Int. J. Electrochem. Sci., 7 **(2012)** 6084 - 6096

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Behavior of Ambroxol and Its Determination in Pharmaceutical Dosage Forms on MWCNT/Nafion Modified Glassy Carbon Electrodes

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Received: 29 May 2012 / *Accepted:* 12 June 2012 / *Published:* 1 July 2012

The electrochemical behavior of ambroxol was investigated on MWCNT/Nafion modified glassy carbon electrodes. In cyclic voltammetry, the compound showed an irreversible oxidation peak. Compared with the results on bare glassy carbon electrode and Nafion modified glassy carbon electrode, significant improvements in the sensitivity were obtained on MWCNT/Nafion modified glassy carbon electrode. The differential pulse voltammetric detection limits were determined to be 3×10^{-8} M, 4×10^{-9} M and 1×10^{-9} M on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode, respectively. At the optimized condition, a sensitive linear differential pulse voltammetric response range for the ambroxol was between 1×10^{-8} and 1.8×10^{-6} M on MWCNT/Nafion modified glassy carbon electrode. Determination of ambroxol in pharmaceutical dosage forms was performed by differential pulse voltammetry. All values were in good statistical agreement with the nominal values.

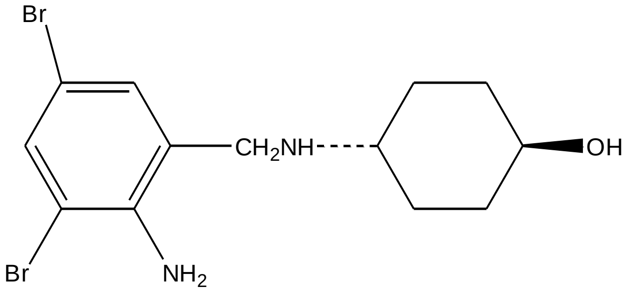
Keywords: cyclic voltammetry; differential pulse voltammetry; ambroxol; Nafion; carbon nanotube

1. INTRODUCTION

Ambroxol (ABX: trans-4-[(2-amino-3,5-dibromophenyl-methyl)amino] cyclohexanol) (see structure in Scheme 1), is a highly substituted aniline that is used as bronchosecretolytic and expectorant drug. It is administered as hydrochloride in daily doses of 30-120 mg and is available commercially as syrups, tablets and granule forms. It is producing good results in the treatment of chronic bronchitis and alveolar proteinosis. Several different methods have been used for the determination of ABX, including ultraviolet spectroscopy,[1] thin layer chromatography,[2] gas chromatography,[3,4] capillary electrophoresis[5] and high-performance liquid chromatography.[6-14] There have been reports on electrochemical methods for the determination of ABX.[15-17] It is generally accepted that ABX is irreversibly oxidized at high positive potentials, giving rise to a product which exhibits a redox couple waves at lower positive potentials.

Both multiwall carbon nanotubes (MWCNTs) and single-wall carbon nanotubes (SWCNTs) have attracted tremendous attention because of their unique structural, mechanical and electronic characteristics.[18-30] Recently, CNTs have been extensively studied as catalyst supports constituting the electrodes of fuel cells.[31,32] Various CNT-modified electrodes have also been used as electrochemical sensors. Several different methods have been employed to fabricate CNT-modified electrodes, including the dispersion of the CNTs along with a binder,[33-36] drop coating without any binder,[37-39] direct growth of the CNTs on an electrode,[40,41] and the abrasion of a pyrolytic graphite electrode on the CNTs.[42] CNT powder mixed with binders such as mineral oil or Teflon was used as a bulk working electrode.[43,44] Nafion is a kind of chemical modifier that possesses ideal properties.[45] This perfluorosulfonated cation exchange resin can be used selectively to preconcentrate positively charged molecules through electrostatic interaction due to the hydrophilic negatively charged sulfonate groups in the polymer structure. To our knowledge, the studies on the voltammetric behaviors of ambroxol and its quantitative determination at CNT/Nafion modified glassy carbon electrodes have not been reported.

The modification of electrode with Nafion is a simple way to improve the electrode performance.[46-50] The electrode performance can be further improved by the combined use of CNT and Nafion.[51-53] In this work, the electrochemical behavior of ABX on bare glassy carbon electrode, Nafion modified glassy carbon and MWCNT/Nafion modified glassy carbon electrode has been investigated by cyclic voltammetry and differential pulse voltammetry. On MWCNT/Nafion modified glassy carbon electrode, a significant improvement in the sensitivity was obtained compared to previous reports.[15-17] The differential pulse voltammetric detection limits were determined to be 3×10^{-8} M, 4×10^{-9} M and 1×10^{-9} M on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode, respectively. The optimum experimental conditions for the determination of ABX containing pharmaceutical products were described in this article.



Scheme 1. Chemical structure of ambroxol.

2. EXPERIMENTAL

2.1. Apparatus

Electrochemical experiments were performed with a CH Instruments model 660B electrochemical analyzer. Glassy carbon electrode of 3.0 mm diameter, was obtained from Bioanalytical Systems Inc.. A Pt wire and Ag/AgCl were used as counter and reference electrodes, respectively. Experiments were carried out in a glass cell designed to suit a three electrode potentiostatic unit. Transmission electron microscopic (TEM) image was obtained using a JEOL EM-2010 microscope at an accelerating voltage of 200 kV. The field emission scanning electron microscopic (FE-SEM) image was obtained using a JSM 6700F microscope (Jeol). IR spectrum was recorded on a Jasco FT/IR 200 spectrophotometer.

2.2. Reagents

The chemicals used for preparation of the solutions were purchased at the highest grade possible. ABX was purchased from Sigma-Aldrich and used without further purification. A stock solution (1×10^{-3} M) of ABX was freshly prepared in deionized water and stored at 4˚C in the dark. A series of standard solutions was then prepared daily by dilution of the stock solution. The Nafion stock solution (5 wt.%) was purchased from aldrich. Nafion solution was diluted to 0.1 wt.% in methanol. Multi wall carbon nanotubes (MWCNT) which have 95 vol.% purity or more, were purchased from Iljin Inc (Seoul, Korea). Water deionized by a Nano Pure System (Barnsted) was used. Syrup and tablets of ABX were obtained from the market.

2.3. Analytical procedures

2.3.1. CNT treatment

0.5 g of MWCNTs was pretreated by refluxing with a 50 ml mixture of concentrated sulfuric and nitric acids (1:1 v/v, 98 and 70 %, respectively) for 10 h. The suspension was diluted with 200 ml deionized water and further treated for 10 h at room temperature with the aid of ultrasonic agitation. Then, the reaction mixture was kept at room temperature for 48 h. Finally, well dispersed upper carbon nanotube suspension was centrifuged for several cycles to neutral pH and dried in an oven at 50 ˚C.

2.3.2. Electrodes

Prior to use, the glassy carbon electrode was polished carefully with wet 0.3 and 0.05 μm alumina slurry on a felt pad successively and rinsed with water. The polished electrode was then cleaned in an ultrasonicating bath for 2 min before use.

The Nafion modified glassy carbon electrode was prepared by pipetting 5 μl of 0.1 wt.% Nafion solution onto the electrode surface and allowing to dry for 10 min under an infrared heat lamp.

1 mg of the chemically oxidized MWNTs was sonicated in a solution containing 1 ml of H_2O, 3.4 ml of ethanol, and 0.1 ml of Nafion solution (5 wt.%, Aldrich) for 15 min, forming a MWNTs dispersion. 5 μl dispersion was applied on the glassy carbon electrode to prepare the MWCNT/Nafion modified electrode. The modified electrode was dried under an infrared heat lamp.

2.3.3. Differential pulse voltammetry

All experiments were carried out at room temperature. It was unnecessary to remove dissolved oxygen from the solution before analysis. The solution mentioned was stirred for a fixed time prior to analysis by differential pulse voltammetry. After a rest period of 5 s, an anodic differential pulse voltammetric scan was made and the peak current was measured. Differential pulse voltammetry was employed for the measurement of ABX under the following conditions: potential range from +0.60 to +1.25 V (vs. Ag/AgCl), scan rate 20 mV/s, pulse amplitude 50 mV, pulse width 50 mV, pulse period 200 ms.

2.3.4. Pharmaceutical sample preparation

An accurately weighted portion of finely powered tablets equivalent to about 100 mg of ABX was transferred into a 50 ml volumetric flask and the content was diluted with 1 M KCl containing 0.1 M HCl. The content of the flask was shaken vigorously on a mechanical shaker for 10 min and sonicated for 10 min. The solution was filtered into a 100 ml calibrated flask, the residue washed several times with 1 M KCl containing 0.1 M HCl. For voltammetric analysis, suitable dilution steps for the solution were made with 1 M KCl containing 0.1 M HCl and the final concentration range obtained for the quantitative analysis on MWCNT/Nafion modified glassy carbon electrode was between 1×10^{-8} and 1.8×10^{-6} M. For ABX syrup, the solution was diluted directly with 1 M KCl containing 0.1 M HCl.

3. RESULTS AND DISCUSSION

3.1. Preparation of the MWCNT/Nafion modified glassy carbon electrode

MWCNTs were pretreated using a mixture of concentrated sulfuric and nitric acids to remove the impurities and to form more defect site. It is known that the higher proportion of edge plane defects may lead to more facile electron transfer.[54-56] After preptreatment, the aqueous dispersion of the chemically oxidized MWCNTs showed good dispersion and wettability and no precipitate occurred for months at room temperature.

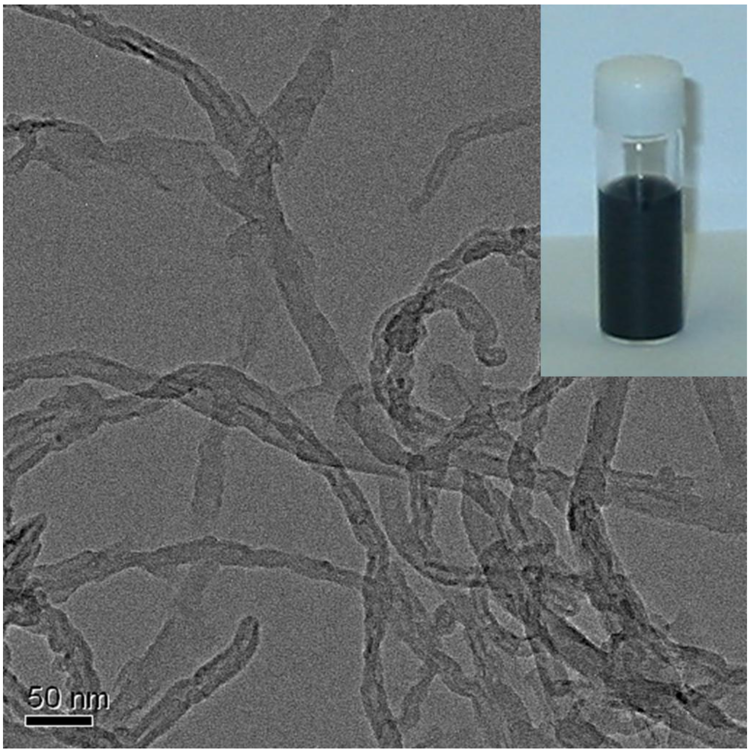


Figure 1. TEM image of the chemically oxidized MWCNTs. The inset in the image is a photograph of the MWCNTs solution.

Figs. 1 shows TEM image of the chemically oxidized MCNTs. The inset in the image is a photograph of the MWCNTs solution. The TEM image shows that the nanotubes have clear hollow channels and are wavy with diameters in the range of 20-30 nm. FT-IR was used to study the chemically oxidized MWCNTs.

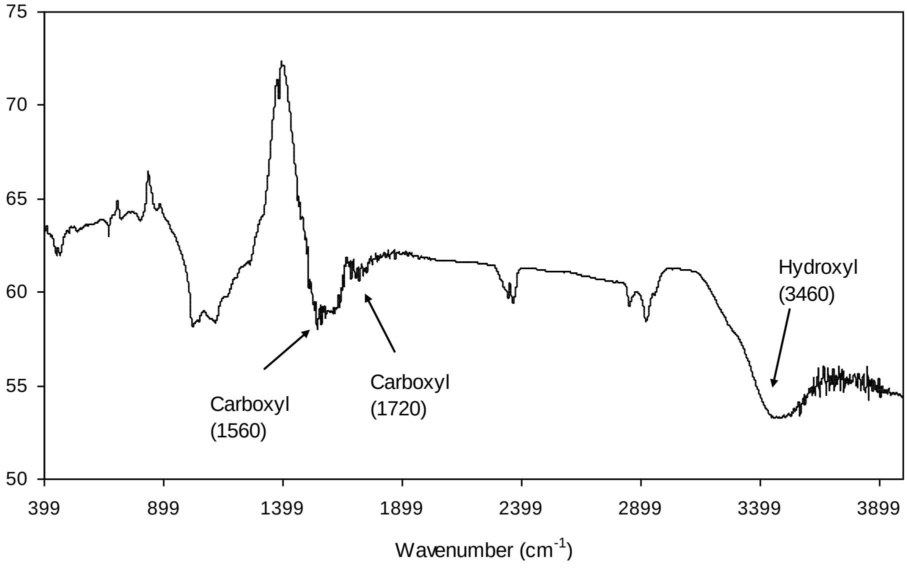


Figure 2. FT-IR spectrum of the chemically oxidized MWCNTs.

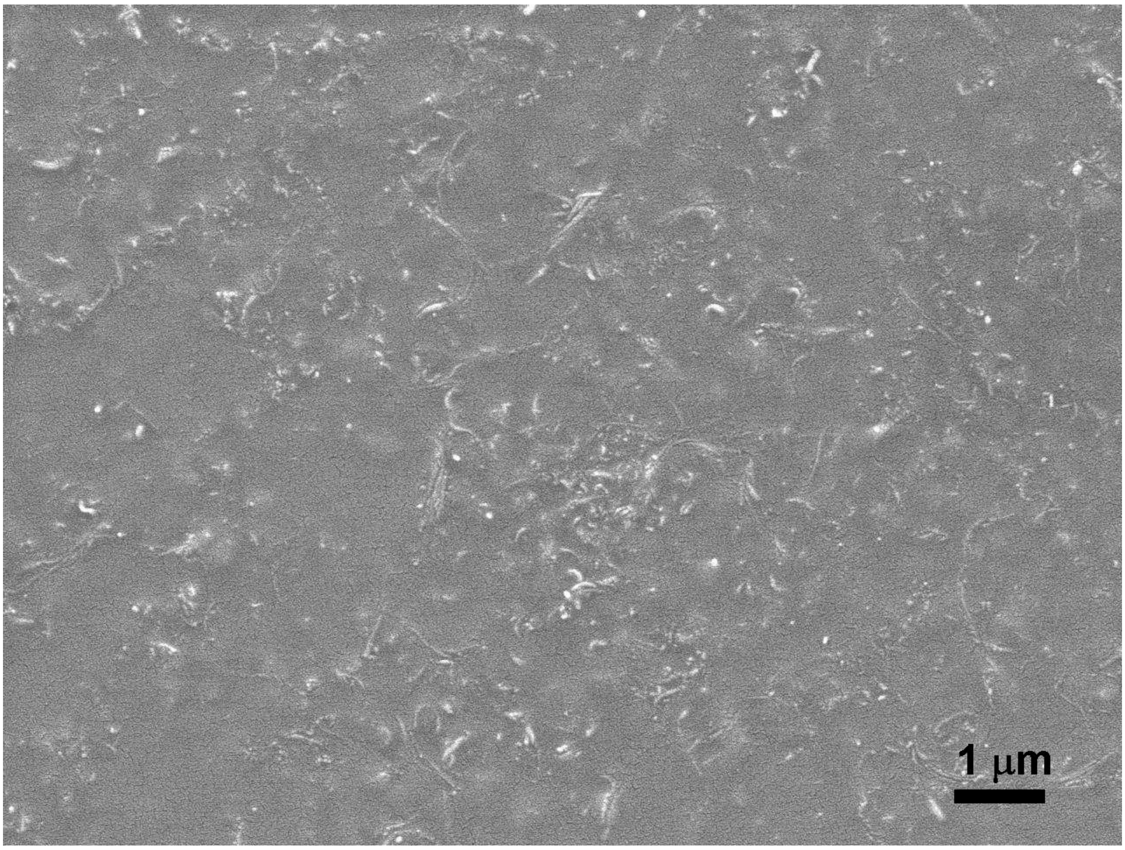


Figure 3. FE-SEM image of the MWCNT/Nafion modified glassy carbon electrode.

As can be seen in Fig. 2, the pretreated MWCNTs exhibites absorbance at about 1720 and 1560 cm^{-1}, indicating the formation of the carboxylic and carboxylate groups in the pretreatment process. These results indicated that the pretreatmented MWCNTs were chemically oxidized and carboxylic functional group was developed.

Fig. 3 shows FE-SEM image of the MCNT/Nafion modified glassy carbon electrode. Noodle shaped carbon nanotubes were found with diameters of 20-30 nm, which were typical diameters of MWCNTs. The FE-SEM image clearly showed that the MWCNTs were well dispersed on the entire glassy carbon electrode surface. The modified layer was very stable under experimental conditions

3.2. Cyclic voltammetry of ABX

The cyclic voltammograms of ABX on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode were recorded. The sweep was started at –0.2 V (vs. Ag/AgCl) in the positive direction and reversed at 1.30 V. Fig. 4 shows the cyclic voltammograms of ABX in different electrodes.

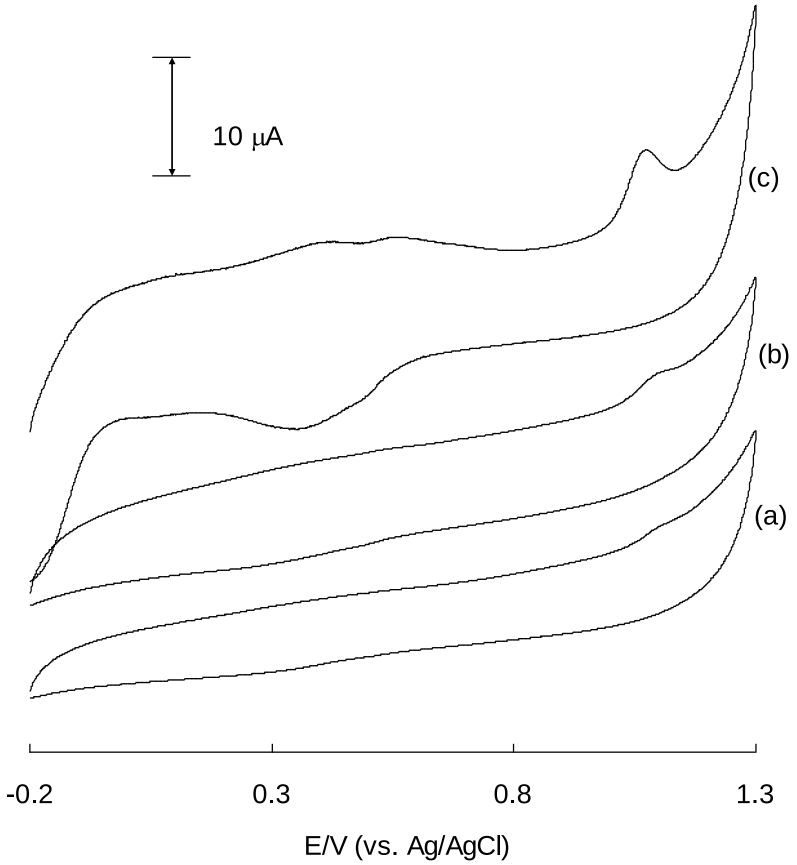


Figure 4. Cyclic voltammograms of 5×10^{-5} M ABX on (a) bare glassy carbon electrode, (b) Nafion modified glassy carbon electrode and (c) MWCNT/Nafion modified glassy carbon electrode. Supporting electrolyte: 1 M KCl solution containing 0.1 M HCl. Potential range: -0.2 to 1.3 V (vs. Ag/AgCl); scan rate: 0.05 V/s.

The oxidation peaks of ABX in KCl solution containing 0.1 M HCl are at 1.13, 1.11 and 1.08 V for bare glassy carbon electrode (Fig. 4a), Nafion modified glassy carbon electrode (Fig. 4b) and MWCNT/Nafion modified glassy carbon electrode (Fig. 4c), respectively. Obviously, the oxidation peak of ABX on MWCNT/Nafion modified glassy carbon electrode is at the most reduced potential. There was a considerable increase in peak current using a MWCNT/Nafion modified electrode compared to bare glassy carbon electrode and Nafion modified glassy carbon electrode. Such ability of MWCNTs to promote the electron transfer between ABX and the electrode thus allows highly sensitive voltammetric detection of ABX with a low limit of detection.

3.3. Differential pulse voltammetry of ABX

Differential pulse voltammetric determination conditions of ABX on each electrode were studied. The results showed that 5 μl of 0.1 % Nafion was the optimum modifying amount on the Nafion modified glassy carbon electrode due to the fact that this amount resulted in the highest peak current. On Nafion modified glassy carbon electrode, the most defined peak was obtained in 0.1 M

HCl. The peak current is improved on Nafion modified glassy carbon electrode due to the pre-concentration of ABX in the Nafion layer on the electrode surface.

The influence of the concentration of MWCNTs in the MWCNT/Nafion dispersion on the electrochemical oxidation of ABX was studied. The concentration of Nafion in the dispersion was fixed at 0.1 % and the concentration of MWCNTs was varied. Through the experimental results, optimum MWCNTs concentration of 0.2 mg/ml in the dispersion was selected with consideration of the sensitivity and electrode stability.

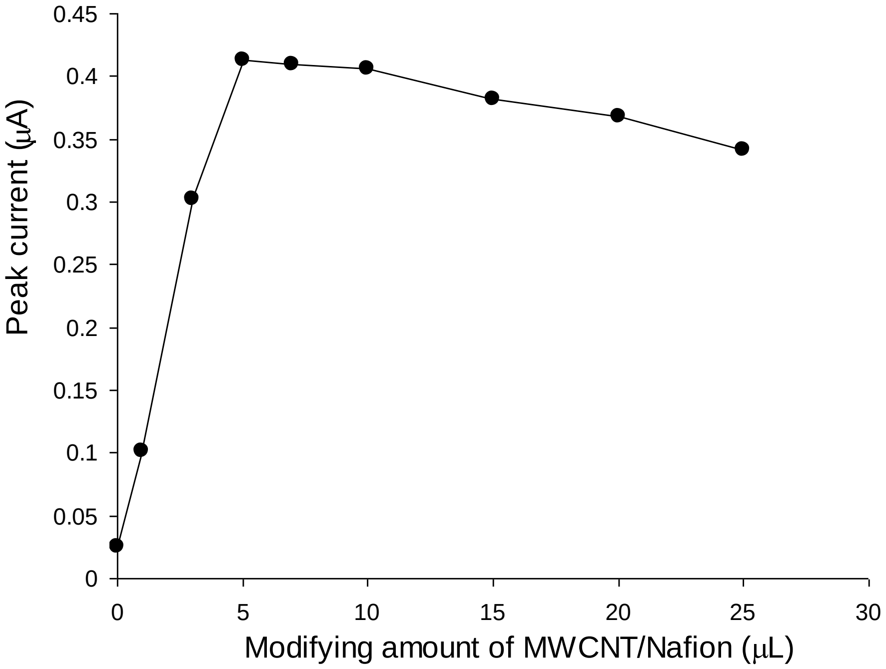


Figure 5. Effects of the modifying amounts of MWCNT/Nafion dispersion on peak current of 1×10^{-7} M ABX in 1 M KCl solution containing 0.1 M HCl. Preconcentration time: 60 s. Scan rate: 0.02 V/s.

The relationship between the amount of MWCNT/Nafion dispersion on the glassy carbon electrode surface and the peak current of ABX were studied and the results are shown in Fig. 5. The peak current is increased with the increase of the modifying amount up to 5 μl. Too much of the amount cause the decrease of the peak current due to the blocking behavior of Nafion. From the above experimental results, 5 μl MWCNT/Nafion dispersion was selected as the modifying amount for the differential pulse voltammetric determination of ABX. Accumulation studies were carried out on a MWCNT/Nafion modified glassy carbon electrode. The peak current of the differential pulse voltammograms increases dramatically when a preconcentraion period preceded the potential scan up to 60 s. So, we select 60s as the accumulation time on MWCNT/Nafion modified glassy carbon electrode. Optimum scan rate for the analysis of ABX by differential pulse voltammetry was fixed at 20 mV/s with consideration of the sensitivity and peak shape.

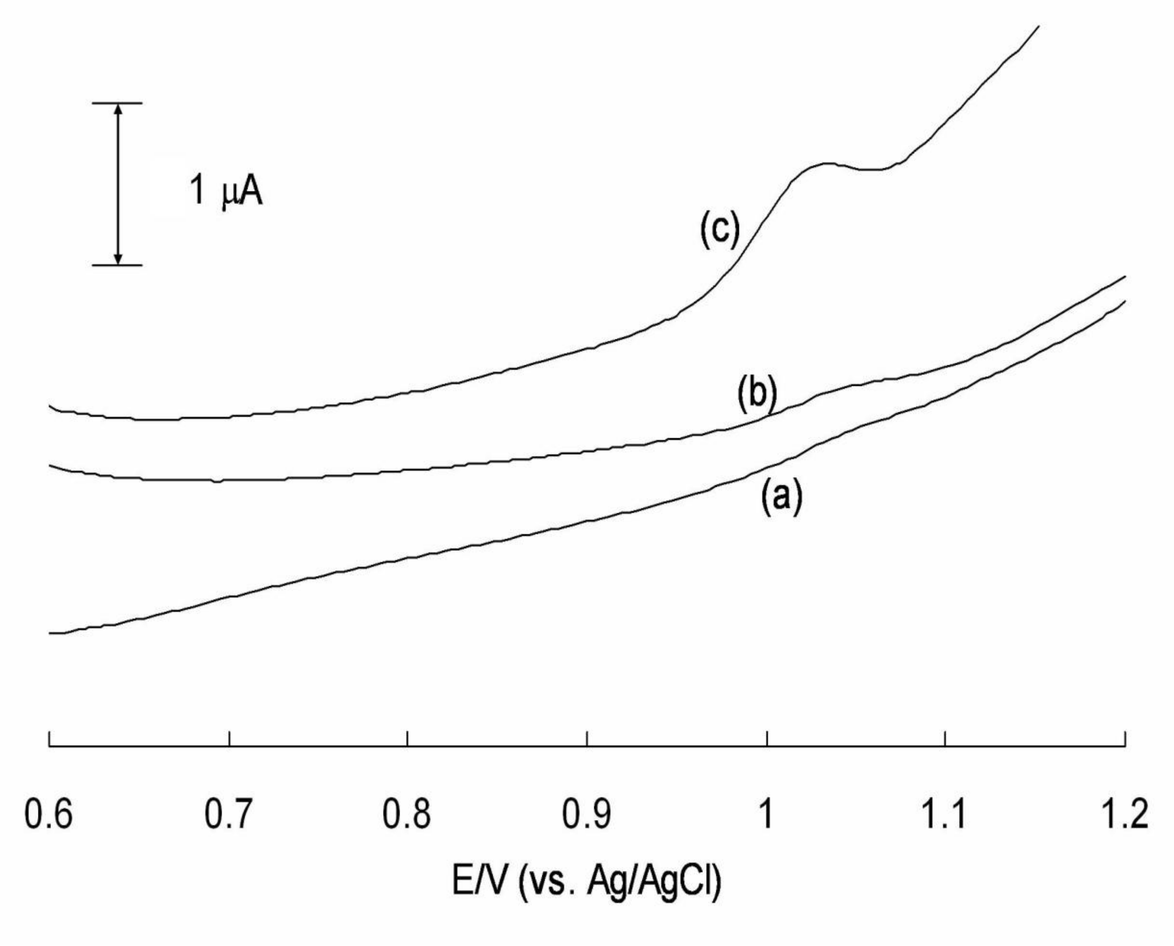


Figure 6. Differential pulse voltammograms of 1×10^{-7} M ABX on (a) bare glassy carbon electrode, (b) Nafion modified glassy carbon electrode and (c) MWCNT/Nafion modified glassy carbon electrode. Supporting electrolyte: 1 M KCl solution containing 0.1 M HCl. Preconcentration time: 60 s. Potential range: 0.6 to 1.2 V (vs. Ag/AgCl); scan rate: 0.02 V/s.

Differential pulse voltammograms of 1×10^{-7} M ABX on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode at the optimized condition are shown in Fig. 6. On bare glassy carbon electrode and Nafion modified glassy carbon electrode, 1×10^{-7} M ABX only yielded a very small oxidation peaks (Fig. 6a and 6b). However, the electrochemical oxidation peak current of ABX on MWCNT/Nafion modified glassy carbon electrode was increased significantly. In addition, a negative shifting in the peak potential in comparision with that on bare and Nafion modified was observed when MWCNTs were presented on the electrode surface. The result suggests that MWCNTs facilitates the electron transfer.

3.4. Linear range and limit of detection of ABX

At the optimized condition, we obtained a linear calibration plot for the analysis of ABX by differential pulse voltammetry on a MWCNT/Nafion modified glassy carbon electrode.

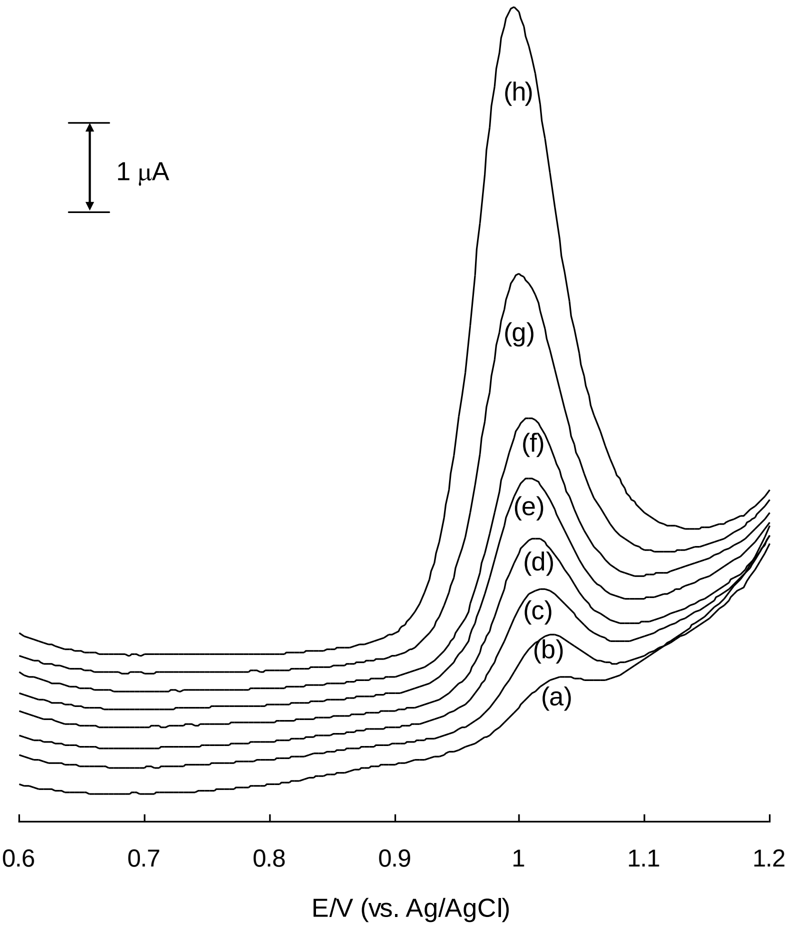


Figure 7. Differential pulse voltammograms of ABX on MWCNT/Nafion modified glassy carbon electrode in the presence of (a) 1×10^{-7} M, (b) 2×10^{-7} M, (c) 3×10^{-7} M, (d) 4×10^{-7} M, (e) 5×10^{-7} M, (f) 6.5×10^{-7} M, (g) 1×10^{-6} M and (h) 1.8×10^{-6} M ABX; electrolyte solution: 1 M KCl solution containing 0.1 M HCl; preconcentration time: 60 s, potential range: 0.6 to 1.2 V (vs. Ag/AgCl); scan rate: 0.02 V/s.

The linear response range for the ABX was between 1×10^{-8} and 1.8×10^{-6} M on MWCNT/Nafion modified glassy carbon electrode. The differential pulse voltammetric detection limits were determined to be 3×10^{-8} M, 4×10^{-9} M and 1×10^{-9} M (S/N=3) on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode, respectively. Fig. 7 shows the differential pulse voltammograms of 1×10^{-7} M, 2×10^{-7} M, 3×10^{-7} M, 4×10^{-7} M, 5×10^{-7} M, 6.5×10^{-7} M, 1×10^{-6} M and 1.8×10^{-6} M ABX on MWCNT/Nafion modified glassy carbon electrode in 1 M KCl solution containing 0.1 M HCl. It is clear that significant improvements in the sensitivity were obtained on MWCNT/Nafion modified glassy carbon electrode. The remarkable improvement is on account of the unusual structure and properties of MWCNTs. The reproducibility of MWCNT/Nafion modified glassy carbon electrode was tested and the relative standard derivation of 2×10^{-7} M ABX on the modified electrode was found to be 4.2 % (n=7).

Various ions were examined to investigate the interference in the determination of ABX. All alkali ions ,ammonium ion, alkaline earth, transition elements (Fe^{3+} , Cu^{2+}, Mn^{2+}) and some anions (CH_3COO^-, Cl^-, CO_3^{2-}, ClO_3^- , NO_3^-, S^{2-}, PO_4^{3-}, SO_4^{2-}) did not cause any interference in the measurement for ABX. This observation was re-confirmed in a separate experiment for the analysis of 2.0×10^{-7} M ABX in the presence of 100-fold excess of these ions by differential pulse voltammetry.

The stability of the MWCNT/Nafion modified glassy carbon electrode was studied by measuring the peak current at a fixed ABX concentration of 2.0×10^{-7} M over a period of 2 weeks. The experimental results showed that the peak current deviated 6.7 %, revealing that the modified electrode possesses long-term stability.

3.5. ABX determination in pharmaceutical dosage forms

Using MWCNT/Nafion modified glassy carbon electrode, the proposed method was applied to the determination of ABX in pharmaceutical dosage forms. For the analysis, the experimental work described in section 2.3 was followed. The recoveries were calculated with reference to the calibration graphs. As can be seen from the results shown in Table 1, the methods give satisfactory recovery data with no significant differences between the declared and experimental data. The standard deviations for the assay results show good precision.

Table 1. Results for ABX in pharmaceutical dosage forms with this method.

Sample	Pharmaceutical forms	Claimed content	Experimental amount (n=5)	RSD (%)
1	Tablets	30.0 mg/tablet	29.6 ± 1.2	2.1
2	Syrup	15 mg/100 ml	14.6 ± 0.8	2.3

4. CONCLUSION

The work described in this paper outlines a detailed study of the electrochemistry of ABX on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode. The addition of a thin MWCNT/Nafion coating to the electrode surface resulted in a large increase in peak current as well as some decrease in the electrochemical oxidation peak potential compared to bare glassy carbon electrode. The differential pulse voltammetric detection limits were determined to be 3×10^{-8} M, 4×10^{-9} M and 1×10^{-9} M on bare glassy carbon electrode, Nafion modified glassy carbon electrode and MWCNT/Nafion modified glassy carbon electrode, respectively. Determination of ambroxol in pharmaceutical dosage forms was performed by this method. All values were in good statistical agreement with the nominal values. The procedure is shown simple, fast and sensitive for the determination of ABX.

ACKNOWLEDGMENT

This work was supported by the Korea Research Council of Fundamental Science and Technology (KRCF) through the project of ‘Development of Characterization Techniques for Nano-materials Safety’ and a grant of the Korea Healthcare Technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A092145). This work was also supported by the Smart IT Convergence System Research Center funded by the Ministry of Education, Science and Technology as Global Frontier Project (0543-20110015).

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