VOLTAMMETRIC DETERMINATION OF MITOMYCIN C USING A CHEMICALLY-MODIFIED GLASSY CARBON ELECTRODE

Wanyun QU[a,b], Hong WANG[a1] and Kangbing WU[a2,*]

[a] *Department of Chemistry, Huazhong University of Science and Technology, Wuhan 430074, P. R. China; e-mail: [1] hongwzy@yahoo.com.cn, [2] wu_kangbing@hotmail.com*

[b] *Department of Chemistry, Hubei Institute for Nationalities, Enshi 445000, P. R. China; e-mail: quwanyun@sohu.com*

Received June 28, 2004
Accepted October 12, 2004

An electrochemical method for the direct determination of mitomycin c (MMC), based on a multi wall carbon nanotube (MWNT)-modified electrode, has been developed. A well-shaped and highly sensitive oxidation peak at 0.79 V was observed on the MWNT-modified electrode for MMC. Further studies show strong evidence that MWNT-modified electrode significantly enhances the sensitivity of determination of MMC since the oxidation peak current of MMC increases remarkably, compared with that with a bare glassy carbon electrode. All the experimental parameters such as pH, scan rate, accumulation conditions, have been examined. A sensitive linear voltammetric response for MMC was obtained over the concentration range of 2.5×10^{-7}–1.0×10^{-4} mol/l; the detection limit is 8×10^{-8} mol/l. Compared with other methods, this new-proposed method possesses advantages such as high sensitivity, fast response, low cost and simplicity. Eventually, the proposed method has been successfully employed to detect MMC in urine samples.

Keywords: Analytical methods; Carbon nanotube; Electrochemistry; Electrooxidation; Mitomycin c; Antitumor agents; Chemically modified electrode; DNA binding molecules; Voltammetry.

Mitomycin c (MMC), with the molecule structure as shown in Fig. 1, is a powerful systemic DNA inhibiting anticancer agent that has been used for many years. MMC is an antitumor antibiotic that has been used in the

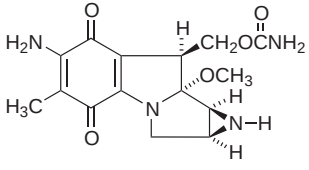

FIG. 1
The molecular structure of MMC

treatment of a number of cancers, including stomach, breast, pancreas and bladder cancer. The anticancer activity of MMC is based on its covalent binding to DNA after chemical or enzymatic reductive activation. Therefore, determination of MMC is of great importance and interest.

Various analytical methods, so far, have been developed for the determination of mitomycin c, and liquid chromatography is the most employed. For example, liquid chromatography with UV-VIS [1,2], differential pulse polarographic[3], dual-electrode coulometric[4] and voltammetric[5] detections were all reported. Recently, limited works concerning the electrochemical determination of MMC have also been published. Wang et al.[6] determined MMC by adsorptive stripping voltammetry at pH 10.2 using an accumulation potential of –0.2 V. Otherwise, Marin et al.[7] reported a stripping voltammetry in combination with the hanging mercury drop electrode for the MMC determination. However, to the best of our knowledge, voltammetric determination of MMC using a carbon nanotube-modified electrode has not been reported.

Carbon nanotubes (CNTs) are molecular-scale wires with high electrical conductivity, extremely high mechanical strength and modulus, which can be divided into two categories: single wall carbon nanotube (SWNT) and multi wall carbon nanotube (MWNT). Since the discovery[8], CNTs have attracted increasing attention due to their extraordinary structural, mechanical, electrical and electrochemical properties as well as their promise in the field of material science. The subtle electronic properties suggest that CNTs have the ability to promote electron transfer, and result in their extensive applications in electrochemistry[9–12]. In this work, a glassy carbon electrode (GCE) coated with a MWNT thin film was first described for the determination of MMC. The electrochemical behavior of MMC indicated that the MWNT film significantly increased the oxidation peak current of MMC. Thus, the sensitivity of the determination of MMC was improved by about ten times. All experimental conditions were examined. Consequently, a voltammetric method has been developed for the measurement of MMC. This newly proposed method possesses following advantages: high sensitivity, rapid response, low cost and simplicity.

EXPERIMENTAL

Reagents

Mitomycin c was from Sigma. Stock solutions of MMC (1×10^{-3} mol/l) were prepared in redistilled water and stored at –18 °C. Standard solutions of MMC were prepared by dilution of a stock solution with redistilled water to give final MMC concentration between 1×10^{-6}

and 1×10^{-4} mol/l. All chemicals were of analytical grade and were used without purification. Redistilled water was used throughout.

The multi wall carbon nanotube was obtained from Chengdu Organic Chemicals Co., Chinese Academy of Sciences. Then, MWNT was refluxed in concentrated HNO_3 for 10 h to cause segmentation and carboxylation[13].

Apparatus

All the electrochemical measurements were carried out with a CHI 830 Electrochemical Workstation (CH Instrument, Austin, U.S.A.). A conventional three-electrode system, including a MWNT-modified glassy carbon working electrode, a saturated calomel reference electrode (SCE) and a Pt wire counter electrode, was employed.

Fabrication of MWNT-Modified GCE

An amount of 5 mg MWNT and 5 mg dihexadecyl hydrogen phosphate (DHP) was dispersed in 5 ml of redistilled water, and then sonicated for about 20 min to give a stable and homogeneous MWNT-DHP suspension. Prior to modification, the GCE was mechanically polished with alumina paste of different grades to a mirror finish, rinsed and sonicated (3 min) in redistilled water. Finally, the GCE was coated with 5 μl of the MWNT-DHP suspension and allowed to evaporate water at room temperature in air. The DHP-modified GCE was prepared by the same procedure as explained above, but without MWNT.

Analytical Procedure

The MWNT-modified GCE was activated in 10 ml of 0.1 M phosphate buffer pH 7.0 placed in the electrochemical cell, by cyclic voltammetric sweeps between 0.50 and 1.10 V until the cyclic voltammograms did not change. Then, the required volume of standard solution of MMC was added with a micropipette, and voltammograms were recorded after 2 min of open-circuit accumulation. The oxidation peak current of MMC at 0.79 V was measured. After every measurement, the MWNT-modified GCE was subjected to cyclic voltammetric sweeps in phosphate buffer pH 7.0 until the voltammograms were stable. Potential cycling removed adsorbed substances and gave a reproducible electrode surface.

RESULTS AND DISCUSSION

Electrochemical Behavior of MMC

Figure 2 shows the cyclic voltammograms of a MWNT-modified GCE in phosphate buffer at pH 7.0 in the absence and presence of MMC. Within the potential window from 0.50 to 1.10 V, no redox peaks were observed for a MWNT-modified GCE (curve *1*, dotted line). However, upon addition of 4×10^{-5} M MMC, a well-shaped and highly sensitive oxidation peak appears at 0.79 V (curve *2*, solid line). There is no corresponding reduction peak during the reverse potential scan from 1.10 to 0.50 V. Moreover, the electrochemical behavior of MMC at different scan rates from 10 to

200 mV/s was also investigated using cyclic voltammetry; only an oxidation peak was observed even at 10 mV/s. This suggests that the electrode reaction of MMC at the MWNT-modified electrode is totally irreversible.

Otherwise, the oxidation peak current of MMC shows a remarkable decrease during successive cyclic voltammetric sweeps. But after the second sweep, the peak current decreases slightly and finally remains unchanged.

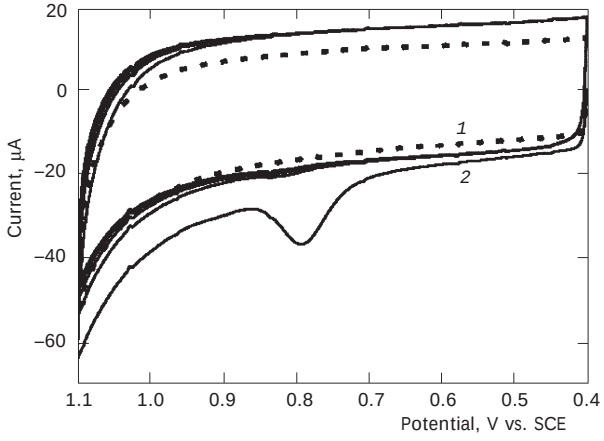

FIG. 2

Cyclic voltammograms of a MWNT-modified GCE in 0.1 M phosphate buffer at pH 7.0 (*1*) and in *1* + 4 × 10^{-5} M MMC (*2*). Scan rate: 100 mV/s

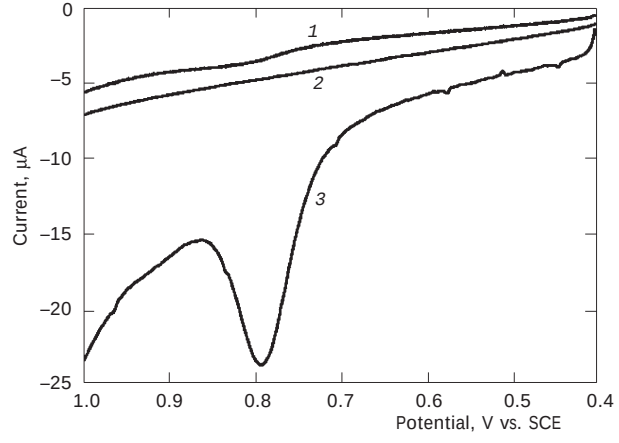

FIG. 3

Linear sweep voltammograms of 1 × 10^{-5} M MMC in phosphate buffer pH 7.0 at three different electrodes after 2-min open-circuit accumulation: bare GCE (*1*), DHP film-modified GCE (*2*), MWNT-DHP film-modified GCE (*3*). Scan rate: 100 mV/s

This phenomenon may be caused by the fact that the adsorption of MMC or its oxidative product occurs at the MWNT-modified GCE surface.

The electrochemical properties of MMC at three different working electrodes (bare GCE, DHP-modified GCE and MWNT-modified GCE) were studied by linear sweep voltammetry (LSV); the results are shown in Fig. 3. At a bare GCE, 1×10^{-5} M MMC a very low oxidation peak yields at 0.81 V in phosphate buffer pH 7.0 after 2-min open-circuit accumulation (curve *1*). Under identical conditions, the oxidation peak of MMC at a DHP-modified GCE almost vanishes (curve *2*). Dihexadecyl hydrogen phosphate forms a perfect thin film on GCE surface, and thus inhibits the electron transfer between MMC and electrode. The oxidation peak current, therefore, decreases compared with that at a bare GCE. However, the oxidation peak current of MMC at the MWNT-modified GCE increases significantly (almost ten times) in comparison with that at a bare GCE. The remarkable peak current enhancement is undoubtedly attributed to the unusual structure and properties of MWNT (such as very large specific area, strong adsorptive ability, subtle electronic properties). In conclusion, a MWNT-modified GCE greatly improves the sensitivity of determination of MMC, as can be seen in Fig. 3.

Choice of Supporting Electrolyte

The electrochemical oxidation behaviors of 1×10^{-6} M MMC in various media, such as phosphate buffers pH 5.0–8.0, sodium citrate–HCl buffers pH 1.0–5.0, Macllvaine buffers pH 2.0–8.0, Britton–Robinson buffers pH 2.0–10.0 (each 0.1 mol/l), were investigated. The best oxidation response was obtained in phosphate buffer pH 7.0 because the peak current is highest and the peak shape is well defined. The influence of pH on the oxidation peak current of MMC was studied in 0.1 M phosphate buffer. As can be seen from Fig. 4, the oxidation peak current of MMC increases gradually with pH increasing from 5.0 to 6.0. However, when pH changes from 6.0 to 8.0, the oxidation peak current alters very slightly. As 7.0 is close to physiological pH value, 0.1 M phosphate buffer at pH 7.0 was chosen as the medium for determination of MMC.

Effect of the Amount of MWNT-DHP Suspension

Generally, the thickness of the MWNT-DHP cast film, which is determined by the amount of MWNT-DHP suspension on the electrode surface, has an obvious effect on the current responses of MMC. Figure 5 depicts the relationship between the oxidation peak current and the amounts of MWNT-

DHP suspension. The oxidation peak current strongly increases when the MWNT-DHP suspension volume increases from 0 to 5 μl. As the MWNT-DHP suspension volume increases further, the peak current changes only slightly. However, when it exceeds 12.5 μl, the peak current decreases. In principle, the oxidation peak current of MMC is almost independent of the

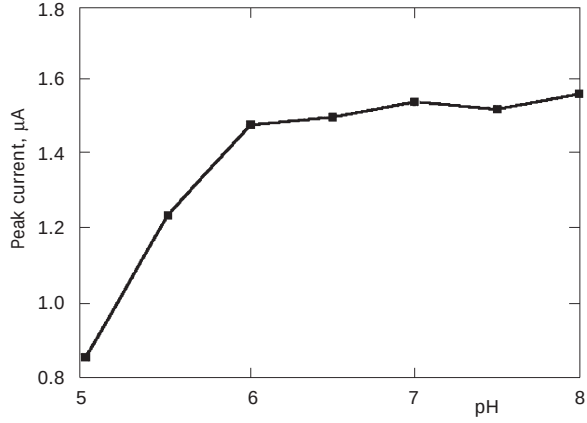

FIG. 4
Effect of pH on the oxidation peak current of 1×10^{-6} M MMC in phosphate buffer. Accumulation time: 2 min, scan rate: 100 mV/s

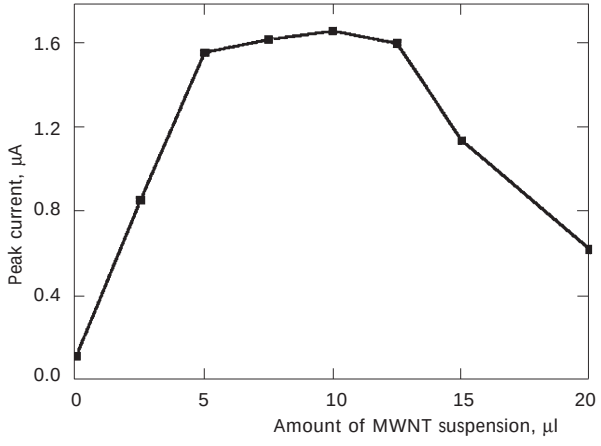

FIG. 5
Influence of the amount of MWNT-DHP suspension on the oxidation peak current of 1×10^{-6} M MMC. Other conditions are the same as in Fig. 4

thickness of MWNT cast film because MWNT is an ideal electrode material. DHP is an insulator, which decreases the electrical conductivity of the cast film, and finally lowers the electron-transfer rate. Hence, the peak current decreases when the MWNT-DHP film is too thick.

Optimization of Accumulation Conditions

The influence of accumulation potential on the oxidation peak current of MMC was examined. The oxidation peak current of 1×10^{-6} M MMC was compared after 2 min of accumulation under different potentials. The peak current almost remained the same for accumulation potentials from –0.40 to 0.40 V, indicating that the accumulation potential had no influence on the oxidation peak current of MMC. Thus, an open-circuit accumulation was used.

Unlike the accumulation potential, the accumulation time has an obvious influence on the oxidation peak current of MMC. Figure 6 illustrates the relationship between the accumulation time and the oxidation peak current of MMC. The oxidation peak current increases greatly within the first 2 min and then levels off, revealing that the MWNT cast film exhibits a rapid accumulation efficiency for MMC.

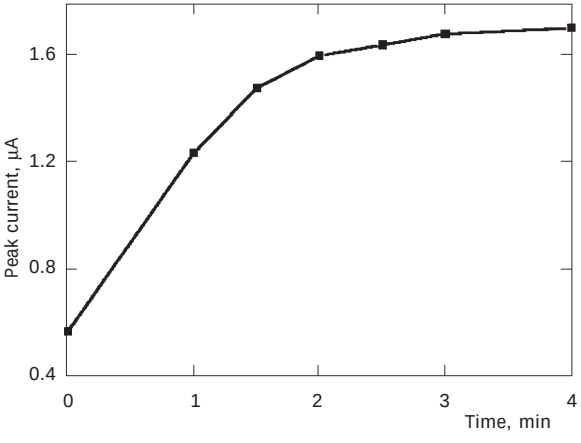

FIG. 6
Dependence of oxidation peak current of 1×10^{-6} M MMC on the accumulation time. Other conditions are the same as in Fig. 4

Effects of Scan Rate

The oxidation peak current of 1×10^{-6} M MMC at different scan rates from 25 to 400 mV/s was measured by LSV. It was found that the oxidation peak current is proportional to the scan rate, indicating that the oxidation of MMC at the MWNT film-coated GCE is adsorption-controlled.

Calibration Graph

The calibration curve for MMC in phosphate buffer pH 7.0 was measured by LSV. The linear segment increases from 2.5×10^{-7} to 1×10^{-4} mol/l with a regression equation of $i_p = 0.08 + 1.44 \times 10^6 C$ ($r = 0.998$, C is the concentration in mol/l, i_p is the peak current in μA). Experiments showed that this method can detect 8×10^{-8} M MMC after 2-min accumulation. The relative standard deviation of 4.9% for 1×10^{-6} M MMC ($n = 5$) showed a good reproducibility.

The long-term stability of the MWNT-modified GCE was evaluated by measuring the current responses at a fixed MMC concentration of 1×10^{-6} mol/l over a period of 2 weeks. The MWNT-modified GCE was used daily and stored in air. The experimental results show that the current responses deviated only 6.2%, suggesting that the MWNT-modified GCE reported in this work possesses long-term stability.

Interferences

To evaluate the interferences of some foreign species on the determination of MMC at 1×10^{-6} mol/l, a systematic study was carried out. It was found that the MWNT-modified GCE can tolerate interferences of other organic compounds. For example, 20-fold concentrations of vitamin B_6, ascorbic acid, uric acid, xanthine, dopamine, vitamin A, and 50-fold concentrations of vitamin E, vitamin B_1, progesterone and caffeine almost do not influence the current response of 1×10^{-6} M MMC (signal change below 5%), revealing that the proposed method has excellent selectivity to MMC.

Analysis of MMC in Urine

The proposed method has been applied to the direct determination of MMC in human urine, but no signal of MMC was detected. Thus, this method was applied to detect MMC in urine samples spiked with MMC at a certain concentration. Urine samples containing MMC were prepared by

spiking urine obtained from healthy volunteers with appropriate volume of MMC stock solution to give final MMC urine concentrations between 5×10^{-7} and 5×10^{-5} mol/l. No sample treatment was performed, except for a urine dilution (1:2) with the phosphate buffer pH 7.0. The MMC concentration was determined by the standard addition method; the results are shown in Table I. The recoveries indicate that the accuracy and repeatability of this voltammetric method are very good. From the results listed in Table I, it is very clear that the novel MWNT-modified electrode has a great potential for MMC determination in practical samples.

CONCLUSION

In this work, a novel chemically modified electrode, MWNT-modified GCE, was easily fabricated for the determination of MMC. Owing to unique properties of MWNT, such as high specific surface area, subtle electronic properties and strong adsorptive ability, the MWNT-modified GCE greatly enhances the oxidation peak current of MMC. As a result, a very sensitive and simple electrochemical method using a MWNT-modified GCE was developed for MMC determination.

TABLE I
Determination of MMC in urine samples

Spiked, mol/l	Found, mol/l	Recovery, %
2.00×10^{-5}	1.98×10^{-5}	99.0
5.00×10^{-6}	5.17×10^{-6}	103.4
7.50×10^{-6}	7.34×10^{-6}	97.9
8.00×10^{-7}	8.24×10^{-7}	103.0
7.50×10^{-6}	7.66×10^{-6}	102.1

Original was 0.00 mol/l in all samples.

REFERENCES

1. Czejka J. M., Jaeger W., Schueler J.: *J. Chromatogr.* **1989**, *497*, 336.
2. Li W. Y., Seah S. K. L., Koda R. T.: *J. Chromatogr., B: Biomed. Appl.* **1993**, *619*, 148.
3. Bennekom W. P. V., Tjaden U. R., Druijn E. A. D., Oosterom A. T. V.: *Anal. Chim. Acta* **1984**, *156*, 289.
4. Ohkubo T., Nambara T.: *J. Chromatogr.* **1990**, *527*, 441.
5. Tjaden U. R., Langenberg J. P., Ensing K., Bennekon A. T. V., Bruijn E. A. D., Oosterom A. T. V.: *J. Chromatogr.* **1982**, *232*, 255.

6. Wang J., Lin M. S., Villa V.: *Anal. Lett.* **1986**, *19*, 2293.
7. Marin D., Perez P., Teijeiro C., Palecek E.: *Anal. Chim. Acta* **1998**, *358*, 45.
8. Iijima S.: *Nature* **1991**, *54*, 56.
9. Britto P. J., Santhanam K. S. V., Ajayan P. M.: *Bioelectrochem. Bioenerg.* **1996**, *41*, 121.
10. Luo H. X., Shi Z. J., Li N. Q., Gu Z. N., Zhuang Q. K.: *Anal. Chem.* **2001**, *73*, 915.
11. Wang Z. H., Wang Y. M., Luo G. A.: *Analyst (Amsterdam)* **2002**, *127*, 653.
12. Musameh M., Wang J., Merkoci A., Lin Y. H.: *Electrochem. Commun.* **2002**, *4*, 743.
13. Tsang S. C., Chen Y. K., Harris P. J. E., Green M. L. H.: *Nature* **1994**, *372*, 159.