J. Braz. Chem. Soc., Vol. 15, No. 6, 803-808, 2004.
Printed in Brazil - ©2004 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Voltammetric Determination of Dipyrone Using a *N,N'*-ethylene*bis*(salicylideneaminato)oxovanadium(IV) Modified Carbon-Paste Electrode

Marcos F. S. Teixeira*[*,a], Luiz H. Marcolino-Júnior[b], Orlando Fatibello-Filho[b], Edward R. Dockal[b] and Éder T. G. Cavalheiro[c]

[a]*Universidade Estadual Paulista, Campus do Litoral Paulista, Unidade São Vicente, Praça Infante D. Henrique s/nº, 11330-900 São Vicente - SP, Brazil*

[b]*Departamento de Química, Universidade Federal de São Carlos, Rod. Washington Luiz, Km 235, 13565-905 São Carlos - SP, Brazil*

[c]*Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, 13560-970 São Carlos - SP, Brazil*

A preparação e a caracterização eletroquímica de um eletrodo de pasta de carbono modificado com o complexo *N,N´*-etileno*bis*(salicilidenoaminato) de oxovanádio(IV) ([VO(Salen)]), como também o seu comportamento eletrocatalítico na oxidação da dipirona foi investigado. O comportamento eletroquímico do eletrodo modificado e a eletrooxidação da dipirona foram estudados usando a voltametria cíclica. A resposta voltamétrica do eletrodo modificado é baseada em duas reações. Uma relacionada com a oxidação eletroquímica do centro metálico do complexo [VO(Salen)] e a outra que envolve o processo redox entre centro metálico do complexo oxidado e a dipirona. A melhor resposta voltamétrica foi observada para uma composição do eletrodo de 25% (m/m) [VO(Salen)] em solução eletrolítica de KCl para pH entre 5,5 a 8,0 e uma velocidade de varredura de 10 mV s^{-1} na presença de dipirona. Uma resposta voltamétrica linear para dipirona foi obtida no intervalo de concentração de 9,9 x 10^{-6} a 2,8 x 10^{-4} mol L^{-1}, com um limite de detecção de 7,2 x 10^{-6} mol L^{-1}. Entre os diversos compostos testados como interferente, somente o ácido ascórbico apresentou alguma interferência. O eletrodo proposto é útil para o controle de qualidade e a análise rotineira de dipirona em formulações farmacêuticas.

The preparation and electrochemical characterization of a carbon paste electrode modified with *N,N'*-ethylene*bis*(salicylideneiminato)oxovanadium(IV) complex ([VO(Salen)]) as well as its behavior as electrocatalyst toward the oxidation of dipyrone were investigated. The electrochemical behavior of the modified electrode and the electrooxidation of dipyrone were explored using cyclic voltammetry. The voltammetric response of the modified electrode is based on two reactions. One electrochemical related to the oxidation of the metallic center of the [VO(Salen)] and the other involving the chemical redox process involving the oxidized form of the complex and the reduced form of dipyrone. The best voltammetric response was observed for a paste composition of 25% (m/m) [VO(Salen)], KCl solution pH from 5.5 to 8.0 as the electrolyte and potential scan rate of 10 mV s^{-1} in the presence of dipyrone. A linear voltammetric response for dipyrone was obtained in the concentration range from 9.9 x 10^{-6} to 2.8 x 10^{-3} mol L^{-1}, with a detection limit of 7.2 x 10^{-6} mol L^{-1}. Among of several compounds tested as potential interference, only ascorbic acid presented some interference. The proposed electrode is useful for the quality control and routine analysis of dipyrone in pharmaceutical formulations.

Keywords: *N,N'*-ethylene*bis*(salicylideneiminato)oxovanadium(IV), modified electrode, voltammetric determination, dipyrone, pharmaceutical formulations

Introduction

The dipyrone (sodium salt of the 1-phenyl-2,3-dimethyl-4-methyl aminomethane sulfonate-5-pyrazolone), is a water soluble white crystalline powder which presents analgesic and antipyretic activity.[1] Dipyrone acts at the central and outlying level simultaneously, and its absorption is fast, uniform and almost complete. About 58% of the dose links to the plasma proteins, and the effect of this drug occur

* e-mail: funcao@csv.unesp.br

approximately fifteen minutes after its administration. The biotransformation of the dipyrone takes place at the hepatic level, and the duration of its effect is approximately 4-6 h, and its elimination is at renal level.[2] The drug can cause occasional or rare reactions as transitory disturbances and inflammation of the renal tissue, mainly in patients with renal disease history or in cases of overdose.[2]

The methods commonly used for dipyrone determination in various pharmaceutical formulations are based on its reaction with iodide.[3,4] The spectrophotometric methods such as ultraviolet-visible absorption,[5] fluorescence,[6] and chemiluminescence[7,8] are frequently reported for dipyrone determination. However, as far as it is known, the determination of dipyrone using electroanalytical techniques has been little investigated. In a previous work, Perez-Ruiz *et al.*[9] employed a glassy carbon electrode as detector in an amperometric flow cell. This flow injection procedure presented a linear response in the dipyrone concentration range from 3.0 x 10^{-6} to 3.0 x 10^{-5} mol L^{-1} in an ammonia buffer solution (pH 9.0) at a potential of 0.4 V *vs*. Ag/AgCl. The flow injection amperometric simultaneous determination of dipyrone, ascorbic acid, dopamine and epinephrine using modified microelectrodes, together with multivariate calibration analysis was proposed by Matos *et al.*[10] It was based on a multi-channel detection system, coupled to a flow cell containing an array of modified microelectrodes. Recently, the same research group developed a flow cell containing a gold electrode from recordable compact discs for the determination of dipyrone in pharmaceutical formulations.[11]

In the present work, the preparation, properties and application of a carbon paste electrode modified with the *N,N*-ethylene*bis*(salicylideneiminato) oxovanadium(IV) complex ([VO(Salen)]) for voltammetric determination of dipyrone in pharmaceutical formulations is reported. Transition metal ion complexes, with *N,N'*-ethylene*bis*(salicylideneaminato) mimics the function of metalloproteins in dioxygen binding, oxidation of olefins and aromatic compounds. The Salen complexes are conformationally flexible and adopt a variety of geometries to generate various active-site environments for different oxidation reactions.[12,13] The influence of several parameters such as pH, potential scan rate and several concomitants as potential interferents on the electrode voltammetric profile is also presented.

Experimental

Apparatus

All the voltammetric measurements were carried out in a 30 mL thermostated glass cell at 25 °C, with a three-electrode configuration: modified carbon paste electrode as the working electrode, saturated calomel reference (SCE) and platinum auxiliary electrodes. During the measurements, the solution in the cell was neither stirred nor aerated. Cyclic voltammetric measurements were performed with an Autolab/PGSTAT-30 (Eco Chemie) potentiostat/galvanostat connected to a microcomputer that was controlled by the software GPES2 version 4.8.

Reagents and solutions

All the solutions were prepared with water from a Millipore (Bedford, MA, USA) Milli-Q system (model UV Plus Ultra-Low Organics Water). All chemicals were of analytical grade and used without further purification. The supporting electrolyte used for all experiments was a 0.10 mol L^{-1} KCl solution at pH 7.0. A 0.010 mol L^{-1} dipyrone stock solution was prepared by dissolution of an appropriate amount of this reagent in 100.0 mL of 0.10 mol L^{-1} KCl solution. The solution was stable for 2 weeks in a refrigerator at 5 °C. Graphite powder (1-2 μm particle size – Aldrich) and mineral oil (Aldrich) of high purity were used in the preparation of the carbon paste.

Synthesis of N,N-ethylenebis(salicylideneiminato) oxovanadium(IV) complex

The Schiff base (Salen) was prepared according to a previously described procedure[14] and used without further purification. The *N,N*-ethylene*bis*(salicylideneiminato) oxovanadium(IV) complex ([VO(Salen)] – Figure 1) was prepared from oxovanadium(IV) sulfate and the ligand Salen, using the method described by Zamian and Dockal,[12] and the complex was purified by Soxhlet extraction using MeCN. No precautions were taken to exclude air from the reaction system, since the complex is stable in the air. The purified complex (green crystals) was dried under vacuum at room temperature for 72 h.

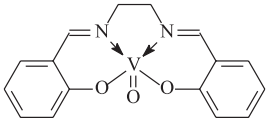


Figure 1. Molecular structure of the *N,N*-ethylene*bis*(salicylideneiminato)oxovanadium(IV).

Carbon paste electrode construction

The modified carbon paste electrode was prepared by mixing 0.250 g of [VO(Salen)] with 0.500 g of graphite powder and subsequently adding 0.250 g of mineral oil.

This mixture was homogenized by magnetic stirring in a 50 mL beaker containing 20 mL of hexane. The final paste was obtained after evaporation of the solvent. The modified carbon paste was packed into an electrode body, consisting of plastic cylindrical tube (o.d. 8 mm, i.d. 6 mm) equipped with a stainless steel rod serving as an external electric contact. Appropriate packing was achieved by pressing the electrode surface against a paper.

Preparation and analysis of pharmaceutical samples

Pharmaceutical formulations such as Novalgina® (Hoechst Marion Roussel), Anador® (Boehringer Ingelheim), Dorflex® (Hoechst Marion Roussel), Cefaliv® (Aché) and Magnopyrol® (Farmasa L.A.F.) containing dipyrone were obtained from local drugstores.

For the analysis of tablet formulation, an accurate amount of 0.2077 g was transferred to a 50.0 mL volumetric flask and this volume completed with 0.10 mol L^{-1} KCl solution at pH 7.0. For liquid formulations, an aliquot of 176 μL was diluted to 50.0 mL with 0.10 mol L^{-1} KCl solution at the same pH. No other treatment of the sample was required. The percentage content of dipyrone in these samples was determined by the standard addition method and compared with the iodimetric procedure.[4]

Results and Discussion

Electrochemical properties of the modified carbon paste electrode (MCPE)

The voltammetric behavior of the modified electrode was investigated in 0.1 mol L^{-1} KCl solution. Figure 2 presents a typical cyclic voltammogram with two peaks at +0.66 V (anodic) and +0.23 V (cathodic) *vs*. SCE at potential scan rate of 25 mV s^{-1}, that remained stable after the second cycle. These processes are usually assumed to be a quasi-reversible single-electron reduction/oxidation of the couple VO^{2+}(Salen)/VO^{3+}(Salen). The electrochemical behavior of oxovanadium(IV) complexes in organic medium has been well-studied.[15-17] The voltammetric profile of the carbon paste electrode modified with [VO(Salen)] complex is in agreement with the results obtained by Balkus *et al.*[16] whom investigated the electrochemical behavior of a composite electrode containing oxovanadium(IV)-Salen. Recently, the effect of the scan rate on the electrochemical behaviour of a carbon paste electrode with [VO(Salen)] in KCl solution was studied.[18,19] The cyclic voltammograms revealed that the peak currents increase and the peak potential shifts as the scan rate increases. The linear correlation of the peak

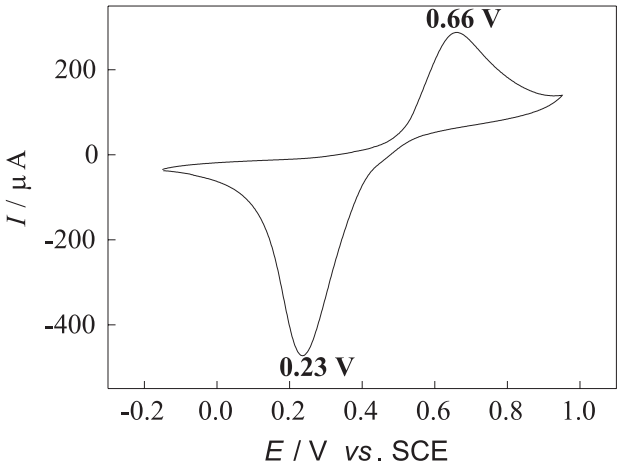


Figure 2. Cyclic voltammogram of the carbon paste electrode modified with *N,N*-ethylene*bis*(salicylideneiminato)oxovanadium(IV) complex in 0.10 mol L^{-1} KCl solution, at a potential scan rate of 25 mV s^{-1} between -0.15 and 0.95 V *vs*. SCE.

current with square root of scan rate showed that the system is similar to the process controlled by diffusion. This behavior suggests a mobility of the counterions of the supporting electrolyte necessary for charge transport or to keep the electroneutrality at the electrode surface during the redox process.[20,21]

The electrochemical behavior of carbon paste electrode modified with [VO(Salen)] complex in 0.10 mol L^{-1} KCl solution was studied over a large pH range between 1.0 and 10.0 using cyclic voltammetry and scanning the potential from -0.15 to 0.95 V (*vs*. SCE) at 25 mV s^{-1} scan rate. The peak potential was independent of pH in the 2.0 to 8.0 range. This behavior suggests that the [VO(Salen)] complex is stable in this pH range. The stability of the MCPE was verified by running 50 cycles in pH 5.0. No significant changes were observed in the peak currents.

Voltammetric determination of dipyrone

Figure 3 shows the cyclic voltammograms obtained for 9.1 x 10^{-5} mol L^{-1} dipyrone solution at a carbon paste electrode unmodified and modified with [VO(Salen)] complex in 0.10 mol L^{-1} KCl solution. At the unmodified electrode the electrochemical oxidation of dipyrone occurred at +0.33 V (peak 1), +0.57 V (peak 2) and +1.0 V (peak 3) *vs*. SCE, as is shown in Figure 3A. From this figure it can be seen that irreversible oxidation peaks represent the oxidation process of dipyrone. Perez-Ruiz *et al.*[9] observed the same electrochemical behavior for dipyrone using a glassy carbon electrode. Figure 3B presents cyclic voltammograms obtained for carbon-paste electrode modified with [VO(Salen)] in 0.10 mol L^{-1} KCl solution in the absence (curve 1) and presence (curve 2) of 9.1 x 10^{-5}

mol L^{-1} dipyrone. With the addition of dipyrone, the anodic peak current increased significantly when compared with that obtained at carbon paste electrode. The voltammetric response of the modified electrode is based in two processes as show the Scheme 1. Initially it begins by electrochemical process (the formation of VO^{3+}) and then a chemical reaction between dipyrone and the oxovanadium(V)

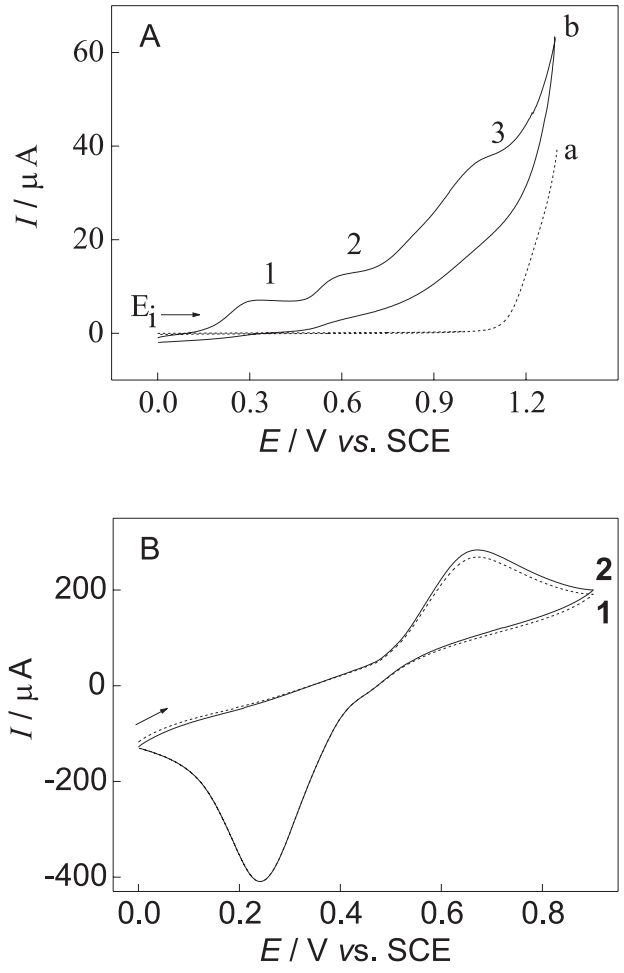
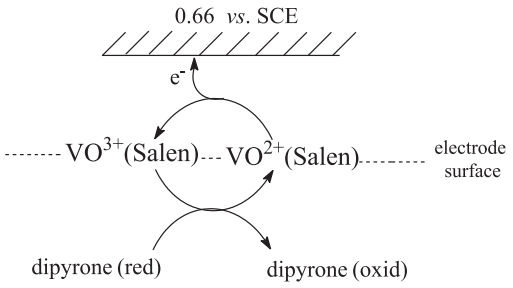


Figure 3. Cyclic voltammograms illustrating the voltammetric determination of dipyrone. **A**) unmodified carbon paste electrode in the absence (curve **a** – dash line) and presence (curve **b** – solid line) of 9.1 x 10^{-5} mol L^{-1} dipyrone in 0.10 mol L^{-1} KCl solution. **B**) carbon paste electrode modified with [VO(Salen)] in the absence (curve **1** – dash line) and presence (curve **2** – solid line) of 9.1 x 10^{-5} mol L^{-1} dipyrone in 0.10 mol L^{-1} KCl solution at 25 mV s^{-1}.

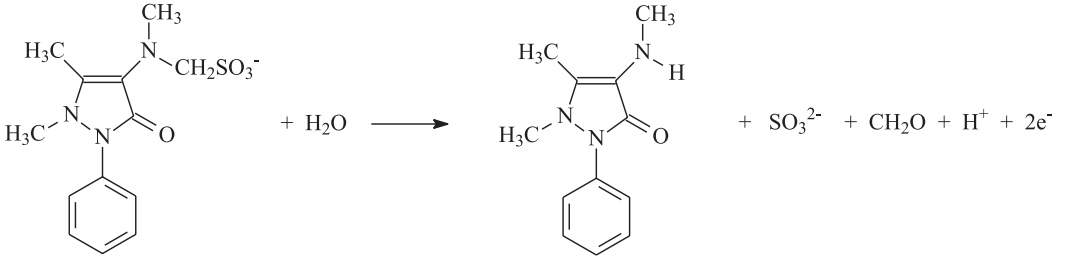


Scheme 1.

produced in the previous step which is regenerated to oxovanadium(IV). In recent works, we studied the electrochemical behavior of the dipyrone in different pHs and we compared it with the aminoantipyrine.[22] The dipyrone and aminoantipyrine present similar structures, differing only by the substituents at the carbon atom in position 4 on the pyrazolone molecule: the dipyrone presents as substituent a methylamino-*N*-methanesulphonate group while the aminoantipyrine, a amine group. We can conclude that the first electrochemical oxidation peak of the dipyrone (Figure 3A) is related with the methylamino-*N*-methanesulphonate group. The variation of anodic peak potential with a exhibited linear slope of -28.8 mV pH^{-1} indicates a mechanism involving the transfer of two electrons and one proton (see Scheme 2). These results essentially corroborate the fact that voltammetric response of the modified electrode is based on the methanesulphonate group oxidation of the dipyrone with central metal complex.

The pH effect on the voltammetric response of the modified electrode in 0.1 mol L^{-1} KCl, containing 9.1 x 10^{-5} mol L^{-1} dipyrone, was evaluated. The voltammetric behavior of the modified electrode was studied from the difference in peak current in relation to the solutions in absence and presence of the dipyrone in solution. The anodic peak current was practically constant in the pH range of 5.0-8.5. The current decrease is observed when the pH of the solution is lower than 5.0. Therefore, a KCl solution of pH between 5.5 and 8.0 was used in subsequent studies.

The effect of the potential scan rates from 2 to 200 mV s^{-1}



Scheme 2.

on the voltammetric response of the carbon paste electrode modified with [VO(Salen)] was investigated for a 3.3 x 10^{-4} mol L^{-1} dipyrone solution. The anodic peak current varied linearly with the square root of the scan rate, suggesting that the dipyrone oxidation follows a diffusion-controlled mechanism according to the linear relation I_{pa} (μA) = 22.1 + 158.8 $v^{1/2}$ ($mV^{1/2}$ $s^{-1/2}$) with correlation coefficient of 0.9994 (n=7). For scan rates above 150 mV s^{-1}, a small decrease in the anodic peak current was observed, indicating the existence of kinetic limitation in the electrochemical reaction between [(VO^{3+}(Salen)] and dipyrone for higher scan rates.[23] From these results, a scan rate of 25 mV s^{-1} was chosen for further studies since it results in voltammograms with better peak definition.

In order to investigate the analytical application of this voltammetric procedure, the effect of the excipients usually present in the pharmaceutical formulations was investigated by carrying out the determination of 1.0 x 10^{-4} mol L^{-1} dipyrone in the presence of each of the different excipients (potassium metabisulfite, saccharine, EDTA, caffeine, ascorbic acid and propylene glycol) at concentrations that can be found in those pharmaceutical formulations. Among those tested substances, only ascorbic acid caused a positive interference (~10%) in the electrode response at concentration ratio of 1/1. Recoveries between 93.8% and 100.8% of dipyrone were obtained using the voltammetric procedure. This is a good evidence of the accuracy of the proposed method.

After optimizing the operating conditions for the carbon paste electrode modified with [VO(Salen)], linear sweep voltammetric measurements were carried out in solutions containing different dipyrone concentrations in order to obtain an analytical curve. Figure 4 shows that the anodic peak was linearly dependent on the dipyrone concentration in the range from 9.9 x 10^{-6} to 2.8 x 10^{-4} mol L^{-1} with a detection limit of 7.2 x 10^{-6} mol L^{-1} in 0.10 mol L^{-1} KCl medium (pH 7.0) (three times the blank standard deviation/slope).[24]

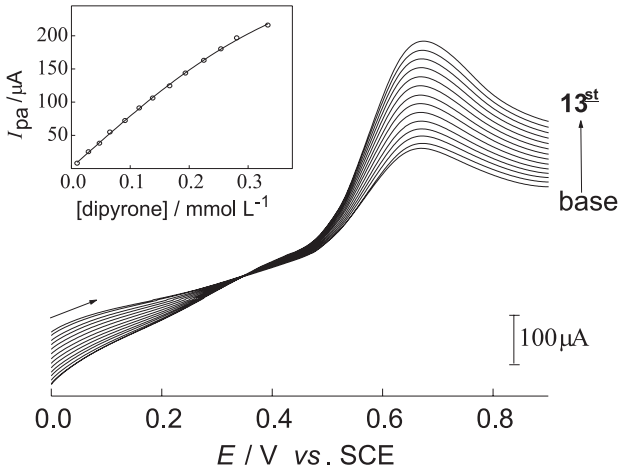


Figure 4. Linear sweep voltammograms obtained at 25 mV s^{-1} for the carbon paste electrode modified with [VO(Salen)] in 0.10 mol L^{-1} KCl solution containing different dipyrone concentrations: The analytical curve obtained from the anodic peak currents is illustrated in detail.

Table 1. Results obtained for dipyrone determination in pharmaceutical formulations by voltammetric and iodometric official methods (ref.4)

Sample	Dipyrone (mg mL^{-1} of solution)			E_r
	Label value	Iodimetric[a]	Voltammetric[a]	
Novalgina®	500	505 ± 2	509 ± 2	+0.8
Magnopyrol®	500	506 ± 3	504 ± 2	–0.4
Anador®	500	486 ± 3	503 ± 2	+3.5
	Dipyrone (mg/tablet)			
	Label value	Iodimetric[a]	Voltammetric[a]	
Dorflex®	500	511 ± 5	510 ± 3	–0.2
Cefaliv®	500	510 ± 2	512 ± 3	+0.4
Novalgina®	500	520 ± 2	527 ± 2	+1.3

[a]mean of three determinations ± SD; E_r = relative error = voltammetric method *versus* official method; Novalgina® composition: dipyrone, sodium benzoate, sodium metabisulfite, potassium sorbate and citric acid; Magnopyrol® composition: dipyrone; Anador® composition: methylparabene, sodium metabisulfite, sorbitol, EDTA and saccharine; Dorflex® composition: dipyrone and orphenadrine citrate; Cefaliv® composition: dipyrone, caffeine and dihydroergotamine mesylate.

Analysis of pharmaceutical samples

The modified electrode developed was applied to the voltammetric determination of dipyrone in six commercial pharmaceutical samples with different compositions. The results obtained using the carbon paste electrode modified with [VO(Salen)] and those obtained using an official method[4] are presented in Table 1. The official method consists in the dipyrone titration in hydrochloric acid medium with a standardized solution of iodine using starch as indicator. The analysis of dipyrone for each sample was realized in triplicate (n=3). The statistical calculations for the results presented in this table suggested good precision for the voltammetric method. According to the *t*-test, there are no significant differences between the results obtained by either procedure at the 95% confidence level, indicating that the modified electrode can be used for voltammetric determinations of dipyrone in such samples.

References

1. Korolkovas, A.; Burckhalter, J. H.; *Química Farmacêutica*, Guanabara Koogan: Rio de Janeiro, 1988, pp.193-195.
2. Jones, S.L.; *Eur. J. Pharmacology* **1996**, *318*, 37.

3. Melentyeva, G.; Antonova, L.; *Pharmaceutical Chemistry*, Mir Publishers: Moscow, 1988, pp.299-309.
4. *Farmacopéia Brasileira*, 3rd ed., Atheneu Editora: São Paulo, 1977, pp.406 – 408.
5. Aburjai, T.; Amro, B.I.; Aiedeh, K.; Abuirjeie, M.; Al-Khalil S.; *Pharmazie* **2000**, *55*, 751.
6. Perez-Ruiz, T.; Martinez-Lozano, C.; Tomas, V.; Carpena J.; *Microchem. J.* **1993**, *47*, 296.
7. Huang, Y.M.; Zhang, C.; Zhang, X.R.; Zhang, Z.J.; *J. Pharm. Biomed. Anal.* **1999**, *2*, 817.
8. Huang, Y.M.; Zhang, C.; Zhang, X.R.; Zhang, Z.J.; *Fresenius J. Anal. Chem.* **1999**, *365*, 381.
9. Perez-Ruiz, T.; Tomas, V.; Martinez-Lozano, C.; *J. Pharm. Biomed. Anal.* **1994**, *12*, 1109.
10. Matos, R.C.; Angnes, L.; Araújo, M.C.U.; Saldanha, T.C.B.; *Analyst* **2000**, *125*, 2011.
11. Munoz, R.A.A.; Matos, R.C.; Angnes, L.; *J. Pharm. Sci.* **2001**, *90*, 1972.
12. Zamian, J.R.; Dockal, E.R.; *Trans. Met. Chem.* **1996**, *21*, 370.
13. Kapturkiewicz, A.; *Inorg. Chim. Acta* **1981**, *53*, L77.
14. Seangprasertkij, R.; Riechel, T.L.; *Inorg. Chem.* **1986**, 25, 3121.
15. Friedrich, A.; Hefele, H.; Mickler, W.; Mönner, A.; Uhlemann, E.; Scholz, F.; *Electroanalysis* **1998**, 10, 244.
16. Balkus, K.J.; Khanmamedova, A.K.; Dixon, K.M.; Bediouri, F.; *Appl. Cat. A: General* **1996**, *143*, 159.
17. Tsuchida E.; Oyaizu, K.; *Coord. Chem. Rev.* **2003**, *237*, 213.
18. Teixeira, M.F.S.; Marino, G.; Dockal E.R.; Cavalheiro, E.T.G.; *Anal. Chim. Acta* **2004**, *508*, 79.
19. Teixeira, M.F.S.; Dockal E.R.; Cavalheiro, E.T.G.; *Sens. Actuat. B*, in press.
20. Munteanu, F.D.; Okamoto, Y.; Gorton, L.; *Anal. Chim. Acta* **2003**, *476*. 43.
21. Calvo-Marzal, P.; Chumbimuni-Torres, K.Y.; Höehr, N.F.; Oliveira Neto G.; Kubota, L.T.; *Sens. Actuat. B* **2004**, *100*, 333.
22. Teixeira, M.F.S.; Marcolino-Júnior, L.H.; Bergamini, M.F.; Fatibello-Filho, O.; *54th Annual Meeting of the International Society of Electrochemistry*, São Pedro, Brazil, 2003, p.205 (805).
23. Pournaghi, M.H.; Razmi-Nerbin, H.; *J. Electroanal. Chem.* **2000**, *488*, 17.
24. Analytical Methods Committee, *Analyst* **1987**, *112*, 199.

Received: March 15, 2004
Published on the web: October 1, 2004

FAPESP helped in meeting the publication costs of this article.