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Electrochemical Sensing of Folic Acid in Presence of Ascorbic acid Using Carbon Paste Nano Composite Modified Electrode

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In the present work, a combination of unique properties of two nanomaterial compounds, MgO nanoparticles (MgO NPs) and multiwall carbon nanotubes (MWCNT), and electrocatalytic activity of an oxadiazole derivative, 3-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-4-methylcyclohexa-1,3-diene-1,2-diol; POM, was used to fabricate a sensitive electrode (POM-MgO NPs-MWCNT-CPE) for electrochemical determination of folic acid. The electrochemical treatment of folic acid was studied in phosphate buffer solution (0.1 M, pH 7) by means of common electrochemical techniques such as cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The sensitive fabricated electrode displayed good operating characteristics such as a wide linear range, 0.08-650.0 μM, and low detection limit, 0.02 μM, for the determination of folic acid. Using the modified electrode, the two well distinguished peaks were recorded at 110.0 and 209 mV for ascorbic acid and folic acid, respectively. The reliability and accuracy of the introduced electrode were studied in pharmaceutical and biological samples.

Keywords: Carbon paste electrode, Oxadiazole derivative, Multiwall carbon nanotubes, Folic acid, Ascorbic acid

INTRODUCTION

Chemically modified carbon paste electrode (CMCPE) is an attractive selection for electrochemical determination of pharmaceutical/biological [1-3] and environmental [4] species.

Using the modified electrode, the redox reaction of an electroactive compound was down at lower potentials and the redox peak current will be increased. The simplicity of refreshing of CMCPE surface is one of the most benefits of carbon paste electrodes toward other carbon electrodes [5].

Nowadays, excellent properties of nanomaterials such as chemical stability, superior conductivity and extensive surface area and electrocatalytic activity, in some cases; MgO NPs make them an attractive compound in the fabrication of electrodes for electrochemical detection of electroactive compounds [6,7].

Many diseases such as anemia, scurvy and racism are caused by a decrease in vitamins. It may be due to their participation in intermediate metabolism, metabolism of certain compounds and biosynthesis. Moreover, they have an important role as a radical scavenger *in vivo*. Therefore, introduction of new methods for the quantification of vitamins is very significant [8].

In the family of water soluble vitamins, ascorbic acid (vitamin C; AA) plays many physiological roles [9] and found in natural and fortified foods and pharmaceuticals. So, the concentration of AA would affect the quality of them and quantitative determination of AA has an important role in quality control of the products [10]. Many articles have been found for the determination of AA such as voltammetry [11-13], colorimetry [14], liquid chromatography [15] and liquid chromatography-mass spectrometry [16].

Folic acid (vitamin B_9; FA) is an important micronutrient that has wide clinical applications. It helps us for our health via keep new cells, avoid the DNA change that can result in cancer and participating in making red

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blood cells to avoid anemias. Also, it is very important for women who may get pregnant. The recommended value of folate intake in the U.S. is 400 µg from foods or dietary supplements daily for adults. So, FA determination is important in pharmaceutical samples and bio-fluids [17]. In literature, various methods have been reported for the determination of FA, such as photoluminescence [18], chromatography [19], voltammetry [20,21] and kinetic spectrophotometry [22].

The application of electrochemical methods in order to detection of biological compounds as an alternative method attracted lots of attention. It is due to its high sensitivity, high rate selectivity, simplicity, and good reproducibility [22-26].

Biological compounds as a kind of electroactive compounds can be determined by voltammetric techniques. Chemically modification of an electrode by introducing a modifier to it can improve the voltammetric response of the electrode than a bare electrode. Therefore, chemically modified electrodes widely used for the electrochemical determination of biological compounds [27-33].

As stated earlier, FA and AA co-exist in bio-fluids such as serum and central nervous system cellular fluid [34]. Therefore, FA determination individually or simultaneously with co-existing species, AA, can be attractive in the field of biomedical and clinical chemistry.

This study pointed to the fabrication of a modified electrode in order to voltammetric determination of FA and AA. The modified electrode was prepared by spiking of MgO NPs and MWCNT to the matrix of carbon paste. The introduced sensor, POM-MgO NPs-MWCNT-CPE, showed excellent electrocatalytic activity in the electrochemical oxidation of FA. The linear range and detection limit were estimated using DPV technique and analytical applicability was tested by carrying out the detection of FA and AA in real samples with different matrices.

EXPERIMENTAL

Apparatus and Reagents

Each electrochemical experiment was done using a potentiostat-galvanostat Palm Sens 3 (Palm Sens, Netherland) armed with PSTrace 4.8 software. The three-electrode cell prepared by a working electrode (3-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-4-methylcyclo-hexa-1,3-diene-1,2-diol (POM) MgO nano particles (MgO NPs) multiwall carbon nanotubes (MWCNT) CPE (POM-MgO NPs-MWCNT-CPE), an auxiliary electrode (a graphite electrode), and a reference electrode (a saturated calomel electrode (SCE)). The data storage and processing were carried out *via* a personal computer. All potentials were measured *vs*. SCE. The pH was monitored by a pH/mV (Metrohm, Swiss).

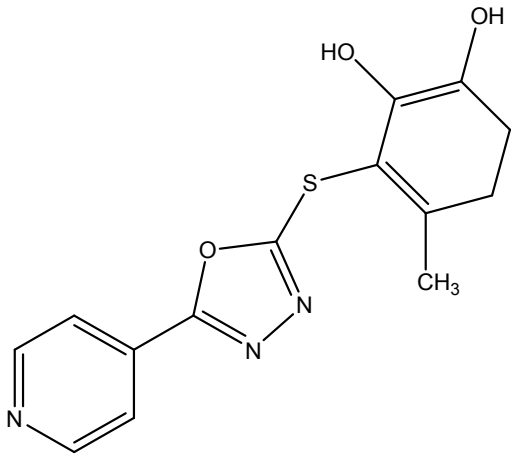
Plasma Chem GmbH was selected to purchase MgO NPs (purity > 99% and mean particle size of 20 nm) and multiwall carbon nanotubes (MWCNT; purity ≥ 97%, length of 5-25 µm and diameter of 10-20 nm). Graphite fine powder (purity > 99%, Fluka) and paraffin oil (DC 350, Merck; d: 0.88 g cm^{-3}) were used for preparing of carbon paste. All analytical grade chemical reagents including FA, AA and others were purchased from Merck (Darmstadt, Germany). The phosphate buffer solution (PBS; 0.1 M, pH 7) was made from ortho-phosphoric acid and NaOH.

Synthesis of Oxadiazole Derivative

The oxadiazole derivative that marked as POM, 3-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-4-methylcyclo-hexa-1,3-diene-1,2-diol (see Scheme 1 for molecular structure), was synthesized using a previous explained process [35]. In brief, through a conformist process, the pre-electrolyzed of 100 ml of PBS (0.15 M, pH 7.2) in a mixture of water and acetonitrile (95/5) was down at 0.2 V *vs*. Ag/AgCl (KCl 3 M) in an undivided cell. The electrolysis was continued at 0.2 V after adding catechols and nucleophiles up to reducing the current to 5% of the initial value. After a day, the solid product was filtered and washed with water. The brownish powder was characterized by MS, ^{1}H NMR and ^{13}C NMR.

Electrode Fabrication

The modified CPE was fabricated as below: graphite fine powder (100.0 mg), MWCNT (0.8 mg), MgO NPs (0.5 mg) and POM (0.4 mg) were blended in a mortar. Paraffin oil was added to the above mixture dropwise and mixed thoroughly to achieve a uniform wetted paste (POM-MgO NPs-MWCNT-CP). To fabricate the modified electrode, the paste was packed to a Teflon rod with a whole (2 mm i.d and 5 mm deep with the length of 10 cm) and



3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-ylthio)-4-methylcyclohexa-1,3-diene-1,2-diol

Scheme 1. Molecular structure of 3-(5-(pyridine-4-yl)-1,3,4-oxadiazole-2-ylthio)-4-methylcyclohexa-1,3-diene-1,2-diol

leveled off by a spatula. To communicate electrically, a copper wire was inserted into the Teflon rod center that has been screwed to potentiostat-galvanostat. Refreshing of the surface electrode was down by polishing it on a weighing paper. Unmodified CPEs were prepared in the same way without adding POM, MgO NPs and MWCNT to the mixture.

Preparation of Real Samples

Pharmaceutical sample preparation. FA tablet in different dosages, AA tablet in existence dosage and AA injection solution were selected as pharmaceutical samples.

FA tablets in dosage 1.0 and 5.0 mg were purchased from the drug store. Ten FA tablets were absolutely grounded before preparing by 5 ml of the 0.01 M NaOH solution. After thorough mixing, the solution was filtered and diluted to the mark in a 50.0 ml volumetric flask.

Five AA tablets in a dose of 250.0 mg that prepared from a local drug store were weighed, grounded and mixed completely. An amount consistent with 100.0 mg of AA was weighed, dissolved in water and filtered. The solution was diluted to the mark in a 100 ml volumetric flask. The AA injection solution (500 mg/5 ml) was diluted in a 0.5 l volumetric flask.

An appropriate volume of each pharmaceutical prepared sample was placed in the electrochemical cell and determined by recording DPV voltammograms.

Biological sample preparation. Human urine and serum were considered biological samples. The samples were collected from persons who had not taken FA and prepared just before the examination.

Urine sample preparation: 10 ml of urine sample were centrifuged for 10 min at 2,000 rpm and filtered by a Whatman filter paper. Then, an appropriate volume of the sample was diluted to the mark with PBS (0.1 M, pH 7) in a 25 ml volumetric flask. The prepared sample was spiked by various amounts of FA and determined by DPV method.

Serum sample preparation: 10 ml of the sample was spiked with various amounts of FA. Purification and pre-concentration of FA were done using C18 cartridge (Supelco Inc., 100 ml) by the recommended procedure [36]. The extracted FA was diluted to the mark in a 10 ml volumetric flask. The suitable amounts of the prepared sample solution were transferred to the electrochemical cell to record the DPV voltammograms.

RESULT AND DISCUSSION

Electrochemical Behaviour of Folic Acid at the Surface of Various Electrodes

The electrocatalytic properties of POM-MgO NPs-MWCNT-CPE were investigated *via* the recording of cyclic

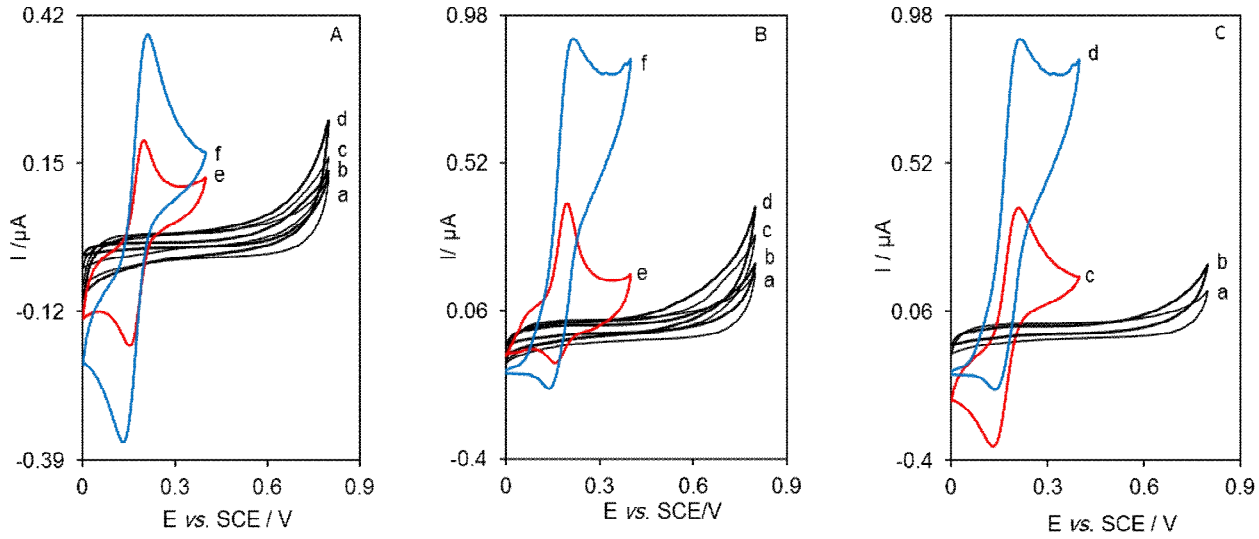


Fig. 1. (A) Cyclic voltammograms of (a) CPE, (b) CPE-MWCNT, (c) CPE-MgO NPs, (d) CPE- MgO NPs-MWCNT, (e) POM-CPE and (f) POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7). (B) Cyclic voltammograms of (a) to (f) were recorded at the surface of the same electrodes of Fig. 1A in the presence of FA 0.1 M. (C) Cyclic voltammograms of (a) MgO NPs-MWCNT-CPE and (c) POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7). (b) as (a) and (d) as (c) were recorded in the presence of FA 0.1 M.

voltammograms of the unmodified electrode (CPE) and various modified electrodes in the lack and existence of 0.1 mM FA (Fig. 1).

Voltammograms (a) to (d) of Fig. 1A recorded by the use of CPE, MWCNT-CPE, MgO Nps-CPE and MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7) at scan rate potential 20 mV s^{-1}, respectively. As it can be seen, at the surface of CPE as a bare electrode (voltammogram a) than spiked CPE with MWCNT (voltammogram b), MgO NPs (voltammogram c) and MgO NPs-MWCNT composite (voltammogram d), redox reaction was not done over the potential range of 0.0-0.8 V. However, a couple of anodic and cathodic peaks was detected at the surface of POM-CPE at potentials 0.24 V and 0.14 V (voltammogram e), respectively. Furthermore, cyclic voltammograms (b) and (c) approved the significance of applying MWCNT and MgO NPs in carbon paste matrix, respectively. Increasing the surface area attributed to MgO NPs and improving the electrical conductivity and surface area resulted to using of MWCNT in CPE nanocomposite gained the peak currents. Also, voltammogram (f) recorded at the surface of POM-MgO NPs-MWCNT-CPE clearly has shown the significance of using the MWCNT and MgO NPs composite in POM-CPE. Increasing the peak current almost twice (voltammogram f) than voltammogram (e), recorded at the surface of POM-CPE can be attributed to the combination of MWCNT and MgO NPs.

Figure 1B depicts cyclic voltammograms of unmodified and modified electrodes in the existence of 0.1 mM of FA at scan rate potential 20 mV s^{-1}. Voltammograms (a) to (d) of Fig. 1B recorded by the use of CPE, as an unmodified electrode, and MWCNT-CPE and MgO NPs-CPE and MgO NPs-MWCNT-CPE, as modified electrodes, in 0.1 mM FA at scan rate potential 20 mV s^{-1}, respectively. As it can be seen, MWCNT, MgO NPs and a combination of them in a matrix of carbon paste can increase the sensitivity. Meanwhile, electrocatalytic redox of FA cannot occur over the range 0.0-0.8 V. Electrocatalytic oxidation of FA was investigated at the POM-CPE surface (voltammogram e) and POM-MgO NPs-MWCNT-CPE (voltammogram f). As it can be seen, in the presence of modifier, POM, FA electrocatalytic oxidation was down in 0.24 V.

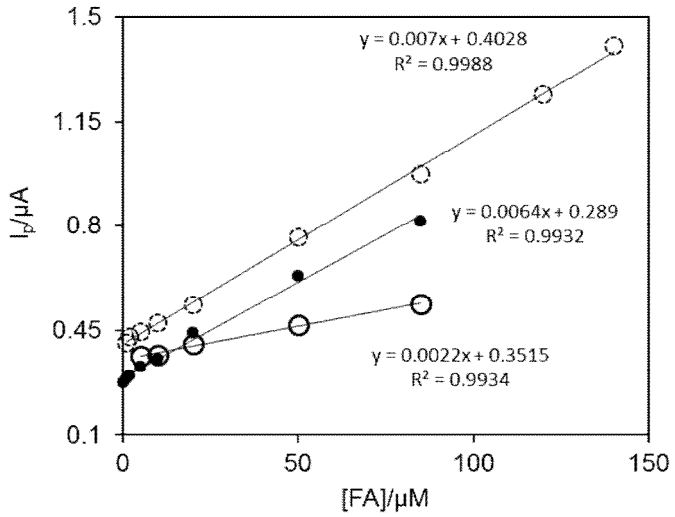


Fig. 2. Effect of pH on the calibration curves of the electrocatalytic oxidation of FA at the POM-MgO NPs-MWCNT-CPE. Curve a, pH 6 in concentration range 5.0-85.0 µM; curve b, pH 7 in concentration range 1.0-140.0 µM and curve c, pH 8 in concentration range 0.3-85.0 µM.

Voltammogram (f) that recorded at the POM-MgO NPs-MWCNT-CPE surface than voltammogram (e) displays the increasing of anodic peak current more than twice. The voltammograms approved the performance of the sensor.

The efficiency of MgO NPs-MWCNT-CPE for electro-oxidation of FA was shown in Fig. 1C. Cyclic voltammograms (a) and (c) recorded at the surface of the modified electrode in PBS (0.1 M, pH 7). Meanwhile, cyclic voltammograms of (b) and (d) related to the same electrodes in 0.1 M FA. Cyclic voltammograms (c) and (d) in comparison of (a) and (b) shown the potential of POM as a modifier obviously. As it was expected, reducing the overvoltage to 0.22 V, occurrence the redox reaction with the anodic peak current of 0.89 µA and reducing the cathodic reduction peak, (voltammogram (d) *vs*. (c)) attributed to the existence of POM in the electrode matrix. Also, dramatically increasing the peak current of FA oxidation at the surface of POM-MgO NPs-MWCNT-CPE (voltammogram d) illustrates the modified electrode is superior for electro-catalytic oxidation of FA.

Effect of pH

The effect of pH on the voltammetric response of electrocatalytic oxidation of FA at the surface of the modified electrode was studied using cyclic voltammetry at different pH over the range 6-8. Fig. 2 shows the calibration curves of FA electrocatalytic oxidation at the POM-MgO NPs-MWCNT-CPE. As it can be seen, the linear concentration range of FA determination at pH 6 is 5.0-85.0 µM that is smaller than the linear range of pH 7 (1.0-140.0 µM) and pH 8 (0.3-85.0 µM). Moreover, the maximum sensitivity, 0.007 µA μM^{-1}, was obtained at pH 7. Therefore, pH 7 was selected for further studies.

Effect of Potential Scan Rate

The effect of potential scan rate on the redox reaction of FA at the modified electrode surface was studied. For this purpose, cyclic voltammograms of 0.1 mM of FA in PBS (0.1 M, pH 7) at different scan rates were recorded (Fig. 3). The obtained results were depicted that the catalytic peak current was increased by increasing the potential scan rate. Also, the linearity of the plot of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) for FA (Fig. 3A) depicted that the oxidation process of FA at sufficient over potential is diffusion controlled. The plot of the scan rate normalized current ($I_p\ v^{-1/2}$) *vs*. the potential scan rate was drawn (Fig. 3B). The typical form of the plot reveals an EC_{cat} process. The shape of the plot shows a

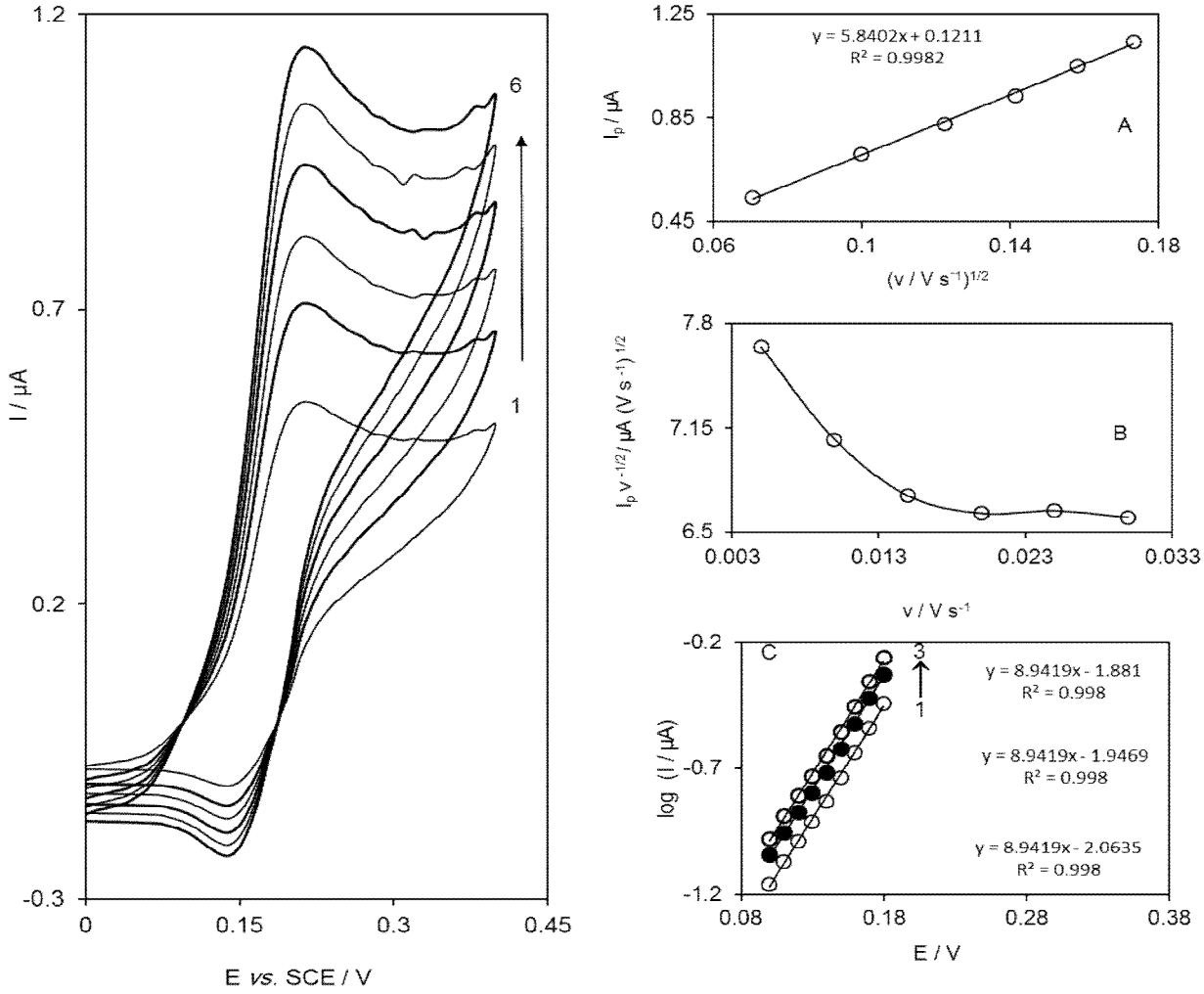


Fig. 3. Cyclic voltammograms of POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7) containing 0. 1 mM of FA at different scan rates. The numbers of 1-6 correspond to scan rates potential of 5, 10, 15, 20, 25 and 30 mV s^{-1}. Insets: (A) Variation of the electrocatalytic peak currents *vs*. the square root of scan rate, (B) variation of the scan rate potential normalized peak current ($I_p v^{-1/2}$) *vs*. the scan rate potential, (C) Tafel plot derived from the current-potential curve recorded at scan rate potentials of 5, 10, 15 mV s^{-1}.

property of an EC_{cat} ($E_rC'_i$) procedure. The obtained outputs demonstrated that electro-oxidation of FA at the POM-MgO NPs-MWCNT-CPE should be controlled through a cross-exchange process between FA and redox site of POM and diffusion of FA at a diffusion layer [37]. The slope of the plot and related equation for irreversible diffusion controlled process [38] was applied for estimation of the number of electrons in the overall reaction. By considering the parameters $(1 - \alpha)n_\alpha = 0.66$ and $D = 9.1 \times 10^{-6}$ cm^2 s^{-1}, the total number of anodic electrons was estimated as $n = 1.89 \approx 2.0$. The obtained value is close to the value stated previously [34].

Regarding Andrieux and Saveant [39] theoretical model of the heterogeneous catalytic process, the mean value of the catalytic rate constant, $k`$, was obtained as $(2.9 \pm 1.1) \times 10^{-3}$ cm s^{-1}. Tafel plot (Fig. 3C) was drawn using the data of

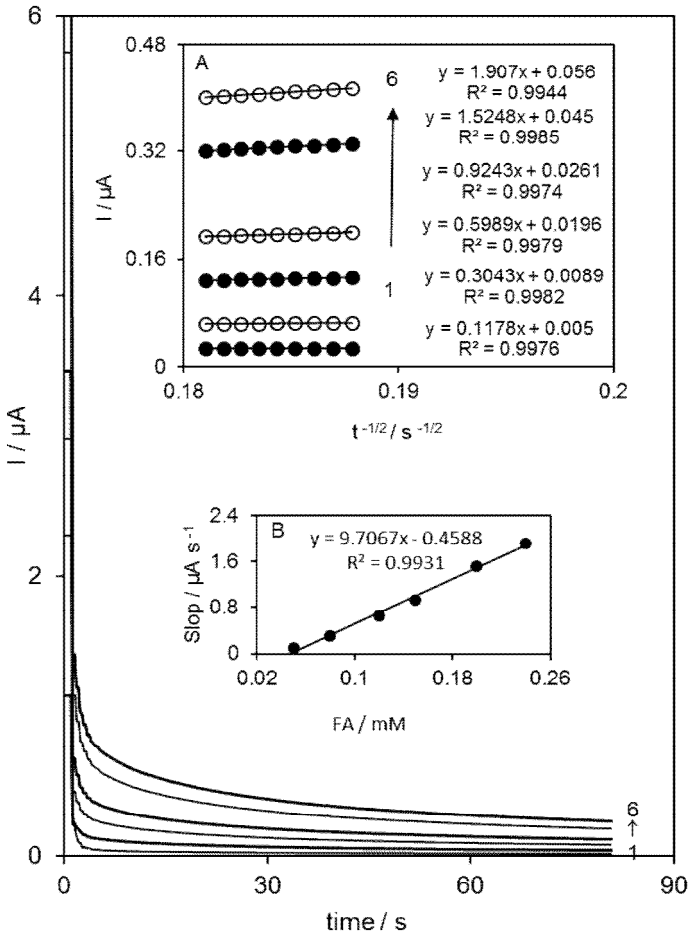


Fig. 4. Chronoamperograms obtained at POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7) for different concentrations of FA. The numbers 1-6 correspond to 0.1, 0.75, 1.2 and 2.0 mM of FA. Insets: (A) Plot of *I vs.* $t^{-1/2}$ obtained from chronoamperograms 1-6. (B) Plot of the slope of the straight lines against FA concentrations.

the rising section of the current-voltage curve of the cyclic voltammograms recorded at potential scan rates of 5, 10 and 15 mV s^{-1}. Using the average Tafel slopes of the potential scan rates which is in agreement with the contribution of one electron at the rate determining step of the electrode process, the average charge transfer coefficient was obtained as $\alpha = 0.34$ for FA. Moreover, the exchange current density, j_0, was calculated from the intercept of the Tafel plot [37]. The j_0 value of FA was obtained $9.2 \times 10^{-3} \pm 0.01$ µA cm^{-2}.

Chronoamperometric Measurements

A chronoamperometric study was applied in order to estimate of FA diffusion coefficient at the surface of the modified electrode. The technique was down by adjusting the working electrode potential on 245 mV *vs*. SCE for different concentrations of FA (Fig. 4) in PBS (0.1 M, pH 7).

Cottrel equation can be used for estimating the diffusion coefficient, D, for an electroactive species [37]:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

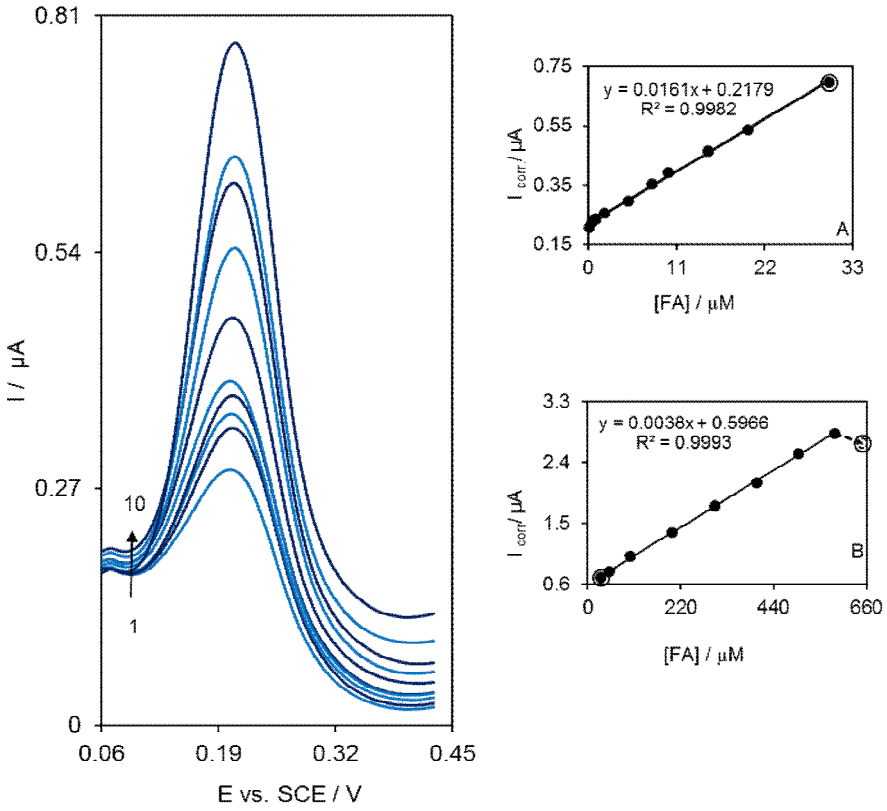


Fig. 5. DPVs of POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7) containing different concentrations of FA. Numbers 1-10 correspond to 0.08, 0.5, 1.0, 2.0, 5.0, 8.0, 10.0, 15.0, 20.0 and 30.0 µM of FA. The Figs. 5B and C show the plot of the corrected peak current as a function of the FA concentration in the range of 0.08-30.0 and 30.0-650.0 µM.

where D is the diffusion coefficient ($cm^2\ s^{-1}$) and other symbols have their conventional meanings. The plot of I *vs.* $t^{-1/2}$ was used with the best fits for different concentrations of FA (Fig. 4, inset A). Then, the slopes of the plotted straight lines were drawn *vs.* FA concentrations (Fig. 3, inset B). Using the resultant slope and the Cottrell equation, the value of D was calculated $9.1 \times 10^{-6}\ cm^2\ s^{-1}$ for FA. The obtained value of D using the proposed method is close to the values reported previously [33,34].

Calibration Plot and Limit of Detection

One of the main goals of electrochemical analysis is introducing of a method capable for quantitative determination of FA. DPV for unique characteristics such as higher resolution than CV and minor charging current portion to the background current is a suitable technique for estimating of linear range, detection limit and individual or simultaneous determination of analytes in real samples. Therefore, DPV experiments were carried out by using POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7) including various concentrations of FA (Fig. 5). The results (Figs. 5B and C) show that FA anodic peak currents were linearly pendent on FA concentration in range of 0.08-650.0 µM including two linear segments (0.08-30.0 and 30.0-650.0 µM with correlation coefficient of 0.9981 and 0.9993, respectively). Also, the limit of detection was calculated as 0.02 µM using the slope (m) of lower concentration range of calibration curve

Table 1. Comparison of Analytical Characteristics of Different Carbon Paste Modified Electrodes for Electrocatalytic Determination of FA

Modifier	Linear range (μM)	Detection limit (μM)	Ref.
Multiwall carbon nanotubes	4.6-152.0	1.1	[40]
ZrO_2 nanoparticles	10.0-170.0	9.86	[41]
NiO/Carbon nanotubes	3.0-550.0	0.9	[42]
Gold nanoparticles	0.06-80.0	2.7×10^{-2}	[43]
Ruthenium(II) Complex-ZnO/ Carbon nanotubes	3.0-700.0	1.0	[44]
Fe_3O_4 nano particles	6.5×10^{-2}-98.0	2×10^{-3}	[45]
Polyortho-methoxyaniline nanostructures	0.5-68.8	0.113	[46]
NiO singlewall carbon nanotubes-1-butyl-3-methylimidazolium methanesulfonate	0.3-350	0.09	[47]
POM-MgO NPs-MWCNT	0.08-585.0	0.02	This work

(m: 0.0162 μA μM^{-1}), the standard deviation of the blank signal (s_b, n = 14) and equation $C_m = 3s_b/m$.

Repeatability and Stability of POM-MgO NPs-MWCNT-CPE

The long-term stability of the POM-MgO NPs-MWCNT-CPE was investigated by recording the cyclic voltammograms after storage of the modified electrode 3-weeks in ambient conditions. The obtained results pointed that the peak potential for FA oxidation at the surface of the modified electrode doesn't have change. Also, the mean value of peak currents and the precision (RSD%) for sequential measurements of 10.0 and 100.0 μM of FA (n = 15) were 0.59 ± 0.02 μM; 3.4% and 1.45 ± 0.03 μM; 2.1%, respectively. The RSD% values indicate that POM-MgO NPs-MWCNT-CPE is completely stable through voltammetric measurements. Table 1 represents the comparison of some of the analytical characteristics obtained for FA determination in this work with those formerly reported in the literature using the modified carbon paste electrode. The parameters that were summarized in Table 1 approved that the introduced modified electrode is preferable in some cases than formerly reported modified carbon paste electrodes.

Simultaneous Determination of FA and AA

As stated earlier, FA and AA are co-existed in bio-fluids. Therefore, another attempt was detecting FA and AA simultaneously in pharmaceutical and biological samples by the recommended DPV procedure *via* the use of POM-MgO NPs-MWCNT-CPE. Therefore, the two analytes, FA and AA, have been detected through a simultaneous increase in concentrations. As it was shown in Fig. 6, two well distinct anodic peaks at potentials of 110 and 209 mV related to the oxidation of AA and FA, respectively were recognized at the surface of the modified electrode. Also, a considerable increase in the peak currents of FA and AA was detected due to an increase in the concentration of them. The inset A of Fig. 6 shows the DPV of a 200.0 μM AA and 100.0 μM FA mixture at MgO Nps-MWCNT-CPE surface as an unmodified electrode. As demonstrated, the unmodified electrode cannot separate the anodic peak currents of AA

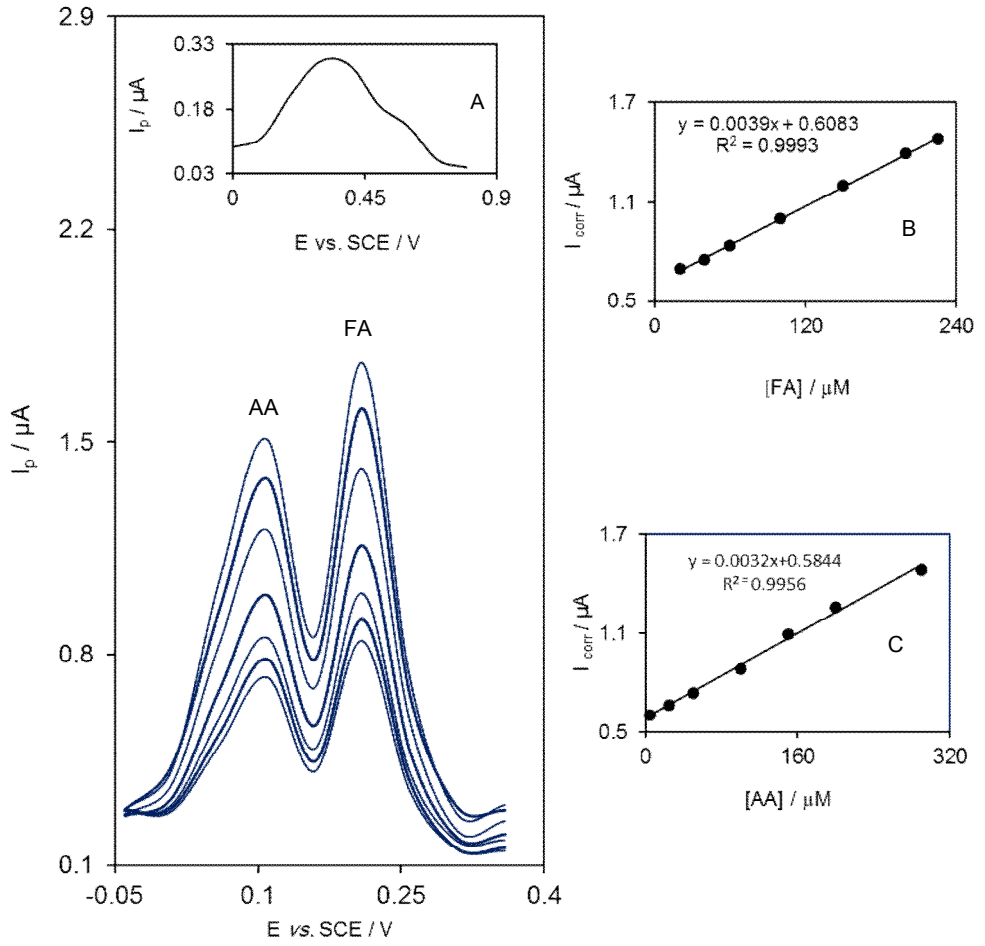


Fig. 6. DPVs of POM-MgO NPs-MWCNT-CPE in PBS (0.1 M, pH 7) containing different concentrations of FA and AA in µM, from inner to outer: 20.0+5.0, 40.0+25.0, 60.0+50.0, 100.0+100.0, 150.0+150.0, 200.0+200.0 and 225.0+290.0 µM, respectively. Inset shows the response of a solution containing 100.0+200.0 µM of FA and AA at the surface of MgO NPs-MWCNT-CPE. (B) and (C) plot of corrected peak current as a function of FA and AA in concentration range 20.0-225.0 µM and 5.0-290.0 µM, respectively.

and FA. Figures 6B and C show the calibration plots of AA and FA in concentration ranges of 5.0-290.0 µM AA and 20.0-225.0 µM FA. Since the slopes of calibration plots of FA in Figs. 5B (that recorded in absence of AA, 0.0038 µA μM^{-1}) and 6B (that recorded in presence of AA, 0.0039 µA μM^{-1}) are nearly similar, concurrent determination of FA and AA enjoy equal efficiency as it's separately determination using POM-MgO NPs-MWCNT-CPE.

Real Sample Analysis

The applicability and reliability of the proposed method were investigated by using it for the quantitative determination of FA and AA in real samples with different matrices.

FA tablet, AA tablet and AA injection solution were considered as pharmaceutical specimens. Sample preparation was performed as discussed formerly. They were spiked with various amounts of FA and AA and the DPVs were recorded. The measurements were carried out

Table 2. Differential Pulse Voltammetric Determination of FA and AA in the Pharmaceutical Samples at the POM-MgO NPs-MWCNT-CPE Surface

Sample	Added (μM)		Found (μM)[a]		Recovery (%)		RSD (%)		Total value[b]		Declared value[c]		RSD (%)		*t* test[d]	
	FA	AA	FA	AA	FA	AA	FA	AA	FA (mg)	AA (mg)	FA (mg)	AA (mg)	FA	AA	FA	AA
FA	-	-	3.6 ± 0.1	-	-	-	2.8	-	0.98 ± 0.03	-	1.0	-	3.0	-	1.15	-
tablet	50.0	-	53. 3 ± 1.4	-	99.4	-	2.6	-	-	-	-	-	-	-	-	-
	100.0	-	104.7 ± 2.6	-	101.1	-	2.5	-	-	-	-	-	-	-	-	-
	200.0	-	201.8 ± 4.6	-	99.1	-	2.3	-	-	-	-	-	-	-		
FA	-	-	8.1 ± 0.2	-	-	-	2.5	-	5.17 ± 0.14	-	5.0	-	2.7	-	2.10	-
tablet	50.0	-	58.6 ± 1.5	-	100.8	-	2.6	-	-	-	-	-	-	-	-	-
	100.0	-	107.1 ± 2.5	-	99.1	-	2.3	-	-	-	-	-	-	-	-	-
	200.0	-	209.8 ± 4.3	-	101.8	-	2.0	-	-	-	-	-	-	-	-	-
AA	-	-	-	100.9 ± 2.4	-	-	-	2. 4	-	254.1 ± 5.9	-	250.0	-	2.3	-	2.28
tablet	-	20.0	-	121.2 ± 2.9	-	100. 2	-	2.3	-	-	-	-	-	-	-	-
	-	100.0	-	198.9 ± 4.1	-	99.0	-	2.1	-	-	-	-	-	-	-	-
	-	200.0	-	303.5 ± 6.6	-	101. 5	-	2. 2	-	-	-	-	-	-	-	-
AA	-	-	-	50.5 ± 1.3	-	-	-	2.6	-	275.6 ± 4.9	-	500.0	-	2.7	-	2.99
injection solution	-	20.0	-	71.7 ± 2.0	-	101. 7	-	2.8	-	-	-	-	-	-	-	-
	-	100.0	-	149.2 ± 4.0	-	99.2	-	2.7	-	-	-	-	-	-	-	-
	-	200.0	-	253.5 ± 5.9	-	101. 2	-	2.3	-	-	-	-	-	-	-	-

[a]Average of three replicate determinations. [b]The total values were calculated by multiplying the determined values by the dilution factor (n = 3). [c]The declared value of AA injection solution is 500 mg/5 ml (equal to 100 mg ml^{-1}). [d]Tabulated *t*-value for 2 degrees of freedom at P (0.95) is 4.30.

Table 3. Differential Pulse Voltammetric Determination of FA and AA in Biological Samples at the POM-MgO NPs-MWCNT-CPE Surface

Sample	Added (μM)		Found (μM)[a]		RSD (%)		Recovery (%)	
	FA	AA	FA	AA	FA	AA	FA	AA
Human urine	-	-	<DL	<DL	-	-	-	-
	25.0	15.0	25.3 ± 0.6	15.4 ± 0.3	2.3	2.3	101.1	102.5
	100.0	100.0	100.8 ± 1.3	99.6 ± 1.2	2.1	2.3	100.8	99.6
	150.0	200.0	149.1 ± 3.0	202.6 ± 1.2	2.0	2.0	99.4	101.0
	200.0	250.0	201.7 ± 3.9	248.9 ± 4.97	2.0	2.0	100.8	99.6
Human serum	-	-	<DL	<DL	-	-	-	-
	25.0	15.0	25.4 ± 0.6	14.9 ± 0.3	2.3	2.4	101.6	99.2
	100.0	100.0	99.1 ± 2.0	102.0 ± 2.2	2.0	2.2	98.1	101.9
	150.0	200.0	150.9 ± 3.0	198.8 ± 3.9	2. 0	2.0	100.6	99.4
	200.0	250.0	202.9 ± 4.1	252.8 ± 4.9	2.0	2.0	101.5	101.1

[a]Average of three replicate determinations ± standard deviation.

using the calibration plots that were presented in Figs. 6B and C. The obtained results were given in Table 2. Regarding the results of Table 2, the total values calculated for FA and AA tablets are close to the declared value on the label. Also, the calculated relative standard deviations (RSD%) and recoveries were approved the modified electrode potential for the determination of FA and AA in pharmaceutical samples. In the following, the accuracy of the results was investigated by a statistical t-test. By referring to the t-test results, the experimental t-value obtained as 1.15 and 2.10 for FA tablet (in dosages of 1 and 5 mg) and 2.28 and 2.99 for AA Tablet and injection solution, respectively. The results approved that the difference of total value and declared value during the determination of FA and AA attributed to random errors (the critical t-value at 95% of confidence limit and two degrees of freedom is 4.30)

Human urine and serum were considered biological samples. Sample preparation was performed as discussed previously. Then, the DPVs of the spiked samples were recorded for estimating FA and AA concentrations using the calibration plots (Figs. 6B and C). The results were represented in Table 3. The calculated values of recovery of the spiked urine (FA: 99.4-101.1; AA: 99.6-102.5) and serum (FA: 98.1-101.6; AA: 99.2-101.9) samples and their relative standard deviations (RSD%) are acceptable. Therefore, POM-MgO NPs-MWCNT-CPE can be satisfactorily applied for quantification of FA in presence of AA in pharmaceutical samples and bio-fluids.

CONCLUSIONS

This work illustrates the fabrication of a POM-MgO NPs-MWCNT-CPE and its application for the determination of FA individually and in presence of AA. The results approved that FA electro-oxidation significantly improved at the POM-MgO NPs-MWCNT-CPE surface than other unmodified electrodes. The kinetic parameters

including the electron transfer coefficient, α, and the heterogeneous catalytic rate constant, $\acute{k}$, for electrocatalytic oxidation of FA at the surface of modified electrode were calculated as 0.34 and 2.9 ± 1.1 × 10^{-3} cm s^{-1}, respectively. The diffusion coefficient of FA was estimated as 9.2 × 10^{-6} cm^2 s^{-1} using chronoamperometric measurements. The DPV catalytic peak currents are linearly dependent over the range 0.08-650.0 μM on FA including two linear segments. The modified electrode effectively separated voltammetric peaks of FA and AA by ≈0.1 V, so that the introduced sensor can be used for the determination of FA and AA selectively. The usefulness of POM-MgO NPs-MWCNT-CPE was approved by using it in real samples with different matrices. The good recovery results approve its applicability to the proposed electrode.

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