*J. Braz. Chem. Soc.*, Vol. 12, No. 2, 202-207, 2001.
Printed in Brazil


0103 - 5053 $6.00+0.00

Article

# Electrochemical Reduction and Cathodic Stripping Voltammetric Determination of Clotrimazole

***Francisco C. Pereira, Nelson R. Stradiotto and Maria Valnice B. Zanoni****

*Instituto de Química, Universidade Estadual Paulista, C. P. 355, 14801-970, Araraquara – SP - Brazil.*

Clotrimazol é reduzido em eletrodo de mercúrio em tampão fosfato pH > 6 através de um processo reversível de um elétron. O processo eletródico apresenta forte efeito de adsorção, o qual pode ser minimizado na presença de Triton X-100. Clotrimazol pode ser determinado em níveis de 50 ng $mL^{-1}$ quando pré-acumulado durante 3 min à –0,20 V. O método proposto foi aplicado para determinação do fármaco em formulação comercial.

The reduction of clotrimazole at a mercury electrode, in phosphate buffer, pH>6, involves a reversible one-electron process. The electrochemical process presents a large contribution from adsorption effects. For the differential pulse polarographic determination the addition of Triton X–100 is recommended. Clotrimazole can be determined by cathodic stripping voltammetry at 50 ng $mL^{-1}$ level when pre-accumulated for 3 min at an accumulation potential of –0.20 V. The proposed method is applied successfully for the determination of clotrimazole in a commercial formulation.

**Keywords:** clotrimazole, cyclic voltammetry, differential pulse polarography, cathodic stripping voltammetry

## Introduction

Clotrimazole, 1-(α-2-chlorotrityl) imidazole (Figure 1) is a chlorinated synthetic imidazole derivative having broad spectrum antifungal and antibacterial properties which is often used as a topical treatment of several infections [1,2].

Several methods have been employed for the quantitative determination of clotrimazole in its pure or in its dosage forms. Titrimetric methods involving titrants such as perchloric acid[2], picric acid[3] and sodium lauryl sulphate[2,4] require high concentration of clotrimazole. Spectrophotometric analysis[5] has been limited due to its low molar absorptivity and some spectrophotometric methods have been based on the ion-pair complex reaction[6,7] or on acid hydrolysis[8] that require extraction procedures, that are time consuming and have low sensitivity. Chromatographic techniques such as thin-layer chromatography[1], gas chromatography[9] and liquid chromatography[10] have been reported and need sophisticated equipment or detailed experimental procedures.

Cathodic stripping voltammetry is a very sensitive technique that has been extensively used for determining pharmaceutical compounds. This technique is based on a preconcentration step of the analytical species on a mercury electrode surface[11] and can be used to monitor trace concentrations of these substances with low cost. The electrochemical investigations of clotrimazole found in the literature have shown that the drug can be determined by DC polarography by using the stability constants of complexes with some metallic ions[12] or by an oscillo-polarographic technique[13]. Nevertheless, the utilization of modern voltammetric techniques in the analysis of clotrimazole has not been reported.

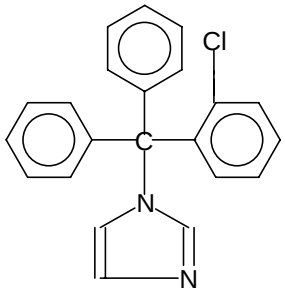



**Figure 1.** Molecular structure of clotrimazole.

The aim of the present work is therefore to investigate the electrochemical reduction of clotrimazole at a mercury electrode and to develop a simple, sensitive and inexpensive procedure for the determination of clotrimazole based on cathodic stripping voltammetry.

## Experimental

*Apparatus*

Voltammetric experiments were performed with a Metrohm Polarecord E 506 linked to a compatible microcomputer, through a Microquimica interface. The multimode electrode Metrohm stand 663 VA was used in both the hanging mercury drop electrode (HMDE) and dropping mercury electrode (DME). The three electrode system was completed by means of an Ag/AgCl (3 mol $L^{-1}$ KCl) reference electrode and a glassy carbon auxiliary electrode. The differential pulse polarograms obtained in the presence of Triton X-100 were recorded in a Polarograph Analyzer 264 A PARC coupled with X-Y recorder RE0091 Houston and a multimode electrode PARC 303A with DME as working electrode, a Ag/AgCl reference electrode and a Pt wire as auxiliary electrode.

Suprapur grade reagents supplied by Merck and de-mineralized water from a Milli-Q system (Milli-pore) were used in the preparation of all solutions. The supporting electrolytes were phosphate buffer prepared by mixing sodium phosphate acid and sodium phosphate diacid (0.2 mol $L^{-1}$) and Britton-Robinson (B-R) buffer, prepared in the usual way by adding appropriate amounts of 0.2 mol $L^{-1}$ sodium hydroxide to orthophosphoric acid, acetic acid and boric acid (0.04 mol $L^{-1}$ in each).

Clotrimazole ($1x10^{-2}$ or $1x10^{-4}$ mol $L^{-1}$) stock solutions were prepared from the dried pure substance (kindly supplied by Bayer S.A.) in methanol. An aliquot of the clotrimazole standard solution to be investigated was added to 20 mL of deaerated phosphate buffer (or B-R buffer) at the appropriate pH. The differential-pulse mode was used with a pulse amplitude of 50 mV and a drop time of 0.8s unless stated otherwise. The cathodic stripping voltammograms were obtained using a step accumulation at -0.2V for 30 s by stirring unless otherwise stated. Following 15s after having stopped the stirring, a cathodic voltammogram was recorded with a 50mV $s^{-1}$ scan rate in the linear scan mode.

Analyses of dosage forms were carried out using a commercial topic solution of Canesten and Dermobene 1% (10 mg $mL^{-1}$). An aliquot of this formulation, after evaporation of the organic solvent under a stream of nitrogen, was diluted with 10 mL of methanol. Aliquots of 20 µL of this solution were transferred into the voltammetric cell containing 20 mL of phosphate buffer (pH 7.0) and the voltammetric curve was recorded.

## Results and Discussion

*Overall characteristics of the reduction of clotrimazole*

Clotrimazole is polarographically reduced in a single wave between pH 6-12. In acidic solution (pH < 3.0), the reduction wave is marked by the electrolyte discharge. Figure 2 shows representative polarograms for 172 µg $mL^{-1}$ clotrimazole in phosphate buffer solution (pH 7.0). The wave shows distortion in the baseline at a more negative potential than peak potential with non symmetrical values in the peak half-width. The height of the reduction peak decreases continuously above pH 7.0 and the peak potential is shifted toward less negative values with increasing pH up to 10.0, as shown in Figure 3. This polarographic behaviour may indicate that the electrode process could involve a strong adsorption phenomenon on the dropping mercury electrode[14]. The effect of the composition of the supporting electrolyte on the voltammetric measurement of clotrimazole was examined in Britton–Robinson buffer pH 6-12 and phosphate buffer pH 6-8. The more convenient supporting electrolyte was phosphate buffer, exhibiting greater voltammetric response and better discrimination against the electrolyte curve.

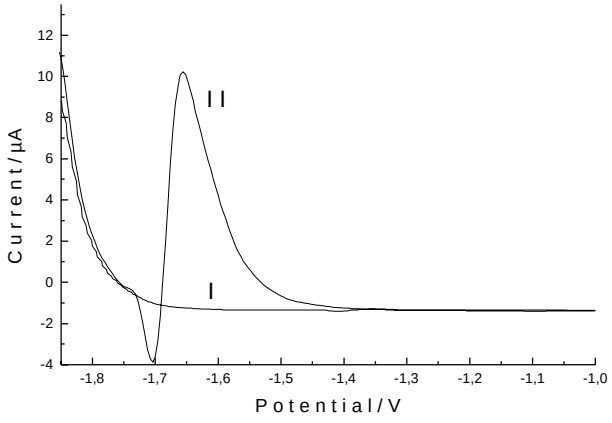



**Figure 2.** Differential pulse polarograms of 172 µg $mL^{-1}$ clotrimazole in phosphate buffer pH 7.0 (curve II). Supporting electrolyte (curve I).

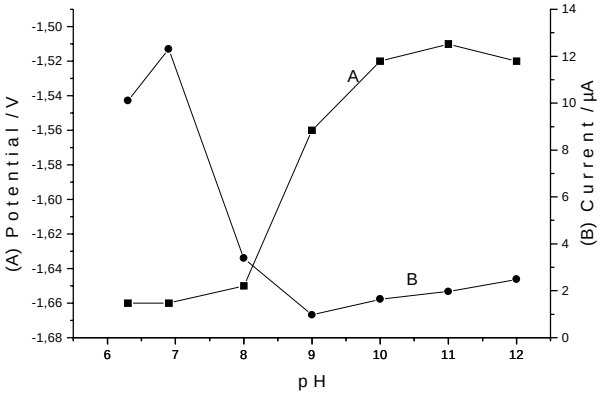



**Figure 3.** Effect of pH on the peak current and peak potentials obtained from 172 µg mL clotrimazole. (A)=Ep vs pH and (B) = ip vs pH.

At pH 7.0, the effect of droping time on the peak current was investigated from 0.4s to 3.0s for 172 μg $mL^{-1}$ of clotrimazole solution. A linear relationship was obtained up to 1.4s from log i *vs* log t, following the equation: log i = 0.60739 +0.86556 log t. An erratic behaviour is verified at these drop time greater than 1.4s, suggesting that the adsorption process is intensified under these conditions.

Differential pulse polarograms obtained for different concentrations of clotrimazole in phosphate buffer solution (pH 7.0) from 103 μg $mL^{-1}$ to 259 μg $mL^{-1}$ are shown in Figure 4. When the concentration is increased the peak potential is shifted to a more negative potential and the baseline of the cathodic part of the peak is completely distorted. Therefore, the corresponding relationship between peak current (taken at less negative potential) and concentration has a linear segment without crossing the origin, following the equation ip(μA)=-0.03012 + 0.00025C (C= μg $mL^{-1}$), indicating an electrode process associated with a strong adsorption phenomenom[15].

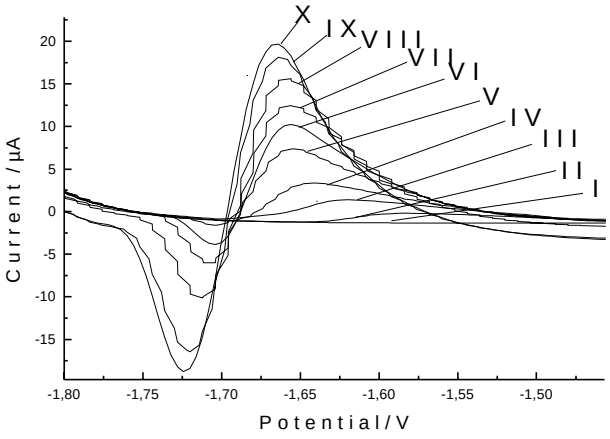



**Figure 4.** Differential pulse polarograms of clotrimazole in phosphate buffer solution (pH 7.0). Curve I= Supporting electrolyte; curve II= 86; curve III=121; curve IV= 138; curve V=155; curve VI= 172; curve VII= 190; curve VIII= 207; curve IX= 241 and curve X=259 (μg $mL^{-1}$).

In order to improve the polarographic curve, the effect of the addition of Triton X-100 on the differential pulse polarograms was studied. Polarograms recorded for a 68.9 μg $mL^{-1}$ clotrimazole solution in the presence of $7.5x10^{-4}$ %(m/v) Triton X-100 have shown the atenuation of the distortion on the baseline (Curve II of Figure 5) and that the wave is shifted to less negative potential. Therefore, 0.001% (m/v) of Triton X-100 was chosen as the best condition to minimize the anomalous behaviour of the polarographic curve without suppressing the current (Curve III of Figure 5).

A pH of 7.0, a drop time of 1.0s and 0.001% Triton X-100 were chosen as optimum for analytical purposes. Under these

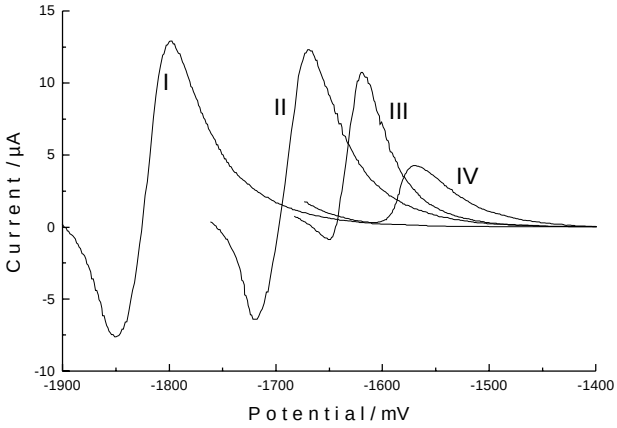



**Figure 5.** Influence of Triton X-100 on differential pulse polarograms of 68.9 μg $mL^{-1}$ clotrimazole in phosphate buffer pH 7.0. Curve I= without; curve II= $7.5x10^{-4}$ % (m/v) Triton X-100 and curve III= $1x10^{-3}$% (m/v) Triton X-100 and curve IV= $2x10^{-3}$% (m/v) triton X-100.

conditions, the peak current was found to be a linear function over the concentration range 1.72 to 93.1 μg $mL^{-1}$, following the equation: ip(μA)= -0.32386 + 52329 C (C= μg $L^{-1}$). This behaviour indicates that the surfactant preferentially covers the mercury electrode surface and promotes the suppression of the adsorption process coupled with the reduction of clotrimazole.

The electroreduction of clotrimazole was also studied by cyclic voltammetry under the same experimental conditions as described for its polarographic reduction at scan rates from 0.010V $s^{-1}$ to 5 V $s^{-1}$. Figure 6 shows a typical cyclic voltammogram of 172 μg $mL^{-1}$ clotrimazole in phosphate buffer solution (pH 7.0) at HMDE. A cathodic peak is seen at potentials corresponding to the polarographic step. An anodic peak resulting from re-oxidation of the generated product was observed during the reverse potential scan at pH 7.0 and pH 12.0, indicating a reversible step in the overall electrode reaction[15]. The difference between the cathodic and the anodic peak potential, ΔE= 50 mV, indicates a one-electron reduction. The ratio of the anodic-to-cathodic peak heights for this redox couple increased with the scan rate from 0.50 at 30 mV $s^{-1}$ to 1.01 at 300 mV $s^{-1}$, indicating that the reversible step is followed by an irreversible chemical reaction[15]. Nevertheless, on plotting ip *vs* υ, a linear relationship was observed for both cathodic and anodic peaks which may indicate an adsorption-controlled process involving the reagent and product. Multicyclic voltammograms repeated at the same mercury drop electrode do not shown any alteration of the height of the cathodic or anodic peak. These results confirm that the product formed remains strongly adsorbed on the electrode surface and can be reversibly reduced. Consequently, the cyclic voltammogram always presents the same shape on successive scans.

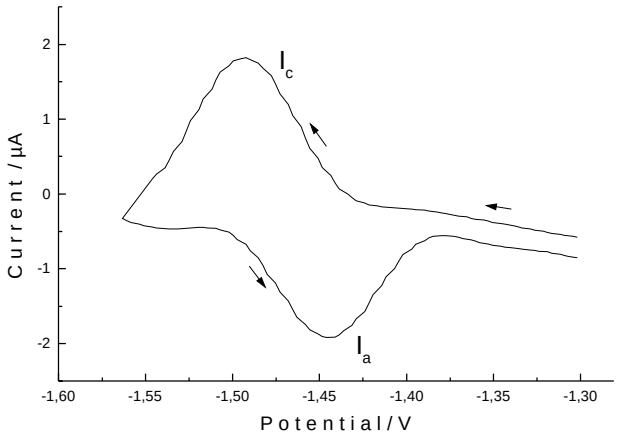
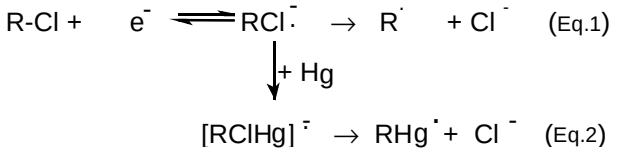



**Figure 6.** Cyclic voltammogram of 172 µg $mL^{-1}$ clotrimazole in phosphate buffer pH 7.0, $\upsilon$= 60 mV $s^{-1}$.

It is known from the literature[16,17] that imidazole is not reducible polarographically in aqueous media in the normally available potential range. Hence, the most probable electrode reaction for clotrimazole is the electroreduction of the carbon-halogen bond, which can be represented as in Scheme 1.




**Scheme 1.** Redution of clotrimazole.

Although reduction with substitution of halogen by hydrogen can take place (Eq. 1), the cathode material plays a very important role and the formation of organometallic compounds prior or after the electron transfer is often the preferred route of electroreduction of halogenated organic compounds on cathods of mercury[16]. Therefore, the overall course of the reduction of clotrimazole probably involves one-electron transfer, but formation of an organometallic species (Eq.2) is competitive with the reductive cleavage of the halide (Eq.1). Hence the direct participation of mercury on the electrode process explains all the anomalous behaviour observed in the polarographic experiments and the reversibility of the cyclic voltammo-grams in the first or successive scans.

*Cathodic stripping voltammetry*

Taking into consideration the existence of the adsorption process at the mercury electrode means that it could be used as an effective pre-concentration step of clotrimazole before voltammetric measurements are made.

Typical linear sweep cathodic stripping voltammograms of a 0.345 µg $mL^{-1}$ solution of clotrimazole in phosphate buffer solution (pH 7.0) after 30 s and 90 s accumulation at –0.2 V are shown in Figure 7. A well defined cathodic peak was observed at all pHs in the range of 6-12 B-R buffer. Nevertheless, maximum signal was observed in phosphate buffer solution between pH 6.0 and 8.0. For analytical purposes, pH 7.0 was therefore chosen for the determination of clotrimazole.

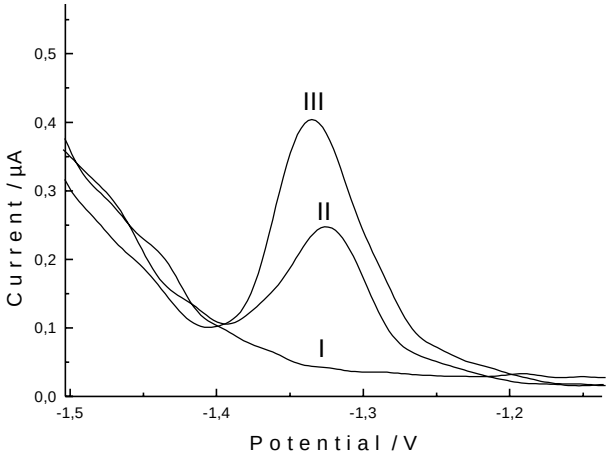



**Figure 7.** Cathodic stripping voltammograms of 0.345 µg $mL^{-1}$ clotrimazole in phosphate buffer pH 7.0. Under accumulation time of 30 s (curve II) and 90 s (curve III). $E_{ac}$= -0.2V; $\upsilon$= 100mV $s^{-1}$. Curve I= blank solution.

The effect of the accumulation potential on the stripping current at potentials from 0 to –1.2 V was investigated. The peak currents are higher from 0 to -0.2 V and an accumulation potential of –0.2V was chosen as satisfactory. Figure 8 shows the dependence of peak currents against different accumulation times at 0.172 µg $mL^{-1}$ and 0.345 µg.$mL^{-1}$ clotrimazole. A linear relationship was obtained from 0 to 4 min and up to 2 min, respectively, for both concentrations investigated.

Calibration graphs obtained using 3 min of accumulation time were linear from 0.05 µg $mL^{-1}$ to 0.17 µg $mL^{-1}$ clotrimazole, following the equation: ip (µA)= -0.0681 + 0.9951 C (C= µg $mL^{-1}$ and r= 0.994). At higher concentrations, the peak current reachs a limiting value suggesting an electrode surface saturation. Nevertheless, the linear range can be extended to 0.07 µg $mL^{-1}$ to 0.35 µg $mL^{-1}$ by pre-concentration for 1 min, equation: ip (µA)= -0.0251 + 0.6872C (C= µg $mL^{-1}$ and r= 0.994). The reproducibility of the cathodic stripping voltammetric response was evaluated for 0.345 µg $mL^{-1}$ of clotrimazole in phosphate buffer (pH 7.0) and the relative standard deviations were calculated to be 4.6%, for 9 determinations. The detection limit was estimated as 0.005 µg $mL^{-1}$ with a 1 min accumulation ( based on a signal-to-noise ratio of 3).

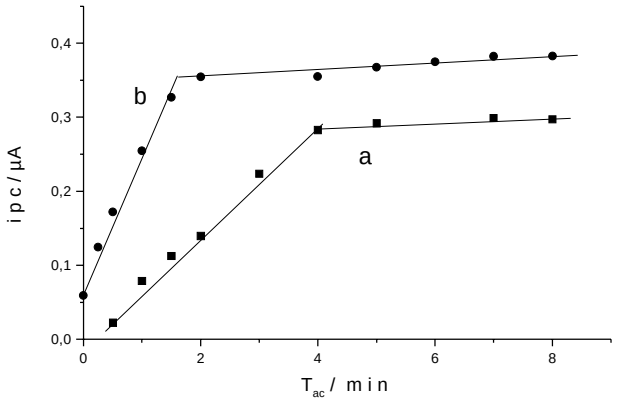



**Figure 8.** Influence of accumulation time on peak current obtained for 0.172 µg $mL^{-1}$ (curve a) and 0.345 µg $mL^{-1}$ (curve b) of clotrimazole in phosphate buffer pH 7.0; $E_{ac}$= -0.2V. υ= 100 mV $s^{-1}$ .

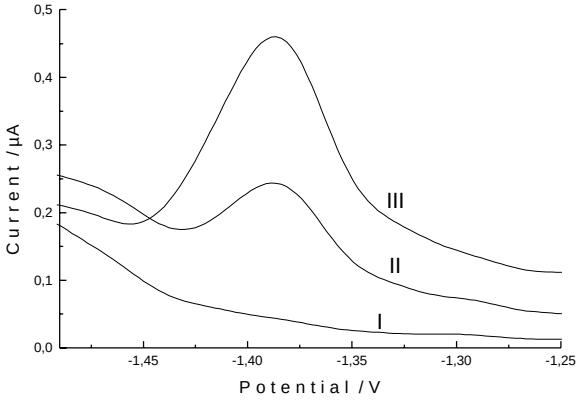



**Figure 9.** Cathodic stripping voltammograms obtained in phosphate buffer pH 7.0; $T_{ac}$ = 60 s; $E_{ac}$ = -0.2V; υ = 100 mV $s^{-1}$. Curve I = Supporting electrolyte, curve II = 0.345µg $mL^{-1}$ of Canesten and curve III = addition of 0.345µg $mL^{-1}$ of clotrimazole.

Thus, clotrimazole can be determined in aqueous solution with good precision and a low detection limit by cathodic stripping voltammetry.

*Analytical applications*

The method described was applied to the determination of clotrimazole in pharmaceutical preparations. Topic solutions commercialised as Canesten 1% containing a nominal quantity of 10 mg $mL^{-1}$ of clotrimazole, were tested. An aliquot of the test solution prepared as described in the experimental section was diluted to 20 mL with 0.002 mol $L^{-1}$ phosphate buffer solution (pH 7.0) and subjected to the optimum voltammetric parameters chosen, accumulation time 60 s, accumulation potential of –0.2 V and scan rate of 100 mV $s^{-1}$. The corresponding voltammetric curve obtained for Canesten and after standard addition of clotrimazole is shown in Figure 9. Table 1 shows the mean values of claimed clotrimazole content by the manufacturers and those given by the proposed method.

Further comparative determination of clotrimazole in the same pharmaceutical preparation[18] using a spectrophotometric method is also shown in Table 1. The results obtained have shown good agreement between the two methods. At a confidence level of 95%, values of 0.352 ± 0.017 µg $mL^{-1}$ and 0.235 ± 0.069 µg $mL^{-1}$ weve obtained for Canesten and Dermobene respectively.

**Table 1.** Analysis of clotrimazole in pharmaceutical formulations by voltammetric determination and spectrophotometric methods.

| Dosage form | Declared amount (µg $mL^{-1}$) | Proposed method (µg $mL^{-1}$) | Reported method[18] (µg $mL^{-1}$) | %Recovery |
|---|---|---|---|---|
| Canesten | 0.35 | 0.35 ± 0.01 | - | 102 ± 0.02 |
| solution | 8.00 | - | 7.96 ± 0.04 | 99.5 ± 0.01 |
| Dermobene | 0.207 | 0.23 ± 0.03 | - | 114 ± 0.12 |
| solution | 8.00 | - | 8.93 ± 0.93 | 112 ± 0.10 |

All values represent the mean of 3 determinations.

## Conclusion

From these studies it was concluded that clotrimazole contains reducible halide and this provides the basis for its electroanalytical determination. The drug is reduced at a mercury electrode above pH 6 involving a reversible one-electron process. Differential pulse polarography can be satisfactorily applied in this work for analytical purposes only in the presence of Triton X-100. The results have shown that improvements in sensitivity can be achieved by using cathodic stripping voltammetry and that this technique can be used as a convenient method for the analysis of clotrimazole in bulk material and in pharmaceutical preparations, namely in solution form.

## Acknowledgements

The authors are grateful for the financial support of FAPESP, CNPq, CAPES and the British Council.

## References

1. Hoogerheide, J. G.; Wyka, B. E. *Analytical Profiles of Drug Substances* **1982**, *2*, 225.
2. *United States Pharmacopeia (USP)*, United States Pharmacopeial Convention Inc., Rockville, 1990, p 334.
3. Volkmann, D. *Fachz. Lab.* **1983**, *27*, 524, *Chem. Abstr.* **1983**, *99*, 58985k.
4. Pellerin, F.; Gautier, J. A.; Demay, D. *Talanta*, **1965**, *12*, 847.

5. Moffat, A. C.; Jackson, J. V.; Moss, M.S.; Widdop, B.(eds.). In *Clarke's Isolation and Identification of Drugs. The Pharmaceutical* Press; London, 1986, p 487.
6. Abdelmageed, O. H.; Khashaba, P. Y. *Talanta* **1993**, *40*, 1289.
7. Ibrahim, M. K.; Nassar, M. W. *J. Pharm. Sci.***1994**, *14*, 184.
8. Bedair, M. M.; Korany, M. A.; Elsayed, M. A. E.; Fahny, T. J. *Assoc. Off. Anal. Chem.* **1989**, *72*, 432.
9. Wallace, S. M.; Shah, V. P.; Riegelman, S.; Epstein, W. *L. Anal. Lett.* **1978**, *11*, 461.
10. Hoogerheid, J. G.; Strusiak, S. H.; Taddei, C. R.; Townley, E. R.; Wyka, B. E. J. *Assoc. Off. Anal. Chem.* **1981**, *64*, 864.
11. Hart, J. P.In *Electroanalysis of Biologically Important Compounds*; Ellis Horwood; Chichester, 1990, p 137.
12. Nsangu, M.; De Rauter, C. J.; Jottier, W. J. *Pharm. Belg.* **1987**, *42*, 118.
13. Fijalek, Z.; Chodkowski, J.; Warowna, M. *Acta Pol. Pharm.* **1992**, *49*, 1.
14. Laviron, E. *J. Electroanal. Chem.* **1974**, *52*, 355.
15. Bard, A. J.; Faulkner, L. R. In *Electrochemical Methods - Fundamentals and Applications*; John Wiley & Sons; New York, 1980, p 519.
16. Baizer, M. M.; Lund, H.;(Eds.). In *Organic Electrochemistry*; 2 edn., Marcel Dekker Inc. New York, 1983, p 259.
17. Fry, A. J. In *Synthetic Organic Electrochemistry*; Harper & Row Publ., New York, 1972, p 172.
18. Osama, A.; Pakinaz, K. *Talanta* **1993**, *40*, 1289.

*Received: January 27, 2000*
*Published on the web: February 19, 2001*
**FAPESP helped in meeting the publication costs of this article.**