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Electrochemical Behavior and Voltammetric Determination of Sulfaguanidine at a Glassy Carbon Electrode Modified With a Multi-Walled Carbon Nanotube

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The electrochemical properties of sulfaguanidine (Sg) is investigated in detail at a glassy carbon electrode (GCE) modified with a multi-walled carbon nanotube (MWCNT). Cyclic voltammetry and chronoamperometry were used as diagnostic techniques. The MWCNT-GCE exhibited excellent electrocatalytic behavior for the oxidation of Sg as evidenced by the enhancement of the 2e-oxidation peak current and the shift in the oxidation potential to less positive values (by 90 mV) in comparison with a bare GCE. A detailed analysis of cyclic voltammograms and chronoamprograms gave fundamental electrochemical parameters including the electroactive surface coverage (Γ), the transfer coefficient (α), the heterogeneous rate constant (k_s) and the diffusion coefficient (D). Under the selected conditions, the peak current shows two dynamic linear ranges10-100 μM and 100–1000 μM with the detection limit of 10μM. The method was successfully applied to analyze Sg in urine and serum samples.

Keywords: Sulfaguanidine, Multi-walled carbon nanotube, Cyclic voltammetry, Determination, Oxidation

1. INTRODUCTION

Sulfonamides which are also called sulfa medicines are used to treat many kinds of infections caused by bacteria and certain other microorganisms. Physicians may prescribe these drugs to treat urinary tract infections, ear infections, frequent or long-lasting bronchitis, bacterial meningitis, certain eye infections, *Pneumocystis carinii* pneumonia (PCP), traveler's diarrhea, and a number of other infections [1, 2]. They are among the priority pollutants to be monitored in animal derived food products, as well as a wide variety of matrices, because their undesirable residues can remain and be

incorporated into waters, soils, crops, animal tissues, and bio-fluids (milk and plasma) [3, 4]. Some sulfonamides such as sulfaguanidine are used in the treatment of gastrointestinal infections particularly bacillary dysentery.

A variety of methods have been used to measure sulfonamides in food and other matrices. Those methods included mainly: high performance chromatography [5-8], gas chromatography [9], colorimetric procedures [10] and immunoassays [11]. Compared with spectroscopic method, the electrochemical assay is simple, reliable and practical with low detection limit and wide dynamic range. Electrode surface modification has been tried as a means to reduce the overvoltage and overcome the slow kinetics of many electrode processes. Carbon nanotube modified electrode form a subcategory of the chemically modified electrodes, which were largely studied [12-16]. Carbon nanotubes are an interesting class of nonomaterial offering high electrical conductivity, high surface area, significant mechanical strength and good chemical stability. One of the most important characteristics of the electron mediators, which are used in modified electrodes, is lowering the overpotential required for electrochemical oxidation of the analyte and enhancement the sensitivity and of course selectivity of the response sensor.

To the best of our knowledge, no study involving a nanoparticle sensor for determining Sg has been reported. Following our recent studies [17-21], in this paper, we present and discuss the electrochemical behavior of sulfaguanidine (Sg) at MWCNT-GCE and introducing a single, fast and inexpensive analytical method for its determination.

2. EXPERIMENTAL PART

2.1. Apparatus

Electrochemical measurements were carried out with a Metrohm model 746VA trace analyzer connected to a 747 VA stand. The working electrode was a glassy carbon electrode (2 mm diameter). Before use, the working electrode was sequentially polished with graded 10 μM alumina powder, and rinsed with doubly distilled water. A platinum wire and a commercial Ag/AgCl saturated KCl electrode from Metrohm were used as auxiliary and reference electrodes, respectively. The scan rate in cyclic voltammetry was 100 mV s^{-1}, with the exception of the experiments in which the influence of this variable was studied.

Chronoamperometric measurements were conducted using different concentrations of Sg in solution at the MWCNT-film modified GCE. The initial potential (E_i), where no electrolysis occurs, and the step potential (E_s), where complete electrolysis occurs, were 0.75 V and 0.93V, respectively (interval time, $\tau = 50$ s).

2.2. Reagents and chemicals

Sulfaguanidine was obtained from Sigma. Multi-walled carbon nanotubes with purity 95% (10-30 nm diameters and 5μm length) were obtained from io-li-tec, Ionic Liquid Technologies. All other

reagents used were of analytical grade without further purification. A stock Britton-Robinson (B-R) buffer solution 0.04 M with respect to boric acid, orthophosphoric acid and acetic acid were prepared from proanalysis reagents. From this stock buffer, solutions with various values of pHs were prepared by the addition of 0.2 M sodium hydroxide solution.

2.3. Preparation of the MWCNT film modified GCE

The glassy carbon electrode was sanded using ultrafine sand paper, polished with 10 μm alumina slurry in sequence and sonicated successively in water and ethanol, respectively for 10 min. MWCNT (4mg) was added to 1 mL DMF. A homogeneous and stable suspension of 4 mg mL^{-1} was achieved with the aid of ultrasonic agitation for about 30 min. The GCE was coated by casting with a 4μL suspension of MWCNT-film modified GCE and dried in the air.

2.4. Procedure for analysis of real samples

1 mL urine or serum sample was collected and diluted to 10 mL buffer solution. Then resulted solution was transferred to the electrochemical cell, and the voltammograms were recorded. To ascertain the validity of the results, the sample was spiked with certain amount of Sg and the recovery rates of the spiked samples were determined.

3. RESULTS AND DISCUSSION

3.1. Electrochemical behavior of Sg at the MWCNT-GCE

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.

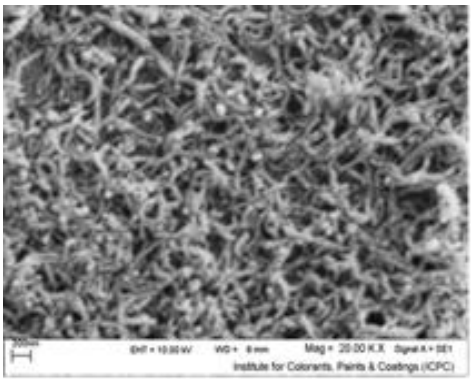


Figure 1. SEM image of the MWCNT-film modified GCE.

Cyclic voltammograms of Sg at a bare GCE and MWCNT-GCE in B-R buffer (pH = 7.0) were shown in Fig 2. In the absence of Sg, no redox peaks were observed at either bare or modified GCE electrodes during the cyclic voltammetric measurments within the potential window of 0.60 V to 0.95 V (Fig 2, curves a, b). Compared with the bare GCE (Fig 2, curve a) a large background current was observed at the MWCNT-GCE (Fig 2, curve b) during the cyclic voltammetric scan, which is probably due to a high double layer capacity [22]. It can be seen that the Sg oxidation peak (0.93V) at the bare GCE was weak and broad due to slow electron transfer (Fig 2, curve c). In comparison, at a MWCNT-GCE, the anodic peak was observed at the less positive potential of 0.84V and clearly showed an increased peak current compared with the bare GCE (Fig 2, curve d). The increased current as well as the negative shift of the anodic peak demonstrated an efficient catalytic oxidation of Sg on the MWCNT-GCE.

It also showed that no reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. Nevertheless, it was found that the oxidation peak current of Sg 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.

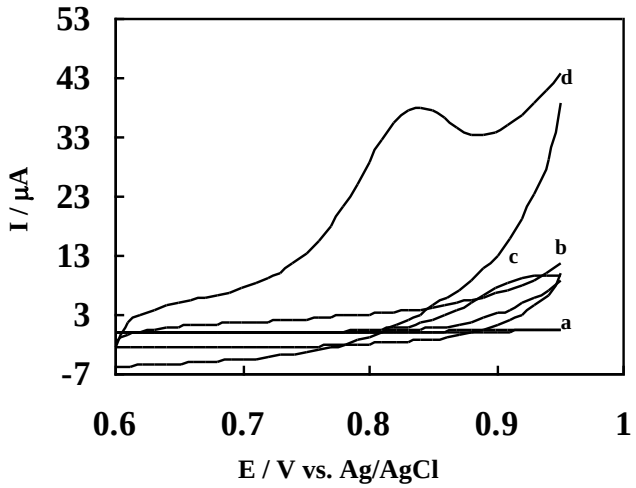


Figure 2. Cyclic voltammograms of (a) Bare GCE in blank solution, (b) MWCNT-GCE in blank, (c) Bare GCE in the presence of 1mM Sg, (d) MWCNT-GCE in the presence of 1mM Sg. B-R buffer (pH=7.0)solution, scan rate: 100 mVs^{-1}.

3.2. Determination of electrochemical active surface area

Electrode reaction rates and most double layer parameters are extensive quantities and have to be referred to the unit area of the interface. Knowledge of the real surface area of electrodes is therefore needed [23]. In order to measure the electrochemically active surface areas of the modified electrode, the chronoamprogram of 0.1 mM potassium ferrocyanide as the redox probe was recorded. In chronoamperometric studies, the current for the electrochemical reaction of ferrocyanide (at a mass-transfer-limited rate) that diffuse to a electrode surface is described by the Cottrell equation [24]:

$$i = nFAD^{1/2}C^{*} / \pi^{1/2}t^{1/2} \quad (1)$$

where *A* is the electrochemical active area, *D* is the diffusion coefficient, C^{*} is the bulk concentration of ferrocyanide and the other parameters have their usual meanings. Under diffusion control, a plot of i vs $t^{-1/2}$ will be linear and from the slope, the value of A can be obtained, since the precise value of the diffusion coefficient of ferrocyanide is well known (6.20×10^{-6} cm^2 s^{-1}). The electrochemically active area of the MWCNT-GCE was 0.09 cm^2.

3.3. The effect of pH

Fig 3A shows the cyclic voltammograms of Sg at different values of pH on the MWCNT-GCE.

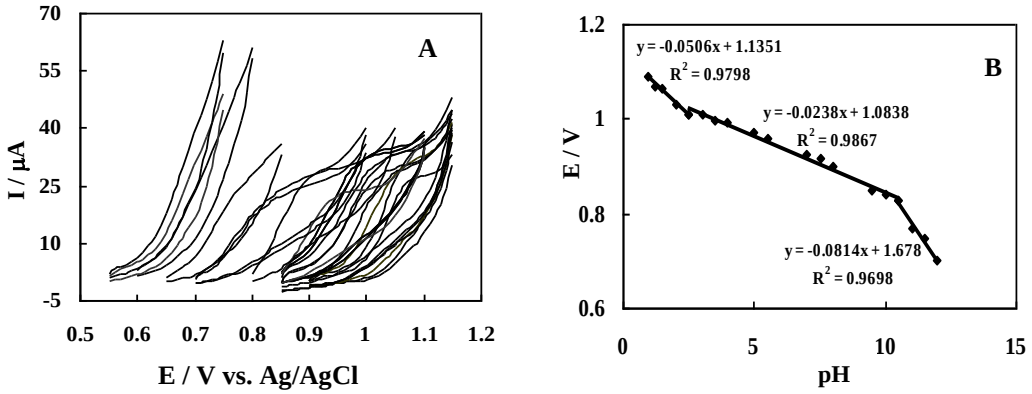


Figure 3. (A) Cyclic voltammograms of 1 mM Sg at MWCNT-GCEfor various pH values (from right to left) 1.0, 1.2, 1.5, 2.0, 2.5, 3.0, 3.5. 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 9.5, 10.0, 11.0, 12.0, 12.5, in B-R buffer, scan rate 100 mVs^{-1}. (B) plot $E^{0'}$ vs pH for MWCNT-GCE.

By increasing the pH the oxidation peak potential of Sg shifts to less positive potentials due to the hindrance of the oxidation at low concentrations of protons. In Sg molecule two acid-base centers, amine and amide are always present. Fig 3B shows two defined breaks at corresponding to the apparently pK_a values, 2.5 and 11.5. The values of pK_a are in excellent agreement with literature [25]. The formal value $E^{0'}$ is linear with pH in acidic media, with a slope 50.6 mV/pH. This value is close to the theaoretical value of 59 mV/pH [24] indicating the participation of the same proton and electron numbers in the electrochemical process.

3.4. The effect of the scan rate

Fig 4A shows the cyclic voltammograms of Sg at the MWCNT-GCE when the scan rate (υ) varies from 10 to 800 mVs^{-1}. As is shown in Fig 4B, the anodic peak current of Sg is proportional to the scan rate, which indicates that the electrode process is surface-controlled. However, at higher scan

rates (more than 150 mV s^{-1}) the plot of I vs. $\upsilon^{1/2}$ was linear, most likely due to the diffusion-controlled Sg oxidation (Fig 4C).

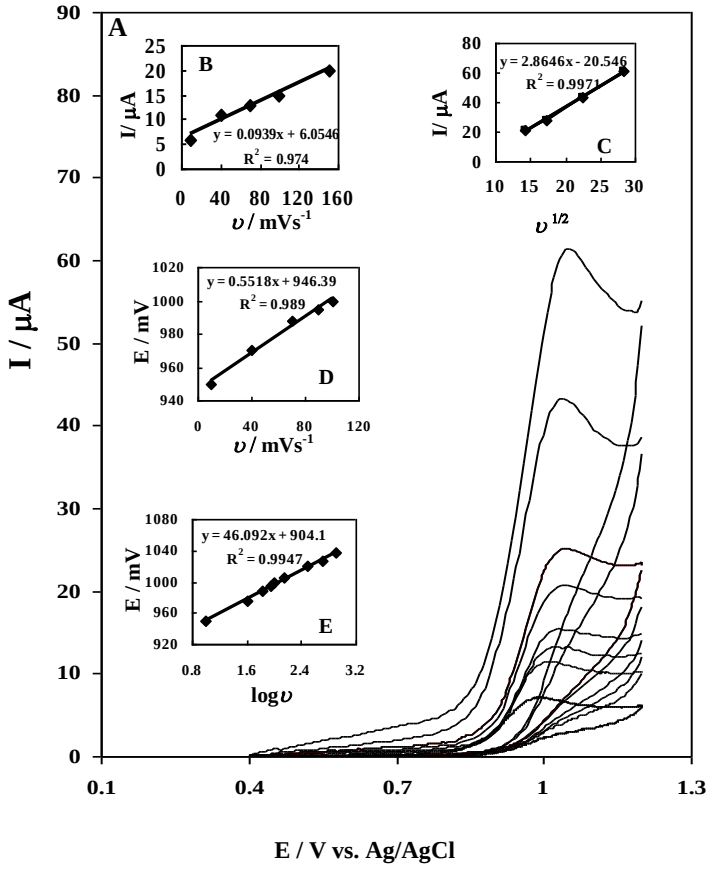


Figure 4. (A) Cyclic voltammetric responses of 1mM Sg at MWCNT-GCE (B-R buffer (pH= 6.0)) at scan rates, (inner to outer) 10, 40, 70, 100, 150, 300, 500, 800 mVs^{-1}. (B and C) The plots of peak currents vs scan rate and square root of scan rate, respectively. (D and E) The variation of peak potential vs υ and log υ, respectively.

From the observations it can be concluded that the electrochemical process consists of a mixture of diffusion and adsorption controlled processes, depending on the scan rate [26-29]. Furthermore, from the slope of the linear plot of *I* vs. υ the surface concentration of the electroactive species (Γ) can be estimated to be about 2.78×10^{-7} $molcm^{-2}$ according to the following equation [30]:

$$i_p = n^2F^2A\Gamma\upsilon/4RT \qquad (2)$$

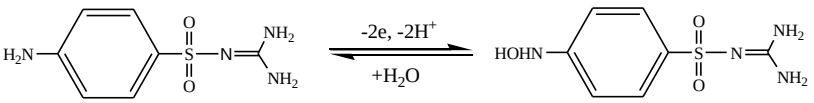
As shown by increasing the scan rate, the peak potential is shifted to a more positive potential. Because of the irreversible electrode process of the oxidation reaction of Sg, the Laviron's equation [31] was used to estimate αn and k_s values as follows:

$$E_p = E^{0'} + (RT/\alpha nF)\,[\ln (RTk_s/\alpha nF) - \ln \upsilon] \quad (3)$$

Where α is the electron transfer coefficient, k_s is the standard rate constant of the surface reaction, υ is the scan rate, n is the electron transfer numbers and E^0 is the formal potential. k_s and αn values can be concluded from the intercept and slope of the linear plot of E_p with respect to ln υ, 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. υ plot on the ordinate by extrapolating the line to $\upsilon = 0$ (Fig 4D). Knowing E^0, and from the

graphical representations of E_p vs. logυ for Sg in the presence of MWCNT (Fig 4E), the values of αn= 1.06±0.04 and k_s=3.125 s^{-1} were obtained from the slope and intercept, respectively. Since for a totally irreversible electron transfer, α was assumed as 0.5, the n was calculated to be 2.12 which indicated that two electrons was involved in the oxidation of Sg on the MWCNT-GCE. As shown before, the total numbers of electrons and protons taking part in the charge transfer was same, the electrochemical reaction process for Sg oxidation at MWCNT-GCEcan be summerized as in scheme 1.



Scheme 1

3.5. Chronoamperometric studies

Chronoamperometry can be used for determination of the diffusion coefficient (D) of Sg. We have obtained chronoamprograms at a fixed potential of 0.93V over 50 s in B-R buffer (pH=7.0) containing different concentrations of Sg (Fig 5A).

From the slope of a plot of I vs $t^{-1/2}$ at different concentrations (Fig 5B), D values were estimated according to the Cotterll equation [24]. The slopes of the resulting straight lines were then plotted vs the Sg concentration (Fig 5C), from whose slope an average value of D = 2.7×10-6 cm^2s^{-1} was obtained. The results showed that the electron transfer is a diffusion-controlled process and the obtained diffusion coefficient value is in good agreement with reported values of diffusion coefficient.

3.6. Analytical application and analytical figures of merit

The effect of the amount of MWCNT on the anodic peak was examined by varying the amount from1 to 10 mg in 1 mL of DMF. The results showed that the peak current reached a maximum at 4 mg and stabilized after that. The effect of the injected volume of MWCNT composite film was also investigated and a maximum current is obtained at 4μL.

Under optimum experimental conditions the peak current of Sg was found to be proportional to its concentration over two dynamic linear ranges of 10-100 μM and 100-2000 μM which can be expressed according to the equation of Y=75.82X+2.66 and Y=33.84X+6.4, respectively. The limit of

detection (LOD) was obtained from $Y_{LOD}=X_B+ 3S_B$, where Y_{LOD} is the signal fat the limit of detection and X_B and S_B are the mean and the standard deviation of the blank signal, respectively. Under optimum conditions the limit of detection (LOD) was 10 μM.

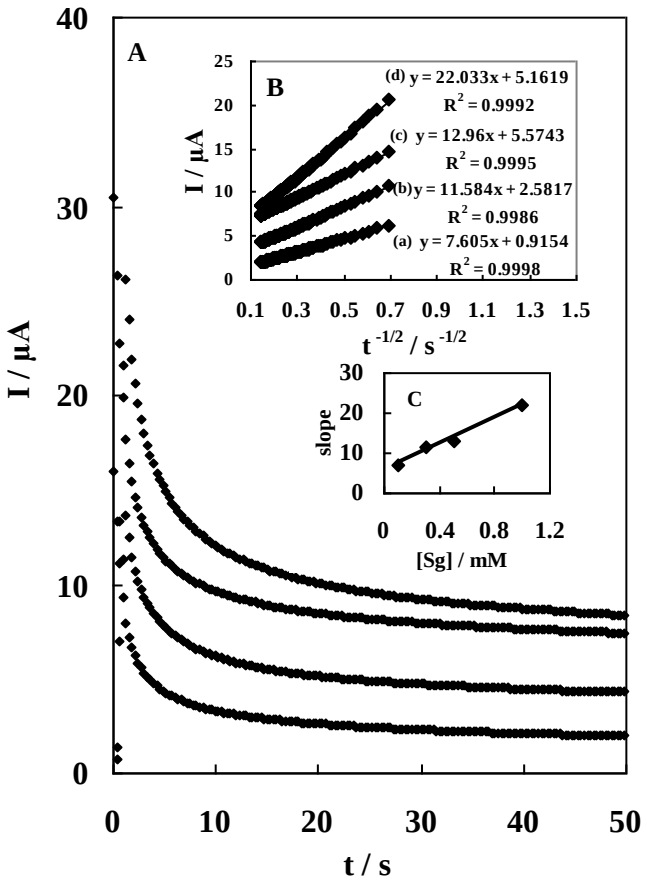


Figure 5. (A) Chronoamperograms of Sg in B-R buffer, pH=7.0, containing (a) 0.1, (b) 0.3, (c) 0.5, (d) 1.0 mM Sg. (B) Plot of I vs $t^{-1/2}$ for different concentrations. (C) The plot of the slope of straight lines against the Sg concentration.

The reproducibility of the method was checked by successive determinations (n=5) of Sg. The relative standard deviations (R.S.D.) were less than 2.45 %. Also on using the MWCNT-GCE after leaving the electrode unused for a period of one week the peak potential of Sg oxidation was unchanged and the current signals showed only less than 5% decrease of the initial response.

3.7. Interferences

Under optimum experimental conditions the effects of some foreign species on the determination of Sg at 1.0 mM were evaluated in detail.100-fold of Ni^{2+}, Sr^{2+}, Cd^{2+}, Ba^{2+}, Li^{+}, Cr^{2+}, NH_4^{+}, Ca^{2+}, Co^{2+}, Bi^{2+}, Cu^{2+}, Mg^{2+}, Na^{+}, K^{+}, NO_3^{-}, SO_4^{2-}, Cl^{-}, PO_4^{3-}, acetate and 50-fold concentration of lysine, L-glutamic acid, L-serine, L-histamine, urea and L-threonine had almost no influence on the

current response of Sg (signal change below 5%). All obtained results clearly represent the efficiency of the method for the determination of Sg in the presence of the potentially interfering species.

3.8. Application to real matrices

To evaluate the applicability of the present method on real matrices, assays were performed in serum and urine samples. To ascertain the validity of the results, the real samples were spiked with certain amount of Sg. The results showed that satisfactory recovery for Sg could be obtained (Table1).

Table 1. Results of analysis of real samples.

Sample	Added mg	Found mg	Recovery (%)	R.S.D. (%) (*n*=3)
Serum	-	0.10	-	-
	1.07	1.11	103	0.56
Urine	-	0.90	-	-
	1.27	1.48	116	2.6

4. CONCLUSIONS

In the present study, an easily prepared MWCNT-GCE was used to investigate the detailed electrochemical behavior of Sg. The reported modified electrode significantly improved the electrochemical response of Sg and clearly demonstrates the excellent electrocatalytic activity of the MWCNT-GCE toward the oxidation of Sg. The electroactive surface coverage (Γ), the transfer coefficient (α), standard rate constant (k_s) and diffusion coefficient (D) were calculated from cyclic voltammetric and chronoamperometric responses. Under optimized conditions, good analytical performance was obtained, including suitable precision, excellent linear dynamic range and good detection and reproducibility. In addition, the results obtained in the analysis of Sg in urine and serum samples demonstrated the applicability of the method for real sample analysis. Furthermore, the proposed method doesn't require expensive instruments or critical analytical reagents.

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