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Voltammetric determination of sulfamethoxazole at a multiwalled carbon nanotube modified glassy carbon sensor and its application studies

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Sulfamethoxazole (SMX) is an anti-bacterial sulfonamide. It prevents the formation of dihydrofolic acid, a compound that bacteria need in order to survive. The present work details the voltammetric analysis of SMX at a multiwalled carbon nanotube (MWCNT)-Nafion modified glassy carbon electrode (GCE). Sulfamethoxazole gives a well defined oxidation peak at 0.74 V in 0.1 M phosphate buffer solution (PBS) of pH 8.0. The experimental parameters such as the amount of MWCNT-Nafion suspension, the pH of the supporting electrolyte and scan rate were optimized and a direct electrochemical method for the determination of SMX was developed. Under optimum conditions the oxidation peak current is linear to the concentration of SMX in the range $1 \times 10^{-2} - 5 \times 10^{-5}$ M with a detection limit of 1×10^{-5} M. The MWCNT/GCE showed good stability, selectivity and was successfully used to quantify SMX in pharmaceutical formulations and urine sample.

Keywords: glassy carbon electrode; multiwalled carbon nanotubes; Nafion; sulfamethoxazole; voltammetry

Introduction

Sulfamethoxazole, 4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (see Figure 1), is a sulfonamide bacteriostatic antibiotic. It competitively inhibits the conversion of *p*-aminobenzoic acid to dihydropteroate, which bacteria need for folic acid synthesis and ultimately purine and DNA synthesis. Humans do not synthesize folic acid but acquire it in their diet, so their DNA synthesis is less affected. Sulfamethoxazole is often used as a part of synergistic combination with trimethoprim in a 5 : 1 ratio. Its primary activity is against susceptible forms of *Streptococcus, Staphylococcus aureus, Escherichia coli, Haemophilus influenza* and oral anaerobes. It is commonly used to treat urinary tract infections. It can also be used as an alternative to amoxicillin-based antibiotics to treat sinusitis.

Several analytical methods have been reported for the determination of SMX in pharmaceutical formulations or biological samples. These include the spectrophotometric method,[1,2] the flow injection spectrophotometric method,[3] the Bratton–Marshall method,[4,5] the titrimetric assay method,[6,7] gas chromatography and gas chromatography-mass spectrometry,[8]* capillary electrophoresis,[5] high-performance liquid chromatography[9] and high-performance thin-layer chromatography.[10] Some of these reported methods require time-consuming sample preparation or expensive instrumentation. Hence it is of immense importance to develop a technique for the determination of SMX with high degree of selectivity, sensitivity and low detection limit. This work explains the development of a voltammetric sensor for the determination of SMX, as voltammetric techniques have been found to be relatively simple and the required sensitivity and selectivity for drug analysis can be achieved easily.[11]

The development of chemically modified electrodes (CMEs) has continued to be of major concern in voltammetric determinations. One of the most important properties of CMEs has been their ability to catalyse the electrode process by significantly reducing overpotential with respect to the unmodified electrode.[12] The operation mechanism of CMEs depends on the properties of the modifier materials that are used to promote selectivity and sensitivity towards the target analytes.[13]

Electrodes based on carbon nanotubes (CNTs) are an attractive research area now. Carbon nanotubes are molecular scale wires with high electrical conductivity, extremely high mechanical strength and modulus.[14–17] The subtle electronic properties suggest that CNTs have the ability to promote electron-transfer reactions when used as an electrode material in electrochemical reactions.[18,19] The major barrier in using CNT is its insolubility in most of the solvents.[20] However Nafion, a sulfonated tetra fluoro ethylene copolymer, was found to be a very good solvent for CNTs. Nafion can efficiently disperse CNTs by its hydrophobic side chains and polar head groups.[21]

An attempt, based on our previous work on drug analysis,[22–25] was made to develop a convenient and sensitive method for the determination of SMX using MWCNT-Nafion modified GCE. In this work, MWCNTs were dispersed into Nafion solution via ultrasonication and then the resulting homogenous suspension of MWCNT was dropped on to GCE. The MWCNT-Nafion modified GCE was obtained by evaporating the solvent. This modified electrode was then used for the determination of SMX in pure form, dosage forms and in urine sample using differential pulse voltammetry (DPV).

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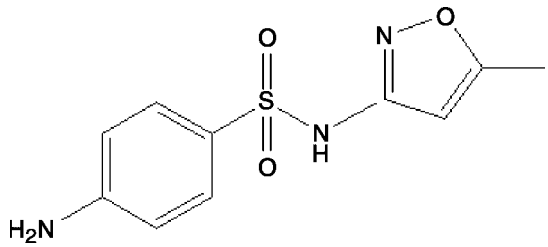

Figure 1. Structure of SMX.

Experimental

Reagents and materials

All chemicals used were of analytical grade and used directly without purification. Pure-grade SMX was obtained as a gift sample. Sulfamethoxazole tablets were purchased from the local market. Double distilled water was used throughout the study. A MWCNT (6–20 nm and 1–5 μm length) and Nafion (5%) were purchased from Aldrich.

Apparatus

Electrochemical measurements were made on a BAS Epsilon Electrochemical analyser (Bioanalytical system, USA) interfaced to a PC. A conventional three-electrode system, including a MWCNT film modified glassy carbon electrode, a platinum wire counter electrode and an Ag/AgCl reference electrode were employed. The pH measurements were carried out with a Metrohm pH meter. Scanning electron microscopic (SEM) images were recorded using JOEL 6300 LV.

Treatment of MWCNT

To eliminate metal oxide catalysts within the nanotubes and segment the MWCNT for easier and better dispersion, the MWCNT was refluxed in 100 mL 6 M HNO_3 for 10 hours. The resulting suspension was then diluted with 200 mL of water and the MWCNT was filtered and washed with double distilled water. The washed nanotube was collected and dried. This treatment caused segmentation and carboxylation of MWCNT.

Preparation of MWCNT-Nafion modified GCE

Multiwalled carbon nanotube (10 mg) was added to 2 mL of 0.5% Nafion water solution and then sonicated for about 1 hour with an ultrasonicator to get a stable and homogenous suspension. Prior to modification, the GCE was polished with alumina and cleaned thoroughly in an ultrasonic cleaner with methanol, 1 : 1 HNO_3 solution, ethanol and double distilled water sequentially. Finally the GCE was coated with 4 μL of the resulting MWCNT/Nafion suspension and the solvent was allowed to evaporate at room temperature in the air to get MWCNT/Nafion modified GCE.

Analytical procedure

The MWCNT/GCE was first activated in 0.1 M PBS by cyclic sweep between 0–1.2 V until stable voltammograms were obtained. The stock solution of SMX (1×10^{-2} M) was prepared in methanol. Standard solutions of the analyte (5×10^{-3} M – 5×10^{-5} M) were prepared by serial dilution of the stock solution by adding supporting electrolyte (0.1 M PBS). A sample solution was taken in the electrochemical cell and then DPV was recorded from 0–1.2 V. An oxidation peak around 0.74 V was measured for SMX. For electrode regeneration, several cyclic scans were carried out in the blank electrolyte solution until a stable voltammogram was obtained.

Results and Discussion

Surface study

$K_3Fe(CN)_6$ (2 mM) was taken as a probe to measure the microscopic areas of the MWCNT modified GCE and bare GCE by cyclic voltammetry (CV) at different scan rates.[26] For a reversible process, the following equation exists:

$$I_p = 2.69 \times 10^5 \, A \, n^{3/2} \, D_R^{1/2} \, c \, \nu^{1/2}$$

where Ip refers to the peak current, n is the electron transfer number, A is the surface area of electrode, D_R is diffusion coefficient, c is the concentration of $K_3Fe(CN)_6$ and ν refers to the scan rate. For $K_3Fe(CN)_6$, n = 1, and $D_R = 7.6 \times 10^{-6}$ cm/s. The surface areas can be calculated from the slope of the i_p versus $\nu^{1/2}$ relation. For bare GCE the surface area was found to be 0.8283 cm^2. There was an enhancement in the effective surface area to 1.0854 cm^2 when GCE was modified with MWCNT. This enlargement in the effective surface area proved that the bare electrode was modified efficiently by MWCNT.

The surface morphology studies of both bare and MWCNT-modified GCE were done using a scanning electron microscope (see Figure 2). SEM images also provided strong evidence for the effective modification of GCE.

Electrochemical behaviour of SMX

The electrochemical behaviour of SMX at bare GCE and MWCNT modified GCE (MWCNT/GCE) was studied by DPV. Sulfamethoxazole gave a well defined oxidation peak at 0.74 V with a peak current of 0.0476 mA at MWCNT/GCE. This peak arises due to the oxidation of the electrochemically active amino group on SMX.[27] Compared with bare GCE, the oxidation peak potential of SMX at MWCNT/GCE has shifted negatively by about 100 mV. The oxidation peak current of SMX has also greatly increased at the MWCNT/GCE (see Figure 3). Thus by modifying GCE with MWCNT, the electrode reactivity was found to be greatly increased as indicated by the enhancement in peak current as well as lowering of peak potential. The remarkable peak current enhancement and the decrease in oxidation potential proved the electrocatalytic activity of MWCNT/GCE in the oxidation of SMX. This may be attributed to the increase in the effective surface area of GCE when modified with MWCNT. No reduction peak is observed for SMX in the reverse sweep of CV indicating an irreversible electrochemical process. The experimental conditions such as pH, scan rate, amount of MWCNT-Nafion dispersion, concentration and supporting electrolyte were optimized to provide precise results.

Effect of supporting electrolyte

The electrochemical behaviour of SMX in various media such as 0.1 M solutions of PBS, H_2SO_4, NaOH, tetra n butyl ammonium chloride and KNO_3 was studied by DPV. The oxidation peak obtained was best defined in 0.1 M PBS. So 0.1 M PBS was taken as the experimental medium for SMX.

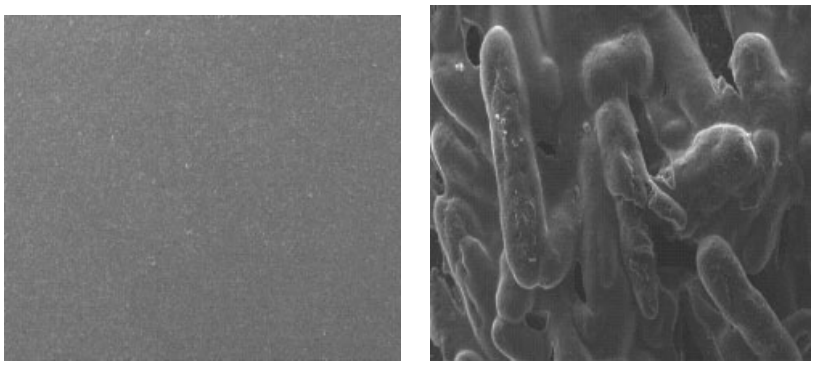

Figure 2. SEM images of (a) bare GCE and (b) MWNT modified GCE.

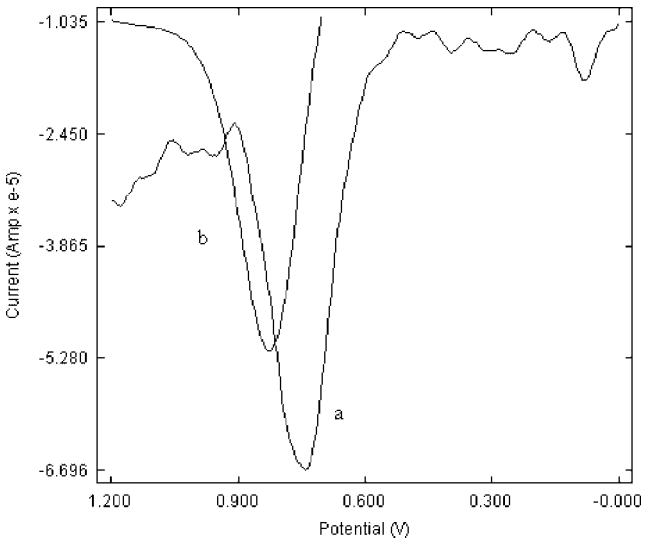

Figure 3. Differential pulse voltammogram of SMX at (a) MWCNT/GCE and (b) bare GCE.

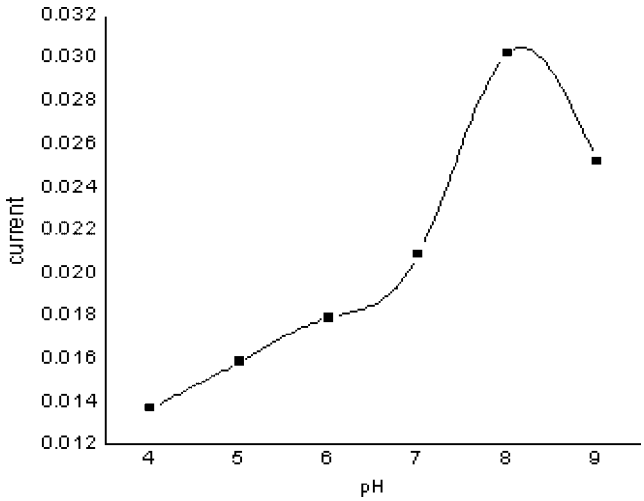

Figure 4. Effect of pH on the anodic current of 3×10^{-3} M SMX.

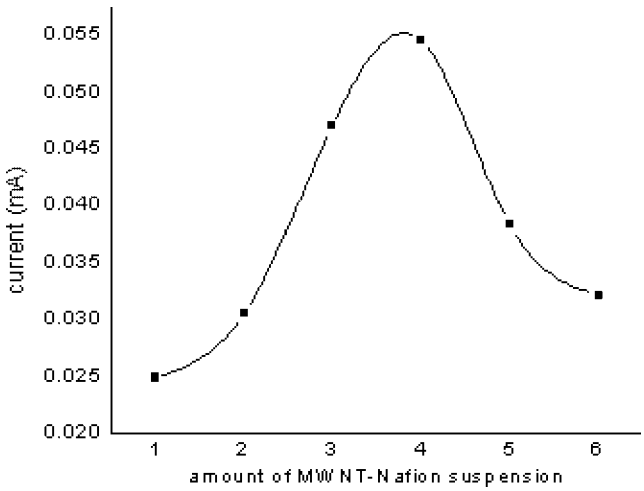

Figure 5. Effect of the amount of MWCNT-Nafion suspension.

Effect of pH

The effect of pH on the anodic peak current of SMX at the MWCNT-Nafion modified GCE was investigated by DPV (see Figure 4). The pH range studied was from 4–9. A well-defined oxidation peak and a high peak current were obtained at pH 8. So pH 8 was selected as the optimal pH.

Effect of the amount of MWCNT-Nafion dispersion

The effect of the amount of MWCNT dispersion on the anodic peak current of SMX was evaluated (see Figure 5). As the amount of MWCNT dispersion was increased up to 4 μL, the oxidation peak current greatly enhanced. The enhancement of the current indicates that the specific surface area and the number of catalytic sites increase with an increase of MWCNT. When the amount of MWCNT dispersion was more than 4 μL, the oxidation peak current decreased slightly. This indicates that the excess of MWCNT blocks the electron transfer of SMX. So the amount of MWCNT dispersion was fixed at 4 μL.

Effect of scan rate

The oxidation peak current of SMX at different scan rates ranging from 10–80 mV/s were measured by DPV. It was found that the anodic peak current increased with an increase in the scan rate. The plot of anodic peak current versus square root of scan rate gave a straight line (see Figure 6), indicating that the oxidation of SMX at the MWCNT-Nafion modified GCE is diffusion controlled.

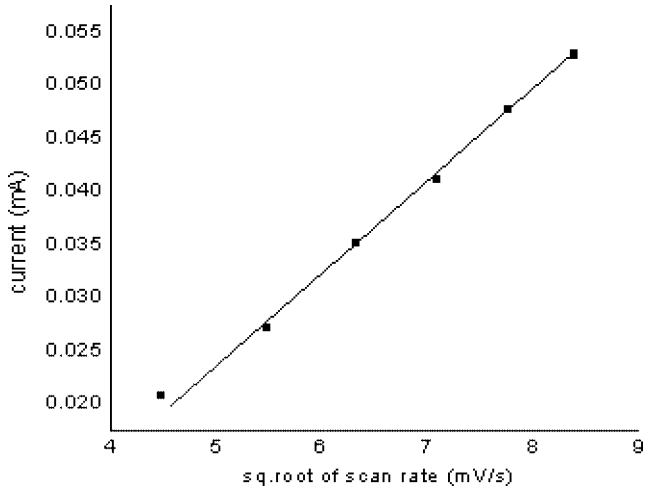

Figure 6. Effect of scan rate.

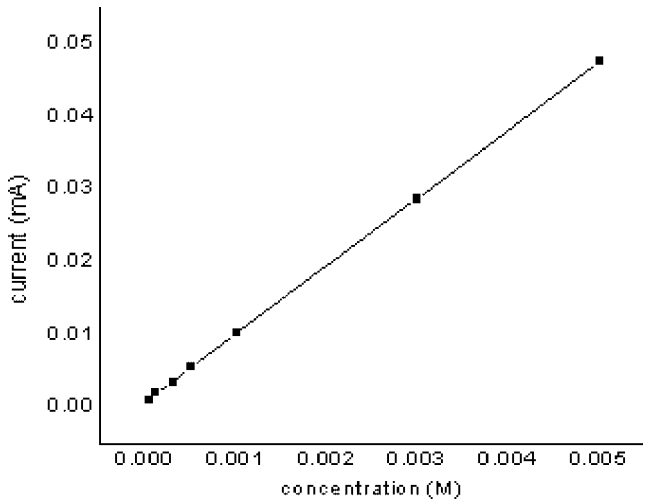

Figure 7. Calibration graph for SMX at MWCNT modified GCE at 25 °C.

Calibration curve

The relationship between the anodic peak current of SMX and its concentration was investigated by DPV (see Figure 7). A linear concentration range was found to occur from 1×10^{-2}–5×10^{-5} M. The detection limit was found to be 1×10^{-5} M.

The reproducibility of the MWCNT-Nafion modified GCE was examined by repetitive measurement of oxidation peak current of 1×10^{-3} M SMX using the same electrode. After each determination, the used modified electrode surface was regenerated as described in the analytical procedure. After several successive measurements, only slight deviation of the peak current was observed suggesting that the MWCNT-Nafion modified GCE has excellent reproducibility.

Interference study

Under optimized experimental conditions, the effects of some foreign species on the determination of SMX (1×10^{-3} M) were evaluated in detail. The results are given in Table 1. Hundredfold concentrations of glucose, lactose, citric acid, Na^+, K^+, SO_4^{2-} and Cl^- have almost no influence on the current response of SMX (signal change below 5%). As SMX is often used as a part of synergistic combination with trimethoprim in tablets, the influence of trimethoprim on the oxidation peak current of SMX was studied. It was found that a tenfold concentration of trimethoprim hardly interfered at all in the determination of SMX.

Table 1. Interference study

Interferent	Concentration (M)	Signal Change (%)
Glucose	0.05	+1.65
Lactose	0.05	−1.32
Citric acid	0.05	−3.33
Trimethoprim	0.01	+4.97
Na^-	0.05	+1.65
K^-	0.05	+1.32
Cl^-	0.05	+1.65
SO_4^{2-}	0.05	+1.32
CH_3COO^-	0.05	+1.25

Table 2. Determination of SMX in tablets

Sample	Declared Amount (mg/tablet)	Found[a] (mg/tablet)	S.D	C.V
Bactrim (Piramal Healthcare, India)	800	800	2.0	0.25
Septran (Burroughs Wellcome, India)	400	402	1.2	0.31

[a] Average of six replicates

Applications

Drug analysis

The developed method was used for the determination SMX in tablets (Bactrim and Septra). The sample solution was prepared as follows. Ten tablets were weighed and ground to a fine powder. An adequate amount of this powder corresponding to the concentration 5×10^{-3} M was weighed and transferred to a beaker. The powder was dissolved in methanol and filtered to a titrimetric flask (50 mL) through a Whatman 41 filter paper. The beaker was washed several times and the washings were collected in the standard flask and then it was quantitatively diluted. Solutions of different concentrations were prepared by serial dilution of the stock with PBS. Differential pulse voltammetry was recorded and the unknown concentrations were determined from the calibration graph. The results are shown in Table 2. The results obtained are in good agreement with the declared SMX content and showed a high degree of precision (coefficient of variation (C.V) is 0.28%).

Determination of SMX in urine sample

The method developed was also applied for the determination of SMX in urine sample. Urine samples (5 mL) were taken in a series of 25 mL standard flasks. Different quantities of SMX (ranging from 4–20 mg) were added to these urine samples and then quantitatively diluted using PBS. The electrochemical behaviour

Table 3. Determination of SMX in urine sample

Added (mg)	Found (mg)	Recovery (%)
6.00	5.89	98.17
10.00	10.22	102.20
14.00	13.83	98.79

of the prepared solutions on MWCNT/GCE was studied by DPV and the unknown concentrations were determined from the calibration graph. The results are shown in Table 3. The recoveries obtained are in the range of 98–100%.

Conclusion

Voltammetric determination of SMX at a MWCNT-Nafion modified sensor was investigated by DPV and CV. The oxidation of SMX was found to be an irreversible process. Multiwalled carbon nanotubes showed electrocatalytic action for the oxidation of SMX, characterized by the enhancement of the peak current and the reduction of peak potential, which was probably due to the larger effective surface area of multiwalled carbon nanotubes and an increase in the number of reaction sites. Thus MWCNT-Nafion film provided a good platform for the determination of SMX in pure form, dosage forms and in urine sample. The method developed is a better alternative for the determination of SMX because it is simple, fast, has low cost and has sufficient precision, accuracy and sensitivity.

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