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Carbon nanofiber screen printed electrode joined to a flow injection system for nimodipine sensing

P. Salgado-Figueroa, C. Gutiérrez, J.A. Squella*

Bioelectrochemistry Laboratory, Chemical & Pharm. Sci. Faculty, University of Chile, Santiago, Chile

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ABSTRACT

A simple and sensitive electrochemical method using pretreated carbon nanofiber screen printed electrodes joined to a flow injection analysis (CNF-SPE-FIA) system was developed to analyze nimodipine (NMD) in pharmaceutical formulations. Low detection limits were achieved by controlling the accumulation time or sample volume. The developed method performed well with a LOD from 0.08 to 0.400 μM for a 6 or 1 mL sample loop and an RSD below 2% for a wide linear range. This method was applicable to both named brand and generic drug tablets containing 30 mg NMD. A contents concentration of 30.2 mg/tablet of NMD with an RSD = 2.0% for named brand and 30.1 mg/tablet with an RSD = 1.9% of NMD in the generic drug was determined. This work could promote the potential application of CNF-SPE-FIA system as a new strategy to be applied to the analysis of any drug derived from nitroaromatic compounds.

1. Introduction

Nimodipine (NMD), chemical name 1,4-Dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylic acid 2-methoxyethyl 1-methylethyl ester (Fig. 1), is a calcium channel blocker.

NMD is used to treat senile dementia and cerebrovascular spasms during the prophylaxis of vascular hemicranias [1–6]. NMD is absorbed rapidly after oral administration, binds over 95% to plasma proteins and is eliminated almost exclusively as their metabolites. Less than 1% is recovered as NMD from urine. The exact action mechanism for NMD in humans is still unknown [2]. However, the clinical use of NMD is severely restricted because of its extensive first-pass effect in the liver [3]. There are both brand name and generic drugs. The generic drug prices allow greater access to medicines. Generic drug occasionally look different because they use different inactive ingredients, such as coloring or flavoring; however, they have the same dosage, strength, performance and use and should have the same quality and safety standards [4–6].

Several methods have been developed to analyze NMD in pharmaceutical formulations and biological samples. The main techniques used are LC–MS/MS [7–9], GC-ECD [10], spectrophotometry [11–15], spectrofluorometry [16], polarography [17], HPLC-UV–vis [18], HPLC-PAD [19], and SWCAdSV [20]. The lowest reported detection limit for NMD analysis has been 0.001 μM using LC–MS/MS. Low detection limits had been similarly obtained via electrochemical techniques using HMDE (0.08 μM) [20] and multiwalled carbon nanotube-modified glassy carbon electrodes (0.02 μM) [21]. Detection limits near 0.24 and 0.45 μM were obtained via HPLC-UV and spectrophotometric methods, respectively. Recently, a nitrogen-doped graphene modified electrode was developed to sensing NMD in commercial tablets with excellent results (0.01 μM) [22].

Screen-printed electrodes (SPE) have recently received increased interest as working electrodes and can be constructed using several materials, including single- and multi-walled carbon nanotubes, carbon nanofibres (CNF), graphene, gold, bismuth and so on [23–27]. Furthermore, the SPE sensor can be improved by chemical modification or activation. Recently, we have reported the enhancement of the electrochemical response of a nitrofuran derivative with a pretreatment of the CNF-SPE in only one step using a Britton-Robinson buffer/DMF solution [29]. On the other hand, the first analytical evidence of combining AdSV with flow injection analysis (FIA) and screen printed carbon electrodes (SPCE) for determination of a nitrofuran derivative drug was described by Mozo et al. [31]. In this paper, we extend this method to other nitrocompounds different from nitrofurans such as nitrobenzene derivatives According to the best of our knowledge no one has previously described a method for determination of nitroaromatics derived from nitrobenzene by using SPE and FIA system via LSV.

In this paper, a method based in pretreated screen printed electrodes joined to a flow injection analysis (SPE-FIA) system was proposed to analyze NMD in pharmaceutical formulations. A low

* Corresponding author. Tel.: +56 2 6782927; fax: +56 2 7371241.
E-mail address: asquella@ciq.uchile.cl (J.A. Squella).

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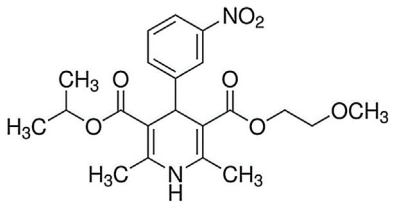

Fig. 1. Nimodipine chemical structure.

detection limit, no sample preparation requirements, low cost, and fast and simple usage are some advantages of SPE-FIA.

2. Experimental

2.1. Reagents

NMD was acquired from Sigma Aldrich; acetic acid, phosphoric acid, N,N-dimethylformamide (DMF), NaCl were purchased from Merck. Boric acid was obtained from Fluka. Two brands of commercial pharmaceutical formulations (tablets) containing 30 mg were purchase from pharmacies ("Regental" from Tecnofarma, Lot: 54688 and "Nimodipino" from Instituto Sanitas, Lot: 0263014).

Britton-Robinson buffer/NaCl (100 mM, pH2), 5.71 mL of acetic acid, 6.74 mL of phosphoric acid, 6.18 g of boric acid and 5.85 g of NaCl, was dissolved in Milli-Q water, sonicated for 10 min and diluted to 1 L. This buffer solution was used as the carrier and supporting electrolyte.

Stock NMD solution (1.0×10^{-3} M): 10.46 mg was weighed and dissolved with approximately 10 mL of DMF in a 25 mL volumetric flask before diluting to the mark with DMF. Solutions with known NMD concentrations were prepared by diluting aliquots of the stock solution and diluting to a convenient volume with a 100 mM buffer Britton-Robinson/NaCl solution. All sample solutions, including the carrier solution, were purged with nitrogen for at least 5 min prior to analysis.

2.2. Apparatus

The linear-sweep voltammetry (LSV) measurements were performed using a portable BiPotentiostat/Galvanostat μStat 400 from DropSens. Disposable carbon nanofiber screen printed electrodes (CNF-SPE), 110CNF, were purchased from DropSens (ceramic substrate: *L*33 mm × *W*10 mm × *H*0.5 mm, working electrode: CNF/carbon, counter electrode: carbon and reference electrode: silver). A glass cell was designed in the lab for batch analysis and use with SPE. The flow injection system included a pumping system Dosimat-715 dispenser, 20 mL burette from Metrohm; methacrylate wall-jet flow cell for screen printed electrodes, DRP-FLWCL (*L*3.3 mm × *W*6.0 mm × *H*3.3 cm) from DropSens; and a six-way manual sample injection valve that included 1 and 6 mL sample loops. All of the flow injection system tubing was polyethylene PE-100 (i.d. 0.034/o.d. 0.060 in.) and supplied by Clay Adams.

SEM Images for carbon nanofiber screen printed electrodes were obtained using a FEI scanning electron microscope, model Inspect F-50. (HV 10.00 kV, 10,000×, HFW 29.8 mm and WD 6.6 mm).

2.3. Methods

2.3.1. CNF-SPE pre-treatment

The active area of each screen-printed electrode was treated with a 100 mM 70/30 (v/v) Britton–Robinson/NaCl buffer/DMF solution before use. Each electrode was vertically immersed for 15 min in a 10 mL beaker, with the buffer/DMF solution covering the working electrode (CNF) area. After this immersion, the electrodes were washed with deionized water and dried in air. All of the electrodes were pre-treated before each measurement.

2.3.2. Sample preparation

2.3.2.1. Composite. Ten tablets were individually weighed and subsequently finely powdered together. Approximately, 40 mg of the tablet powder was required to make a 10 mL NMD stock solution at 1.0×10^{-3} M in DMF. Aliquots of 500 μL from this solution were diluted to 25 mL with Britton-Robinson buffer/NaCl (100 mM, pH 2). Ten samples from two different pharmaceutical formulations were prepared. The NMD was quantified using an external calibration curve.

2.3.2.2. Individual tablets. Ten tablets were individually weighed and finely powdered as generic pharmaceutical formulations (*Instituto Sanitas*). Approximately, 40 mg of the tablet powder was required to obtain a 10 mL NMD stock solution at 1.0×10^{-3} M in DMF. Aliquots of 500 μL of the clear solution were diluted to 25 mL with Britton-Robinson buffer/NaCl (100 mM, pH 2). The diluted solutions were prepared according the procedure described above.

2.3.3. NMD electrochemical behavior

A 1.0×10^{-4} M NMD solution in 100 mM Britton-Robinson buffer/NaCl in a batch system was used to study the pH and electrochemical behavior of NMD via cyclic voltammetry.

2.3.4. SPE-FIA method validation

The instrument was validated based on its linearity, limits of detection (LOD) and quantification (LOQ), precision and accuracy.

2.3.5. Linearity

NMD solutions with 10 different concentrations (4.0, 8.0, 16.0, 24.0, 32.0, 40.0, 48.0, 56.0, 64.0, and 72.0 μM) were prepared and injected into the SPE-FIA system for the linearity study. The responses were measured as the current peak (i_p) by LSV.

2.3.6. Detection and quantification limits

Seven solutions with known concentrations in the lower linear region were analyzed using the SPE-FIA system and LSV to study the LOD and LOQ. The LOD was calculated according to Eq. (1):

$$\mathrm{LOD} = t_{(n-1,\alpha=0.99)} \times (s), \quad (1)$$

where $t_{(n-1,\alpha=0.99)}$ is the one-sided *t*-statistic based on the number of samples used to determine (s) at the 99% level.

The LQD was estimated from LOD according to Eq. (2):

$$\mathrm{LQD} = 3 \times \mathrm{LOD} \quad (2)$$

2.3.7. Precision

Seven solutions with known concentrations were prepared in the middle of the linear range. The method precision was studied based on the repeatability and intermediate precisions. Both were expressed as percent relative standard deviations (%RSDs). To study the repeatability, seven measurements were performed on the same day. To determine the intermediate precision, seven solutions were measured over several days and using different SPE.

2.3.8. Accuracy

The method accuracy was evaluated based on seven replicate NMD solutions in the middle of the linear range using LSV and SPE-FIA. The method accuracy was expressed as the percent recovery (% Recovery).

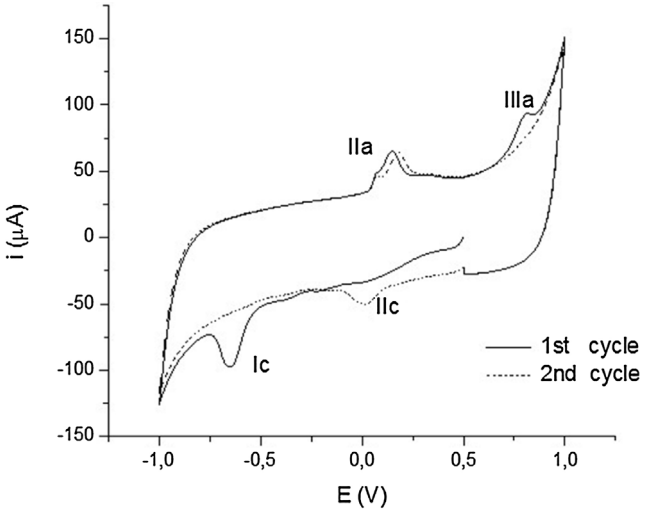

Fig. 2. Cyclic voltammogram for 1.0×10^{-5} M NMD at pH 2 in 100 mM aqueous Britton–Robinson buffer/NaCl with a 3 min accumulation time.

3. Results and discussion

3.1.1. Batch system

Before developing the FIA system for NMD, the electrochemical behavior and optimal pH for NMD were determined using a batch system with pretreated CNF-SPE and cyclic voltammetry. Four signals were observed from 1.0×10^{-5} M NMD during the CV scan (Fig. 2), at pH 2 in 100 mM aqueous Britton-Robinson buffer/NaCl with a 3-min accumulation time.

The cathodic signal (I_c) was due to the nitro group reduction in NMD to the corresponding hydroxylamine derivate via a four electron transfer according to Eq. (3):

$$\text{Ar-NO}_2 + 4 = \text{e} + 4\text{H+} \rightarrow \text{Ar-NHOH} + \text{H}_2\text{O} \quad (3)$$

$$\text{Ar-NHOH} \leftrightarrow \text{Ar-NO} + 2\text{e}^- + 2\text{H}^+ \quad (4)$$

During the backward scanning, the hydroxylamine derivate oxidized to its nitroso derivate (IIa). The redox couple (IIa/IIc) formed during the second cycle resulted from the reduction-oxidation of the nitroso-hydroxylamine derivative, according to Eq. (4). The anodic signal (IIIa) corresponds to the 1,4 dihydropyridine ring oxidation to the corresponding pyridine derivative.

This voltammetric behavior is analogous to the previously described for a similar nitrocompound, i.e. nitrendipine, on carbon nanotubes [28].

The voltammetric behavior was pH-dependent. Cyclic voltammograms obtained between pHs 2 and 10 in a batch system with a 3-min accumulation time are shown in Fig. 3.

The peak current corresponding to the Ic signal was recorded. Based on the pH study, a pH 2 was selected as the optimal condition for reducing the nitro group. For a basic pH, values over 8 disadvantaged the nitro reduction, and the i_p values decreased drastically. The cathodic I_c signal was selected for analytical purposes.

In order to analyze the effect of the analytical procedure on the electrode surface we use SEM microscopy. In Fig. 4, it can be seen the comparison of the surface of the CNF-SPE before the analytical procedure and after 30 consecutive measurements.

The sensitivity and reproducibility of the signal intensity is affected by the continued use of the electrode. After 30 measurements the electrode lose part of the electroactive sites, effect that is shown by the SEM images obtained for both a new and used screen printed electrode. In the case of a new screen printed electrode (Fig. 4a), the entire electrode surface is covered by the carbon

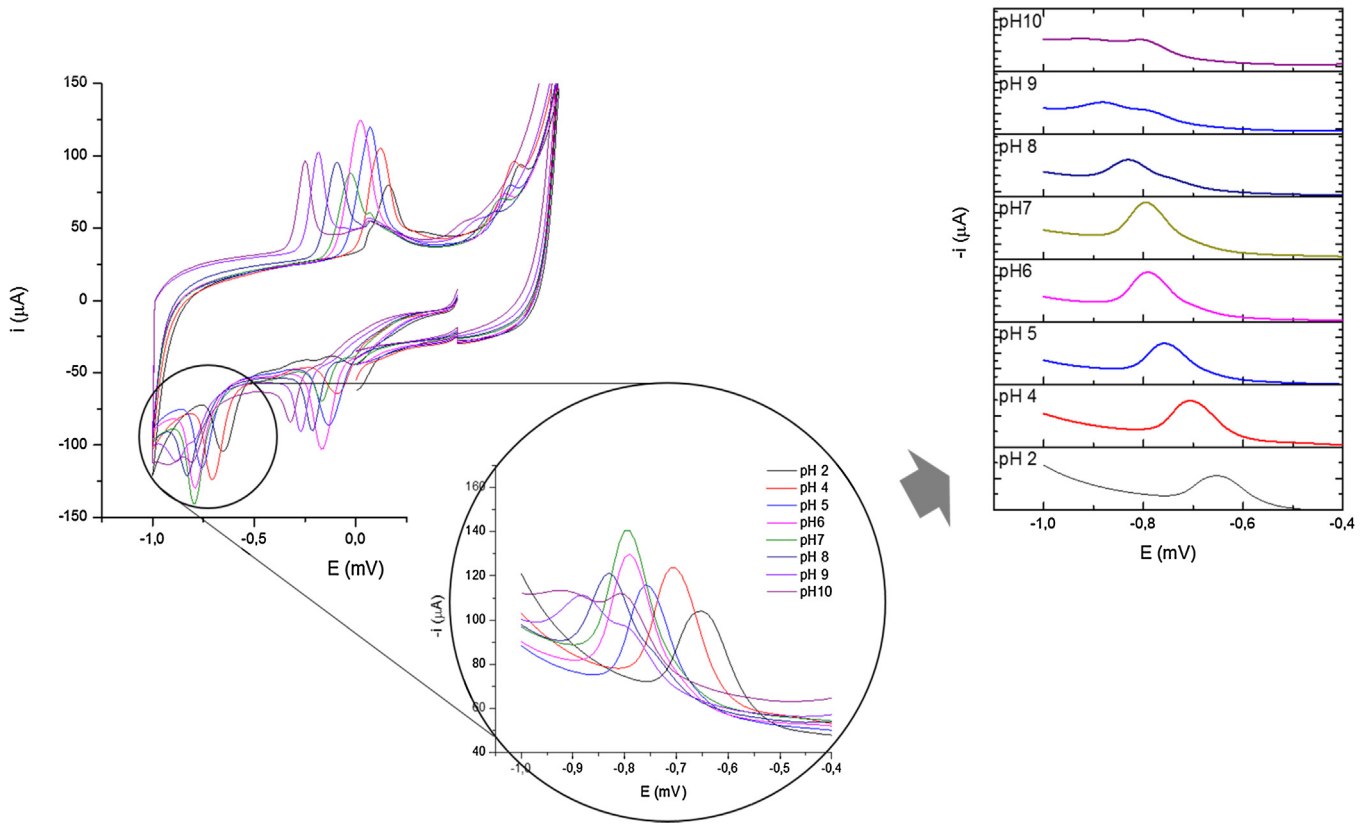

Fig. 3. Nimodipine pH behavior from pH 2 to 10 via cyclic voltammetry in a batch system with a 3-min accumulation time in 100 mM Britton–Robinson buffer/NaCl.

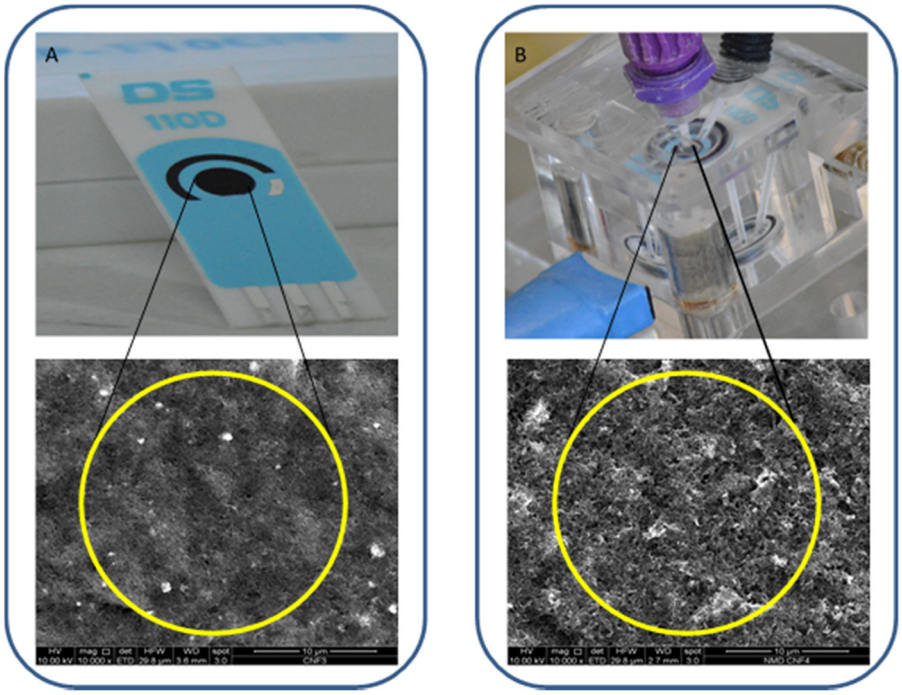

Fig. 4. SEM images for electroactive area for screen printed electrodes obtained by a Scanning electron microscope FEI, model Inspect F-50 (HV 10.00 kV, magnification of 10,000×, HFW 29.8 mm and WD 6.6 mm). (A) Inspection of electroactive area for a new SPE without pretreatments. (B) Image for a used SPE in the FIA system after approximately 30 measurements.

nanofibers while in Figure 4b an SEM image of an electrode screen printed carbon nanofiber after 30 measurements showed the lose of electroactive sites.

The accumulation time was determined by increasing the 1×10^{-5} M NMD in 100 mM Britton-Robinson buffer/NaCl media contact time with the working electrode from 0 to 20 min. The solution was stirred (400 rpm) until just before the linear sweep voltammetry (LSV) measurement. The current intensity was higher for higher accumulation times until it plateaued after 10 min, as shown in Fig. 5.

The optimal accumulation time can vary and depends on the required analytical sensitivity. When low detection limits are required, the accumulation time must be adequate to increase the method's response and sensitivity.

Moreover, the high analyte concentration for drugs makes it unnecessary to use long accumulation times to obtain appropriate current responses. The pH results and accumulation time for the NMD were used to implement the analytical methods using a flow injection analysis system.

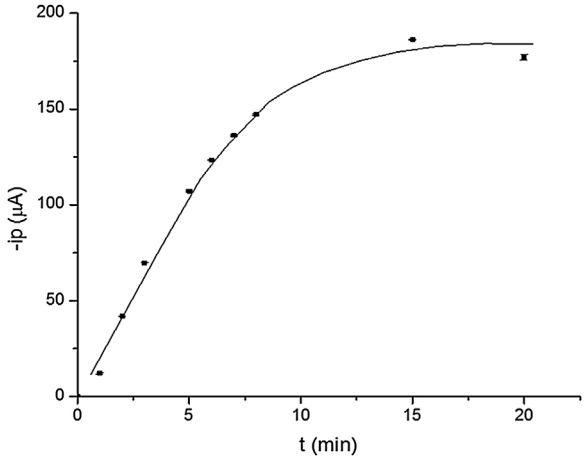

Fig. 5. Current intensity versus accumulation time for 1×10^{-5} M NMD in 100 mM Britton–Robinson buffer/NaCl at pH 2 based on linear sweep voltammetry.

3.2. Flow injection analysis system

Our FIA system contained a pump system, wall-jet flow cell for screen printed electrodes, pre-treated CNF-SPE and a six-way manual sample injection valve with a 1 mL sample loop joined to a potentiostat. The carrier solution, 100 mM Britton-Robinson buffer/NaCl, was transported by the pump into the system. The samples were diluted with 100 mM Britton-Robinson buffer/NaCl and injected into the valve using a 5 mL syringe. The carrier solution was always deaerated by bubbling with N_2. The samples were deaerated 5 min before the analysis. A 1 mL sample loop was used to inject the sample into the stream, and a 30-s accumulation time was used because it directly related to the sample volume.

3.3. Validation

Tto.exe [30,31] software was used to correct the base current to facilitate the peak integration. This program allows for correcting the baseline slope in the voltammograms and smoothly transitioning into the ordinate. This correction is accomplished using a second-order polynomial algorithm and subtracting a straight line that fits the voltammogram area (in the neighborhood of the peak), where only the base signal exists. Tto was used for all of the voltammograms from the method validation and NMD quantification using CNF-SPE-LSV-FIA.

The analytical method validation determined the linearity and working linear range, both detection and quantification limits, precision and accuracy based on the experimental pH and accumulation time conditions established for the batch system (pH 2 and 0 s, respectively).

A concentration study was developed to establish the linear and working range for this method. NMD solutions with 10

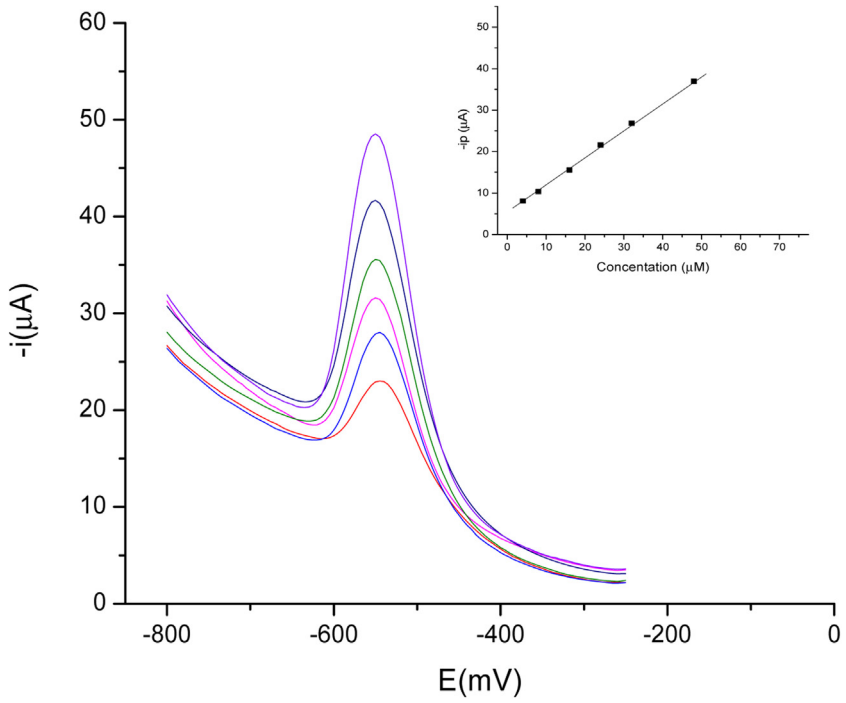

Fig. 6. NMD linear range in 100 mM Britton–Robinson buffer/NaCl at pH 2.

different concentrations were prepared in 100 mM Britton-Robinson buffer/NaCl (4.0, 8.0, 16.0, 24.0, 32.0, 40.0, 48.0, 56.0, 64.0, and 72.0 μM) and injected into the SPE-FIA system. The responses were measured as the current peak (i_p) by LSV. The observed linear range went up to 56.0 μM. The linear working range can be situated in this interval based on the analytical needs as shown in Fig. 6.

In our case, the interval from 4.0 to 40.0 μM was selected as the linear working range. A correlation coefficient above 0.995 was the criterion for establishing curve linearity.

In the lower linear range, ten 2.0 μM NMD solutions in 100 mM Britton-Robinson buffer/NaCl were analyzed via CNF-SPE-LSV-FIA for the LOD and LOQ study. The value of $t_{(9,\alpha=0.99)} = 2.82$ was considered the LOD based on Eq. (1) (in Section 2). The detection limits obtained for a 1 mL loop and 30 s accumulation time was LOD = 0.4 μM. Consequently, the quantification limit according to equation calculated from Eq. (2) (in experimental section) was LOQ = 1.2 μM. Additionally, the limit of detection for this method using a 6 mL loop was calculated to improve the sensitivity by increasing the accumulation time (the sample volume incremented directly with the accumulation time increase). The calculated detection limit was LOD = 0.08 μM based on seven 0.1 μM NMD samples in 100 mM Britton-Robinson buffer/NaCl at pH 2 via CNF-SPE-LSV-FIA in the lower levels of the linear range. A 270 s accumulation time and 6 mL sample volume loop were used with $t_{(6,\alpha=0.99)} = 3.14$. Several LOD for nimodipine quantification using different methodologies are compared in Table 1. The LOD obtained by CNF-SPE applied to pharmaceutical formulations was similar to the LOD using HMDE and better than HPLC-UV and spectrophotometric methodologies.

The first three techniques show an excellent LOD from 0.001 to 0.02 μM, respectively, but a low precision is reported for them with values from 2.9% to 6.1% and values of precision lower than 1.5% are required by the pharmacopeia. In the same way, NGE modified electrode show excellent results of LOD however it is very time consuming. It is necessary to prepare the nitrogen-doped graphene (NGE) in a process of 10 h of reflux. The construction of NGE modified electrode required of 1 h of sonication. Additionally, 1 h of sonication is necessary for the sample pretreatment too. The complete analysis can be performed in about 14 h, this long period of time became a major disadvantage of the method when is used to perform the analysis of a large number of samples. CNF-SPE-FIA does not require many steps for the development of the methodology, the longest period spent during the analysis corresponds to the accumulation time and can be controlled according to the expected concentration in the sample, higher accumulation time will be used to get lower LOD.

Seven 20 μM solutions in 100 mM Britton-Robinson buffer/NaCl at pH 2 were prepared in the middle of linear working range to study the repeatability. The analyses were performed on the same day using CNF-SPE-FIA and LSV with the same electrode, analyst and analytical conditions. The relative standard deviation (%RSD) was used to calculate the intra-day precision. A satisfactory RSD of 1.5% was obtained for determining NMD in pharmaceutical formulations. This RSD allowed for NMD quantification below the USP requirements, which indicates that the NMD concentration in the pharmaceutical formulations should have an error of ±1.5% (pharmaceutical NMD formulations contain no less than 98.5% and no more than 101.5% NMD [5]).

The intermediate precision was determined identically to the repeatability; however, the solutions were analyzed on different days using different SPEs. The RSD was 4.8% and was considered acceptable for an analytical method using carbon nanofiber electrodes based on previous work.

The method accuracy was evaluated using seven replicates of 20 μM NMD in 100 mM Britton-Robinson buffer/NaCl at pH 2. The concentration was selected from the middle of the linear working range. The analysis was developed using CNF-SPE-LSV-FIA. The method accuracy was expressed as the percent recovery (% recovery). The recoveries were between 99.3% and 102.6%, and the average recovery was 100.8%, with an RSD = 1.31%.

3.4. Pharmaceutical sample analysis

3.4.1. Composite tablets

To establish the applicability of the proposed analytical method, two brands of 30 mg NMD formulations were analyzed. These

Table 1
Comparison of LOD and performance values for nimodipine using different methodologies applied to pharmaceutical formulations and human plasma.

Technique	LOD (μM)	Precision (%)	Recovery (%)	Sample matrix
LC–MS/MS [8]	0.001	6.1	97	Human plasma
Nitrogen-doped grapheme [22]	0.01	4.7	102.8	Tablet
MWCNT-GC [21]	0.02	2.9	97.6	Tablet
CNF-SPE (This paper)	0.08	1.5	100.8	Tablet
HMDE [20]	0.08	1.0	100.1	Tablet
HPLC-UV [19]	0.24	1.1	99.0	Tablet
Spectrophotometric [15]	0.45	1.6	100.4	Tablet

formulations, one of which was a generic drug (Instituto Sanitas, Lot: 0263014), were purchased from pharmacies. To prepare the samples, ten tablets were first individually weighed and then finely powdered as a composite. Approximately, 38 mg of the tablet powder was required to obtain a 10 mL stock solution of 1.0×10^{-3} M of NMD in DMF for tablets from the Instituto Sanitas, and 46 mg of the tablet powder yielded the same NMD concentration for tablets from Tecnofarma in 10 mL of DMF. All solutions were allowed to settle for one hour before taking any aliquots. Diluting 500 μL of the clear solutions to 25 mL with Britton-Robinson buffer/NaCl (100 mM, pH 2) yielded a theoretical concentration of 20 μM. Every sample was evaluated using CNF-SPE-LSV-FIA. A 1-mL loop was used with a 30-s accumulation time. The NMD content in the tablets were quantified via an external calibration curve across the range from 4 to 30 μM. The measured drug concentrations ranged from 29.3 to 31.1 mg for NMD from *Tecnofarma*, and the average concentration was 30.2 mg/tablet of NMD with an RSD = 2.0%. The NMD content for the generic formulation ranged from 29.1 to 30.9 mg for NMD from *Instituto Sanitas* and the average concentration was 30.1 mg/tablet of NMD, with an RSD = 1.9%.

According to these results, the proposed method is suitable for quantifying the nimodipine content in commercial pharmaceutical formulations. Because the common excipients and active species in these tablets did not interfere with the quantification, the external calibration curve provided excellent results.

3.4.2. Individual tablets

Ten generic NMD tablets (Instituto Sanitas) were individually finely powdered and weighed to define the NMD homogeneity in each. Approximately, 38 mg from each powdered tablet was weighed and dissolved in 10 mL of DMF in an amber volumetric flask. The solutions were shaken vigorously and allowed to decant for one hour before removing an aliquot. The diluted samples were prepared by diluting 500 μL of each clear solution to 25 mL with Britton-Robinson buffer/NaCl (100 mM, pH 2) to obtain a theoretical concentration of 20 μM. Every sample was evaluated via CNF-SPE-LSV-FIA using a 1-mL loop and 30-s accumulation time. The NMD contents for these tablets were quantified using an external calibration curve ranging from 8 to 40 μM. The NMD contents for eight tablets ranged from 28.5 to 31.3 mg. The NMD contents of two samples were 36.6 and 37.6 mg. The calculated relative standard deviation, RSD, was 11.0% based on all samples. The RSD being higher for individual tablets than the composite indicates that the NMD in each tablet is not homogeneous, despite the average concentration being 31.4 mg.

4. Conclusions

The proposed analytical method was suitable for analyzing NMD in pharmaceutical formulations. No interferences were observed from the excipients for the active species in the tablets. Therefore, an external calibration curve could be used to quantify the NMD by CNF-SPE-LSV-FIA. A 30-s accumulation time was sufficient to obtain reproducible and accurate results and demonstrated that controlling the accumulation time could decrease the detection limits from 0.08 to 0.400 μM, as shown in this work. CNF-SPE is simple to use and cheap disposables that can be reused over 30 times before discarding. The CNF and FIA systems are technique sensitive and accurate with low detection limits. The sample requires no pretreatment before analyzing, which is a significant advantage over techniques that require several pretreatment or cleanup steps such as HPLC. The NMD concentration in both the brand name and generic drugs agreed with the pharmacopeia [5], and the concentrations were accurate and precise, with RSDs below 2%.

The good performance of the methodology based on CNF-SPE-FIA applied to nitrofurans and reported in previous publications together with the results obtained in the current study with nitrobenzene derivative (nimodipine) allows us to conclude that this methodology can be generalized to the analysis of any nitroaromatic compound.

We recommend the proposed method to be used as a rapid screening method for detecting nitro compounds to subsequently apply the techniques approved by the respective institutions as is the case of the FDA or the pharmacopoeia.

Acknowledgement

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Biographies

P. Salgado-Figueroa is a collaborator professor at the Chemical & Pharmaceutical Sciences, Faculty of the University of Chile. She received the Ph.D. in chemistry from University of Chile in 2013. She works on the development of chemical sensors.

C. Gutierrez is currently a Ph.D. in chemistry at University of Chile. She works on the development of chemical sensors.

J.A. Squella is a full professor of electrochemistry at University of Chile, Santiago, Chile. He is currently Dean at the Chemical & Pharmaceutical Sciences, Faculty of the University of Chile. Current research interest focuses on molecular electrochemistry, organic electrochemistry and electroanalysis.