

Advanced Nanostructure Amplified Strategy for Voltammetric Determination of Folic Acid

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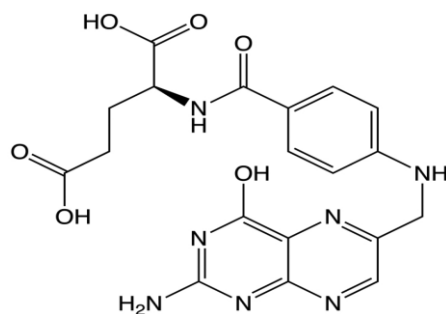
Received: 13 August 2019 / Accepted: 1 October 2019 / Published: 29 October 2019

In this paper for determination of folic acid in food and tablet samples, some highly conductive MgO nanoparticles (MgO NPs) are used as amplifier for modification of the carbon paste electrode (CPE) as a sensitive tool. The MgO NPs are synthesized by a simple chemical precipitation method and are characterized with the XRD method. The oxidation of folic acid appeared at potential ~782 mV with current ~78.8 μA at surface of the CPE/MgO-NPs to better show the experimental results compare to the CPE with potential ~810 mV and current ~42.2 μA . At pH=9.0, the oxidation of folic acid is detected at dynamic range 0.09–1000 μM with a detection limit of 0.04 μM (3δ). The CPE/MgO-NPs showed high performance capability for analysis of folic acid in food and tablet samples.

Keywords: Amplified Sensor, Folic Acid Analysis, MgO Nanoparticles, Voltammetry

1. INTRODUCTION

Folic acid (scheme1) is one of the important and necessary forms of a water-soluble B vitamin for the human body. Folic acid is introduced as an important biological compound for human health. Several roles have been recognized for folic acid, such as the production of DNA and RNA, growing of cells and helping to pregnant women. Coupling this vitamin with vitamin B₁₂ is necessary for making and controlling the red blood cells [1]. With regard to the importance of folic acid, it is suggested as a food additive for improving food quality and must be present in the dietary supplement. Due to the cheating of some food and pharmaceutical illegal manufacturers, determination of folic acid is an important topic in these industries. For determination of folic acid in food additives and pharmaceutical samples, Thermogravimetry [2, 3], Spectrophotometry [4-6], Capillary Electrophoresis [7], High Performance Liquid Chromatography (HPLC) [8], Chemiluminescence [9, 10], Enzyme-Linked Ligand Sorbent Assay [11, 12] and Electrochemical methods [13-19] were suggested.



Scheme 1. Molecular structure of folic acid

Because of simplicity, low toxicity and low detection limit compared to the other analytical techniques, the electrochemical-based methods have drawn more attentions for food and drug analysis [20-26]. The amplified electrodes used with conductive materials have created a new approach for fabrication of electrochemical sensors and biosensors with excellent selectivity [27-35]. Nanomaterials were suggested for amplification of electrodes because of superior characteristics such as high surface area, unique optical properties and good electrical conductivity [36-44]. According to reported papers and scientific investigations [45-49], the nanomaterials can reduce the charge transfer resistance of electrodes and increase sensitivity of modified sensors. In these papers, because of their high electrical conductivity the Magnesium oxide nanoparticle were suggested as amplifiers for modification of electrode surface. Lu et al. [50] reported the hydrogen peroxide biosensor based on the MgO nanoparticles (MgO NPs) - chitosan composite matrix. Karimi-Maleh et al. [51] utilized an ionic liquid–MgO NPs modified carbon paste electrode for determination of methyldopa in pharmaceutical and patient human urine samples.

In this paper, the CPE/MgO-NPs is suggested as a renewable and sensitive voltammetric sensor for determination of folic acid in pharmaceutical and food samples. The MgO nanoparticle amplified the electrical conductivity of carbon paste sensor and exhibits improved characteristics for trace level analysis of folic acid in real samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

An electrochemical analyzer (Autolab PGSTAT 302 N; Ag|AgCl|KCl (3 M) as reference electrode) was used for recording the electrochemical signals by using GPES software. Folic acid and magnesium nitrate hexahydrate were prepared of Sigma-Aldrich (USA) origin and used without further purification. Graphite powder (particle diameter = 0.10 mm) from Merck (New Jersey, US) was used as the working electrode substrate. All other reagents were of analytical grade. The MgO nanoparticle was synthesized according to Karimi-Maleh et al. recommended procedure [51].

2.2. Preparation of sensor

MgO NPs/MCPE was prepared by hand mixing of 0.9 g of graphite powder, 0.1 g MgO NPs and suitable amount of paraffin and mixed well for 50 min until a uniformly wetted paste was obtained. A portion of prepared paste was then packed into a glass tube with a copper wire as electrical contact.

2.3. Preparation of real samples

The 10 tablet of folic acid were purchased from local pharmacy and become powder inside the mortar. Then suitable amount of tablet powder dissolved in buffer solution pH=9.0. The juice samples purchased from local market, centrifuged at 2000 rpm, and filtered. The obtained juice was used directly for real sample analysis.

3. RESULTS AND DISCUSSION

3.1. MgO NPs characterization

MgO NPs powders were analyzed by XRD analysis. Fig. 1 depicts the XRD patterns of MgO NPs. The peaks are found in the lattice planes (hkl) of (111), (200), (220), (311) and (222) at the 2θ values of 36.1, 43.2, 62.5, 74.5, and 78.5° respectively. The grain size of the MgO NPs was calculated 44.0 nm using the Scherrer's equation [52]. The XRD pattern of MgO NPs powders is similar for MgO NP with JCPDS Card no. 89-7746 and previous publish papers [53, 54].

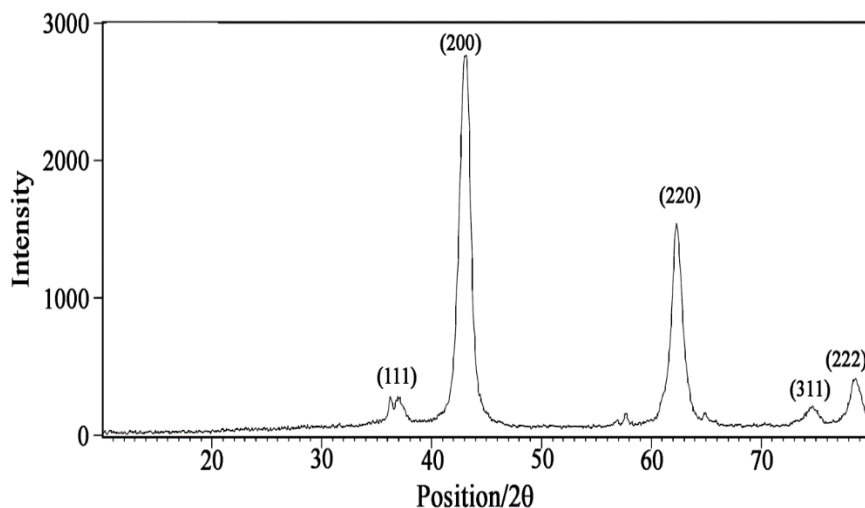


Figure 1. XRD patterns of as-synthesized MgO NPs

3.2. Electrochemical studies

The values of active surface areas for CPE and CPE/MgO-NPs were estimated ~ 0.284 and 0.30 cm² using Randles-Sevcik equation in the presence standard solution of 1 mM Fe(CN)₆^{3-/4-} [55]. The

presence of MgO/NPs with large surface area at carbon paste matrix causes the increment of active surface area. This point showed the high performance ability of MgO NPs for fabrication of electrochemical sensors [51].

Current density plots for CPE and CPE/MgO-NPs are presented in Figure 3. As pictured, the MgO/NPs can increase electrode conductivity for fabricated electrode if mixed by 150 μM folic acid. In Figure 2, curves (a) and (b) show the electro-oxidation signals for CPE/MgO-NPs and CPE when mixed by 150 μM folic acid, respectively.

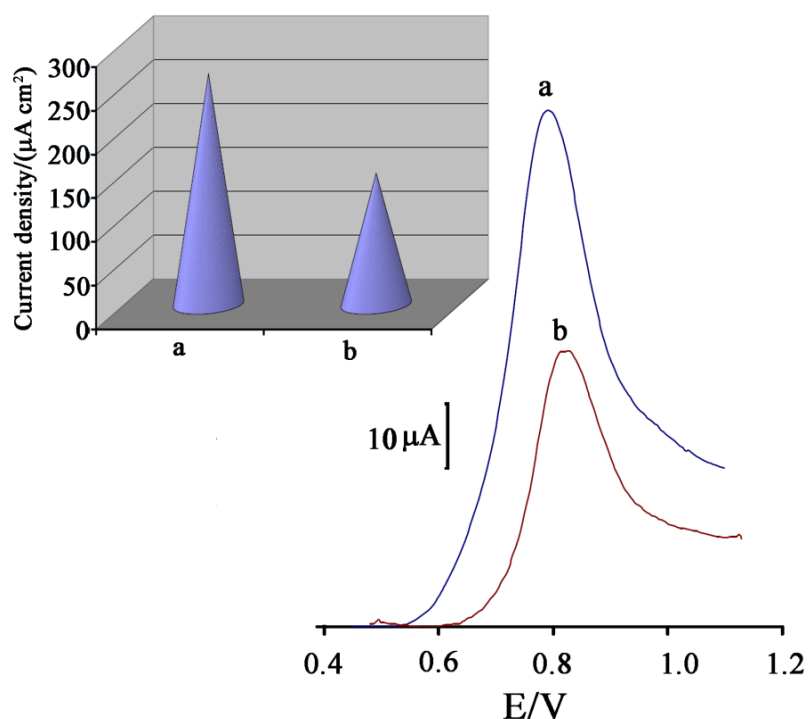


Figure 2. SWV of (a) CPE/MgO-NPs and (b) CPE mixed by 150 μM folic acid at pH 9.0. Insert; peak current density data for CPE/MgO-NPs and (b) CPE mixed by 150 μM folic acid

As pictured in this figure, adding the MgO/NPs to the carbon paste matrix leads to decrement in overvoltage for folic acid and meanwhile increment in oxidation signal. This phenomenon confirms the ability of MgO nanoparticle for amplification of electrode surface for trace level analysis [51]. The negative shift in oxidation potential and increasing in redox current of electroactive analytes after modification of electrode with nanomaterials is relative to high surface area and good electrical conductivity of nanostructure materials. Due to this properties, the nanostructure compounds are useful for many applications such as electrochemical sensors and etc. [56-67].

Due to the presence of amine group in folic acid structure and according to previous published report [51], the oxidation reaction of folic acid is relative to value of pH solution. The relation between oxidation current of 150 μM folic acid and changing of pH are given in Fig. 3. According to this figure, by changing the pH value in the surface of CPE/MgO-NPs, the current increases up to 78 μA (around pH=9.0) and later decreases after this point. Hereinafter, the pH=9.0 is chosen as the optimum pH for the experiments.

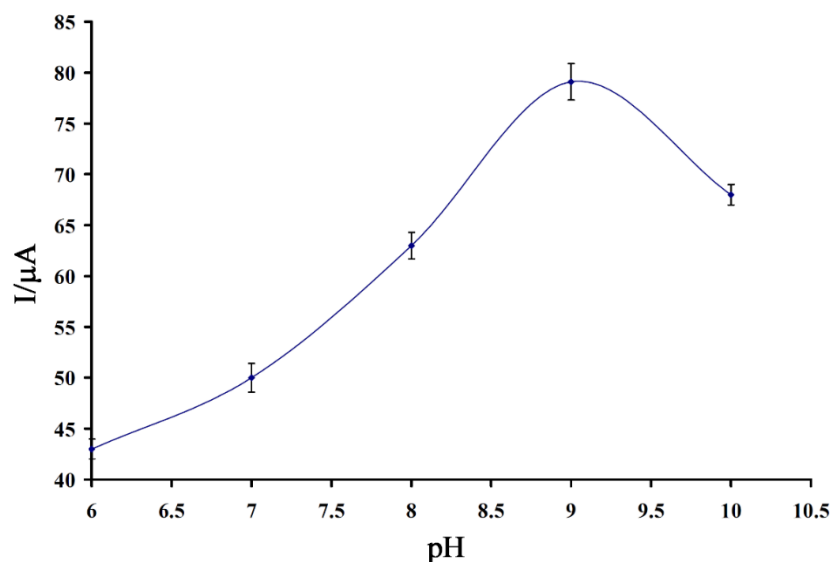


Figure 3. Current–pH curve for electrooxidation of 150 μM folic acid at MgO NPs/MCPE

For determination of electron transfer coefficient as a kinetic parameter, the plot of $\log I$ vs. E (Tafel plot) for the folic acid was recorded at a surface of CPE/MgO-NPs (Fig. 4). Using the Tafel slope and Tafel equation ($n(1-\alpha)F/2.3RT$), we calculated $\alpha \sim 0.78$. This value of electron transfer coefficient confirm an irreversible behavior for electro-oxidation of folic acid at surface of CPE/MgO-NPs.

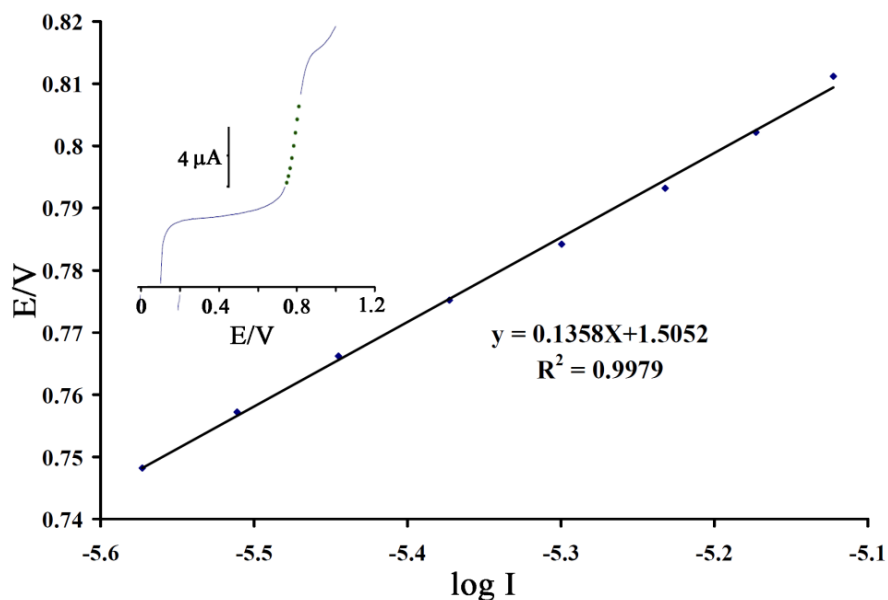


Figure 4. Tafel plot for MgO/MCPE in 0.1 M PBS (pH 9.0) with a scan rate of 10 mVs^{-1} in the presence of 200 μM folic acid.

Chronoamperometric method was used to determine the diffusion coefficient of folic acid at a surface of CPE/MgO-NPs by applied potential 900 mV (Fig. 5). The diffusion coefficient of folic acid, D , was determined by using the cottrell equation:

$$I = n F A D^{1/2} C \pi^{-1/2} t^{-1/2} \quad (1)$$

According to equation (1), the diffusion coefficient was calculated $1.93 \times 10^{-5} \text{ cm}^2/\text{s}$ for folic acid.

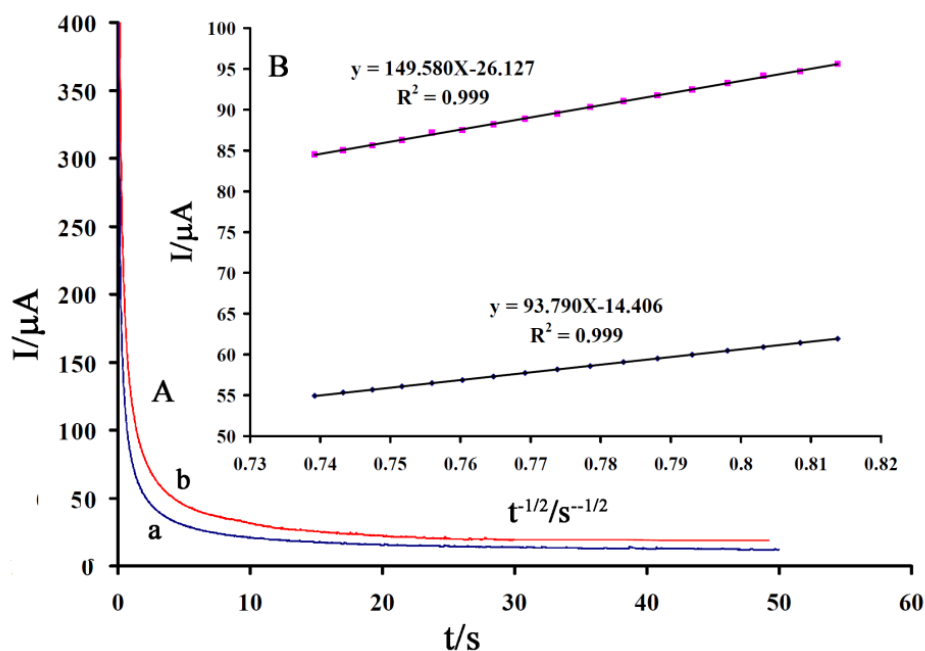


Figure 5. (A) Chronoamperograms obtained at MgO NPs/MCPE when mixed by (a) 800 and (b) 900 μM folic acid in the buffer solution (pH 9.0). (B) Cottrell's plot for the data from the chronoamperograms.

Figure 6 shows the effect of folic acid concentration on SWVs of the CPE/MgO-NPs. As shown in this figure, the height of the anodic peak increases with increasing folic acid concentration. It clearly shows the linear relation between the peak current and folic acid concentration from 0.09 to $1.0 \times 10^3 \mu\text{M}$. The linear regression equation is $I (\mu\text{A}) = 0.526 C_{\text{folic acid}} (\mu\text{M}) - 2.2299$ ($R^2 = 0.9925$). The limit of detection (LOD) of folic acid was determined $0.04 \mu\text{M}$ when the signal to noise ratio was 3. The linear dynamic range and limits of detection in the proposed sensor is comparable with another published electrochemical-reported sensor (table 1). As can be seen, the limit of detection or linear dynamic range for CPE/MgO-NPs is better compare to previous electrochemical suggested sensors that confirm good modification process for suggested sensor. The CPE/MgO-NPs showed better dynamic range compare to previous electrochemical sensor and in some cases the better limit of detection for determination of folic acid.

3.3. The interference studies

The effects of some compounds in the pharmaceutical or food can be easily found in oxidation signal of $10.0 \mu\text{M}$ folic acid when investigated at a surface of MgO NPs/MCPE. The results are shown in table 2, which shows good selectivity of MgO NPs/MCPE for analysis of folic acid without any important interference.

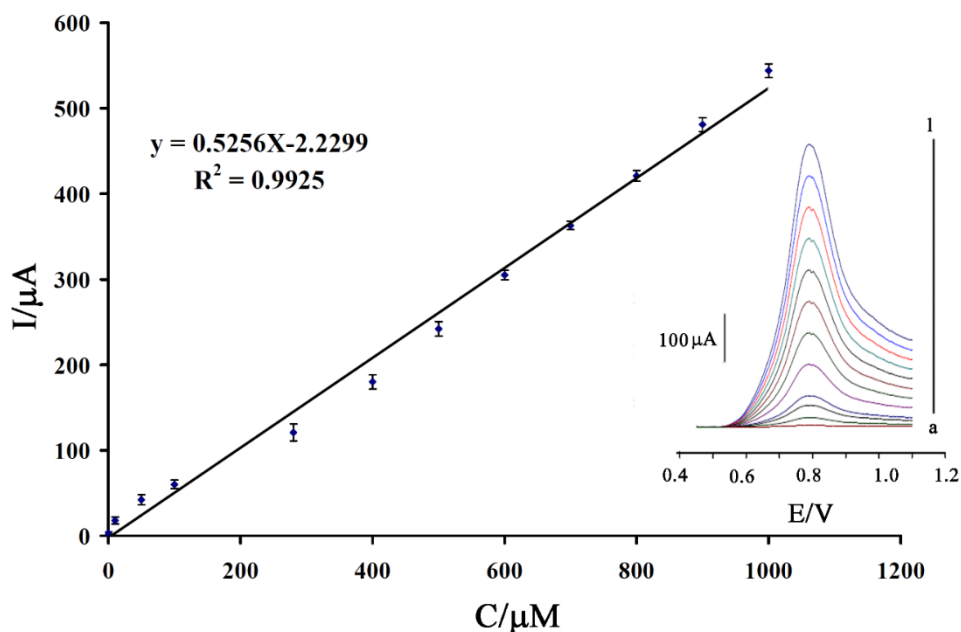


Figure 6. The plots of the electrooxidation peak current as a function of folic acid concentration. Inset; SWVs of MgO NPs/MCPE in 0.1 M PBS (pH 9.0) containing different concentrations of folic acid in μM . (from a-i): 0.09; 10.0; 50.0; 100.0; 280.0; 500.0; 700.0; 900.0 and 1000.0, respectively.

Table 1. The analytical data obtained by some published electrochemical sensors for folic acid determination.

Electrode	Mediator	Linear dynamic range (μM)	Limit of detection (μM)	Ref.
Carbon paste	ZnO/nanoparticles+1-butyl-3-methylimidazolium hexafluorophosphate	0.05-550	0.01	[68]
Carbonaceous electrode	-----	0.25-40	0.011	[69]
Pencil graphite	ds-DNA	0.1-10.0	0.01	[70]
Gold	Gold	0.01-1.0	0.079	[71]
Glassy Carbon	Nanoparticles Single wall carbon nanotubes	0.02-4.0	0.01	[72]
Gold	multi-walled carbon nanotube	0.02-1.0	0.01	[73]
Glassy Carbon	Single wall carbon nanotubes	0.01-100	0.001	[74]
carbon fibre microelectrode	---	0.02-1.0	0.01	[75]
Carbon paste	MgO NPs	0.09-1000	0.04	This work

3.4. Real sample analysis

In this step, the ability of MgO NPs/MCPE for determination of folic acid in pharmaceutical and food samples has been investigated. The obtained data for proposed sensor was also compared with a published method [54] with real sample analysis in Table 3. The results indicate that the determination of folic acid using MgO NPs/MCPE is effective and can be applied for their detection of folic acid with real samples. The statistical tests confirm the powerful ability of suggested sensor in real samples analysis.

Table 2. Interference study for the determination of 10.0 μM folic acid.

Species	Tolerant limits ($W_{\text{Substance}}/W_{\text{folic acid}}$)
Glucose,	1000
Glycine, Methionine, Alanine, Valine, Histidine, ascorbic acid	600
Uric acid, Vitamin B ₂ , Vitamin B ₆	400
Starch	Saturation

Table 3. Determination of folic acid in real samples (n=3).

Sample	Found (folic acid) Proposed method (μM)	Found (folic acid) Other method (μM)	F_{ex}	F_{tab}	t_{ex}	$t_{\text{tab}}(95\%)$
Tablet	20.35 \pm 0.65	20.76 \pm 0.95	8.9	19.0	2.5	3.8
Orange juice	11.73 \pm 0.55	12.01 \pm 0.75	7.9	19.0	2.1	3.8
Apple juice	9.52 \pm 0.43	9.78 \pm 0.51	5.3	19.0	1.6	3.8

4. CONCLUSION

In this work the MgO NPs/MCPE was fabricated as a novel sensor for folic acid analysis. MgO NPs were synthesized by using direct chemical precipitation method and characterized by XRD method. The electro-oxidation behaviors of folic acid on the MgO NPs/MCPE were further studied with electrochemical method. The MgO NPs/MCPE was successfully applied to the folic acid detection in real samples.

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