 80/20).

four-electron reduction of the nitro group, and the obtained results, we can assume that the reduction mechanism of PA-824 also would correspond to the 4-electron reduction of the nitro group, according to:



Furthermore considering that some studies (6) reveal that probably the action mechanism of PA-824 would be similar to the metronidazole one i.e., involving a nitro reduction step, it is interesting to note that both compounds present very similar reduction potentials meaning similar reduction capabilities from the electrochemical point of view.

UV Spectroscopy

With the aim of learn more about the chemistry in solution of PA-824, an UV-vis Spectrophotometric study was conducted (Figure 7).

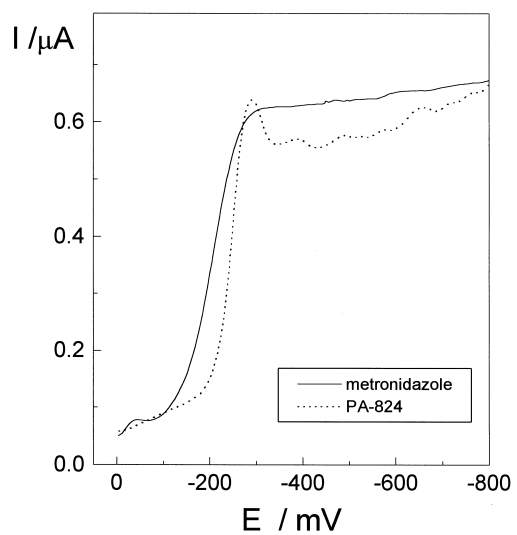


Figure 6. Tast polarograms of 0.1 mM PA-824 solution (dotted line) and 0.1 mM of metronidazole (solid line) in 0.4 M Britton-Robinson/ethanol, 80/20 at pH 7.

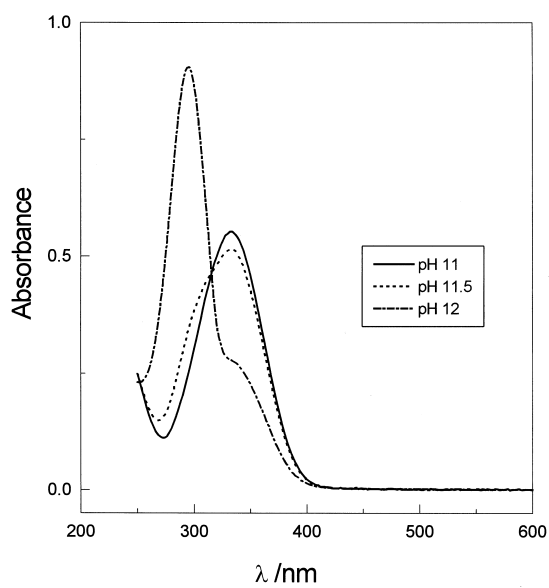


Figure 7. UV spectra of 0.08 mM PA-824 solution at differential pH.

PA-824 presents an absorption band with a maximum at 332 nm, in a wide range of pH (2–11) and it was found that this compound shows a pH-independent behavior inside this pH range. But, there is a dramatic change between pH 11–12, with a hypsochromic shifting and a new maximum at 295 nm. Surprisingly this change is irreversible i.e., when the pH solution was returned to pH 11, from the more alkaline media, the spectrum is not the same that was obtained initially at pH 11. This result indicates that the observed change in the spectrum is not due to an acid-base equilibrium, but a probable hydrolysis of PA-824 occurring at pH higher than 11. This fact also explains the decrease in the polarographic limiting current observed at this pH in Figure 4C. The data obtained in UV-Vis study were not useful to obtain an apparent pK_a from PA-824. Probably, the value of pK_a for the imidazole nitrogen is below to pH 2.

Quantitative Application

According to the above-obtained results by electrochemical studies, it was possible to apply the electrochemical techniques to the quantitative analysis of PA-824. pH 7 solutions and DPP and DPV modes were selected for this quantitative study.

In order to provide a DPP and DPV quantitative procedure, the dependence between PA-824 concentration and peak current (I_p) was conducted. A linear relation in the wide concentration range between 1×10^{-6} and 7.5×10^{-5} M was found (Figure 8). Above this concentration (7.5×10^{-5} M) a loss of linearity was observed, probably due to the adsorption of PA-824 on the electrode. In spite of the above we can use the linear zone to quantitative analysis. For quantitation the calibration plot method, with concentration ranging between 1×10^{-6} and 1×10^{-5} M of PA-824 solutions in ethanol/Britton–Robinson buffer (pH 7), was used. The calibration plots are described by the following regression curve:

$$I_p[\mu\text{A}] = 18497.39 \times C[\text{M}] - 8.99 \times 10^{-3} \quad (r = 0.9975, n = 10) \quad \text{DPP}$$
$$I_p[\mu\text{A}] = 13056.45 \times C[\text{M}] - 7.07 \times 10^{-3} \quad (r = 0.9909, n = 10) \quad \text{DPV}$$

where I_p is the peak current and C is the PA-824 concentration. The repeatability of the measurement was calculated from ten independent runs and the detection limit were calculated as is described by Quattrocchi (14). The obtained results are summarized in Table 1. By comparing the above results of detection limits with other previously informed for other nitroaromatic drug, i.e., nimesulide (L.D. = 2.5×10^{-6} M) (15), we can conclude that with the present method we have improved the detection in about one order of

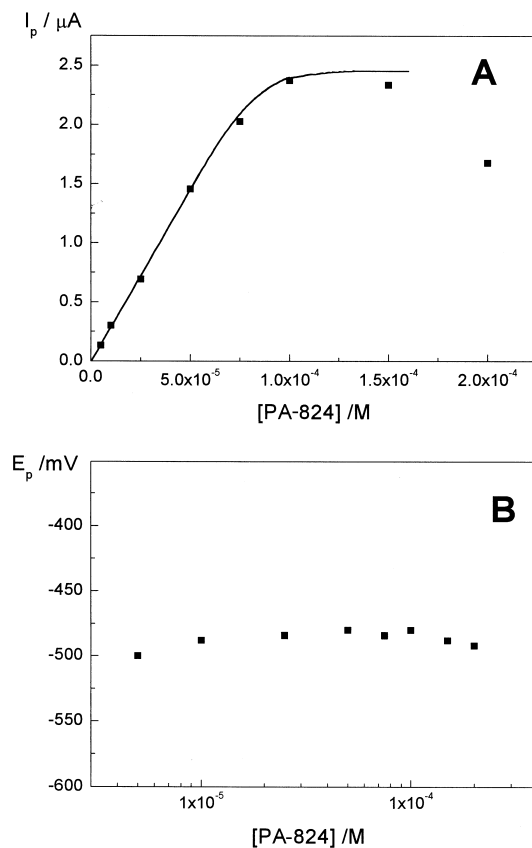


Figure 8. Variation of current peak (A) and potential peak (B) with the concentration of PA-824 (0.4 M Britton-Robinson/ethanol, 80/20, pH 7).

magnitude. On the other hand if we compare with the previously informed detection limit for the polarographic determination of metronidazole (13) we have improved the detection limit about 3 times.

In order to validate and to obtain the precision and accuracy of the developed methods we have also carried out a recovery study. The obtained results are summarized in Table 2. These results reveal that the proposed methods have adequate precision and accuracy and consequently can be validated to the determination of PA-824.

On the other hand, with comparative purposes we have also developed an alternative UV-spectrophotometric method, selecting the 295 nm band to develop the quantitative analysis. The calibration plot method, with

Table 1. Results of the Repeatability and Detection and Quantification Limits Studies

	DPP	VPD	UV
Repeatability, %	0.68	1.97	0.94
Detection limit, M	2.23×10^{-7}	2.2×10^{-7}	1.18×10^{-5}
Quantification limit, M	4.48×10^{-7}	3.9×10^{-7}	2.74×10^{-5}

Table 2. Results of the Recovery Study of PA-824

	DPP	VPD	UV
Average, %	101.1	99.6	102.2
s.d., %	2.3	1.9	2.2
C.V., %	2.3	1.9	2.1

concentrations ranging between 1×10^{-5} and 1×10^{-4} M of PA-824 solutions in ethanol/Britton–Robinson buffer (pH 7), was used. The calibration plot is described by the following regression equation:

$$A = 6393.3 \times C[M] + 0.0071 \quad (r = 0.999, n = 10)$$

The results of repeatability and detection and quantification limits studies are summarized in Table 1. On the other hand also a recovery study was developed and the results are summarized in Table 2.

In conclusion differential pulse polarography and differential pulse voltammetry can be a good alternative for the analytical determination of PA-824 because it has sufficient precision, accuracy, sensitivity, selectivity, is not time consuming, is not expensive and show better sensitive than the UV-spectrophotometric method. Furthermore, from the electrochemical point of view, PA-824 shows similar reduction capabilities that metronidazole supporting the hypothesis that both drugs could share a similar reductive metabolism.

ACKNOWLEDGMENTS

This work was supported by FONDECYT, Grant No 8000016. C.Y Thanks FONDECYT for a post-doctoral project N°3000043. We also acknowledge PathoGenesis Corporation (Seattle, USA) for providing PA-824 pure.

REFERENCES

1. Dye, C.; Scheele, S.; Dolin, P.; Pathania, V.; Raviglione, M.C. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality my country. WHO Global surveillance and monitoring project. *J. Am. Med. Ass.* **1999**, *282*, 677–686.
2. Bloom, B.R.; Small, P.M. The evolving relation between humans and *Mycobacterium Tuberculosis*. *N. Engl. J. Med.* **1998**, *338*, 677–678.
3. Kaufman, S.; Van Embden, J. Tuberculosis: a neglected disease strikes back. *Trends Microbiol.* **1993**, *1*, 2–5.
4. Bloom, B.R.; Murray, C.J. Tuberculosis: commentary on a reemergent killer. *Science* **1992**, *257*, 1055–1064.
5. Fischl, M.A. et al. Clinical presentation and outcome of patients with HIV infection and tuberculosis caused by multiple-drug-resistant bacilli. *Ann. Intern. Med.* **1992**, *117*, 184–190.
6. Strover, C.K.; Warren, P.; VanDevanter, D.R.; Sherman, D.R.; Arain, T.M.; Langhorne, M.H.; Anderson, S.W.; Towell, J.A.; Yuan, Y.; McMurray, D.N.; Kreiswirth, B.N. A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. *Nature* **2000**, *405*, 962–966.
7. Edwards, D.I. Mechanism of antimicrobial action of metronidazole. *J. Antimicrob. Chemother.* **1979**, *5*, 499–502.
8. Barety, D.; Resibois, B.; Vergoten, G.; Moschetto, Y. Electrochemical behaviour of nitroimidazole derivatives in dimethylsulphoxide. *J. Electroanal. Chem.* **1984**, *162*, 335–341.
9. Dumanovic, D.; Jovanovic, J.; Suznjevic, D.; Erceg, M.; Zuman, P. Polarographic and electrochemical studies of some aromatic and heterocyclic nitro compounds, Part IV: polarographic reduction of 2-alkylnitroimidazoles and aminonitroimidazoles. *Electroanalysis* **1992**, *4*, 889–898.
10. Radi, A.; El-Laban, S.; El-Kourashy, A.G. Electrochemical reduction of secnidazole and its determination in tablets. *Electroanalysis* **1997**, *9*(8), 625–628.
11. Bollo, S.; Núñez-Vergara, L.J.; Bontá, M.; Chauviere, G.; Périé, J.; Squella, J.A. Polarographic reduction of megalol and derivatives, and its polarographic, UV spectrophotometric and HPLC determination. *Electroanalysis* **2001**, in Press.
12. Gibson, G.G.; Skett, P. Introduction to drug metabolism (2nd Edn), Blackie Academic: Norwich, 1994.
13. La-Scalea, M.; Serrano, S.; Gutz, I. Voltammetric behaviour of metronidazole at mercury electrodes. *J. Braz. Chem. Soc.* **1999**, *10*, 127–135.

14. Quattrocchi, O.; De Andrizzi, S.; Laba, R.; “Introducción a la HPLC, Aplicación y práctica”, 1992.
15. Álvarez-Lueje, A.; Vásquez, P.; Núñez-Vergara, L.J.; Squella, J.A. Voltammetric study of nimesulide and its DPP determination in pharmaceuticals. *Electroanalysis* **1997**, *9*, 1209–1213.