Determination of Nifuroxazide by Flow Injection Linear Adsorptive Stripping Voltammetry on a Screen-Printed Carbon Nanofiber Modified Electrode

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Abstract
A new, sensitive, reproducible and easy-to-use flow-injection method for determining nifuroxazide based on adsorptive stripping linear sweep voltammetry over screen-printed carbon electrodes is presented. The presented method is the first report of Nifuroxazide determination using a screen printed electrode and FIA and it is useful for pharmaceutical quality control, specifically, it could be adopted as an effective portable tool for on the shelf quality control at pharmacies.Limits of quantification (*LOQ*) and detection (*LOD*) as low as 42 and 10 ng/mL, respectively, were found. An average recovery of 100.7 %, and a standard deviation of 2.5 % indicates acceptable accuracy and precision validating the proposed method.

Keywords: Nifuroxazide, Flow injection analysis, Adsorptive stripping, Screen-printed carbon electrode

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1 Introduction

Nifuroxazide (Figure 1) is a nitrofuran derivative that is a useful component of drugs used to treat gastrointestinal disorders caused by bacteria. In commercial formulations, it is usually accompanied by other active components that promote absorption by pathogens, which improves their pharmacological activity.

The analytical determination of nifuroxazide in pharmaceutical compositions, urine and plasma, has been published by several authors using different electrochemical [1–5], spectrophotometric [6], and chromatographic [7,8] analytical systems. The electrochemical methods involved diferential pulse polarographic (DPP) determination of nifuroxazide in capsule and tablets [3,5], as well as adsorptive stripping voltammetry (AdSV) at mercury and carbon paste electrodes for determination of nifuroxazide in urine and human serum [1,4]. Also the electrooxidation of nifuroxazide was investigated by cyclic (CV) and differential pulse voltammetry (DPV) at both, unmodified and sephadex-modified carbon paste electrodes [2].

AdSV is a technique that is extensively used for inorganic and organic determination in both batch and flow systems [9]. Specifically there are a lot of applications combining FIA with AdSV analysis to the determination of pharmaceuticals. Some selected examples are the sequential-injection analysis of nifuroxime using DNA-modified electrodes [10], the determination of curcumin using FIA and AdSV at a multiwalled carbon nanotube (MWCNT) modified electrode [11] and the determination of fluoxetine in pharmaceuticals and serum using FIA and AdSV at a hanging mercury drop electrode (HMDE) [12]. On the other hand, the use of screen printed electrodes is a growing and attractive electroanalytical tool for quality control of organic compounds i.e. pharmaceuticals. Some pharmaceutical applications using this type of electrodes are: the quantitative analysis of paracetamol on an untreated screen-printed carbon electrode (SPCE) using amperometric detection coupled to a FIA system [13], the determination of lamotrigine by differential pulse adsorptive stripping voltammetry (DPAdSTV) using SPCE [14], and the electrochemical determination of nitrazepam at a SPCE using AdSV [15].

At the best of our knowledge no references combining AdSV with flow injection analysis (FIA) and screen printed carbon electrodes (SPCE) for determination of nifuroxazide have been described.

Buchberger et al. [1] have studied in detail the electrochemical behavior of nifuroxazide on both carbon paste and mercury drop electrodes based on the 4-electron re-

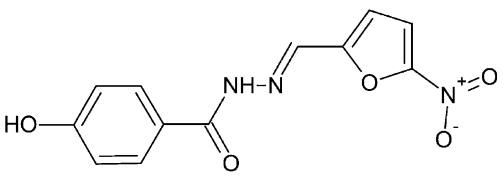

Fig. 1. Nifuroxazide molecular structure.

duction of the nitro group, and they reported the ability to preconcentrate the analyte on the electrode under certain conditions.

According to the current technological advancements, it is probable that nifuroxazide has the ability to adsorb onto the SPCE now commercially available that possess attractive features relative to conventional electrodes. Therefore, a reproducible, inexpensive and sensitive analytical method can be developed and adapted to the flow operation conditions required to automate routine determinations.

The aim of this work was to develop an FIA system with enhanced sensitivity that allows preconcentration by adsorptive stripping voltammetry over SPCE chips. The operating conditions and electrode type were tested to optimize the analytical parameters of the method.

2 Experimental

2.1 Reagents

Pure nifuroxazide was acquired from Sigma-Aldrich as an analytical standard (Cod no: 46494). All other reagents were of analytical reagent grade and were used as supplied. Commercial tablets containing nifuroxazide were purchased from a local authorized supplier. Imecol™ (Laboratorio Andromaco S.A., Santiago /Chile, Tablets containing 200 mg Nifuroxazide, 500 mg Ftalylsulfatiazol plus excipients), Diaren (Laboratorio Saval S.A., Santiago/Chile, Tablets containing 200 mg Nifuroxazide, 500 mg Atapulgite plus excipients), and Enterol (Instituto Sanitas S.A., Santiago/Chile, Tablets containing 200 mg Nifuroxazide, 350 mg phtalylsulfacetamide plus excipients) were used as commercial tablets.

The carrier solution was a pH 2.0 aqueous solution containing 0.1 M Britton-Robinson buffer and 0.1 M NaCl. The NaCl was added to this solution to complete the reference of the screen-printed electrode used. This solution was also used as the supporting electrolyte in the batch experiments.

Stock solutions of nifuroxazide were prepared by dissolving exactly 5 mg in 50.0 mL of absolute ethanol PA (Merck) and protected from light in an amber volumetric flask. Sample solutions of convenient concentration were prepared by diluting aliquots of the stock solution to 2 mL with ethanol and then diluting further to 15.0 mL using the carrier buffer solution.

The sample solutions were purged with nitrogen for at least 5 min prior to injection, and the carrier solutions were purged before pumping. High-resistivity water, 18.2 MΩ/cm, was used for all solutions.

2.2 Apparatus

A CHI-440A model electrochemical analyzer (CH Instruments, Austin, Texas) was used to control the electrode potential and to measure current response. A Dosimat-715 dispenser from Metrohm (Metrohm AG, Herisau, Switzerland), equipped with a 20 mL burette, was used as the pumping system. An electrochemical wall-jet flow-cell, DRP-FLWCL by DropSens (DropSens S.L., Oviedo, Spain), was used as the electrochemical detector. Screen-printed carbon electrodes (SPCE), model DRP-C110, and its multiwalled carbon nanotube, DRP—C110CNT (CNT-SPCE), and carbon nanofiber, DRP-C110CNF (CNF-SPCE), analogs (modified by DropSens) were used in the detector. A six-way injection valve similar to the 1106 model by Omnifit (Diba Industries Ltd., Cambridge, UK) equipped with a 2 mL sample loop was used. All tubing was 0.03×0.09 inch flexible Tygon S-54-HL by Norton Plastics and Synthetics Division and was supplied by VWR.

2.3 Calibration Procedure

Aliquots of the alcohol stock solution of nifuroxazide were diluted to 15 mL by the aqueous buffer carrier solution to obtain a concentration range of 0.01 to 10 μg/mL; absolute ethanol was added when necessary to maintain a constant ethanol content of 13 % (v/v). The sample (2 mL) was injected into the flow system, and a linear sweep voltammogram was obtained after a preconcentration period. We have used the following optimal conditions for the analysis: flow rate = 1.5 mL/min, scan rate = 200 mV/s, deposition time = 80 s, preconcentration potential = 800 mV. The measurement procedure was repeated until reproducible results were obtained. The background currents were corrected and the mean value of the peak height was plotted against the corresponding concentration.

2.4 Determination Procedure for Nifuroxazide in Tablets

Recovery: We prepared the synthetic samples, which contained 200 mg nifuroxazide plus excipients, in accordance with the commercially available formulation. A quantity of the powder equivalent to 10.0 mg of nifuroxazide was dissolved in 50.0 mL of absolute ethanol as a stock solution. For the measurements, 125 μL of this solution was diluted to 25 mL with the carrier solution and enough ethanol to yield a 13 % (v/v) final concentration.

Composite: Ten tablets of the commercial formulations were weighed, powdered and mixed thoroughly. A quantity of the powder equivalent to 10.0 mg of nifuroxazide was weighed, and then, a procedure similar to the composite analysis was followed.

Individual tablet assay (ITA): Analyses of the nifuroxazide content of a single tablet were also performed by the procedure described above.

3 Results and Discussion

3.1 Electrochemical Behavior at the SPCE

The SPCE are flat ceramic strips that contain the requisite three electrodes in a miniaturized configuration. The

working electrode surfaces used were carbon and carbon modified with either carbon nanotubes or carbon nanofibers.

Figure 2 shows the cyclic voltammograms over both positive and negative potential ranges for the first and second scan at the SPCE after an accumulation period. The anodic scan (Figure 2a) shows an oxidation signal at approximately 1.0 V for the first scan, while the second scan shows no signal. The cathodic scan (Figure 2b) shows a reduction peak at approximately −0.280 V in the first scan that disappeared in the second one. The anodic peak corresponds to the oxidation of the phenolic hydroxyl group at nifuroxazide molecule [2]. The cathodic peak corresponds to the well-known four electron reduction of the aromatic nitro group to the corresponding hydroxylamine derivative [3]. We selected this reduction peak as the analytical signal.

As expected, the reduction behavior of nifuroxazide over the SPCEs, both with and without CNT/CNF, was similar to that described by other authors for carbon paste electrodes [1]. The nifuroxazide showed the ability to adsorb onto the carbon surface with different saturation times, which depended on the solution concentration, pH and electrode potential. This property of nifuroxazide allows the sensitivity of the analytical method to be enhanced using adsorptive stripping voltammetry.

The first studied condition was pH. In Figure 3 we can observe the strong dependence between peak current and pH. While pH increase until pH 6 the peak current decrease, after this pH value the solution turns light yellow and the peak current continue decreasing up to totally vanish near pH 10. According to this behavior we selected pH 2 as the optimal working condition.

The experimental conditions that obtained the best results for the SPCEs were similar to those published for carbon paste electrodes [1]. A series of batch experiments were performed to confirm this result by immersing the screen-printed electrode in solutions of nifuroxazide at several concentrations with the buffer solution as the supporting electrolyte. The measured current increased linearly over the applied accumulation potential range of 0 to 800 mV. More positive potentials were avoided because the nifuroxazide oxidatively decomposes, and no reduction signal was observed.

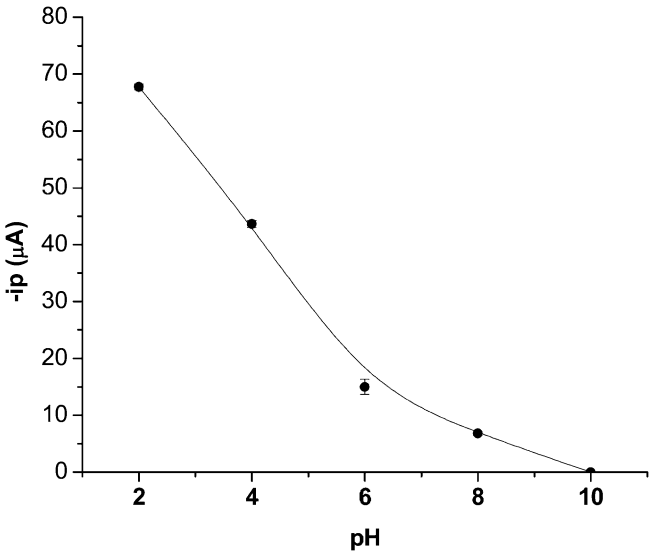

Fig. 3. pH dependence with peak current for a 2 μg/mL nifuroxazide solution in 0.1 M Britton-Robinson buffer +0.1 M NaCl. Operation conditions were: flow rate = 1.5 mL/min, scan rate = 200 mV/s, deposition time = 80 s, preconcentration potential = 800 mV, working electrode CNF-SPCE.

In Figures 4a and 4b we can observe the dependence of peak current with both preconcentration time and potential, respectively.

The best results were obtained by applying a preconcentration potential of 800 mV for 80 s, which is adequate for a solution with pH 2. After the preconcentration period the solution was submitted to linear sweep voltammetry (LSV), whose scan rate was 200 mV/s. We also try with pulse techniques such as differential pulse voltammetry (DPV) but inexplicably the sensibility of the signal was considerably smaller. Probably this fact is related

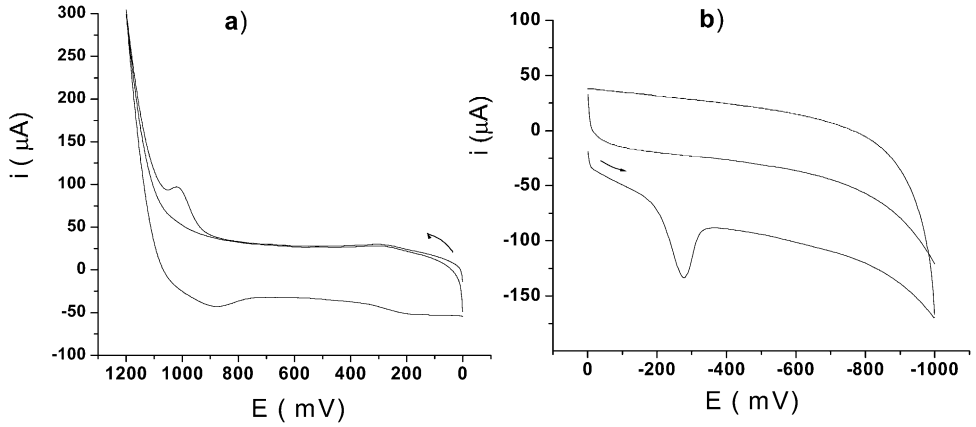

Fig. 2. Cyclic voltammograms, first and second scan in the a) anodic direction and b) cathodic direction for the nifuroxazide adsorbed onto SPCE.

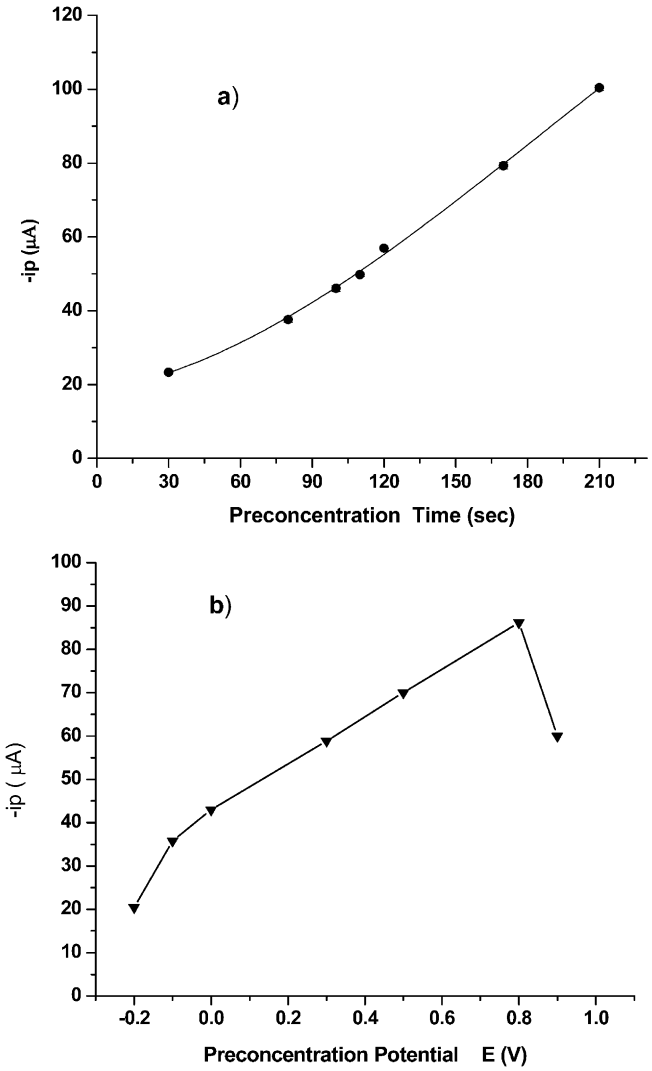

Fig. 4. Dependence of peak current with both preconcentration time (a) and preconcentration potential (b). Operation conditions (a): flow rate = 1.5 mL/min, scan rate = 200 mV/s, preconcentration potential = 800 mV, pH 2, nifuroxazide concentration 1 μg/mL. Operation conditions (b): flow rate = 1.5 mL/min, deposition time = 80 s, scan rate = 200 mV/s, pH 2, nifuroxazide concentration 2 μg/mL. Working electrode CNF-SPCE.

with the strong adsorptive character of the nifuroxazide signal.

In Figure 5 we show comparative voltammograms obtained using both DPV and LSV.

In all cases, a unique peak with a height linearly related to the concentration of nifuroxazide was found when all other conditions remained constant. The potential of this peak is always approximately −280 mV. To obtain the height of this peak, it is necessary to correct for the base-current present in the recorded voltammogram. The procedure used in this work, presented in Figure 6, was fitted to a second-order polynomial and adapted to the curved shape of the base-current in the neighborhood of the peak. Tto.exe [16] software for visualizing and processing

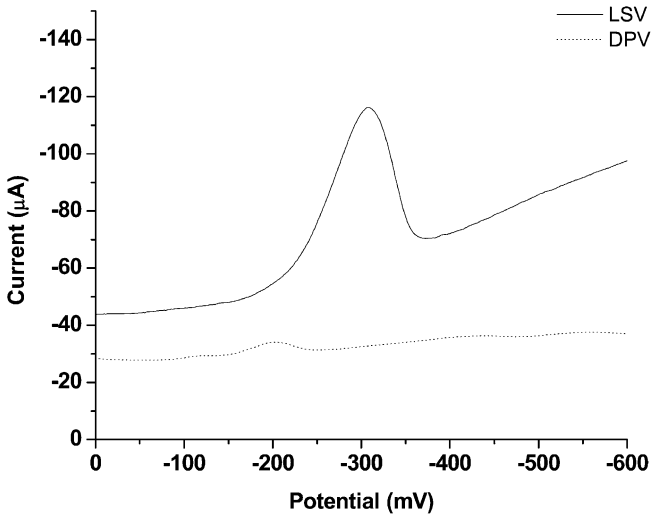

Fig. 5. Comparative voltammograms in the FIA system obtained using both DPV and LSV. Operation conditions were: flow rate = 1.5 mL/min, deposition time = 80 s, preconcentration potential = 800 mV, pH 2, nifuroxazide concentration 2 μg/mL. DPV conditions: Pulse height = 50 mV, pulse period = 500 ms, scan rate = 10 mV/s, sample with = 20 ms; LSV conditions: scan rate = 200 mV/s. Working electrode CNF-SPCE.

electrochemical data was used to compute and subtract the polynomial base-line.

This correction procedure was compared to those implemented by the commercial software on the electrochemical devices, such as the current reading with respect to the base-line extension of the peak, and we found that the obtained results were a clear improvement in terms of the large dynamic range of the detector, lower limit of quantification and lower relative standard deviation.

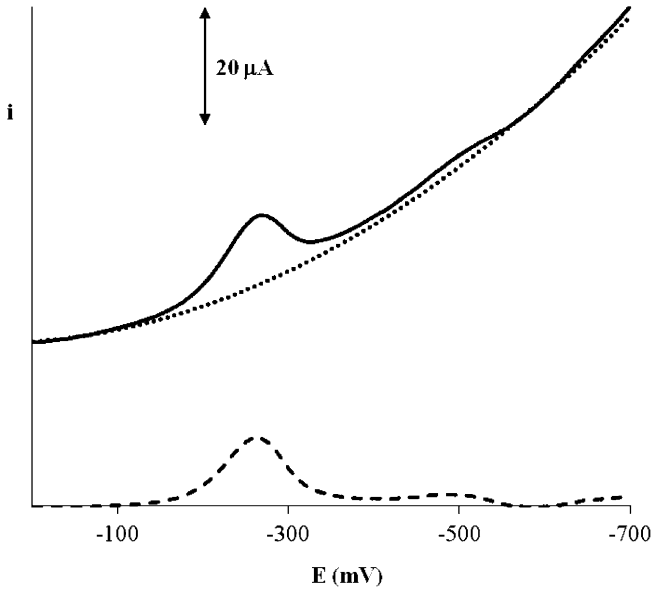

Fig. 6. A base-current correction procedure applied to voltammograms. (—) Original current response for nifuroxazide 1 μg/mL in the FIA system, (·····) adjusted second-order polynomial correction and (----) corrected current response.

Also, the calibration process of this procedure is easily automated.

3.2 The FIA System

In an attempt to automate the nifuroxazide detector, a flow-injection analysis (FIA) system was adapted to use the adsorptive stripping voltammetry technique described previously. The flow system was contained within a single channel manifold that includes the carrier reservoir, pump, six-way injection valve and electrochemical flow detector. The flow carrier served as the buffer solution, which also acted as the supporting electrolyte. Samples containing nifuroxazide were injected in 2 mL quantities into the stream, and the programmed potential sweep was then initiated upon injection. The sample volume was set to match the time it took the sample to pass over the electrode while the preconcentration potential was applied. Therefore, a 80 s preconcentration time and 1.5 mL/min flow rate were used.

By the time the preconcentration time ended, the entire sample had flowed over the electrode and a proportional quantity of the nifuroxazide had been adsorbed. At this moment, the potential sweep started to both reduce and desorb the nifuroxazide and obtain the analytical response while the buffer solution flowed over the electrode. No analytes are in the solution during the voltammetric analysis to avoid any contributions of the dissolved species to the analytical signal and to favor the elimination of the reduced species.

Notably, the carrier flow is pulseless as a piston pump was used, which was free of dissolved oxygen; therefore, a very low background noise was obtained, which allowed for low detection and quantification limits. The volume of the injection loop and the flow rate determine the accumulation time, which can be easily varied over a wide range to adapt the method to different concentration ranges. The used system permits the reproducible control of the adsorption time.

3.3 Validation

In Figure 7 we can observe the comparative voltammograms between the different SPCE. From this result it is clear that CNF-SPCEs produced the highest signal.

Furthermore, as can be seen in Figure 8, the best performance was obtained by the carbon nanofiber-modified SPCE, which had a sensitivity that was approximately twice that of the other electrodes. The calibration curve obtained for the range 0.5–3 μg/mL was $i_p(A) = 1.45 \times 10^{-6} + 3.941 \times 10^{-5}$ (μg/mL) ($r = 0.9999$).

The stability of the electrode was also checked by repeating the experiments over time, and differences were found between the SPCEs, CNT-SPCEs and CNF-SPCEs. The CNF-SPCEs produced reproducible signals even after 60 consecutive measurements with only a slight change in the sensitivity; however, the response signal of CNT-SPCEs tended to diminish by 5% after only one series of four measurements, showing a memory effect. It is possible to recover the initial sensitivity of the CNT-SPCE by injecting 2 mL of the buffer solution after three or four sample measurements and applying the measurement procedure described earlier (doing this eliminates the adsorbed wastes on the electrode surface and regenerates it).

A complete characterization of the proposed flow analysis system (using the CNF-SPCEs) was made, and we found that the dynamic range of determination covered

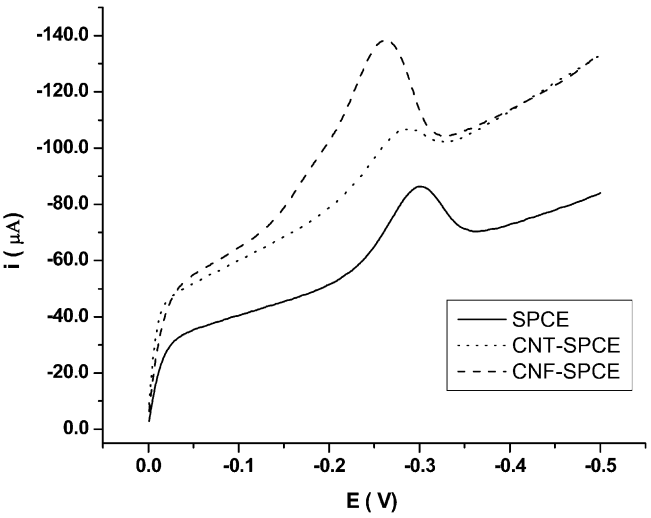

Fig. 7. Comparative voltammograms in the FIA system obtained using different working SPCE. Flow rate = 1.5 mL/min, deposition time = 80 s, preconcentration potential = 800 mV, pH 2, nifuroxazide concentration 1 μg/mL, scan rate = 200 mV/s.

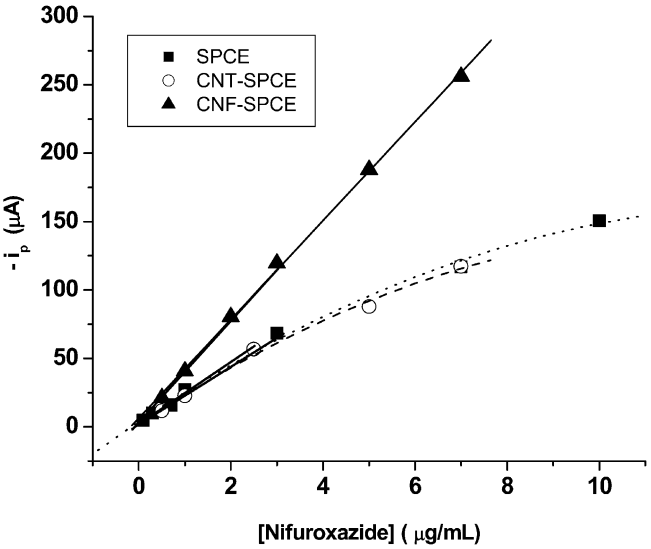

Fig. 8. The linear calibration curve of the nifuroxazide FIA detector. The dark lines are the least-squares adjusted curves over the 0.1 to 3.0 μg/mL range. Points are the experimental data for (■) SPCE, (○) CNT-SPCE and (▲) CNF- SPCE. Operating conditions: Flow rate = 1.5 mL/min, deposition time = 80 s, preconcentration potential = 800 mV, pH 2, scan rate = 200 mV/s.

more than one decade from the limit of quantification (LOQ) at 0.1 μg/mL to the limit of linearity (LOL) at 3.0 μg/mL (see Figure 8). The peak from the nifuroxazide reduction is observed at concentrations as low as10 ng/mL; however, the limit of detection (LOD) at the 99% confidence level is 42 ng/mL. The LOD and LOQ were calculated by 3 s_{lq}/m_{CC} and 10 s_{lq}/m_{CC}, respectively [17], where s_{lq} is the standard deviation of the lower quantified concentrations and m_{CC} is the slope of the calibration curve.

The repeatability of this method, in terms of relative standard deviation (RDS), was 2.5% over the entire range of quantification, and the accuracy was determined by the recovery analysis of nifuroxazide from 1.0 and 0.7 μg/mL hydro-alcoholic solutions. These recoveries were found to be $100.7 \pm 2.5\%$, ($n = 15$).

The analytical throughput of this system was 20 peaks per hour. A total volume of 5 mL of the carrier solution and only 2 mL of the sample are needed for each measurement.

Because the surface of the SPCEs is rough, their specific surface area is different for each electrode. The parameter of the analytical method most affected by this variation is the sensitivity. Therefore, a new calibration curve must be obtained every time the electrode is replaced, and the samples must be analyzed just after. The sensitivity obtained for a set of five CNT electrodes has an average value of 22 μA/ppm and a standard deviation of 1.3%.

As shown in Figure 8, the sensitivity of this method is similar for SPCEs and CNT-SPCEs. The improved electrochemical behavior of the CNTs seems to have no effect on the adsorptive capacity for nifuroxazide and its subsequent desorptive reduction. Furthermore, with respect to nifuroxazide, it seems that the screen-printed carbon electrodes surface has a similar electrochemical and adsorptive activity with and without CNTs used to modify them. However the CNF-SPCEs behave differently, possibly because a three-dimensional network of the fibers form a high surface area with better adsorption-desorption properties. The continuous flow of fresh buffer solution between the different measurements is important to efficiently restore the working electrode.

The recovery study for synthetic samples containing 200 mg of nifuroxazide plus excipients according to the manufacturer's batch formulas revealed an average recovery of 100.7%, and a standard deviation of 2.5%. This result indicates acceptable accuracy and precision validating the proposed method. The excipients do not interfere with the nifuroxazide determination.

3.4 Application

The nifuroxazide content for the following three pharmaceutical formulations was determined: Imecol (Laboratorios Andrómaco), Diaren (Laboratorios Saval-Eurolab) and Enterol (Instituto Sanitas). The averaged results of the proposed FIA method for nifuroxazide measured from these commercial tablets are as follows. Composite analysis from the tablets containing 200 mg of nifuroxazide was 205.2 ± 8.0 mg (Imecol), 200.4 ± 11.8 mg (Diaren) and 188.8 ± 11.6 mg (Enterol). The result of the individual tablet assay (ITA) was 207.6 ± 9.2 mg (Imecol), 203.2 ± 18.6 mg (Diaren) and 189.4 ± 11.8 mg (Enterol). According to these results, it is possible to conclude that this method is suitable for determining the nifuroxazide content in commercial formulations. Furthermore, common excipients and active species found in the tablets did not interfere in the quantification.

4 Conclusions

With respect to the adsorption and reduction conditions, the electrochemical behavior of nifuroxazide on screen-printed carbon electrodes was similar to that previously described for carbon paste electrodes. The chip SPCEs are inexpensive, stable and suitable for the routine determination of nifuroxazide. In addition, CNT modified electrodes did not improve the performance of this system, whereas CNF doubled the method's sensitivity.

A flow-injection system was developed for use with adsorptive stripping linear sweep voltammetry, and the operating parameters were optimized. Concentrations ranging from 0.1 to 3.0 μg/mL can be determined with an analytical throughput for the flow system of 20 peaks per hour with a low consumption of both reactants and solvents.

The technique, including the calibration, measurement, data processing and reading of the results, can be fully automated.

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References

[1] W. Buchberger, G. Niessner, R. Bakry, *Fresenius J. Anal. Chem.* **1998**, *362*, 205.
[2] A. Radi, *Fresenius J. Anal. Chem.* **1999**, *33*, 1229.
[3] J. A. Squella, I. Lemus, G. Lonza, L. J. Núñez-Vergara, *Bol. Soc. Chil. Quim.* **1990**, *36*, 109.
[4] A. Radi, M. A. El Ries , *Anal. Sci.* **1999**, *15*, 385.
[5] A. Radi, S. El Laban, I. M. M. Kenawi, *Anal. Sci.* **1998**, *14*, 607.
[6] M. I. Toral, M. Paine, P. Leyton, P. Richter, *J. OAOC Int.* **2004**, *87,* 1323.
[7] P. R. Guinebault, M. Broquaire, R. A. Braithwaite, *J. Chromatogr.* **1981**, *204*, 329.
[8] M. Broquaire, P. R. Guinebault, *J. Liq. Chromatogr.* **1981**, *4*, 2039.
[9] A. Economou, *Anal. Chim. Acta* **2010**, *683*, 38.

[10] N. Diab, A. AbuZuhri, W. Schuhmann, *Bioelectrochemistry* **2003**, *61*, 57.
[11] P. Danesghar, P. Norouzi, A. Akvar, M. Reza, E. Haghsenas, F. Dousty, M. Farhadi, *J. Appl. Electrochem.* **2009**, *39*, 1983.
[12] H. P. Nouws, C. Delerue-Matos, A. A. Barros, J. A. Rodriguez, A. Santos Silva, F. Borges, *Anal. Lett.* **2007**, *40*, 1131.
[13] P. Fanjul-Bolado, P. J. Lamas-Ardisama, D. Hernandez-Santos, A. Costa García, *Anal. Chim. Acta* **2009**, *638*, 133.
[14] O. Domínguez-Enedo, M. E. Burgos, M. J. Arcos-Martinez, *Sensors* **2008**, *8*, 4201.
[15] N. McGuire, K. Honeychurch, J. Hart, *Electroanalysis* **2009**, *21*, 2165.
[16] J. D. Mozo, Tto.exe software for electrochemical data processing. **2010**, http://www.uhu.es/giea/help.htm
[17] IUPAC, *Pure Appl. Chem.* **1995**, *67*, 1699.

