Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(7): 99-109

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Copper Nanoparticles/Poly-Neutral Red Modified Pencil Graphite Electrode for Electroanalysis of Folic Acid

Saeid Soltani[1], Reza Davarnejad[2*], Faezeh Rahimi[1], Tayeb ab Matin[1], Elahe Ahmadi[1]

[1]Department of Chemistry, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran
[2]Department of Chemistry, Arak Branch University, Arak, Iran

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ABSTRACT

In this work, an electrochemical sensor was created based on pencil graphite electrode (PGE) modified by copper nanoparticles (CuNPs) and poly- neutral red (poly-NR) for folic acid (FA) analysis. The morphological properties of the structured sensor (CuNPs/poly-NR/PGE) were characterized by an electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDS). Subsequently, various parameters which affect the electrochemical performance of the modified electrode toward folic acid analysis such as the pH of buffer solution, type and concentration of buffer solution, and amount of modifiers on the electrode surface were discussed. The modified electrode showed excellent electrocatalytic ability toward FA oxidation with an irreversible behavior in pH 11 Britton-Rabinson (BR) buffer solution. Square wave voltammetry (SWV) was used toward FA determination. A calibration graph was plotted in optimized conditions, by the alteration of anodic peak currents as a function of FA concentration in the linear range of 1-1000 µM with a detection limit of 300 nM (S/N = 3). The CuNPs/poly-NR/PGE was applied for the determination of FA in blood serum sample as real sample and the good accuracy of the sensor that was corroborated by admissible betterment values.

Keywords: Copper nanoparticles; Graphite electrode; Electroanalysis; Folic acid

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INTRODUCTION

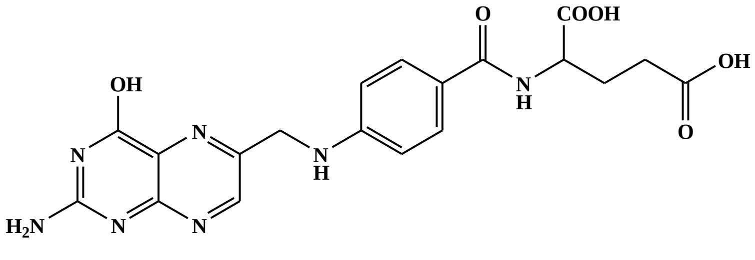
Folic acid (FA, Scheme 1) is one of the water-soluble vitamin B groups that is a tasteless and odorless yellowish orange crystal. Folate has been recognized as one of the most significant vitamin in human's body for normal functioning metabolism. The amount of folic acid perception is appropriate with neural tube defects (NTD) in infants, cardiovascular diseases, colon cancer and certain anemia [1, 2]. Also, folic acid is essential for cell growth, division and participates in lots of reactions in body and mainly in synthesis of nucleic acid and some important substances. It promotes the synthesis of protein from amino acid, in human body. Folate is not synthesized in humans, thus it should be obtained from dietary sources [1].

Different kinds of analytical methods such as, high-performance liquid chromatography (HPLC) [3, 4], HPLC–MS [5], colorimetry [6], spectrophotometry [7], chemiluminescence [8], flouorimetric [9] and capillary electrophoresis [10] have been reported for the determination of folic acid. However, these methods suffer from some limitations such as long chromatographic run times, low sensitivity and consumption large sample volumes; require chemical derivatization before determination and time-consuming sample pretreatment stages prior to test. Electrochemical sensors have all the merits and represent low cost alternatives. Their ease, great reproducibility, rapid response time, often associated low detection limits generated extensive interest. In order to overcome these problems, many efforts have been devoted into the development of novel electrode materials or FA sensing [11, 12]. There are several literature reports for FA sensing based on various electron mediators such Graphene/MWCNTs [13], Single-walled carbon nanotube-ionic liquid modified glassy carbon electrode (SWCNT-IL/GCE) [14], α-Polyoxometalate-polypyrrole-Au nanoparticle modified gold electrode (PPy-α-POM-AuNPs/Au) [15], Gold nanoparticle modified carbon paste electrode (AuNPs/CPE) [16], modified grapheme oxide-methylene blue modified glassy carbon electrode (GCE/rGO/methylene blue) [17], ZnO nanoparticle modified carbon ionic liquid paste electrode (ZnO/NP/CILPE) [18], Pt:Co/ionic liquid/carbon paste electrode (Pt:Co/IL/CPE) [19] and Carbon nanohorns supported in titanate nanotubes [20].

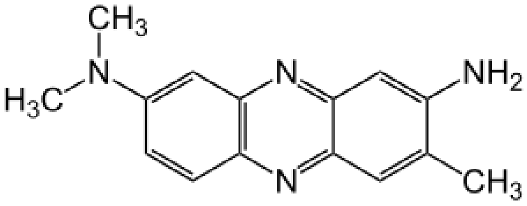
Nanostructures due to their extraordinary physicochemical properties have gained in the area of biosensing, recently. Among them, metallic nanoparticles have attracted considerable attention from scientific communities in recent years because of their fascinating physical and chemical properties distinct from the bulk materials [21-23]. The engrossing optoelectronic properties of coinage metals nanoparticles (Cu, Ag, Pt, Au NPs and so on) present attention of researchers for diverse applications in biosensing, biomedical devices, catalytic and printed electronics [24-26]. Since copper is less expensive nature than other metal nanoparticles and also concurrently has the similar properties of surface plasmon resonance (SPR), so CuNP can be a promising alternative than other metal nanoparticles for an outspread of low-cost nanotechnology-based therapeutics and diagnostics [27]. The use of copper nanoparticles to modification of electrodes including carbon electrodes makes it possible to construct sensors to determine of biomolecules at the respective level. The electrochemical and electrocatalytic activity of CuNPs and its composites has been used to the detection of important compounds in relevant biological and pharmaceutical systems [28-32]. Pencil graphite leads were used as working electrodes which currently known as pencil graphite electrodes (PGEs). PGEs are cheap, easy to use and more convenient with no time-consuming electrode surface cleaning/polishing step. Using of PGEs along with various types of voltammetric methods to measure different of analytes from a wide range of samples, can be obtained well-defined voltammetric peaks and reproducible signals. The use of PGEs has given stability, sensitivity, reproducibility, renewable and economical tool [33- 35].

Neutral red (NR) (scheme 2) is a phenazine dye that used for electrochemical investigations of the biological system [36]. The structure of neutral red is similar to other planar dyes such as acridine, thiazine and xanthenes. Neutral red can as a redox mediator exhibits outstanding electrocatalytic activities in enzyme biosensors. Neutral red can be made easily by electropolymerization on the surface of electrode [37-41]. In this work, neutral red was electropolymerized on the surface of PGE to obtain poly-NR modified PGE.

Herein, the CuNPs formed by electrochemical deposition method was employed for the electrochemical detection of FA. By the above regards, we investigate CuNPs/poly-Neutral red modified PGE for the sensitive and selective electrochemical detection of FA. The optimum conditions such as supporting electrolyte, NR concentration, cycle number, potential range for electropolymerization process were investigated. We will show that the better electrochemical performance of this electrode allowed for the direct electrochemical oxidation of FA at E = 0.3 V vs. Ag/AgCl with a detection limit of 10 nM. The prepared modified electrode was successfully applied for voltammetric determination of low level of FA in a blood serum sample.



Scheme 1: Molecular structure of folic acid



Scheme 2: Molecular structure of neutral red

METHODOLOGY

Materials

All the chemicals used were of analytical grade and were used as purchased from Merck (Darmstadt, Germany) without further purification. The pencil ‐ lead rods (0.5 mm in diameter) were purchased from local bookstore. In these experiments, Britton-Robinson (BR) buffer solution was prepared from 0.04 M of glacial acetic acid, phosphoric acid and boric acid. Double distilled water was used throughout the experiments. All experiments were accomplished at ambient temperature (25±1◦C).

Apparatus

A typical three-electrode system was utilized, with pencil graphite electrode (PGE, 0.5 mm diameter) or modified PGEs, a platinum wire, a silver/silver chloride (Ag/AgCl) in saturated potassium chloride were used as working, counter and reference electrodes, respectively. Square wave voltammetry (SWV) and cyclic voltammetry (CV) experiments were performed using an Autolab PGSTAT30 Potentiostat/Galvanostat (Eco Chemie, Utrecht, and the Netherlands) with NOVA 1.10 software. All potentials in this study referred to the reference electrode. A Metrohm 827 pH meter was used for pH measurements. The surface morphology of modified electrodes was characterized with a scanning electron microscope (SEM) and an X-ray energy dispersive analysis device (EDS analysis) (Bruket AXS). An accelerating voltage between 5 kV (nitrogen detection) and 15 kV was used. square wave voltammograms (SWV) were obtained by scanning the potential from -0.5 to 1.0 V with the following pulse amplitude; 30 mV/s, pulse width; 50 ms. All electrochemical experiments were carried out in 0.04 M BR (pH=11.0), unless otherwise specified.

Preparation and activation of PGE

The pencil lead's frame was rolled with Teflon tape, and an electrical connection was made through a copper wire at one end of it. Another end of cross section of pencil graphite that contacted with solution was polished on a weighing paper before each use to a smoothed finish. The PGE was placed vertically in the electrochemical cell. The oxidation signal of FA at untreated PGE does not be seen; this may due to the presence of existed impurities on the PGE surface. So the polished PGE was immersed vertically in 10 mL buffer solution (0.04 M Britton-Robinson) and a potential of 1.0 V was applied for 300 s without stirring the solution to activate PGE [42].

Preparation of Modified Electrodes

Prior to electrochemical measurements, the modified electrodes were prepared by recording successive cyclic voltammograms of Cu and NR on PGE. To prepare the Cu nanoparticles modified electrode by the electrochemical deposition method, the PGE was performed by the cyclic voltammetry in the potential range -0.8 to 0.3 V in 0.05 M H_2SO_4 and 10 μM $CuSO_4$ at a scan rate of 30 mVs^{-1} versus Ag/AgCl for 20 cycles [43].

After the CuNPs film was formed on PGE, the neutral red were electrochemically deposited on the CuNPs/PGE by cyclic voltammetry. The poly-NR modified PGE (PNR/CuNPs/PGE) was prepared by cycling CuNPs/PGE for several cycles in 0.1 M NaOH aqueous solution containing 0.01 mM NR. Electropolymerization was achieved by cyclic sweeping between -1.2 to 1.2 V with the scan rate of 30 mVs-1 for 18 cycles [44].

After the polymerization process, the electrode was washed with BR solution and was stored at ambient temperature until use. In order to obtain the best optimized response of modified electrodes towards the FA oxidation, the effect of the supporting electrolyte, NR concentration, cycle number and anodic potential limit during the electropolymerization of Cu and NR on the oxidation of FA was investigated. For this purpose PGE surface was polished on a weighing paper to a smooth finish before each use and then cleaned via sonicating in ethanol and double distilled water. Then, the cyclic voltammetric procedure in optimum conditions (supporting electrolyte: 0.05 M $H2SO4$ and 0.1 M NaOH for electropolymerization of CuNPs and poly-NR, respectively; concentration: 10 µM $CuSO4$ and 0.01 mM NR; cycles number: 20 and 18 for electropolymerization of CuNPs and poly-NR, respectively; potential range: -0.8 to 0.3 V and -1.2 to 1.2 V vs. Ag/AgCl for electropolymerization of CuNPs and poly-NR, respectively) was used for the electrode modification. These modified electrode were used for the investigation of electrooxidation of FA after rinsing with BR buffer solution (pH =11).

Electrochemical Measurements

The electrochemical measurement was carried out using square wave (SW) and cyclic voltammetry (CV). SWV was performed in solution containing 0.04 Britton-Robinson as the buffer solution (pH =11) and sweeping the electrode potential between -0.5 V and 1.0 V using a pulse amplitude of 30 mV. After each run, the CuNPs/poly-NR/PGE was refreshed by successive cyclic voltammetric sweeps in BR buffer solution (between -1.0 and 1.0 V at 50 mV s-1) to get a reproducible electrode surface. All potentials reported in this paper are referenced to an Ag/AgCl.

Preparation of Real Sample

In order to measurement of FA in blood serum sample, this sample was obtained from healthy volunteer. 0.5 mL of methanol was added to a 1.0 mL serum sample to remove serum protein. After vortexing for 1 min of the serum sample, the precipitated proteins were pulled apart by centrifugation for 3 min at 10000 rpm. Finally, the transparent liquid lying above a solid residue was filtered and diluted to a definite volume.

RESULTS AND DISCUSSIONS

Surface Morphology Study

For investigation of the surface structure and morphology of the structured sensor, SEM images of bare and modified electrodes were distinguished. Fig. 1 represents the SEM images of a) bare PGE, b) PGE coated with copper nanoparticles and c) poly-NR/CuNPs/PGE. As can be seen that the copper nanoparticles (b) have a spherical shape with a diameter ranged from 25 to 50 nm and its morphology is different from that observed for bare PG electrode (a). Different morphology that was observed for poly-NR/CuNPs/PGE in Fig. 1C indicates the presence of the poly-NR at nanosize and its immobilization on the surface of the CuNPs/PG electrode. The presence of poly-NR leads to an increase in the surface area for more FA adsorption and analysis. Fig. 2 corresponds to the EDS (Energy-dispersive X-ray spectroscopy) analysis of the surface of electrodes in each step. It becomes clear that CuNPs and poly-NR films are strongly attached on the surface of electrode. The overlapping of both nanostructures is important as it will assure a good connection and conducting network, thus facilitating electron transfer. Also, the EDS spectra image data in Fig. 3 (a), (b) and (c) were shown for three step of modification. EDS makes use of the X-ray spectrum emitted by a solid sample bombarded with a focused beam of electrons to obtain a localized chemical analysis. Qualitative analysis involves the identification of the lines in the spectrum and is fairly straightforward owing to the simplicity of X-ray spectra. The pencil graphites contain of graphite and kaolin. Fig.3a showed the presence of carbon with significant fraction at low degree of oxygenation as well as of silicon. Also find small amounts of other elements such as potassium, calcium, titanium and iron. Therefore, these data revealed that PGE contained mainly elemental carbon as major composition with oxygen indicating pure form of graphite. Fig. 3b is clearly visible the presence of Cu in the film composite on the surface of electrode. No other metallic impurities other than copper were observed, which confirmed the presence of the Cu nanoparticles in the film composite. As shown in Fig.3c, the fraction of carbon and oxygen with polymerization of electrode with NR decreased and plainly indicates the presence of Cu.

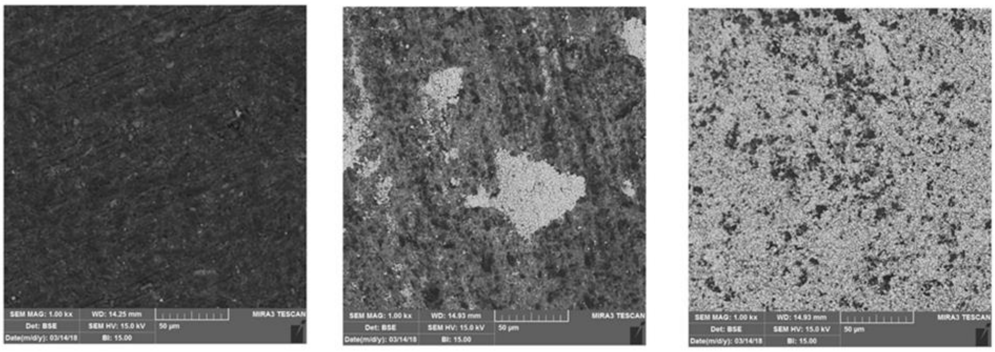


Figure 1: SEM images of a) bare PGE, b) PGE coated with copper nanoparticles and c) poly-NR/CuNPs/PGE

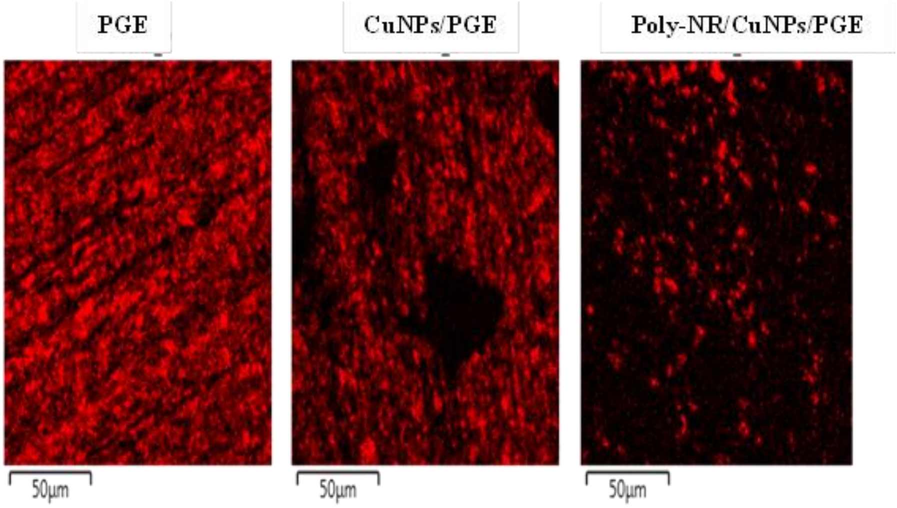


Figure 2: Energy-dispersive X-ray spectroscopy analyses of bare PGE, CuNPs/PGE and poly-NR/CuNPs/PGE

Electrochemical Characterization of the Electrodes

The poly-NR/CuNPs/PGE was also characterized by electrochemical impedance spectroscopy. In this method was used [Fe(CN)6]3-/4- as an electrochemical redox indicator. In EIS measurements, the semicircle diameter of impedance equals the electron transfer resistance (Rct), which actually represents the control of the electron transfer kinetic of the redox indicator on the electrode surface. Fig. 4 exgibits the Nyquist plots of the bare PG (curve a), CuNPs/PG (curve b) and poly-NR/CuNPs/PG electrodes (curve c) in a solution containing 3 mM [Fe (CN) 6]3-/4- and 1 M KCl. For the PGE, its Nyquist plot (curve a) is a semicircle domain with Rct is about 790 Ω and an almost straight line which is a characteristic of diffusion limited step of the electrochemical process. About the CuNPs/PGE, the hape of the Nyquist plot (curve b) is the same as that of the PGE, but with a smaller semicircle diameter, and the value of Rct is about 291 Ω. The lower Rct at the CuNPs/PGE surface depicts that CuNPs can promote electron transfer, and thus accelerates the diffusion of ferricyanide to the surface of electrode. Due to the assembling of poly-NR the resistance of electrode interface was increased (560Ω). Thus, an increase of interfacial resistance proved that the formation of poly-NR on the PGE surface.

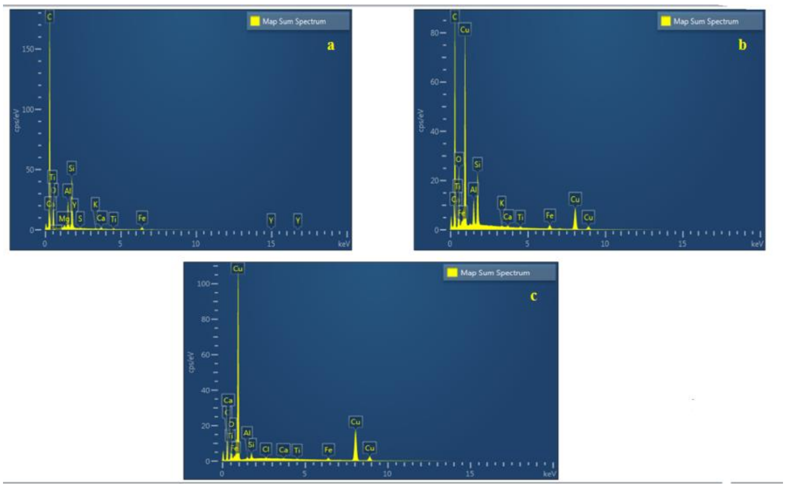


Figure 3: EDS spectra image data of a) bare PGE, b) CuNPs/PGE and c) poly-NR/CuNPs/PGE

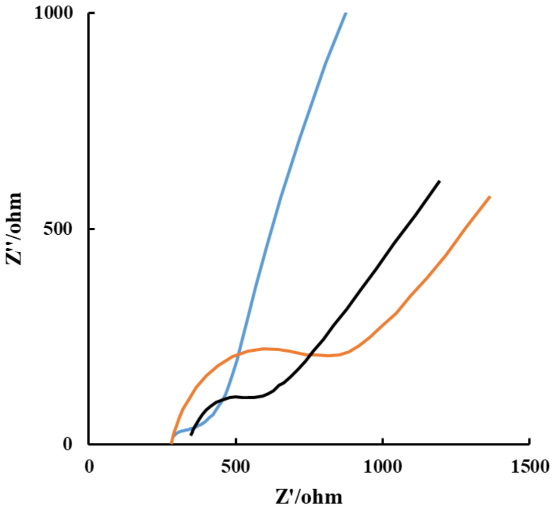


Figure 4: Nyquist plots of 0.1 M KCl containing 1 mM K3[Fe(CN)6] at: a) bare PGE, b) CuNPs/PGE and c) poly-NR/CuNPs/PGE. EIS conditions: initial potential, 0.2 V; amplitude voltage, 10 mV; frequency range, 100 kHz to 0.1 Hz

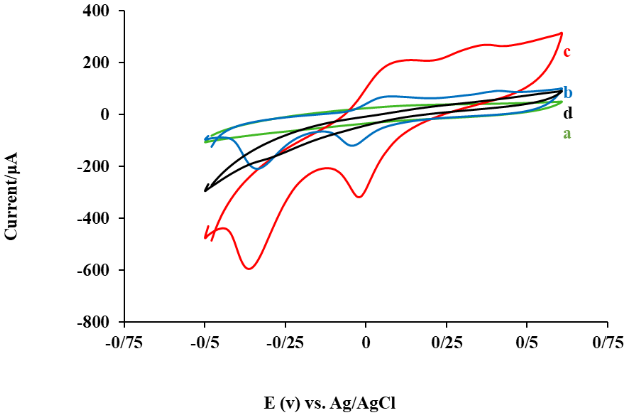


Figure 5 The CVs of 0.04 M B-R buffer solution (pH 11) containing 15 µM FA at bare PGE (curve a), CuNPs/PGE (curve b) and poly-NR/CuNPs/PGE (curve c); scan rate 100 mV s-1

Electrochemical Behavior of FA at Poly-NR/CuNPs/PGE

Fig. 5 shows the cyclic voltammograms (CVs) of 15 µM FA in BR buffer solution (0.04 M, pH = 11) at the poly-NR/CuNPs/PG (curve a), CuNPs/PG (curve b), and PG (curve c) electrodes. As can be seen, FA's response at the surface of bare PGE is rather poor and only a weak oxidation peak is observed at 0.38 V (curve c). On the other hand, using CuNPs/PGE (curve b) a well-defined anodic peak with a considerable enhancement in the peak current is obtained. However, the current obtained at the poly-NR/CuNPs/PGE AT 0.34 v (curve a) is significantly larger than that obtained at the bare PGE, which can be related to the increased active surface area of the modified electrode and accumulation of more amounts of FA on the surface of the modified electrode. Such a large enhancement of anodic current revealed that by lowering the anodic overpotential of the electrode process, the kinetics of electron transfer for FA improves remarkably at the modified electrode. During the cyclic voltammetric studies, no cathodic peak is resulted for FA during the reverse scan, which suggests a totally irreversible behavior for the electrode process.

Fig. 6A shows the cyclic voltammograms of FA at the poly-NR/CuNPs/PGE in BR buffer (pH = 11) at different potential sweep rates. It can be noted from this Fig. that the peak potential for the oxidation of FA shifts to more positive potentials as the sweep rate is increased suggesting the involvement of a kinetic limitation in the reaction between the poly-NR/CuNPs/PGE and FA. Also was shown in Fig. 6B the anodic peak currents varied linearly with the square root of the scan rate in the range of 10–900 mV s-1, suggesting that the FA oxidation follows a diffusion controlled process which is the reflection of the relatively slow diffusion of counter ions into the electrode surfaces. The linear variation of FA peak potentials, Ep, as a function of log v suggests that the electrode process can be regarded as a totally irreversible reaction (Fig. 7C). For an irreversible electrode process, according to Laviron theory [45] the electron transfer rate constant (ks) and transfer coefficient (α) can be determined by the variation of the peak potential with scan rate according to the following equation:

$Ep = E0 + (2.303RT/\alpha nF) \log (RTks) + (2.303RT/\alpha nF) \log \upsilon$ (1)

The value of αn can be easily calculated from the slope of peak potentials versus the logarithm of the scan rate. The slope of Ep versus log υ plot is 0.12. Using the Eq. (1) and the one electron transferred for FA, the transfer coefficient (α) 0.49 was obtained. The value of ks can be determined from the intercept of peak potentials versus the logarithm of the scan rate. The value of E0 in Eq. (1) can be obtained from the intercept of the E versus υ curve by extrapolating to the vertical axis at υ = 0 [46]. In this system the intercept for Ep versus log υ plot was 0.9379 and E0 was obtained to be 0.779 V. By introducing these parameter values in the Eq. (1), the electron transfer rate constant of 36.8 s-1 was obtained.

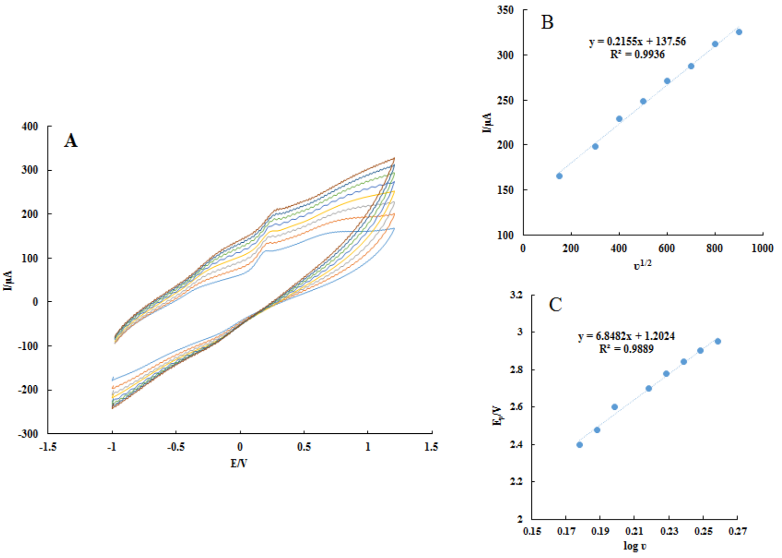


Figure 6: The CVs of 15 µM FA in B-R buffer solution (pH 11) at the surface of poly-NR/CuNPs/PGE at various scan rates (10-900 mV s-1). Insets: B) Plot of peak current versus square of scan rate and C) Plot of peak potential versus logarithm of scan rate

Influence of Type of Salts

The effect of two types of the salts as supporting electrolyte including KCl and KNO3 on the voltammograms peak current of FA was investigated by the experimental solution. To monitor the concentration of the each supporting electrolytes used, the concentration of salts was changed from 0.0

to 1.0 M in the experimental solution. These salts unfolded that the peak current and also sensitivity of this method were increased by increasing salt concentration the KCl upto 0.1 M. Accordingly, subsequent experiments were done by addition of 1ml of 0.1 M of KCl.

Effect of pH

The effect of solution pH on the electrochemical behavior of FA on the poly-NR/CuNPs/PGE was investigated using 0.04 M Britton–Robinson buffer solution in the pH range of 3–11 by the CV technique (Fig. 7). The pH of the solutions was adjusted with addition of sufficient amounts of HCl or NaOH. The effect of pH on the peak current of 150 µM FA in 0.04 M Britton–Robinson buffer solution shows the maximum FA signal was obtained in pH=11. Therefore, the Britton–Robinson buffer solution of pH=11 was selected as the optimum pH in subsequent studies. In the pH range of 3–11, there is a linear decrease of Ep with pH, being approximated by the expressions

Ep = -0.01pH + 0.29 (r = 0.989). This behavior suggests that the redox system obeys the involvement of the proton transfer mechanism in the overall electrode reaction. This slope is in agreement with the theoretical Nernstian slope of −0.0296 V pH–1 for redox reactions involving a ratio between the number of electrons and protons equal to 2:1. Thus, the electrooxidation reaction for folic acid involved twice as many electrons as protons. This information is completely in accordance with the electrooxidation mechanism previously reported for folic acid [14, 47].

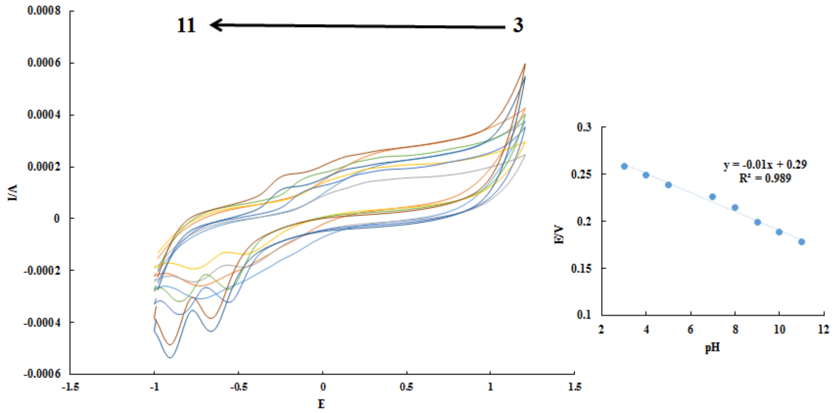


Figure 7: The effect of pH on the peak current of 15 µM FA at the poly-NR/CuNPs/PGE; scan rate 100 mV s-1. Inset: dependence of the peak potentials on pH solution

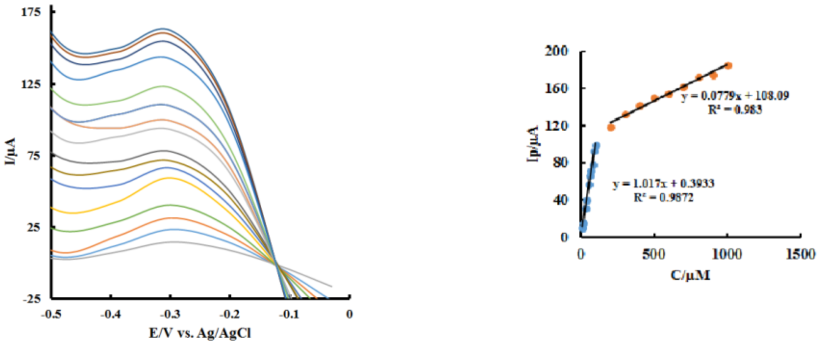


Figure 8: Effects of various concentrations of FA (from bottom to top, 10-1000 µM) on the response of the fabricated sensor and its calibration curves in B-R buffer solution (pH 11) under optimal conditions: accumulation potential 100 mV;accumulation time 300 s; scan rate 60 mV s-1

Calculation of the Effective Surface Area of the Poly-NR/CuNPs/PGE

To obtain the effective surface area of the prepared sensor, cyclic voltammetry and the Randles-Sevcik equation were used [48]. The CV experiment was done using a poly-NR/CuNPs/PGE in 1 mM Fe(CN)6 3-/4- containing 1 M KCl solution. By using cyclic voltammetry and gaining Ip, the effective surface area can be calculated according to the Randles–Sevcik equation:

$Ip= 2.65\times10^{5}n^{3/2}ACD^{1/2}v^{1/2}$ (2)

Where, n is the number of transferred electrons of potassium hexacyanoferrate and equals to one. D is the diffusion coefficient of $Fe(CN)_6{}^{3-/4-}$ and equals to 0.76×10^{-5} cm^2 s-1 [49] and C is the concentration of $Fe(CN)_6{}^{3-/4-}$ that is 1 mM. By applying eq. (2), the effective surface area of the poly-NR/CuNPs/PGE was calculated to be 0.0057 cm2, which was approximately five times higher than the geometric area of the PGE (0.0012 cm^2).

Diagnostic Performances

The analytical performance of the poly-NR/CuNPs/PGE was investigated toward the determination of FA by square wave voltammetry. The peak current dependence of the FA concentration is shown in Fig. 8. Well defined peak was obtained over a flat background. The calibration graph is depicted in the inset of Fig. 8. The linear dependence among the SWV signal and FA concentration over the range of 10-1000 µM was apperceived. Two linear segments between the SWV signal and FA concentration over the range of 10-100 and 200-1000 µM was observed. The detection limit was acquired as three times the ratio between the blank signal and the sensitivity, which was 300 nM. This sensor provides a comparable and even better detection limit with good linearity range for FA determination, compared with those obtained using previous modified electrodes (Table 1). Because of extremely easy, quick and low cost preparation of this modified electrode, it seems that this electrode can be a great utility for making a voltammetric sensor toward FA detection in real samples.

Table 1: Comparison of different modified electrodes for the FA detection

Methods	Linear range (µM)	LOD (nM)	Conditions	Real sample	References
Gr/MWCNTs[a]	15-175	92	PBS (pH=7.0)	Urine	[13]
SWNT-GCE [b]	0.008-9	3	PBS (pH=5.5)	Wheat flour, Fruit juice, Milk	[14]
PPy–α-POM–AuNPs-AuE[c]	0.02-0.49	0.15	BRS (pH=6.0)	Humanserum	[15]
GNPs/CPE [d]	5.9-28	38	PBS (pH=6.0)	Urine	[16]
MB/ERGO/ GCE[e]	5-176	0.8	PBS(pH=7.4)	Tablet	[17]
ZnO/NP/CILPE[f]	0.09-580	20	PBS(pH=9.0)	Urine, Tablet, Apple Juice	[18]
Pt:Co/IL/CPE[g]	0.5-505	48	PBS(pH=9.0)	Food, Tablet, Mint	[19]
TNTs/CNHs/GCE[h]	0.00002-59	0.0028	PBS(pH=7.0)	Tablet	[20]
poly-NR/CuNPs/ PGE[i]	10-1000	320	BRS(pH=11.0)	Human serum	This work

[a]Graphene/ multi-walled carbon nanotubes; [b]A single-walled carbon nanotubes/glassy carbon electrode;

[c]α-polyoxometalate–polypyrrole/Aunanoparticles/Au electrode; [d]gold nanoparticles-modified carbon paste electrode; [e]methylene blue onto electrodeposited/reduced graphene oxide/glassy carbon electrode; [f]ZnO/nanoparticles (NPs) modified carbon ionic liquid paste electrode; [g]ionic liquid (n-hexyl-3-methylimidazolium hexafluoro phosphate) Pt:Co carbon paste electrode; [h]Titanate nanotubes/carbon nanohorns/glassy carbon electrode; [i]Copper nanoparticles/poly-neutral red modified pencil graphite electrode

Table 2: Determination of FA in real sample (n = 5)

Sample	Added (nM)	Found (nM)	Recovery (%)	R.S.D. (%)
Serum	-	285	-	3.13
	580	785	99.7	2.99
	1000	1895	108.0	3.21

Analytical Application

In order to evaluate the application of the proposed method for voltammetric determination of FA in the real sample, FA was measured in the serum sample. After sample preparation as described in Section 2.6, the SWV method was applied to determination of the FA by the standard addition method and these results was summarized in Table 2. The obtained results in Table 2 show that the recoveries of the spiked samples are acceptable. Therefore, the manufactured sensor can be efficiently used to determination of FA in real samples with various matrices.

Interference Study

To use the possibility of analytical application of the method, the effects of some common interference on the determination of FA in optimum conditions were tested. Some pharmaceutical and/or in biological common substances were examined on the determination of the 15 µM FA. The tolerance limit was defined as the maximum concentration of the materials which reduced an error of less than 5% for measurement of FA. The results showed that when a 500-fold Na^+, K^+, Mg^{2+}, Ca^{2+}, Cl^-, Li^+, Al_3^+, NH_4^+, dopamine, ascorbic acid and uric acid were present in the synthetic mixture, the average recovery in the determination of FA was 100.4%. These results illustrated that the selectivity of the method is admissible and so, this procedure appropriate for an analysis of FA in real samples with different matrices. The possibility to analytically quantify the presence of additional compounds is high.

CONCLUSIONS

A pencil graphite electrode modified by poly-NR/CuNPs was applied to voltammetric determination of FA. The limit of detection and linear range of the proposed method were compared with the works for the analysis of FA previously reported, and the results are summarized in Table 1. FA was determined by the standard addition method in blood serum sample. As can be seen in the Table 1, the analytical parameters are comparable to the results presented for FA determination. The reliability and applicability of the poly-NR/CuNPs/PGE were examined by spiking FA into real samples and good recovery values demonstrated this study.

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