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ORIGINAL ARTICLE

Voltammetric Determination of Nimesulide Using Multiwalled Carbon Nanotubes Modified Carbon Paste Electrode

Çok Duvarlı Karbon Nanotüp ile Modifiye Edilmiş Karbon Pasta Elektrot Kullanılarak Nimesulidin Voltametrik Miktar Tayini

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ABSTRACT

A multiwalled carbon nanotubes (MWCNTs) modified carbon paste electrode (CPE) was prepared for voltammetric determination of anti-inflammatory drug nimesulide (NIM). The electro-oxidation of NIM was exhibited irreversible and diffusion controlled process with MWCNTs modified CPE. The linear response between peak current and concentration in the quantitative determination of NIM by differential pulse voltammetry in 0.1 M phosphate buffer solution (PBS) at pH 5.0 obtained in the range of the concentration from 6×10^{-8} -1×10^{-5} M with limit of detection (LOD) 1.07×10^{-9} M and limit of quantification (LOQ) 3.24×10^{-9} M. Differential pulse voltammetry was developed according to linear response of NIM with high selectivity, precision, accuracy using modified electrode was successfully applied to the determination of NIM in pharmaceuticals and human serum samples.

Key words: Carbon paste electrode, Determination, Multiwalled carbon nanotubes, Nimesulide, Voltammetry

ÖZ

Antienflamatuvar ilaç etken maddesi nimesulidin (NIM) voltametrik miktar tayini için çok duvarlı karbon nanotüp ile modifiye edilmiş karbon pasta elektrot hazırlanmıştır. NIM'in çok duvarlı karbon nanotüp ile modifiye edilmiş karbon pasta elektrot ile elektro-oksidasyonu tersinmez ve difüzyon kontrollü bir özellik göstermiştir. NIM'in diferansiyel puls voltametrisi ile 0.1 M fosfat tampon çözeltisinde pH 5.0 de miktar tayininde derişim ve pik akımı arasındaki doğrusallık 6×10^{-8} 1×10^{-5} M derişim aralığında, saptama sınırı 1.07×10^{-9} M ve tayin alt limiti 3.24×10^{-9} M olarak bulunmuştur. Çok duvarlı karbon nanotüp ile modifiye edilmiş karbon pasta elektrodu kullanarak NIM'nin doğrusal cevabına göre yüksek seçicilik, kesinlik, ve doğrulukla geliştirilen diferansiyal puls voltametrisi, NIM'nin farmasötik preparatlardan ve insan serum numunelerinden miktar tayinine başarılı bir şekilde uygulanmnıştır.

Anahtar kelimeler: Karbon pasta elektrot, Tayin, Çok duvarlı karbon nanotüp, Nimesulid, Voltametri

INTRODUCTION

Nimesulide, N-(4-nitro-2 methanosulfonanilid) (Figure 1), is a new non-steroidal anti-inflammatory drug that is selective for cyclooxygenase-2 and effective in reducing the pain which is associated with rheumatoid arthritis and osteoarthirits (1). pK_a value of NIM is 6.46 that is very important for gastric tolerability, and this avoids the back diffusion of the hydrogen ions that are liable for tissue damage. NIM is nearly completely biotransformed to 4-hydoxynimesulide in free and conjugated forms and it provides to promote to the anti-inflammatory activity of NIM (2,3).

There are several reports on the determination of NIM in the literature for example HPLC (high performance liquid chromatography) (4), with spectrophotometry UV (ultraviolet) (5,6), capillary electrophoresis (7). Most of this methods appear as time-consuming, expensive, complicated and lengthy procedures. Electrooxidation of NIM at gold electrode (8), electroreduction of NIM using glassy carbon electrode modified with SiC (silicon carbide) (9) and MWCNTs (multiwalled carbon nanotubes) modified glassy carbon electrode (10) have been reported in the literature. Electrochemical methods have certain advantages for example fast response, low cost and high sensitivity compare to other analytical methods (11,12).

Carbon nanotubes (CNTs) can be used as electrode material for electrochemical and bioelectrochemical applications

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because of their unique characteristics and useful properties for example high chemical stability, conductivity, aspect ratio, and extremely high mechanical strength and modules (13-16). CNTs are largely used as working electrode modification material for drug analysis due to they have the capability for promoting electron transfer reactions and developing sensitivity in electrochemistry (17).

In this study, a sensitive MWCNTs modified CPE was prepared for electroanalytical determination and it used to investigate electro-oxidative behavior of NIM with cyclic and differential pulse voltammetry. The prepared MWCNTs modified CPE was exhibited rapid response, high selectivity, sensitivity, low detection limit, and good reproducibility and successfully used electroanalytical determination of NIM.

EXPERIMENTAL

Instrumentation

Voltammetric measurements were carried out with a computer-controlled Autolab Pgstat128n potentiostat/galvanostat with Nova 10.0 software (Metrohm-Autolab, The Netherlands). A three-electrode electrochemical cell analyzer contains a carbon paste electrode with modification

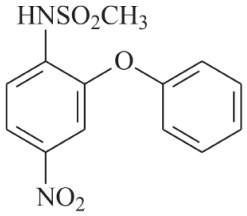

Figure 1. Molecular structure of nimesulide

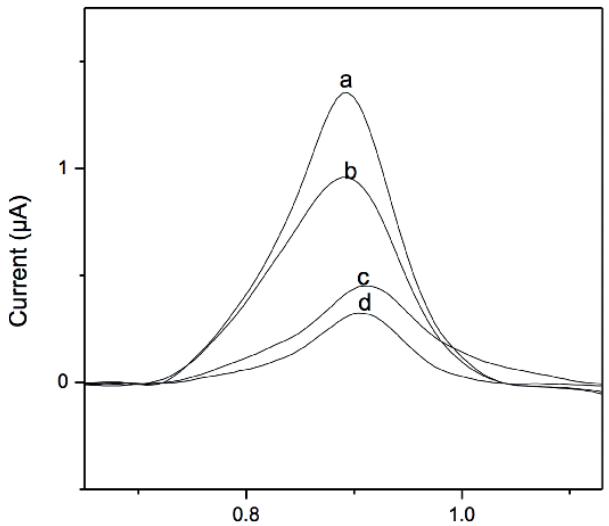

Figure 2. Differential pulse voltammograms 10 µM of NIM in 0.04 M Britton-Robinson buffer at pH 7.0, (a) 0.2%, 2.5 µL; (b) 0.5%, 1 µL; (c) 0.5%, 3 µL, (d) 0.2%, 5 µL of MWCNTs

MWCNTs as working electrode, a platinum wire as the counter electrode and Ag/AgCl electrode as the reference electrode. The pH measurements were carried out using model Hanna HI2211 pH meter (Romania) with an accuracy of ±0.05 pH at room temperature.

Reagents

Nimesulide and its pharmaceutical dosage form tablet (100 mg per tablet) were supplied by Sanovel-Turkey. They were used without further purification. Stock solutions of NIM (1×10^{-3} M) were prepared in methanol and stored at +4°C away from light. NIM working solutions for voltammetric investigation were prepared by the direct dilution of the stock solution with selected supporting electrolyte containing a constant amount of methanol (20% (v/v)). Graphite powder (d=2.2 g/mL, Merck, Germany) and paraffin oil (d=0.84 g/mL, Aldrich, U.S.A) as the binding agent were used for preparing the pastes. MWCNTs was purchased from NanoLab. U.S.A, with purity 95%, 30±10 nm diameter, and 1-5 µm lengths.

Phosphate buffer solutions (PBS) (0.1 M) were prepared from phosphoric acid (Merck, Germany) for pH 4.0 and disodium hydrogen phosphate (Aldrich, U.S.A.), sodium dihydrogen phosphate (Merck, Germany) for pH 5.0-8.0. Britton-Robinson (BR) buffer solutions (0.04 M) were prepared at pH 3.0-9.0 from 0.04 M phosphoric acid (Merck, Germany), 0.04 M boric acid (Aldrich, U.S.A.) and 0.04 M acetic acid (Merck, Germany). Acetate (AT) buffer solutions (1 M) at pH 3.5, 4.5 were prepared from 1 M acetic acid (Merck, Germany). pH was adjusted with 5 M sodium hydroxide (Aldrich, U.S.A.) solution.

Sartorius Arium proUV nanopure water (resistivity ≥18 MΩ cm), and analytical reagents were used for the preparation

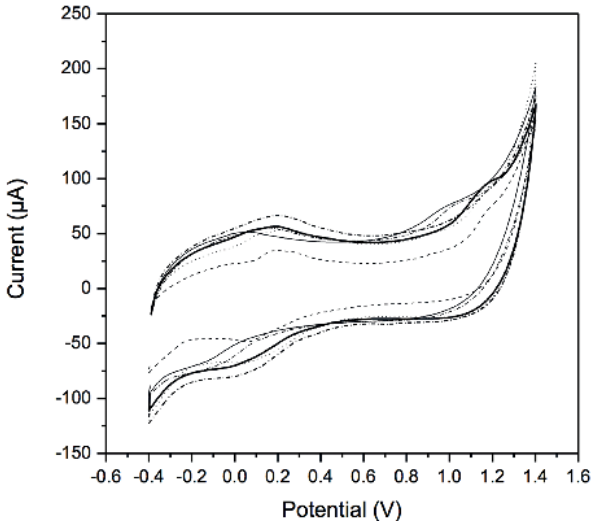

Figure 3. Cyclic voltammograms of NIM in 1 M acetate buffer at pH 3.5 (-----), 0.1 M phosphate buffer at pH 4.0 (·····), pH 5.0 (▬), 0.04 M Britton-Robinson buffer pH 7.0 (–·–·–), pH 9.0 (—) with MWCNTs modified CPE. Short dash dot line 0.1 M phosphate buffer at pH 5.0; NIM concentration: 100 µM; scan rate 100 mV/s

of solutions. All of the experiments were performed at room temperature (25±1°C).

Preparation of bare and MWCNTs modified carbon paste electrodes

The ratio of graphite powder and paraffin oil to binder were optimized for NIM, and then the carbon paste electrode was prepared homogeneous paste by thoroughly hand-mixing the from optimized graphite powder and paraffin oil in the ratio of 75:25 (w/w). A portion of the homogeneous paste was packed into the cave of the teflon tube. A copper wire inserted into the carbon paste provided the electrical contact. When necessary, a new surface was obtained by pushing an excess of the paste out of the tube and polishing with a weighing paper.

The MWCNTs were dispersed in DMF with loading 0.2% (w/v) and sonicated for 4h to obtain a homogeneous mixture. A selected 2.5 µL of the dispersion was dropped directly on the surface of CPE. The resulting modified electrode was named as MWCNTs modified CPE. The MWCNTs modified CPE electrode dried for overnight at room temperature.

Pharmaceutical assay

Ten tablets (each tablet contains 100 mg NIM) were first weighed and then finely powdered. The required amount of powder equivalent to 10^{-3} M of NIM was diluted to 100 mL with methanol and sonicated for 15 min. The analyzed solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with the selected supporting electrolyte NIM working solutions for voltammetric investigation were prepared by the direct dilution of the stock solution with selected supporting electrolyte containing a constant amount of methanol (20% v/v).

Analysis of serum

Drug-free human serum samples were obtained from healthy people and stored frozen in the dark until assay. An aliquot volume of serum sample was fortified with NIM dissolved in methanol to achieve final concentration of 1×10^{-3} M and treated with acetonitrile to removing serum proteins effectively. Blank and stock solution of NIM were transported to ultrasonic bath and agitated for 15 min and subsequently centrifuged for 15 min at 5000 rpm to separate serum protein residues and supernatant. Appropriate volumes of this supernatant were taken carefully and transferred into the volumetric flask and diluted up to the required volume with the selected supporting electrolyte containing a constant amount of methanol (20% v/v).

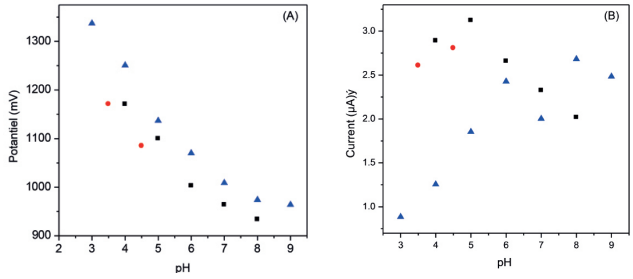

Figure 4. Plots of peak potential, (E_p), versus pH (A) and peak current (I_p), versus pH (B) from cyclic voltammetry voltammograms of 100 µM of NIM with MWCNTs modified CPE. Squares indicate 0.1 M phosphate buffer solution, tringle 0.04 M Britton-Robinson buffer solution and circles 1 M acetate buffer solution

Validation of the analytical methods

The ruggedness, precision, and accuracy of the studied methods, were checked by assaying five replicate samples on the same day and on different days over a week. Relative standard deviations (%) were also calculated to check the ruggedness and precision of the method. The accuracy of the methods was expressed as bias (%) (18,19). Each of the solutions was freshly prepared just before the experiments and protected from the light. All of the measurements were carried out at room temperature (25±1°C). The calibration equation for differential pulse voltammetry method was constructed by plotting the peak current against NIM concentration.

RESULTS AND DISCUSSION

Effect of volume variations on the peak current was investigated at two different concentrations of MWCNTs suspension (0.2% and 0.5% in DMF) to optimize MWCNTs volume for determination of NIM (Figure 2). The suspension amounts of 2.5 µL and 5 µL for 0.2% of MWCNTs, 1 µL and 3 µL for 0.5% of MWCNTs suspension were studied for 10 µM NIM with CV and DPV. As shown in Figure 1, the peak current reaches its maximum value when the suspension amount is 2.5 µL for 0.2% MWCNTs. So, 2.5 µL was chosen as the optimized amount for 0.2% MWCNTs suspension.

The electro-oxidation behavior of NIM on MWCNTs modified CPE was studied by CV at a scan rate of 100 mV/s between

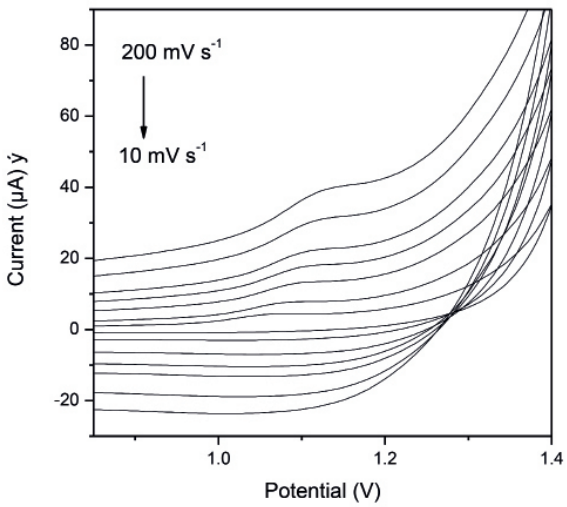

Figure 5. Cyclic voltammograms of 100 µM of NIM in 0.1 M phosphate buffer solution at pH 5.0 at scan rates of 10, 25, 50, 75, 100, 150 and 200 mV/s with MWCNTs modified carbon paste electrode

pH 3.0 and 9.0 in different buffer solutions. The cyclic voltammetric measurements (Figure 3) performed for 100 µM NIM solution exhibit that NIM has irreversible electrochemical oxidation behavior on MWCNTs modified CPE.

Effect of pH on the anodic peak current and peak potential of 100 µM NIM were analyzed with cyclic and differential pulse voltammetry in different buffer solutions between pH 3.0 and 9.0 using MWCNTs modified CPE electrode. Due to detection responses for CV and DPV are similar, only cyclic voltammetry responses of NIM were exhibited in Figure 4. As shown in Figure 4, NIM show irreversible anodic peak in the studied all pH values. The peak potential in the oxidation process of NIM shifted to less positive potentials (Figure 4A) with increasing pH. Anodic peak of NIM exhibited a pH dependent behavior between pH 3.0 and 7.0 with linear relationship (equation 1).

E_p (mV)= 1298-64.1pH; r=0.992 (between pH 3.0 and 7.0) (equation 1)

The observed pH dependence in the electro-oxidation behavior of NIM indicated that the methylsulfonamide group (electroactive group) corresponding to the NIM main oxidation peak was in acid-base equilibrium with pK_a of about 7.0. The breaking point of the curve was close to the pK_a value of NIM, at about 6.56 (20). The obtained slope value for plot of peak potential versus pH was close to theoretical value of 59 mV/pH in the Nerst equation. This corresponds to the oxidation process of NIM involves equal number of electrons and protons (21,22). The peak potential of NIM nearly was pH independent (Figures 4A), above pH 7.0. This attributed because of a change in the protonation-deprotonation process of the methylsulfonamide and the oxidation potential of NIM remains pH independent and before the electron transfer rate-determining step there are no proton transfer steps. The conjugate base must be formed by rapid dissociation of the protonated form at pH<pK_a. The plot of peak current versus pH is shown in Figure 4B. The maximum peak current and well peak shape of oxidation NIM were obtained in 0.1 M PBS at pH 5.0. Thus, electroanalytical determination of NIM and further studies were studied in 0.1 M PBS at pH 5.0.

The effect of scan rate over the range of 10.0-200.0 mV/s on the peak potential and peak current was studied by CV in PBS at pH 5.0. The peak potential of 100 µM NIM in 0.1 M PBS at pH 5.0 is moved to the anodic direction with the scan rate increasing (Figure 5). The plot of logarithm of peak

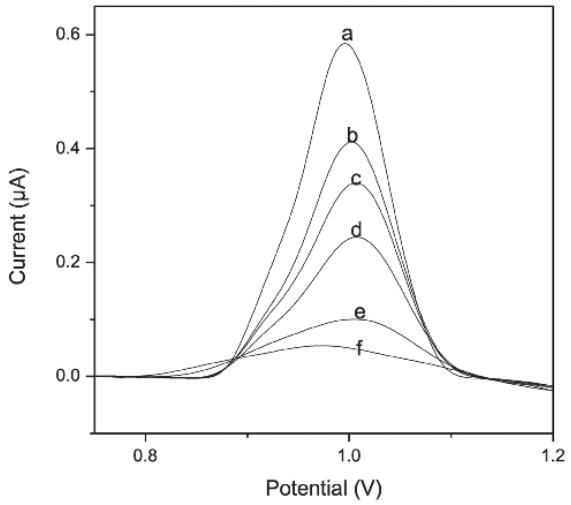

Figure 6. Differential pulse voltammograms (a) 10, (b) 8, (c) 6, (d) 4, (e) 1, (f) 0.4 µM of NIM solution in 0.1 M phosphate buffer solution at pH 5.0 with MWCNTs modified carbon paste electrode

Table 1. Validation data of calibration lines for the quantitative determination of NIM by DPV for MWCNTs modified carbon paste electrode in 0.1 M PBS at pH 5.0 and serum samples

	MWCNTs modified carbon paste electrode	
	Supporting electrolyte	Spiked serum
	DPV	DPV
Peak potential (V)	1.006	1.012
Linearity range (µM)	0.06-10	0.4-40
Slope (µA/µM-1)	89055	29531
Intercept (µA)	+0.1008	+0.0672
Correlation coefficient	0.9922	0.9977
Limit of detection (µM)	0.00107	0.0363
Limit of quantification (µM)	0.00324	0.1101
Repeatability of peak current (R.S.D.%)	0.581	0.424
Repeatability of peak potential (Relative standard deviation %)	0.418	0.255
Reproducibility of peak current (Relative standard deviation %)	0.808	0.669
Reproducibility of peak potential (Relative standard deviation %)	0.961	0.566

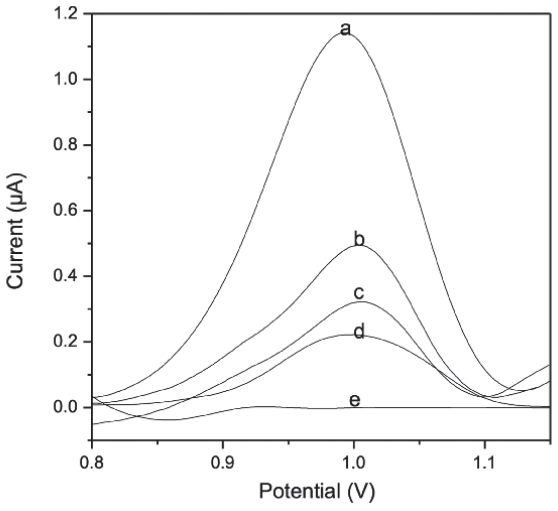

Figure 7. Differential pulse voltammograms (a) 40, (b) 20, (c) 8, (d) 6 µM of NIM solution (f) blank solution in spiked serum 0.1 M phosphate buffer solution at pH 5.0 with MWCNTs modified carbon paste electrode

current versus the logarithm of scan rate showed a linearity with a slope value of 0.341 (equation 2) and the plot of peak current versus square root scan rate exhibited a straight line with a slope value of 0.166 (equation 3). These values of the slopes were found to be close to the theoretical value of 0.5. This contributes that the electro-oxidation process of NIM is diffusion controlled process on the MWCNTs modified CPE (23). Related equations are noted below:

$\log I_p = 0.341 \log v - 0.339$ r=0.991 n=8 (equation 2)

$I_p = 0.166\ v^{1/2} + 0.519$ r=0.991 n=8 (equation 3)

Validation of the analytical procedure

Voltammetric studies for determination of the NIM were carried out by DPV. DPV was selected due to the peaks are sharper and better determined at lower concentration of NIM than the peaks derived by CV. The anodic peak current increased linearly with increasing concentration of NIM in the DP voltammograms, as shown in Figure 6. The MWCNTs modified CPE showed linearity in the range from 0.06 and 10 µM of NIM for DPV.

The related validation parameters for DPV and characteristics of the calibration equation are reported in Table 1. The developed DPV was validated according to standard validation procedures (24,25). Limit of detection and limit of quantification were calculated according to 3 s/m and 10 s/m, respectively, by using the standard deviation of the anodic peak response (s) and the slope value of the calibration curve (m) (26). The limit of detection value that was obtained in this study was the lowest value than the reported value in the literature for the electroanalytical determination of NIM.

We have investigated repeatability, reproducibility, precision, recovery, bias%, and selectivity for validation NIM with MWCNTs modified CPE. All validation results for NIM with MWCNTs modified CPE were repetitive, selective, reproducibility measurements, as shown in Table 1. The validation results demonstrate good precision, accuracy, repeatability and reproducibility (Table 1).

Table 2. The results for the determination of NIM from tablet dosage forms and recovery experiments in 0.1 M PBS buffer at pH 5.0 by DPV for MWCNTs modified carbon paste electrode

	Tablet (mg)
	DPV
Labeled claim (mg)	100
Amount found (mg)*	100.41
Relative standard deviation %	0.278
Bias %	+0.406
Added (mg)	33.3
Found (mg)*	34.16
Average recovered (%)	100.64
Relative standard deviation % of recovery	0.817
Bias %	+0.627

Determination of NIM in pharmaceutical dosage forms

The MWCNTs modified CPE was applied for the determination of NIM in Nimes® tablet dosage form. Each NIM tablet in pharmaceutical dosage form contains 100 mg NIM and inactive ingredients. The developed DPV was carried out to direct determination of NIM in pharmaceutical dosage form, using the related calibration straight line. Pretreatment such as evaporation, extraction was not required for tablet dosage form. The results obtained from the tablet dosage form are listed in Table 2. The proposed method could be successfully applied for NIM assay in tablet dosage form without any interference.

Determination of NIM in spiked human serum samples

The differential pulse voltammetry optimized was successfully carried out to the voltammetric determination of NIM in protein-free spiked human serum samples. Acetonitrile was used as a serum precipitating agent. No evaporation or extraction other than centrifugal protein separation at 5000 rpm was required before analyse for the drug. The calibration equation parameters and validation parameters were shown in Table 1. Obtained recovery results of human serum samples were given in Table 3.

Differential pulse voltammograms of 40 and 6 µM of NIM obtained serum spiked were exhibited in Figure 7. As shown in Figure 7, no oxidation or noise peaks were present in

Table 3. Results of obtained for NIM determination from spiked serum

	DPV
Added concentration (µM)	8.00
Obtained concentration (µM)	8.02
Number of experiments	5
Average recovered (%)	99.83
Relative standard deviation % of recovery	0.692
Bias %	+0.25

Table 4. Electrochemical detection of NIM at different modified electrodes

Electrode	Method	Linear range (µM)	Limit of detection (µM)	Reference
Glassy carbon electrode modified by cysteic acid/CNTs	DPV	0.1-10	0.05	27
Barium doped zinc oxide nanoparticles modified glassy carbon electrode	DPV	0.1-10	0.0018	28
Gold electrode	DPV	0.2-1.2	0.0011	8
Multiwalled carbon nanotubes modified carbon paste electrode	DPV	0.06-10	0.00107	This work

the potential range where the analytical peak was formed analytical peak and determination of NIM was successfully applied in human serum samples.

Serum samples was kept in +4˚C in darkness and the stability of serum samples was studied by five consecutive analyses of the serum samples over a period of, approximately, five hours. The peak currents and peak potentials of NIM was not shown significant changes between the first and last measurements.

As it is shown in Table 4, the MWCNTs modified CPE was compared to other modified electrode in the literature according to their linear range and limit of detection. The linear range for determination of NIM with MWCNTs modified CPE at this method are better than other electrochemical methods reported in literature (27, 28, 8). When compared to limit of detection values for NIM with gold electrode (10), barium doped zinc oxide nanoparticles modified electrode glassy carbon electrode (28) and MWCNTs modified CPE (this study), this study has lowest limit of detection value.

CONCLUSION

Carbon paste electrode was modified with multiwalled carbon nanotubes and optimized for NIM. The prepared MWCNTs modified CPE was used electroanalytical determination of NIM CV and DPV. The MWCNTs modified CPE for electroanalytical determination of NIM using DPV was carried out highly selectively, simply and stably from pharmaceutical dosage forms and human serum samples. Additionally, simplicity of the electrode preparation is very practical. Thus, multiwall carbon nanotubes modified carbon paste electrode is a practical sensor and very useful for the voltammetric determination of NIM.

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