

# Electrochemical behavior of adriamycin at an electrode modified with silver nanoparticles and multi-walled carbon nanotubes, and its application

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**Abstract** An electrochemical sensor has been constructed for the determination of adriamycin (ADM) that is based on a glassy carbon electrode modified with silver nanoparticles and multi-walled carbon nanotubes with carboxy groups. The modified electrode was characterized by scanning electron microscopy and exhibits a large enhancement of the differential pulse voltammetric response to ADM. Signals are linear with the concentrations of ADM in the range from  $8.2 \times 10^{-9}$  M to  $19.0 \times 10^{-9}$  M, with a detection limit of  $1.7 \times 10^{-9}$  M. The sensor is highly reproducible and exhibits excellent stability. It was to detect calf thymus DNA.

**Keywords** Adriamycin · Calf thymus DNA · Multi-walled carbon nanotubes · Silver nanoparticles

## Introduction

Adriamycin is an antibiotic of the family of anthracyclines with a wide spectrum of chemotherapeutic applications and

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anti-neoplastic action widely used as an antitumor agent, and exhibits antitumor activity against solid tumors, such as breast and lung cancers [1]. Its strong DNA binding properties lead the discoverers of the antibiotics to suggest that the drugs receptor site is DNA [2, 3]. Most of the available evidence indicates that it binds to double helix mainly at CG-GC steps, the aminosugar being determinant for intercalation to occur [4].

Up to now, many techniques for the determination of the drug and DNA have been developed, for example, high performance liquid chromatography method [5]; microbiological assay method [6]; chemiluminescent [7]; spectrophotometric [8]; photochemical-fluorimetric [9]; electroanalytical techniques [10–13] etc. Among these techniques, electrochemical techniques are becoming increasingly important in the determination of compounds of pharmaceutical interest due to its simple, rapid, and low cost. Recently, nano-materials have attracted much attention and have been widely used in material, electronic, physical fields for various purposes due to their unique properties. Carbon nanotubes (CNT), discovered in 1991 by Iijima [14], represent an important group of nanoscale materials. It had been widely used as a new support for catalysts [15–19]. In recent years, many attempts have been conducted to enhance the electron transfer between electroactive species and electrodes based on deposit various metal particles onto the surface of CNTs, such as platinum, palladium, copper, nickel, ruthenium, silver, gold and so on, have been deposited on the CNTs successfully [20–28].

In this work, a silver nanoparticles ( $Ag_{\text{nano}}$ )/multi-walled carbon nanotubes (MWCNTs-COOH) modified electrode was fabricated and first applied for detecting ADM. The experiments results showed the proposed electrode exhibited a large enhancement of the differential pulse

voltammetric response to ADM and had a low detection limit and a good sensitivity.

## Experimental

### Apparatus and reagents

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI650C electrochemical workstation (Shanghai Chenhua Instruments Co, China, <http://chi.instrument.com.cn>). The three-electrode system was used in the experiment with a bare GCE or modified electrode as working electrode, a saturated calomel electrode (SCE) as reference electrode, and a platinum wire as counter electrode. All electrochemical measurements were carried out in a 10 mL cell at room temperature, where oxygen was removed with high-purity nitrogen for 30 min. All measurements were performed under a nitrogen atmosphere. All the potentials given in this paper were referred to SCE.

The UV/Vis absorbance spectra were obtained by using U-3010 spectrofluorometer (Hitachi, Japan). All of the spectroscopic work was performed at room temperature.

Calf thymus DNA (ctDNA) (sodium salt, type I) was obtained from Sigma (USA, <http://www.sigmaaldrich.com>) and was used without further purification, and its purity was confirmed by UV/Vis absorption, which produced  $A_{260}/A_{280}$  of approximately 1.8–1.9, suggesting the DNA sample was free of proteins. The stock solution of DNA was directly dissolved in water and stored at 4 °C. The DNA concentration per nucleotide (DNA-P) was determined spectrophotometrically [29] at 260 nm by using the extinction coefficient  $6,600 \text{ cm}^{-1} \text{ M}^{-1}$ . Adriamycin was purchased from Alfa Aesar (Tianjing, China, <http://alfaesar1.blog.sohu.com>), silver nitrate ( $\text{AgNO}_3$ ) was purchased from Sigma Aldrich (<http://www.sigmaaldrich.com>), multi-walled carbon nanotubes with carboxy groups (20–30 nm diameters, 30 mm length and >95% purity) were purchased from the Chengdu Institute of Organic Chemistry (<http://www.timesnano.com>). 0.1 M phosphate buffer solutions with different pH values were prepared by mixing the stock standard solution of  $\text{Na}_2\text{HPO}_4$  and  $\text{NaH}_2\text{PO}_4$  and the pH was adjusted with  $\text{H}_3\text{PO}_4$  or NaOH solution. All chemicals were of analytical reagent grade. All solutions were prepared with doubly distilled water.

### Preparation of the $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}$ modified electrode

Prior to modification, the bare GCE was mechanically polished to a mirror-like surface with 1.0, 0.3 and 0.05  $\mu\text{m}$   $\alpha\text{-Al}_2\text{O}_3$ . The electrode was rinsed with doubly distilled

water, and then successively ultrasonically washed with nitric acid (obtained by mixing nitric acid with doubly distilled water according to the volume ration 1:1), ethanol and doubly distilled water for 1 min, respectively, at last allowed to dry at room temperature. The MWCNTs-COOH modified electrode was obtained by dropping 5  $\mu\text{L}$  of the suspension (obtained by dispersing 0.1 mg MWCNTs-COOH into 2 mL alcohol) onto the surface of clean GCE and it was dried at room temperature, the obtained electrode was taken as MWCNTs-COOH/GCE. The  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}$  electrode was obtained by immersing the MWCNTs-COOH/GCE into  $\text{AgNO}_3$  solution for 15 min to adsorb and form silver complexes depended on the electrostatic and complexation interaction of silver ion with the carboxy groups of MWCNTs-COOH [30], after rinsing thoroughly with 0.5 M  $\text{KNO}_3$  solution, the MWCNTs-COOH modified electrode with silver ions was transferred into 0.5 M  $\text{KNO}_3$  solution and  $\text{Ag}_{\text{nano}}$  were grown by a chronoamperometry method according to the previous report [27]. The potential was stepped from  $-0.5$  to 0 V (vs. SCE) for 30 s, where oxygen was removed with high-purity nitrogen for 30 min.

## Results and discussion

The characterization of the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$

Figure 1 displays the typical morphology of the MWCNTs-COOH (Fig. 1a) and the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}$  modified electrode (Fig. 1b) was characterized by scanning electron microscopy (SEM). For the Fig. 1a, the MWCNTs-COOH distributed homogeneously on the electrode and displayed a special three-dimensional structure. For the Fig. 1b, it can be obviously observed that the  $\text{Ag}_{\text{nano}}$  have been successfully covered on MWCNTs-COOH.

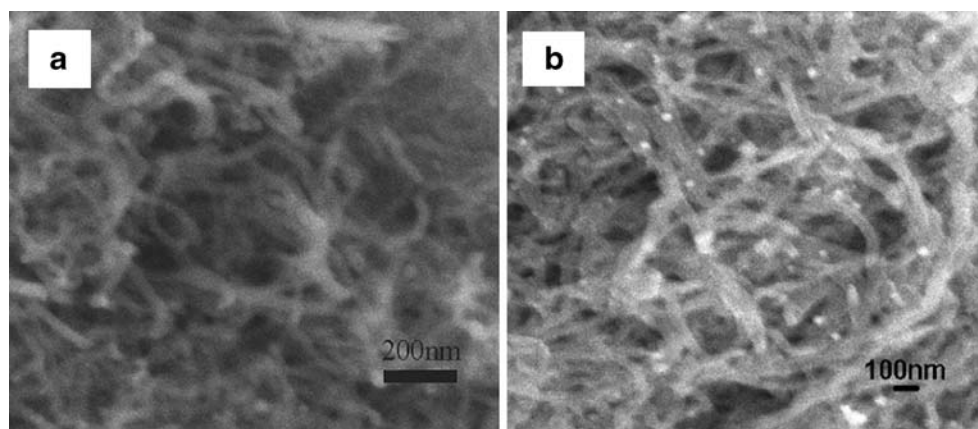
Electrochemical behavior of the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$

Figure 2a shows the CVs of the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}$  modified electrode with different scan rate in  $\text{KNO}_3$  (0.5 M). A pair of redox peak was observed at 0.22 and  $-0.04$  V, which was ascribed to the redox of silver. The Fig. 2b showed the peak current was linear with scan rate.

Electrochemical behaviors of ADM at the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$

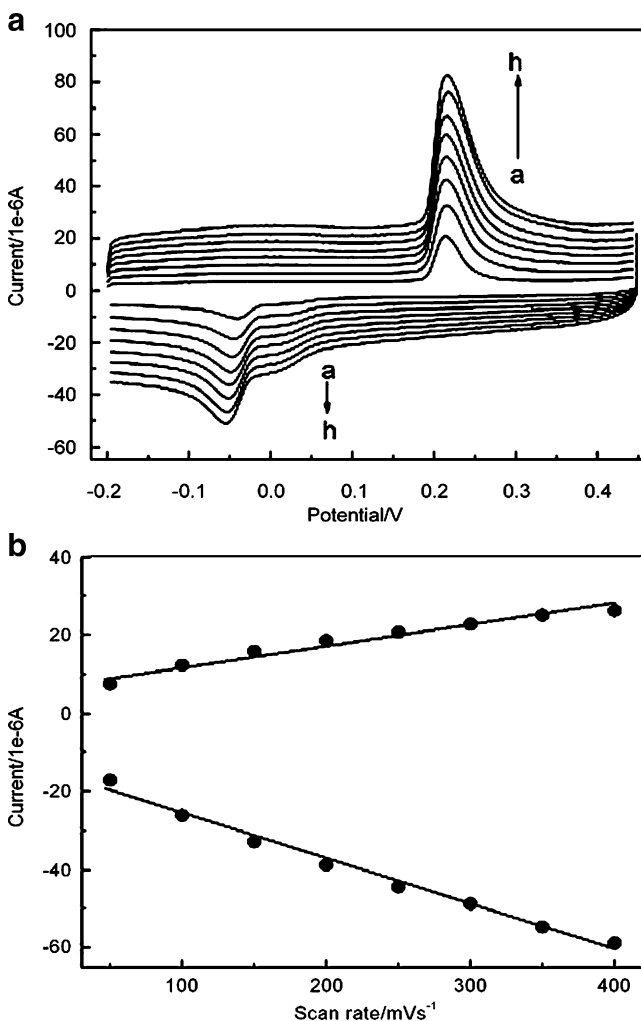
Figure 3 shows the CVs of ADM at the bare GCE, the MWCNTs-COOH/GCE, and the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  in 0.1 M PBS (pH 7.0). At the bare GCE (Fig. 3a), the CV response of ADM was poor. When MWCNTs-COOH

**Fig. 1** The SEM images of the MWCNTs-COOH (a), the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}$  (b) modified GCE

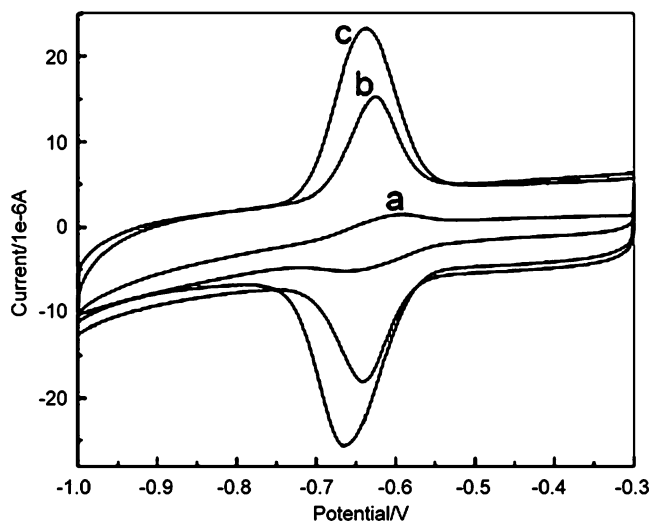


was modified on GCE (Fig. 3b), the CV response of ADM was enhanced, meanwhile the peak potential negatively shifted due to MWCNTs-COOH having a large specific surface area and good electronic properties. For the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  (Fig. 3c), the peak current of ADM

was further enhanced and the peak potential further negatively shifted. This may attribute to the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}$  composite film can adsorb much more ADM. Meanwhile we can conclude the electrode reaction of ADM at the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  was reversible based on the CVs. In addition, the effect of the scan rate on the peak current of ADM at the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  was investigated by CV in 0.1 M PBS (pH 7.0). The result showed that the peak currents were proportional to scan rates from 20 to  $580 \text{ mV}\cdot\text{s}^{-1}$ , which suggest that the electrode reaction of ADM at the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  is an adsorption-controlled process. The effect of the solution pH on electrochemical response of ADM at the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  was also investigated by CV in 0.1 M PBS (pH 7.0) (The figure was not shown). The experiments results showed that the peak potentials shifted negatively with increasing in the solution pH, which indicated that protons take part in the electrochemical process. And the peak currents increased



**Fig. 2** (a) CVs of the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  in  $\text{KNO}_3$  (0.5 M) with different scan rate: 50, 100, 150, 200, 250, 300, 350 and  $400 \text{ mV}\cdot\text{s}^{-1}$  (a-h). (b) Plots of the peak current vs. the scan rate



**Fig. 3** CVs responses of  $5.5 \times 10^{-6} \text{ M}$  ADM at different electrodes: the bare GCE (a); the MWCNTs-COOH/GCE (b); the  $\text{Ag}_{\text{nano}}/\text{MWCNTs-COOH}/\text{GCE}$  (c) in 0.1 M PBS (pH 7.0). Scan rate:  $100 \text{ mV}\cdot\text{s}^{-1}$

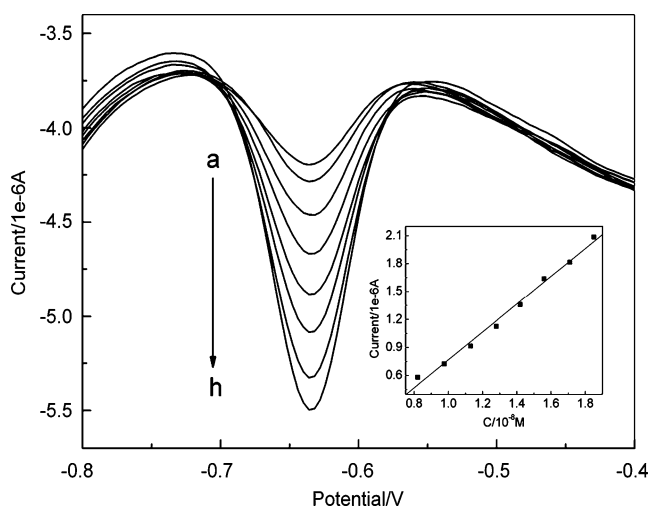
with the solution pH increased in the range of 3.0–7.0. When the pH > 7.0, the peak current decreased. So we selected pH 7.0 PBS as the support electrolyte in this paper.

#### Determination of ADM

DPV was used to determine ADM. The reduction peak current of ADM was used as the analytical signal. The results were shown in Fig. 4, it could be observed that the peak currents increased with the concentration of ADM increased, and were linear with the concentration of ADM in the range of  $8.2 \times 10^{-9}$  M– $19.0 \times 10^{-9}$  M (shown in inset of the Fig. 4), the linear regression equation was  $i(10^{-6}A) = -0.73 + 1.49c(10^{-8}M)$ , with a correlation coefficient  $r=0.9957$ . The detection limit was  $1.7 \times 10^{-9}$  M ( $S/N=3$ ).

#### The stability and reproducibility

The stability of the  $Ag_{nano}/MWCNTs-COOH$  modified GCE was studied by CV in 0.1 M PBS (pH 7.0) containing  $1.0 \times 10^{-6}$  M ADM, and scanning for 30 continuous cycles at the potential between  $-0.3$  and  $-1.0$  V (vs. SCE) with a scan rate of 100 mV/s, the peak heights of the cycle voltammogram showed a negligible change. After the modified electrode was stored in PBS (pH 7.0) for 3 days, the peak current of the same concentration ADM decreased less than 8%. The reproducibility of the  $Ag_{nano}/MWCNTs-COOH$  modified GCE was also investigated. The fabrication of five modified electrodes, made independently, showed a well reproducibility with a relative standard deviation (R.S.D) of 7.6% for the currents detection of  $1.0 \times 10^{-6}$  M ADM. Therefore, the  $Ag_{nano}/MWCNTs-COOH$  modified GCE exhibited an excellent stability and reproducibility.



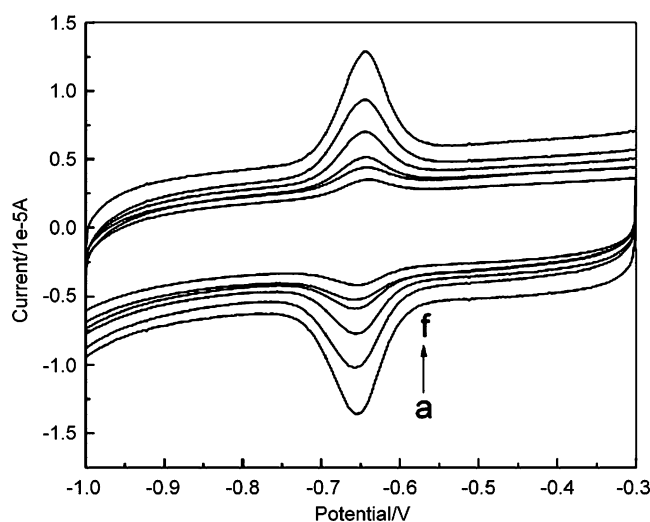
**Fig. 4** DPVs responses of the different concentrations ADM at the  $Ag_{nano}/MWCNTs-COOH/GCE$  in 0.1 M PBS (pH 7.0); the inset: the DPV reduction peak current vs. the concentration of ADM

#### Detection of ctDNA

The UV/Vis absorbance spectrum was used to investigate the interaction between ctDNA and ADM in 0.1 M PBS (pH 7.0). The obtained experiment result is according with the report [12], which indicated the ctDNA can react with ADM. In this work, we used the change of CV of ADM to detect ctDNA. Figure 5 shows the CVs of ( $3.4 \times 10^{-6}$  M) ADM in 0.1 M PBS solution in the presence of ctDNA with different concentrations. As it could be seen, with adding ctDNA into ADM solution, the peak currents of ADM gradually decreased, which is the similar with the previous report [12]. Furthermore, the reduction peak currents were linear with the concentrations of ctDNA in the range from  $8.60 \times 10^{-6}$  to  $4.3 \times 10^{-5}$  g·mL<sup>-1</sup>, the linear regression equation was  $i_{pc}(10^{-6}A) = 5.45 - 1.07c_{ctDNA}(10^{-5}M)$ , the correlation coefficient ( $r$ ) was 0.9855, which could be used to determine ctDNA.

#### Conclusions

Herein, we have prepared an advanced  $Ag_{nano}/MWCNTs-COOH$  modified GCE and investigated the electrochemical behaviors of ADM at the modified electrode. The results showed that the modified electrode could effectively enhance the electrochemical response of ADM, and had high sensitivity for detection of ADM, the detection limit was  $1.7 \times 10^{-9}$  M. In addition, the sensor exhibited an excellent reproducibility and stability. Furthermore, CV signal of ADM can be applied to determine ctDNA.



**Fig. 5** CVs of  $3.4 \times 10^{-6}$  M ADM in 0.1 M PBS solution in the presence of ctDNA with different concentrations: 0 (a),  $8.6 \times 10^{-6}$  M (b),  $1.7 \times 10^{-5}$  M (c),  $2.6 \times 10^{-5}$  M (d),  $3.4 \times 10^{-5}$  M (e),  $4.3 \times 10^{-5}$  M (f)

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