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ORIGINAL PAPER

Electrochemical oxidation behavior of methotrexate at DNA/SWCNT/Nafion composite film-modified glassy carbon electrode

Yanwei Wang · Hui Liu · Fei Wang · Yumei Gao

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Abstract Glassy carbon electrode modified with DNA-functionalized single-walled carbon nanotube (DNA/SWCNT) and Nafion composite film was developed for the detection of methotrexate. The characteristics of the modified electrode were examined by transmission electron microscopy and cyclic voltammetry. Compared with a bare glassy carbon electrode and Nafion- and SWCNT/Nafion-modified electrodes, the DNA/SWCNT/Nafion-modified one exhibited the more superior ability of detecting methotrexate, including the higher sensitivity and the lower overpotentials, due to the synergetic DNA-functionalized SWCNT and Nafion. Also, the dependence of the current on pH, nature of buffer, instrumental parameters, accumulation time, and potential was investigated to optimize the experimental conditions in the determination of methotrexate. Under the selected conditions, the modified electrode in pH=2.78 Britton–Robinson buffer solutions showed a linear voltammetric response to methotrexate within the concentration range of 2.0×10^{-8}–1.5×10^{-6} mol L^{-1}, with the detection limit of 8.0×10^{-9} mol L^{-1}. The method was also applied to detect methotrexate in medicinal tablets and spiked human blood serum samples.

Keywords Methotrexate · DNA/SWCNT/Nafion · Electrochemical oxidation · Square wave voltammetry

Y. Wang (✉) · F. Wang · Y. Gao
Department of Material and Chemistry Engineering,
Henan Institute of Engineering,
Zhengzhou 450007, People's Republic of China
e-mail: ywwang2012@163.com

H. Liu
Department of Chemistry, Zhengzhou Normal University,
Zhengzhou 450044, People's Republic of China

Introduction

Methotrexate is an antifolate in a class of folic acid analogs that have demonstrated effective antineoplastic activity for the treatment of disorders of cell proliferation. The structure of methotrexate is shown in Fig. 1. Clinical trials conducted during the last few decades have demonstrated the efficacy of methotrexate for the treatment of various human neoplastic disorders, including childhood acute leukemia [1], head and neck cancer [2], and micrometastases of osteosarcoma [3]. Thus, the study of its active mechanism and development of a rapid, simple, and sensitive method in the determination of methotrexate is of great importance and interest. So far, some analytical methods, including spectrophotometry [4], fluorimetry [5, 6], chemiluminescence [7], high-performance liquid chromatography [8–10], and capillary electrophoresis [11–13], have been applied to determine methotrexate. Recently, interest has been increasing in the application of simple, sensitive, rapid, and inexpensive electrochemical methods in the analysis of pharmaceutical drugs. These methods do not require tedious pretreatment and involve limited preseparation and consequently reduce the cost and time of analysis. Moreover, the techniques also help for identifying the redox of drug compounds and provide important information about pharmacological actions. At present, the report regarding the electrochemistry and voltammetric determination of methotrexate is raised [14–17] but very limited.

Carbon nanotubes (CNTs), discovered by Iijima [18], have attracted much attention in the perspective of electrochemical sensor design because of their unique geometrical, mechanical, electronic, and chemical properties [19]. However, the major challenge for developing such CNT-based devices is the insolubility of CNTs in all solvents. To resolve the problem and to broaden the scope of analytes to be

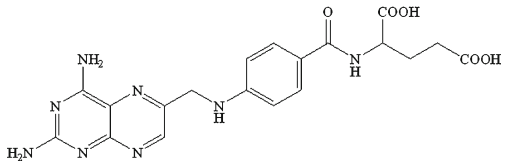

Fig. 1 The chemical structure of methotrexate

detected, the most common route is to functionalize the surface of CNTs with specific bio/chemical molecules [20], and many research activities have addressed the generation of DNA-functionalized CNTs hybrids [21–23], which further expanded the scope of CNTs in sensor applications [24–31].

In this work, a novel electrochemical sensor, based on the DNA-functionalized single-walled carbon nanotube (DNA/SWCNT) and Nafion composite film-modified glassy carbon electrode (GCE), was developed and used for the detection of trace amounts of methotrexate. Here, Nafion can increase the immobilization stability of DNA/SWCNT on the GCE surface due to its excellent film-forming ability, and Nafion can also attract the positive-charged methotrexate to the electrode surface effecting as accumulation. The electrochemical response of methotrexate at the modified electrode was investigated by cyclic voltammetry (CV), square wave voltammetry (SWV), and chronocoulometry (CC), and the results reveal that DNA/SWCNT/Nafion cast film can remarkably enhance the electrooxidation signal of methotrexate as well as its determining sensitivity. Based on these, a simple and sensitive electrochemical method was proposed for detecting the methotrexate in medicinal tablets and spiked human blood serum samples.

Experimental

Apparatus and reagents

Model CHI 650A electrochemical system (CHI Instrumental, Shanghai, China) was employed for electrochemical techniques. A transmission electron microscope (Tecnai $G^2$20S-TWIN, FEI Company, Holland) was employed to observe the morphology. A standard three-electrode electrochemical cell was used with a glassy carbon electrode (d=3 mm) or modified GCE as the working electrode, platinum (Pt) wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode (the internal solution was saturated KCl solution). All the pH measurements were made with a PHS-3 C precision pH meter (Leici Devices Factory of Shanghai, China), which was calibrated with a standard buffer solution at 25±0.1 °C everyday.

All reagents were of analytical grade and were used as received. Double-distilled water was used for all preparations. Methotrexate was purchased from Fluka (Buchs, Switzerland), and fish sperm dsDNA was supplied by Shanghai Sangon Company (Shanghai, China). Stock solutions (5.0×10^{-4} mol L^{-1}) of methotrexate were prepared with 0.2 mol L^{-1} NaOH and stored at 4 °C in the dark. SWCNT (95 % purity) was obtained from Beijing Nachen S&T Ltd. Nafion (5 %, *w*/*v* in alcoholic solution) was purchased from Alfa and was diluted to 0.2 % (*w*/*v*) with ethanol before use. Human blood samples were obtained from healthy volunteers and treated prior to usage.

Fabrication of the DNA/SWCNT/Nafion composite film-modified GCE

The wrapping of SWCNT by dsDNA was prepared according to the literature [20]. First, 2 mg SWCNT was added into 2 mL dsDNA (2 mg mL^{-1} in 0.1 mol L^{-1} NaCl) and then was sonicated in an ice-water bath for 2 h; the mixture was then centrifuged for 1.5 h at 10000 rpm to remove the very large aggregates that were not dispersed during the initial sonication, leaving a DNA/SWCNT hybrid solution. Finally, 1.0 mL DNA/SWCNT solution was added into 1.0 mL 0.2 % Nafion solution, followed by ultrasonication for 0.5 h to form a homogenous mixture of DNA/SWCNT/Nafion.

Before modification, the bare GCE was polished to a mirror-like appearance with 0.3- and 0.05-μm slurries; then washed successively with redistilled deionized water, anhydrous alcohol, and redistilled deionized water in an ultrasonic bath; and dried by N_2 blowing. Then, 5 μL of DNA/SWCNT/Nafion solution was deposited on the freshly prepared GCE surface. After the solvent evaporated, the electrode surface was thoroughly rinsed with redistilled deionized water and dried in the air. The obtained electrode was noted as DNA/SWCNT/Nafion/GCE. For comparison, Nafion- and SWCNT/Nafion-modified GCEs were fabricated with the similar procedure, marked as Nafion/GCE and SWCNT/Nafion/GCE.

Tablets assay procedure

The sample powder was obtained by grinding one tablet (Shanghai Sine Pharmaceutical Co., Ltd.). The powder was extracted with 20 mL of 0.1 mol L^{-1} NaOH for 30 min in an ultrasonic bath. Then, the solution was filtered into a 100-mL volumetric flask through an ordinary filtration paper, and then the solution was diluted to the exact volume with distilled water. Sample solution was stored in the dark. Just

before each measurement, the sample solution was diluted quantitatively with the supporting electrolyte.

Analysis of spiked serum samples

Serum samples of healthy individuals (after having obtained their written consent) were stored frozen until assay. Acetonitrile removes serum proteins more effectively; to add 1.5 volumes of serum is enough to remove the proteins. After vortexing for 45 s, the mixture was then centrifuged for 30 min at 5000 rpm to get rid of serum protein residues and the supernatant was taken carefully. Suitable volumes of this supernatant were transferred into the volumetric flask and diluted up to the volume with distilled water. Sample solution was stored in the dark. Just before each measurement, the sample solution was diluted quantitatively with the supporting electrolyte.

Results and discussion

Transmission electron microscope characterization of DNA/SWCNT/Nafion

Transmission electron microscope (TEM) was employed to observe the morphology of DNA/SWCNT/Nafion film. The distinct structure of SWCNT and DNA was observed in Fig. 2. These data indicate that the DNA/SWCNT/Nafion composite film was successfully obtained just as designed.

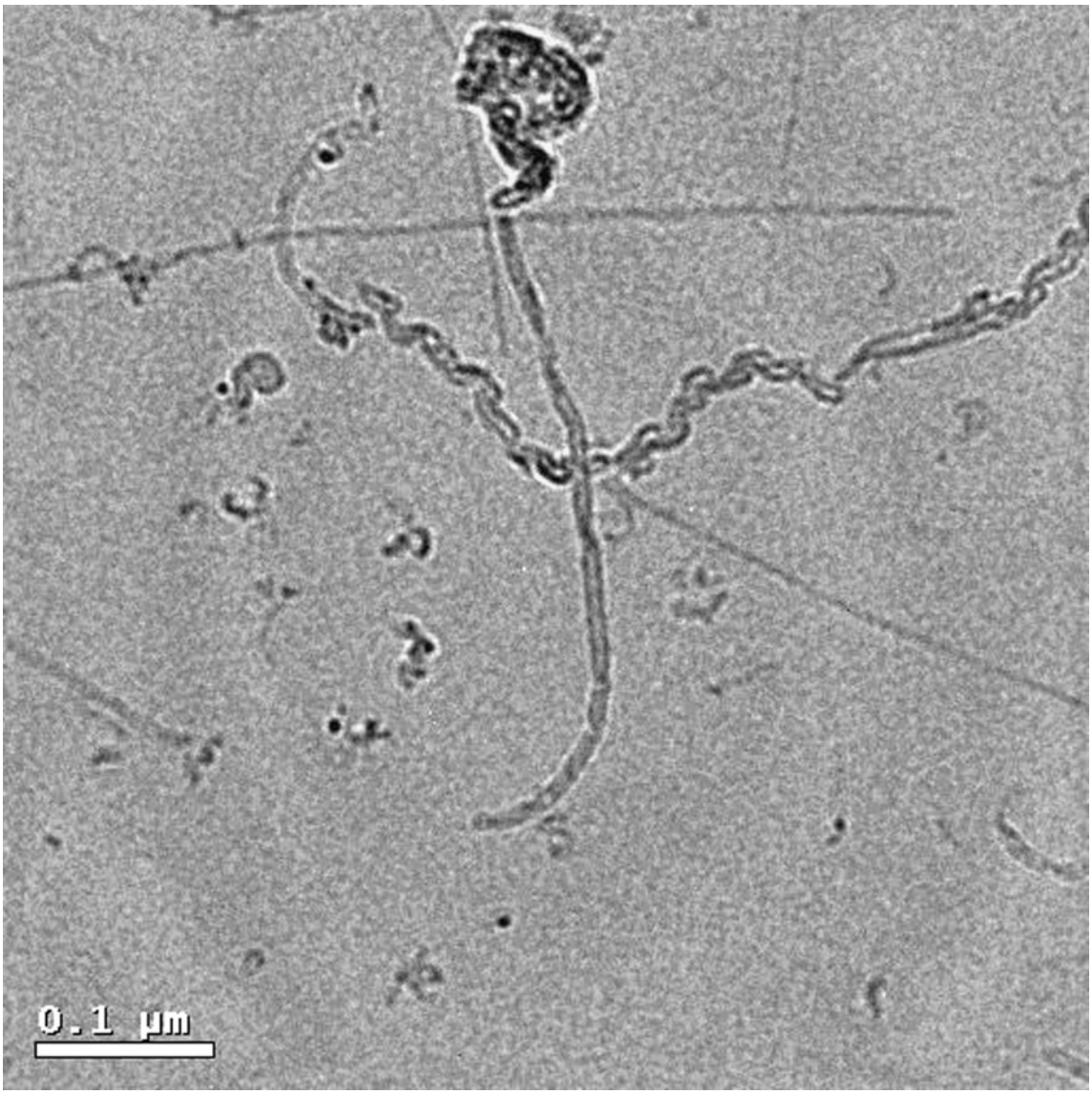

Fig. 2 TEM image of DNA/SWCNT/Nafion film (acceleration voltage, 200 kV; metallization of the surface, copper)

Electrochemical characterization of the electrode

Figure 3 shows the cyclic voltammograms of the bare GCE (curve a), Nafion/GCE (curve b), SWCNT/Nafion/GCE (curve c), and DNA/SWCNT/Nafion/GCE (curve d) obtained in 2×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$ solution containing 0.1 mol L^{-1} KCl. Well-defined CV, a characteristic of a diffusion-limited and reversible electron transfer redox process, was observed at the bare GCE. When the electrode was coated with Nafion, the redox peaks disappeared completely, which could be attributed to the negatively charged Nafion, blocking the diffusion of $Fe(CN)_6^{3-}$ from the solution to the electrode surface. Nevertheless, after SWCNT was immobilized on the Nafion/GCE, the redox peak current increased significantly compared with Nafion/GCE, indicating that SWCNT can effectively increase the electron transfer rate of $Fe(CN)_6^{3-}$ due to its large surface area and good conductivity. Compared with SWCNT/Nafion/GCE, the peak current of DNA/SWCNT/Nafion/GCE decreased but the peak-to-peak potential separation (ΔE_p) increased obviously, demonstrating that DNA had been immobilized on the surface of the SWCNT. The negatively charged phosphate skeletons of DNA immobilized on the SWCNT had a repulsive force to $[Fe(CN)_6]^{3-/4-}$ anion, and therefore, the current response of $[Fe(CN)_6]^{3-/4-}$ at the DNA/SWCNT/Nafion/GCE decreased significantly.

Electrochemical oxidation behavior of methotrexate at the DNA/SWCNT/Nafion/GCE

The cyclic voltammograms of the background (curve a) and 5.0×10^{-7} mol L^{-1} methotrexate (curve b) in pH=2.78 Britton–Robinson (B-R) buffer solutions were shown in the inset of Fig. 4. It is clear that there is only one anodic peak when the potential is scanned from 0.5 to 1.2 V. The oxidation process is not accompanied by a reduction wave, which indicates that the oxidation reaction is totally irreversible, and the voltammograms recorded from multicycles show that the peak current decreases with increasing number of cycles and tends to disappear finally. This fact may be partly attributed to the poor solubility of the oxidized product of methotrexate [15], which accumulated on the electrode surface during the electrochemical process. At the same time, we find that if the working electrode is kept in the electrolyte solution for 5 min, the working electrode surface will be restored to the initial state so that a new voltammogram then exhibits the same characteristics as those of the first cycle in the inset of Fig. 4. Therefore, in the following discussion, the peak current is taken from the first cycle.

Figure 4 compared the electrochemical responses of methotrexate (5.0×10^{-7} mol L^{-1}) at bare GCE (curve a), Nafion/GCE (curve b), SWCNT/Nafion/GCE (curve c), and DNA/SWCNT/Nafion/GCE (curve d). Methotrexate showed an electrochemical activation on all these four electrodes. At bare GCE, Nafion/GCE, and SWCNT/Nafion/GCE, a weak anodic peak could be discerned (Fig. 4, curves a, b, c). In contrast, a distinct well-defined anodic peak appeared at the DNA/SWCNT/Nafion/GCE under the same experimental condition (Fig. 4, curve d), of which the i_p is about 20-, 6-, and 2-fold higher than that of bare GCE, Nafion/GCE, and SWCNT/Nafion/GCE, respectively, and the E_p at the DNA/SWCNT/Nafion/GCE is the lowest. This result indicated that DNA/SWCNT/Nafion/GCE can

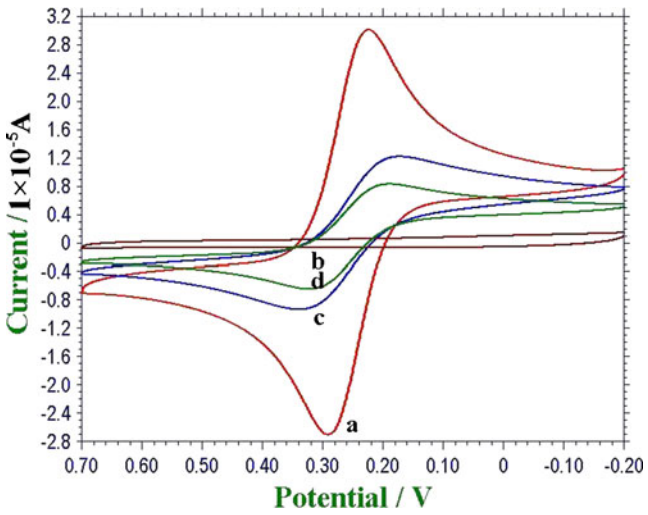

Fig. 3 Cyclic voltammograms of 2.0×10^{-3} mol L^{-1} $K_3[Fe(CN)_6]$+ 0.1 mol L^{-1} KCl solution at a clean, freshly polished bare GCE (*curve a*), Nafion/GCE (*curve b*), SWCNT/Nafion/GCE (*curve c*), and DNA/SWCNT/Nafion/GCE (*curve d*) with scan rate v=0.10 Vs^{-1}

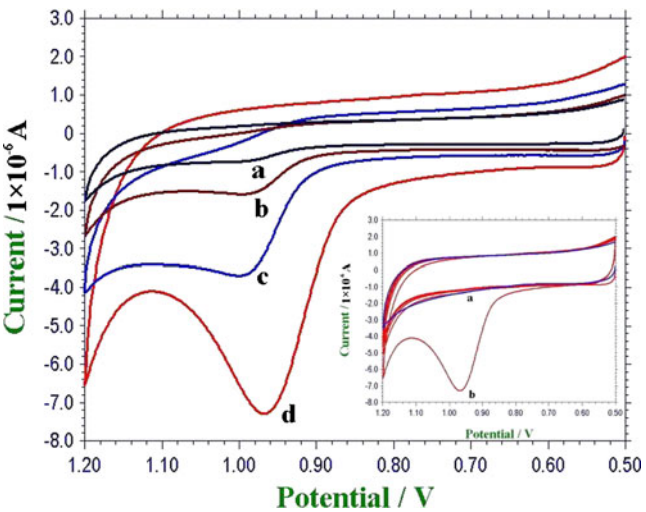

Fig. 4 Cyclic voltammograms of methotrexate (5.0×10^{-7} mol L^{-1}) at bare GCE (*curve a*), Nafion/GCE (*curve b*), SWCNT/Nafion/GCE (*curve c*), and DNA/SWCNT/Nafion/GCE (*curve d*) in pH=2.78 B-R buffer solutions with scan rate v=0.05 Vs^{-1}; the *inset* shows cyclic voltammograms of the background (*curve a*) and methotrexate (5.0×10^{-7} mol L^{-1}, *curve b*) in buffer solutions at the DNA/SWCNT/Nafion/GCE

significantly improve the response of methotrexate. We calculate that the reasons for the notable sensitivity of the methotrexate reaction at the DNA/SWCNT/Nafion/GCE may be summarized as follows: (1) methotrexate becomes positively charged in pH=2.78 B-R buffer solutions and the DNA/SWCNT/Nafion/GCE contains the cation exchanger of Nafion, which has an enriched ability for methotrexate due to an electrostatic interaction and (2) the interfusion of DNA/SWCNT into Nafion would improve the electrochemical response of methotrexate, and due to plenty π electrons, hydrogen bond binding sites, the large surface area, and good conductivity were provided [30, 31]. Without any doubt, the synergetic DNA-functionalized SWCNT and Nafion make contributions to the higher current response and detected sensitivity of methotrexate.

To further elucidate the electrode reaction of methotrexate at the DNA/SWCNT/Nafion/GCE, the influence of the potential scan rate (v) on i_{pa} of 5.0×10^{-7} mol L^{-1} methotrexate was studied by CV in different sweep rates from 50 to 300 mV s^{-1} (see Fig. 5). The peak currents of methotrexate grow with increasing v, and there are good linear relationships between i_{pa} and v, indicating that the oxide process of methotrexate at the DNA/SWCNT/Nafion/GCE was an adsorption-controlled irreversible reaction. Furthermore, the oxidation peak potentials shift positively with the increasing v, and good linear relationships are observed between oxidation peak potential (E_{pa}) and ln v (see the insets), with a good linear equation of E_{pa} (in volts)=1.045+0.042 ln v (in volts per second), R=0.992. According to Laviron's theory [32], from the slope of the straight line of E_p against ln v, αn=0.61 could be obtained. Here, according to the equation, $i_{pa}=n^2F^2vA\Gamma/4RT=nFQv/4RT$, n was calculated as 1, meaning that 1e transfer was involved. Thus, the value of α was 0.61. Meanwhile, the value of k_s of 1.75 s^{-1} was calculated from the intercept of the straight line of E_{pa} vs. ln v. The value of E^0 was determined to be 0.943 V from the intercept of E_{pa} vs. v plot on the ordinate by extrapolating the line to be v=0.

For the variation of solution pH from 2.0 to 5.0, it was found that the E_{pa} shifted to lower values as the pH increased, indicating an intervention of the protons in the electrochemical oxidation of methotrexate. The relationship between the E_{pa} and pH could be fitted into the regression equation: E_{pa} (in volts)=1.010−0.064 pH (R=0.999). The slope of d_{Ep}/d_{pH} plot as 64 mV pH^{-1} is close to 60 mV pH^{-1}

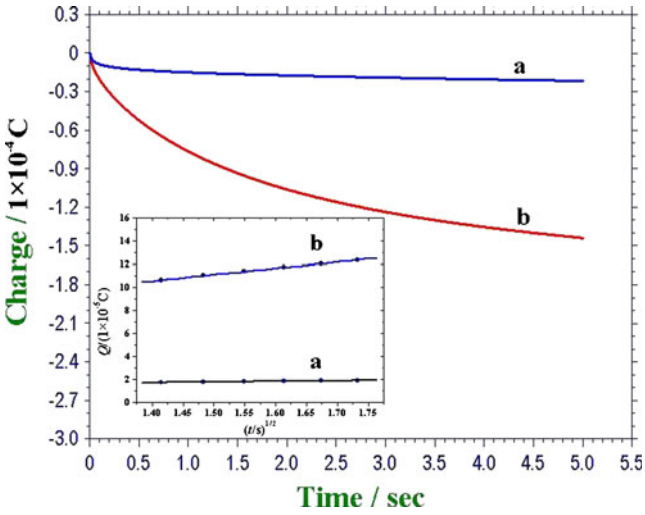

Fig. 6 Chronocoulometric curves of the background (*curve a*) and methotrexate (5.0×10^{-5} mol L^{-1}) (*curve b*) in pH=2.78 B-R buffer solutions at DNA/Nafion/SWNT/GCE. The *inset* shows the corresponding Q–$t^{1/2}$ plots

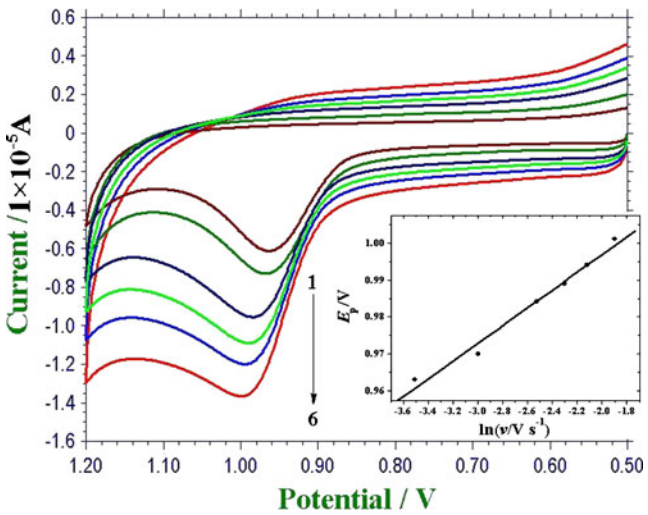

Fig. 5 CV curves of 5.0×10^{-7} mol L^{-1} methotrexate at the DNA/SWCNT/Nafion/GCE at different scan rates (from 1 to 6: 0.03, 0.05, 0.08, 0.10, 0.12, 0.15 Vs^{-1}); the *inset* shows the relationship of the peak potential E_{pa} against ln v; the other experimental conditions are the same as those described in Fig. 4

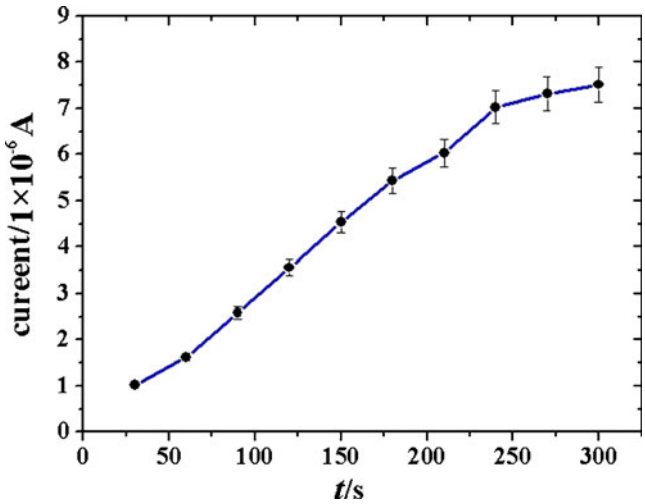

Fig. 7 Square wave anodic stripping voltammograms peak current vs. accumulation time plots for 5.0×10^{-7} mol L^{-1} methotrexate following accumulation under open circuit. Other experimental conditions are the same as those described in Fig. 4

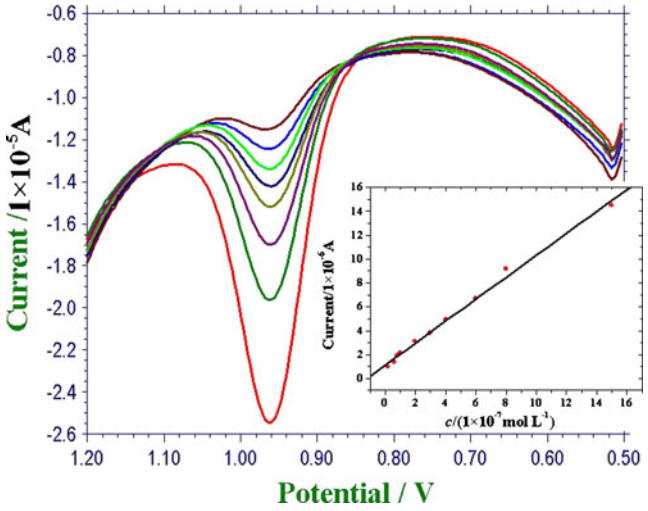

Fig. 8 Square wave anodic stripping voltammograms and their associated calibration plot (*inset*) for increasing concentrations of methotrexate at DNA/SWCNT/Nafion/GCE under optimum conditions; the methotrexate concentrations used are the following: (1) 2.0×10^{-8} mol L^{-1}, (2) 6.0×10^{-8} mol L^{-1}, (3) 8.0×10^{-8} mol L^{-1}, (4) 1.0×10^{-7} mol L^{-1}, (5) 2.0×10^{-7} mol L^{-1}, (6) 3.0×10^{-7} mol L^{-1}, (7) 4.0×10^{-7} mol L^{-1}, (8) 6.0×10^{-7} mol L^{-1}, (9) 8.0×10^{-7} mol L^{-1}, (10) 1.5×10^{-6} mol L^{-1}

is expected for the 1 H^+ per electron stoichiometry. Here, methotrexate belongs to pteridine groups, and the amino group at the C2 position on the pyrimidine ring is electroactive [15]. Therefore, the mechanism of the oxidation peak detected in this experiment should also correspond to the oxidation of the amino group at the C2 position on the pyrimidine [15].

Next, elctrooxidation of methotrexate at the DNA/SWCNT/Nafion/GCE was investigated by employing chronocoulometry (CC) for the determination of the saturating absorption capacity. The DNA/SWCNT/Nafion/GCE was immersed in a methotrexate solution (5.0×10^{-5} mol L^{-1}) for several minutes to achieve a saturated absorption, and then, a step potential from 0.5 to 1.2 V was applied. *Q–t* curve was recorded (Fig. 6, curve b) to calculate the saturated absorption capacity. For control, *Q–t* curve was also recorded in a blank solution (Fig. 6, curve a). The corresponding $Q–t^{1/2}$ plots were also performed and shown as the inset in Fig. 6.

According to the formula given by Anson [33],

$$Q = \frac{2nFAc(Dt)^{1/2}}{\pi^{1/2}} + Q_{dl} + Q_{ads}$$

where Q_{dl} is the double-layer charge and Q_{ads} is the Faradaic charge due to the oxidation of adsorbed methotrexate. Using Laviron's theory of $Q=nFAI^*$ and intercept difference between curves a and b, a I^* value of 7.33×10^{-10} mol cm^{-2} was obtained.

Analytical applications and method validation

Supporting electrolytes

To optimize the determination conditions of methotrexate, the current responses of 5.0×10^{-7} mol L^{-1} methotrexate were estimated by SWV in different supporting electrolytes such as phosphate buffer, acetate buffer, borate buffer, tartaric acid–sodium tartrate, and B-R buffer solution. The results showed that higher peak current and better peak shape could be obtained in B-R buffer solution. Therefore, B-R buffer solution was adopted. The effect of the B-R buffer solution pH, which ranged from 1.0 to 8.0, on the response of 5.0×10^{-7} mol L^{-1} methotrexate was investigated. The results showed that the i_{pa} of methotrexate increased gradually with the decrease of pH value, but the i_p and stability of the modified electrode changed badly in the pH <2. We think that the reason is DNA tends to denature in the pH <2 and blocks the oxidation of methotrexate. Therefore, pH 2.78 was chosen for the determination of methotrexate.

Instrumental parameters

In order to obtain a much more sensitive peak current, the SWV was employed in the determination of methotrexate. The optimum instrumental conditions (pulse-amplitude E_{sw}, frequency *f*) were studied for a 5.0×10^{-7} mol L^{-1} methotrexate solution following accumulation time (t_{acc}) of 300 s under open circuit. The results

Table 1 Comparison of the determination of methotrexate by different sensing methods reported

Electrode	Methods	Linear range	Detection limit (mol L^{-1})	Reference
Hanging mercury-drop electrode	DPASV	1.0×10^{-8}–1.0×10^{-5}	2.0×10^{-9}	[14]
Bare GCE	SWASV	8.0×10^{-7}–2.0×10^{-5}	3.5×10^{-7}	[15]
DNA LB-modified GCE	SWASV	2.0×10^{-8}–4.0×10^{-6}	5.0×10^{-9}	[16]
DNA/SWCNT/Nafion/GCE	SWASV	2.0×10^{-8}–1.5×10^{-6}	8.0×10^{-9}	This work

DPASV differential pulse anodic stripping voltammetry, *SWASV* square wave anodic stripping voltammetry

Table 2 Determination results of methotrexate in the tablets by SWASV and UV

SWASV (n=5)					UV (n=3)	
Amount found (mg tablet^{-1})	R.S.D. (%)	Standard added (mg L^{-1})	Total found (mg L^{-1})	Recovery (%)	Amount found (mg tablet^{-1})	R.S.D. (%)
2.416	2.1	2.0	4.408	99.6	2.451	1.1

indicated that the i_{pa} increased with the increasing of square wave amplitude from 10 to 50 mV or square wave frequency in the range of 10–40 Hz, but the peak potential shifted to more positive values, and the peak changed unshapely. So 30 mV were chosen as the optimum amplitude and 35 Hz were chosen as the optimum frequency.

Accumulation conditions

For consideration of the adsorption of methotrexate at the DNA/SWCNT/Nafion/GCE surface, SWV technique coupled with accumulation procedure was used for the study. With an increase in accumulation time (t_{acc}), the peak current increased. When the t_{acc} was 240 s, peak current achieved a maximum value in the methotrexate solution of 5.0×10^{-7} mol L^{-1} (see Fig. 7). A plateau appeared for prolonging the t_{acc} afterwards, and the accumulation potential (0.1–0.7 V) had a little effect on the peak current. So the t_{acc} of 240 s was used for further studies, and the accumulation of methotrexate was carried out under open circuit.

Calibration curve, detection limit, reproducibility, and stability

Figure 8 displays the response of different concentration of methotrexate in the optimized working conditions by square wave anodic stripping voltammetry (SWASV) using DNA/SWCNT/Nafion/GCE. A linear relationship could be established between i_{pa} and the concentration of methotrexate in the range of 2.0×10^{-8}–1.5×10^{-6} mol L^{-1} (the inset of Fig. 8). The linear regression equation and correlation coefficient are

$$i_{pa} = 1.123 + 0.919 \times 10^{7}C, R = 0.998$$

where i_{pa} was the oxidation peak current in microampere and C was the concentration of methotrexate in moles per liter. Standard deviations for the slope and intercept of the calibration curve were 0.00142 and 0.0174, respectively. Based on the signal-to-noise ratio of 3 [34], the detection limit was obtained as 8.0×10^{-9} mol L^{-1}.

The repeatability of the DNA/SWCNT/Nafion/GCE was checked by SWASV in the 8.0×10^{-7} mol L^{-1} methotrexate+pH=2.78 B-R buffer solutions; no change was observed for five successive detections. Similarly, 10 different methotrexate solutions (same concentration) were determined, and the relative standard deviation observed was 3.7 %. For testing the stability of the DNA/SWCNT/Nafion/GCE, the same methotrexate solution was detected on day 1 and a month later. The peak currents of methotrexate recorded on day 1 and a month later changed about 5.3 %. These experiments indicate that the DNA/SWCNT/Nafion/GCE has good repeatability and stability for the determination of methotrexate.

The proposed DNA/SWCNT/Nafion/GCE for methotrexate determination was compared with other methods reported, and the results were listed in Table 1. It is clear that mercury electrodes can achieve the lowest detection limit but the toxicity of mercury limits their use. At the same time, DNA Langmuir–Blodgett films-modified GCE also shows lower detection limit and potential uses for the detection of methotrexate. However, in this method, a Langmuir–Blodgett instrument was required. Thus, compared with the previous report, the proposed DNA/SWCNT/Nafion/GCE was simple and quick. At the same time, it could offer a reasonable linear range for methotrexate detection and a detection limit of 8.0×10^{-9} mol L^{-1} using 240-s preconcentration. The lower detection limit to the method mainly depends on the amount of substance, which is transferred to the electrode surface during accumulation. The synergetic properties of DNA-functionalized SWCNT and Nafion composite film, endowed the capability to strongly adsorb a target substance, enhanced the surface concentration. This research suggests also that the

Table 3 Application of the SWASV method to the determination of methotrexate in spiked human blood serum samples

Sample	Original found (1.0×10^{-7} mol L^{-1})	Added (1.0×10^{-7} mol L^{-1})	Total found[a] (1.0×10^{-7} mol L^{-1})	R.S.D. (%)	Recovery (%)
1	–[b]	1.00	1.10	2.3	110.0
2	–[b]	3.00	3.04	1.7	101.3
3	–[b]	5.00	4.89	3.1	97.8
4	–[b]	7.50	7.24	2.9	96.5

[a]Average of three determinations

[b]Below the detection limit

modified electrode as voltammetric sensors might be a very promising direction in trace analysis of electrochemistry.

Interference studies

For the possible analytical application of the proposed method, various possible interfering species were evaluated, with a fixed methotrexate concentration of 1.0×10^{-6} mol L^{-1}. The tolerance limit for a foreign species was taken as the largest amount yielding a relative error<±5 % for the determination of methotrexate. The experiment results showed that no interference could be observed for the following organic compounds: glucose (100), glutamic acid (100), uric acid (100), epinephrine (70), dopamine (50), and norepinephrine (65), where the data in parentheses denote the molar ratio of interfering compound to methotrexate. At the same time, the inorganic species, such as K^+, Na^+, Ca^{2+}, Al^{3+}, NH_4^+, Cl^-, PO_4^{3-}, and Ac^-, did not interfere, and at least 20-fold concentrations of Hg^{2+}, Pb^{2+}, Zn^{2+}, and Cd^{2+} did not interfere with the oxidation signal of methotrexate. The results indicate that the present method was adequate for the determination of methotrexate in real samples.

Determination of methotrexate in the medicinal tablets

To evaluate the practical applicability of the proposed method, it was employed to the detection of methotrexate in the medicinal tablets. Five parallel samples were analyzed with a relative standard deviation (R.S.D.) of 3.1 % (Table 2). After determining the content of methotrexate, some standard methotrexate was added in the five samples respectively, and the total contents of methotrexate were determined again to calculate the recovery (Table 2). For testing the accuracy of proposed method, the same medicinal tablets were analyzed using UV method and the results were also listed in Table 2 [4]. The results showed that there is no significant difference between them, which indicates that the method is reliable for the quantitative determination of methotrexate in the medicinal tablets.

Determination of methotrexate in human blood serum

The applicability of the SWASV to the determination of methotrexate in spiked human blood serum samples was investigated. The direct determination of methotrexate in spiked human blood serum samples was found to be possible by employing a high dilution of the sample with the supporting electrolyte. The determination of methotrexate concentration was performed by the calibration curve method. The results of the determination are listed in Table 3. The mean recovery of methotrexate was 101.4 %.

Conclusions

In the current report, we described a simple, sensitive, and selective SWV method for the quantitative determination of methotrexate based on the DNA-functionalized SWCNT and Nafion composite film-modified GCE (DNA/SWCNT/Nafion/GCE) as voltammetric sensors. The data reported here showed that the DNA/SWCNT/Nafion/GCE was highly stable and consistent in detecting methotrexate and the method can be used successfully to assay the methotrexate in the medicinal tablets and spiked human blood serum samples.

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