



Original scientific paper

## Voltammetric folic acid sensor based on nickel ferrite nanoparticles modified-screen printed graphite electrode

Peyman Mohammadzadeh Jahani<sup>1</sup> and Mohammad Reza Aflatoonian<sup>2,✉</sup>

<sup>1</sup>School of Medicine, Bam University of Medical Sciences, Bam, Iran

<sup>2</sup>Leishmaniasis Research Center, Kerman University of Medical Sciences, Kerman, Iran

Corresponding author: ✉ [m.aflatoonian97@gmail.com](mailto:m.aflatoonian97@gmail.com)

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### Abstract

*In this study, an electrochemical sensor for the quantification of folic acid with voltammetric detection in physiological conditions was constructed. For this purpose, nickel ferrite (NiFe<sub>2</sub>O<sub>4</sub>) nanoparticles were used to modify the surface of a screen-printed graphite electrode (NiFe<sub>2</sub>O<sub>4</sub>/SPGE) and applied in the determination of folic acid. The modified electrode displays a strong electrochemical response to folic acid. Folic acid was determined electrochemically using the differential pulse voltammetry (DPV) technique with a detection limit of 0.09±0.001 μM in 0.2–147.0 μM linear range in phosphate buffer solution (PBS) at pH 7.0 with this NiFe<sub>2</sub>O<sub>4</sub>/SPGE sensor, which has the best electron transfer rate. Also, the sensitivity of the modified electrode was obtained as 0.1139 μA μM<sup>-1</sup>. The NiFe<sub>2</sub>O<sub>4</sub>/SPGE sensor was successfully applied for the determination of folic acid in real samples.*

### Keywords

Modified electrode, electrochemical sensor, electrocatalytic activity, magnetic nanoparticles

### Introduction

Vitamins are a group of organic compounds essential in very small amounts for the body's normal functioning [1]. Folic acid (N-[p-{{(2-amino-4-hydroxy-6-pteridiny) methyl} amino}benzoyl]-L-glutamic acid), also called pteroylglutamic acid (PteGlu), is a water-soluble vitamin of B complex family. It is most commonly referred to as vitamin B<sub>9</sub> [2,3]. Folic acid is an important substance for keeping the activity and health of critters and is essential for cell growth and division of the human body. It participates in lots of bodily reactions and mainly in the synthesis of nucleic acid and some important substances and promoting the synthesis of protein from amino acid [4-6]. Research over the past decades has shown that deficiency in folate concentration leads to neural tube defects in newborns and an increased risk of megaloblastic anemia, cancer, coronary heart disease, Alzheimer's disease, neurological disorders, and cardiovascular disease in children and adults. Furthermore, the requirement of folate increases during periods of rapid cell division and it is essential for pregnant women [7,8]. Hence, analytical methods for the determination of this important bioelement are needed.

Several methods have been proposed for the determination of folic acid in real samples, including spectrophotometry [9], high-performance liquid chromatography [10], capillary electrophoresis [11], fluorimetric [12], colorimetry [13], and flow injection chemiluminescence method [14]. However, in most of the cases reported above, prior steps are required before the actual determination of folic acid. Also, these techniques consume a long time for analysis, are subject to interferences and require expensive reagents. These disadvantages do not make them applicable for rapid analytical determination.

It is well known that electrochemical methods are simple and inexpensive, in which analytical techniques require a small amount of sample. Electrochemical sensors have attracted wide attention due to their facile fabricating processes, quick surface renewal, high sensitivity, selectivity, low background current, fastness, a wide range of potential ranges, compatibility and reproducibility [15-29].

The concept of modified electrodes is an exciting development in the field of electrochemistry. The electrocatalysis of slow electron transfer reactions is perhaps the most important feature of chemically modified electrodes. Such electrodes enhance the electron transfer rate by reducing the overpotential associated with a reaction. The importance of modified electrodes in electrochemical sensors is because of their high electron transfer rate, high sensitivity, selectivity and stability in analyzing the electrochemical behavior of the analyte. So it is very important to develop highly sensitive and precise analytical methods and material with good conductivity to modify electrode to detect the concentration of analyte effectively [30-41].

During the last years, the research outcomes related to nanomaterials have increased in different application fields due to the development in the preparation and application of these new materials [42-46]. Nanomaterials offer unique and specific electroanalysis properties only found in nanoscale materials. These properties derive from the enhanced diffusion of the target analyte based on convergent rather than linear diffusion, with a high surface area, enhanced selectivity, catalytic activity, and a high signal-to-noise ratio [47-51].

Magnetic nanoparticles provide significant levels of new functionality for electrochemistry due to their high surface area, effective mass transport, catalysis and control over the local microenvironment [52,53].

Magnetic nanoparticles (NPs) with the general formula  $MFe_2O_4$  ( $M = Fe, Ni, Co, Cu, Mn, etc.$ ) are the most popular materials in analytical biochemistry, medicine, removal of heavy metals and biotechnology and have been increasingly applied to immobilize proteins, enzymes, and other bioactive agents due to their unique advantages. Nickel-ferrite is one of the most malleable and important spinel compounds due to its typical ferromagnetic properties and high electrochemical stability. Moreover,  $NiFe_2O_4$  NPs exhibit a high surface area and low mass transfer resistance. It is expected that  $NiFe_2O_4$  could also be used as an electrocatalyst apart from its electronic and magnetic applications due to its conducting nature [54-56].

In this work, a screen-printed graphite electrode modified with the  $NiFe_2O_4$  magnetic nanoparticles was used for sensitive voltammetric determination of folic acid and the modified electrode exhibited excellent electrocatalytic activity to folic acid.

## Experimental

### *Apparatus and chemicals*

All the electrochemical measurements were carried out on a PGSTAT302N potentiostat/galvanostat Autolab. The measurement cell consisted of SPGE (DropSens; DRP-110: Spain) containing a graphite counter electrode, a graphite working electrode, and a silver pseudo-reference electrode.

Solution pH values were determined using a 713 pH meter combined with a glass electrode (Metrohm, Switzerland). All chemicals used were of analytical grade and were used as received without any further purification and were obtained from Merck. Orthophosphoric acid was utilized to prepare the phosphate buffer solutions (PBS), and sodium hydroxide was used to adjust the desired pH values (pH range between 2.0 and 9.0).

### Preparation of modified electrode

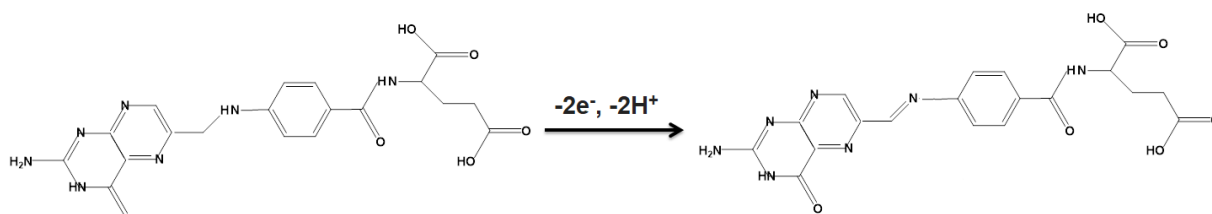
NiFe<sub>2</sub>O<sub>4</sub>/SPGEs were prepared by modifying the bare working electrode of an SPGE using the drop-casting method. Briefly, 4  $\mu$ L of the solution of NiFe<sub>2</sub>O<sub>4</sub> NPs (1 mg/mL) were dropped onto the working electrode surface and dried at room temperature. The obtained electrode was noted as NiFe<sub>2</sub>O<sub>4</sub>/SPGE.

The surface area of NiFe<sub>2</sub>O<sub>4</sub>/SPGE and the bare SPGE were obtained by CV using 1 mM K<sub>3</sub>Fe(CN)<sub>6</sub> at different scan rates. Using Randles-Ševčík formula for NiFe<sub>2</sub>O<sub>4</sub>/SPGE, the electrode surface was found to be 0.109 cm<sup>2</sup> which was about 3.5 times greater than bare SPGE.

## Results and discussion

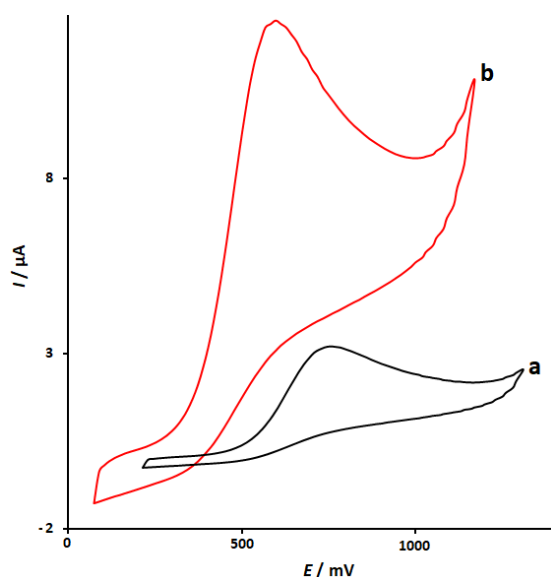
### Electrochemical behavior of folic acid at the surface of various electrodes

The effect of the electrolyte pH on the oxidation of 100.0  $\mu$ M folic acid was investigated at NiFe<sub>2</sub>O<sub>4</sub>/SPGE using DPV measurements in the PBS in the pH range from 2.0 to 9.0. According to the results, the oxidation peak current of folic acid depends on the pH value and increases with increasing pH until it reaches the maximum at pH 7.0 and then decreases at higher pH values. The optimized pH corresponding to the higher peak current was 7.0, indicating that protons are involved in the reaction of folic acid oxidation. Scheme 1 demonstrates the electrooxidation process of folic acid.



**Scheme 1.** The electrooxidation reaction of folic acid

Figure 1 displays cyclic voltammograms from the electrochemical oxidation of 100.0  $\mu$ M folic acid at the surface of NiFe<sub>2</sub>O<sub>4</sub>/SPGE (curve b) and bare SPGE (curve a).

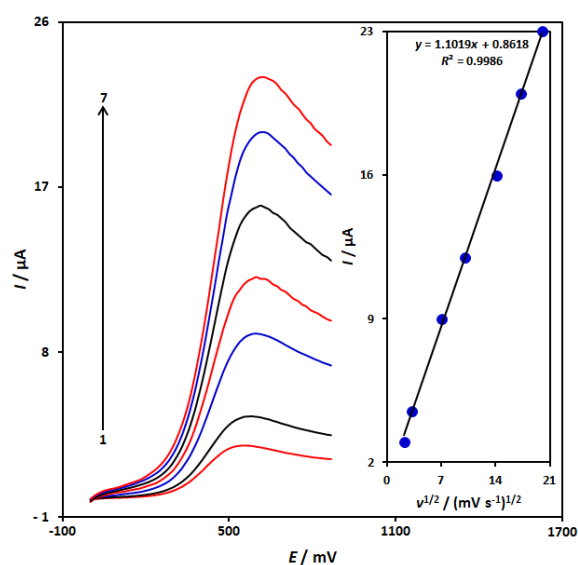


**Figure 1.** Cyclic voltammograms of a) bare SPGE, b) NiFe<sub>2</sub>O<sub>4</sub>/SPGE in the presence of 100.0  $\mu$ M folic acid in 0.1 M phosphate buffer solution, pH 7.0

The results showed that the oxidation of folic acid is very weak on the surface of the bare SPGE, but the presence of  $\text{NiFe}_2\text{O}_4$  NPs in SPGE could enhance the peak current and decrease the oxidation potential (decreasing the overpotential). A substantial negative shift of the currents starting from oxidation potential for folic acid and a dramatic increase of the current indicates the catalytic ability of  $\text{NiFe}_2\text{O}_4/\text{SPGE}$  to folic acid oxidation. The results showed that the use of  $\text{NiFe}_2\text{O}_4$  nanoparticle (curve b) definitely improved folic acid oxidation characteristics, partly due to the excellent characteristics of  $\text{NiFe}_2\text{O}_4$  NPs, such as good electrical conductivity and high chemical stability.

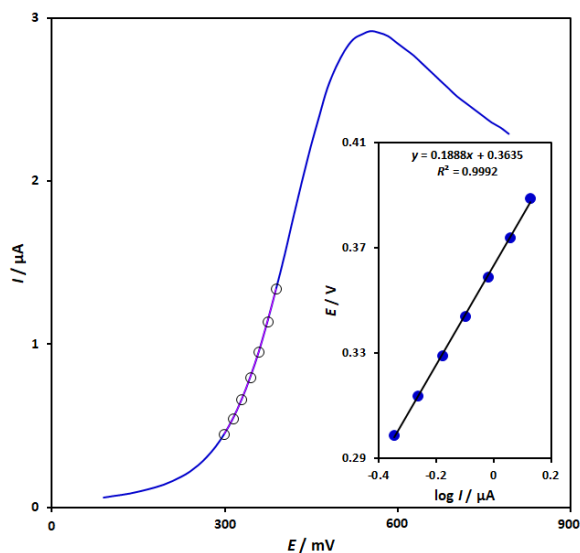
#### Effect of scan rate on the determination of folic acid at $\text{NiFe}_2\text{O}_4/\text{SPGE}$

The influence of potential scan rate ( $\nu$ ) on  $I_p$  of  $70.0 \mu\text{M}$  folic acid at the  $\text{NiFe}_2\text{O}_4/\text{SPGE}$  was studied by linear sweep voltammetry (LSV) at various sweep rates (Figure 2). As shown in Figure 2, the peak currents of folic acid grow with the increasing scan rates and there are good linear relationships between the peak currents and  $\nu^{1/2}$  (square root of scan rate) (Figure 2 inset). The regression equation is  $I_{pa} = 1.1019 \nu^{1/2} + 0.8618$  ( $R^2 = 0.9986$ ), indicating the oxidation process of  $70.0 \mu\text{M}$  folic acid at the  $\text{NiFe}_2\text{O}_4/\text{SPGE}$  was diffusion-controlled.



**Figure 2.** Linear sweep voltammograms of  $70.0 \mu\text{M}$  folic acid at  $\text{NiFe}_2\text{O}_4/\text{SPGE}$  at different scan rates, 1-7 correspond to 5, 10, 50, 100, 200, 300 and  $400 \text{ mV s}^{-1}$  in  $0.1 \text{ M}$  phosphate buffer solution,  $\text{pH } 7.0$ . Inset shows the plot of  $I_{pa}$  versus  $\nu^{1/2}$  for the oxidation of folic acid at  $\text{NiFe}_2\text{O}_4/\text{SPGE}$

To obtain further information on the rate-determining step, the Tafel plot for oxidation of  $70.0 \mu\text{M}$  folic acid at the surface of  $\text{NiFe}_2\text{O}_4/\text{SPGE}$  using the data derived from the raising part of the current-voltage curve has been recorded in Figure 3.

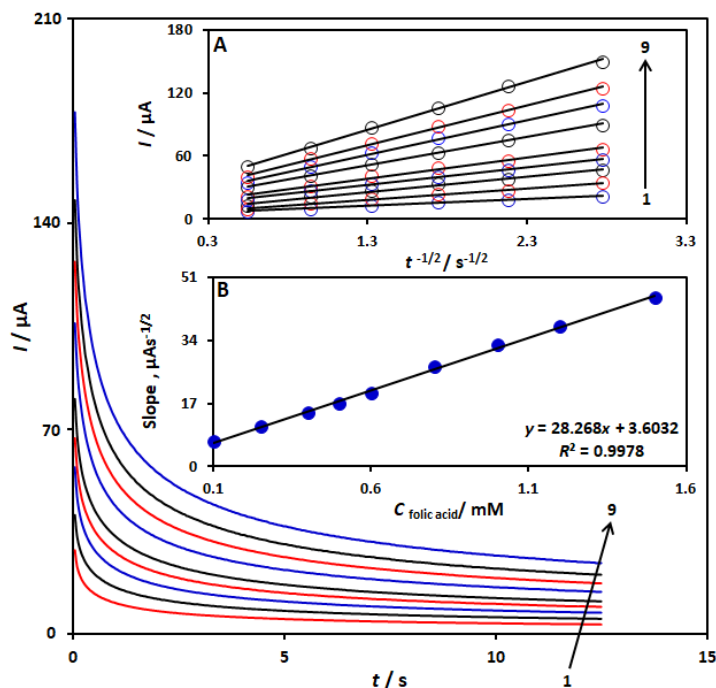


**Figure 3.** Linear sweep voltammograms for  $70.0 \mu\text{M}$  folic acid with  $5 \text{ mV s}^{-1}$  scan rate. Inset: The Tafel plot derived from the rising part of the corresponding voltammogram

Using the slope of Tafel at a scan rate of 5 mV/s, the value of the electron transfers coefficient ( $\alpha$ ) was determined as 0.68.

### Chronoamperometric studies

The electrochemical oxidation of folic acid by a NiFe<sub>2</sub>O<sub>4</sub>/SPGE was also studied by chronoamperometry. Chronoamperometric measurements of different concentrations of folic acid at NiFe<sub>2</sub>O<sub>4</sub>/SPGE were done by setting the working electrode potential at 650 mV (Figure 4).



**Figure 4.** Chronoamperograms obtained at the NiFe<sub>2</sub>O<sub>4</sub>/SPGE in 0.1 M phosphate buffer solution, pH 7.0 for different concentrations of folic acid. Numbers 1-9 correspond to 0.1, 0.25, 0.4, 0.5, 0.6, 0.8, 1.0, 1.2 and 1.5 mM of folic acid. (A) Plots of  $I$  vs.  $t^{-1/2}$  for electrooxidation of folic acid obtained from chronoamperometry. (B) Plot of the slope of the straight lines against folic acid concentration

In chronoamperometric studies, we have determined folic acid's diffusion coefficient,  $D$ . The experimental plots of  $I$  versus  $t^{-1/2}$  with the best fits for different concentrations of folic acid were employed (Figure 4 A). The slopes of the resulting straight lines were then plotted versus the folic acid concentrations (Figure 4 B), from whose slope and using the Cottrell equation (1):

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

We calculated a diffusion coefficient of  $6.6 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  for folic acid.

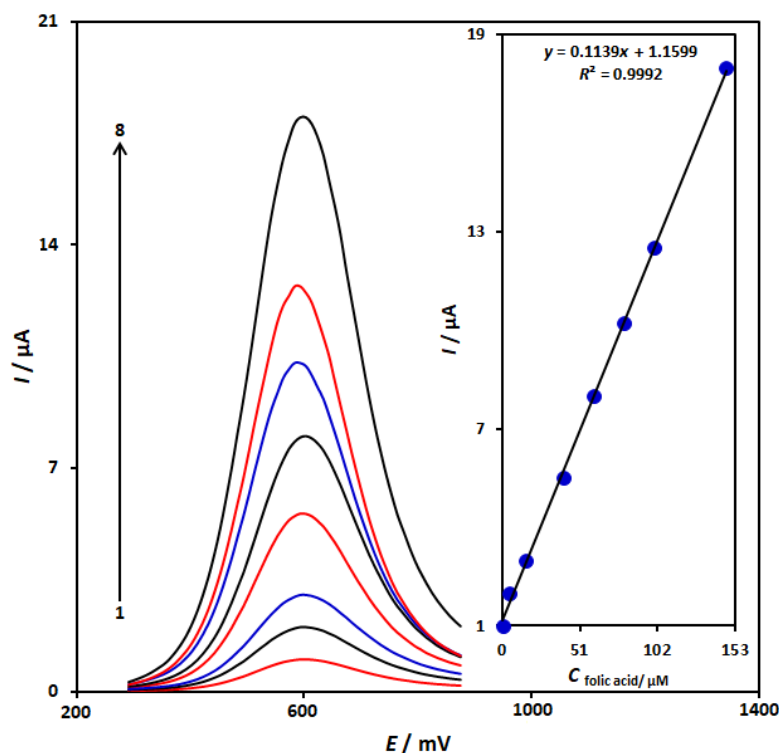
### Calibration curve and limit of detection

Since DPV has a much higher current sensitivity than cyclic voltammetry, we used the DPV method for the determination of folic acid. Figure 5 (inset) shows DPVs of different concentrations of folic acid and the obtained calibration curves (step potential = 0.01 V and pulse amplitude = 0.025 V). The results showed a linear segment for folic acid concentration from 0.2 to 147.0  $\mu\text{M}$  folic acid (Figure 5), with a regression equation of  $I_p = 0.1139C_{\text{folic acid}} + 1.1599$  ( $R^2 = 0.9992$ ,  $n = 8$ ). The detection limit, LOD, was obtained by using the equation (2):

$$\text{LOD} = 3S_b / m \quad (2)$$

where  $S_b$  is the standard deviation of the blank response ( $n = 15$ ) and  $m$  is the slope of the calibration plot. The limit of detection was determined to be  $0.09 \pm 0.001 \mu\text{M}$  for folic acid.

In addition, Table 1 shows that the NiFe<sub>2</sub>O<sub>4</sub>/SPGE can compete with other sensors for the determination of folic acid [4, 57-60].



**Figure 5.** Differential pulse voltammograms of the NiFe<sub>2</sub>O<sub>4</sub>/SPGE in 0.1 M phosphate buffer solution (pH 7.0) containing different concentrations of folic acid. Numbers 1 to 8 correspond to 0.2, 5.0, 15.0, 40.0, 60.0, 80.0, 100.0 and 147.0 μM of folic acid. (B) plot of the voltammetric peak current as a function of folic acid concentration

**Table 1.** Linear range and LOD obtained at the NiFe<sub>2</sub>O<sub>4</sub>/SPGE for the determination of folic acid compared with other sensors

Electrochemical sensor	Method	Linear range, μM	LOD, μM	Ref.
Multi-wall carbon nanotube/glassy carbon electrode	Square wave stripping voltammetry	0.3 - 80.0	0.134	4
Gold nanoclusters/activated graphene/multi-wall carbon nanotube nanocomposite/glassy carbon electrode	Square wave voltammetry	10.0 - 170.0	0.09	56
Poly(o-methoxyaniline)-gold (POMA-Au) nanocomposite/glassy carbon electrode	Differential pulse voltammetry	0.5 - 900.0	0.090	57
Platinum nanoparticles/graphene nanoplatelets/multi-walled carbon nanotubes/β-cyclodextrin composite/carbon glass electrode	Cyclic voltammetry	20.0 - 500.0	0.48	58
Fe <sub>3</sub> O <sub>4</sub> nanoparticles@molecularly imprinted polymer-graphene oxide/carbon paste electrode	Square-wave adsorptive voltammetry	2.5 - 48.0	0.65	59
NiFe <sub>2</sub> O <sub>4</sub> /SPGE	Differential pulse voltammetry	0.2 - 147.0	0.09	This work

**Real sample analysis**

To investigate the applicability of the proposed sensor for the voltammetric determination of folic acid in real samples, we selected urine and folic acid tablet samples for the analysis of folic acid contents. The folic acid contents were measured after sample preparation using the standard addition method. The results are given in Table 2. According to the table, the recovery values within 97.5 to

103.3 % confirm the powerful ability of NiFe<sub>2</sub>O<sub>4</sub>/SPGE for the determination of folic acid in real samples.

**Table 2.** Application of NiFe<sub>2</sub>O<sub>4</sub>/SPGE for determination of folic acid in real samples (n=3)

Sample	C / $\mu\text{M}$		Recovery, %	RSD, %
	Spiked	Found		
Urine	0	-	-	-
	4.0	3.9	97.5	2.2
	8.0	8.1	101.2	3.0
Folic acid tablet	0	4.0	-	3.4
	2.0	6.2	103.3	1.9
	3.0	6.9	98.6	2.7

## Conclusion

In this work, a simple, rapid and sensitive electrochemical detection method has been developed for the determination of folic acid. NiFe<sub>2</sub>O<sub>4</sub> nanoparticles modified SPGE as a voltammetric sensor to improve the detection sensitivity. The sensitivity ( $0.1139 \mu\text{A } \mu\text{M}^{-1}$ ), detection limit ( $0.09 \pm 0.001 \mu\text{M}$ ) and linear response range (0.2 to 147.0  $\mu\text{M}$ ) for the NiFe<sub>2</sub>O<sub>4</sub>/SPGE modified electrode make it an efficient way for determination of folic acid. Real sample applications were carried out to prove the applicability and precision of the novelty-produced electrode. The amount of folic acid in real samples was obtained satisfactorily with high recovery values by the standard addition method.

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