Scientific paper

Modified Screen Printed Electrode for Selective Determination of Folic Acid

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Abstract

Folic acid (FA) as one of the water-soluble vitamins contributes to the construction of healthy cells, as FA deficiency is one of the leading causes of anaemia. Based on reports, reduced folate level can lead to development of carcinogenesis. Hence, its analysis from the clinical point of view is highly demanded. In the present work, NiFe2O4 nanoparticles was successfully synthesized and used for modified novel voltammetric sensor for determination of folic acid. Differential pulse voltammetry response shows the linear increment of oxidation signals with an increase in the concentration of folic acid in the range of 1.0 × 10−7–5.0 × 10−4 M with limit of detection 3.4 × 10−8 M. The modified electrode displays an excellent selectivity towards the determination of FA even in the presence of various interfering species. Finally, the screen printed electrode (SPE) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode modified by NiFe2O4 nanoparticles (NFO) was applied for FA determination folic acid tablet and urine samples whose accuracy was attested by means of addition and recovery assays (97.0–103.5%) as well as by differential pulse voltammetry.

Keywords: Folic acid; NiFe2O4 nanoparticles; screen printed electrodes; voltammetry; real sample; electrochemical sensor.

1. Introduction

Application of screen-printed electrodes (SPEs) has a main advantage of miniaturization compared to the conventional electrodes including carbon paste or glassy carbon electrodes.1 SPEs offer attractive advantages in electrochemical analysis featuring disposability, low cost, flexible in design, ease of chemical modification, and rapid response.2–4

Magnetic nanoparticles (NPs) 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.5–9 NiFe2O4 nanoparticles (NiFe2O4 NPs) have attracted an increasing interest in construction of sensors and biosensors because of their good biocompatibility, strong super paramagnetic property, low toxicity, easy preparation and high adsorption ability. The quantitative cytotoxicity test verified that NiFe2O4 nanoparticles had nontoxicity. Moreover, NiFe2O4 NPs exhibit high surface area and low mass transfer resistance.10–12

Folic acid (FA) is a kind of water-soluble vitamin and can act as coenzyme in the transfer and utilization of one-carbon groups and in the regeneration of methionine from homocysteine.13

This vitamin has lately received considerable attention due to its believed antioxidant activity and use for cancer prevention. While present in a wide variety of nutrition and pharmaceutical formulations, the human metabolism is unable to produce folic acid.14–16 The decrease in concentration of folic acid can cause however serious complications such as leucopenia, giantocytic anemia, psychosis, devolution of mentality and increasing possibility of heart attack and stroke. Hence, the development of sensitive and fast methods for the determination of folic acid has attracted considerable attention.17–19 Some analytical methods have been reported for the determination of FA with high performance liquid chromatography,20 spectrophotometry,21 chemiluminescence,22 spectrofluorometric23 and Enzyme-linked ligand sorbent test methods.24 But these techniques have many disadvantages, such as high cost from the equipments and disposable chemi-
ed, complicated and time-consuming pretreatments, and so on. Electrochemical techniques are the most preferred ones considering their simplicity, rapid response, good stability, low cost, high sensitivity and excellent selectivity which are widely used in the field of food, drug, biological and environmental analysis.\textsuperscript{25–29}

The present study is aimed at the synthesis of the NiFe\textsubscript{2}O\textsubscript{4} nanoparticles and its application in the form of

EDX microanalysis.

\begin{itemize}
  \item [29x735]NiFe\textsubscript{2}O\textsubscript{4} nanoparticles and its application in the form of
  \item [48x628]EDX microanalysis.
  \item [48x322]samples were collected on JSM, 6380 LV equipped with an
  \item [48x333]Diffraction Standards (JCPDS) card. SEM images of the
  \item [48x345]pattern was indexed using Joint Committee on Powder
  \item [48x357]verify the formation of products. The X-ray diffraction
  \item [48x369]measurements.
  \item [48x376]NiFe\textsubscript{2}O\textsubscript{4} nanoparticles were synthesized in the pres-
  \item [402x404]solvent was evaporated in room temperature.
  \item [416]were completely grounded and homogenized before
  \item [428]impracticality, and a wide linear range of folic acid concentrations
  \item [432]FT-IR infrared spectrometer. FT-IR spectra in the range 4000–
  \item [440]of aliquots of the NiFe\textsubscript{2}O\textsubscript{4} suspension solution was cast on
  \item [447]of folic acid at the NiFe\textsubscript{2}O\textsubscript{4} nanoparticles. Low detection limit, high sensitiv-
  \item [624]ucts. The resulting products were dried in a vacuum oven
  \item [652]were thus obtained.
\end{itemize}

2. Experimental

2. 1. Apparatus and Chemicals

Fourier transform infrared (FT-IR) spectra were recorded in transmission mode with a Perkin Elmer BX FT-IR infrared spectrometer. FT-IR spectra in the range 4000–400 cm\textsuperscript{-1} were recorded in order to investigate the nature of the chemical bonds formed. X-ray powder diffraction (XRD) analysis was conducted on a Philips analytical PC-APD X-ray diffractometer with graphite monochromatic CuK\textalpha radiation \((\alpha_1, \lambda_1 = 1.54056 \text{Å}, \alpha_2, \lambda_2 = 1.54439 \text{Å})\) to verify the formation of products. The X-ray diffraction pattern was indexed using Joint Committee on Powder Diffraction Standards (JCPDS) card. SEM images of the samples were collected on JSM, 6380 LV equipped with an EDX microanalysis.

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with the General Purpose Electrochemical System software. The screen-printed electrode (DropSens, DRP-110, Spain) consists of three main parts which are a graphite counter electrode, a silver pseudo-reference electrode and a graphite working electrode, unmodified. A Metrohm 710 pH meter was used for pH measurements.

Folic acid and all other reagents were of the analytical grade, and they were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts over the pH range of 2.0–9.0.

2. 2. Synthesis of NiFe\textsubscript{2}O\textsubscript{4} Nanoparticles

NiFe\textsubscript{2}O\textsubscript{4} nanoparticles were synthesized in the presence of urea using a hydro/solvothermal method. Solution of urea were dissolved in 60 mL of deionized water and then 20 mL polyethylene glycol was added to solution to form brown homogeneous solutions. Then 10 mL FeCl\textsubscript{3}·

3. 1. Morphology and Structure of NiFe\textsubscript{2}O\textsubscript{4}

Nanoparticles

The vibration frequencies in the infrared spectrum of a molecule were considered to be a unique physical

3. Result and Discussion

3. 1. Morphology and Structure of NiFe\textsubscript{2}O\textsubscript{4}

Nanoparticles

The vibration frequencies in the infrared spectrum of a molecule were considered to be a unique physical
property and were a characteristic of the molecule. Fig. 1 shows two persistent absorption bands corresponding to the vibration of tetrahedral and octahedral complexes at 599 cm$^{-1}$ and 465 cm$^{-1}$, respectively. Those bands confirmed the formation of spinel nickel ferrite structure. As can be seen from FT-IR spectra the normal mode of vibration of tetrahedral cluster (599 cm$^{-1}$) is higher than that of octahedral cluster (465 cm$^{-1}$). This is due to the shorter bond length of tetrahedral cluster than the octahedral cluster. 

\[ t = \frac{0.9 \lambda}{\beta \cos(\theta)} \]

The morphology of the product was examined by SEM. Fig. 3A depicts the SEM pictures of NiFe$_2$O$_4$ nanoparticles. From the graph, it was observed that the nanoparticles, which are nearly spherical, are not agglomerated and they are seen as less than 10 nm.

The EDX analysis was performed to further confirm the composition of the obtained products. Fig. 3B shows that the products are composed of Ni, Fe and O. The C peak in the spectrum is attributed to the electric latex of the SEM sample holder.

3.2. Electrochemical Behaviour of Folic Acid at The Surface of Various Electrodes

The electrochemical behaviour of folic acid depends on the pH value of the aqueous solution. Therefore, the pH optimization of the solution seems to be necessary in order to obtain the best results for electro-oxidation of folic acid. Thus, the electrochemical behaviour of folic acid was stud-
ied in 0.1 M PBS in different pH values (2.0–9.0) at the surface of NFO/SPE by voltammetry. It was found that the electro-oxidation of folic acid at the surface of NFO/SPE was more favoured under neutral conditions than in acidic or basic medium. Here pH 7.0 was chosen as the optimum pH for electro-oxidation of folic acid at the surface of NFO/SPE.

Fig. 4 depicts the CV responses for electro-oxidation of 100.0 μM folic acid at the unmodified SPE (curve b) and NFO/SPE (curve a). The peak potential occurs at 670 mV due to the oxidation of folic acid, which is about 70 mV more negative than the unmodified SPE. Also, NFO/SPE shows much higher anodic peak currents for the oxidation of folic acid compared to the unmodified SPE, indicating that the modification of the unmodified SPE with NiFe₂O₄ nanoparticles has significantly improved the performance of the electrode towards folic acid oxidation.

3. 4. Chronoamperometric Measurements

Chronoamperometric measurements of folic acid at NFO/SPE were carried out by setting the working electrode potential at 0.75 V vs. Ag/AgCl/KCl (3.0 M) for various concentrations of folic acid (Fig. 6) in PBS (pH 7.0). For electroactive materials (folic acid in this case) with a diffusion coefficient of D, the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation:

\[ I = n F A D^{1/2} C_b \pi^{-1/2} t^{-1/2} \] (1)

where D and C_b are the diffusion coefficient (cm² s⁻¹) and the bulk concentration (mol cm⁻³), respectively. Experimental plots of I vs. t⁻¹/² were employed with the best fits for different concentrations of folic acid (Fig. 6A). The slopes of the resultant straight lines were then plotted against folic acid concentrations (Fig. 6B). From the resultant slope and the Cottrell equation, the mean values of D were found to be 1.3 × 10⁻⁵ cm²/s for folic acid.

3. 5. Calibration Plots and Limits of Detection

The electro-oxidation peak currents of folic acid at the surface of NFO/SPE can be used to determine folic acid in the solution. Since differential pulse voltammetry (DPV) has the advantage of having an increase in sensitivity and better characteristics for analytical applications,
DPV experiments were performed by using NFO/SPE in 0.1 M PBS containing various individual concentrations of folic acid (Fig. 7). The results show that the electrocatalytic peak currents of folic acid oxidation at the surface of NFO/SPE were linearly dependent on folic acid concentrations over the range of 1.0–500.0 µM, while the detection limit (3σ) was obtained as 0.023 µM. These values are comparable with values reported by other research groups for electrocatalytic oxidation of levodopa at the surface of chemically modified electrodes (see Table 1).

3.6. Interference Study

We investigated the effect of various interfering species on measuring 20.0 µM FA. The tolerance limit was adjusted as the concentration of foreign ions causing ±5% error in the determination. Based on the obtained results, the tolerance limit for Na+, Cl− and K+ was 0.1 M; for Mg²⁺ and Ca²⁺ it was 0.05 M; for L-lysine, glucose, sucrose, lactose, citric acid, fructose methanol, ethanol, L-asparagine, alanine, phenylalanine, glycine and NADH it was 0.004 M.

3.7. Real Sample Analysis

In order to evaluate the analytical applicability of the proposed method, it was applied to determine folic acid in folic acid tablets and urine samples by using the standard addition method. The results for the determination of the folic acid in real samples are given in Table 2. Satisfactory recoveries of the experimental results were found for folic

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**Table 1.** Comparison of analytical parameters for the determination of folic acid by various electrodes.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Modifier</th>
<th>LOD (M)</th>
<th>LDR (M)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon paste</td>
<td>multiwall carbon nanotubes</td>
<td>$1.10 \times 10^{-6}$</td>
<td>$4.6 \times 10^{-6} - 152.0 \times 10^{-6}$</td>
<td>33</td>
</tr>
<tr>
<td>Carbon paste</td>
<td>(DEDE) and NiO/CNTs nanocomposite</td>
<td>$0.90 \times 10^{-6}$</td>
<td>$3.0 \times 10^{-6} - 550.0 \times 10^{-6}$</td>
<td>34</td>
</tr>
<tr>
<td>Carbon paste</td>
<td>ZrO₂ nanoparticles</td>
<td>$9.86 \times 10^{-6}$</td>
<td>$2.0 \times 10^{-5} - 2.5 \times 10^{-3}$</td>
<td>35</td>
</tr>
<tr>
<td>Glassy carbon</td>
<td>graphene/MWCNT nanocomposite</td>
<td>$0.09 \times 10^{-6}$</td>
<td>$10.0 \times 10^{-6} - 170.0 \times 10^{-6}$</td>
<td>36</td>
</tr>
<tr>
<td>Carbon paste</td>
<td>Ruthenium(II) Complex-ZnO/CNTs nanocomposite</td>
<td>$1.00 \times 10^{-6}$</td>
<td>$3.0 \times 10^{-6} - 700.0 \times 10^{-6}$</td>
<td>37</td>
</tr>
<tr>
<td>Screen printed</td>
<td>NiFe₂O₄ nanoparticles</td>
<td>$3.40 \times 10^{-8}$</td>
<td>$1.0 \times 10^{-7} - 5.0 \times 10^{-4}$</td>
<td>This Work</td>
</tr>
</tbody>
</table>
Table 2. Determination of folic acid in folic acid tablet and urine samples. All the concentrations are in μM (n=5).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked</th>
<th>Found</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Folic acid tablet</td>
<td>0</td>
<td>15.0</td>
<td>–</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>17.8</td>
<td>101.7</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>19.5</td>
<td>97.5</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>7.5</td>
<td>23.3</td>
<td>103.5</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>24.8</td>
<td>99.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Urine</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>10.3</td>
<td>103.0</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>20.0</td>
<td>19.9</td>
<td>99.5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>29.1</td>
<td>97.0</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>40.0</td>
<td>40.5</td>
<td>101.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

acid. The reproducibility of the method was demonstrated by the mean relative standard deviation (RSD).

4. Conclusion

In this work, NiFe₂O₄ nanoparticles have been employed as a modifier in the modification of SPEs. A novel sensor has been developed, which provides an extremely sensitive and selective method for determination of folic acid. At the optimum pH of 7.0, the oxidation of FA occurs at a potential about 670 mV which is about 70 mV more negative than the unmodified SPE. Based on differential pulse voltammetry (DPV), the oxidation of LD exhibited a dynamic range between 1.0 × 10⁻⁶ and 5.0 × 10⁻⁵ M and a detection limit (3σ) of 3.4 × 10⁻⁸ M. The proposed protocol demonstrated a novel, simple, portable, inexpensive, and easy-to-use fabrication method to measure folic acid concentrations in folic acid tablet and urine samples with good analytical performance.

5. References

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