

Adsorptive Voltammetric Determination of Nimesulide at Glassy Carbon Electrode

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SUMMARY. A voltammetric method is described for the determination of nimesulide based on the reduction of the nitro group at glassy carbon electrode. The voltammetric behavior of the drug was investigated in Britton-Robinson buffer (pH 2.0-12.0) applying cyclic voltammetry technique. One cathodic and one anodic peaks were observed. The comparison of peak heights and potentials indicated that these peaks are quasi-reversible. The determination of nimesulide in pure form was performed using adsorptive linear sweep voltammetry. The cathodic peak current varied linearly in the range 4.0×10^{-7} - 5.0×10^{-5} M (0.116 - $14.65 \mu\text{g mL}^{-1}$). The limits of detection (LOD) and quantification (LOQ) were 3.2×10^{-8} and 1.06×10^{-7} mol L⁻¹, respectively. The proposed method was applied to pharmaceutical formulations with percent recoveries in the range 98.00-101.60%, and a relative standard deviation of 0.61-1.46%. The validity of the method was performed to the determination of nimesulide in human serum with acceptable results for biological samples. No sample pre-treatments or solvent extraction procedures were needed.

INTRODUCTION

Nimesulide, (4-nitro-2-phenoxyethanesulfonamide, Fig. 1), is a non-steroidal anti-inflammatory drugs (NSAIDs), which inhibits cyclooxygenase (COX), the key enzyme in the biosynthesis of prostaglandins. This drug is not a classical NSAID, and it has weak action against prostaglandin synthesis (COX-2 inhibitor) but has a potent anti-inflammatory, antipyretic, and analgesic activities in addition to low toxicity, a moderate incidence of gastric side effects, and a high therapeutic index ¹. Besides, it is better tolerated and causes fewer adverse effects than other currently used non-

steroidal anti-inflammatory drugs. Also, nimesulide seems to express less severe gastrointestinal side effects ².

Many chromatographic methods have been employed for the determination of nimesulide such as LC ^{3,4}, HPLC ⁵⁻⁸, HPLC/MS ⁹, HPTLC ¹⁰ and reversed-phase HPLC ¹¹. Other methods as spectrophotometry ¹², second derivative spectrometry ¹³, and capillary zone electrophoresis ¹⁴ were also applied for its determination.

There are only a few electroanalytical methods described in the literature for the detection and quantification of nimesulide. A flow amperometric method ¹⁵ has been described for its determination in pharmaceuticals. Differential-pulse voltammetry technique was performed for determination of nimesulide at glassy carbon electrode modified with cysteic acid/CNTs ¹⁶ based on electrochemical oxidation of l-cysteine. The same technique was applied for determination of this drug by using a Plackett-Burman matrix ¹⁷. The electrochemical reduction of nimesulide has been studied at mercury elec-

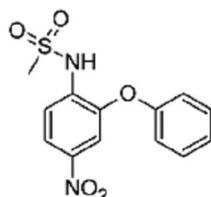


Figure 1. Molecular structure of nimesulide.

KEY WORDS: Cyclic voltammetry, Glassy carbon electrode, Linear sweep, Nimesulide, serum.

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trode^{18,19} and on modified glassy carbon electrode²⁰ for analytical purposes. An electrochemical sensor²¹ has been also developed for the determination of nimesulide based on the Nimesulide-molybdophosphoric acid (MPA) as the electroactive material in PVC matrix. No analytical methods were reported for the studying or determination of nimesulide at the glassy carbon electrode.

The aim of this study was to establish the experimental conditions, in order to investigate the voltammetric behavior of nimesulide using cyclic and linear sweep voltammetric techniques. This work was also aimed to develop new, fully validated, rapid, selective and simple voltammetric methods for the direct determination of nimesulide in raw materials, pharmaceutical dosage forms and spiked human serum samples without any time-consuming extraction, separation or evaporation steps prior to drug assay.

EXPERIMENTAL

Apparatus

Voltammetric and stripping measurements were carried out using a potentiostat model 273 A (Advanced Analytics, USA) with a software model HQ 2030. A cell containing three electrodes: a glassy carbon electrode ((IJ Cambria Scientific Ltd of a 3 mm diameter) as the working electrode, a platinum wire counter electrode and an Ag/AgCl (3.5 mol/L KCl) reference electrode were employed. The pH values were measured using a pH-meter model HI 8014, Hanna Instruments (Italy). All measurements were made at room temperature.

Materials and reagent solutions

Nimesulide was kindly supplied by Alkan Pharma. Nimesulide® (Alkan Pharma, 6th of October City, Egypt) and Nimalox® (Sigma Pharmaceutical Industries, Egypt) commercial tablets were obtained and used as received. All chemicals were of analytical-reagent grade or better and de-ionized water was used throughout. 0.1 mol L⁻¹ Britton-Robinson buffer (B-R buffer) (acetic acid-boric acid-phosphoric acid) for polarographic experiments was used, and desired pH (2.0-12.0) was adjusted with 0.2 mol L⁻¹ solutions of NaOH. Standard stock solutions (1x10⁻² mol L⁻¹) were prepared by dissolving an appropriate amount of electroactive species in water and ethanol. Stock solutions were protected from light through out the experimental procedures. Desired concentrations of solutions

were prepared daily from the stock solutions by dilution with de-ionized water and ethanol, keeping the final concentration of water:ethanol 9:1 (v/v).

General Procedure

A 10-ml of the electrolyte solution was transferred into the voltammetric cell. After measurement of the blank solution, the appropriate amount of nimesulide standard solution was added and the cyclic or cathodic potential sweep was carried under different operational parameters. The working solution contained a final percentage of 10% (v/v) ethanol to ensure complete solubility of the drug during analysis. The solution was de-gassed by passing purified nitrogen gas prior to the measuring step. Before each run, the working electrode was immersed in 95% ethanol for 2 min with continuous stirring and then washed with de-ionized water. Before each set of experiments, the electrode was polished by alumina (0.5 µm in particle mean diameter). Finally, the electrode was rinsed with de-ionized water and dried with a non-abrasive tissue paper.

Cyclic voltammetry was carried out from +0.4 to -1.5 V with a scan rate v varying from 10 to 500 mV s⁻¹. For linear sweep adsorptive voltammetric measurements, the accumulation potential was applied to the working electrode while the solution was continuously stirred. Stirring was discontinued and, after 5 s, a negative potential scan was initiated and the resulting voltammogram was recorded. Peak heights were evaluated as the difference between each voltammogram and the background electrolyte voltammogram.

Procedure for tablets

Ten tablets were weighed and the average mass per tablet was determined. The Sulide® or Nimalox® tablets (containing 100 mg/tablet) were finely powdered and a portion of this powder equivalent to 0.7075 mg of nimesulide (corresponding to 1x10⁻⁴ mol/L) was accurately weighed. The sample was shaken with ethanol for about 10 min and completed to a final volume of 25 mL with the same solvent. Appropriate aliquot of the clear supernatant liquor was then transferred into a voltammetric cell containing 8 mL of B-R buffer (pH 5.3) to yield the desired final concentration of nimesulide at a final volume of 10 mL containing ethanol : water (90:10). The stripping linear sweep voltammograms were subsequently recorded at optimal

conditions. The content of the drug in tablet was determined using the standard addition method.

Procedure for serum

Drug-free human blood, obtained from healthy volunteers was centrifuged at 5000 rpm for 15 min at room temperature, and separated serum samples were stored frozen till assay. 0.1 mL of serum sample was transferred into a 10-mL volumetric flask and a suitable volume of the drug solution was added and completed to the volume with ethanol. After vortexing for 1 min, the mixture was then centrifuged for 10 min at 5000 rpm to eliminate serum protein residues, and the supernatant was taken carefully. Appropriate volumes of the supernatant were transferred into a 10-mL volumetric flask, diluted to the volume with buffer solution of pH 5.3 and ethanol. The solution was analyzed applying adsorptive linear sweep voltammetry (AdLSV) by using standard addition method under optimum conditions.

Method validation

The proposed method was validated by determining the following parameters: linearity, range, accuracy, precision, and selectivity.

Linearity and range

The calibration curve was constructed by plotting the concentration of different standard solutions of nimesulide versus corresponding cathodic peak height. Ten different concentrations of the drug solution were used and analyzed in triplicate. The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method.

Accuracy

The accuracy was evaluated by the assay of three concentrations of the sample solution (0.58, 4.64, and 9.28 $\mu\text{g mL}^{-1}$) in triplicate. The percent recovery and the relative error % were determined.

Precision

The precision were checked in the same day ($n = 5$). The results were expressed as relative standard deviation (R.S.D.) of the measurements.

Selectivity

The specificity was evaluated by analyzing solutions containing the excipients employed

for the preparation of nimesulide commercial tablets. The proposed procedure was also applied to the determination of different concentrations of the drug spiked in human serum after deproteinization to examine the effect of the natural components of serum on the analyte signal.

Limits of detection (LOD) and limits of quantification (LOQ)

The LOD and LOQ for the proposed method were calculated²² according to the equation: $\text{LOD} = 3S_a/a$ and $\text{LOQ} = 10S_a/a$, where S_a represents standard deviation of the slope (a).

RESULTS AND DISCUSSION

Cyclic voltammetry behavior

The reversibility of the reduction process of nimesulide was investigated at GCE by the use of cyclic voltammetry in B-R buffer of different pH values. Typical voltammograms of nimesulide at different pH values without accumulation are illustrated in Figure 2. A well defined reduction peak was obtained at all pH values used corresponding to the reduction of the nitro group²³, while in the anodic direction a remarkably smaller anodic peak was observed. The values of $E_{p_c} - E_{p_a}$ (about 640 mV) and I_{p_a}/I_{p_c} (about 0.18) indicates that the reduction process of nimesulide at GCE is quasi-reversible²⁴. On increasing the pH of the test solution, the cathodic peak potential shifted to more negative potentials indicating the involvement of hydrogen ions in the reduction process. The results in Figure 2. show that the anodic wave in the reverse sweep is nearly disappeared on increasing the pH.

The reduction peak current I_{p_c} is proportional to the scan rate (Fig. 3), indicating that the reduction of nimesulide at the GCE is ad-

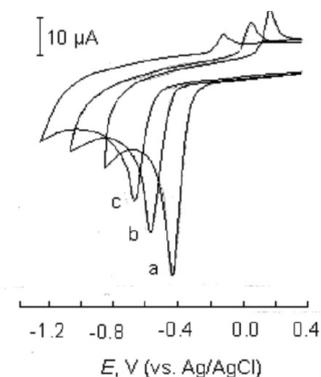


Figure 2. Effect of pH on 1×10^{-4} mol L^{-1} nimesulide at scan rate 500 mVs^{-1} , pH= **a:** 2.50, **b:** 4.45 and **c:** 7.25.

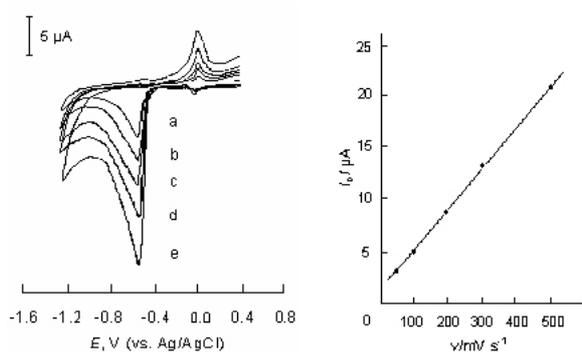


Figure 3. Cyclic voltammograms of 1×10^{-4} mol L⁻¹ nimesulide in B-R buffer solution of pH 4.6 at different scan rates (a: 50, b: 100, c: 200, d: 300, e: 500 mV s⁻¹).

sorption controlled. The reduction peak is attributed to the four-electron reduction of nitro group to the corresponding hydroxylamine according to the currently accepted mechanism for the electro-reduction of aromatic and hetero-aromatic nitro compounds²⁵⁻²⁸.

Linear sweep voltammetry

Cyclic voltammetry experiments showed that nimesulide shows a well defined reduction peak which can be used for quantitative determination of this drug. The experimental design was used as a tool for the optimization of the intensity of that peak using adsorptive linear scan voltammetry. Different media with different pH values were examined as supporting electrolytes for the reduction process applying linear sweep voltammetry. These supporting electrolytes were B-R buffer (2.0-12.0), acetate buffer (2.3-5.6), HClO₄ (0.1 mol L⁻¹), NaClO₄ (0.1 mol L⁻¹), and KCl (0.1 mol L⁻¹). A good reduction peak due to the reduction of nitro group at about -0.7 V was obtained at B-R buffer of pH 5.3, so it was used as the optimum supporting for further measurements.

The ability of nimesulide to be accumulated on the glassy carbon electrode was examined. The effect of accumulation time (t_{acc}) on the peak current of the drug was studied at two concentration levels of nimesulide: 1.0×10^{-6} and 5.0×10^{-5} mol L⁻¹. At $t_{acc} = 90$ s, an equilibrium surface concentration was occurred and the peak height reached the maximum value (Fig. 4). Accordingly, a pre-concentration time of 90 s was adopted for the adsorptive stripping analysis of nimesulide.

The dependence of the stripping reduction peak current on the accumulation potential (E_{acc}) was studied from +100 to -400 mV for

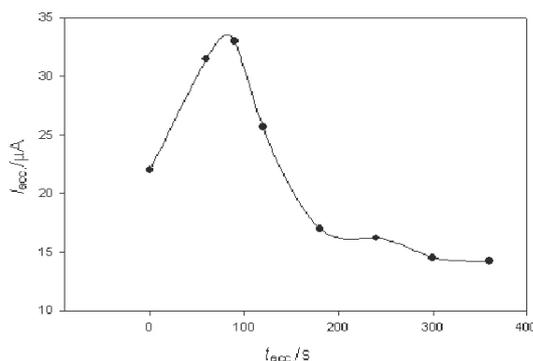


Figure 4. Effect of accumulation time on 5×10^{-5} mol L⁻¹ nimesulide in B.R. buffer of pH 5.3 at $E_{acc} = -400$ mV and $v = 500$ mV s⁻¹.

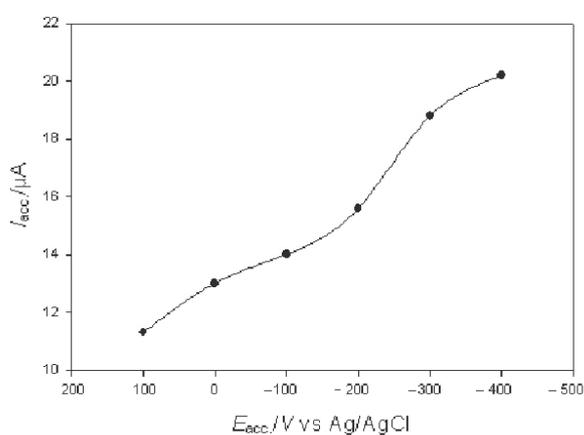


Figure 5. Effect of accumulation potential on 5×10^{-5} mol L⁻¹ nimesulide in B.R. buffer of pH 5.3 at $t_{acc} = 240$ s, $v = 500$ mV s⁻¹.

5×10^{-5} mol L⁻¹, (Fig. 5). From the results obtained, the cathodic current was increased with increasing the accumulation potential in the cathodic direction. Therefore, an accumulation potential of -400 mV was chosen for the rest of the present measurements.

Validation of the analytical method

Under the optimum conditions, the calibration graph (Fig. 6) for the determination of nimesulide is obtained in the concentration range of 4.0×10^{-7} – 5.0×10^{-5} mol L⁻¹ (0.116 – 14.65 μ g mL⁻¹) of nimesulide with a correlation coefficient of 0.9997. The analytical parameters of the adsorptive linear sweep voltammetry are collected in Table 1. The regression equation: $-I_p = 0.18 + 308.33 C$ was obtained ($n = 8$), where C is the concentration of nimesulide in m moles and I_p is the peak currents of standard solutions in μ A.

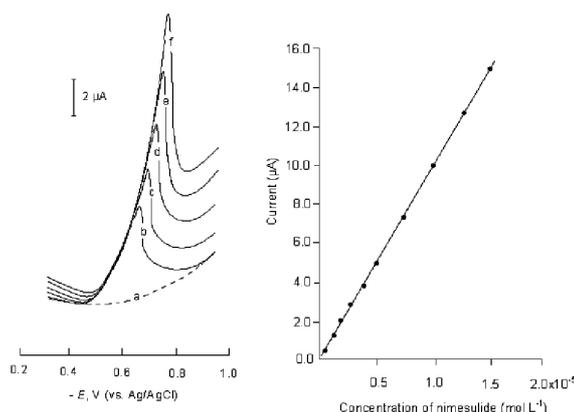


Figure 6. AdLSV for nimesulide in B-R buffer under optimum conditions. (a) blank, (b) 5.0×10^{-6} , (c) 7.5×10^{-6} , (d) 1.0×10^{-5} , (e) 1.25×10^{-6} , (f) 1.5×10^{-5} mol L⁻¹ nimesulide.

Parameter	AdLSV
Optimum pH	5.3
Optimum t_{acc} (s)	90
Optimum I_{acc} (mV)	-400
Optimum v (mVs ⁻¹)	500
Concentration range: (mol L ⁻¹)	4.0×10^{-7} - 5.0×10^{-5}
$\mu\text{g mL}^{-1}$	0.116 - 14.65
LOD	3.2×10^{-8} mol L ⁻¹
LOQ	1.06×10^{-7} mol L ⁻¹
Correlation coefficient (r)	0.9997
Slope (m mol L ⁻¹)	308.33
Intercept (μA)	0.18
%Error	0.69

Table 1. Analytical parameters for adsorptive Linear sweep voltammetric determination of nimesulide.

Pharmaceutical Formulation (tablets)	Taken (mol L ⁻¹)	Found ^a (mol L ⁻¹)	Recovery (%)	R.S.D. ^b
Sulide®	8.0×10^{-7}	7.92×10^{-7}	99.90	1.46
	1.0×10^{-6}	0.99×10^{-6}	99.00	0.94
	1.2×10^{-6}	1.22×10^{-6}	101.60	1.05
	1.5×10^{-6}	1.48×10^{-6}	98.66	1.32
	5.0×10^{-6}	5.00×10^{-6}	100.00	0.89
Nimalox®	8.0×10^{-7}	7.95×10^{-7}	99.37	1.20
	1.0×10^{-6}	1.01×10^{-6}	101.00	0.71
	2.5×10^{-6}	2.47×10^{-6}	98.80	1.43
	5.0×10^{-6}	4.97×10^{-6}	99.40	0.95
	1.0×10^{-5}	1.01×10^{-5}	101.00	0.61

Table 2. Assay and recovery of nimesulide in dosage forms. ^a Average of five determinations. ^b Relative Standard Deviation.

Analytical applications

The proposed procedure was applied to the analysis of nimesulide in commercial tablets. The precision was estimated for different concentrations of the drug ranging from 8.0×10^{-7} to 1.0×10^{-5} mol L⁻¹ of the drug in contained in the tablets using the standard addition method. Recovery studies were carried out after addition of known amounts of the pure drug to various pre-analyzed formulations of nimesulide. The obtained mean percentage recoveries and the relative standard deviations (% R.S.D.) based on the average of five replicate measurements were found to be between 98.66 and 101.66 and between 0.61 and 1.46, respectively (Table 2). The small value of relative standard deviation indicates that the proposed method is highly accurate, precise and reproducible.

In order to investigate the matrix effect of a

complex sample on the method of analysis, experiments were performed to determine the feasibility of using the proposed method to measure nimesulide in spiked serum samples. Recovery studies were conducted with samples containing 1, 2, 3, 4 and 5 $\mu\text{g mL}^{-1}$ of nimesulide. The results of the recovery studies are summarized in Table 3. Excellent recovery was observed indicating that the constituents of the serum samples do not interfere in any way with the detection of this drug. Therefore, the proposed voltammetric method could be used for the determination of nimesulide in serum samples.

CONCLUSION

The optimized and validated method shows good accuracy and precision, wide linearity range and low concentration limits. The pro-

Added conc. ($\mu\text{g mL}^{-1}$)	Found conc. ^a ($\mu\text{g mL}^{-1}$)	Recovery (%)	R.S.D. ^b
1.0	0.98	98.0	1.88
2.0	2.05	102.5	1.41
3.0	3.06	102.0	0.98
4.0	3.92	98.0	1.37
5.0	5.08	101.6	0.94
mean		100.22	
S.D. ^b		2.69	

Table 3. Application of the proposed AdLSV method to determination of nimesulide in spiked human serum samples. ^a Average of five determinations. ^b Relative Standard Deviation.

posed method is simple, fast and can be applied for routine determination of nimesulide in pharmaceutical formulations. The procedure did not require any time-consuming extraction steps prior to the assay of the drug. It seems also appropriate for application to the determination of the drug in biological fluids.

REFERENCES

- Davis, R. & R.N. Brogden (1994) *Drugs* **48**: 431-54.
- Gupta, S. K., R.K. Bhardwaj, P. Tyagi, S. Sen-gupta & T. Velpandian (1999) *Pharmacol. Res.* **39**: 137-41.
- Maltese, A., F. Maugeri & C. Bucolo (2004) *J. Chromatogr. B* **804**: 441-3.
- Ferrario, P. & M. Bianchi (2003) *J. Chromatogr. B* **785**: 227-36.
- Nagaralli, B.S., J. Seetharamappa, B.G. Gowda & M.B. Melwanki (2003) *J. Anal. Chem.* **58**: 778-80.
- Khaksa, G. & N. Udupa (1999) *J. Chromatogr. B: Biomed. Sci. Appl.* **727**: 241-4.
- Ptacek, P., J. Macek & J. Klima (2001) *J. Chromatogr. B: Biomed. Sci. Appl.* **758**: 183-8.
- Panusa, A., G. Multari, G. Incarnato & L. Gagliardi (2007) *J. Pharm. Biomed. Anal.* **43**: 1221-7.
- Carini, M., G. Aldini, R. Stefani, C. Marinello & R.M. Facino (1998) *J. Pharm. Biomed. Anal.* **18**: 201-11.
- Patravale, V. B., S. D'Souza & Y. Narkar (2001) *J. Pharm. Biomed. Anal.* **25**: 685-8.
- Tubic, B., B. Ivkovic, M. Zecevic & S. Vladimirov (2007) *Acta Chim. Slov.* **54**: 583-90.
- Nagaraja, P., H.S. Yathirajan, H.R. Arunkumar & R.A. Vasantha (2009) *J. Pharm. Biomed. Anal.* **29**: 277-82.
- Altinoz, S. & O.O. Dursun (2000) *J. Pharm. Biomed. Anal.* **22**: 175-82.
- Chen, Y.L. & S.M. Wu (2005) *Anal. Biomed. Chem. Anal.* **381**: 907-12.
- Catarino, R.I.L., A.C.L. Coneicao, M.B.Q. Garcia, M.L.S. Concalves, J.L.F.C. Lima & M.M.C. dos Santos (2003) *J. Pharm. Biomed. Anal.* **33**: 571-80.
- Wang, C., X. Shao, Q. Liu, G. Yang & X. Hu (2006) *J. Pharm. Biomed. Anal.* **42**: 237-44.
- Furlanetto, S., S. Orlandini, G. Aldini, R. Gotti, E. Dreassi & S. Pinzauti (2000) *Anal. Chim. Acta* **413**: 229-39.
- Reddy, R. & P. Reddy (1996) *Ind. J. Pharm Sci.* **58**: 96-9.
- Alvarez-Lueje, A., P. Vasquez, L.J. Nunez-Vergara & J.A. Squella (1997) *Electroanalysis* **9**: 1209-13.
- Wang, C., X. Shao, Q. Liu, Q. Qu, G. Yang & X. Hu (2006) *J. Pharm. Biomed. Anal.* **42**: 237-44.
- Kumar, K.G., P. Augustine & S. John (2007) *Portog. Electrochem. Acta* **25**: 375-81.
- Miller, J.N. & J.C. Miller (2000) *Statistics and Chemometrics for Analytical Chemistry*, 4th ed., Prentice Hall, Harlow, pp. 120-3.
- Lund, H. (1990) *Cathodic Reduction of Nitro and Related Compounds*, in *Organic Electrochemistry* (H. Lund and M.M. Baizer, eds.), p. 411, M. Dekker Inc. New York 3rd Ed.
- Brett, C.M.A. & A.M.O. Brett (1994) *Electrochemistry, Principles, Methods and Applications*, Oxford University Press, New Yourk, 2nd Edn., 183-5.
- Zuman, P. & Z. Fijalek (1990) *J. Electroanal. Chem.* **296**: 589-93.
- El-Jammal, A., J.C. Vire, G.J. Patriarche & O.N. Palmeiro (1992) *Electroanalysis* **4**: 57-64.
- Lu, S., K. Wu, X. Dang & S. Hu (2004) *Talanta* **63**: 653-7.
- Carbajo, J., S. Bollo, L.J. Nunez-Vergara, A. Campero & J.A. Squella (2002) *J. Electroanal. Chem.* **531**: 187-94.