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A Sensitive Simultaneous Determination of Epinephrine, Mefenamic Acid and Acetaminophen Using a Nickel Hydroxide Nanoparticles/Multiwalled Carbon Nanotubes Modified electrode

Ali Babaei,* Elham Rezaei, Masoud Sohrabi and Omid Karbalaei Hasani

Department of Chemistry, Faculty of Science, Arak University, Arak 38156-8-8349, Iran

* Corresponding Author, Tel.: +98 863 4173401; Fax: +98 863 4173406

E-Mail: ali-babaei@araku.ac.ir

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Abstract- The electro-oxidation of epinephrine (EPI)**,** acetaminophen (ACT) and Mefenamic acid (MEF) has been investigated by application of nickel hydroxide nanoparticles / multi-walled carbon nanotubes modified glassy carbon electrode (MWCNT-NHNPs/GCE) using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and chronoamperometry (CA) methods. The modified electrode showed suitable electrochemical responses for EPI, ACT and MEF determination. Under the optimum conditions the electrode provides a linear response versus EPI, ACT and MEF concentrations in the range of 0.5-180 µM, 0.1-180 µM, and 0.1-85 respectively using the DPV method. Application of CA method showed linear responses for EPI and ACT concentrations in the range of 1-600 µM and 1-500 µM respectively. The CA results for MEF showed two linear range of 1-50 µM and 60-600 µM. The modified electrode was used for determination of EPI, ACT and MEF in human serum and urine with satisfactory results.

Keywords- Epinephrine, Mefenamic acid, Acetaminophen, Multi-Walled Carbon Nanotubes, Nickel Hydroxide Nano-particles

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1. INTRODUCTION

The application of nanomaterials in various fields of science and technology has been extensively developed due to the unique properties of these materials [1–3]. Metal nanoparticle has received considerable attention in recent years. It has unique chemical, electrical properties due to its size-dependent properties. Hence there is currently an intense interest in the use of nanoparticles for the fabrication of modified electrodes and a wide range of bioscience applications [4]. Many electrodes have been modified by Ni, NiO_2, $Ni(OH)_2$ particles and nanoparticles on traditional electrode surfaces such as diamond [5], gold [6], carbon or graphite [7,8]. In contrast to Ni nanomaterials which are unstable and easily oxidized in air and solution, hydroxide (or oxide) of these materials are relatively stable [8,9]. Many precipitation methods for preparing nickel hydroxide nanoparticles (NHNPs) have been reported. However the method of coordination homogeneous precipitation (CHP) is new and facile [10]. This method doesn't need expensive raw materials or equipment, also it is easy for mass production, and can be extended to synthesize other hydroxide or oxide nanocrystals. Therefore in this work, CHP method for synthesis of NHNPs was used.

Carbon nanotubes (CNTs), have been used in various fields such as catalysis of redox reactions [11–13], nanoelectronics [14], electrochemical sensors [15,16] etc., due to their unique structure, electrical and mechanical characteristics. Sensors based on CNTs have received a lot attention and have largely improved the voltammetric response of a variety of biological, clinical and environmental compounds [17].

Epinephrine (EPI, 1-(3, 4-dihydroxyphenyl)-2-methyloaminoethanol or adrenaline) is a hormone and it plays important roles as a neurotransmitter. A great number of analytical methods have been applied such as: spectrophotometry [18,19], fluorimetry [20], liquid chromatography [21-24], capillary electrophoresis [24,25], thermal lens microscopy [26], chemiluminescence [27], electrochemiluminescence [28]. However, these methods suffer from some disadvantages such as requirement for sample pretreatment, low sensitivity or selectivity, high costs, the use of organic solvents and long analysis times. In contrast, electrochemical techniques are less time consuming, rapid, simple, without tedious procedures, inexpensive, and with high sensitivity. Several electrochemical methods for the determination of EPI have been proposed [29-33].

Acetaminophen (ACT, Paracetamol, or N-acetyl-p-aminophenol) is a long-established substance being one of the most extensively employed drugs in the world. It is also found that overdoses of ACT will damage liver and kidney. A great number of analytical methods such as: spectrophotometry [34,35], high-performance liquid chromatography [36], near infrared transmittance spectroscopy [37], spectrofluorimetry [38] and capillary electrophoresis [39], have been developed for the determination of ACT in pharmaceutical formulations and biological fluids. There are several reports on electrochemical determination of ACT [40-43].

Mefenamic acid (MEF) is used to relieve the symptoms of many diseases such as rheumatoid arthritis, non-articular rheumatism, and sport injuries [44]. It is used to treat mild to moderate pain, including headache, dental pain, post-operative and post-partum pain, dysmenorrhoea, as well as musculoskeletal disorders and joint disorders such as osteoarthritis [45]. Overdoses of MEF produce toxic metabolite accumulation that causes acute hepatic necrosis, inducing morbidity and mortality in humans [46]. Due to the vital importance of the assay of MEF for pharmaceutical formulations and biological fluids, several analytical methods have been developed for the quantitative determination of this drug in both pharmaceutical and biological samples [47,48].

It has been found that there are an antagonism relationship between the metabolic responses induced by EPI, ACT and MEF. ACT and MEF inhibit prostaglandin synthesis. Otherwise, EPI stimulates prostagladin release [49,50], Therefore it would be useful to study simultaneous determination of EPI , ACT and MEF. To the best of our knowledge, no study has been reported so far on the simultaneous electrochemical determination of EPI, ACT and MEF. In this study, we report the preparation and application of a nickel hydroxide nanoparticle / multi-walled carbon nanotube modified glassy carbon electrode (MWCNT-NHNPs/GCE) as a sensor for simultaneous determination of EPI, ACT and MEF. The modified electrode showed good sensitivity, lower detection limit with wide linear dynamic range. The analytical performance of the modified electrode in quantification of EPI, ACT and MEF in human serum and urine is evaluated with satisfactory results.

2. EXPERIMENTAL

2.1. Reagents and Synthesis of NHNPs

Multiwalled carbon nanotubes (purity more than 95%) with number of walls 3-15, and tube length 1–10 micro meters were purchased from PlasmaChem GmbH Company. EPI, ACT and MEF were obtained from S.D. fine-Chem limited and Sigma chemical companies, respectively. The reagents were analytical grade and used without any further purification.

All solutions were freshly prepared with triply distilled water. Phosphate buffer solutions (PBS) were prepared from stock solution of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4. PH was adjusted using concentrated HCL or NaOH solutions. Electrochemical experiments on EPI, ACT and MEF were carried out in 0.1 mol L^{-1} Phosphate buffer at pH of 7.0. Fresh human serum and blood sample was purchased from Razi Institute of Vaccine and Serum Company (Tehran, Iran). The serum and blood sample was filtered and diluted 40 times using a 0.1 M Phosphate buffer solution of pH 7.0 and used for determination of spiked EPI, ACT and MEF in the serum.

NHNPs were synthesized using CHP procedure as previously reported [51]. Briefly, by adding concentrated ammonia (28 wt.%) to nickel nitrate solution (1 M), a deep blue colored nickel hexamine complex solution was formed. The solution was added into a given amount

of distilled water, the reaction was carried out under magnetic stirring for 1 h at 70 °C. Finally, light green sediments were formed. The precipitate was separated by centrifuge and rinsed with distilled water and ethanol three times respectively to remove the adsorbed ions, then dried in a vacuum oven at 80 ˚C for 12 h to form a green powder of NHNPs. The product obtained without the use of surfactant in the reaction process had a platelet-like shape.

2.2. Instrumentation

Electrochemical measurements were performed with a Palm Sense instrument/potentiostat (EcoChemie, The Netherlands) with a conventional three-electrode cell. A circular 3 mm diameter modified glassy carbon electrode (Metrohm) and a platinum wire are used as the working electrode and counter electrode, respectively. All the cell potentials were measured with respect to that of an Ag/AgCl/3 M KCl reference electrode. Differential pulse voltammetry (DPV) experiments were carried out with pulse amplitude of 50 mV, scan rate of 10 mV $s^{-1}$and a pulse interval of 0.2 s. All the measurements were carried out at room temperature. pH measurement were performed with a Metrohm 744 *pH* meter using a combination glass electrode.

2.3. Preparation of MWCNT-NHNPs/GCE Modified Electrode and General Procedure

The effect of composition of MWCNTs and NHNPs for modification of the GCE was tested using the cyclic voltammetry method (not shown). The anodic and cathodic peaks in 0.1 M PBS solution could be due to the Ni^{+2}/Ni^{+3} redox couple. Similar behavior for NHNPs has been reported previously [9]. The proportion of NHNPs influences the sensitivity of the biosensor. It was found that as the proportion by mass of NHNPs increased from 2 to 5%, the response of the electrode improved and when the proportion was more than 5%, the response decreased with larger background current, which resulted in poor measure for EPI, ACT and MEF (not shown).

Prior to Modification, the GCE was first polished with 0.3 and 0.05 μm aluminum oxide aqueous slurry and rinsed thoroughly with triply distilled water. It was then cleaned by sonication for 5 min, first in ethanol and then distilled water, and then dried under a nitrogen gas flow.

A stock solution of MWCNT–NHNPs in DMF was prepared by dispersing weighed amounts of MWCNT and NHNPs (95:5% w/w) in 1 mL DMF using an ultrasonic bath and 20 μL of the prepared homogeneous suspension was cast on the electrode with a microsyringe. The electrode was then dried at room temperature to obtain the modified electrode. This fabricated MWCNT-NHNPs/GCE was placed in the electrochemical cell

containing 0.1 mol L^{-1} Phosphate buffer and several cycles in the potential windows of -0.3to 0.8 V were performed using the CV method to obtain stable responses.

The general procedure used to obtain voltammograms was as follows. Each sample solution (10 mL) containing 0.1 M Phosphate buffer solution (pH 7.0) and appropriate amount of analytes was pipetted into a voltammetric cell. The differential pulse voltammograms showed oxidation peak potentials about 0.19, 0.37 and 0.57 V corresponding to EPI, ACT and MEF compounds, respectively. The amounts of EPI, ACT and MEF were obtained using corresponding peak heights. The modified electrode was regenerated by thoroughly washing the electrode with triply distilled water and then 2% sodium hydroxide solution. The electrode was finally rinsed carefully with distilled water to remove all adsorbates from the electrode surface and provide a fresh surface for the next experiment. Electrochemical impedance spectroscopy (EIS) was performed in a solution containing 5 mM of each of $Fe(CN)_6^{3-}$ and $Fe(CN)_6^{4-}$ and 0.1 M KCl with the frequency swept from 10^5 to 0.01 Hz at the condition potential of 0.25 V.

3. RESULTS AND DISCUSSION

3.1. Characterization of MWCNT-NHNPs/GCE

Different parts of the electrode surface were observed by scanning electron microscopy (SEM). Figure 1 shows a typical image of the MWCNTs and NHNPs. The result shows a platelet-like nanostructure with a dimension in the range of 50–100 nm. It can be seen that the particles are quite uniform in size. In addition to well distributed small particles, large agglomerated particles are also observed.

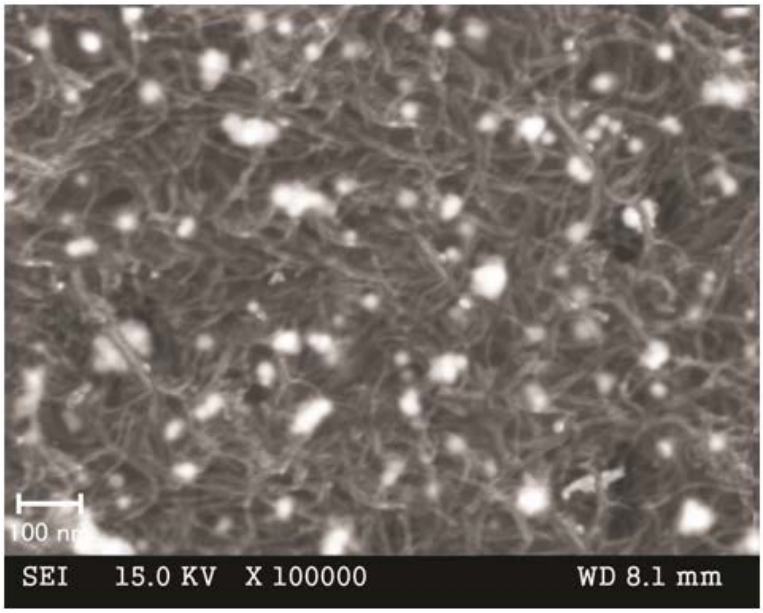

Fig. 1. SEM image of the MWCNT-NHNPs composite

Electrochemical impedance spectroscopy (EIS) can provide some information on impedance changes of the electrode surface as a result of the modification process. Figure 2 shows the Nyquist plots *(-z″ vs. z′)* for MWCNT-NHNPs/GCE (Fig. A) and GCE (Fig. B) electrodes obtained when the electrodes were immersed in a 0.1 M KCl solution containing 5 mM in both $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$. As can be seen, the diameter of the semicircle for the MWCNT-NHNPs/GCE is smaller than that of the GCE, which suggests the MWCNT-NHNPs composite modification of the electrode provides lower resistance. This phenomena could be due to higher surface area of the modified electrode as verified by the next experiments.

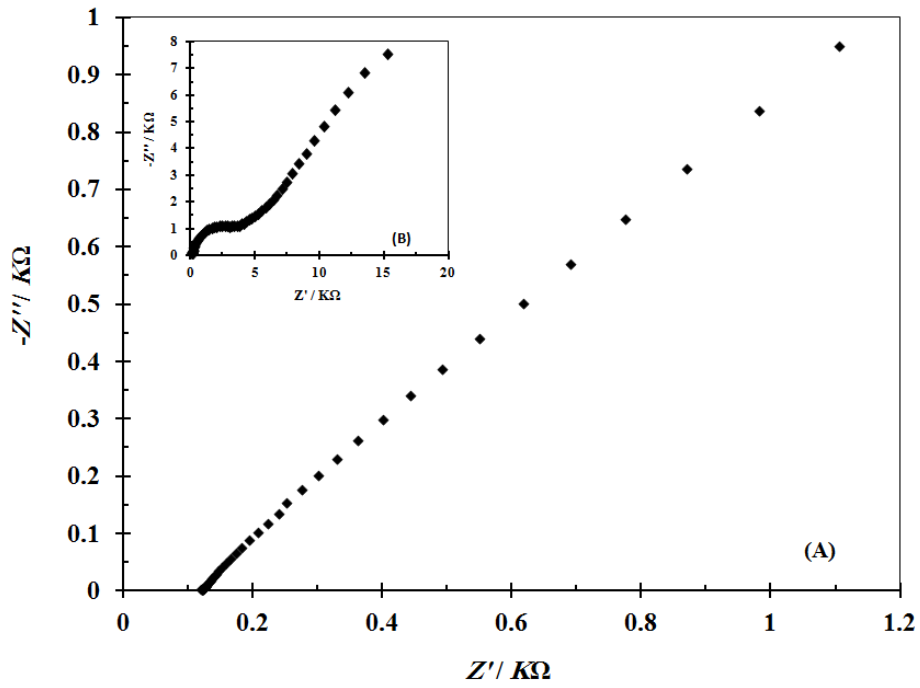

Fig. 2. Nyquist plots for MWCNTs-NHNPs/GCE (Fig. A) and GCE (Fig. B) electrodes obtained when the electrodes immersed into solutions of 5 mM $K_3[Fe(CN)6]/K_4[Fe(CN)_6]$ and 0.1 M KCl solution

The effect of modification of the electrode on active surface area was characterized by cyclic voltammograms using MWCNT-NHNPs/GCE, MWCNT/GCE and GCE in 4 mM potassium ferricyanide with Phosphate buffer solution (pH of 7.0) [52]. $K_3Fe(CN)_6$ exhibited a pair of reversible redox peaks at a bare and modified GC electrode. However for the modified electrodes the redox peak currents are larger than for the GCE. On the other hand, under the same conditions, the cathodic peak currents were linear with the square root of scan rate ($v^{1/2}$) on the GCE and other modified GCE electrodes. The obtained regression equations for the three electrodes are as follows:

$I_{pa}(\mu A) = 28.74\nu^{1/2}$ (V $s^{-1})^{1/2}$ + 4.24 (R^2 = 0.990) **GCE**

$I_{pa}(\mu A) = 550.9\nu^{1/2}$ (V $s^{-1})^{1/2}$ + 24.4 (R^2= 0.999) **MWCNT/GCE**

$I_{pa}(\mu A) = 651.9\nu^{1/2}$ (V $s^{-1})^{1/2}$ + 21.83 (R^2= 0.999) **MWCNT-NHNPs/GCE**

A reversible system should satisfy the Randles-Sevcik equation [53]:

$I_p = 2.69\times10^5\ n^{3/2}\ A\ D^{1/2}\ \nu^{1/2}\ C$

The apparent area of the MWCNT-NHNPs/GC and MWCNT/GC modified electrodes were estimated about 22.7 and 19.2 times as large as that of the GC electrode, respectively. It can be concluded that the application of a MWCNT-NHNPs composite leads to higher electrochemically active surface area than MWCNT/GCE and GCE.

3.2. Optimization of Operational Parameters

The amount of anodic peak currents of EPI, ACT and MEF were measured in different media, namely phosphate buffer solution, Britton-Robinson buffer solution, ammonium buffer solution and acetate buffer solutions at pH of 7. The best sensitivity was obtained in phosphate buffer solution. The effect of the pH value on the voltammetric behavior of EPI, ACT and MEF at the MWCNT-NHNPs/GCE was carefully investigated in the pH range 5–9 (not shown). The oxidation peak current of EPI, ACT and MEF increased gradually from pH 5.0 to 7.0 and then decreased with pH change from 7 to 11. So, the ammonium buffer with a pH of 7.0 was selected as the optimum supporting electrolyte for the simultaneous determination of EPI, ACT and MEF compounds.

The anodic peak currents of EPI, ACT and MEF improve with accumulation time, but after 20 s for EPI, ACT and MEF remained almost stable (not shown). This may be due to saturation of the amount of EPI, ACT and MEF adsorbed on the modified electrode surface. Thus, the accumulation time of 60 s was selected as an optimum time for subsequent experiments.

3.3. Electrochemical Studies of EPI, ACT and MEF on the Modified Electrode

The electrochemical behaviors of 100 µM of EPI, 50 µM of ACT and 50 µM MEF were investigated by the cyclic voltammetry method at bare GCE, MWCNT/GCE and MWCNT-NHNPs/GCE in the PBS (Figure 3). Voltammogram **a** displays the EPI, ACT and MEF data at the GCE. Voltammograms **b** and **c** show results of EPI, ACT and MEF under the same conditions at the MWCNT/GCE and MWCNT-NHNPs/GCE, respectively. It is obvious that the MWCNT-NHNPs/GCE exhibits enhanced electrocatalytic oxidation with higher peak

current for the oxidation of EPI, ACT and MEF in comparison to the bare GCE and MWCNT/GCE. Therefore, it was concluded that MWCNT-NHNPs/GCE can be used for a highly sensitive simultaneous electrochemical determination of EPI, ACT and MEF.

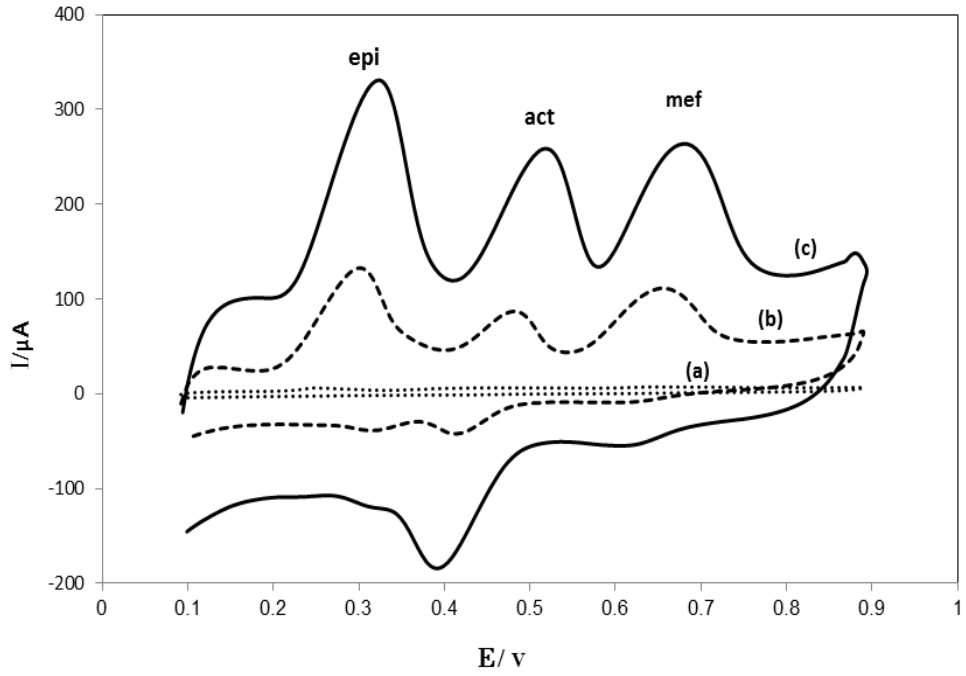

Fig. 3. Cyclic voltammograms of 100 µM EPI , 50 µM ACT and 50 µM MEF at (a) GC, (b) MWCNTs /GCE and (c) MWCNTs-NHNPs / GCE in 0.1 M phosphate buffer solution (*pH* 7.0). At scan rate of 50 mV s^{-1}

The influence of scan rate on the oxidation peak potential (E_{pa}) and current of EPI, ACT and MEF at the MWCNT-NHNPs/GCE in 0.1 M phosphate (pH 7.0) were studied by cyclic voltammetry (Figure 4). The E_{pa} shifted to more positive potentials with increasing scan rate (v), confirming the kinetic limitation of the electrochemical reaction. The anodic peak current of 100 µM of EPI, 50 µM of ACT and 50 µM MEF was proportional to the scan rate over the range of 10 to 100 mV s^{-1} with linear regression equations:

$I_{pa}(\mu A) = 2117.6v + 32.945$ (V s^{-1}) ($R^2 = 0.9926$) **EPI**

$I_{pa}(\mu A) = 997.1v + 36.936$ (V s^{-1}) ($R^2 = 0.9958$) **ACT**

$I_{pa}(\mu A) = 1979.1v + 23.357$ (V s^{-1}) ($R^2 = 0.9939$) **MEF**

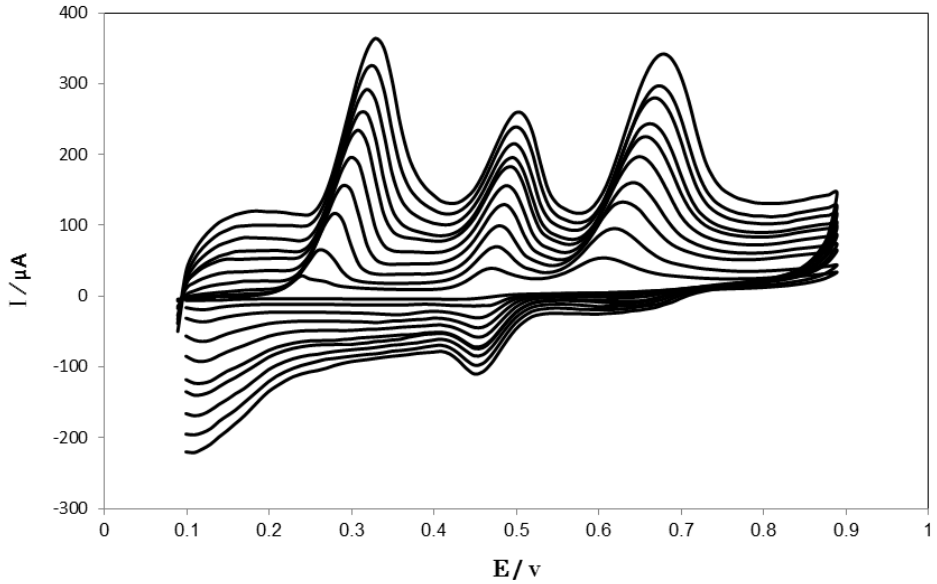

Fig. 4. Cyclic voltammograms of EPI, ACT and MEF at different scan rates (from A to J) 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1 V s^{-1}. Insets: dependence of peak currents *vs*. scan rate

These phenomena indicate oxidations of EPI, ACT and MEF are adsorption controlled processes at those scan rates. At sweep rates from 100 to 320 mV s^{-1} values, the plot of currents *vs*. scan rate deviate from linearity and the peak currents relate linearly with the square root of scan rate ($v^{1/2}$). The results indicate diffusion-controlled mechanisms with linear regression equations:

$I_{pa}(\mu A) = 635.49v^{1/2} + 47.563$	$(V\ s^{-1})^{1/2}$	$(R^2 = 0.993)$	**EPI**
$I_{pa}(\mu A) = 291.39v^{1/2} + 62.522$	$(V\ s^{-1})^{1/2}$	$(R^2 = 0.9898)$	**ACT**
$I_{pa}(\mu A) = 635.37v^{1/2} + 7.9431(V\ s^{-1})^{1/2}$		$(R^2 = 0.9898)$	**MEF**

Figure 5. exhibits the differential pulse voltammograms (DPVs) obtained for EPI, ACT and MEF mixture on MWCNT-NHNPs modified GCE in phosphate buffer by synchronously changing the concentrations of EPI, ACT and MEF. The peak currents of EPI were proportional to the concentration in the range of 0.5-180 µM (I_p(µA)=0.3634c (µM) -2.0158) with a correlation coefficient of 0.9958 (Fig. 6A). For ACT the oxidation peak current increased linearly with concentration in the range of 0.1-180 µM (I_p(µA)=0.2055c (µM)-0.6659) with correlation coefficient of 0.9915 (Fig. 6B). The oxidation peak current of MEF was increased linearly with concentration in the range of 0.1-85 µM (I_p(µA)=0.3668c (µM) +1.5202) with correlation coefficient of 0.9971 (Fig. 6C) . Considering signal-to-noise ratio (S/N) of 3, the detection limits for EPI, ACT and MEF were obtained as 0.03 µM, 0.06 µM and 0.04 µM, respectively.

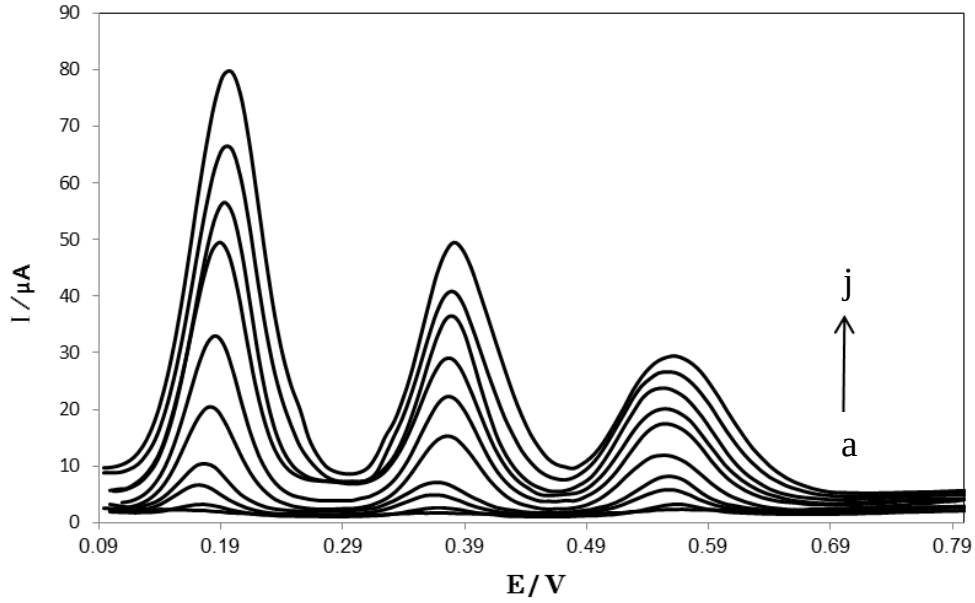

Fig. 5. Differential pulse voltammograms for different concentrations of EPI , ACT and MEF mixture as (a) 0.5+0.1+0.1 , (b) 9+8.5+3, (c) 18+17.5+6, (d) 36+35.5+20, (e) 60+59.5+25, (f) 85+90+40, (g) 120+119.5+48, (h) 144+143.5+65, (i) 160+160+65, (j) 180+180+85, respectively, in which the first value is the concentration of EPI in µM , the second value is the concentration of ACT in µM and third is value is the concentration of MEF in µM. Other conditions: Open circuit, t_{acc}=60 s, pulse amplitude=50 mV, scan rate=10mV s^{-1}, interval time=0.5 s, modulation time=0.2 s and step potential=5 mV.

The chronoamperometry method was employed for investigation of the electro-oxidation of EPI, ACT and MEF at MWCNT-NHNPs/GCE (Figure 7). The peak currents of EPI were proportional to concentration between 1 and 600µM with regression equation of *Ip*(µA)=0.3924c (µM)+0.916 (R^2=0.997). The calibration plot (not shown) of ACT is linear between 1 and 500 µM with regression equation of *Ip*(µA)=0.2391c (µM)+2.7436 (R^2=0.9915) and for MEF is linear between 1-50 and 60-600 µM with regression equation of *Ip*(µA)=0.3541c (µM)+0.1653 (R^2=0.9968) and *Ip*(µA)=0.1792c (µM)+0.1163 (R^2=0.9949) . The corresponding detection limits were 0.17 µM, 0.28 µM and 0.19 µM for EPI, ACT and MEF, respectively.

3.5. Stability and Repeatability of the MWCNT-NHNPs/GCE

To evaluate the repeatability of the MWCNT-NHNPs/GCE, the peak currents of 8 successive measurements by DPV in a mixture solution of 100µM EPI, 100µM ACT and 50 µM MEF were determined. The relative standard deviation (R.S.D.) of 0.82%, 0.42% and 0.94% was obtained for EPI, ACT and MEF, respectively. The results indicate excellent

repeatability of MWCNT-NHNPs/GCE, which could be due to this fact that the electrode surface is not subject to surface fouling by the oxidation products.

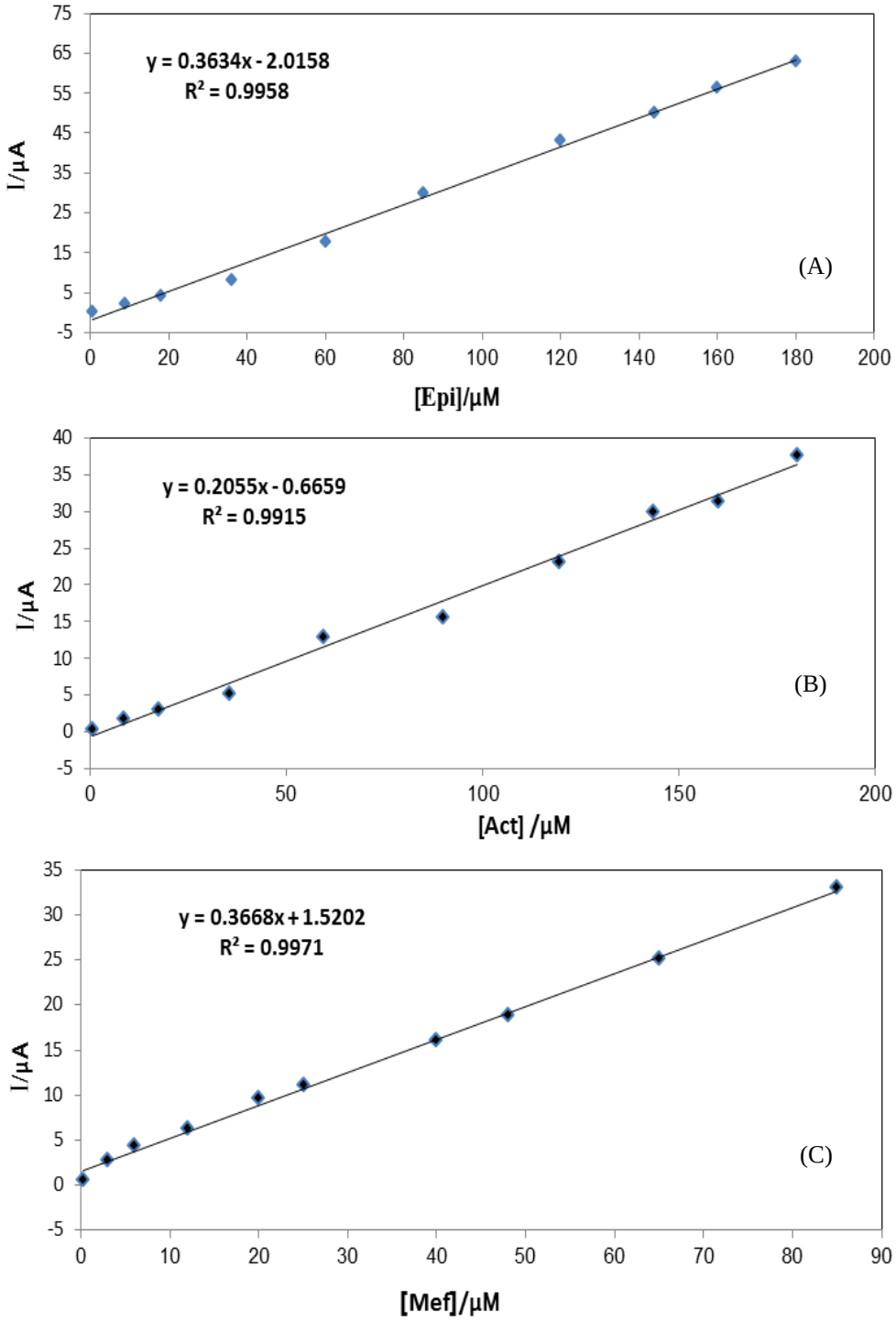

Fig. 6. (A) Plot of peak currents as a function of EPI concentration. (B) Plot of the peak currents as a function of ACT concentration. (C) Plot of the peak currents as a function of MEF concentration

The stability of the modified electrode was measured by determining the decrease in peak currents during repetitive DPV measurements of EPI, ACT and MEF after storing the electrode in 0.1 M phosphate buffer (pH 7.0). When the modified electrode was subjected to an experiment in specific period of time for ten times, after 12 h it gave no more than 7.4, 8.7 and 9.1% decrease in the current response for EPI, ACT and MEF, respectively. However, storing the modified electrode in air for 10 days gave only about 6.75, 6.71 and 6.41% decrease in oxidation peak current for EPI, ACT and MEF, respectively. The results showed that the MWCNT-NHNPs/GCE has very good stability to use for detection of these compounds.

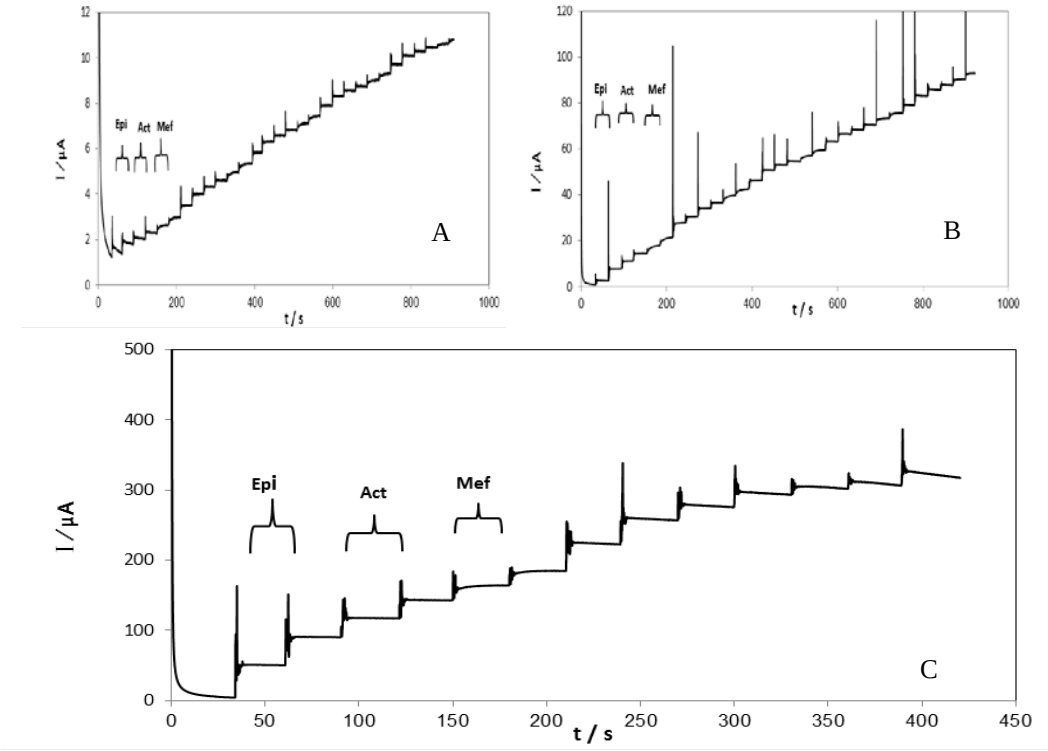

Fig. 7. Amperometric response at rotating MWCNTs-NHNPs/GCE (rotating speed 2000 rpm) held at 0.63 V in PBS for simultaneous determination of by successive additions of (A) 1+1+1 µM, (B) (10+10+10 µM and (C) 100+100+100 µM of EPI , ACT and MEF respectively

3.6. Effect of Interferences and Analytical Applications

The effects of common interfering species, which may be coexist in body, in solutions of 100 µM EPI, 100 µM ACT and 50 µM MEF were investigated in the optimum measurement conditions. Table 1 lists the tolerance limit for each potential interferent, which is defined as the concentration of the interferent that gives an error of ≤10% in the determination of EPI,

ACT and MEF. The data show that interferences are only significant at relatively high concentrations, confirming that the proposed method is likely to be free from interferences from common components of biological samples.

Table 1. Maximum tolerable concentration of interfering species

Interfering species	**EPI** C_{int}**/ (µM)**	**ACT** C_{int}**/ (µM)**	**MEF** C_{int}**/ (µM)**
Ascorbic acid	250	350	350
L-glutamic acid	400	300	500
L-alanin	700	600	600
Aspartic acid	1500	1300	1800
Aspirin	1000	1500	1300

C_{int} refers to interfering compound concentration

The proposed method was successfully applied to the simultaneous determination of EPI, ACT and MEF in human urine and blood at optimum conditions by differential pulse voltammetry method (Table 2 and Table 3). The samples were diluted 40 times before analysis and spiked with appropriate amounts of EPI, ACT and MEF. The concentrations of EPI, ACT and MEF were calculated by using standard additions method in order to prevent of any matrix effect. Good recoveries were obtained for spiked samples providing further evidence that this is a reliable method for the direct determination of EPI, ACT and MEF in serum and urine samples. These confirm that the proposed method can be used for the consistent simultaneous determination of these compounds in biological fluids.

Table 2. Estimation of EPI, ACT and MEF diluted (40-fold) human urine

Spiked (µM)			**Found (µM)**			**[a]R.S.D. (%)**			**Recovery**		
EPI	ACT	MEF	EPI	ACT	MEF	EPI	ACT	MEF	EPI	ACT	MEF
30	30	10	31.5	28.9	9.89	3.3	1.9	2.2	105	96.3	98.9
40	40	20	38.8	41.2	19.6	2.8	1.5	1.8	97	103	98

[a] Average of five determinations at optimum conditions

Table 3. Estimation of EPI, ACT and MEF diluted (40-fold) urine

Spiked (μM)			Found (μM)			[a]R.S.D. (%)			Recovery		
EPI	ACT	MEF	EPI	ACT	MEF	EPI	ACT	MEF	EPI	ACT	MEF
25	30	10	25.2	28.3	10.7	3.5	3.4	2.7	100.8	94.3	105
35	40	20	34.1	39.1	18.3	3.1	2.9	1.3	97.4	97.7	95.5

[a] Average of five determinations at optimum conditions

4. CONCLUSION

The proposed MWCNT-NHNPs/GCE exhibited a good electrocatalytic performance for determination of EPI, ACT and MEF due to combination of NHNPs and MWCNTs. The electrode also shows high stability in repetitive experiments. The interfering study of some species showed no significant interference with determination of EPI, ACT and MEF. Application of the proposed sensor for the determination of EPI, ACT and MEF in some real samples gave satisfactory results, without the necessity of sample pretreatments or time-consuming extractions. The simple fabrication procedure, high speed, repeatability, high stability, wide linear dynamic range, and high sensitivity, suggest that the proposed sensor is an attractive candidate for practical applications.

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REFERENCES

[1] N. F. Atta, M. F. El-Kady, and A. Galal, Sens. Actuators B 141 (2009) 566.
[2] J. Heo, Y. W. Lee, M. Kim, W. S. Yun, and S. W. Han, Chem. Commun. 15 (2009) 1981.
[3] H. Parham, and N. Rahbar, J. Hazard. Mater. 177 (2010) 1077.
[4] S. Shahrokhian, M. Ghalkhani, M. Adeli, and M. K. Amini, Biosens. Bioelectron. 24 (2009) 3235.
[5] K. Ohnishi, Y. Einaga, H. Notsu, C. Terashima, N. Rao, S. G. Park, and A. Fujishima, Electrochem. Solid State Lett. 5 (2002) D1.
[6] I. G. Casella, and M. Gatta, Anal. Chem. 72 (2000) 2969.
[7] Q. Li, L. S. Wang, B. Y. Hu, C. Yang, L. Zhou, and L. Zhang, Mater. Lett. 61 (2007) 1615.

[8] G. R. Fu, Z. A. Hu, L. J. Xie, X. Q. Jin, Y. L. Xie, Y. X. Wang, Z. Y. Zhang, Y. Y. Yang, and H. Y. Wu, Int. J. Electrochem. Sci. 4 (2009) 1052.
[9] A. Safavi, N. Maleki, and E. Farjami, Biosens. Bioelectron. 24 (2009) 1655.
[10] G. Xiao-Yan, and D. Jian-Cheng, Mater. Lett. 61 (2007) 621.
[11] J. J. Davis, R. J. Coles, H. Allen, and O. Hill, J. Electroanal. Chem. 440 (1997) 279.
[12] H. Luo, Z. Shi, N. Li, Z. Gu, and Q. Zhuang, Anal. Chem. 73 (2001) 915.
[13] J. M. Nugent, K. S. V. Santhanam, A. Rubio, and P. M. Ajayan, Nano Lett. 1 (2001) 87.
[14] S. J. Tans, A. R. M. Verschueren, and C. Dekker, Nature 393 (1998) 49.
[15] J. J. Gooding, Electrochim. Acta 50 (2005) 3049.
[16] J. Kong , N. R. Franklin, C. Zhou, M. G. Chapline, S. Peng, K. Cho, and H. Dai, Science 287 (2000) 622.
[17] A. Babaei, M. Afrasiabi, S. Mirzakhani, and A. R. Taheri, J. Braz. Chem. Soc. 22 (2011) 334.
[18] M. H. Sorouraddin, J. L. Manzoori, E. Kargarzadeh, and A. M. H. Shabani, J. Pharm. Biomed. Anal. 18 (1998) 877.
[19] J. J. B. Nevado, J. M. L. Gallego, and P. B. Laguna, J. Pharm. Biomed. Anal. 14 (1996) 571.
[20] A. Tzontcheva, and N. Denikova, Clin. Chim. Acta 297 (2000) 217.
[21] T. Kawada, T. Yamazaki, T. Akiyama, T. Sato, T. Shishido, M. Sugimachi, M. Inagaki, J. Alexander, and K. Sunagawa, J. Chromatogr. B 714 (1998) 375.
[22] H. B. He, C. M. Stein, B. Christman, and A. J. J. Wood, J. Chromatogr. B 701 (1997) 115.
[23] E. C. Y. Chan, and P. C. Ho, Rapid Commun. Mass Spectrum. 14 (2000) 1959.
[24] D. Chen, D. Z. Zhan, C. W. Cheng, A. C. Liu, and C. Chen, J. Chromatogr. B 750 (2001) 33.
[25] M. Chicharro, A. Zapardiel, J. A. Bermejo, J. A. Perez, and L. Hemandez, J. Chromatogr. 622 (1993) 103.
[26] M. H. Sorouraddin, A. Hibara, and T. Kitamori, F. J. Anal. Chem. 371 (2001) 91.
[27] J. Michalowski, and P. Halabura, Talanta 55 (2001) 1165.
[28] F. Li, and H. Cui, Anal. Chim. Acta 471 (2002) 187.
[29] R. C. Matos, L. Angnes, M. C. V. Araujo, and T. C. B. Saldanha, Analyst 125 (2000) 2011.
[30] S. Shahrokhian, M. Ghalkhani, and M. K. Amini, Sens. Actuators B 137 (2009) 669.
[31] Y. Z. Zhou, L. J. Zhang, S. L. Chen, S. Y. Dong, and X. H. Zheng, Chin. Chem. Lett. 20 (2009) 217.
[32] Y. Gong, L. Ye, H. X. Ju, and H. Y. Chen, Chem. J. Chin. Univ. 21 (2000) 202.
[33] M. Marazuela, L. Agui, A. Gonzalez-Cortes, P. Yanez-Sedeno, and J. M. Pingarron, Electroanalysis 11 (1999) 1333.

[34] A. P. Periasamy, Y. J. Chang, and S. M. Chen, J. Bioelectrochem. 80 (2011) 114.
[35] H. Beitollahi, and I. Sheikhshoaie, J. Electroanal. Chem. 661 (2011) 336.
[36] H. S. El-Desoky, and M. M. Ghoneim, Talanta 84 (2011) 223.
[37] R. Olive-Monllau, C. S. M-Cisneros, J. Bartoli, and M. Baeza, J. Sens. Actuators B 151 (2011) 416.
[38] M. H. Sorouraddin, J. L. Manzoori, E. Kargarzadeh, and A. M. H. Shabani. J. Pharm. Biomed. Anal. 18 (1998) 877.
[39] J. J. B. Nevado, J. M. L. Gallego, and P. B. Laguna, J. Pharm. Biomed. Anal. 14 (1996) 571.
[40] A.Tzontcheva, and N. Denikova, J. Clin. Chim. Acta 297 (2000) 217.
[41] T. Kawada, T. Yamazaki, T. Akiyama, T. Sato, T. Shishido, M. Sugimachi, M. Inagaki, and J. Jr. Alexander, J. Chromatogr. B 2 (1998) 375.
[42] H. B. He, C. M. Stein, and B. Christman, J. Chromatogr. B 1 (1997) 115.
[43] A. Babaei, M. Farshbaf, M. Afrasiabi, F. Bamdad, and A. Dehdashti, Anal. Bioanal. Electrochem. 5 (2013) 381.
[44] P. A. Milligan, J. Chromatogr. 576 (1992) 121.
[45] D. Cerretani, L. Micheli, A. L. Fiaschi, and G. Giorgi, J. Chromatogr. B 614 (1993) 103.
[46] L. Liu, and J. Song, Anal. Biochem. 354 (2006) 22.
[47] M. R. Rouini, A. Asadipour, Y. H. Ardakani, F. Aghdasi, J. Chromatogr. B 800 (2004) 189.
[48] A. Babaei, M. Afrasiabi, and M. Babazadeh, Electroanalysis 22 (2010) 1743.
[49] M. Fransoice, and S. Harbon, J. Mol. Pharmacol. 10 (1974) 457.
[50] M. A. Badgujar, and K. V. Mangaonkar, J. Chem. Pharm. Res. 3 (2011) 893.
[51] G. Xiao-Yan, and D. Jian-Cheng, Mater. Lett. 61 (2007) 621.
[52] A. Babaei, M. Sohrabi, and M. Afrasiabi, Electroanalysis 24 (2012) 2387.
[53] A. J. Bard, and L. R. Faulkner, Electrochemical Methods, Fundamentals and applications, 2nd ed. Wiley, New York (2001) pp. 229-231.