Original Paper

Application of a Single-Wall Carbon Nano-Tube Film Electrode to the Determination of Trace Amounts of Folic Acid

Chenghang Wang, Chunya Li, Li Ting, Xiuling Xu, and **Changfa Wang***

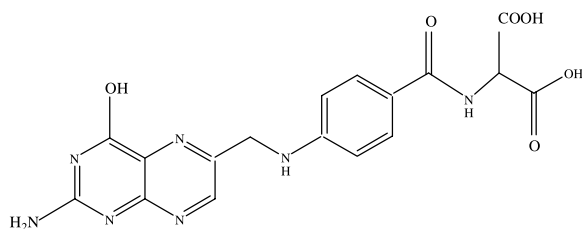Department of Chemistry, Wuhan University, Wuhan 430072, China

Abstract. Single-wall carbon nano-tubes were used to modify the surface of a glassy carbon electrode (GC) and applied in the determination of folic acid with voltammetry. The experiments demonstrated that the presence of a carbon nano-tube film on the electrode greatly increased the reduction peak current of folic acid. Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were used in a comparative investigation of the electrochemical reduction of folic acid with the film electrode. Effects of pH on the peak current and the peak potential were studied in the pH range of 4.0–8.0 with Britton-Robinson buffer solution. The reduction peak current was found to be linearly related to folic acid concentration over the range of 1×10^{-8} to $1 \times 10^{-4} \, \text{mol} \, \text{L}^{-1}$ with a detection limit of $1 \times 10^{-9} \, \text{mol} \, \text{L}^{-1}$ after 5 min accumulation. The film electrode provides an efficient way for eliminating interferences from some inorganic and organic species in the solution. The high sensitivity, selectivity and stability of the film electrode demonstrate its practical application from a simple and rapid determination of folic acid in tablets.

Key words: Single-wall carbon nano-tube; folic acid; modified electrode; voltammetry.

  * Author for correspondence. E-mail: chenghangwangwhu@163.com

Carbon nanotube (CNTs) is molecular-scale wires with high electrical conductivity, extremely high mechanical strength and can be divided into two categories: single-wall carbon nanotubes (SWNTs) and multi-wall carbon nanotubes (MWNTs). Compared with MWNTs, SWNTs is a well-defined system in terms of electronic properties. Individual SWNTs can be regarded as quantum wires [1]. However, the electrochemistry of SWNTs is less studied so far. Some is reported about the cast films of SWNTs on Pt and Au electrodes, but the films did not show well-resolved voltammograms [2]. SWNTs are treated with nitric acid during the purification process, and subsequently cast on a glassy carbon (GC) electrode to form a carbon nano-tube film electrode. The film electrode shows very stable electrochemical behavior and can be used to catalyze the electrochemical reaction of some biomolecules such as 4-amiophenol [3] and hemoglobin [4].

Folic acid, N-[p-[[(2-Amino-4-hydroxy-t-pteridinyl) methyl]amino]-benzoyl]-L]glutamic acid [5] (Fig. 1), is vitamin B found in some enriched food and vitamin pills. Folic acid deficiency is believed to be the most common vitamin deficiency in the world due to processing, food selection, and intestinal disorders. In the body, folic acid may be converted into any of the active forms of folic acid. Folic acid acts as a co-enzyme in several single electron transfer reactions to synthesize DNA, RNA and protein components.

Fig. 1. Schematic of folic acid

Table 1. Determination of the content of folic acid in the tablets

No.	Found (10^{-6} mol L^{-1})	Added (10^{-6} mol L^{-1})	Average (n = 5) (10^{-6} mol L^{-1})	Recovery (%)
1	0.5636	0.25	0.8131	99.8
2	0.3120	0.10	0.4147	102.7
3	38.37	10.0	48.17	98.03

Folic acid is a hematopoietic vitamin occurring free or combined with one or more additional molecules of l-glutamic acid in liver, kidney, mushrooms, spinach, yeast and green leave grasses [6]. Folic acid deficiency causes anemia. In view of the preceding, analytical methods for determination of this important bioelement are needed.

In the literature, various methods of folic acid determination have been reported, for example, fluorometric [7], spectrophotometric [8, 9], high-performance liquid chromatography (HPLC) [10–12], Capillary electrophorese (CE) [13]. Electrochemical methods have also been used to detect folic acid and to investigate its redox properties [14–16]. Cathodic stripping voltammetry has been used previously to detect low nanomolar levels of folic acid in weakly acidic solution and synthetic electrolytes [17, 18].

In this work, SWNTs was dispersed in DHP solution and cast on a GC electrode to form a carbon nano-tube film electrode. The film electrode can used to catalyze the electrochemical reduction of folic acid. An electrochemical procedure for the determination of folic acid based on the film electrode has been proposed. The results suggest that the procedure possesses following advantages: rapid response, low detection limit, excellent reproducibility, low cost and simplicity.

Experimental

Reagents

Folic acid was obtained from Sigma Chemical Company (USA). A Stock standard solution of folic acid (1.0 × 10^{-3} mol L^{-1}) was prepared from 0.2 mol L^{-1} NaOH and stored in a refrigerator in the dark until used. Working solution was prepared by the dilution of the standard solution with 0.1 mol L^{-1} Britton-Robinson (B-R) buffer solution (pH = 5.5). Dihexadacyl hydrogen phosphate (DHP) was purchased from Fluka. Other chemicals were of analytical grade, and all compounds were used without further purification. All solutions were prepared with redistilled water.

Single-wall carbon nano-tubes (obtained from Institute of nanometer materials, central China normal university) was synthesized by a catalytic pyrolysis method and treated with nitric acid during purification.

Apparatus

Electrochemical data were obtained with a three-electrode system using a CHI 660A electrochemical workstation (Shanghai Chenhua Co., China), including a SWNTs-film electrode as the working electrode, a saturated calomel electrode as the reference electrode (SCE) and a Pt wire as the counter electrode.

Preparation of SWNTs-film Electrode

0.5 mg of SWNTs and 1.0 mg of DHP were dispersed in 5 mL of redistilled water by ultrasonication agitation for about 25 min to give 0.1 mg mL^{-1} blank suspension. Prior to modification, GCE was mechanically polished to mirror finished with polish paper and 0.3–0.5 μm alumina slurry, and cleaned thoroughly in an ultrasonic cleaner with 1:1 nitric acid solution, alcohol and redistilled water. Finally, GCE was coated with 10 μL of 1 mg mL^{-1} SWNTs-DHP suspension and allowed to evaporate water at room temperature in the air. The DHP-film coated GCE was prepared by the same procedure without SWNTs.

Procedure

10 mL 0.1 mol L^{-1} B-R buffer solution (pH = 5.5) containing a specific amount of samples was added to the cell. The accumulation step was carried out under open-circuit with stirring for 5 min, then linear sweep voltammograms from −0.3 to −1.2 V were recorded after 25 s quiet time, and the reductive peak current was measured at −0.660 V. After each measurement, the SWNTs-film electrode undergoes successive cyclic voltammetric sweeping in pH 5.5 B-R buffer (between 0.4 to −1.4 V at 100 mV s^{-1}) to give reproducible electrode surface.

Sample Preparation

Folic acid tablets were obtained from Changzhou Pharmaceutial factory Co. LTD. (Guangzhou, China). 2.0 tablets was triturated and distilled by the appropriate solution of NaOH. After centrifugation for 5 min at 3000 rpm, the extract was filtered with 0.45 μm membrane filters, and then the solution was diluted to 100 mL with B-R buffer solution and stored in a refrigerator in dark until used.

Results and Discussion

Electrochemical Behavior of Folic Acid

Figure 2 shows voltammograms of folic acid at bare GCE (curve a) and the SWNTs-film electrode (curve b).
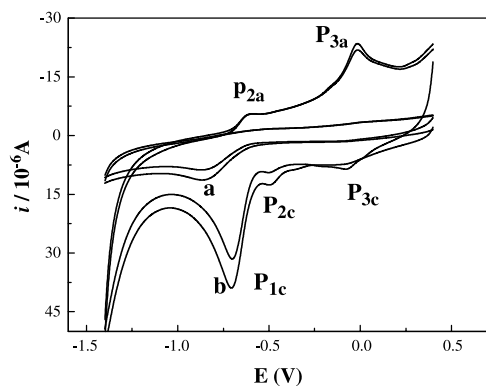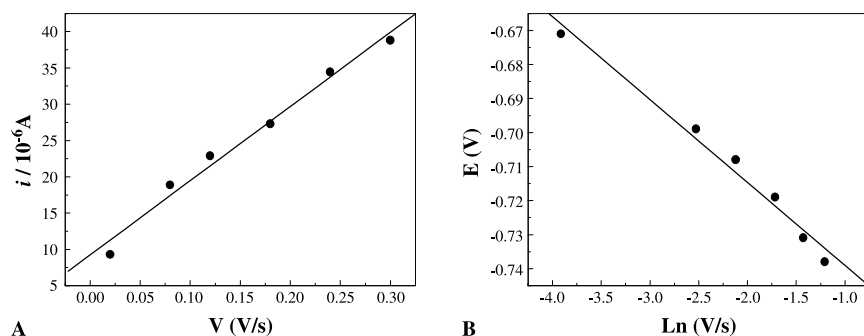
Fig. 2. Cyclic voltammograms for 1.0×10^{-4} mol L^{-1} folic acid at bare GCE (a) and the SWNTs-film electrode (b) Scan rate 100 mV s^{-1}

Fig. 3. Linear sweep voltammograms of 1.0×10^{-4} mol L^{-1} folic acid at the SWNTs-film electrode with different scan rate (v). Curve a to f is corresponding to 20, 50, 80, 100, 120, 180, 200, 300 mV s^{-1}
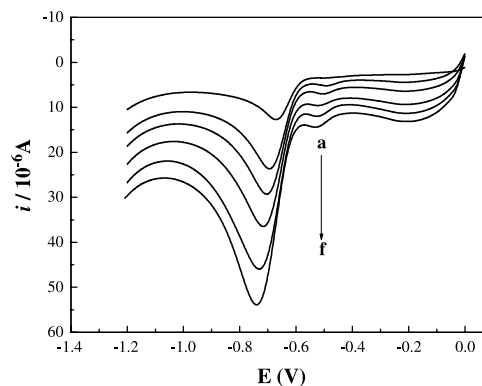
At the bare GC electrode, a single broad reduction peak was observed at -0.71 V, indicating an irreversible electrochemical process. As shown in curve b, two couples of redox peaks between 0.4 and -1.4 V can be observed at the film electrode except for an irreversible reduction peak. At the film electrode, the peak current has increased greatly. This might be attributed to the surface volume ration of SWNTs and its catalytic ability towards folic acid. Another contribution would be the increase of the active surface of the electrode when modified with carbon nano-tube film. Compared with that obtained at the bare GCE, the reduction peak potential (E_{p1c}) has shifted positively about 150 mV and the reduction overpotential decreased significantly, indicating that SWNTs film possesses catalytic property towards folic acid. Thus, in the further work, the study was focused mainly on the reduction peak (E_{p1c}) due to its high sensitivity.

Influence of Scan Rate

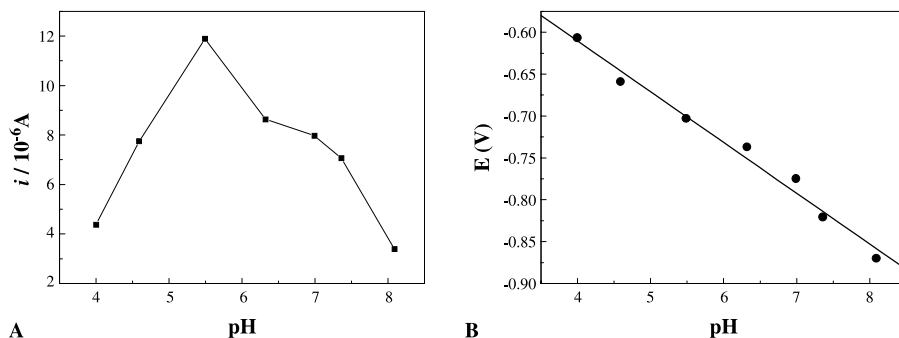Influence of scan rate on the reduction peak potential and current at the SWNTs-film electrode was investigated in the range of 20–300 mV s^{-1} for 1.0×10^{-4} mol L^{-1} folic acid by linear sweep voltammetry (Fig. 3). It was found that the reduction peak current was directly linear to scan rate from 20 to 300 mV s^{-1} (Fig. 4A), indicating that the electrochemical reduction process of folic acid at the film electrode is an adsorption-controlled process. With scan rate increasing, the peak potential (E_{p1c}) shifted negatively. According to Laciron's conclusion [19], the relationship between E_{p1c} and v was examined. It was found that E_{p1c} depends linearly on the logarithm of v as following equation: $E = -0.7634 - 0.02439 \ln v$ (Fig. 4B). The value of αn_a can be calculated from the slope of the plot (b) according to $b = RT/\alpha n_a F$, where α of the totally irreversible electrode process is assumed as 0.5, thus, the calculated n_a value should be 2.07. It indicates that two electrons are involved in the reduction process of folic acid. The result is consistent well with other reports [20, 21] However, for scan rates higher than 300 mV s^{-1}, the plot became non-linear, showing an upward curvature as result of the more intense peak current from the values predicted for this dependence.

Fig. 4. Effect of scan rate on the reduction peak current. Other conditions are the same as in Fig. 3

Influence of pH

Dependence of pH values on the reduction peak potential and peak current of folic acid at the film electrode was investigated by linear sweep voltammetry. With the increase of pH, the reduction peak potential shifted negatively, indicating the participation of the protons in the electrochemical process. For pH level between 4.0 and 8.09, the linear dependence fits the equation: $E_{\mathrm{p1c}} = -0.368 + 0.061\mathrm{pH}$ (Fig. 5A). According to Nernst equation: $E_{\mathrm{p}} = E^{0} - 2.303RTm\mathrm{pH}/\alpha nF$ [22], where m is the proton transfer numbers and n is the electron transfer numbers. 0.061 V per pH unit suggests that the equitation of electron and proton numbers have been transferred in the electrochemical process. The result means that two protons have taken part into the reduction of folic acid at the film electrode. Curve B in Fig. 5 shows the reduction peak current change in the given pH range. The peak current increased first with following decrease and a maximum value was obtained at pH 5.49. Therefore, pH = 5.49 was selected for the determination of folic acid at the film electrode.

Influence of the Film Thickness

Influence of the thickness of the SWNTs-film on the reduction peak current was investigated. When 10 μL SWNTs-DHP suspensions were used to cast on the surface of the electrode, the reduction peak current can reach its maximum value. Further increasing the amount of SWNTs-DHP suspension, the peak currents change slightly. However, when the amount of SWNTs-DHP suspension exceeds 20 μL, the peak current conversely decrease. SWNTs are an ideal electrode material with excellent electrical conductivity and high aspect ratio. In principle, the reduction peak current is almost independent of the SWNTs film thickness. However, DHP is an insulator so that it can produce uncompensated resistive effects and prohibit the charge transfer of folic acid at the film electrode. Therefore, the reduction peak current decreases when the film is too thick.

Influence of the Accumulation Time

Accumulation step is usually a simple and effective way to enhance the sensitivity. Accumulation potential and time are two crucial parameters for the accumulation step. The reduction peak current of $4.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ folic acid was compared after 5 min accumulation at different accumulation potential by LSV. The peak current almost does not change with the accumulation potential shifting from -300 to 600 mV, revealing that the accumulation potential has no influence on the reduction peak current of folic acid at the SWNTs-film electrode. Thus, the accumulation of folic acid was carried out under open circuit.

Unlike the accumulation potential, accumulation time severely influences the reduction peak current, and is illustrated in Fig. 6. The reduction peak current increases significantly in the first 5 min, and the levels
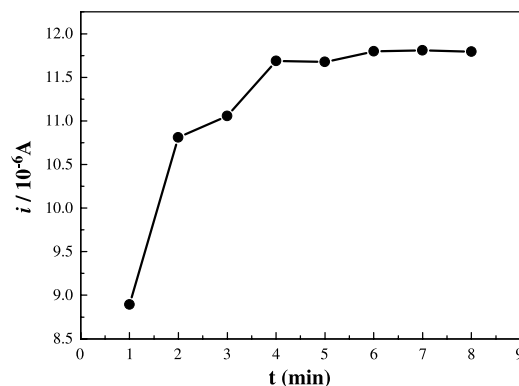
Fig. 6. Influences of accumulation time on the reduction peak current of $4.0 \times 10^{-5}\,\mathrm{mol\,L^{-1}}$ of folic acid at the SWNTs-film electrode. Other conditions are the same as in Fig. 3
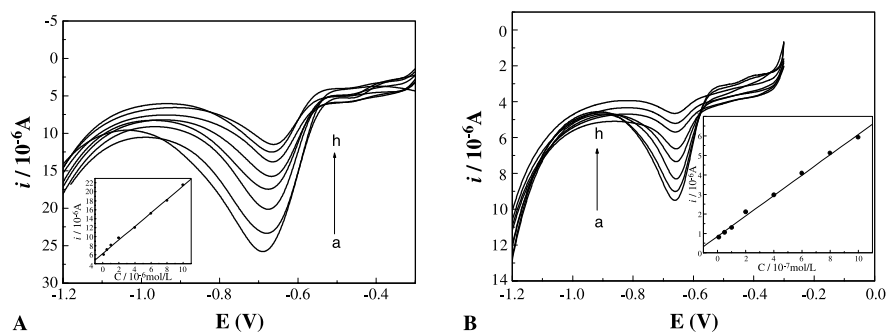
Fig. 7. Linear sweep voltammograms for the different concentration of folic acid at the SWNTs-film electrode. The insert graphs are the relationship between the reduction peak current and the folic acid concentration

off. This may be caused by the saturated adsorption of folic acid on the surface of the SWNTs-film electrode. Thus, the accumulation step in this study was performed under open-circuit for 5 min.

Calibration Graph

The calibration curve for the determination of folic acid was obtained with linearly sweep voltammetry (LSV). The linear sweep voltammograms of various concentrations of folic acid are illustrated in Fig. 7. Under the optimized conditions, the reduction peak current is linear over the range from 1.0×10^{-4} to $1.0 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ with a regression equation: $I_p\,(\mu A) = 6.3067 + 1.4995\,C$ $(C = 10^{-5}\,\mathrm{mol\,L^{-1}},\ r = 0.9984)$. Then, For concentration level between 1.0×10^{-6} to $1.0 \times 10^{-8}\,\mathrm{mol\,L^{-1}}$, the linear dependence fits the equation: $I_p\,(\mu A) = 0.8518 + 0.5223\,C$ $(C = 10^{-7}\,\mathrm{mol\,L^{-1}},\ r = 0.9963)$. The detection limit for the determination of folic acid at this film electrode is $1.0 \times 10^{-9}\,\mathrm{mol\,L^{-1}}$ after 5 min accumulation.

Reproducibility and Stability

A repetitive experiment was carried out with $1.0 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ folic acid solution. The relative standard derivation for eight repetitive measurements is 3.41%. The results indicate that the film electrode displays excellent reproducibility. The film electrode that was left to exposure in the air is stable for at least two weeks. The reduction peak current of $1.0 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ folic acid remained almost 96.3% of its original response. On the other hand, the porous structure and large surface area of the film electrode encourage folic acid to penetrate the interfacial layer easily, which is not beneficial for the subsequent determination. Therefore, for practical applications, the electrode must be cleaned thoroughly and simply

in the electrochemical determination of folic acid. During the experiments, a reproducible film electrode can be obtained by cyclic voltammetric scan in the potential range from 0.4 to $-1.3\,\mathrm{V}$ in a blank buffer solution.

Interferences

To evaluate the interference of foreign compounds on the determination of folic acid at the $1 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$ level, a systematic study was carried out. It was found that Cu^{2+}, Fe^{3+}, Zn^{2+}, Ca^{2+}, ascorbic acid, dopamine and uric acid at 100-fold concentration almost does not interfere the current response of $1 \times 10^{-6}\,\mathrm{mol\,L^{-1}}$, due to its peak current changes below 5%. These results indicated that the film electrode has good selectivity for the determination of folic acid. However, 20-fold concentration of adenine, 8-azaguanine interferes the determination of folic acid severely, because they possess the same electroactive group.

Application

In order to testify its practical application, the film electrode was used to determine the content of folic acid in the tablets. The folic acid concentration was determined by a standard addition method. A volume of folic acid sample solution was added into a cell containing 10 mL of a B-R buffer solution (pH = 5.49), and the peak current of folic acid were measured by linear sweep voltammetry after 5 min accumulation. Then, specific amount of folic acid standard solution was added and the peak current was measured again. From the difference of the peak currents, the concentration of folic acids in the samples can be obtained. The results are demonstrated in Table 1, and the recoveries are in the range from 98.03 to 102.7%.

Conclusions

Single-wall carbon nano-tubes are easily cast onto the surface of a glass carbon electrode to form a SWNTs-film electrode. The film electrode displays a strongly electrocatalytic activity towards the redox reaction of folic acid. Based on this property, the concentration of folic acid has been determined directly by voltammetry with excellent sensitivity. Sufficient experimental results demonstrated that the film electrodes might be a good electrochemical sensor for the direct measurements of folic acid.

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