Full Paper

A Graphite-Polyurethane Composite Electrode for the Analysis of Furosemide

Felipe S. Semaan,[a,b] *Edilson M. Pinto*,[b] *Éder T. G. Cavalheiro*,[a] *Christopher M. A. Brett*[b]*

[a] Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos-SP, Brazil
[b] Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal
*e-mail: brett@ci.uc.pt

Received: May 8, 2008
Accepted: August 12, 2008

Abstract
A graphite-polyurethane composite electrode has been used for the determination of furosemide, a antihypertensive drug, in pharmaceutical samples by anodic oxidation. Cyclic voltammetry and electrochemical impedance spectroscopy were used to characterize the electrooxidation process at +1.0 V vs. SCE over a wide pH range, with the result that no adsorption of analyte or products occurs, unlike at other carbon-based electrode materials. Quantification was carried out using cyclic voltammetry, differential pulse voltammetry, and square-wave voltammetry. Linear ranges were determined (up to 21 μmol L^{-1} with cyclic voltammetry) as well as limits of detection (0.15 μmol L^{-1} by differential pulse voltammetry). Four different types of commercial samples were successfully analyzed. Recovery tests were performed which agreed with those obtained by spectrophotometric evaluation. The advantages of this electrode material for repetitive analyzes, due to the fact that no electrode surface renewal is needed owing to the lack of adsorption, are highlighted.

Keywords: Composite electrode, Graphite-polyurethane electrode, Furosemide

DOI: 10.1002/elan.200804329

1. Introduction

Furosemide, also called frusemide (4-chloro-*N*-furfuryl-sulfamoylantranilic acid) is a sulfonamide initially described as an antibacterial agent but, due to its intense and fast diuretic effect, also became used as an antihypertensive drug, being currently sold in the form of tablets and as parenteral solutions [1, 2]. The structure of the neutral form of furosemide is shown in Figure 1; the protonated form has four ionizable acid groups [3, 4]. It is soluble in alkaline media, as well as in organic solvents such as acetone and methanol; however, it has a very low solubility in water and acidic aqueous solutions [2].

A wide range of reports has been published concerning the determination of this analyte and/or its products, after reactions such as hydrolysis, oxidation, complexation, or diazotization, in commercial samples and in biological matrices. Chromatographic separation has been followed by detection methods such as UV-vis spectrophotometry [5–7], or amperometric detection at glassy carbon [8] or carbon fiber microelectrodes [9]. Additionally, optical methods based on absorption or emission [10–40], direct electrochemical [41, 42], and titrimetric procedures [43–45] have also been used, with a clear predominance of spectrophotometric compared to electrochemical methods. Reviews presenting the 'state of the art' for furosemide determination [46–48], reported lowest limits of detection of 3×10^{-10} mol L^{-1} using HPLC-MS and 2.1×10^{-8} mol L^{-1}, employing micellar liquid chromatography with a diode array detector.

A comparison between the analytical parameters obtained by various electrochemical procedures is presented in Table 1. A limit of detection close to 1.5×10^{-7} mol L^{-1} is found at glassy carbon electrodes, but with the necessity of renewal of the electrode surface after each measurement, in order to achieve reproducible responses [41]; limits of detection of 1.7×10^{-7} mol L^{-1} (using flow injection analysis) and 5.5×10^{-7} mol L^{-1} using HPLC with electrochemical detection were found in [8] at glassy carbon electrodes. In the latter case, the oxidation peak potential had a relatively high value, close to +1.25 V vs. Ag/AgCl (1 M KCl), electrode surface pre-treatment being needed before each measurement. For electrochemical determinations, the main problem which causes most difficulties is adsorption of the analyte or its reaction products on the electrode surface.

Apart from solid carbon electrodes, such as glassy carbon, composite electrodes have also been investigated for use in oxidative electroanalytical procedures. The first description of carbon paste composites, by Adams, dates from 1958 [49], when new composite materials began to be evaluated in order to substitute mercury and extend the use of electrochemical techniques to potential ranges where mercury is not useable. A composite was defined by Tallman and Petersen [50] as a mixture of components, each with different properties, leading to a new material with new

Table 1. Electroanalytical procedures for determination of furosemide in the literature.

Detection	Media	*LOD* (mol dm^{-3})	Comments	References
Amperometric detection at a glassy carbon electrode (+1.3 V vs. Ag/AgCl) coupled to HPLC	Water-acetonitrile (30:70)	4.5×10^{-8}	Liquid-liquid extraction needed (ethyl acetate).	[8]
Amperometric detection at carbon fiber microelectrodes (+1.25 V vs. Ag/AgCl) coupled to HPLC and FIA	Acetonitrile-water (25:75) with 5 mmol L^{-1} NaH_2PO_4 (HPLC), and 5 mmol L^{-1} NaH_2PO_4 pH 6.5 (FIA)	5.5×10^{-7} (HPLC), 1.7×10^{-7} (FIA)	Surface fouling, electrochemical treatment needed after each measurement	[9]
Voltammetric detection at glassy carbon electrodes (+1.2 V vs. Ag/AgCl)	Methanol-water (10:90)	1.5×10^{-7}	Surface fouling, surface renewal by polishing followed by methanol and water, liquid-liquid extraction needed (ethyl acetate).	[41]

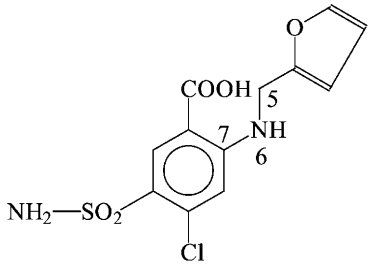

Fig. 1. Structure of the neutral form of furosemide.

properties. Among the components it is necessary to have at least one insulator and one conductor.

This work reports the use of a composite electrode with graphite as conducting phase in a castor-oil polyurethane resin of vegetable origin as insulating phase for the analysis of furosemide in pharmaceutical samples. This graphite-polyurethane composite began to be studied in 2002 [51], in which the best conditions of preparation, cure and use were described. Analytical applications to the determination of hydroquinone in photographic developers [52], of imipramine [53] and atenolol [54] in pharmaceutical formulations, of dopamine in synthetic cerebrospinal fluid [55] and of indoleacetic acid in the environment [56] have been demonstrated. Advantages of such composites are the possibility of manufacture in different shapes and sizes, the easy addition of chemical modifiers to the composite, applicability over a wide range of pH and in different solvents, and low cost of production.

The application of this composite as an electrode material in the analysis of furosemide, without problems of adsorption of furosemide itself or of its oxidation products, is demonstrated.

2. Experimental

2.1. Reagents and Solutions

All reagents were of analytical grade and were used as received; solutions were prepared by direct dissolution of the salts in Millipore Milli-Q ultrapure water (resistivity >18 MΩ cm). A 1.00 mmol L^{-1} stock solution of furosemide was prepared in 1.0 mmol L^{-1} NaOH, and was diluted as necessary on the day of use.

Table 2. Buffer supporting electrolyte solutions, final concentration 0.1 mol dm^{-3}, made from component solutions of concentration 0.2 mol dm^{-3} with dilution by a factor of two.

Composition	pH
HCl + KCl	1.2
HCl + KCl	2.0
HOAc + NaOAc	3.3
HOAc + NaOAc	4.0
HOAc + NaOAc	5.3
$Na_2HPO_4 + NaH_2PO_4$	5.8
$Na_2HPO_4 + NaH_2PO_4$	6.9
$Na_2HPO_4 + NaH_2PO_4$	8.0
$Na_2B_4O_7.10H_2O$ + NaOH	9.3
NaOH + KCl	12.2
NaOH + KCl	13.0

Commercial tablets, containing a nominal amount of 40 mg of furosemide per tablet, were purchased in Brazil (Furosix, Neosemid, Pharlab, and Teuto). Twenty tablets were triturated, according to the procedure outlined in the United States Pharmacopeia [57, 58] until a fine and homogeneous powder was obtained, from which chosen, weighed amounts were taken and dissolved in 1.0 mmol L^{-1} NaOH solution to give a final concentration of ca. 1 mmol L^{-1} furosemide. No previous separation or preparation step was needed.

Buffer solutions (pH from 1.2 to 13.0) were prepared according to Table 2 for characterizing the electrooxidation process at different pH values and for evaluating the best conditions for the analytical determination of furosemide.

2.2. Composite Electrode Preparation

Polyurethane resin was prepared by mixing 0.85 parts of prepolymer A-249 and 1.0 part of polyol B-471 (Poliquil,

Brazil). Suitable amounts of graphite powder, 1–2 μm diameter (Aldrich, USA) were added in order to reach 60% in mass. This mixture was homogenized in a mortar during 15 minutes and then extruded as 3.0 mm diameter rods [50]. The rods were left to cure during 24 hours and then cut into 1 cm sections. Contacts were made using silver epoxy glue and copper wire, and the assembly was sealed in non-conducting epoxide resin. The surfaces were polished using abrasive paper followed by α-Al_2O_3 of 1.0 μm diameter (Arotec, Brazil).

The electroactive surface area was determined by cyclic voltammetry, using 5 mmol L^{-1} hexacyanoferrate(II) in 0.5 mol L^{-1} KCl and different potential scan rates (10–75 mV s^{-1}): and applying Equation 1:

$$I_{pa}\ (A) = 2.95 \times 10^5\ n^{3/2}\ A\ D^{1/2}\ c_\infty\ v^{1/2} \quad (1)$$

where n is number of electrons involved in the oxidation, A is the electroactive area (cm^2), D is the diffusion coefficient of hexacyanoferrate(II) in $cm^2\ s^{-1}$ ($7.7 \times 10^{-6}\ cm^2\ s^{-1}$ in 0.5 mol L^{-1} KCl [59]), c_∞ the bulk concentration of hexacyanoferrate(II) in mol cm^{-3}, and v the potential scan rate, in V s^{-1}. From a plot of peak current vs. scan rate the electroactive area was found to be ca. 0.043 cm^2, corresponding to 61% of the geometric area.

2.3. Apparatus

Voltammetric experiments were carried out with a computer-controlled μ-Autolab Type II potentiostat/galvanostat with GPES 4.9 software (Eco Chemie, Netherlands). A three-electrode cell, with 20 mL capacity, was used; the reference electrode was a saturated calomel electrode (SCE) and the counter electrode was platinum foil (area 1 cm^2).

Electrochemical impedance measurements were carried out in the same electrochemical cell with a PC-controlled Solartron 1250 Frequency Response Analyzer coupled to a Solartron 1286 Electrochemical Interface using ZPlot 2.4 software (Solartron Analytical, UK), frequency scans were from 65000 Hz down to 0.1 Hz with ten measurements per frequency decade, using a sinusoidal voltage perturbation of 10 mV rms. For comparative studies by UV-visible spectrophotometry, a Specord S100 Carl Zeiss spectrophotometer was used, at a wavelength of 271 nm. All measurements were carried out at room temperature ($25 \pm 1\,^\circ C$) without deaeration of solutions.

2.4. Procedures

Cyclic voltammetry was carried out in different buffer solutions of pH between 1.2 and 13.0, from −1.0 to +1.2 V (vs. SCE). Five sequential cycles were recorded and the difference in response between blank and spiked solutions (6.5×10^{-5} mol L^{-1}) was measured.

Quantification was carried out by cyclic, differential pulse and square-wave voltammetry. In cyclic voltammetry, scan rates from 10 to 100 mV s^{-1} were tested. For DPV, an effective scan rate of 10 mV s^{-1} (10 mV scan increment) was applied for amplitude modulation optimization (10, 25, 50, and 100 mV), then the optimum amplitude was fixed in order to find the best apparent scan rate (10, 25, 50, and 100 mV s^{-1}). In the case of SWV, the frequency (10, 25, 50 and 100 Hz), and step potential (1, 2, and 5 mV) were both varied as well as the amplitude (10 to 50 mV).

Electrochemical impedance spectroscopy studies were done in order to evaluate the electrode process and the occurrence of any adsorption phenomena, first at +1.0 V vs. SCE, for blank and spiked solutions (0.12 mmol L^{-1}, pH 3.3). Spectra were then also recorded at different furosemide concentrations (8 and 16 μmol L^{-1}) and potentials (+0.5, +0.75, +1.0, and +1.1 V vs. SCE).

2.5. Spectrophotometric Reference Procedure

The samples were analyzed by UV-vis spectrophotometry, and compared with a solution of known concentration using NaOH as extractor solution, with detection at 271 nm. The details of this procedure are described in the United States Pharmacopoeia [57]. Although the most recent USA Pharmacopoeia suggests the use of HPLC procedures [58], in the present case the UV-vis procedure was chosen due to its simplicity and low waste generation.

3. Results and Discussion

3.1. Influence of pH and Mechanistic Studies

Cyclic voltammetry experiments were carried out in different buffer solutions from pH 1.2 to 13.0 in order to choose the most suitable value of pH for the electroanalytical measurements. The best definition of signals was in pH 3.3 acetate buffer solution, with an oxidation peak at +1.0 V vs. SCE at pH 3.3 (see Fig. 2). Figure 3a shows the relationship between pH and peak potential, up to pH 6 (above this pH no signal was obtained), with slope close to 30 mV up to pH 4 which suggests the involvement of two electrons for each proton in the electrochemical oxidation of furosemide. The relationship between log I_{pa} and log scan rate with slope close to 0.5 shows a diffusion-controlled mechanism, with no adsorption (see Fig. 3b).

The oxidation was also studied by differential pulse voltammetry and square-wave voltammetry. The oxidation peak half-width, $W_{1/2}$, is close to 70 mV, suggesting a two-electron irreversible process, confirmed by the fact that the SWV backward component current shows no cathodic peak (data not shown). These results are in agreement with those from cyclic voltammetry here and in [41], where values for αn were shown to be in agreement with two-electron processes.

In order to propose a possible electrooxidation mechanism, it is necessary to consider the different species which exist as a function of pH. The determination of the pK_a of a substance with multiple deprotonation steps and low solubility is not easy to perform [3]. Values have been given for deprotonation involving the successive reactions:

$$H_3A^+ \overset{pK_1}{\rightleftharpoons} H_2A \overset{pK_2}{\rightleftharpoons} HA^- \overset{pK_3}{\rightleftharpoons} A^{2-} \overset{pK_4}{\rightleftharpoons} A^{3-}$$

related to the protonated nitrogen at position 6, the carboxylic group, a second proton from the nitrogen at position 6 and the sulfonamide group, respectively (see Fig. 1 for the structure of the neutral form H_2A).

Experimental values obtained by different techniques estimate p$K_1 \approx 0.5$ from octanol/water partition measurements [3] and p$K_2 = 3.9$; 3.8; 3.65 or 3.6, determined by aqueous solution titration; mixed solvents titration; octanol/water partition and solubility methods respectively [3]. Using liquid chromatography a 3.9 value has been assigned to the carboxylic acid dissociation [4].

These data confirm that in the pH range studied where a signal is obtained, up to pH 6, the species occurring are the neutral species shown in Figure 1 and that corresponding to ionization of the carboxyl group. The potential at which oxidation occurs suggests that it is of the amine at position 6, which will initially give rise to a cation radical. In order to explain the experimental observations (loss of 2 electrons and one proton), this must then be immediately followed by loss of a second electron and of a proton. The highly reactive species which are produced, could dimerize and undergo other reactions. It is extremely likely that the cation radical undergoes a homogeneous reaction very quickly because otherwise the effects of the following chemical reaction would be seen in the cyclic voltammograms [60] and/or a strong interaction with the electrode surface would occur, leading to blocking adsorption, as occurred in [41, 42].

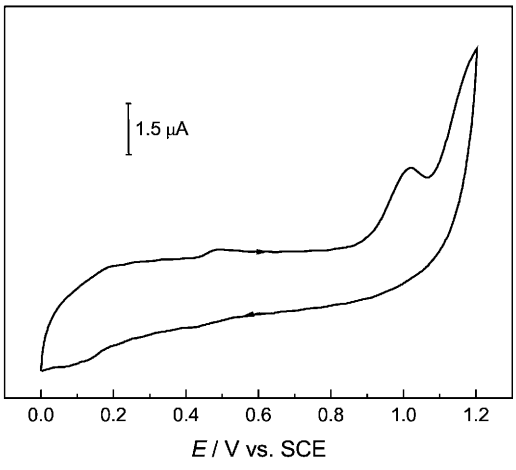

Fig. 2. Cyclic voltammogram of 1.3×10^{-4} mol L^{-1} furosemide in pH 3.3 acetate buffer solution, scan rate 100 mV s^{-1}.

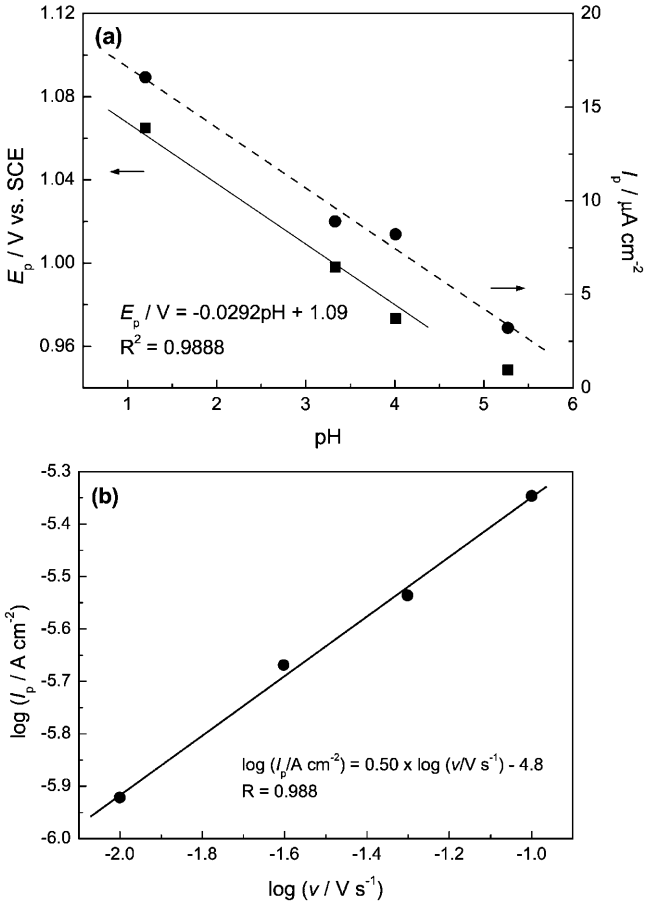

Fig. 3. a) Dependence of cyclic voltammetric peak current, I_p, (●) and peak potential, E_p, (■) on pH for 1.3×10^{-4} mol L^{-1} furosemide; scan rate (v) 100 mV s^{-1}. b) Plot of log I_p vs. log v for 6.5×10^{-5} mol L^{-1} furosemide in pH 3.3 acetate buffer electrolyte.

3.2. Electrochemical Impedance Spectroscopy

Electrochemical impedance spectroscopy experiments were done with the aim of evaluating the possibility of analyte adsorption on the electrode surface. Impedance spectra were recorded in blank solutions, as well as before and after fifteen successive potential cycles in 0.12 mmol L^{-1} furosemide – the electrode then being washed with water without polishing, and replaced in the cell.

Impedance spectra, recorded at potentials lower and higher than the oxidation peak (+0.50, +0.75 and +1.10 V vs. SCE), in the absence of furosemide and after cycling in solutions spiked with 8 and 16 µmol L^{-1} furosemide, are shown in Figure 4. An equivalent electrical circuit with a cell resistance, R_Ω, in series with a parallel combination of a Constant Phase Element, CPE, and a charge transfer resistance was used to fit the curves. In all cases, the $CPE = \{C\ (i\omega)^\alpha\}^{-1}$ models a nonideal capacitor. The CPE was found to be necessary because of the heterogeneous nature of the electrode, expressed through the exponent α. A typical value of ca. 0.80 was obtained for all spectra, independent of the presence of furosemide. The cell

resistance R_Ω was around 192 Ω cm^2, a value expected for a composite electrode.

At +0.5 V, the general features of the spectra are similar in the absence or presence of furosemide, and are characterized by an inclined straight-line shape. The observed effect can be considered as a non-ideal capacitor, due to the intrinsic characteristic of the electrode material. At +0.75 and +1.0 V (vs. SCE), the same tendencies in the curves were observed. Important information obtained, comparing all spectra, is that the values of imaginary impedance (Z'') do not change significantly with increasing potential, indicating that the electrode surface has a well-defined behavior.

At +1.1 V (vs. SCE), close to the positive limit of the potential window, the spectra shape becomes similar to that of a semicircle, with a corresponding decrease in R values. At this potential, the capacitance values are more or less constant. The reason is the beginning of solvent decomposition.

Since no significant differences in the spectra were observed after exposure to furosemide at any of these potentials the conclusion is that there was no adsorption of analyte or its oxidation products. The differences observed in charge transfer are due only to the increase in the applied potential.

3.3. Electroanalytical Procedures

Electroanalytical procedures were developed using cyclic, differential pulse and square-wave voltammetry and the results are presented in Table 3.

Using cyclic voltammetry at pH 3.3, , the electrode was scanned at 100 mV s^{-1} between 0 and +1.2 V vs. SCE; for blank, and three successive sample additions, see Figure 5. A linear dependence of peak current on furosemide concentration, was observed, illustrated for furosemide from Neosemid, between 8 and 21 μmol L^{-1} (I_p (A) = 0.12 $C + 3 \times 10^{-6}$, $R^2 = 0.9996$, $n = 5$); limits of detection and quantification were 2.8 and 8.4 μmol L^{-1}, respectively. Recovery tests led to values between 96 and 101%.

Differential pulse voltammetry, Figure 6, was also used to quantify the analyte. In this case, the best determination conditions were found using an amplitude of 100 mV, and an effective scan rate of 25 mV s^{-1}, scanning the potential from +0.5 to +1.2 V (vs. SCE). A peak was observed at +0.9 V. The linear range was from 0.75 to 6.5 μmol L^{-1} (I_p (A) = 0.2 $C + 1.5 \times 10^{-6}$, $R^2 = 0.998$, $n = 6$, where C is the concentration of furosemide); limits of detection and quantification were 0.15 and 0.46 μmol L^{-1}, respectively. Teuto commercial tablet samples were analyzed in order to evaluate recovery, showing recoveries from 98 to 102%.

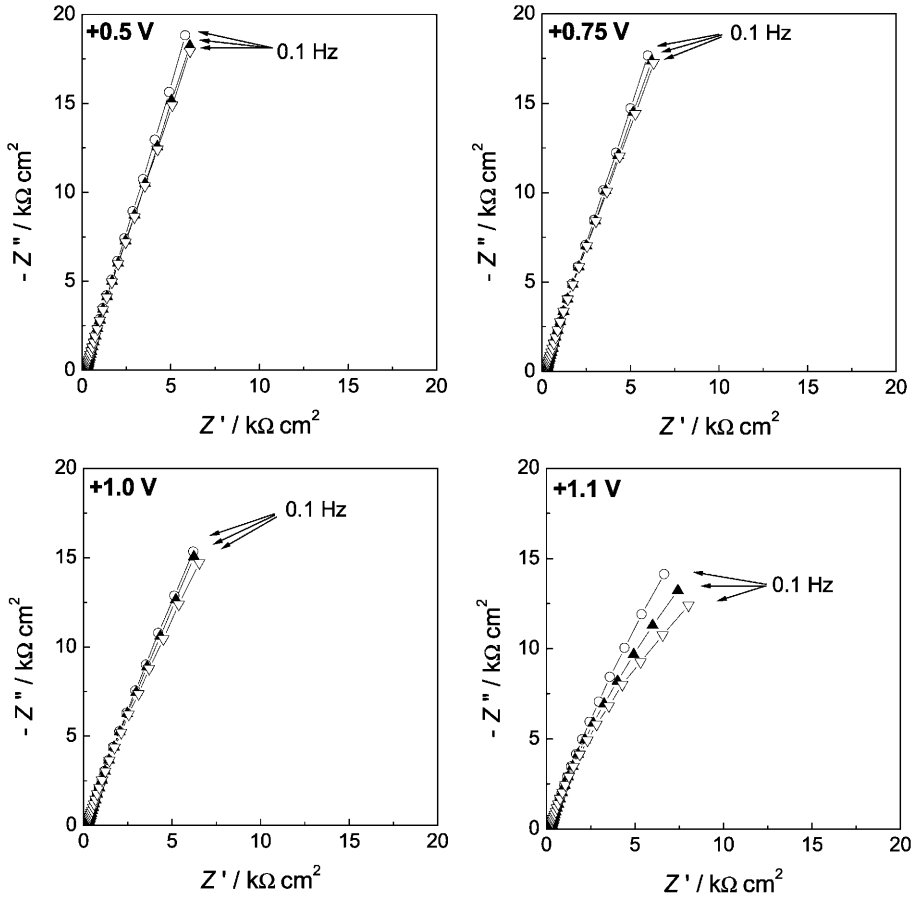

Fig. 4. Electrochemical impedance spectra for (○) blank, (▲) 8, and (▽) 16 μmol L^{-1} furosemide spiked solutions at +0.5, 0.75, 1.0 and 1.1 V vs. SCE.

Table 3. Analysis of furosemide (mg per tablet) in commercial samples, declared amount of 40 mg per tablet.

	CV [a]	DPV [a]	SWV [a]	Spectrophotometric method [b]
Furosix	41.2 ± 1.1	40.2 ± 2.0	41.6 ± 0.3	42.4
Neosemid	42.2 ± 1.2	42.1 ± 0.3	38.8 ± 0.3	43.3
Pharlab	40.5 ± 2.0	41.3 ± 2.0	40.4 ± 0.4	39.5
Teuto	41.9 ± 0.4	38.1 ± 0.6	41.5 ± 0.4	41.8

[a] $n = 3$
[b] according to [57]

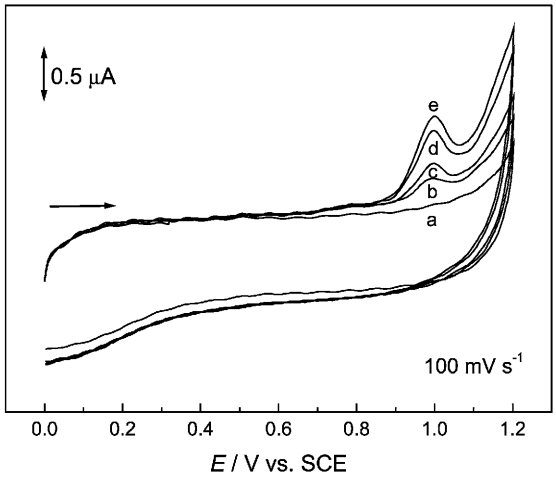

Fig. 5. Cyclic voltammograms for the analysis of Neosemid, under optimized conditions: a) blank, b) sample, and plus c) 8.0, d) 16.0, e) 20.0 μmol L^{-1} furosemide.

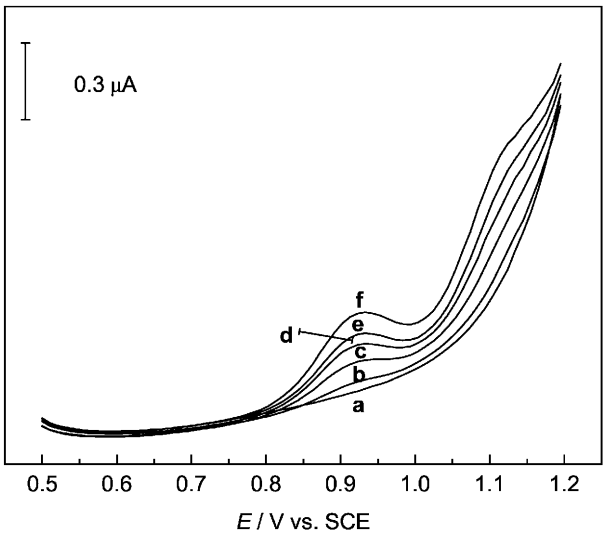

Fig. 6. Differential-pulse voltammograms for the analysis of Furosix, under optimized conditions: a) blank, b) sample, and plus c) 2.2, d) 3.7, e) 5.1, f) 6.5 μmol L^{-1} furosemide.

Experiments using square-wave voltammetry were also carried out. Best conditions were found using 10 Hz frequency and 5 mV potential increment, with 25 mV amplitude, scanning from +0.6 to +1.2 V vs. SCE, the oxidation peak appearing at +0.96 V. The linear range was from 3 to 9 μmol L^{-1} (I_p (A) = 0.1 C + 1.5 × 10^{-7}, R^2 = 0.988, $n = 6$); limits of detection and quantifications of 0.96 and 3.2 μmol L^{-1}, respectively, were found. Recovery tests with furosemide from Pharlab tablets led to results from 103 to 104%.

Careful examination of the results obtained suggests that differential pulse voltammetry is probably the technique which offers the best results in terms of linear range, detection limit and recovery.

3.4. Statistical Evaluation and Comparison with the Reference Method

In order to compare the applicability of the developed methods, student *t*-paired tests were done, showing agreement at the 95% confidence level for Furosix, Neosemid, and Pharlab samples, and 99% confidence level for Teuto. Results were also compared with those found using the spectrophotometric reference method, reaching the same conclusions concerning applicability to the analysis of commercial samples.

4. Conclusions

A graphite-polyurethane composite electrode has been used for voltammetric quantification of furosemide in commercial samples without adsorption of furosemide or its oxidation products. A possible mechanism for furosemide oxidation is suggested. On the graphite-polyurethane electrode material there is no analyte adsorption, shown by voltammetric techniques and by electrochemical impedance, thus demonstrating its viability for repetitive determinations without electrode surface regeneration or renewal.

This new experimental procedure is faster and more reproducible than previously-developed electrochemical detection methods. It can be expected that similar advantages of avoiding adsorption by using this composite electrode will be found for other pharmaceutical compounds, thus enhancing the applicability of such electroanalytical procedures.

5. Acknowledgements

Financial support from the Brazil/Portugal Bilateral Agreement (CAPES/FCT 177/07), FAPESP-Brazil (04/08550-0

and 05/04297-1), and ICEMS-Coimbra, Research Unit 103 (FCT), is gratefully acknowledged. FSS thanks CAPES for a PhD and post-doctoral grant (2670/06-2 and 3183/07-6) and EMP thanks FCT for a PhD grant (SFRH/BD/31483/2006).

6. References

[1] J. N. Delgado, W. A. Remers, *Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 9th ed., J. B. Lippincot, Philadelphia, **1991**.
[2] S. S. Budavari, M. J. O'Neil, A. Smith, P. E. Heckelman, J. F. Kinneary, *The Merck Index*, 13th ed., Merck, Whitehouse Station, New Jersey **2001**.
[3] N. Sistovaris, Y. Hamachi, T. Kuriki, *Fresenius J. Anal. Chem.* **1991**, *340*, 345.
[4] M. Manderscheid, T. Eighinger, *J. Chromatogr. Sci.* **2003**, *41*, 323.
[5] C. D. Mills, C. Whitforth, L. P. Rybbak, C. M. Henley, *J. Chromatogr. B* **1997**, *701*, 65.
[6] Y. S. El-Saharty, *J. Pharm. Biomed. Anal*. **2003**, *33*, 699.
[7] F. S. Semaan, A. J. Santos-Neto, F. M. Lanças, E. T. G. Cavalheiro, *Anal. Lett.* **2005**, *38*, 1651.
[8] M. B. Barroso, R. M. Alonso, R. M. Jimenez, *J. Liquid Chromatogr. Related Technol.* **1996**, *19*, 231.
[9] A. Guzmán, L. Agüí, M. Pedrero, P. Yáñez-Sedeño, J. M. Pingarrón, *J. Pharm. Biomed. Anal.* **2003**, *33*, 923.
[10] S. Agatonovic-Kustrin, L. Zivanovic, D. Radulovic, D. Pecanac, *J. Pharm. Biomed. Anal.* **1990**, *8*, 983.
[11] M. S. Garcia, C. Sánchez-Pedreño, M. I. Alberto, V. Ródenas, *J. Pharm. Biomed. Anal.* **1997**, *15*, 453.
[12] Y. Rao, X. Zhang, G. Luo, W. R. G. Bayens, *Anal. Chim. Acta* **1999**, *396*, 273.
[13] S. V. Beltyukova, E. I. Tselik, A. V. Egorova, *J. Pharm. Biomed. Anal.* **1998**, *18*, 261.
[14] P. C. Ioannon, V. Rusakova, D. A. Andrikoupoulo, K. M. Glynou, V. Tzompanaki, *Analyst* **1998**, *123*, 2839.
[15] N. A. Georges, *Anal. Chim. Acta* **2003**, *476*, 149.
[16] P. Parimoo, A. Bharati, K. Padma, *Ind. J. Pharm. Sci.* **1995**, *57*, 126.
[17] E. Casassas, J. L. Fabregas, *Anal. Chim. Acta* **1979**, *106*, 151.
[18] M. C. F. Ferraro, P. M. Castellano, T. S. Kaufman, *J. Pharm. Biomed. Anal.* **2001**, *26*, 443.
[19] I. L. T. Dias, J. L. S. Martins, G. O. Neto, *Anal. Lett.* **2005**, *38*, 1159.
[20] J. S. Millership, C. Parker, D. Donnelly, *Il Farmaco* **2005**, *60*, 333.
[21] M. I. Toral, S. Pope, S. Quintanilla, P. Richter, *Int. J. Pharm.* **2002**, *249*, 117.
[22] S. Gangwal, P. Trivedi, *Ind. Drugs* **1998**, *35*, 412.
[23] R. Panchagnula, K. Kaur, A. Sood, I. Singh, *J. Pharm. Sci.* **1997**, *3*, 425.
[24] M. H. Abdel-Hay, *Int. J. Pharm.* **1993**, *99*, 333.
[25] H. Salem, M. El-Maamii, M. El-Sadek, A. Kheir, *Spectrosc. Lett.* **1991**, *24*, 451.
[26] F. S. Semaan, R. A. Sousa, E. T. G. Cavalheiro, *J. Flow Injection Anal.* **2005**, *2*, 34.
[27] P. B. Isospoulos, Fresenius *J. Anal. Chem.* **1989**, *334*, 554.
[28] C. S. P. Sastry, T. N. V. Prassad, B. S. Tata, B. S. Sastry, S. Bhetanabhotla, E. V. Rao, *Analyst* **1988**, *113*, 255.
[29] L. Zivanovic, S. Agatonovic-Kustrin, D. Radulovic, *Pharmazie* **1990**, *45*, 935.
[30] L. Zivanovic, S. Agatonovic-Kustrin, D. Radulovic, *Mikrochim. Acta* **1990**, *1*, 49.
[31] P. Mishra, D. Katrolia, R. K. Agrawal, *Curr. Sci.* **1989**, *58*, 503.
[32] J. Shah, M. R. Jan, M. A. Khan, *J. Chin. Chem. Soc.* **2005**, *52*, 347.
[33] A. Sevillano-Cabeza, P. Campins-Falco, M. Serrador-Garcia, *Anal. Lett.* **1997**, *30*, 91.
[34] J. S. Esteve-Romero, E. F. Simo-Alfonso, M. C. Garcia-Alvarez-Coque, G. Ramos-Ramos, *Talanta* **1993**, *40*, 1711.
[35] C. S. P. Sastry, K. R. Srinivas, K. M. M. K. Prassad, *Ind. J. Pharm. Sci.* **1996**, 58, 120.
[36] C. S. P. Sastry, M. V. Suryanarayana, A. S. R. P. Tipirneni, B. S. Sastry, *Ind. Drugs* **1989**, *26*, 714.
[37] T. N. V. Prasad, B. S. Sastry, E. V. Rao, C. S.P. Sastry, *Pharmazie* **1987**, *42*, 135.
[38] R. Matsuda, Y. Takeda, M. Ishibashi, M. Uchiyama, M. Suzuki, S. Takitani, *Busenki Kagaku* **1986**, *35*, 151.
[39] T. Takeuchi, Y. Kabasawa, R. Horikawa, T. Tanimura, *Analyst* **1988**, *113*, 1673.
[40] H. Ichiba, M. Morishita, T. Yajima, *Chem. Pharm. Bull.* **1988**, *36*, 5009.
[41] M. B. Barroso, R. M. Alonso, R. M. Jimenes, *Anal. Chim. Acta* **1995**, *305*, 332.
[42] I. L. T. Dias, G. Oliveira-Neto, D. C. Vendramini, C. Sommer, J. L. S. Martins, L. T. Kubota, *Anal. Lett.* **2004**, *37*, 35.
[43] S. A. Kulichenko, G. M. Schevchenko, *Anal. Bioanal. Chem.* **2003**, *375*, 255.
[44] S. A. Kulichenko, S. A. Fesenko, *J. Anal. Chem.* **2002**, *57*, 231.
[45] K. Basavaiah, U. Chandraschekar, P. Nagegowda, *Ind. J. Chem. Technol.* **2005**, *12*, 401.
[46] I. L. T. Dias, G. Oliveira-Neto, J. L. S. Martins, *Lecta* **2004**, *1*, 19.
[47] F. S. Semaan, E. T. G. Cavalheiro, *Anal. Lett.* **2006**, *39*, 2557.
[48] M. J. Ruíz-Rangel, A. Berthod, S. Carda-Broch, M. C. Garcia-Álvarez-Coque, *Sep. Purif. Rev.* **2006**, *35*, 39.
[49] R. N. Adams, *Anal. Chem.* **1958**, *30*, 1576.
[50] D. E. Tallman, S. L. Petersen, *Electroanalysis* **1990**, *2*, 499.
[51] R. K. Mendes, S. Claro-Neto, E. T. G. Cavalheiro, *Talanta* **2002**, *57*, 909.
[52] R. K. Mendes, P. Cervini, E. T. G. Cavalheiro, *Talanta* **2006**, *68*, 708.
[53] R. A. Toledo, M. C. Santos, K. M. Honório, A. B. F. da Silva, E. T. G. Cavalheiro, L. H. Mazo, *Anal. Lett*. **2006**, *39*, 507.
[54] P. Cervini, L. A. Ramos, E. T. G. Cavalheiro, *Talanta* **2007**, *72*, 206.
[55] R. A. Toledo, M. C. Santos, E. T. G. Cavalheiro, L. H. Mazo, *Anal. Bioanal. Chem.* **2005**, *381*, 1161.
[56] R. A. Toledo, C. M. P. Vaz, *Microchem. J.* **2007**, *86*, 161.
[57] T*he United States Pharmacopeia*, Vol. XXI, United States Pharmacopeial Convention, Rockville, MD **1985**.
[58] T*he United States Pharmacopeia*, Vol. XXVIII, United States Pharmacopeial Convention, Rockville, MD **2003**.
[59] M. Stackelberg, M. Pilgram, V. Toome, *Z. Electrochem.* **1953**, *57*, 342.
[60] L. Nadjo, J. M. Saveant, *J. Electroanal. Chem*. **1973**, *48*, 113.

