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A novel vitamin B9 sensor based on modified screen-printed electrode

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Abstract

In the field of determination of vitamin B9 (folic acid, FA), we have described the development of a sensitive electrochemical sensor through promoting the screen-printed electrode (SPE) and taking the advantage of zinc ferrite magnetic nanoparticles (ZnFe$_2$O$_4$MNPs). Cyclic voltammetry (CV) experiments demonstrated the powerful activity of ZnFe$_2$O$_4$MNPs/SPE for electrooxidation of FA by showing the prominent oxidation peak at 600 mV vs. Ag/AgCl. By differential pulse voltammetry (DPV) measurements, a linear relation between current response and concentration of vitamin B9 was determined in the range of 1.0–100.0 μM, and detection limit is found to be 0.3 μM (S/N=3). Except high sensitivity, the developed sensor demonstrated high stability, reproducibility and repeatability, and was also successfully applied to specify FA in real samples of vitamin B9 tablets and human urine.

Keywords
Voltammetric sensor; folic acid; modified electrode; ZnFe$_2$O$_4$ magnetic nanoparticles

Introduction

Vitamin B9 is folic acid (FA), N-[4-[[(2-amino-4-oxo-1,4-dihydropteridin-6-yl)methyl]amino] benzoyl]-L-glutamic acid, which can be dissolved in water and commonly found in fresh fruits, vegetables, liver, and yolk [1,2]. FA is necessary in many biological functions and is a significant compound in the process of repair of DNA, synthesis and methylation of DNA, and synthesis of red blood cells in human's body [3-5]. The deficiency of FA in human's body can cause many disorders such as higher risk of neural tube defects, hypomethylation, colorectal cancer and, it can induce proto-oncogene expression causing cancer [6-8]. Hence, the specification of FA is considered necessary in pharmaceutical, food, and clinical samples.

Most of literature investigations on determination and quantification of FA are using fluorimetry [9], capillary electrophoresis [10], spectrophotometry [11], flow injection analysis [12],

high performance liquid chromatography [13] and chemiluminescence analysis [14]. All these techniques are commonly expensive, show low sensitivity, take long time for each testing, and require sample pretreatment by organic solvent extraction. Therefore, using new alternative techniques is supposed to be very useful.

In recent years, electrochemical methods and particularly voltammetry, have attracted significant attention due to their benefits like sensitivity, simplicity, low cost, accuracy and selective specification of electroactive species [15-28]. In addition, the usage of electrochemical approach towards detection of FA is generally promising because FA is the electrochemically active compound [29,30].

Among many potentially useful electrodes, screen-printed electrodes (SPEs) provide many benefits for electrochemical sensors, such as low cost, portability, ease of chemical modification, rapid response, and flexible design [31-36]. For determining the trace level of FA, however, the signal to noise ratio of the bare, *i.e.* unmodified SPE is not high enough. Therefore, the scientists tried to modify bare SPE surfaces by different sorts of modifiers. Among modifiers, nano-materials can be a good choice due to their high surface-to-volume ratio which will significantly increase the voltammetric response. Also, presence of nanomaterials leads to faster electron transfer between the electrode and analyte [37-48].

In order to construct electrochemical sensors and biosensors, many nanomaterials like metal oxides, metals, semiconductor NPs, and carbon-based materials have already been employed [49-51]. Among them, $ZnFe_2O_4$ magnetic NPs showed promising electronic and magnetic characteristics and thermal stability, which are commonly appropriate to a broad range of usage like drug delivery technology, magnetic resonance imaging (MRI), and gas sensing [52-55].

The main goal of the present work is to take advantages of both SPE and $ZnFe_2O_4$ magnetic NPs and combine them in a sensor for specifying the amounts of vitamin B9 (FA) in various real samples.

Experimental

Chemicals and apparatus

Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands) was utilized for electrochemical measurements. General Purpose Electrochemical System (GPES) software was used for controlling experimental conditions. SPE (DropSens; DRP-110: Spain) possessed three electrodes, *i.e.* an unmodified graphite working electrode, graphite counter electrode and Ag/AgCl reference electrode. Metrohm 710 pH meter was utilized in order to determine pH.

FA and all the remaining reagents were of analytical grade and provided by Merck (Darmstadt, Germany). Orthophosphoric acid and the related salts were utilized to prepare the buffer solutions of pH in the range of 2.0–9.0. $ZnFe_2O_4$ nanoparticles were synthesized according to the literature [56].

Preparation of real samples

Drug-free human urine specimens were stored in a refrigerator immediately after collection. Firstly, 10 ml of each sample was used and centrifuged at 2000 rpm for 15 minutes and the supernatant passed through 0.45 μm filter. Next, various quantities of the treated urine samples were taken and transferred to a flask and diluted by utilizing a phosphate buffer solution (PBS) with pH of 7.0. For these samples, various quantities of FA were spiked. The standard addition approach was utilized to determine FA concentrations.

Five FA tablets labelled as 100 mg per tablet, Tehran Chemie Pharmaceutical Co., Iran) were firstly grounded to a powder. Afterwards, the tablet solution was prepared *via* dissolving 500 mg of the powder in 25 ml water through ultra-sonication. Various volumes of the diluted solution have been transported to 25 ml volumetric flasks and diluted to the marks with PBS, pH 7.0. FA contents were analysed by the recommended technique *via* the standard addition method.

Results and discussion

Electrochemical profile of FA at ZnFe$_2$O$_4$ MNPs/SPE

The mechanism of FA oxidation is schematically drawn in Figure 1 where electrochemical oxidation of FA has been proposed as 2e$^-$/2H$^+$ reaction, what suggests pH dependent electro-chemical response [57]. Therefore, finding of the optimum pH value would be required for achieving precise outputs. The modified electrodes were tested at pH values ranging between 2.0 and 9.0. The most acceptable output for FA electrooxidation was observed at pH 7.0.
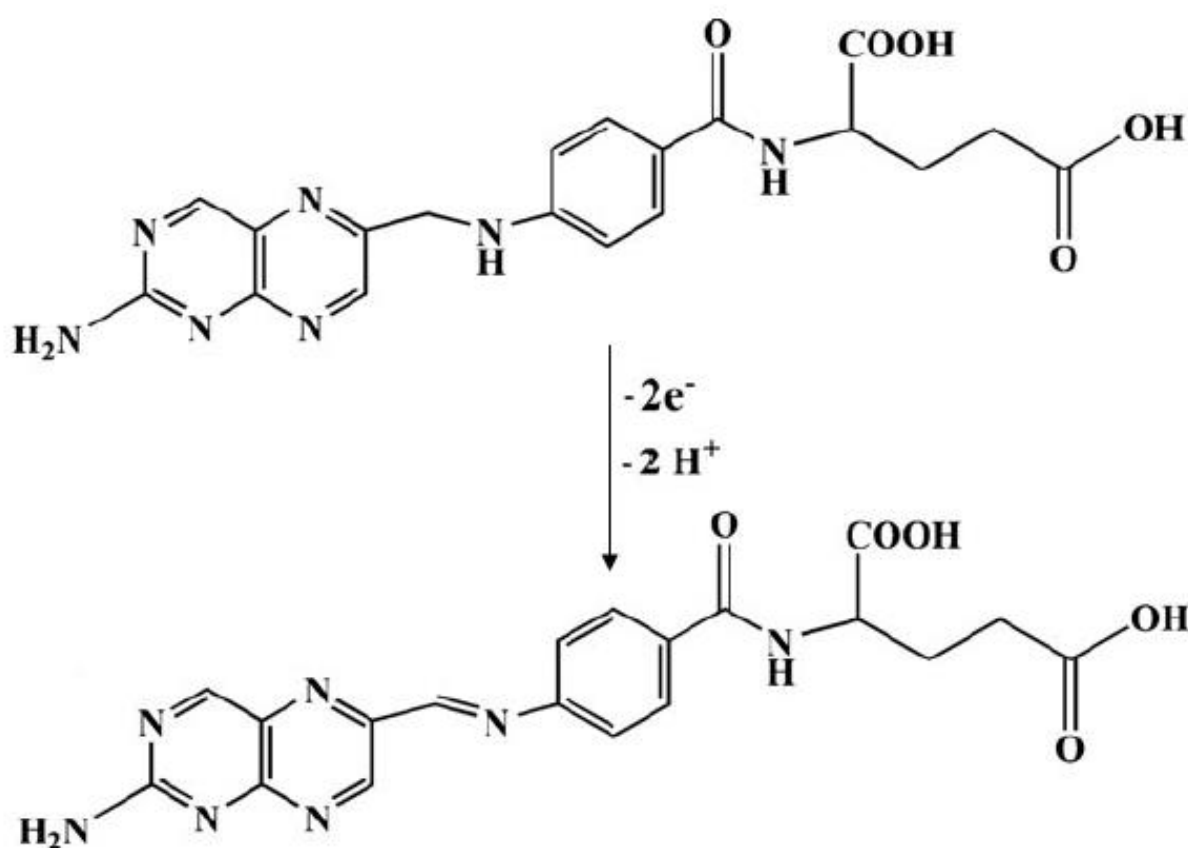
Figure 1. *Electrochemical mechanism for oxidation of folic acid [57].*

Figure 2 represents cyclic voltammograms (CV) of bare SPE (curve a) and ZnFe$_2$O$_4$ MNPs modified SPE (curve b) in 0.1 M PBS (pH 7.0) containing 60.0 μM of FA. Based on CV outcomes, it may be stated that much higher oxidation peak current of FA was recorded for ZnFe$_2$O$_4$ MNPs/SPE, proving thus higher sensitivity of modified electrode than bare SPE. Also, peak potential for modified SPE occurred at 590 mV, what is about 70 mV more negative in comparison to the unmodified SPE. This suggests that modifying SPE with ZnFe$_2$O$_4$ MNPs improved the catalytic activity of the electrode towards FA.
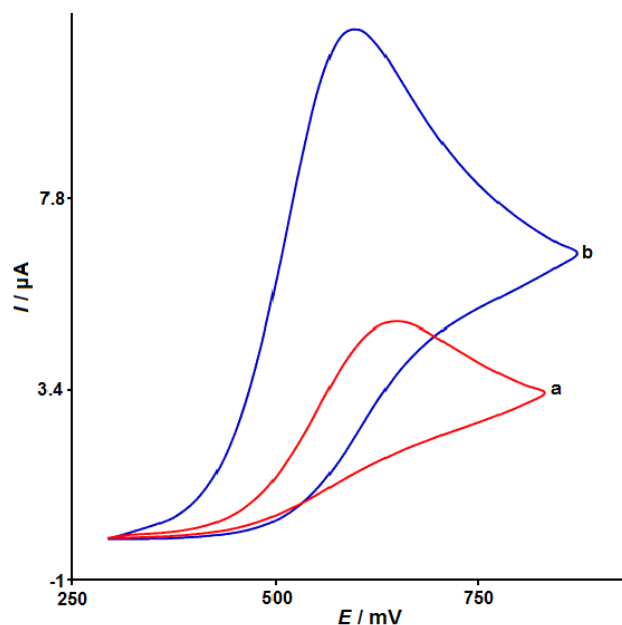
Figure 2. *CVs (50 mV s^{-1}) of a) SPE and b) ZnFe$_2$O$_4$ MNPs/SPE in presence of 60.0 µM of FA in 0.1 M PBS (pH 7.0).*

Effect of scan rate

Figure 3 presents the effect of potential scan rate changes on the oxidation currents of vitamin B9 (FA), recorded by linear sweep voltammetry (LSV). The results show increase of anodic currents with potential scan rate, and only minor shifts of peak potential values. It can be observed in the inset of Figure 3 that the peak current (I_p) and the square root of the potential scan rate ($v^{1/2}$) are linearly associated, what demonstrates that the oxidation process of FA is fast and almost diffusion-controlled process.
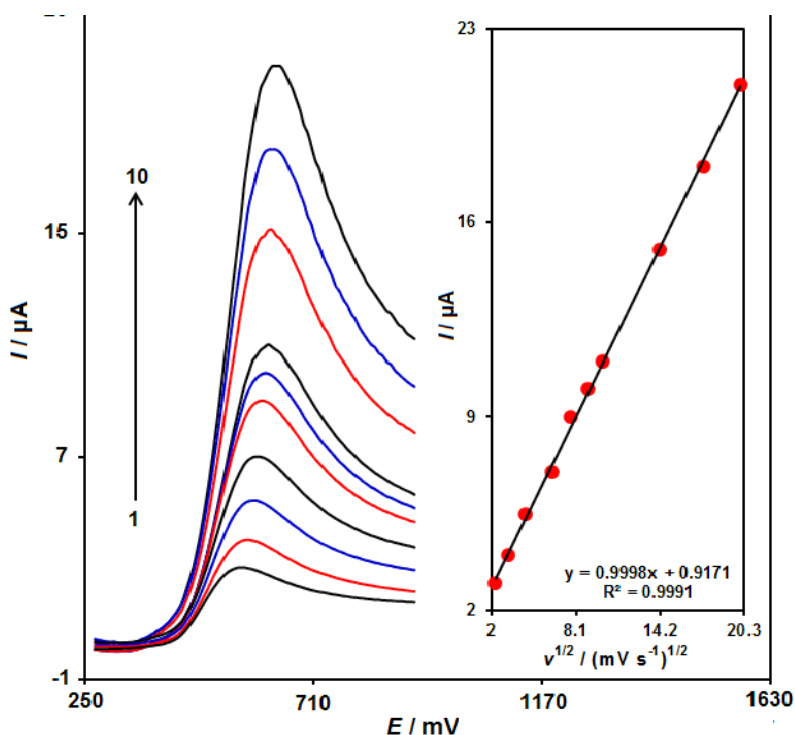

Figure 3. *LSVs of ZnFe$_2$O$_4$ MNPs/SPE in 0.1 M PBS (pH 7.0) containing 40.0 µM FA at different scan rates (1-10 correspond to v = 5, 10, 20, 40, 60, 80, 100, 200, 300 and 400 mV s^{-1}). Inset: variation of anodic peak current vs. v$^{1/2}$.*

Chronoamperometric analysis

For chronoamperometric analysis, the working electrode potential was set at 0.64 V for each sample solution containing different concentration of vitamin B9 in 0.1 M PBS (pH 7.0), and the outputs are shown in Figure 4. Generally, the current for an electroactive material such as vitamin B9 at the mass transport limited condition, could be illustrated by Cottrell's equation:

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \tag{1}$$

In eq. (1), n, C_b, D and A refer to the number of electrons in redox reaction, bulk concentration of analyte (mol cm^{-3}), diffusion coefficient (cm^{2}s^{-1}) and surface area of the electrode (cm^2), respectively. The optimized fitting results of I vs. $t^{-1/2}$ linear plots based on the experiments are, for different vitamin B9 samples, presented in Figure 4A. The slopes of the obtained straight lines vs. concentration of vitamin B9 are shown in Figure 4B.
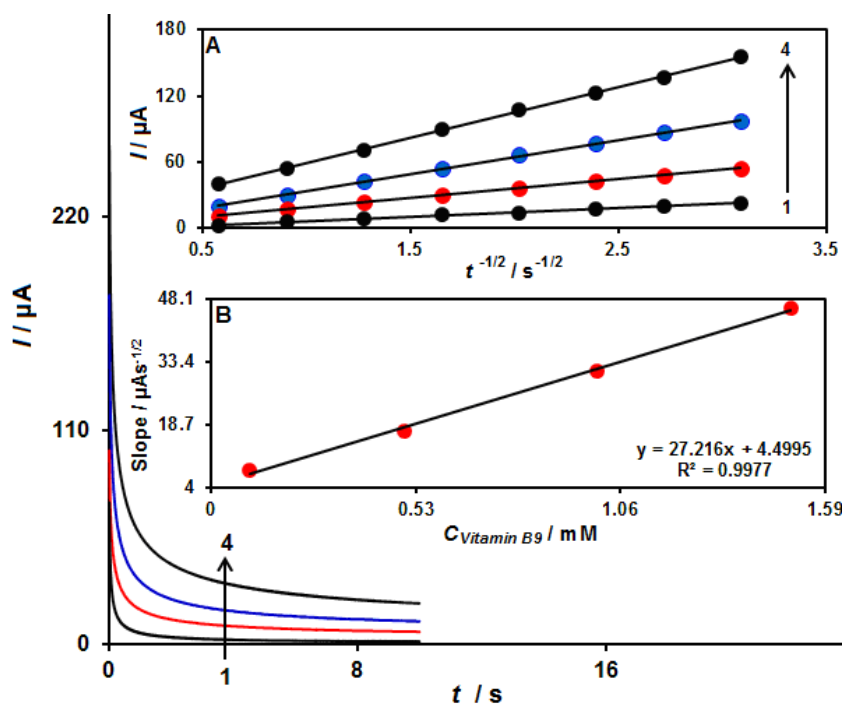

Figure 4. *Chronoamperograms of ZnFe$_2$O$_4$ MNPs/SPE in 0.1 M PBS (pH 7.0) for various concentrations of vitamin B9 (1 to 4 correspond to 0.1, 0.5, 1.0, and 1.5 mM of FA). Inset (A): current vs. t$^{-1/2}$ plots achieved from chronoamperograms 1 to 4. Inset (B): slopes of straight lines in Fig. 4B vs. vitamin B9 concentrations.*

Considering Cottrell equation (1), the resulting slopes in Figure 4, n=2 (Figure 1), and geometric surface area of WE of 0.0341 cm^2, D value for FA in PBS solution was calculated equal to 6.3×10^{-5} cm^2 s^{-1}.

Calibration plot and limit of detection

Vitamin B9 (FA) was quantitatively analyzed using differential pulse voltammetry (DPV), because DPV technique offers several benefits, like better sensitivity for the analytical utilization. DPVs of the modified electrode (ZnFe$_2$O$_4$ MNPs/SPE) for different concentrations of vitamin B9 (1.0 to 100.0 µM) in 0.1 M PBS (pH 7.0) are shown in Figure 5. It is obvious from the inset of Figure 5 that the oxidation peak current and concentration of vitamin B9 are linearly correlated in the concentration range between 1.0 to 100.0 µM, with the correlation coefficient 0.9994. Limit of detection (LOD) is found equal to 0.3 µM.
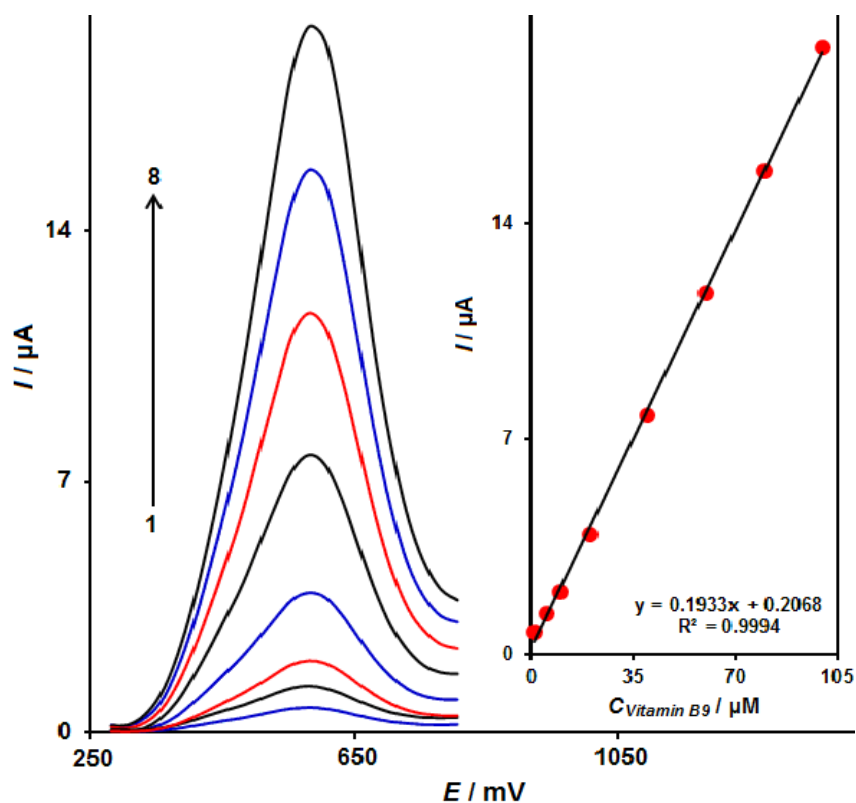
Figure 5. *DPVs of ZnFe$_2$O$_4$ MNPs /SPE in 0.1 M PBS (pH 7.0) containing diverse concentrations of vitamin B9 (1 to 8 correspond to 1.0, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0 and 100.0 μM) Inset: peak current plot vs. vitamin B9 concentrations from 1.0-100.0 μM.*

Stability and repeatability of ZnFe$_2$O$_4$ MNPs/SPE

Stability of ZnFe$_2$O$_4$MNPs/SPE was tested by maintaining the proposed sensor electrode for 15 days in PBS solution of pH 7.0. Prior and after immersion, cyclic voltammograms were recorded in the solution containing 30.0 μM of FA to obtain information on the stability of the prepared sensor electrode. A slight decrease in voltammetric response equal to 2.1 % was observed comparing with the initial response. This confirms that ZnFe$_2$O$_4$MNPs/SPE is highly stable sensor electrode.

The anti-fouling characteristic for the oxidation of FA were tested by cyclic voltammetry of the modified SPE before and after its use in the presence of FA. Cyclic voltammograms were recorded after 15 potential cycles performed at 50 mV s^{-1} in the presence of FA. It was found that the current values were reduced less than 2.3 %, while the peak potential is not changed. These experimental results approved high electrode repeatability.

Analysis of real samples

For assessing the usefulness of here proposed ZnFe$_2$O$_4$ MNPs/SPE sensor for FA determination, the analyses in some real samples were performed. Quantifications of FA in vitamin B9 tablets and human urine samples were performed using the standard addition method, and the obtained analytical results are presented in Table 1. It must be added here that the recovery of FA was found satisfactory, as well as reproducibility which was demonstrated on the basis of mean relative standard deviation (RSD) (1.7-3.5).

Table 1. *Analytical results of ZnFe$_2$O$_4$ MNPs/SPE for determination of FA in vitamin B9 tablet and urine samples (n=5).*

Sample	Concentrations, µM		Recovery, %	RSD, %
	Spiked	Found		
Vitamin B9 tablet	0	6.0	-	2.9
	2.5	8.6	101.2	3.4
	5.0	10.8	98.2	1.8
	7.5	14.0	103.7	2.2
	10.0	15.9	99.4	3.0
Urine	0	-	-	-
	5.0	4.9	98.0	1.7
	10.0	10.3	103.0	2.2
	15.0	15.2	101.3	3.5
	20.0	19.8	99.0	2.6

Conclusions

A disposable electrochemical sensor for detection of vitamin B9 (folic acid, FA) was developed using a screen-printed electrode (SPE) modified by ZnFe$_2$O$_4$ magnetic nanoparticles (NPs). Based on the advantages of SPE technology in combination with excellent electrocatalytic properties of magnetic ZnFe$_2$O$_4$NPs, a highly suitable sensor for FA was developed. Modified SPE showed significantly improved oxidation peak current in comparison with unmodified SPE. The electrochemical efficiency of the modified sensor was assessed using DPV, showing a wide linear range (1.0-100.0 µM) and low detection limit of 0.3 µM FA. Testing performed with the developed ZnFe$_2$O$_4$ MNPs/SPE showed appropriate sensitivity, reproducibility, stability and repeatability of sensor electrode for exact determination of FA. Determination of vitamin B9 in some real samples (vitamin B9 pharmaceutical tablets and human urine) performed using the developed sensor and standard addition method, showed very acceptable results.

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