Int. J. Electrochem. Sci., 10 **(2015)** 8738 - 8749

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Screen-printed Electrode Based Electrochemical Sensor for the Detection of Isoniazid in Pharmaceutical Formulations and Biological Fluids

Rosa A.S. Couto, José L.F.C. Lima and M. Beatriz Quinaz[*]

LAQV/REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Portugal
[*]E-mail: bquinaz@ff.up.pt

Received: 14 July 2015 / *Accepted:* 18 August 2015 / *Published:* 26 August 2015

This work focuses on the development of a novel and sensitive electrochemical sensor for the tuberculostatic drug INH. The use of INH is frequently associated to numerous side effects and poisoning incidents, being the assay of INH levels essential for effective therapeutic dosages. Herein we propose an electrochemical method based on the use of Nafion modified-SPCEs for the study of the electro-oxidation process of INH and its determination in pharmaceutical formulations and biological fluids. The modified electrodes showed high stability, good sensitivity and reproducibility, enabling the use of micro-volumes of samples for the determination of the drug in complex matrixes. The study of the electrochemical behaviour of INH was performed by CV and SWV and the method was successfully applied to the determination of the drug in tablets and in human urine and serum samples in 0.1M PBS pH 7.0 using 100 µL of samples and no pre-treatment steps. The proposed method exhibited numerous advantages, such as simplicity, rapid analysis procedures and great sensitivity, offering a promising alternative for the analysis of INH using disposable Nafion-SPCE that can be used in portable sensors for decentralized analyses of this analyte.

Keywords: Electrochemical detection; Electrochemistry; Isoniazid; Pharmaceutical analysis; Screen-printed electrodes (SPEs)

1. INTRODUCTION

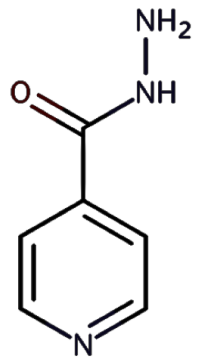
The use of screen-printing technology in the serial production of disposable low-cost screen-printed electrodes (SPEs) for the electrochemical determination of a wide range of substances is currently undergoing widespread growth [1-5]. Screen-printing techniques offer high-volume production of inexpensive, highly reproducible and reliable sensors, providing precise control over the SPEs dimensions, excellent uniformity, high reproducibility and the potential for mass production [6].

A SPE consists of a chemically inert substrate on which the three electrodes, namely the working, reference and counter electrodes (WE, RE and CE, respectively) are printed through screen-printing methodology [7]. Different substrates can be employed in SPE devices and the extensive range of modifications to SPEs opens numerous fields of applications [8]. Particularly, the use of these electrodes on the analytical detection of pharmaceuticals in a wide range of samples can provide important advantages, such as no extensive sample processing, low detection limits, simplicity, low cost, portability and potential for miniaturization [9].

Carbon materials are often used in the construction of the WE of the SPEs (screen-printed carbon electrodes, SPCEs) due to their low background current, readily renewable surface and wide potential window [10,11]. These materials offer several advantages since they are inexpensive, versatile and chemically inert, being used in both bare (unmodified) or modified forms [12]. Actually, the great versatility presented by the SPCEs lies in the different ways in which the WEs surface may be modified [13,14]. The modification of the SPEs surface can be made with a wide range of substrates, resulting in an enhancement of their functions and applications [15]. Particularly, the SPEs surface modification with polymers, such as Nafion films, for the detection of pharmaceutical compounds has been described in many works [16-18]. Nafion is a sulfonated tetrafluoroethylene based copolymer with cationic exchange properties that allows the easy production of modified electrodes, showing many advantageous chemical and physical characteristics, such as good electrical conductivity, high partition coefficients of many redox compounds and good biocompatibility [18]. The use of Nafion enhances the analytical signal intensity and minimizes the interference of electroactive species, playing an important role on the protection of the surface of the working electrodes [13,19].

Tuberculosis is a multisystemic disease caused by *Mycobacterium tuberculosis* and one of the oldest infectious diseases affecting the humankind [20]. Currently, tuberculosis is considered the second leading cause of death from infectious disease, causing millions of deaths worldwide [21]. Isoniazid or isonicotinic acid hidrazide (INH) (Scheme 1) is a tuberculostatic agent effective against *Mycobacterium tuberculosis* that has been used since 1952 and is currently used as front-line drug in the prophylaxis and treatment of tuberculosis [20,21]. However, INH is frequently associated to numerous side effects and poisoning incidents, which may occur due to unintentional ingestion, suicidal intent, or extra dosage taking as compensation for missed doses [20]. Thus, the assay of INH levels is essential for effective therapeutic dosages and has encouraged the development of many methods for its determination, such as high-performance liquid chromatography (HPLC) [22,23], HPLC/mass spectrometry (MS) [24], liquid chromatography [25], LC/MS [26-30], chemiluminescence [31,32], capillary electrophoresis [33,34], micellar electrokinetic capillary chromatography (MEKC) [35], colorimetry [36,37] and fluorimetry [38]. However, these reported methods usually require complex pretreatment steps, long response times and expensive apparatus, being necessary to develop a simple, robust and cost-effective method for the sensitive analytical detection of INH. In this context, several electrochemical approaches have been suggested to the detection of this drug [39-43]. However, the design and production of an electrochemical sensor based on SPEs for the detection of INH in complex matrixes by using small volumes of samples is highly required. In this work, the development of a disposable electrochemical sensor using SPCEs modified with Nafion for the study

of the voltammetric behaviour of INH is described for the first time. The proposed method was applied to the direct determination of INH in pharmaceutical formulations, human urine and serum samples.



Scheme 1 Chemical structure of INH.

2. EXPERIMENTAL

2.1. Chemicals and materials

All solutions were prepared with water from a Milli-*Q* system (conductivity ≤ 0.1 μS cm^{-1}) and chemicals of analytical reagent grade quality. Reagents were not subject to any further purification.

Nafion perflourinated resin solution – 5 wt % in mixture of lower aliphatic alcohols and water, with 45% water was purchased from Sigma-Aldrich (St. Louis, MO, USA). Working solutions of 0.01%, 0.025%, 0.05%, 0.10% and 0.15% were prepared by suitable dilutions with deionised water.

Phosphate buffer solution (PBS) 0.1 M was used as supporting electrolyte. pH values (1.5, 3.0, 5.0, 7.0 and 10.5) assayed were adjusted by using small volumes of concentrated HCl or NaOH solutions.

Isoniazid (INH) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of INH were daily prepared by dissolving of suitable quantities of INH in deionised water. Working standard solutions were prepared by suitable dilutions of the stock solutions with 0.1M PBS pH 7.0.

2.2. Apparatus

Electrochemical measurements were performed with a PC-controlled 910 PSTAT mini potentiostat controlled by a PSTAT software for data acquisition and experimental control. Disposable SPCEs with a 4 mm diameter carbon WE, a carbon CE and silver RE were purchased from Metrohm (ref. 6.1208.110, Herisau, Switzerland). The electrochemical measurements were performed in both bare (unmodified) and modified (with Nafion) carbon WEs. A specific electrode cable and a connector for PSTAT mini were also supplied by Metrohm. All experiments were performed at 25.0 ± 0.5^0C.

2.3. Modified SPCEs preparation

Disposable SPCEs purchased from Metrohm comprised a traditional three-electrode system printed on an inert support (L34 x W10 x H0.5 mm). The three electrodes were screen-printed on a ceramic substrate and exposed to high-temperature curing and a protective ink coating was used to insulate the conductive tracks from the electrodes. For the surface modification of the WE, different concentrations (0.010%, 0.025%, 0.050%, 0.10% and 0.15%) of Nafion solutions were prepared by dilution of the Nafion perflourinated resin solution 5 wt % in deionised water. The modified electrodes were prepared by dropping of different volumes (10μL, 15μL and 20μL) of the prepared Nafion solutions over the carbon WEs followed by complete solvent evaporation achieved at 75^0C for 2 hours.

2.4. Analytical procedure

The electrochemical behaviour of INH was investigated at the surface of both bare (unmodified) and modified Nafion-SPCEs by CV and SWV. Measurements were performed with a 100□L drop of solution, covering the surface of the three-electrode system. The electrode surface was washed with deionised water before the measurement of different samples.

2.4.1. Analysis of INH in pharmaceutical formulations

Isoniazid Labesfal tablets labeled to contain 300 mg isoniazid were used. Ten tablets were accurately weighed and the average weight was calculated. These ten tablets were finely powdered and a stock solution of 1.0×10^{-3} M in INH was prepared by weighing the corresponding quantity of powder and dissolving it in deionised water. The working solutions were obtained by dilution of the respective stock solution in 0.1M PBS pH 7.0 and a 100μL drop of the obtained solution was analysed by CV and SWV at the surface of the Nafion-SPCEs. No additional pre-treatment steps of the samples were required.

2.4.2. Analysis of INH in human urine and serum samples

Aliquots of human urine and serum samples were diluted (1:100 and 1:10, respectively) with 0.1M PBS pH 7.0 and spiked with INH in order to reach the desired final concentrations. A 100μL drop of each sample was used to perform the CV and SWV studies on the surface of the Nafion-SPCEs. No additional pre-treatment steps of the samples were required.

3. RESULTS AND DISCUSSION

3.1. Influence of the pH

The effect of the pH on the oxidation peak current and potential (I_{pa} and E_{pa}, respectively) of INH at the surface of the SPCEs was investigated by CV and SWV in the presence of 4.0×10^{-5} M

INH over a pH range between 1.5 and 10.5. In all instances, the cyclic voltammograms for INH exhibited an irreversible oxidation peak. By SWV, it was observed that the oxidation peak shifts towards more positive potentials as the pH increased and at pH values higher than 7.0 the oxidation peak appeared adjacent to the oxidation barrier, avoiding the observation of a well-defined peak. In addition, for both CV and SWV techniques, the highest I_{pa} values were found for the INH solution in PBS pH 7.0 (Figure 1), as observed by other authors [40,44], being this pH value selected for the determination of INH in pharmaceutical formulations and biological fluids samples.

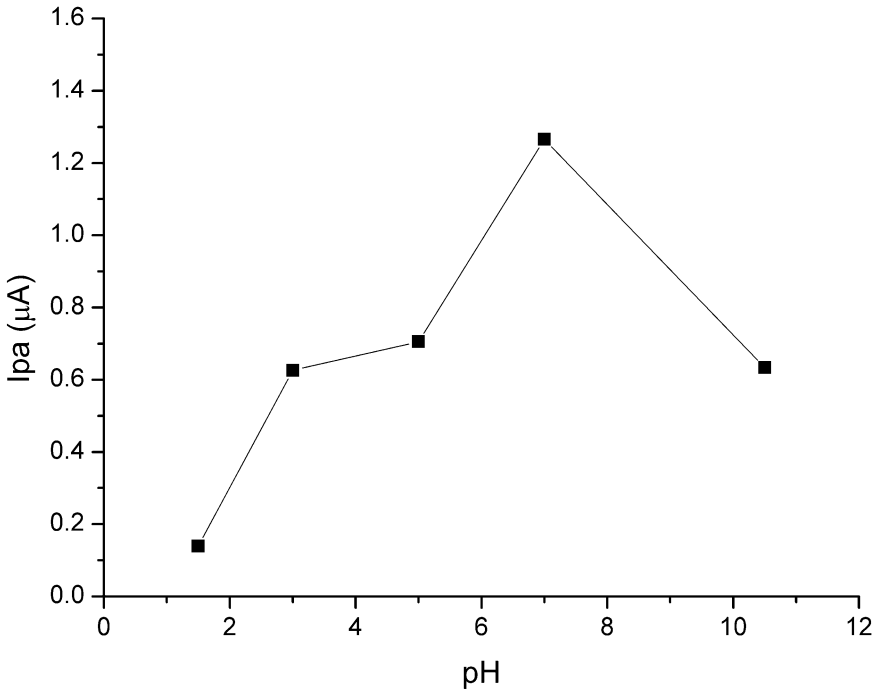


Figure 1. Plot of I_{pa} vs. pH obtained by SWV of a 4.0×10^{-5} M INH solution in 0.1 M PBS at different pH values: 1.5, 3.0, 5.0, 7.0 and 10.5 at the surface of unmodified SPCEs. Frequency: 10 Hz.

3.2. SPCE surface modification

3.2.1. Optimization of the SPCE surface coating

The polymeric coating of the surface of the carbon WE was performed using the solvent evaporation method, which consists on the deposition of a small volume of the polymer solution on the active surface of the electrode, followed by the evaporation of the solvent to obtain a thin coating film [16]. The concentration of the polymer solution defines the film thickness, which influences the sensibility and repeatability of the developed electrode [19]. In this work, five different concentrations of Nafion were tested, namely 0.01%, 0.025%, 0.05%, 0.10% and 0.15%. The coating procedure consisted on the deposition of 20 μL of the modifier solution at the surface of the carbon WE followed by a dry step at 75^0C for 1 hour to ensure the complete evaporation of the solvent. The performance of the obtained modified electrodes was evaluated with CV by means of I_{pa} current for a 6.0×10^{-5} M

INH solution in PBS pH 7.0. It was observed that the intensity of the analytical signal decreases with the increasing of the Nafion concentration, suggesting that the formation of thicker films for higher concentrations of Nafion difficults the diffusion of the analyte to the electrode surface (Figure 2).

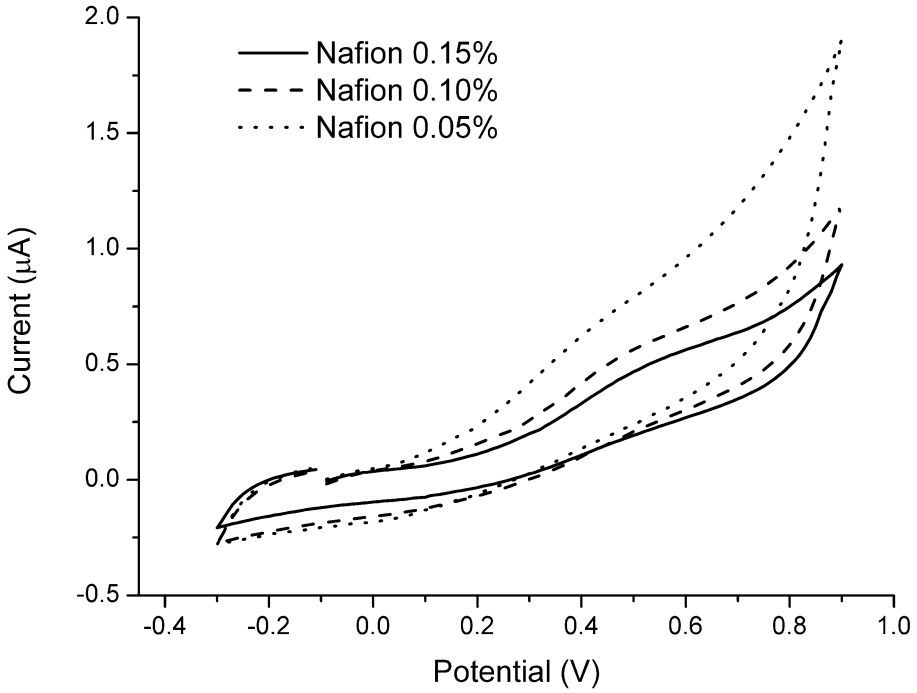


Figure 2. Cyclic voltammograms of a 6.0×10^{-5} M INH solution in PBS pH 7.0 at SPCEs modified with 0.15%, 0.10% and 0.05% Nafion solutions. Scan rate: 0.10 V s^{-1}.

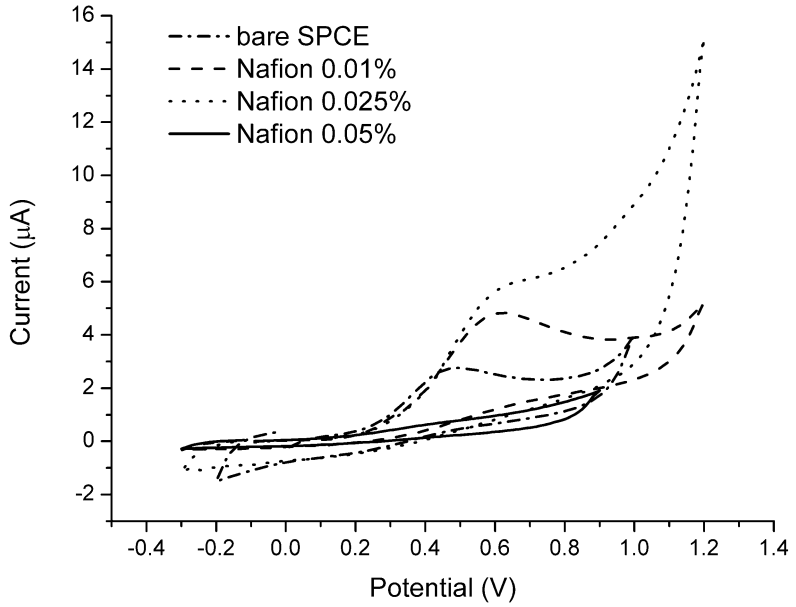


Figure 3. Cyclic voltammograms of a 6.0×10^{-5} M INH solution in PBS pH 7.0 at bare SPCE and SPCEs modified with 0.01%, 0.025% and 0.05% Nafion solutions. Scan rate: 0.10 V s^{-1}.

The change in the I_{pa} and E_{pa} values observed for INH oxidation in the SPCEs modified with different concentrations of Nafion can be attributed to different accessibility of the analyte within the film to the electrode surface [16]. It was observed that the charge transfer is facilitated for lower concentrations of Nafion and the best results (*i.e.,* the highest I_{pa} values) were found for the lower concentrations tested – 0.01%, 0.025% and 0.05% (Figure 3). For these concentrations, 3 different application volumes were tested, namely 10 μL, 15 μL and 20 μL. The modification with 20 μL of 0.025% Nafion solution exhibited the best performance, being selected to the quantification of INH in pharmaceutical formulations and biological fluids (*i.e.,* human urine and serum). Compared with other methods reported, the proposed Nafion-SPCE for INH determination exhibited good sensitivity, which mainly depended on the quantity of the modifier on the SPCE surface that influences the amount of substance that is transferred to the electrode surface during the oxidation process [45]. A well-defined oxidation signal, characteristic of a diffusion-controlled electron transfer process, was observed for both bare and Nafion-coated electrodes by CV, which is in accordance with previous reports [16, 17]. Nonetheless, the oxidation peak current significantly increased with the Nafion coating, compared with the bare SPCE, demonstrating that Nafion can effectively increase the electron transfer rate due to its electric properties. The prepared Nafion-SPCEs were conditioned at room temperature and washed with deionised water before the first measurement and whenever it was necessary. No further treatment steps were used.

3.2.2. Characterization of the modified SPCE

The study of the voltammetric behaviour of INH at the surface of the modified SPCEs with 20 μL Nafion 0.025% was carried out. By performing 10 consecutive determinations of a 1.0×10^{-4} M INH standard solution, it was shown that the coated electrode allowed the attainment of I_{pa} and E_{pa} values with good repeatability (relative standard deviation (RSD) of 6.6% and 1.7%, respectively), being possible to conclude that the electrode coating with Nafion film allowed the detection of INH with good repeatability, preventing the passivation of the working electrode surface.

The reproducibility of the coating procedure was also studied. The electrochemical behaviour for INH was studied at the surface of three different SPCEs prepared in the same conditions. According to the results, it was concluded that the modifying process was reproducible since, for the same INH solution, similar values of I_{pa} were obtained (with RSD of 2.5%). In addition, the measurement of INH at the surface of the same modified Nafion-SPCE on three different days was performed and similar values were obtained for I_{pa} and E_{pa} (with RSD lower than 5% and 2%, respectively). Despite the disposability of the SPCEs, the coated SPCEs exhibited good stability, being possible to conclude that the same Nafion-modified SPCE could be used to carry out up to 100 determinations without loss of reproducibility (with a RSD lower than 10% for the I_{pa} values). Hereupon, the unique characteristics of the Nafion material described above endowed the capability to strongly promote the charge transfer of INH, improving the sensitivity of the method, thus suggesting that a sensor based on Nafion-modified SPCEs might be a promising strategy for the electrochemical detection of this drug.

3.3 Influence of the scan rate

Preliminary cyclic voltammetric experiments were performed to study the INH electrochemical behaviour at the surface of the Nafion modified-SPCEs. Scan rate studies were than carried out to assess whether the oxidation process occurred under diffusion or adsorption control. Figure 4 allows the comparison of a series of cyclic voltammograms for INH at different scan rates from 0.01 to 0.25 V s^{-1} in 0.1M PBS pH 7.0. The solution concentration was 8.0×10^{-5} M INH. A well-defined voltammetric response, characteristic of a diffusion-controlled reaction, is observed in the potential range of -0.2 to 1.0 V. Cyclic voltammograms of INH gave one well-defined anodic peak, which can be attributed to the oxidation of the amide moiety of the INH molecule. There is no peak on the reverse scan indicating the irreversibility of the process. The effect of the potential scan rate between 0.01 and 0.25 V s^{-1} on the I_{pa} and E_{pa} of INH was evaluated. The obtained cyclic voltammograms indicated that the I_{pa} increases with increasing of the scan rate and shows a linear relationship with the square root of the scan rate over the wide range of scan rates tested with a correlation coefficient value greater than 0.99 (Inset Figure 4 A). It was also observed that the E_{pa} shifts into more positive values with increasing of the scan rate, with a good linear dependence of the E_{pa} upon the logarithm of the scan rate (Inset Figure 4 B), which confirms the irreversibility of the process. The obtained results suggest that the anodic oxidation of INH on the surface of the Nafion-SPCEs is a typical diffusion-controlled electrochemical process.

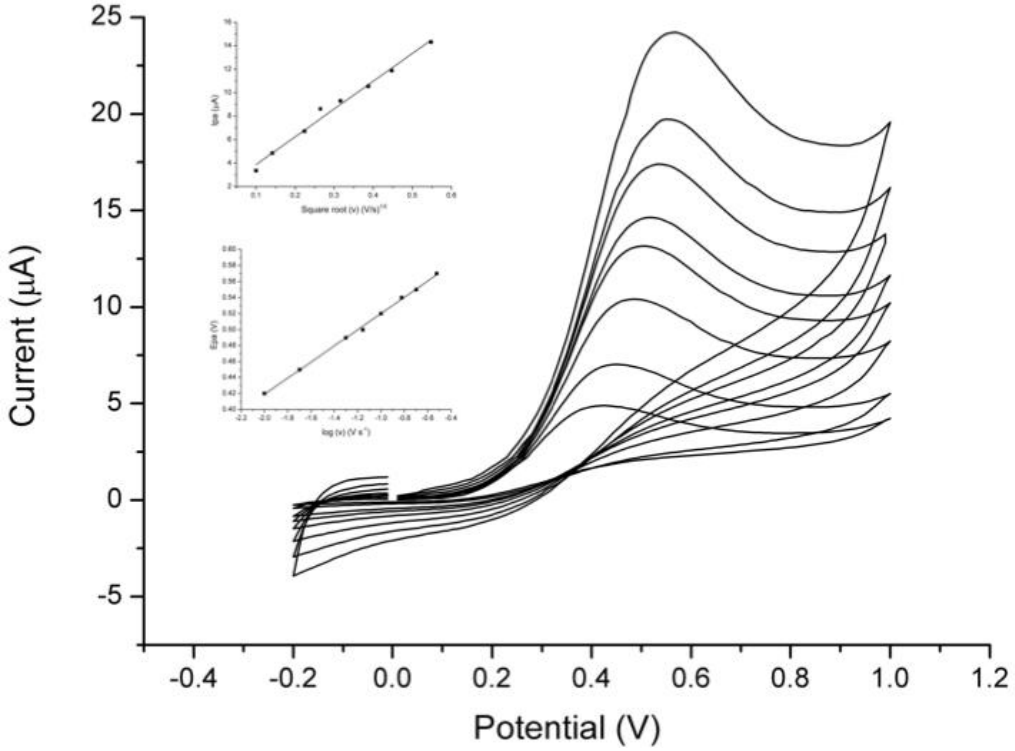


Figure 4. Cyclic voltammograms of a 6.0×10^{-5} M INH solution in PBS pH 7.0 at the Nafion-SPCE at different scan rates: 0.01, 0.02, 0.05, 0.07, 0.1, 0.15, 0.2 and 0.25 V s^{-1}. Inset A: linear relationship between the peak currents and the square root of the scan rates. Inset B: linear relationship between the peak potentials and the logarithm of the scan rates.

3.4. Optimization of the SWV parameters

In order to establish a SWV procedure for the quantification of INH, several parameters were optimized, namely the frequency, the potential step and the potential amplitude, since the I_{pa} values obtained by SWV technique largely depends on the combined effect of such parameters. In this work, a well-defined voltammetric profile was obtained for a potential step value of 0.01 V and potential amplitude of 0.01 V. In addition, several values of frequency were applied (1.0, 2.0, 5.0, 7.5, 10.0, 12.5 and 15.0 Hz). It was observed a linear increase in the peak current with the increasing of the frequency and a value of 10 Hz was selected since a great peak current and a well-defined oxidation peak were simultaneously obtained using this frequency. Thus, the determination of INH by SWV was performed using a frequency of 10 Hz, a potential step of 0.01 mV and a potential amplitude of 50 mV. Using these conditions, several concentrations of INH were analysed by SWV (Figure 5) and a calibration curve over a range of 2.5×10^{-5} M to 2.0×10^{-4} M was obtained (Inset Figure 5), which fitted the equation $I_{pa}(\mu A)=5.91e3\times[INH](M)–0.055$, $r^2=0.99$. Limits of detection (three times the standard deviation of the intercept/slope) and quantification (ten times the standard deviation of the intercept/slope) of 1.4×10^{-5} M and 4.7×10^{-5} M, respectively, were obtained.

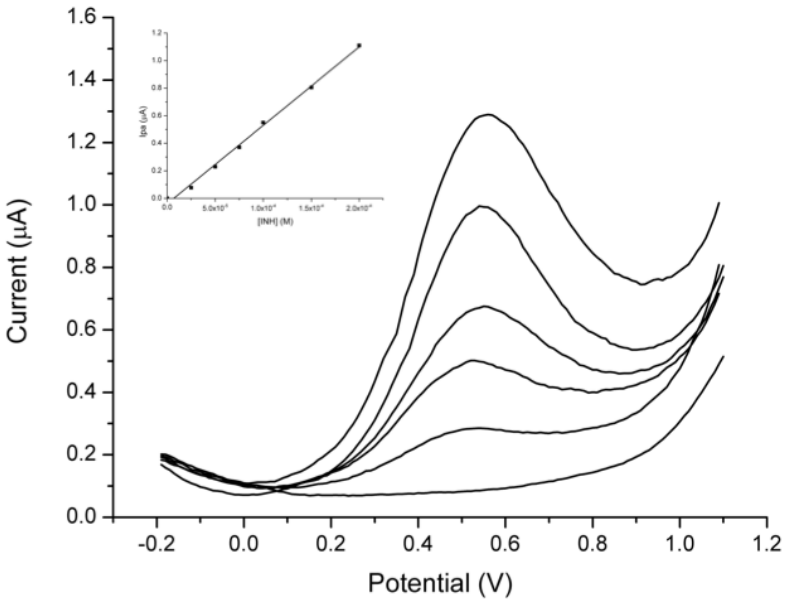


Figure 5. Square wave voltammograms obtained at under optimized conditions in 0.1 M PBS pH 7.0 containing 0.0, 2.5×10^{-5}, 5.0×10^{-5}, 7.5×10^{-5}, 1.0×10^{-4}, 1.5×10^{-4} M of INH. Inset: Analytical curve.

3.5. Analytical application

To evaluate the applicability of the proposed method to the determination of INH in complex matrixes, pharmaceutical formulations (tablets) and human urine and serum samples were analysed. Firstly, the matrix effect of the human urine and serum on the voltammetric signal was studied. Different ratios of dilution (sample/0.1M PBS pH 7.0) were tested for both human urine and serum

samples. In the case of human urine samples, dilutions of 1:10, 1:50, 1:100 and 1:150 were examined, being observed that for lower dilutions (1:10 and 1:50) the oxidation signal of INH almost disappeared and for dilutions greater than 1:100 a decreasing in the recovery value was attained. Thus, the ratio of 1:100 was established for the voltammetric determination of INH in human urine samples. For the human serum samples, dilutions of 1:1, 1:10 and 1:50 were tested, being found that the voltammetric response decreased for the ratio 1:1 and well-defined oxidation peaks were achieved for both dilutions 1:10 and 1:50, being the ratio 1:10 chosen for the determination of INH in human serum samples. These results suggest that endogenous compounds present in both human urine and serum samples can adsorb at the surface of the modified-SPCEs, acting as barrier in the INH oxidation process. The determination of INH in tablets and human urine and serum samples was performed as described in the Experimental section, without further pretreatment steps. A 100 μL drop of each sample spiked with different concentrations of INH was used to perform the square wave voltammograms and the obtained results are presented in Table 1. Recoveries from 96.81% to 101.81%, 94.84% to 101.81% and 95.18% to 97.18% of INH were obtained for tablets, human urine and serum samples, respectively, suggesting the accuracy of the proposed method for the determination of INH in complex matrixes. Indeed, the modification of the SPCEs with Nafion largely improved the disposable electrodes' performance, thus opening a promising window to the rapid, effective and low-cost determination of this drug in both pharmaceutical and biological samples, requiring no pre-treatment steps.

Table 1. Determination of INH in tablets and human urine and serum samples.

Sample	Repetition	I_{pa} (nA)	[INH] (M)		Recovery (%)	Relative error (%)
			Found	Added		
Tablet	1	289.412	5.83E-05	6.00E-05	97.18	-2.82
Tablet	2	287.109	5.79E-05	6.00E-05	96.53	-3.47
Tablet	3	282.291	5.71E-05	6.00E-05	95.18	-4.82
Urine	1	350.952	6.87E-05	7.00E-05	98.18	-1.82
Urine	2	365.981	7.13E-05	7.00E-05	101.81	1.81
Urine	3	345.292	6.78E-05	7.00E-05	96.81	-3.19
Serum	1	505.326	9.48E-05	1.00E-04	94.84	-5.16
Serum	2	520.949	9.75E-05	1.00E-04	97.49	-2.51
Serum	3	543.675	1.01E-04	1.00E-04	101.33	1.33

4. CONCLUSIONS

SPEs are highly versatile devices that are very convenient to use, as they are disposable and easy to produce, aiming the development of portable sensors able to be mass-produced and provide inexpensive and rapid detection of a wide range of drugs in several complex matrixes.

In this work, we have successfully applied a rapid, sensitive and cost effective method for the determination of INH in small volumes of samples (*i.e.*, pharmaceutical formulations and biological fluids samples) based on the use of modified SPCEs. The reported method is based on the use of Nafion coated-SPCEs, which showed high stability, reproducibility and repeatability. Additionally, the conductivity of the SPCEs was largely improved by the polymer coating with Nafion, which allowed the enhancement of the analytical signal intensity and minimized the interference of the complex matrixes used, protecting of the surface of the electrodes and allowing the determination of INH with low detection limits and notable reuse performance, without the need of extensive sample pre-treatment. The proposed method exhibited numerous advantages, such as simplicity, rapid analysis procedures and great sensitivity, offering a promising alternative for the analysis of INH in micro-volumes of complex matrixes using disposable Nafion-SPCE that can be used in portable sensors for decentralized analyses of this analyte.

ACKNOWLEDGMENTS
This work received financial support from the European Union (FEDER funds through COMPETE) and National funds (FCT, Fundação para a Ciência e Tecnologia) through LAQV - Laboratório Associado para Química Verde UID/QUI/50006/2013

References

1. R.A.S. Couto, J.L.F.C. Lima and M.B. Quinaz, *Talanta* (2015), http://dx.doi.org/10.1016/j.talanta.2015.06.011.
2. C. Zhu, G. Yang, H. Li, D. Du, Y. Lin, *Anal. Chem.*, 87(2015)230.
3. A.M. Burke, A.A. Gorodetsky, *Nat. Chem.*, 4(2012)595.
4. M.M. Rahman, A.M. Asiri, S.B. Khan, *Int. J. Electrochem. Sci.*, 9(2014)6896.
5. G. He, X. Yang, Y. Hu, Y. Hu, F. Zhang, *Int. J. Electrochem. Sci.*, 9(2014)6962.
6. J.P. Metters, E.P. Randviir, C.E. Banks, *Analyst*, 139(2014)5339.
7. A. Hayat, J.L. Marty, *Sensors (Basel)*, 14(2014)10432.
8. Y. Liu, X. Dong, P. Chen, *Chem. Soc. Rev.*, 41(2012)2283.
9. V.K. Gupta, R. Jain, K. Radhapyari, N. Jadon, S. Agarwal, *Anal. Biochem.*, 408(2011)179.
10. R.L. McCreery, *Chem. Rev.*, 108(2008)2646.
11. A.T. Lawal, *Talanta*, 131(2015)424.
12. R.T. Kachoosangi, G.G. Wildgoose, R.G. Compton, *Analyst*, 133(2008)888.
13. L. Agüí, P. Yáñez-Sedeño, J.M. Pingarrón, *Anal. Chim. Acta*, 622(2008)11.
14. N.A. Pchelintsev, P.A. Millner, *Anal. Chim. Acta*, 612(2008)190.
15. T. Asefa, C.T. Duncan, K.K. Sharma, *Analyst*, 134(2009)1980.
16. M.L. Silva, M.B. Garcia, J.L. Lima, E. Barrado, *Anal. Chim. Acta*, 573(2006)383.
17. F. Wang, J. Zhou, Y. Liu, S. Wu, G. Song, B. Ye, *Analyst*, 136(2011)3943.
18. R. García-González, M.T. Fernández-Abedul, A. Costa-García, *Talanta*, 107(2013)376.
19. W. Putzbach, N.J. Ronkainen, *Sensors (Basel)*, 13(2013)4811.
20. V. Bernardes-Génisson, C. Deraeve, A. Chollet, J. Bernadou, G. Pratviel, *Curr. Med. Chem.*, 20(2013)4370.
21. M. Pinheiro, A.S. Silva, S. Pisco, S. Reis, *Chem. Phys. Lipids*, 183(2014)184.
22. M.C. Gennaro, R. Calvino, C. Abrigo, *J. Chromatogr. B. Biomed. Sci. Appl.*, 754(2001)477.

23. H.I. Seifart, W.L. Gent, D.P. Parkin, P.P. van Jaarsveld, P.R. Donald, *J. Chromatogr. B. Biomed. Appl.*, 674(1995)269.
24. P.F. Fang, H.L. Cai, H.D. Li, R.H. Zhu, Q.Y. Tan, W. Gao, et al, *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.*, 878(2010)2286.
25. A. Espinosa-Mansilla, M.I. Acedo-Valenzuela, A. Muñoz de la Peña, F. Cañada Cañada, F. Salinas López, *Talanta*, 58(2002)273.
26. K.H. Hee, J.J. Seo, L.S. Lee, *J. Pharm. Biomed. Anal.*, 102(2015)253.
27. Z. Zhou, X. Wu, Q. Wei, Y. Liu, P. Li, A. Ma, et al, *Anal. Bioanal. Chem.*, 405(2013)6323.
28. K.Y. Ng, H. Zhou, Y.L. Zhang, B. Hybertson, T. Randolph, U. Christians, *J Chromatogr B Analyt. Technol. Biomed. Life Sci.*, 847(2007)188.
29. S.H. Song, S.H Jun, K.U. Park, Y. Yoon, J.H. Lee, J.Q. Kim, et al, *Rapid Commun. Mass Spectrom.*, 21(2007)1331.
30. X. Chen, B. Song, H. Jiang, K. Yu, D. Zhong, *Rapid Commun. Mass Spectrom.*, 19(2005)2591.
31. J. Abolhasani, J. Hassanzadeh, *Luminescence*, 29(2014)1053.
32. J. Xi, B. Shi, X. Ai, Z. He, *J. Pharm. Biomed. Anal.*, 36(2004)237.
33. Y. Liu, Z. Fu, L. Wang, *Luminescence*, 26(2011)397.
34. X. Zhang, Y. Xuan, A. Sun, Y. Lv, X. Hou, *Luminescence*, 24(2009)243.
35. M.I. Acedo-Valenzuela, A. Espinosa-Mansilla, A. Muñoz De La Peña, F. Cañada-Cañada, *Anal. Bioanal. Chem.*, 374(2002)432.
36. A. Csiba, *Acta Pharm. Hung.*, 59(1989)205.
37. A.M. el-Brashy, S.M. el-Ashry, *J. Pharm. Biomed. Anal.*, 10(1992)421.
38. R.A.S. Lapa, J.L.F.C. Lima, J.L.M. Santos, *Anal. Chim. Acta*, 419(2000)17.
39. M.S. Quintino, L. Angnes, *J. Pharm. Biomed.*, 42(2006)400.
40. M.M. Ghoneim, K.Y. el-Baradie, A. Tawfik, *J. Pharm. Biomed. Anal.*, 33(2003)673.
41. G. Yang, C. Wang, R. Zhang, Q. Qu, X. Hu, *Bioelectrochemistry*, 73(2008)37.
42. Z.N. Gao, X.X. Han, H.Q. Yao, B. Liang, W.Y. Liu, *Anal. Bioanal. Chem.*, 385(2006)1324.
43. S. Cheemalapati, S.M. Chen, M.A. Ali, F.M. Al-Hemaid, *Colloids Surf. B. Biointerfaces*, 121(2014)444.
44. S. Cheemalapati, S. Palanisamy, S. Chen, *Int. J. Electrochem. Sci.*, 8(2013)3953.
45. M.F. Bergamini, D.P. Santos, M.V.B. Zanoni, *Bioelectrochemistry*, 77(2010)133.