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Electroanalysis of trimethoprim on metalloporphyrin incorporated glassy carbon electrode

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Trimethoprim (TMP) is a bacteriostatic antibiotic mainly used in the prophylaxis and treatment of urinary tract infections. It belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors. Its use is associated with idiosyncratic reactions, including liver toxicity and agranulocytosis. In order to determine TMP electrochemically, a metalloporphyrin modified glassy carbon electrode was prepared by coating [5,10,15,20- tetrakis(4-methoxyphenyl) porphyrinato]Mn (III)chloride (TMOPPMn(III)Cl) solution on the surface of the electrode. The electrochemical behaviour of TMP in Phosphate buffer solution (PBS) on TMOPPMn(III)Cl modified glassy carbon electrode (TMOPPMn(III)Cl/GCE) was explored using differential pulse voltammetry (DPV). The voltammograms showed enhanced oxidation response at the TMOPPMn (III)Cl/GCE with respect to the bare GCE for TMP, attributable to the electrocatalytic activity of TMOPPMn(III)Cl. Electrochemical parameters of the oxidation of TMP on the modified electrode were analyzed. The electro-oxidation of TMP was found to be irreversible, pH dependent and adsorption controlled on the modified electrode. It is found that the oxidation peak current is proportional to the concentration of TMP over the range $6 \times 10^{-8} - 1 \times 10^{-6}$ M with a very low detection limit of 3×10^{-9} M at 2 min open circuit accumulation. The repeatability expressed as relative standard deviation (RSD) for n=9 was 3.2% and the operational stability was found to be 20 days. Another striking feature is that equimolar concentration of sulfamethoxazole did not interfere in the determination of TMP. Applicability to assay the drug in urine and tablet samples has also been studied.

Keywords: trimethoprim; metalloporphyrin; glassy carbon electrode; differential pulse voltammetry; electrooxidation

Introduction

Trimethoprim (TMP), chemically 5-(3,4,5-trimethoxybenzyl) pyrimidine-2,4-diamine, the structure of which is shown in Figure 1A, belongs to the class of chemotherapeutic agents known as dihydrofolate reductase inhibitors. It is used in prophylaxis treatment and urinary tract infections. TMP is a synthetic antibiotic that interferes with the production of tetrahydrofolic acid (a necessary chemical for bacteria and human cells to produce proteins), by inhibiting the enzyme responsible for making tetrahydrofolic acid from dihydrofolic acid. Tetrahydrofolic acid is an essential precursor in the *de novo* synthesis of the intermediate thymidine monophosphate which is a precursor for DNA metabolite thymidine triphosphate. Bacteria cannot take up folic acid from the environment and are dependent on their own *de novo* synthesis. TMP inhibits the bacterial enzyme more than the corresponding human enzyme. TMP was commonly used in combination with sulphamethoxazole. The use of this combination, known as 'co-trimoxazole', was restricted in 1995. Still it is prescribed for some infections viz., prophylaxis in HIV-affected patients at risk of pneumocystic jirovecii pneumonia, Whipples disease and for those with some hematological malignancies. Use of TMP is contraindicated during pregnancy, especially in the first trimester, and for sufferers of certain blood disorders. It can also reduce the clearance of creatinine at renal tubules. Additionally, it can also lead to dangerously low levels of thrombocytes (cells that help blood clot) by lowering folic acid levels and associated bone marrow blood cell formation. Thus the determination of TMP is of great importance and various methods have been developed. They include spectrophotometry,[1] molecular imprinting chemiluminiscence,[2] polarography,[3] and voltammetry.[4] But most of these methods lack selectivity and sensitivity for the determination of TMP. Hence it is of immense importance to develop a technique for the determination of TMP with a high degree of selectivity and sensitivity and a low detection limit.

Immobilization of chemical microstructures onto electrode surfaces has been a major thrust area in electrochemistry in recent years.[5–7] Modification of electrodes with suitable biocompatible materials enables the electrochemistry of the redox biological compounds to proceed without hindrance, which results in increased selectivity and sensitivity of determination. Certain transition metal complexes with phthalocyanins, porphyrins, and schiff bases can catalyze via reduction of their central metal ions, thus enabling electro-oxidation of some chemical and biological compounds.[8–10]

Porphyrins are a class of naturally occurring macrocyclic compounds that play an important role in the metabolism of living organisms,[11,12] which is structured on 16 atom rings containing 4 nitrogen atoms. They are of perfect size to bind nearly all metal ions. Metalloporphyrins and related macrocycles provide an extremely versatile synthetic base on which to

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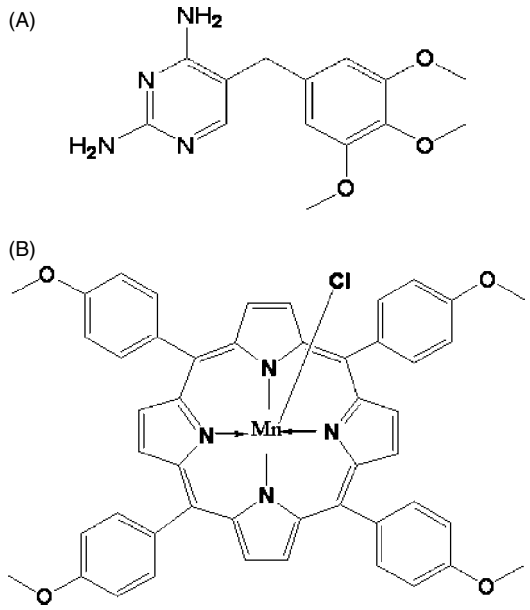

Figure 1. Structure of (A) TMP; and (B) TMOPPMn(III)Cl.

design physical and chemical properties. Porphyrins can be molecularly engineered to get desirable molecular and material properties.[13,14] They are good sensing materials and find use as catalysts, semiconductors, and anticancer medicine.[15] As porphyrins possess the amphibilic properties, they are used in membrane studies.[16,17] Porphyrin as a redox system exhibits interesting electrochemical properties.[18–20] Metalloporphyrins as drug-sensing materials are one of the promising classes of compound to be used as voltammetric sensors.[21] Reports have shown that a tetraphenylporphyrin-coated glassy carbon electrode was used for anodic stripping voltammetry of heavy metals.[22] Simultaneous electrochemical determination of uric acid and ascorbic acid on Co(II)tetrakisphenylporphyrin-modified glassy carbon electrode is also reported.[23]

In continuation of our work on drug analysis,[24–28] an attempt has been made to develop a convenient method for the determination of the drug, Trimethoprim (TMP). This work details an electrochemical method for the determination of TMP using [5,10,15,20-tetrakis(4-methoxyphenyl)porphyrinato]Mn(III)chloride film modified glassy carbon electrode (TMOPPMn(III)Cl/GCE).

Experimental

Chemicals and solvents

All reagents used in the investigation were of analytical reagent grade. Double-distilled water was used for preparing all aqueous solutions. Porphyrin and metalloporphyrin were prepared according to the literature procedure.[29,30] Pyrrole and anisaldehyde were purchased from Sisco Research Laboratories Ltd Mumbai, (India) and were freshly distilled prior to use. $(CH_3COO)_2$ $Mn.4H_2O$ was obtained from S.D. Fine Chemicals Pvt. Ltd (Mumbai, India). Pure TMP was obtained as a gift sample. The stock solution of TMP (1×10^{-2} M) was prepared in methanol. The supporting electrolyte used was phosphate buffer solution (PBS), which was prepared using $NaH_2PO_4.2H_2O$ and Na_2HPO_4. Standard solutions of the analyte were prepared by serial dilution of the stock solution using PBS. Nafion (5%) was purchased from Sigma Aldrich Saint Louis, MO. Dosage forms containing TMP was prepared in the lab.

Instrumentation

All electrochemical experiments were performed on an electrochemical analyzer (BAS Epsilon West Lafayette, Indiana. Bioanalytical System, USA), interfaced to a PC. A three electrode system which consists of a TMOPPMn(III)Cl/GCE as working electrode, an Ag/AgCl reference electrode, and a Pt wire auxiliary electrode was 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 Vario EL III CHNS analyzer.

Synthesis of 5,10,15,20- tetrakis(4-methoxyphenyl) porphyrin (TMOPP)

TMOPP was synthesized according to the Adler method.[29] 15 mmol of freshly distilled pyrrole (1.04 mL) and anisaldehyde (1.82 mL) were added to 80 mL of refluxing reagent grade propionic acid. After refluxing for 30 min, the solution was ice-cooled, filtered, and the filter cake washed thoroughly with methanol. After a hot water wash, the resulting purple crystals were air dried. The product was purified by column chromatography.

Elemental analysis of TMOPP

Calcd (%): C, 78.0; H, 5.1; N, 7.6
Found (%): C, 77.99; H, 5.0; N, 7.51
IR (KBr), γ (cm^{-1}): 3363 (NH); 3000 (CH)
UV-Visible spectrum in CH_2Cl_2, λ (nm): 364, 417, 516, 557,651
^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 8.8 (s, 8H, pyrrolic β H), 3.39 (s, 12 H, $0CH_3$), 3.5 (s, 2H, NH), 8.2-7.3 (m, 16 H, aromatic)

Synthesis of [5,10,15,20-tetrakis (4-methoxyphenyl) porphyrinato] Manganese(III)chloride (TMOPPMn(III)Cl)

TMOPPMn(III)Cl was prepared according to the literature procedure.[30] The ligand TMOPP (2 g, 3.22 mmol) and $Mn(CH_3COO)_2.4H_2O$ (2 g, 9.40 mmol) were refluxed in 500 ml glacial acetic acid for 72 h. The solvent was then stripped off and the residue was then extracted into 250 mL methanol. The filtered methanol solution was treated with 250 mL concentrated HCl and with 250 mL of distilled water. A green precipitate was formed immediately, which was isolated by filtration, washed with 250 mL of distilled water and air dried. The crude product was dissolved in 100 mL of benzene and filtered and 400 mL of petroleum ether were added. The cooled solution yielded lustrous green crystals and pure product was isolated by filtration and air drying yielded 1.5 g. The molecular structure of TMOPPMn(III)Cl is displayed in Figure 1B.

Elemental analysis of TMOPPMn(III)Cl

Calcd (%): C, 70.0; H, 5.0; N, 7.1
Found (%): C, 69.03, H, 4.88, N, 6.44
UV-Visible spectrum in CH_2Cl_2, λ (nm): 236, 388, 405, 481, 582, 621
IR (KBr), γ (cm^{-1}): 3363 (NH); 3000 (CH); 455 (M-N).
^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 8.9 (s, 8H, pyrrolic β H), 4 (s, 12 H, $0CH_3$), 8.2-7.3 (m, 16 H, aromatic)

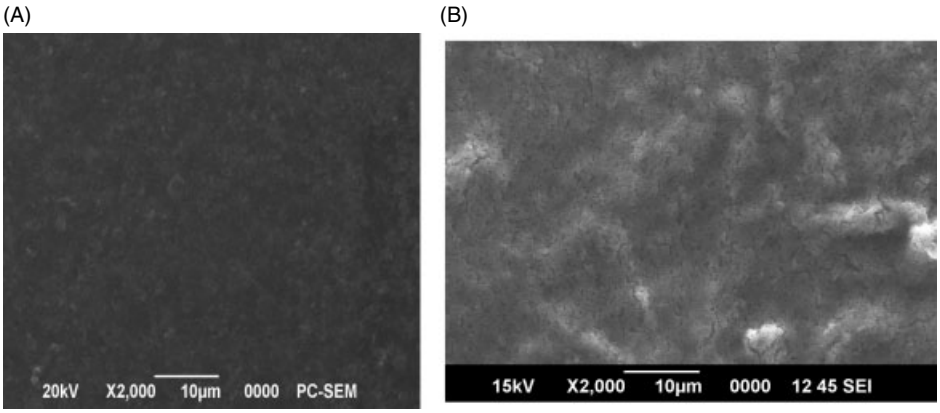

Figure 2. SEM images of (A) bare GCE; and (B) TMOPPMn(III)Cl/GCE.

Preparation and characterization of TMOPPMn(III)Cl modified glassy carbon electrode

TMOPPMn(III)Cl (2 mg) was dissolved in a mixture of 3 mL nafion and 2 mL ethanol. The solution was then agitated ultrasonically for about half an hour to get a stable and homogeneous solution. The bare glassy carbon electrode (GCE) was mechanically polished with alumina slurry until obtaining a mirror-like surface. The electrode was then rinsed thoroughly with redistilled water and was ultrasonically cleaned in methanol. Finally it was sonicated in 1:1 HNO_3, acetone, NaOH solution, and doubly distilled water for 5 min, respectively. The electrode was then allowed to dry. The TMOPPMn(III)Cl/GCE was prepared by dropping 3 μL of TMOPPMn(III)Cl solution onto the clean GCE surface and evaporating the solvent at room temperature.

Surface morphological observations of TMOPPMn(III)Cl/GCE were carried out by scanning electron microscopy (SEM). Figures 2A and 2B depict the SEM images of the bare GCE and TMOPPMn(III)Cl/GCE. The comparison points to the effective modification of the bare GCE. Cyclic voltammetry of 2 mM potassiumferricyanide solution was carried out at both bare GCE and TMOPPMn(III)Cl/GCE at different scan rates to calculate the effective surface area of them.

Preparation of sample

PBS (pH 5, 0.1 M) was used as the supporting electrolyte for the determination of TMP. Stock solution of TMP (1×10^{-2} M) was prepared in methanol. Standard solutions of the analyte (1×10^{-3} M–1×10^{-8} M) were prepared by serial dilution of the stock solution using PBS.

Analysis of sample

Sample solution was taken in the electrochemical cell. The accumulation step was carried out under open circuit while stirring the solution for 2 min, then the differential pulse voltammograms from 0 to 1.30 V at 20 mVs^{-1} were recorded and finally the peak current at about 1.080 V was measured for TMP. Prior to and after each measurement, the TMOPPMn(III)Cl/GCE was activated by successive cyclic voltammetric sweeps between 0 to 1.3 V at 100 mVs^{-1} in PBS (pH 5,0.1 M) until the voltammograms kept unchangeable.

Results and Discussion

Comparison of surface area of bare GCE and TMOPPMn(III)Cl/GCE

Cyclic voltammetry of 2 mM potassiumferricyanide solution was carried out at both bare GCE and TMOPPMn(III)Cl/GCE at different scan rates. The obtained current was plotted against the square root of scan rates in both the cases. The slopes of the straight lines were determined. By using the Randles-Sevcik equation for reversible reaction,

$$i_p = (2.687 \times 10^5) n^{3/2} \upsilon^{1/2} D^{1/2} A c \quad (1)$$

(i_p refers to peak current, n is the number of electrons transferred, D is the diffusion coefficient, A is the surface area of the electrode, c is the concentration of potassium ferricyanide solution and υ stands for scan rate), effective surface area of bare GCE and TMOPPMn(III)Cl/GCE were calculated, Taking n=1 and D= 7.6×10^{-6} cms^{-1}, the effective surface area of bare GCE and TMOPPMn(III)Cl/GCE was calculated to be 0.06685 cm^2 and 0.2112 cm^2 respectively. The large surface area of the modified GCE compared to bare GCE is evident from the resultant values.

Electrochemical behaviour of TMP and the optimization of the developed method

The electrochemical behaviour of TMP at a TMOPPMn(III)Cl/GCE has been investigated using differential pulse voltammetry (DPV). Figure 3 shows the comparison of oxidation peak of 1×10^{-3} M TMP in PBS (pH 5) at a scan rate of 20 mVs^{-1} with pulse width 50 ms, pulse period 200 ms and pulse amplitude 50 mV at bare GCE and TMOPPMn(III)Cl/GCE. At the bare GCE, TMP yields an irreversible very low oxidation peak at 1.160 V (curve a). Under the same conditions, a well defined irreversible oxidation peak appears at 1.088 V for the TMOPPMn(III)Cl/GCE (curve b). Obviously, the anodic peak current of TMP at the TMOPPMn(III)Cl/GCE increases significantly and the peak potential shifts towards a more negative potential compared to that of a bare GCE. The increase in the peak current and the negative shift of oxidation potential may be attributed to the electrocatalytic activity of TMOPPMn(III)Cl. Further electrochemical studies of TMP on TMOPPMn(III)Cl/GCE were carried out.

The electrochemical properties of 10^{-3} M TMP in various medium such as 0.1 M PBS, H_2SO_4, HCl, KCl, tetra-n-butyl ammonium chloride, KNO_3, acetate buffer, NaOH were investigated

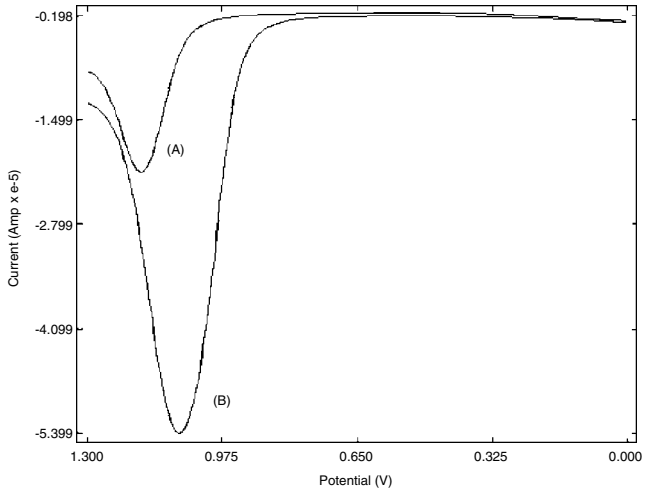

Figure 3. Differential pulse voltammogram of 1×10^{-3} TMP at (A) bare GCE; and (B) TMOPPMn(III)Cl/GCE.

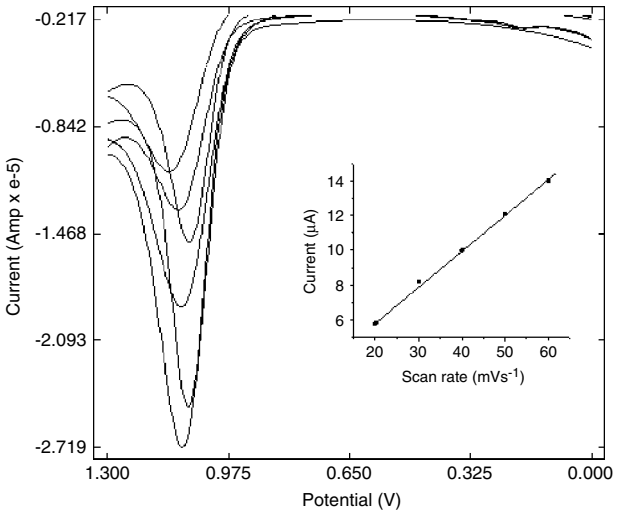

Figure 4. Differential pulse voltammograms of 10^{-6} M TMP at scan rates 20, 30, 40, 50, 60, 70, 80 mVs^{-1} (from top to bottom). Inset is the variance of anodic peak current of TMP with scan rate in the range 20–60 mVs^{-1}.

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 TMP.

The electrochemical studies of 10^{-3} M TMP in PBS were carried out in the pH range of 3 to 10 using DPV. The best oxidation response was obtained in pH 5 as the peak current is the highest. Thus pH 5 was fixed as optimal pH.

The amount of TMOPPMn(III)Cl solution on the GCE directly determines the thickness of the TMOPPMn(III)Cl film. It is found that the oxidation peak current of TMP increases, while gradually increasing the volume of TMOPPMn(III)Cl solution from 1 to 3 µL. The enhancement of current indicates that the number of catalytic sites increases with the increase of the amount of TMOPPMn(III)Cl. Further increasing the volume of TMOPPMn(III)Cl solution results in the decrease of the peak current. This is because nafion, used as one of the solvents, is a kind of insulator that blocks the electron transfer. Due to uncompensated resistive effects or lowering of the charge transfer rate, the peak current is conversely decreased. Hence, the volume of TMOPPMn(III)Cl solution was fixed to be 3 µL.

The accumulation step is usually a simple and effective way of enhancing the sensitivity. The influence of the accumulation time on the oxidation peak current of 10^{-6} M TMP was tested using DPV. The oxidation peak current increased gradually within the first 2 min indicating the enhancement of TMP concentration at the electrode surface. After that, as the accumulation time increases, the peak current tends to level off showing that the adsorptive equilibrium is reached. These results indicate that electrochemical oxidation of TMP on TMOPPMn(III)Cl/GCE is adsorption controlled.

The influence of the scan rate on the oxidative peak current and potential of 10^{-6} M TMP were investigated. Figure 4 describes the variation of anodic peak current with scan rate 20–80 mVs^{-1} and the inset shows the linear behaviour of anodic peak current with the scan rate in the range 20–60 mVs^{-1}. This again points to the fact that the behaviour of TMP during the electrode reaction is controlled by adsorption rather than diffusion. Potential plotted against ln scan rate gave a linear plot, slope of which is 0.0135. Based on the Laviron's equation,[31] the slope of this plot equals to $RT/\alpha nF$, where α is assumed to be 0.5 for the totally irreversible electrode process. The calculation gave the number of electrons involved in the electrochemical reaction (n_a) to be 3.8 (close to 4).

Earlier report with respect to the electrochemical oxidation of TMP illustrates the identification of products using various techniques like measurement of melting point, TLC, IR and mass spectroscopy, the results of which point to the formation of mononitroso and dinitroso derivatives. TMP was electro-oxidized in the pH range 3–10 to mononitroso derivative by 4e mechanism. The mononitroso derivative underwent electro-oxidation to dinitroso derivative by 4e mechanism at pH less than 3.[32] Based on our experimental observation of the involvement of 4e and on the previous report of identification of products of electro-oxidation of TMP, we propose a possible mechanism for it (Figure 5). The amino group at position 4 of the pyrimidine ring may be more easily oxidized than the one at position 2 which is a part of stable pyrimidine system. Again, a 4e oxidation of the mononitroso derivative can lead to the formation of dinitroso derivative.[32] But then, there should be two distinct peaks in the voltammogram corresponding to the formation of mono and dinitroso derivatives. In the present investigation, only a single oxidation peak for TMP is obtained in the pH range 3–10, which indicates the formation of mononitroso derivative by a 4e process, thus ruling out the possibility of 8e oxidation of TMP to dinitroso derivative. Thus a 4e oxidation for TMP is proposed.

Limit of detection of TMP

The effect of concentration of TMP in PBS (pH 5) on the modified GCE was studied using DPV. Figure 6 shows differential pulse voltammograms of TMP on TMOPPMn(III)Cl/GCE at various concentrations (10^{-3}–10^{-8} M). The results show that the oxidative peak current has a linear relationship with the concentration in the range $6 \times 10^{-8} - 1 \times 10^{-6}$ M which is shown in the inset of Figure 6. The linear regression equation is

$$i_p = 2.1102 + 0.1634C \; (r = 0.9855, \; C \text{ in M}, \; i_p \text{ in } \mu A) \qquad (2)$$

The lower detection limit of TMP is 3×10^{-9} M.

Comparison with the standard method

The developed method was compared with the standard potentiometric titration method for the determination of TMP.[33]

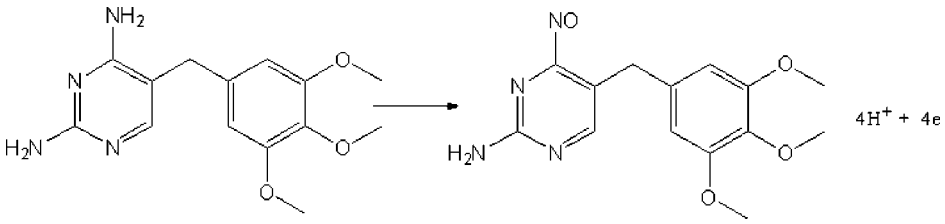

Figure 5. Mechanism of oxidation of TMP.

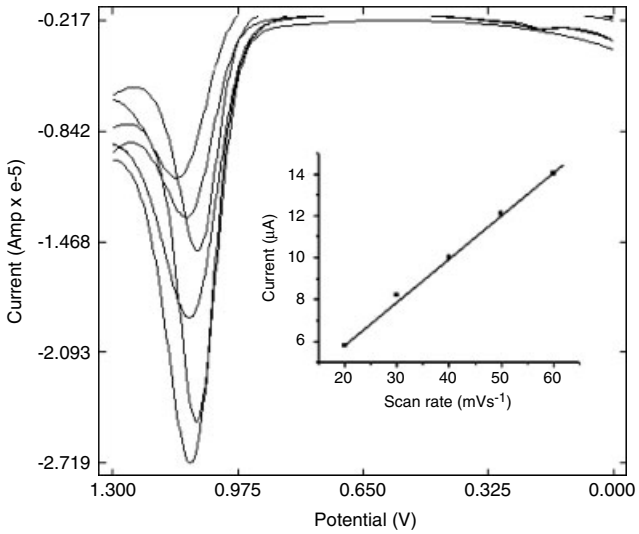

Figure 6. Differential pulse voltammograms of concentrations 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} M of TMP (from bottom to top). Inset shows the dependence of peak current (i_p) on the concentrations of TMP in the range 6×10^{-8}–1×10^{-6} M.

TMP in anhydrous acetic acid was titrated against standard perchloric acid. Compared to the standard method, a very low detection limit of TMP could be achieved with the developed sensor.

Reproducibility and stability

The reproducibility of the electrode was examined by repetitive voltammetric determination of 1×10^{-3} M TMP using the same TMOPPMn(III)Cl/GCE. Comparable results were obtained with relative standard deviation (RSD) of 3.2% for n=9 suggesting that the TMOPPMn(III)Cl/GCE has good reproducibility. After each determination, the modified electrode was run in PBS (pH=5). Moroever, TMOPPMn(III)Cl/GCE exhibited stable behaviour for 20 days.

Selectivity

Table 1 lists the influence of other substances on the oxidation signal of TMP. A 100-fold concentration of KCl, NaCl, citric acid, dextrose, lactose, urea, and K_2SO_4 have no influence on the signals of 1×10^{-3} M TMP, with deviation below 5%. Since sulphamethoxazole is often used as part of a synergistic combination with TMP in tablets, the influence of sulphamethoxazole on the oxidation peak current of TMP was studied. It was found that same concentration of sulphamethoxazole did not interfere in the determination of TMP.

Table 1. Influence of 10^{-1} M of foreign species on the oxidation peak current of 1×10^{-3} M TMP

Interferent	Signal change, %
Potassium chloride	−2.50
Sodium chloride	−4.40
Ascorbic acid	25
Citric acid	1.30
Dextrose	0.63
Lactose	−3.70
Urea	3.04
Sulphamethoxazole	4.15
Glycine	−8.24
Potassium sulphate	0.03

Applicational studies

A tablet of TMP was prepared by mixing TMP and sulphamethoxazole in the ratio 1 : 1. 50 mL of 1×10^{-5} M of the tablet solution was prepared in methanol and the solution was quantitatively filtered and transferred to a volumetric flask (50 mL). Solutions of different concentrations were prepared by serial dilution of the stock solution with PBS (pH 5). The diluted solutions were determined by DPV at the TMOPPMn(III)Cl/GCE and the unknown concentrations were determined from the calibration graph. The recoveries are in the range 98–99% and the relative standard deviation is found to be 0.252% (≤3%) for six determinations of the sample. These results indicate that the method provides a potential tool for the exclusive determination of TMP in presence of sulphamethoxazole in their mixture.

An adequate amount of TMP corresponding to 10^{-3} M was added to the urine sample. This solution was quantitatively diluted using PBS to obtain various concentrations (10^{-5} M–10^{-6} M). DPV at TMOPPMn(III)Cl/GCE were recorded and the unknown concentration was determined from the calibration graph. The recovery obtained lie in the range of 96–99%.

Conclusions

The voltammetric behaviour of TMP was investigated at a TMOPPMn(III)Cl/GCE by DPV. TMOPPMn(III)Cl film provides a good platform for the oxidation of TMP. The results showed that TMOPPMn(III)Cl could efficiently accelerate the electron transfer rate of TMP and hence can act as an electrocatalyst for the oxidation of TMP. The TMOPPMn(III)Cl/GCE is convenient to fabricate and is characterized by remarkable enhancement of the peak current and the reduction of anodic peak potential with the detection limit of 3×10^{-9} M. The proposed sensor was utilized for the analysis of TMP in tablets and urine and was found to perform successfully. The

proposed method is an advanced substitute for the determination of TMP as certain predominant characteristics vested in this work put forth: low detection limit, simple process, accelerated speed of detection, cost effectiveness good reproducibility, stability and excellent sensitivity for TMP.

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References

[1] A. L. El-Ansary, Y. M. Issa, W. Selim, *Anal. Lett.* **1999**, *32*, 955.
[2] M. Polas ek, M. Jambor, *Talanta* **2002**, *58*, 1253.
[3] M. A. Brooks, J. A. F. de Silva, L. M. D. Arconte, *Anal. Chem.* **1973**, *45*, 263.
[4] H. M. Carapuca, D. J. Cabral, L. S. Rocha, *J. Pharm. Biomed. Anal.* **2005**, *38*, 364.
[5] R. Vittal, H. Gomathi, K. K. Jin, *Adv. Colloid. Interfac.* **2006**, *119*, 55.
[6] J. Wang, *Electroanalysis* **1991**, *3*, 255.
[7] N. R. Stradiotto, H. Yamanaka, M. V. B. Zanoni, *J. Braz. Chem. Soc.* **2003**, *14*, 159.
[8] M. Biesaga, K. Pyrzynska, M. Trojanowicz, *Talanta* **2000**, *51*, 209.
[9] T. Malinski, Z. Taha, *Nature* **1992**, *358*, 676.
[10] Q. Deng, S. Dong, *Analyst* **1996**, *121*, 1123.
[11] V. K. Gupta, D. K. Chauhan, V. K. Saini, S. Agarwal, M. M. Antonijivec, H. Lang, *Sensors* **2003**, *3*, 223.
[12] V. K. Gupta, S. Chandra, D. K. Chauhan, R. Mangla, *Sensors* **2002**, *2*, 164.
[13] K. S. Suslick, S. D. Jeffries, *Supramolecular Chemistry*, Elsevier: Oxford, **1996**.
[14] P. Bhyrappa, J. K. Young, J. S. Moore, K. S. Suslick, *J. Am. Chem. Soc.* **1996**, *118*, 5708.
[15] M. Patel, B. J. Day, *Tre. Pharmacol. Sci.* **1999**, *20*, 359.
[16] E. M. G. Santos, A. N. Araujo, C. M. C. M. Couto, M. C. B. S. M. Montenegro, *J. Pharm. Biomed. Anal.* **2006**, *42*, 535.
[17] S. S. M. Hassan, W. H. Mahmoud, M. A. F. Elmosallamy, M. H. Almarzooqi, *Anal. Sci.* **2003**, *19*, 675.
[18] D. T. Gryko, C. Clausen, J. S. Lindsey, *J. Am. Chem. Soc.* **1999**, *121*, 8635.
[19] J. L. Sessler, V. L. Capuano, A. J. Harriman, *J. Am. Chem. Soc.* **1993**, *115*, 4618.
[20] J. S. Manka, D. S. Lawrence, *Tetrahedron Lett.* **1989**, *30*, 6989.
[21] F. C. Gong, X. B. Zhang, C. C. Guo, G. L. Shen, R. Q. Yu, *Sensors* **2003**, *3*, 91.
[22] H. H. Frey, C. J. McNeil, R. W. Keay, J. V. Bannister, *Electroanalysis* **1998**, *10*, 480.
[23] C. X. Li, Y. L. Zeng, Y. J. Liu, C. R. Tang, *Anal. Sci.* **2006**, *2*, 393.
[24] R. Joseph, K. Girishkumar, *Drug Test. Analysis* **2010**, *2*, 278.
[25] S. Issac, K. Girishkumar, *Drug Test. Analysis* **2009**, *1*, 350.
[26] R. Joseph, K. Girishkumar, *Anal. Lett.* **2009**, *42*, 2309.
[27] K. Girishkumar, P. Augustine, S. John, *J. Appl. Electrochem.* **2009**, *40*, 65.
[28] B. Saraswathyamma, M. Pajak, J. Radecki, W. Maes, W. Dehaen, K. Girishkumar, H. Radecka, *Electroanalysis* **2008**, *20*, 2009.
[29] A. D. Adler, F. R. Longo, J. D. Finarelli, J. Goldmacher, J. Assour, *J. Org. Chem.* **1967**, *32*, 476.
[30] R. R. Gaughan, D. F. Shriver, L. J. Boucher, *Proc. Nat. Acad. Sci. USA* **1975**, *72*, 433.
[31] E. Laviron, *J. Electroanal. Chem.* **1974**, *52*, 355.
[32] R. N. Goyal, A. Kumar, *Electroanalysis* **1990**, *2*, 539.
[33] The United States Pharmacopoeia, 21st revision, United States Pharmacopeial Convention: Rockville, **1985**.