Talanta 122 (2014) 187–194

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Multi-walled carbon nanotubes/Nafion composite film modified electrode as a sensor for simultaneous determination of ondansetron and morphine

Biljana Nigović *, Mirela Sadiković, Miranda Sertić

University of Zagreb, Faculty of Pharmacy and Biochemistry, A. Kovacica 1, 10000 Zagreb, Croatia

ARTICLE INFO

Article history:
Received 19 November 2013
Received in revised form
17 January 2014
Accepted 22 January 2014
Available online 31 January 2014

Keywords:
Ondansetron
Morphine
Multi-walled carbon nanotubes
Nafion
Modified glassy carbon electrode

ABSTRACT

The electrochemical behavior of ondansetron was studied on the multi-walled carbon nanotubes/Nafion polymer composite modified glassy carbon electrode (MWCNTs–Nafion/GCE). The oxidation peak potential was shifted from 1.32 V to 1.18 V compared to the bare electrode indicating excellent electrocatalytic activity of immobilized film toward drug molecule. The modified electrode exhibited a remarkable enhancement effect on voltammetric response due to the synergistic effect of nanomaterial and cation-exchange polymer on the electron transfer rate, the effective electrode area and the accumulation capability. After optimizing the experimental parameters, adsorptive stripping procedure was used for the determination of ondansetron in pharmaceutical formulation. The results were satisfactory in comparison with those obtained by high-performance liquid chromatography. In addition, the MWCNTs–Nafion/GCE exhibited high selectivity in the voltammetric measurements of ondansetron and co-administrated drug morphine with potential difference of 430 mV. The response peak currents had linear relationship with drug concentration in the range of 1.0×10^{-7}–5.0×10^{-6} M and 1.0×10^{-7}–4.0×10^{-6} M with detection limits 3.1×10^{-8} and 3.2×10^{-8} M for ondansetron and morphine, respectively. The electrode was successfully applied for simultaneous electrochemical sensing of both drugs in human serum samples after selective accumulation at the electrode surface.

1. Introduction

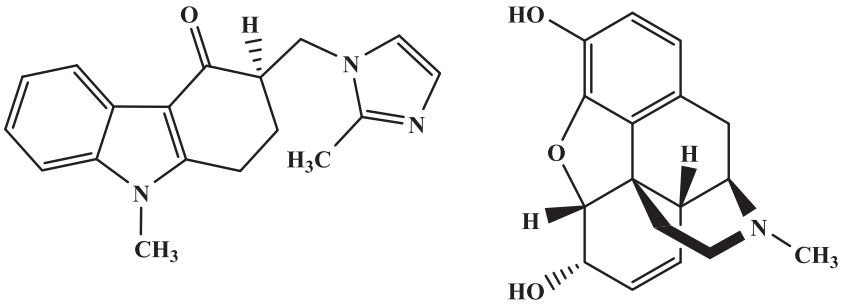
Ondansetron (OND) is a selective 5-HT_3 receptor antagonist widely used in the treatment of emetogenic side effects accompanying cancer chemotherapy or radiotherapy (Scheme 1). It has also become the first line therapy for the prevention of post-operative nausea and vomiting [1]. In addition, OND is used to ameliorate pruritus, irritable bowel syndrome, diarrhea associated with cryptosporidiosis or diabetes, chronic refractory diarrhea, anxiety and sleep disorders, alcohol dependency, opiate withdrawal syndrome, vertigo, Tourette's syndrome and psychosis in advanced Parkinson's disease [2]. The studies on the use of OND in new indications continue and, therefore, the development of a sensitive analytical method to evaluate the quality of its pharmaceutical product or to determine drug concentration in biological fluids is highly required.

At present, several analytical methods have been employed for the determination of OND in biological samples or pharmaceutical preparations, such as HPLC combined with mass spectrometry [3–5] or UV detection [6–8], supercritical fluid chromatography/tandem mass spectrometry [9], HPTLC [10], spectrophotometry [11], radioimmunoassay [12] and capillary zone electrophoresis [13]. However, most of these methods are time-consuming, solvent-usage intensive, expensive and involve tedious sample preparations or, on the other hand, suffer from poor selectivity.

Morphine (MOR) is a widely used and highly effective analgesic agent for the treatment of acute and chronic pain associated with cancer, recommended by the World Health Organization. OND often coexists with MOR in biological fluids as these drugs are co-administered for control of pain, nausea and vomiting in patients undergoing surgical procedure and chemotherapy. To prevent overdose-induced toxicity, monitoring of the therapeutic levels of OND and MOR concentrations in biological samples of patients is a critical issue in clinical medicine. Therefore, it is necessary to establish simple, rapid and sensitive analytical methods for detection and quantitation of OND and MOR in biological matrix for clinical, toxicological and pharmacological studies. Recently, some new electrochemical methods have been proposed for morphine detection [14–19]. However, up to now, only one analytical method has been reported in the literature on the simultaneous determination of OND and MOR [20]. Two analytes were separated within 20 min and determined in

* Corresponding author. Tel.: +385 1 6394 453; fax: +385 1 4856 201.
E-mail address: bnigovic@pharma.hr (B. Nigović).

0039-9140/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2014.01.026



OND MOR

Scheme 1. Chemical structures of ondansetron (OND) and morphine (MOR).

pharmaceutical dosage form by HPLC method with UV detection. Consequently, a cheap, highly sensitive and selective method is of great demand for simultaneous determination of both drugs.

Electrochemical detection of an analyte is a very elegant method in pharmaceutical analysis due to its high sensitivity, rapid response, simple operations and low cost [21,22]. In spite of that, the redox behavior and electrochemical determination of OND in pharmaceutical formulation or human fluids have not been reported in the literature to this date. Our preliminary results showed that the voltammetric response of OND at the glassy carbon electrode (GCE) was not satisfactory because of slow heterogeneous electron transfer. In the light of these findings, it was a challenge to find a new electrode material for determination of OND using voltammetric technique.

In recent years, carbon nanotubes (CNTs) have attracted increasing attention in the electrode preparation due to their unique structure and extraordinary electronic properties [23]. CNTs have huge surface area, significant mechanical strength, high electrical conductivity and efficient electrocatalytic activity. On the other hand, Nafion has been extensively applied as an electrode modifier due to its excellent antifouling capacity, high permeability to cations and strong adsorption ability. The hydrophilic negatively charged sulfonate group in Nafion film enables selective preconcentration of positively charged drug molecules through electrostatic interaction, whereas, the hydrophobic fluorocarbon network of the polymer gives a selectivity for the hydrophobic part of the molecule [24].

Based on the above advantages of nanomaterial and cation-exchange polymer, a simple and sensitive electrochemical sensor was developed for simultaneous trace analysis of OND and MOR. To take advantage of the remarkable properties of MWCNTs in electrochemical sensing application, the MWCNTs should be properly functionalized and uniformly immobilized at electrode surface. However, the MWCNTs are inclined to form agglomerates due to strong Van der Waals interactions when they are dispersed in water or organic solvents [23]. To overcome this difficulty, Nafion was selected as the binder in electrode fabrication procedure. In the presence of Nafion, the MWCNTs were homogeneously dispersed into ethanol via ultrasonication. Consequently, a stable and uniform film was easily achieved at the electrode surface after organic solvent evaporation.

The electrochemical behavior of OND on the multi-walled carbon nanotubes/Nafion polymer composite modified glassy carbon electrode (MWCNTs–Nafion/GCE) strongly revealed selective interfacial accumulation of positively charged drug molecule and excellent electrocatalytic activity toward target, thus improving the sensitivity and voltammetric signal-to-noise ratios as well as the stability of the resulting film. The proposed method was applied for the quantification of OND in real drug samples. The ability of the modified electrode for voltammetric response of MOR was also evaluated. Finally, the MWCNTs–Nafion/GCE was successfully used as an electrochemical sensor for the simultaneous determination of OND and MOR at trace levels in biological samples.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using a μ-Autolab potentiostat (Eco Chemie, Utrecht, The Netherlands) controlled by GPES 4.9 software. A conventional three-electrode system was employed, comprising a bare GCE (3-mm diameter, Metrohm, Switzerland) or MWCNTs/Nafion film modified GCE as a working electrode, a platinum wire as a counter electrode and an Ag/AgCl/3 M KCl (Metrohm) as the reference electrode. All electrochemical experiments were carried out at room temperature (23 ± 1 °C). When required, stirring was applied using a computer-controlled stirrer at ca. 300 rpm.

Scanning electron microscopy (SEM) measurement was performed on a Jeol JSM-7000 F microscope (Jeol Ltd., Tokyo, Japan). High-performance liquid chromatographic experiments were carried out using an Agilent 1100 Series LC system equipped with a diode array detector (Agilent Technologies, Waldbronn, Germany).

2.2. Chemicals

OND, kindly donated by Pliva (Zagreb, Croatia), was used as received without any further purification. Ondantor® (Sandoz) film-coated tablets, containing ondansetron hydrochloride dihydrate equivalent to 8 mg of OND, were supplied by local pharmacy. MOR was obtained from the Agency for Medicinal Products and Medical Devices (Zagreb, Croatia). Multi-wall carbon nanotubes ($>98\%$, O.D. 6–13 nm, length 2.5–20 μm) and Nafion (5 wt% solution in a mixture of lower aliphatic alcohols and water) were from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of analytical grade quality. Ultra-pure water used for the preparation of standard solutions and buffers was obtained by a Milli-Q system (Millipore, Bradford, USA).

2.3. Preparation of MWCNTs/Nafion electrode

Before use, the received MWCNTs were sonicated in concentrated HNO_3 for 4 h to generate carboxylic acid-functionalized MWCNT surface. The suspension was filtered through a cellulose nitrate membrane with a 200 nm pore size. The solid powders were washed thoroughly with double distilled water to remove any residual acid and then dried under vacuum at room temperature. The immobilizing suspension was prepared by dispersing 1 mg of functionalized MWCNTs in 1 mL of 0.5% (m/v) Nafion solution prepared in ethanol. The mixture was then sonicated for

30 min to get a stable and well-distributed MWCNTs–Nafion suspension. Prior to modification, the GCE was polished with aqueous slurry of 0.05 μm alumina powder on a smooth polishing cloth, thoroughly rinsed with water and then ultrasonically cleaned in water for 30 s. Finally, the electrode was washed with purified water and dried. The cleaned GCE was uniformly coated with 5 μL of the MWCNTs–Nafion suspension and air-dried at room temperature. Then, the modified electrode was carefully rinsed with purified water. At the beginning of experiment, the modified electrode was scanned by two successive cyclic voltammetric sweeps between 0 and 1.4 V at 100 mV/s in a blank solution of 0.1 M H_2SO_4. The relative electrochemical surface areas of the MWCNTs–Nafion/GCE and bare GCE were determined by cyclic voltammetry (CV) between −0.2 and 0.7 V in 1×10^{-3} M ferricyanide solution as a redox probe in 0.1 M KCl electrolyte at different scan rates (v). From the slope of the plot of anodic peak current versus $v^{1/2}$, the electro active area was calculated. The modified MWCNTs–Nafion/GCE showed a surface area 5.2 times greater than the bare GCE. The MWCNTs–Nafion/GCE can be stored in air or kept in distilled water for several weeks without the loss of activity. For comparison, a Nafion modified GCE was prepared using the same procedure described previously, but without MWCNTs, and denoted as Nafion/GCE.

2.4. Electrochemical measurement procedures

Stock solution of OND (1×10^{-3} M) was prepared in purified water and stored under refrigeration. Stock solutions of MOR (1×10^{-3} M) were freshly prepared for daily use with purified water. The working standard solutions of OND and MOR were obtained by serial dilution of stock solutions with a supporting electrolyte just before voltammetric measurements. The studies were carried out in 0.1 M H_2SO_4 solution and Britton–Robinson buffer (0.04 M in each of acetic, phosphoric and boric acids) adjusted to the desired pH with 0.2 M sodium hydroxide solution. The oxidative behavior of OND was investigated by CV in the scan range from 0.0 V to 1.5 V. Square-wave voltammetry (SWV) was used for the determination procedures of OND and MOR. The sensor was immersed in the sample solution and preconcentration of both analytes was carried out at potential −0.5 V with a 360 s accumulation step. After 5 s equilibrium period, the voltammogram was recorded in the SWV mode (frequency, 75 Hz; potential step, 8 mV; amplitude, 25 mV) from 0.7 to 1.4 V. Following each measurement, the MWCNTs–Nafion/GCE was transferred to the solution of supporting electrolyte to regenerate the electrode surface by applying single positive-going SWV potential scan from 0.7 to 1.4 V.

2.5. Pharmaceutical dosage form assay procedure

To prepare the solutions of OND commercial pharmaceutical product, ten tablets were weighted and crushed to a fine powder. An accurately weighted powdered sample of the drug formulation equivalent to 3.6 mg of active ingredient was transferred into a 10.0 mL calibrated flask and dispersed in water. The tablet solution was sonicated for 15 min to provide complete dissolution of the active ingredient. After sonification, the sample was filtered through 0.45 μm Acrodisc GHP filters (Gelman, Ann Arbor, USA). An aliquot of filtrate was then transferred into a calibrated flask and diluted with water to yield a final drug concentration of 1.0×10^{-4} M. A series of dilutions were made with the supporting electrolyte to cover the working concentration range. Solutions were subjected to voltammetric measurements as previously described and the content of OND in the pharmaceutical preparations was determined by the standard addition method. For recovery studies, aliquots of the OND standard solutions were added to real samples prepared from tablets.

For the comparison study, HPLC experiments were carried out using a chromatographic column Zorbax C18, 250 × 4.1 mm, particle size 5 μm (Agilent Technologies, Waldbronn, Germany). The mobile phase, consisted of acetonitrile, ultra-pure water and tetraethylammonium hydroxide at the ratio of 50:50:1 (v/v/v), was filtered through cellulose nitrate filter (0.45 μm, Sartorius, Goettingen, Germany). The HPLC analyses were carried out at constant temperature (25 °C) with a flow-rate of 0.7 mL/min under isocratic conditions. For chromatographic analysis, standard solutions of OND were made by dilution with the mobile phase and filtered through a 0.2 μm Acrodisc GHP filters (Gelman, Ann Arbor, USA). The 10 μL aliquots were injected into the HPLC system for analysis. The DAD detector recorded UV spectra in the range from 190 to 400 nm and chromatogram was obtained at 248 nm. All chromatographic data acquisition and processing was performed using ChemStation software (Agilent Technologies, Waldbronn, Germany).

2.6. Determination of OND and MOR in biological samples

Human serum samples were obtained from healthy volunteers abstained from any medications during the week preceding the study and were stored frozen until assay. Serum samples were fortified with the appropriate aliquot volume of OND and MOR standard solutions to achieve final concentrations (5.0×10^{-7} and 1.0×10^{-7} M) that are found in serum after the treatment with therapeutic daily doses of 16 and 80 mg for OND and MOR, respectively [25,26]. An aliquot of serum sample containing OND and MOR was mixed with acetonitrile (1:1) to remove serum proteins effectively. After vortexing for 60 s, the mixture was then centrifuged for 6 min at 6000 rpm. Appropriate volumes of this supernatant were transferred into the volumetric flask and diluted with the supporting electrolyte and analyzed in the voltammetric cell. Quantitations in biological fluids were performed using the standard addition method by adding three successive aliquots of drug standard solutions.

3. Results and discussion

3.1. Electrochemical response of OND at GCE

In this paper, the redox behavior of OND was studied for the first time and initially, we started with the investigation of the electrochemical oxidation of drug molecule at a bare GCE by cyclic voltammetry. A poorly defined anodic current response was observed during the scanning from 0 to 1.5 V (Fig. 1, curve a). On the reverse potential scan, there was no corresponding reduction peak, suggesting that the electrode reaction of OND is totally irreversible. The voltammetric response was broad due to slow electron transfer. In addition, the peak potential of OND obtained at GCE had a relatively high value, close to +1.32 V in 0.1 M H_2SO_4 solution, which resulted in the merging of the signal current with the background current. Therefore, the effect of pH on the electrochemical response of OND was studied over the pH range of 2.0–9.0 in BR buffer. However, the peak potential of the oxidation process remained almost constant in the pH interval 2.0–7.0 (E_p (V) = 1.37–0.009 pH). As the pH value was increased above 7, the oxidation peak shifted to less positive potentials, but it was split into two broad and overlapped waves, together with a decrease in peak currents.

The effect of scan rate on the oxidative peak currents was also evaluated. Variation of the scan rate in the range of 20–500 mV/s resulted in a linear relationship between the anodic peak current

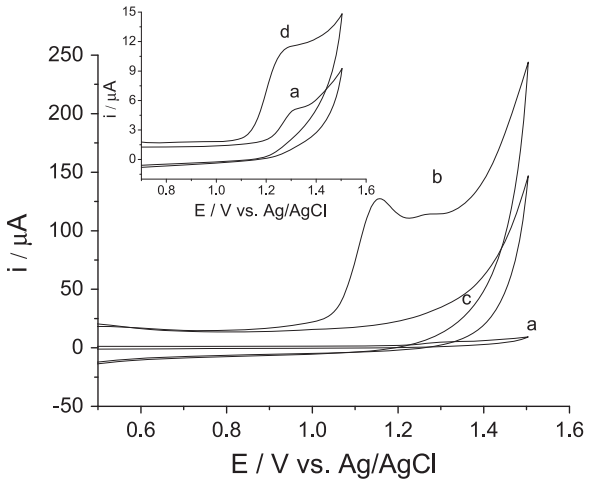


Fig. 1. Cyclic voltammograms of ondansetron (1×10^{-4} M) at a bare GCE (a) and the MWCNTs–Nafion/GCE (b) together with corresponding background recording (c) in 0.1 M H_2SO_4. Inset: cyclic voltammograms of ondansetron at a bare GCE (a) and the Nafion/GCE (d). Scan rate: 100 mV/s.

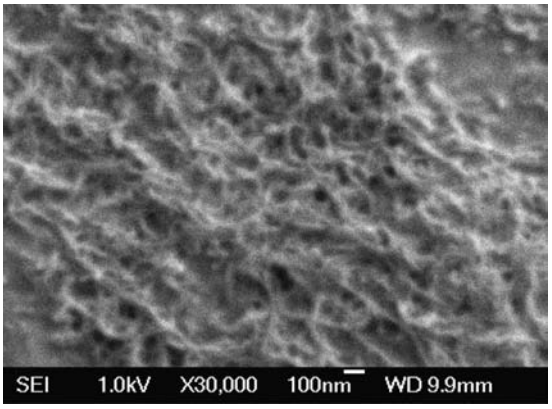


Fig. 2. The surface image of MWCNTs–Nafion/GCE produced by scanning electron microscopy. Scale bar: 100 nm.

and the square-root of a scan rate. This indicates that the electrode process was controlled by diffusion rather than adsorption. Additionally, a plot of logarithm of peak current versus logarithm of scan rate gave a straight line $\log i=0.456\log\nu$ –0.326 ($r=0.993$) with a slope very close to the theoretical value of 0.5, an expected value for an ideal reaction of solution species [27]. Therefore, the preliminary results clearly revealed that the voltammetric response of OND at bare GCE was not satisfactory for the analytical application due to slow electron transfer reaction, reduced sensitivity since the adsorption of drug molecule could not be purposely used as a preconcentration step, electrode fouling problems and unstable analytical signal.

3.2. Electrochemical behavior of OND on modified electrode

To improve the electrochemical response of OND, the electrode was modified with nanomaterial and cation-exchange polymer. The surface morphology of MWCNTs–Nafion/GCE was characterized by SEM. As shown in Fig. 2, the MWCNTs were well dispersed in the Nafion and entrapped in the polymer matrix. The nanocomposite film deposited at the surface of the GCE formed a three-dimensional structure and reticular formation revealing a much larger surface area than electrode geometric area.

The cyclic voltammograms of 1.0×10^{-4} M drug solution in 0.1 M H_2SO_4 obtained at the MWCNTs–Nafion/GCE, Nafion/GCE and a bare GCE with a scan rate of 100 mV/s are presented in Fig. 1. When the bare GCE surface was coated with 0.5% Nafion film, a well-shaped oxidation wave ($i_p=9.6$ μA) appeared at 1.29 V (inset of Fig. 1). Nafion as a cation-exchanger attracted positively charged drug molecules from the bulk solution to enhance the anodic signal. However, the electroanalytical performance of the MWCNTs–Nafion /GCE for measuring OND was advantageous over that observed at the Nafion/GCE and bare GCE. Compared with Nafion/GCE, the voltammetric response of OND intensively increased at MWCNTs–Nafion/GCE with a current of 30.5 μA and a peak potential of 1.18 V. The oxidation peak potential of OND was shifted to less positive values by 140 mV compared with that of using a bare GCE indicating faster electron transfer reaction at the electrode surface and excellent electrocatalytic activity of immobilized film toward drug molecule. The experimental results clearly indicated a remarkable enhancement effect on the electrochemical response of OND obtained from the combined contribution of Nafion and CNTs. This is due to the larger surface area of the modified electrode as well as the synergistic effect of the electronic conductivity and electrocatalytic activity of MWCNTs with ionic conductivity and ion-exchange capacity of Nafion. OND is a weak basic compound with pK_a 7.4 and therefore, the molecule is completely protonated in the experimental conditions under which voltammetric measurements were performed. It is evident that Nafion enhances the preconcentration of drug molecules through electrostatic interaction with negatively charged sulfonate group in Nafion polymer structure, leading to a considerable improvement in the analytical sensitivity. Another effect of Nafion observed here was fixing CNTs onto GCE surface tightly and enhancing the stability of the electrochemical sensor. On the other hand, the sensor exhibited a higher sensitivity for OND determination owing to the excellent properties of MWCNTs, thus indicating an improvement in the electrode kinetics and a decrease in the oxidation potential substantially. The OND oxidation observed at the high potential using GCE was more facile at the MWCNTs–Nafion/GCE due to the shift in peak potentials to less positive values, thus placing the electrochemical oxidation outside of the solvent window.

For evaluating the reaction character of OND at the MWCNTs–Nafion/GCE, cyclic voltammetry was performed in a 5.0×10^{-5} M OND solution with different scan rates. The peak potential was positively shifted from 1.116 to 1.241 V when increasing the scan rates from 20 to 500 mV/s. The dependence of the peak potential is linear with logarithm of the scan rate, which could be expressed as a regression equation: E_p (V)=0.0927 log ν (mV/s)+0.989 ($r=0.996$), allowing the calculation of $\alpha n=0.638$ (inset of Fig. 3). Using the value of the charge transfer coefficient (0.66) obtained from the difference between the peak potential (E_p) and the half wave potential ($E_{p/2}$), taking the equation $\alpha=(47.7/E_p-E_{p/2})$ [28], the number of electrons exchanged was calculated to be $n=0.96$. The voltammetric response of OND at the modified electrode is probably initiated by one-electron transfer to form the cation radical at nitrogen atom in the imidazole ring. On the other hand, peak currents were linearly increased with the scan rates from 20 to 200 mV/s, and their relationship obeyed the following regression equation i_p (μA)=0.227 ν (mV/s)+7.838 ($r=0.997$). This result indicated that the electrochemical reaction of OND at the MWCNTs–Nafion/GCE was a surface-controlled process [27]. Therefore, to obtain the highest sensitivity of the method, the adsorption of OND at the modified electrode surface was purposely used in subsequent analytical measurements as an effective accumulation step prior to the voltammetric quantitation of the drug.

3.3. Selection of the experimental conditions

The electrochemical oxidation of OND was examined in different electrolyte solutions using CV. Various types of the supporting

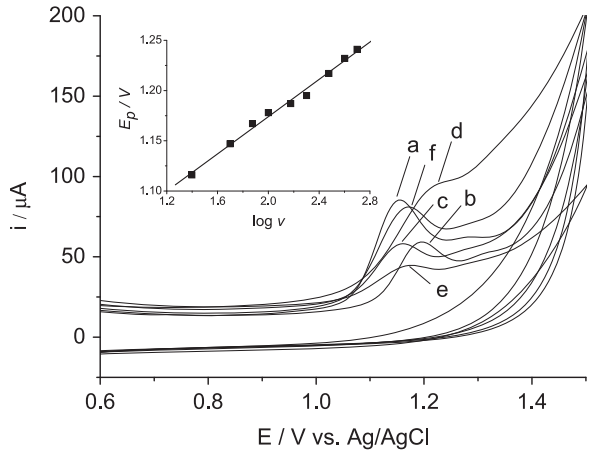


Fig. 3. Influence of supporting electrolyte on ondansetron oxidation at the MWCNTs–Nafion/GCE: 0.1 M H_2SO_4 (a), 0.5 M H_2SO_4 (b), Britton–Robinson buffer pH 2 (c) and pH 4 (d), 0.1 M CH_3COOH (e) and 0.01 M HCl (f). Scan rate: 100 mV/s; $c = 1 \times 10^{-5}$ M. Inset depicts peak potential vs. logarithm of scan rates from 20 to 500 mV/s in 0.1 M H_2SO_4.

electrolytes, including BR buffer pH 2.0–6.0, H_2SO_4, HCl and CH_3COOH, were tested for the electroanalytical measurements, as shown in Fig. 3. The comparison of voltammograms showed that only in lower pH values OND could exhibit higher peak current. The drug molecule is completely protonated on the nitrogen atom in the imidazole ring and could be more effectively attracted to the modified electrode surface. A lower peak potential and highest peak current was achieved in 0.1 M H_2SO_4 and, therefore, this solution was selected as the suitable supporting electrolyte for the drug determination using the MWCNTs–Nafion/GCE.

To improve the sensitivity, SWV was employed for measuring OND due to the most favorable response compared to other voltammetric modes such as differential pulse voltammetry and linear-sweep voltammetry. Variation of the peak current for OND recorded at the MWCNTs–Nafion/GCE was monitored while changing instrumental parameters, i.e. frequency (25 Hz $\leq f \leq$ 100 Hz), pulse amplitude (5 mV $\leq a \leq$ 50 mV) and potential step (2 mV $\leq \Delta E_s \leq$ 10 mV). The most favorable response was achieved using a combination of a frequency of 75 Hz, a pulse amplitude of 25 mV and a potential step of 8 mV.

The thickness of the cast film on the GCE surface, which was determined by the amount of MWCNTs–Nafion suspension, had certain effects on the current response of OND. The volume of MWCNTs–Nafion suspension coated on the electrode changed the properties and functions of the electrode surface. When the volume of MWCNTs–Nafion suspension added to the GCE surface increased from 3 to 5 μL, the oxidation peak current enhanced notably, but with further increase of the amount of MWCNTs–Nafion suspension to 9 μL, the oxidation peak current conversely showed gradual decline. The Nafion film became thicker and lowered the electrical conductivity of MWNTs and, consequently, retarded the electron transfer rate of OND oxidation as well as the mass transportation between the drug and the electrode resulting in the higher electric resistance. In the beginning, on increasing the amount of the modifier, the sites of electron exchange increased and the adsorption of OND was enhanced. However, the accumulation capacities of the film depend on the amount of Nafion, leading to an increment of the ion-exchange capacity of the film up to a certain limit, which is determined by the nature of the analyte and its diffusion through the film. The voltammetric signal increased up to 0.5% of Nafion concentration in the immobilizing suspension. The SWV response of OND reached the maximum when the composite film was uniformly coated on the electrode surface using 5 μL of homogenous MWCNTs–Nafion suspension.

3.4. Adsorption characteristics of OND on modified electrode

Since the modification of the electrode surface with a MWNTs–Nafion layer led to a strong interfacial accumulation of OND, the possibility of the analyte preconcentration before voltammetric measurement was investigated in order to obtain lower detection limits. Fig. 4 shows square-wave voltammograms for 2.5×10^{-6} M solutions of OND without accumulation and after a 360 s accumulation step at the MWCNTs–Nafion/GCE. To achieve the maximum sensitivity and the optimum conditions for the maximum adsorption, the influence of accumulation potential on the stripping peak current was evaluated from −1.0 to 0.9 V using SWV mode. When the accumulation potential shifted from 0 to 0.9 V, the stripping peak current greatly decreased. The results showed that the oxidation peak current increased as the accumulation potential became more negative. The optimum deposition potential of −0.5 V was selected for subsequent sensitive determination of OND. Square-wave voltammograms with increasing accumulation times (between 30 s and 480 s) were recorded for solutions containing OND at concentration level of 1×10^{-6} M. The accumulation time significantly affected the voltammetric response of OND at the MWCNTs–Nafion/GCE. The oxidation peak current enhanced greatly with the increase of the accumulation time within 360 s (Fig. 5). The linear relationship between the voltammetric response and the deposition time pointed to a constant adsorption of positively charged drug molecules attracted on the surface of the MWCNTs–Nafion/GCE. Further increment of accumulation time period showed a deviation of the peak current from linearity, thus indicating surface adsorption saturation. Therefore, the value of 360 s was considered as optimum to achieve the highest possible sensitivity in acceptable analysis time.

3.5. Simultaneous determination of OND and MOR

To develop a new electroanalytical method for simultaneous determination of OND and MOR, the voltammetric response of MOR was also studied at the MWCNTs–Nafion/GCE. Fig. 6 shows the SWV responses from the electrochemical oxidation of OND and MOR in 0.1 M H_2SO_4 solution following preconcentration for 360 s at −0.5 V. The proposed sensor exhibited potent electron mediating behavior followed by well-separated oxidation peaks towards OND and MOR at 1.17 and 0.74 V, respectively. The large

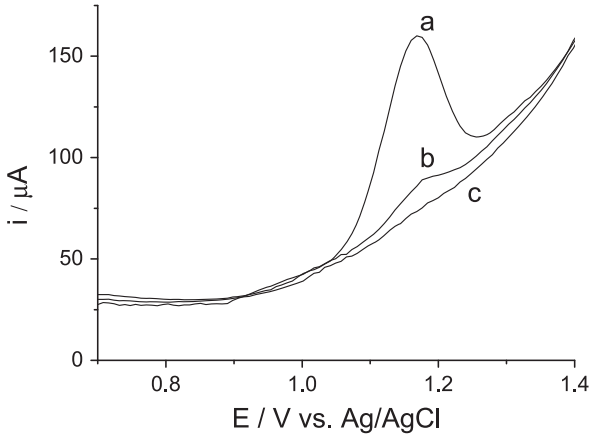


Fig. 4. Square-wave voltammograms of ondansetron (2.5×10^{-6} M) at the MWCNTs–Nafion/GCE in 0.1 M H_2SO_4 after accumulation time of 360 s (a), without accumulation (b) and blank solution (c). SWV settings: frequency of 75 Hz, amplitude of 25 mV and potential step of 8 mV, $E_{acc} = -0.5$ V.

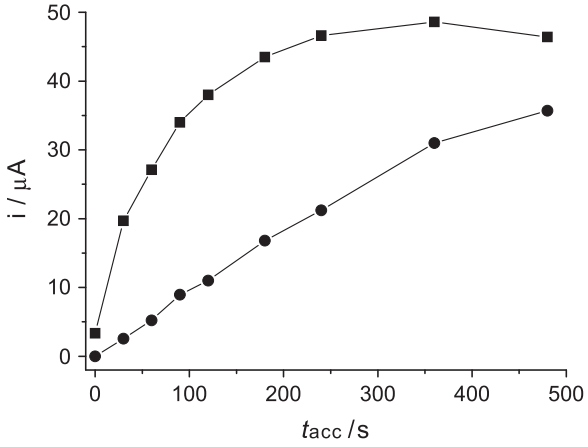


Fig. 5. Effect of the accumulation time on the oxidation peak current for ondansetron (1×10^{-6} M) (circles) and morphine (1×10^{-6} M) (squares). SWV settings and accumulation conditions same as in Fig. 4.

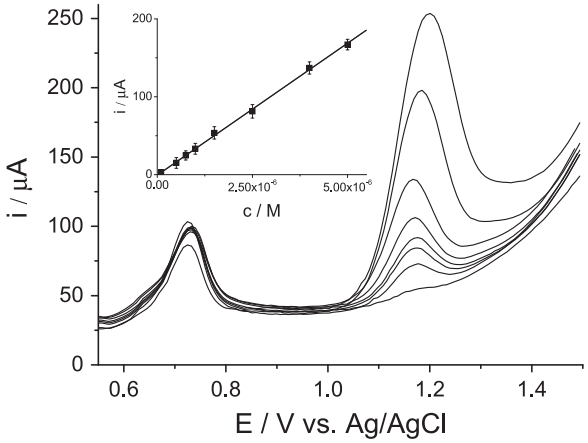


Fig. 6. Adsorptive stripping square-wave voltammograms of ondansetron at the MWCNTs–Nafion/GCE recorded in 0.1 M H_2SO_4 at different concentrations (1×10^{-7}–5×10^{-6} M) in the presence of morphine (1.5×10^{-6} M). SWV settings and accumulation conditions same as in Fig. 4. Inset shows a calibration graph for the quantification of ondansetron.

separation of the peak potentials allows simultaneous determination of OND and MOR in their mixture.

On the basis of previous investigation of MOR oxidation mechanism using GCE, the anodic voltammetric signal of MOR at the MWCNTs–Nafion/GCE can be ascribed to oxidation of phenolic group which involves one electron transfer [29]. The tertiary amine of MOR has a pK_a of 8.4, and the phenolic group has a pK_a of 9.4. Therefore, MOR was also completely in the cationic form under used voltammetric condition and MOR molecule, like OND, showed adsorption at the MWCNTs–Nafion/GCE. As shown in Fig. 5, the accumulation time of 120 s provided for MOR the largest peak current in the linearity range indicating faster adsorption of MOR then OND on the surface of the modified electrode. However, the value of 360 s was used as the optimum for simultaneous quantification of both drugs because the peak current of MOR increased up to a maximum at $t_{acc}=360$ s which was selected previously as the optimal value for OND.

The oxidation peak of OND at 1.17 V in 0.1 M H_2SO_4 solution after 360-second accumulation time showed a linear response at the MWCNTs–Nafion/GCE for concentration in the range of 1.0×10^{-7}–5.0×10^{-6} M. The calibration plot is described by the following regression curve: i_p (μA)$=3.38\times10^7 c$ (M)–0.43, $r=$ 0.999. Since the presence of MOR may affect the quantification of OND due to its competitive adsorption, the variation of adsorptive stripping SWV peak current versus OND concentration in the presence of MOR (1.5×10^{-6} M) was also checked using MWCNTs–Nafion/GCE (Fig. 6). The sensitivity of the modified electrode towards the oxidation of OND in the presence of MOR is 3.27×10^7 μA M^{-1}, which is very close to the value observed in the absence of MOR (3.38×10^7 μA M^{-1}), indicating that the oxidation of OND and MOR at the MWCNTs–Nafion/GCE, as well as their adsorption at electrode surface in the accumulation step, are independent. The detection limit (LOD) and the quantification limit (LOQ) estimated from the calibration curve as LOD$=3s/b$ and LOQ$=10s/b$, where s is the standard deviation of the intercept and b is the slope of the calibration curve, were calculated to be 3.1×10^{-8} M for LOD and 9.6×10^{-8} M for LOQ. Compared with other methods, the linearity range obtained at proposed electrochemical sensor for OND was in the similar concentration range as for HPLC methods with UV detection and HPTLC [6,8,10]. The LOD and LOQ values are better than those obtained in capillary electrophoresis and spectrophotometry [11,13], but are higher than LODs reported for chromatographic methods coupled to mass spectrometry [3–5]. However, the LC–MS analytical procedures demand expensive and sophisticated equipment that is not available in many laboratories. The proposed voltammetric method using the MWCNTs–Nafion/GCE offers several advantages over chromatographic techniques applied only to the quantitative determination of a drug, including short analysis time, simplicity of operation and lower running cost. It should be mentioned once again that in the literature there are no studies on electrochemical behavior of OND or its voltammetric determination.

Fig. 7 shows the square-wave voltammograms for the solutions containing various concentrations of MOR and constant concentration of OND (1.5×10^{-6} M). It can be seen that the related peak currents increased by increasing MOR concentration, while there are no significant changes in the peak current and potential of OND. The calibration graph obtained for anodic peak current of MOR at the MWCNTs–Nafion/GCE was linear within the concentration range of 1.0×10^{-7}–4.0×10^{-6} M (inset of Fig. 7). The regression equation was expressed as i_p (μA)$=2.90\times10^7 c$ (M)$+$ 11.99 with a correlation coefficient of 0.999. The calculated LOD and LOQ values were found to be 3.2×10^{-8} and 9.8×10^{-8} M, respectively.

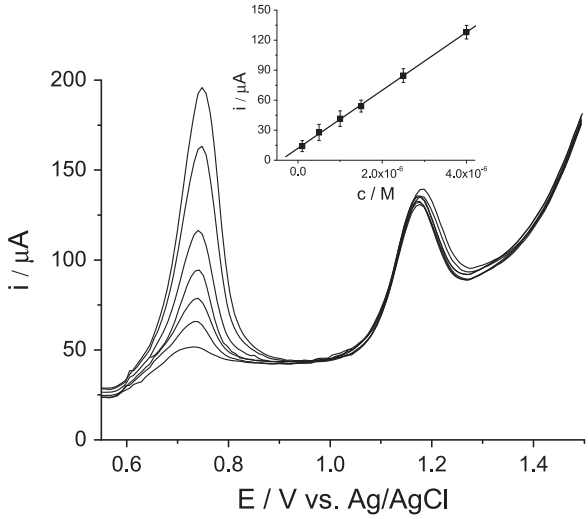


Fig. 7. Adsorptive stripping square-wave voltammograms of the mixture containing morphine at different concentrations (1×10^{-7}–5×10^{-6} M) and ondansetron (1.5×10^{-6} M) recorded at the MWCNTs–Nafion/GCE in 0.1 M H_2SO_4. SWV settings and accumulation conditions same as in Fig. 4. Inset shows a calibration graph for morphine.

In comparison to HPLC–UV procedure developed for the simultaneous quantification of MOR and OND mixtures in 0.9% sodium chloride injection, the MWCNTs–Nafion/GCE showed wider linear range for the quantification of both drugs [20]. The detection and quantification limits of OND and MOR obtained at this sensor are of the same order as for the HPLC method, but the proposed electroanalytical method is simpler, cheaper and faster. In HPLC method the separation time of 20 min was necessary for the analysis. In addition, the HPLC method requires a high percentage of organic solvent making it less environmental friendly.

3.6. Stability and reproducibility of the modified electrode

The stability of the MWCNTs–Nafion/GCE was checked by measuring the adsorptive stripping SWV response in 2.5×10^{-6} M OND and MOR solution over a period of four weeks. Before measurements, the modified electrode was scanned between 0.5 and 1.4 V in the solution of supporting electrolyte until the SWV response was stable. When the nanocomposite modified electrode was stored in the air at room temperature for 10 days, the peak potential of both drugs was unchanged and the current response of OND and MOR decreased by about 2.6% and 3.2%, respectively. The high mechanical strength of the MWCNTs-interspersed in the polymer film could be the reason for the high stability of the proposed electrochemical sensor. Repeating the experiment after a longer time period, it was found that the current responses decreased about 15% in three weeks for both drugs.

To evaluate the repeatability of the electrode response, the same modified electrode was used for six successive measurements of 1.0×10^{-6} M OND and MOR solution. After each measurement, the surface of the MWCNTs–Nafion/GCE was regenerated by applying single positive-going SWV potential scan from 0.5 to 1.5 V in a blank solution of 0.1 M H_2SO_4. The recorded oxidation peak potentials for OND (mean $E_p=1.17$ V) and MOR (mean $E_p=0.74$ V) were unchanged. The RSD values of the peak current for OND (mean $i_p=35.4$ μA) and MOR (mean $i_p=36.9$ μA) were 1.9% and 1.2%, respectively. These results indicated that the MWCNTs–Nafion/GCE was stable in repeated measurements for selective determination of OND and MOR compounds. The reproducibility of the modified electrode was characterized by three replicate measurements of 1×10^{-6} M OND and MOR over three days using freshly prepared standard solutions. The RSDs of the anodic peak current for OND and MOR were not greater than 2.5% and 1.7%, respectively. Additionally, a series of five sensors prepared repeatedly in the same manner were also tested and the RSDs of peak potentials and currents for both drugs were in the range 0.5–0.7% and 2.5–3.1%, respectively, indicating good fabrication reproducibility.

All experiments confirmed that the prepared MWCNTs–Nafion composite film has good durability, homogeneity in deposition, reproducibility, more active sites and strong adherence to electrode surface. These characteristics are suitable for long-term electrochemical sensing applications.

3.7. Interference

The influence of potential interfering species, which are likely to be in biological samples or are co-formulated with the active pharmaceutical ingredient, on the voltammetric responses of OND and MOR was examined. A fixed amount of 1×10^{-6} M OND and MOR, spiked with various excess amounts of interfering species, was evaluated under optimized experimental conditions described previously. The tolerance limit was defined as the maximum concentration of the interfering substance that caused an error less than ±5% in the determination of both drugs. The presence of Na^+, Cl^- (2000 fold), K^+, NO^{3-}, SO_4^{2-}, HPO_4^{2-} (1000 fold), Zn^{2+}, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}, Na_2EDTA, manitol (500 fold), L-alanine, L-glycine, L-aspartic acid, L-glutamic acid, citric acid (400 fold), lactose, sucrose, starch (300 fold) and L-cysteine (200 fold), had no influence on the peak current of both drugs. Also, nitrite (400 fold) did not interfere with voltammetric response of both drugs although nitrite ions were oxidized at +0.75 V, very close to MOR potential. However, the MWCNTs–Nafion composite film allows facile accumulation of the positively charged MOR molecule, while prevent accumulation of negatively charged oxidizable nitrite ions onto the electrode surface. The experiments displayed that the oxidation peak current of OND and MOR at the MWCNTs–Nafion/GCE did not change after adding 400-fold concentration of ascorbic acid and 300-fold of glucose indicating that the selectivity of the method could be satisfactory for the quantification of both drugs in biological fluids where these endogenous substances are always present. When 200-fold concentration of uric acid was added into the solution containing 1×10^{-6} M OND and MOR, no obvious interference was observed for OND quantification, but the current response of MOR increased significantly due to the oxidation of uric acid at +0.65 V. Selectivity of the developed procedure was also investigated by observing any interference encountered from dopamine. The results showed that dopamine is oxidized at potential +0.49 V, however, in the presence of equal concentration do not cause a competitive adsorption onto the surface of modified electrode since a decrease in the currents of both drugs was not greater than 5%.

3.8. Applications of electrochemical sensor

The applicability of the MWCNTs–Nafion/GCE was examined via quantitation of OND in pharmaceutical dosage form. OND was analyzed in commercial film-coated tablets using the standard addition method in order to eliminate matrix effects. The results obtained using the proposed sensor are in good agreement with the claimed amount (Table 1). The analysis of OND in its pharmaceutical formulation exhibited the mean recovery of 99.7% with the relative standard deviation of 1.3% indicating good accuracy and precision as well as the suitability of the proposed sensor for this purpose. To evaluate possible interactions with excipients, recovery experiments were carried out by spiking the formulation solution samples with known amount of standard OND solution. The mean recovery of 98.1% indicated that excipients have no interference effect on the analysis of OND, therefore a separation step can be avoided.

The film-coated tablets were also analyzed by the reverse-phase HPLC method. The developed HPLC method with UV-detection was validated according to standard procedure [30]. The results obtained

Table 1
Analysis of ondansetron in film-coated tablets by the proposed adsorptive stripping SWV and HPLC methods.

Technique	AdSWV	HPLC
Stated content (mg)	8.00	8.00
Detected content (mg)[a]	7.97	8.02
RSD %	1.25	0.39
Added (mol L^{-1})	5.00×10^{-7}	2.00×10^{-5}
Found (mol L^{-1}) [b]	4.91×10^{-7}	1.97×10^{-5}
Recovery %	98.1	98.5
RSD %	3.46	0.24
F [c]	0.10	–
t [c]	0.37	–

[a] Each value is the mean of five experiments.
[b] Each value is the mean of three experiments.
[c] The theoretical values of F and t-test at 95% confidence limit are 6.39 and 2.31, respectively.

with the proposed sensor were compared to those obtained by the HPLC method (Table 1). Statistical analysis of the results obtained using voltammetric and HPLC procedures showed no significant difference between the performance of the two methods regarding accuracy and precision, as revealed by the student *t*-test and variance ratio *F*-test. However, the electroanalytical method does not require separation, degassing and expensive solvents that are needed for an HPLC procedure.

Finally, the MWCNTs–Nafion/GCE was applied for simultaneous determination of OND and MOR in biological samples. The sensitivity of the proposed method complies with the expected serum concentration level for both drugs after the treatment with the therapeutic daily dose. However, the quantitation of OND and MOR in human serum at the therapeutic concentration range could not be feasible without accumulation of protonated drug molecules on the MWCNTs–Nafion/GCE surface. Furthermore, the MWCNTs–Nafion film contains sulfonate groups and such surface layer prevents accumulation of negatively charged oxidizable substances found in biological fluids onto the electrode surface. The recovery studies of both drugs in serum samples were performed using the standard addition method to nullify any remaining interference. The mean recovery of $98.7 \pm 1.5\%$ for OND and $102.6 \pm 2.7\%$ for MOR was achieved in this type of matrix. From the experimental results, it is obvious that the proposed electrochemical sensor has great potentials for practical analysis of biological samples.

4. Conclusions

The paper describes, for the first time, the oxidative behavior and electrochemical determination of OND. A simple, but powerful, approach was applied for the fabrication of stable CNT-based electrochemical sensor. The MWCNTs–Nafion/GCE exhibited a remarkable enhancement effect on the voltammetric response of OND due to excellent electrocatalytic activity and adsorption capability of the immobilized film toward the drug molecule. This work provides a new and convenient electroanalytical method for direct quantitation of OND in film-coated tablets. In comparison to HPLC method, the proposed sensor possesses numerous advantages such as high sensitivity, rapid response, low cost and simplicity. In addition, the MWCNTs–Nafion/GCE exhibited high selectivity in the voltammetric measurements of OND and co-administrated drug MOR with potential difference of 430 mV. The electrode was successfully applied for the simultaneous determination of both drugs in human serum samples after selective accumulation at the electrode surface.

References

[1] A. Markham, E.M Sorkin, Drugs 45 (1993) 931–952.
[2] J.H. Ye, R. Ponnudurai, R. Schaefer, CNS Drug Rev. 7 (2001) 199–213.
[3] F. Musshoff, B. Madea, F. Stuber, U.M. Stamer, J. Anal. Toxicol. 34 (2010) 581–586.
[4] K. Liu, X.J. Dai, D.F. Zhong, X.Y. Chen, J. Chromatogr. B 864 (2008) 129–136.
[5] Y. Dotsikas, C. Kousoulos, G. Tsatsou, Y.L. Loukas, J. Chromatogr. B 836 (2006) 79–82.
[6] R. Sheshala, Y. Darwis, N. Khan, Chromatographia 70 (2009) 75–81.
[7] V. Zizkovsky, R. Kucera, J. Klimes, J. Pharm. Biomed. Anal. 44 (2007) 1048–1055.
[8] D. Chandrasekar, S. Ramakrishna, P.V. Diwan, Arzneim. –Forsch. 54 (2004) 655–659.
[9] Y. Hsieh, L. Favreau, J. Schwerdt, K.C. Cheng, J. Pharm. Biomed. Anal. 40 (2006) 799–804.
[10] P.B. Raval, M. Puranik, S.J. Wadher, P.G. Yeole, J. Pharm. Sci. 70 (2008) 386–390.
[11] A. Raza, A.S. Ijaz, Atta-ur-RehmanU. Rasheed, J. Chin. Chem. Soc. 54 (2007) 223–227.
[12] S.A. Wring, R.M. Rooney, C.P. Goddard, I. Waterhouse, W.N. Jenner, J. Pharm. Biomed. Anal. 12 (1994) 361–371.
[13] C. Arama, A. Varvara, C.M. Monciu, Farmacia 59 (2011) 34–43.
[14] K.C. Ho, C.Y. Chen, H.C. Hsu, L.C. Chen, S.C. Shiesh, X.Z. Lin, Biosens. Bioelectron. 20 (2004) 3–8.
[15] A. Salimi, R. Hallaj, G.R. Khayatian, Electroanalysis 17 (2005) 873–879.
[16] F. Li, J. Song, C. Shan, D. Gao, X. Xu, L. Niu, Biosens. Bioelectron. 25 (2010) 1408–1413.
[17] N.F. Atta, A. Galal, R.A. Ahmed, Electroanalysis 23 (2011) 737–746.
[18] N.F. Atta, A. Galal, S.M. Azab, Analyst 136 (2011) 4682–4691.
[19] A. Babaei, M. Babazadeh, H.R. Momeni, Int. J. Electrochem. Sci. 6 (2011) 1382–1395.
[20] T.G. Venkateshwaran, J.T. Stewart, D.T. King, J. Liq. Chromatogr. Relat. Technol. 19 (1996) 1329–1338.
[21] B. Nigović, S.B. Hocevar, Electrochim. Acta 58 (2011) 523–527.
[[22] B. Nigović, J. Spajić, Talanta 86 (2011) 393–399.
[23] G.A. Rivas, M.D. Rubianes, M.C. Rodrıguez, N.F. Ferreyra, G.L. Luque, M.L. Pedano, S.A. Miscoria, C. Parrado, Talanta 74 (2007) 291–307.
[24] B. Nigović, M. Marušić, S. Jurić, J. Electroanal. Chem. 663 (2011) 72–78.
[25] R. de Wit, J.H. Beijnen, O. van Tellingen, J.H. Schellens, M. de Boer-Dennert, J. Verweij, Br. J. Cancer 74 (1996) 323–326.
[26] F. Tagliaro, D. Franchi, R. Dorizzi, M. Marigo, J. Chromatogr. B 488 (1989) 215–228.
[27] E. Laviron, J. Electroanal. Chem. 112 (1980) 1–9.
[28] A.J. Bard, L.R. Faulkner, Electrochemical Methods Fundamentals and Applications, 2nd ed., Wiley, New York, 2004.
[29] J.M.P.J. Garrido, C.D. Matos, F. Borges, T.R.A. Macedo, A.M.O. Brett, Electroanalysis 16 (2004) 1419–1426.
[30] International Conference on Harmonization (ICH), Validation of Analytical Procedures: Text and Methodology Q2 (R1), 2005.