Research Article

Drug Testing and Analysis

Received: 22 January 2010 Revised: 8 March 2010 Accepted: 9 March 2010 Published online in Wiley Interscience: 20 April 2010

(www.drugtestinganalysis.com) DOI 10.1002/dta.129

Differential pulse voltammetric determination and catalytic oxidation of sulfamethoxazole using [5,10,15,20- tetrakis (3-methoxy-4-hydroxy phenyl) porphyrinato] Cu (II) modified carbon paste sensor

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Sulfamethoxazole (SM) is a sulfonamide bacteriostatic antibiotic. Its primary activity is against susceptible forms of streptococcus, staphylococcus aureus, escherichia coli, haemophilus influenzae, and oral anaerobes. It is commonly used to treat urinary tract infections. In addition it can be used as an alternative to amoxicillin-based antibiotics to treat sinusitis. It can also be used to treat toxoplasmosis. In the present work, a metalloporphyrin modified carbon paste sensor was fabricated and the electrochemical behaviour of SM was studied using differential pulse voltammetry (DPV). Cu (II) complex of 5,10,15,20-tetrakis (3-methoxy-4-hydroxy phenyl) porphyrin (TMHPP Cu (II)) was used as the active material. Compared with bare carbon paste electrode (CPE), the TMHPP Cu (II) modified CPE exhibits excellent enhancement effect on the electrochemical oxidation of SM. A well-defined oxidation peak of SM occurs at −140 mV in 0.1M phosphate buffer solution (PBS) of pH 6. All the experimental parameters were optimized and it was found that under optimum conditions the oxidation peak current was linear to the concentration of SM in the range of 1.0×10^{-2}–1.0×10^{-8} M with a detection limit of 1.5×10^{-9}M. The developed sensor has been successfully applied for the determination of SM in pharmaceutical formulations and urine sample.

Keywords: sulfamethoxazole; metalloporphyrin; differential pulse voltammetry; electrochemical oxidation; carbon paste sensor

Introduction

Sulfamethoxazole, 4-amino-N-(5-methylisoxazol-3-yl)-benzene-sulfonamide, (Figure 1A) is a sulfonamide used in the treatment of urinary tract infections, pneumocystis pneumonia, chronic bronchitis, meningococcal meningitis, acute otitis media, and toxoplasmosis. It is also used in veterinary practices. At present, the standard methods for the determination of SM are the spectrophotometric method,[1,2] the flow injection spectrophotometric method,[3] the Bratton–Marshall method,[3–5] the titrimetric assay method,[6,7] gas chromatography and gas chromatography-mass spectrometry,[8]* capillary electrophoresis,[5] high performance liquid chromatography,[4] high performance thin layer chromatography,[9] liquid chromatography-mass spectrometry,[10] and voltammetry.[11] Among these, electrochemical detection is one of the promising approaches. To the best of our knowledge, voltammetric determination of SM using TMHPP Cu (II) modified CPE has not been reported.

The use of chemically modified electrodes (CME) in analytical applications[12–15] continues to be an area of vigorous research activity. A broad range of approaches have been pursued including electrostatic accumulation, coordination effects, and precipitation.[16,17] Electrode modification has also been employed to prevent electrode fouling or to enhance selectivity (or both). Moreover, chemically modified electrodes also offer the possibility of adjustable physical and chemical properties (i.e. charge, polarity, surface area, permeability, etc.). Among the many electrodes used, the use of carbon paste electrodes (CPEs) appears especially advantageous because of the ease of electrode preparation and regeneration as well as low background currents. Modification of CPEs with suitable materials facilitates the electrochemical reactions of the redox compounds to proceed without hindrance.[18,19] This phenomenon generally results in increased selectivity and sensitivity of the determinations.

Recently, synthetic metalloporphyrins have attracted attention in relation to chemical and biological recognition.[20,21] Electrodes prepared by incorporating metalloporphyrins are prospecting candidates for various applications, because they have excellent thermal, chemical, electrochemical, and photochemical stability.[22,23] The coordinated metal, the peripheral substituents, and the conformations of the macrocyclic skeleton influence the coordination and the related sensing properties of these compounds. Furthermore, they have also been used extensively as catalysts, semiconductors, anticancer medicine, etc.[24,25] Various metalloporphyrins have shown potentiometric response to anions with selectivity sequences solely dependent on the centrally bonded

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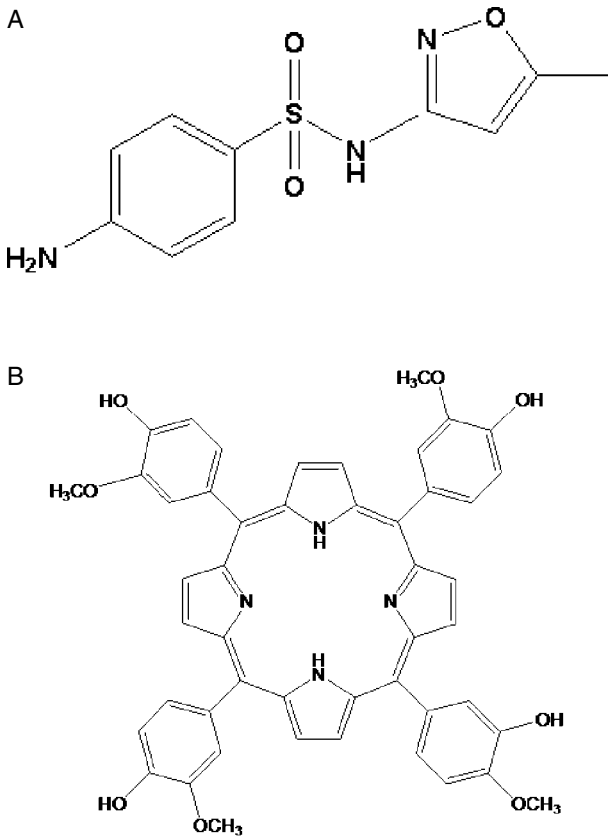

Figure 1. A. Structure of Sulfamethoxazole. B. Structure of 5,10,15,20-tetrakis (3-methoxy-4-hydroxy phenyl) porphyrin.

metal.[26] Metalloporphyrins as drug-sensing materials appear to be one of the promising classes of compound to be used as voltammetric sensors.[27] Reports have shown that CPE modified with tetraphenylporphyrin was used for voltammetric detection of heavy metals.[28]

In continuation of our work on drug analysis,[29–31] a voltammetric sensor based TMHPP Cu (II) has been developed for the selective determination of SM. The electrochemical behaviour of SM suggests that TMHPP Cu (II) modified CPE exhibits obvious electrocatalytic activity to the oxidation of SM, since it greatly enhances the oxidation peak current of SM as well as lowering its oxidation potential compared to bare CPE. After optimizing the experimental parameters, a differential pulse voltammetric method was developed for the direct measurement of SM. The developed sensor has been successfully applied for the determination of SM in commercially available tablets and urine samples. This newly proposed method possesses several advantages such as high sensitivity, rapid response, low cost, and simplicity. The enhanced detection limit (10^{-9} range), and wide concentration window is the striking advantage of the present method over the reported sensor method.[11]

Experimental

Reagents and materials

All reagents used in the investigation were of analytical reagent grade. Porphyrin and metalloporphyrin were prepared. Pyrrole and 3-methoxy-4-hydroxy benzaldehyde were purchased from Sisco Research Laboratories Ltd (Mumbai India) and were freshly distilled prior to use. $CuCl_2.2H_2O$ was obtained from s.d fine chemicals Pvt. Ltd (Mumbai, India). Pure SM was obtained as a gift sample. The stock solution of SM (1×10^{-2} M) was prepared in methanol. Double distilled water was used throughout the experiments.

Apparatus

All electrochemical experiments were performed on an electrochemical analyzer (BAS Epsilon Bioanalytical system, West Lafayette USA), interfaced to a PC. A three-electrode system which consists of a TMHPP Cu (II) modified CPE as working electrode, an Ag/AgCl reference electrode, and a platinum wire auxiliary electrode were employed. The pH measurements were carried out in a Metrohm pH meter. The UV-visible spectrum was recorded using Spectro UV-Visible Double beam UVD-3500 instrument. The FT-IR spectra of the powdered samples were recorded on JASCO 4100 FT IR spectrometer using KBr discs. ^{1}H NMR spectra were recorded using JEOL GSX 400 NB FT NMR spectrometer. Elemental analysis was perfomed with a Vario EL III CHNS analyzer.

Synthesis of 5,10,15,20 TMHPP

The synthesis was performed according to the Alder method.[32] Freshly distilled pyrrole (1.04 ml, 0.015 mol) and 3-methoxy-4-hydroxy benzaldehyde (2.282 g, 0.015 mol) were added to 30 ml of boiling propionic acid. The mixture was refluxed for 30 min and was allowed to cool for a few minutes. The filter cake was washed thoroughly with methanol. The resulting purple crystals were further purified by column chromatography. The yield was found to be 10%. Elemental analysis of the product gave the following results.
Calcd (%): C, 72.18; H, 4.76; N, 7.01
Found (%): C, 72.08; H, 4.66; N, 6.97
IR (KBr), ν (cm^{-1}): 3363 (NH); 3000 (CH); 3539 (OH)
UV-Visible spectrum in CH_2Cl_2, λ (nm): 411, 445, 514, 647
^{1}H NMR (500 MHz, $CDCl_3$) ppm: δ = 8.9 (s, 8H, pyrrollic $-\beta$- H), 5.9 (s, 4H, OH), 3.9 (s, 12H, OCH_3), −2.7 (s, 2H, NH), 8.2 - 7.3 (m, 12H, aromatic)
The structure of TMHPP is shown in Figure 1B.

Synthesis of [5,10,15,20 tetrakis (3-methoxy-4-hydroxy phenyl) porphyrinato] Cu (II)

The ligand TMHPP (2.5 g, 0.003 mol) and $CuCl_2.2H_2O$ (1.6 g, 0.009 mol) were refluxed in 500 ml N, N-dimethylformamide for 30 min. The solvent was then stripped off and the residue extracted into 250 ml water. A red precipitate was formed immediately, which was isolated by filtration, washed with 250 ml of distilled water and air dried. The yield was found to be 4%. Elemental analysis of the product gave the following results.
Calcd (%): C, 61.43; H, 3.25; N, 6.51.
Found (%): C, 61.20; H, 2.85; N, 6.45.
IR (KBr), ν (cm^{-1}): 3363 (NH); 3000 (CH); 3539 (OH); 455 (M-N).
UV-visible spectrum in DMSO, λ (nm): 405, 481, 530, 621.

Preparation of TMHPP Cu (II) modified CPE

Bare CPE was prepared by thoroughly mixing analytical grade graphite and paraffin liquid (plasticizer) in a 70 : 30 (w/w %) ratio. TMHPP Cu (II) modified CPE was prepared by mixing different

percentages of graphite powder, paraffin liquid, and TMHPP Cu (II). This was mixed in a mortar for at least 10 min to become a homogeneous mixture. This paste was packed into one end of a teflon holder in which electrical contact was made with a copper rod that runs through the centre of the electrode body. The electrode surface was polished using a butter paper to produce a reproducible working surface. Electrochemical behaviour of SM at these different electrodes was studied using differential pulse voltammetric technique. Best results were obtained at 70 : 22:9 (w/w %) ratio of graphite powder, paraffin liquid and TMHPP Cu (II). This optimized electrode composition was then used for the voltammetric determination of SM.

Analytical procedure

Standard solutions of the analyte (1×10^{-3} M - 1×10^{-8} M) were prepared by serial dilution of the stock solution using PBS. Sample solution was taken in the electrochemical cell and then de-aerated with N_2 for 10 min. Differential pulse voltammograms from 900 to −1500 mV at 20 mV/s were recorded and finally the peak current at −140 mV was measured for SM.

Results and Discussion

Electrochemical behaviour of SM

The electrochemical behaviour of SM at a TMHPP Cu (II) has been investigated using DPV. Para amino substituted sulfonamides can be electrochemically oxidized at the $-NH_2$ group, but the reduction of the $-SO_2$ group is very difficult to achieve. The group attached to −NH functional group has little or no influence on the oxidation potential.[33] Previous investigations[34–36] have addressed the electrochemical behaviour of sulfonamides, and proposed an irreversible two-electron pH-dependent reaction for their oxidation in aqueous solutions. In the present case, one oxidation peak is obtained and this is attributed to the two-electron oxidation of amino group in SM to the corresponding iminobenzoquinone according to the currently accepted mechanism[36] and is shown in Figure 2. According to Lacirons conclusion,[37] the relationship between peak potential and scan rate (v) was examined. It was found that peak potential depends linearly on the logarithm of v as following equation:

$$E = -0.7634 - 0.02439 \ln v \quad (1)$$

The value of αn_a can be calculated from the slope of the plot (b) (potential versus ln v) according to $b = RT/\alpha n_a F$, where α of the totally irreversible electrode process is assumed as 0.5, thus, the calculated n_a value is 2.05. It indicates that two electrons are involved in the oxidation process of SM.

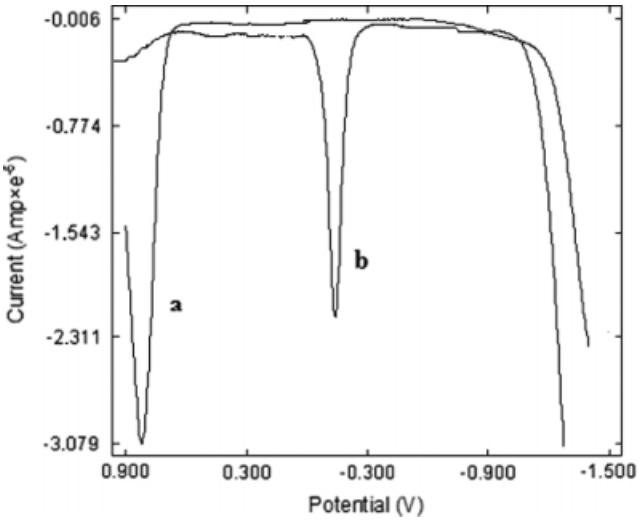

Figure 3. Differential pulse voltammogram of sulfamethoxazole at (a) Bare CPE (b) TMHPP Cu (II) modified CPE.

Figure 3 displays the comparison of oxidation peak of 1×10^{-3} M SM in PBS (pH 6) at bare CPE and TMHPP Cu (II) modified CPE. At the bare CPE, SM yields a very low oxidation peak at 816 mV (curve a). Under the same conditions, a well-defined oxidation peak appears at −140 mV for the TMHPP Cu (II) modified CPE (curve b). Compared with bare CPE, there was also an enhancement of oxidation peak current for SM from 0.0169 mA to 0.0208 mA at TMHPP Cu (II) modified CPE. The tremendous decrease in oxidation potential was remarkable, and additionally, an increase in the oxidation peak current of SM can be distinguished when the CPE electrode is modified with TMHPP Cu (II). This behaviour, which was observed at different concentrations of SM and at several potential scan rates, clearly demonstrates that the mediator functions electrocatalytically towards SM.

Infuence of supporting electrolyte

The supporting electrolyte plays an important role in the electrochemical response of SM. Its choice can modify the thermodynamics and kinetics of electrochemical processes, as well as mass transfer within the cell.Therefore 0.1 M concentrations of PBS, sulfuric acid, hydrochloric acid, potassium chloride, acetate buffer, tetra-n-butyl ammonium chloride and sodium hydroxide were tested as supporting electrolytes for SM oxidation by DPV. It was observed that the peak current is highest and the peak shape is well defined in PBS. Hence PBS was chosen as the experimental medium for the voltammetric studies of SM.

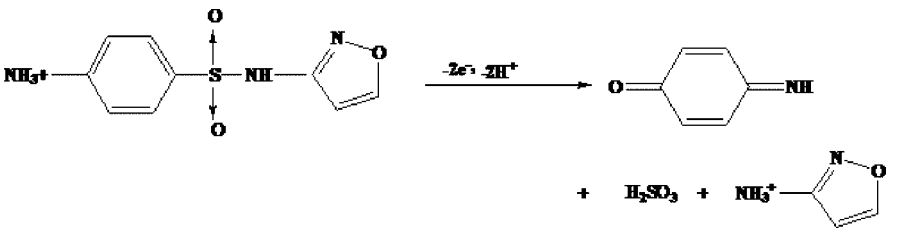

Figure 2. Mechanism of oxidation of amino group in sulfamethoxazole to iminobenzoquinone.

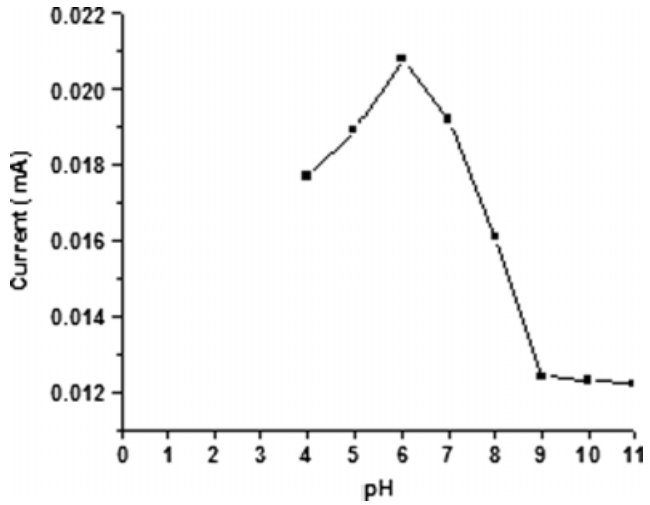

Figure 4. Effect of pH.

Effect of pH

The effect of pH on the anodic peak current of 1×10^{-3} M SM at the TMHPP Cu (II) modified CPE was investigated by DPV. The pH range was studied from 3–10. Figure 4 clearly depict the effect of pH on the anodic peak current of SM at the TMHPP Cu (II) modified CPE. The best oxidation response was obtained in pH 6 as the peak current is the highest. Thus pH 6 was fixed as optimal pH.

Effect of scan rate

The effect of scan rate on the oxidation peak current was studied by DPV. It was found that oxidation peak current of 1×10^{-3} M SM shows a linear relationship with scan rate in the range 10–150 mV/s. The results are illustrated in Figure 5. The oxidation peak current varies linearly with square root of scan rate (Figure 5) indicating that the oxidation of SM at the TMHPP Cu (II) modified electrode is diffusion controlled.

Calibration curve

Under the optimized experimental conditions, the calibration curve for SM in PBS (pH 6) at TMHPP Cu (II) modified CPE was characterized by DPV. The results are illustrated in Figure 6. The results show that the oxidative peak current has a linear relationship with the concentration in the range $1 \times 10^{-3} - 1 \times 10^{-8}$ M. The detection limit of SM was 1.5×10^{-9} M. The reproducibility of the electrode was examined by repetitive measurement of 1×10^{-3} M SM using the same TMHPP Cu (II) modified CPE. After several successive measurements, comparable results were obtained suggesting that the TMHPP Cu (II) modified CPE has good reproducibility.

Interference study

The possible interfering species examined are listed in Table 1. A 100-fold concentration of glycine, sodium chloride, potassium chloride, dextrose, lactose, urea, and trimethoprim has no influence on 1×10^{-3} M SM signals, with deviation below 5%. Since trimethoprim is often used as a part of synergistic combination with SM in tablets, the influence of trimethoprim on the oxidation peak current of SM was studied. It was found that same concentration of trimethoprim did not interfere in the determination of SM. These results showed that the TMHPP Cu (II) modified CPE has good selectivity for the determination of SM.

Determination of SM in pharmaceutical formulation (tablet)

Ten tablets were weighed, crushed, and ground into fine powder. An adequate amount of this powder, corresponding to the

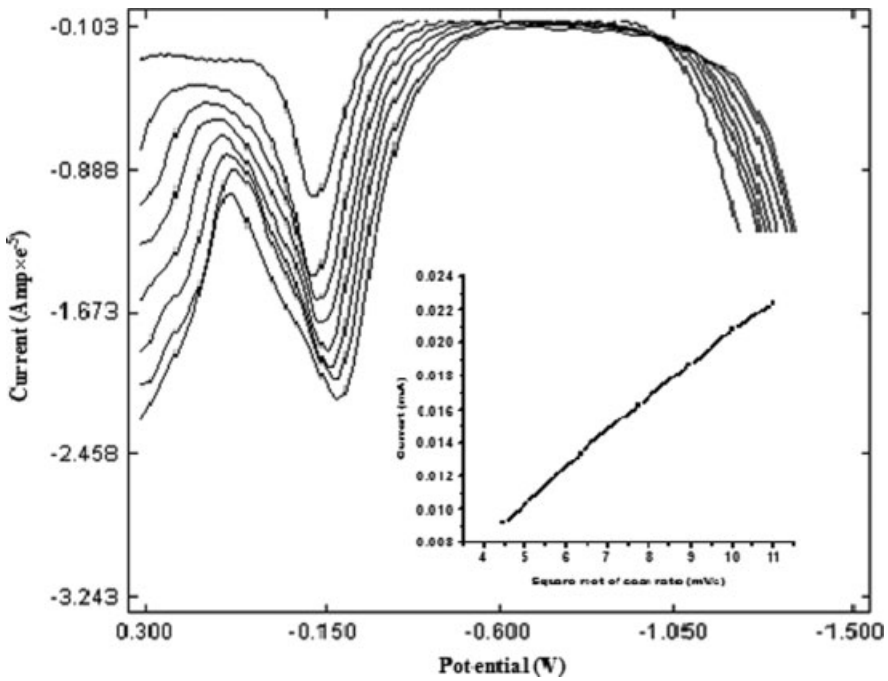

Figure 5. Differential pulse voltammogram of sulfamethoxazole at different scan rates in phosphate buffer solution. Inset is the plot of current versus square root of scan rate.

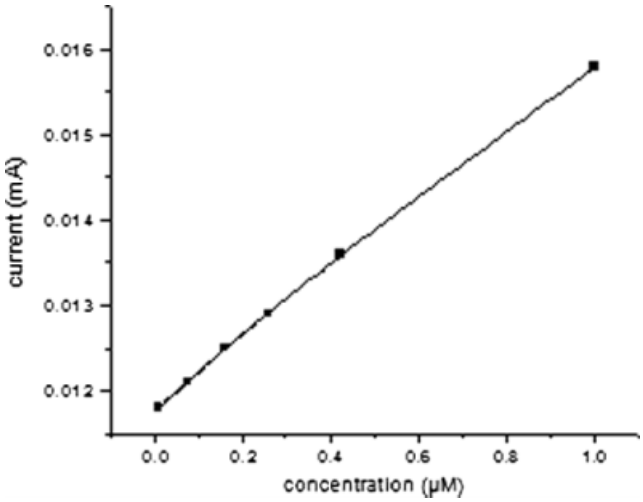

Figure 6. Effect of concentration.

Table 1. Interference study

Interferent	Concentration (M)	Signal change %
Glycine	1×10^{-1}	2.05
Sodium chloride	1×10^{-1}	2.00
Potassium chloride	1×10^{-1}	2.09
Trimethoprim	1×10^{-1}	1.98
Dextrose	1×10^{-1}	2.10
Lactose	1×10^{-1}	2.13
Urea	1×10^{-1}	1.99

Table 2. Determination of SM in tablet

Sample	Declared amt (mg/tablet)	Found* (mg/tablet)	SD*	CV*
Septran (Burroughs Wellcome, India)	400.0	396.0	1.41	3.5×10^{-3}

* Average of six replicates.

concentration 5×10^{-3} M was taken, dissolved in dilute methanol and filtered into a 100-ml titrimetric flask and made up to volume. Solutions of different concentrations were prepared by serial dilution of the stock with PBS. DPV was recorded and the unknown concentrations were determined from the calibration graph. The results shown in Table 2 are in good agreement with the declared SM content and showed a high degree of precision (relative standard deviation (RSD) is 0.28%).

Determination of SM in urine sample

The developed sensor was applied for the determination of the drug in urine samples. Sulfamethoxazole, after metabolism, gives five products in urine. The metabolism of SM involves acetylation and oxidation at the N_4 nitrogen atom leading to N_4- acetyl sulfamethoxazole[38] (43.5 ± 5.6%) and N_4- hydroxy sulfamethoxazole (5.3±1.0%).[39,40] Hydroxylation also takes place at the C_5 methyl group, leading to 5- hydroxy sulfamethoxazole (3.0 ± 1.0%) and N_4- acetyl -5- hydroxysulfamethoxazole[40–42]

Table 3. Determination of SM in urine sample

Added (M)	Found (M)	Recovery* (%)	C.V*
$(1.00–9.00)\ 10^{-6}$	$(1.00–9.00)\ 10^{-6}$	99.6	0.84

* Average of six replicates.

(2-3%). Glucuronidation occurs at the N_1 nitrogen atom, forming sulfamethoxazole- N_1- glucuronide (9.8±2.6%).[43,44] It is reported that around 10% of the drug is excreted as such without absorption.[45] As the mechanism of the present analytical method is the oxidation of the amino group, the present method may not be useful in determining these metabolic products. However, the applicability of the method in determining the free drug in urine samples is established by the standard addition method. Urine samples of 5 ml were taken in different 25 ml standard flasks. An adequate amount of SM corresponding to 1×10^{-3} M was added to the urine samples. This solution was quantitatively diluted using PBS to obtain various concentrations. The prepared solution was analyzed for SM using TMHPP Cu (II) modified CPE by DPV method and the unknown concentrations were determined from the calibration graph. The results are shown in Table 3. Coefficient of variance (C.V) of six replicates shows that the results are reproducible.

Conclusion

A voltammetric sensor based on TMHPP Cu (II) has been developed for the determination of SM. The oxidation of SM was found to be an irreversible process. The TMHPP Cu (II) modified CPE showed electrocatalytic action for the oxidation of SM, characterized by the enhancement of the peak current and the reduction of peak potential. The TMHPP Cu (II) modified CPE was successfully applied as a selective and very sensitive voltammetric sensor for the detection of micromolar amounts of SM in pharmaceutical formulations and urine samples. The performance characteristics of the modified electrode, as well as the simplicity of its preparation and the renewability of its surface by simply polishing, demonstrate its analytical utility as a sensor for the determination of SM.

Acknowledgements

The authors are grateful to the Defence Research and Development Organisation (DRDO), India for the financial assistance in the form of project, IIT Chennai, India, and to the Sophisticated Test and Instrumentation Centre (STIC), Cochin University, India for providing help with the analysis of compounds.

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