Electrochemical Oxidation of Sulfamethazine on Multi-Walled Nanotube Film Coated Glassy Carbon Electrode

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Abstract

The electrochemical oxidation of sulfamethazine (SMZ) has been studied at a multi-walled carbon nanotubes modified glassy carbon electrode (MWCNT-GCE) by cyclic voltammetry. This modified electrode (MWCNT-GCE) exhibited excellent electrocatalytic behavior toward the oxidation of SMZ as evidenced by the enhancement of the oxidation peak current and the shift in the anodic potential to less positive values (170 mV) in comparison with the bare GCE. The formal potential, \(E^0\), of SMZ is pH dependent with a slope of 54 mV per unit of pH, close to the anticipated Nerstian value of 59 mV for a 2-electron and 2-proton oxidation process. A detailed analysis of cyclic voltammograms gave fundamental electrochemical parameters including the electroactive surface coverage (\(\Gamma\)), the transfer coefficient (\(\alpha\)), the heterogeneous rate constant (\(k_h\)). Under the selected conditions, the peak current shows two dynamic linear ranges of 10-200 \(\mu M\) and 300-3000 \(\mu M\) with the detection limit of 6.1 \(\mu M\). The method was successfully applied to analyze SMZ in serum sample.

1. Introduction

Sulfonamides are a common class of antibiotics in bacterial and livestock diseases such as gastrointestinal and respiratory in veterinary and human medicine practice. As a member of the sulfonamides family, sulfamethazine is a widely used antimicrobial agent added to the feed of meat-producing animals treated infections [1]. The inappropriate use in food can cause the undesirable residues in edible foodstuffs and it is suspected to be carcinogenic [2]. The best scientific information available indicates that SMZ is an enzyme inhibitor that blocks thyroid hormone synthesis and results in secondary stimulate of thyroid cell growth [3].
After administration, a high fraction of SMZ is excreted without metabolism via urine or feces, and then discharged into the manure or sewage, finally entered into the environment [4]. In order to monitor the level of SMZ, selective and sensitive qualitative method is required. A variety of analytical methods have been developed for its determination. Those methods included mainly: high performance chromatography [5], gas chromatography [6], spectrofluorimetry [7], biosensors and immunoassays [8, 9]. Electrochemical methods such as sensors are simple, speedy, sensitive and inexpensive. In the field of sensors, nanopatricles modified electrodes have exhibited strong and stable electrocatalytic response towards drugs [10-12]. Carbon nanotubes are an interesting class of nonmaterial offering high electrical conductivity, high surface area, significant mechanical strength and good chemical stability. They have been known to promote electron transfer reactions when used as electrode modifying material. In the present work, we reported the electrocatalytical properties of MWCNT modified GCE toward the oxidation of SMZ. The MWCNT could effectively facilitate the electron transfer of SMZ to the electrode therefore it was applied to developing a sensor for its determination.

2. Experimental procedure

Electrochemical measurements were carried out with a Metrohm model 746 VA trace analyzer connected to a 747 VA stand. The working electrode was a glassy carbon electrode (2 mm diameter). A platinum wire and a commercial Ag/AgCl saturated KCl electrode from Metrohm were used as auxiliary and reference electrodes, respectively.

Sulfamethazine was obtained from Alfa Aesar. All other reagents used were of analytical grade without further purification. Multi-walled carbon nanotubes with purity 95% (10-30 nm diameters and 5 µm length) were obtained from io-li-tec, Ionic Liquid Technologies.

MWCNT (4 mg) was added to 1 mL DMF. A homogeneous and stable suspension of 4 mg mL⁻¹ MWCNT was achieved with the aid of ultrasonic agitation for about 30 min.

Prior to the immobilization, glassy carbon electrode was sanded using ultrafine sand paper, polished with 10 µm alumina slurry in sequence and sonicated in water for 10 min. The MWCNT-GCE was prepared by casting 6 µL of the suspension of MWCNT on the surface of a GCE, which was dried in air for 30 min at room temperature.

3. Results and discussion

The distribution of MWCNT over the surface of modified GCE is shown by SEM in Fig 1. As shown in Fig 1, porous MWCNT film has large surface area. The SEM image also reveals that the MWCNT are well distributed on the surface and that most of the MWCNT are in the form of small bundles or single tubes.

Fig. 1. SEM image of MWCNT-GCE.

Fig 2 shows cyclic voltammograms of SMZ on the bare GCE and MWCNT-GCE in B-R buffer.
solution (pH 6.0). In the absence of SMZ, no redox peaks were observed at bare electrode during the cyclic voltammetric measurements within the potential window of 0.50 V to 1.20 V (Fig 2, curve a). As it is shown in Fig 2, curve b, the SMZ oxidation peak at the bare GCE showed only a small and broad anodic peak occurs at about 1.08 V and no corresponding oxidation peak was observed in the reverse scan, which indicates that the electrochemical behavior of SMZ at unmodified GCE is an irreversible and sluggish electron transfer process due to the 2-electron oxidation of the -NH$_2$ group to hydroxylamine [13]. However, at a MWCNT-GCE, the anodic peak was observed at the less positive potential of 0.91 V with an increased peak current compared with the bare GCE (Fig 2, curve c) due to electrocatalytic effect of MWCNT [12]. Nevertheless, it was found that the oxidation peak current of SMZ showed a remarkable decrease during the successive cyclic voltammetric sweep at bare GCE, while at the MWCNT-GCE the peak current decreased slightly and finally remained unchanged.

The pH dependence of the SMZ reduction was systematically studied in the pH range of 1.5-9.0 in B-R buffer solution at MWCNT-GCE. As it is shown in Fig 3A by increasing the pH the oxidation peak potential of SMZ shifts to less positive potentials due to the hindrance of the oxidation at low concentrations of protons. Since SMZ molecule has two acid-base centers, amine and amide, two defined breaks at corresponding to the apparently pK$_a$ values, 2.7 and 7.4 which are in excellent agreement with literature [14]. The formal value E$_0'$ is linear with pH in acidic media, with a slope 54 mV/pH (Fig 3B). This value is close to the theoretical value of 59 mV/pH [15] indicating the participation of the same proton and electron numbers in the electrochemical process.

**Fig 2.** Cyclic voltammograms of (a) bare GCE in blank solution, (b) bare GCE in the presence of 2mM SMZ, (c) MWCNT-GCE in the presence of 2mM SMZ. B-R buffer (pH 6.0) solution, scan rate: 100 mVs$^{-1}$.

**Fig 3.** (A) Cyclic voltammograms of 2 mM SMZ at MWCNT-GCE for various pH values (from right to left) 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0, 9.0 in B-R buffer, scan rate 50 mVs$^{-1}$. (B) plot E0' vs. pH for MWCNT-GCE.

Useful information involving electrochemical mechanism usually can be acquired from the relationship between peak current and scan rate. Thus, the cyclic voltammograms of SMZ at various scan rates were recorded on the surface MWCNT-GCE (Fig 4A). As is shown in Fig 4B, the anodic peak current of SMZ is proportional to the scan rate which indicates that the electrode process is adsorption-controlled. Furthermore, from the slope of the linear plot of I versus $\nu$ the
surface concentration of the electroactive species (Γ) can be estimated to be about 5.15×10^{-6} \text{ mol cm}^{-2} can be obtained at the MWCNT-GCE according to the following equation [16]:

\[ i_p = n^2F^2 \nu \Delta \Gamma / 4RT \]

(1)

where \( n \) represents the number of electrons involved in reaction, A is the surface area (0.0314 cm\(^2\)) of the GCE, Γ (mol cm\(^{-2}\)) is the surface coverage and other symbols have their usual meaning. However, at higher scan rates the plot of \( I \) vs. \( \nu^{1/2} \) was linear, most likely due to the diffusion-controlled SMZ oxidation (Fig 4C).

As shown by increasing the scan rate, the peak potential is shifted to a more positive potential. Because of the irreversible electrode process of the oxidation reaction of SMZ, the Laviron’s equation [17] was used to estimate \( \alpha n \) and \( k_s \) values as follows:

\[ E_p = E^0 + (RT/\alpha nF)[\ln(RTk_s/\alpha nF) - \ln \nu] \]

(2)

where \( \alpha \) is the electron transfer coefficient, \( k_s \) is the standard rate constant of the surface reaction, \( \nu \) is the scan rate, \( n \) is the electron transfer numbers and \( E^0 \) is the formal potential. \( k_s \) and \( \alpha n \) values can be concluded from the intercept and slope of the linear plot of \( E_p \) with respect to \( \ln \nu \), if the value of \( E_0 \) is known.

The \( E^0 \) value at MWCNT-GCE can be deduced from the intercept of \( E_p \) vs. \( \nu \) plot on the ordinate by extrapolating the line to \( \nu = 0 \) Fig 4D. Knowing \( E^0 \) and from the graphical representations of \( E_p \) vs. \( \log \nu \) for SMZ in the presence of MWCNT (Fig 4E), the values of \( \alpha n = 1.12 \) and \( k_s = 525.5 \text{ s}^{-1} \) were obtained from the slope and intercept, respectively. Since for a totally irreversible oxidation of SMZ \( n \) is equal to 2, the \( \alpha \) was calculated to be 0.56. As shown before, the total numbers of electrons and protons taking part in the charge transfer was same; the electrochemical reaction process for SMZ oxidation at MWCNT-GCE can be summarized as in Scheme 1.

![Scheme 1](image)

Fig 4. (A) Cyclic voltammetric responses of 2mM SMZ at MWCNT-GCE (B-R buffer (pH 6.0)) at scan rates, (inner to outer) 20, 30, 40, 50, 60, 80, 100, 120, 150, 200, 300 mVs\(^{-1}\). (B and C) The plots of peak currents vs scan rate and square root of scan rate, respectively. (D and E) The variation of peak potential vs \( \nu \) and \( \log \nu \), respectively.

In preliminary experiments, it became obvious that both the amount and injected volume of MWCNT affect the voltammetric analysis. Hence, these parameters were studied and optimized. The effect of the MWCNT amount on the anodic peak was examined by varying the amount of MWCNT from 1 to 10 mg in 1 mL of DMF. The results showed that the peak current reached a maximum
at 4 mg and decreased after that. The effect of the injected volume of MWCNT composite film was also investigated and a maximum current was obtained at 6 μL. By increasing the pH value, I increased and then decreased; and reached its maximum at pH 6. An optimal pH value of 6.0 was therefore chosen.

Under the optimized conditions two linear ranges were obtained for SMZ over the 10-200 μM and 300-3000 μM (Fig 5). The detection limit of 6.1 μM was calculated according to the $Y_{LOD}=X_B+3S_B$, where $Y_{LOD}$ is the signal at the limit of detection and $X_B$ and $3S_B$ are the mean and the standard deviation ($n=8$) of the blank signal, respectively.

The reproducibility of the method was evaluated by successive determinations ($n=10$) of SMZ. A relative standard deviations (R.S.D.) value of less than 1.36 was obtained.

Under the optimum conditions, the possible interferences of several ions such as NH₄⁺, Li⁺, Al³⁺, Ca²⁺, Cd²⁺, Cu²⁺, CO₃²⁻, Sn²⁺, Na⁺, K⁺, Ni²⁺, Fe²⁺, Zn²⁺, Ba²⁺, Sr²⁺, Pb²⁺, Cr³⁺, Cl⁻, SO₄²⁻, NO₃⁻, HSO₄⁻, HPO₄²⁻ were investigated by premixing 0.5 mM SMZ solution with the foreign substances. Amino acids such as, L-lysine, L-arginine, L-serine, L-histidine, L-glutamic acid, L-theronine and urea were investigated but did not show any interference effect.

To evaluate the applicability of the present method on real matrices, assays were performed on serum samples. Recovery experiments were carried out by adding standard solutions of SMZ to serum matrices. According to the results, satisfactory recovery for SMZ could be obtained. Table 1 summarizes the data obtained for SMZ assays performed on the matrices, which were studied using the optimized experimental methodology.

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4. Conclusion
In the present study, an easily prepared MWCNT-film modified GCE was used to investigate the detailed electrochemical behavior of SMZ. The reported modified electrode significantly improved the electrochemical response of SMZ and clearly demonstrates the excellent electrocatalytic activity of the MWCNT-film modified GCE toward the oxidation for the oxidation of SMZ. Some electrochemical parameters such as the standard rate constant of the surface reaction, transfer coefficient and surface concentration of the electroactive species have been calculated. The bioconjugates of carbon
nanotubes and DNA have potential applications in many areas due to the combination of the unusual structure of carbon nanomaterials and bioactivity of DNA reported in the literatures as bellow: The $\pi-\pi$ interactions between the nanotube sidewalls and nucleic acid bases, wrapping of CNTs in DNA, and attachment of oligonucleotides to the sidewalls of CNTs through hydrophobic interactions.

Under optimized experimental conditions, good analytical performance was obtained, including suitable precision and excellent linear dynamic range and detection limit. The method is sensitive enough for the analysis of lower concentrations of SMZ in serum.

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References