Anal. Bioanal. Electrochem., Vol. 7, No. 4, 2015, 426-438

Analytical & Bioanalytical Electrochemistry

2015 by CEE
www.abechem.com

Full Paper

Electrochemical Characterization and Determination of Theophylline at a Graphite Pencil Electrode using Cetyltrimethyl Ammonium Bromide as an Enhancing Agent

Prashant A. Magdum, Vijay P. Pattar and Sharanappa T. Nandibewoor*

P. G. Department of Studies in Chemistry, Karnatak University, Dharwad 580003, India

* Corresponding Author, Tel.: +918362215286; Fax: +91836 2747884

E-Mail: stnandibewoor@yahoo.com (S. T. Nandibewoor)

Received: 29 April 2015 / Received in revised form: 17 July 2015 / Accepted: 21 July 2015 / Published online: 31 August 2015

Abstract- The oxidation of theophylline (TP) was studied at a graphite pencil electrode (GPE) in the presence of cetyltrimethyl ammonium bromide (CTAB) by cyclic and differential pulse voltammetry. The results indicated that the electrochemical responses of theophylline are apparently improved by cetyltrimethyl ammonium bromide, due to the enhanced accumulation of theophylline at pencil graphite electrode surface. Key experimental parameters such as pH of supporting electrolyte, scan rate, concentration and interferents were investigated. Under optimal conditions, the peak current was proportional to theophylline concentration in the range of 1.0×10^{-7} to 1.3×10^{-6} M with a detection limit of 2.63×10^{-9} M by differential pulse voltammetry. The proposed method was successfully applied to the determination of theophylline in tablet and urine samples.

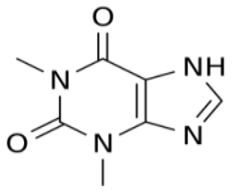
Keywords- Graphite pencil electrode, Electroanalysis, Oxidation, Surfactant, Theophylline

1. INTRODUCTION

Drug analysis is an important tool for drug quality control. The development of a simple, sensitive, rapid and reliable method for the determination of drugs is of great importance. The graphite pencil electrode (GPE) has been successfully used as a biosensor in modern electroanalytical field due to its high electrochemical reactivity, good mechanical rigidity,

low cost, low technology and ease of modification, renewal and low background current [1,2]. The GPE has good application in the analysis of neurotransmitter and detection of traces of metal ions and drugs.

Theophylline (1, 3-dimethylxanthine, TP) as shown in scheme 1, is a member of xanthine-based alkaloids, has a stimulating effect on respiration. TP is a widely used bronchodilator drug employed in the management of various asthmatic conditions. The main mechanism of action of TP is that of adenosine receptor antagonism. TP is a non-specific adenosine antagonist, antagonizing A1, A2, and A3 receptors almost equally, which explains many of its cardiac effects and some of its anti-asthmatic effects. TP in-vitro can restore the reduced histone deacetylase activity that is induced by oxidative stress, returning steroid responsiveness towards normal. The plasma level generally accepted for effective bronchodilation in adults is 5-20 $mgmL^{-1}$ [3,4]. Levels below this range are usually non-therapeutic; while higher levels may cause toxicity [5]. The rate of metabolism of theophylline varies considerably from one individual to another. As a consequence of the variation of pharmacokinetics between patients, it is necessary to monitor concentration of drugs in individual patients to ensure the maximum clinical response and to avoid undesirable side effects



Scheme 1. Structure of Theophylline

Several methods have been used to determine TP, including spectrophotometry [6], high-performance liquid chromatography [7,8]. The main problems encountered in using such methods are time consuming extraction and separation procedure. In the literature, various electrochemical techniques are reported [9-16]. These voltammetric methods are not as good as our method, since in the previous methods, Poly (AHNSA)/GCE, Xanthine oxidase electrode, Nefion/lead-ruthenium oxide pyrochlore chemically modified electrode, Multi-wall carbon nanotube modified GCE in which expensive chemicals are used and the modification is not easy. Hence in our method, the electrode used is of low cost, renewable and ease of modification.

Surfactants are a kind of amphiphilic ion or molecule with a hydrophilic head compatible with water on one side and long hydrophobic tail compatible with oil on the other side. They have been widely used in the field of electrochemical and electro analytical chemistry [17-19]. The surfactant can change the electrochemical process through adsorption at interfaces

or aggregation into supramolecular structure [20]. Many groups have successfully employed surfactants for the analysis of some bio-molecules in their work [21,22]. They got very good results which indicated that the electrochemical responses of analyzed objects were remarkably enhanced in the presence of surfactants.

Survey of literature revealed that no electro analytical method for determination of theophylline at a GPE using CTAB was reported. The aims of this study are to establish the experimental conditions and to optimize the conditions for the determination of this compound in pharmaceutical dosage form and in human biological fluids using cyclic and differential pulse voltammetric techniques.

2. EXPERIMENTAL

2.1. Materials and reagents

The pencil-lead rods (HB 0.5 mm in diameter and 6 cm length) were purchased from local bookstore. Pure TP in powdered form was obtained from S. D. Fine Chemicals. A stock solution (1.0 mM) of TP was prepared in doubly distilled water. The TP containing tablets (Theo-Asthalin (100 mg per tablet)) were purchased from a local pharmacy. All the surfactants obtained from Hi-Media Pvt. Ltd. were dissolved in doubly distilled water. Phosphate buffer solutions (Ionic strength=0.2 M) were prepared according to the literature method [23]. All other reagents used were of analytical grade. All solutions were prepared in double distilled water.

2.2. Instrumentation and analytical procedure

Electrochemical measurements were carried out on a CHI630D electrochemical analyzer (CH Instruments Inc., USA). The voltammetric measurements were carried out in a 10 mL single compartment three-electrode glass cell with Ag/AgCl as a reference electrode, a platinum wire as counter electrode and a GPE/CTAB as working electrode. All the potentials are given against the Ag/AgCl (3.0 M KCl). PH measurements, were performed with an Elico LI 120 pH meter (Elico Ltd.,India). All experiments were carried out at an ambient temperature of 25±0.1◦C.

The parameters for differential pulse voltammetry (DPV) were initial potential E: 0.20 V; final potential E: 0.50 V; sample interval: 0.001 V; amplitude: 0.05 V; frequency: 15 Hz; quiet time: 2s; sensitivity: 1.0×10^{-7} A/V.

2.3. Area of the electrode

The area of the electrode was obtained by the cyclic voltammetric method using 1.0 mM $K_4Fe(CN)_6$ as a probe, at different scan rates. For a reversible process, the following Randles-Sevcik formula was used [24].

$$i_p = (2.69x10^5)n^{3/2}AD_o^{1/2}C_o\,\upsilon^{1/2} \quad (1)$$

Where i_p refers to the anodic peak current, n is the number of electrons transferred, A is the surface area of the electrode, D_0 is diffusion coefficient, *v* is the scan rate and C_0 is the concentration of $K_4Fe(CN)_6$. For 1.0 mM $K_4Fe(CN)_6$ in 0.1 M KCl electrolyte, n=1, $D_0=7.6\times10^{-6}$ cm^2s^{-1}. Then from the slope of the plot of i_p *vs* $\upsilon^{1/2}$, the electro active area was calculated. In our experiment, electro active area for GPE/CTAB was found to be 0.228 cm^2 and for carbon paste electrode was 0.099 cm^2. Electro active area of GPE/CTAB was higher than the electro active area of CPE. Hence greater response of peak current was observed for GPE/CTAB towards theophylline.

2.4. Tablet sample preparation

Ten pieces of TP containing tablet, Theo-Asthalin (100 mg per tablet) were powdered in a mortar. A portion equivalent to a stock solution of a concentration of about 1.0×10^{-3} M was accurately weighed and transferred into a 100 ml calibrated flask and completed to the volume with double distilled water. The contents of the flask were sonicated for 15 minutes to get complete dissolution. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquid and diluting them with the phosphate buffer solution. Each solution was transferred to the voltammetric cell and analyzed by standard addition method. The differential pulse voltammograms were recorded between 0.2 and 0.5 V after open-circuit accumulation for 120 s with stirring. The content of the drug in the tablet was determined referring to the calibration graph.

2.5. Recovery experiment from tablet

To study the accuracy of the proposed method used in the dosage forms, recovery experiments were carried out by the standard addition method. This study was performed by the addition of known amounts of theophylline to known concentration of the tablet sample. The resulting mixture was analyzed as in pure theophylline.

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetric behavior of TP

The electrochemical behavior of TP at GPE in the presence of CTAB was investigated using cyclic voltammetry. The results are shown in Fig. 1. No apparent cyclic voltammetric signals were observed in the phosphate buffer solution in the presence of CTAB (curve c), which indicates that CTAB is an electrochemically inactive material in the working potential range. The TP exhibits an anodic peak at about 1.27 V (curve b) at the bare GPE. After the addition of 2.0×10^{-5} M CTAB, the oxidation peak current of TP increases greatly (curve a).

This indicates that CTAB can make the electron transfer of TP more easily and show obvious enhancement effect to the oxidation of TP. The peak current enhancement was undoubtedly attributed to the interaction of CTAB with TP and GPE. It is well known that surfactants can be adsorbed on a hydrophobic surface to form surfactant film, which may alter the over voltage of the electrode and influence the rate of electron transfer. In the presence of CTAB, the electrode surface may form a hydrophilic film with positive charge. This hydrophilic layer may increase the concentration of TP on the surface of electrode. No reduction peak was observed in the reverse scan, suggesting that the electrochemical reaction was a totally irreversible process. The voltammograms corresponding to the first cycle were generally recorded.

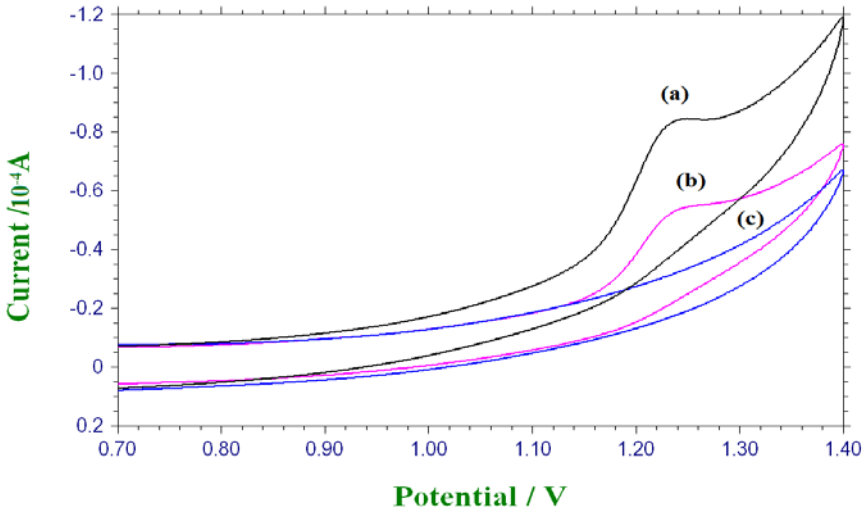


Fig. 1. Cyclic voltammograms with pH 3, 0.2 M PBS, υ=50 mVs^{-1} at (a) GPE /CTAB with TP of 1.0 mM (b) GPE with TP of 1.0 mM (c) GPE /CTAB without TP

3.2. Effect of concentration of CTAB

The effect of CTAB concentration on the oxidation TP was as shown in Fig. 2. It is well known that surfactants can be adsorbed on a hydrophilic surface to form surfactant film, which may alter the over voltage of electrode and influence the rate of electron transfer. In the presence of CTAB, the electrode surface may form a hydrophilic film with positive charge. This thickness of CTAB film at GPE determines the current sensitivity of the electrode. When the concentration of CTAB was increased from 0 to 2.5×10^{-5} M, the peak current increased to a maximum at 2.0×10^{-5} M which may be due to the inadequate surface coverage. When we used the surfactant above 2.0×10^{-5} M, the thickness of CTAB film at GPE surface increased and the electron transfer kinetics became sluggish. Hence 2.0×10^{-5} M CTAB was used in the further experiments.

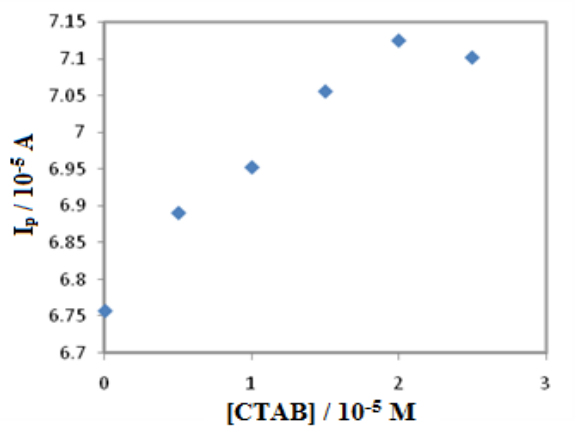


Fig. 2. Effect of concentration of surfactant on the peak current of TP

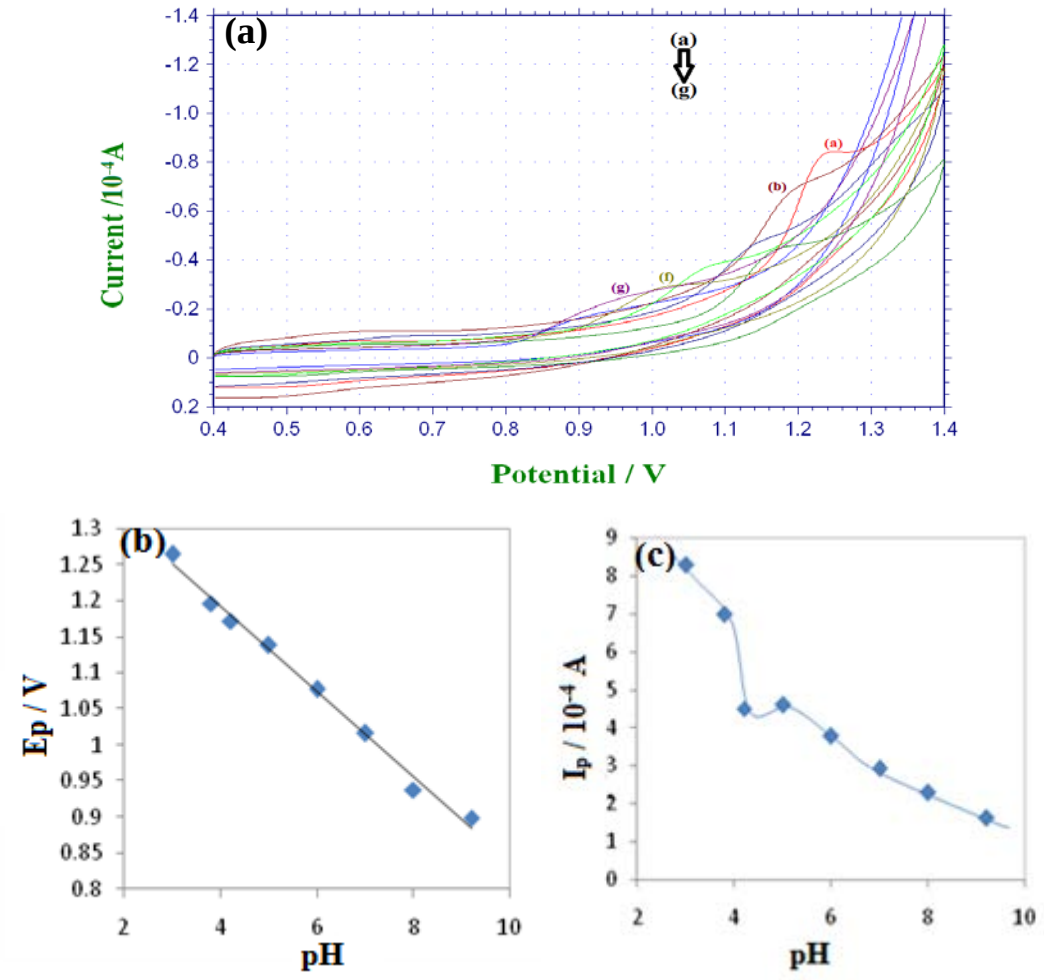


Fig. 3. a) Effect of pH on the peaks in phosphate buffer solution at (a) pH 3.0, (b) pH 4.2, (c) pH 5.0, (d) pH 6.0, (e) pH 7.0, (f) pH 8.0 and (g) pH 9.2 with potential scan rate 50 mVs^{-1}. Other conditions are as in Fig. 1; **b)** Effect of pH on the peak potential of TP; **c)** Effect of pH on the peak current of TP

3.3. Effect of pH

The influence of solution pH on the response of 1.0×10^{-3} M TP was examined over the pH range 3.0–9.2 in phosphate buffer solution by CV at a scan rate of 50 mV s^{-1}. With increase in the solution pH, the potential of the peak is shifted to less positive values (Fig. 3a).

This indicates that hydrogen ions were concerned with the electrode reaction. The linear relationship between E_p and pH (Fig. 3b) can be expressed as follows:

$$E_p(V)=1.428-0.063\ pH \qquad r = 0.992 \qquad (2)$$

The slope of 0.063 per pH unit suggested that the number of electron transfer is equal with that of hydrogen ions taking part in the electrode reaction [25].The solution pH influenced the peak current considerably. The peak current decreased with the increase of solution pH (Fig. 3c).

However, the best result with respect to sensitivity accompanied with sharper response was obtained with pH 3.0, so pH 3.0 was selected for further experiments.

3.4. Effect of scan rate

The effect of scan rate on the electro oxidation of TP was examined by cyclic voltammetry (Fig. 4a). With an increase in scan rate, the peak potential shifted to a more positive value, and a linear relationship was observed in the range 0.05 to 0.5 Vs^{-1}.

The influence of the square root of the scan rate on the peak current showed a linear relationship (Fig.4b), which is of typical diffusion controlled process [26] and the equation can be expressed as follows:

$$i_p(A)=0.614\upsilon^{1/2}(V^{1/2}s^{-1/2})+1.664 \qquad r=0.987 \qquad (3)$$

A linear relationship was observed between log i_p and log υ (Fig.4c), corresponding to the following equation:

$$\log i_p(A)=0.389\ \log \upsilon\ (Vs^{-1})+0.125 \qquad r=0.970 \qquad (4)$$

The slope of 0.39 was close to the theoretically expected value of 0.5 for a purely diffusion controlled process [27], which, in turn, further confirms that the electro oxidation of TP was diffusion controlled.

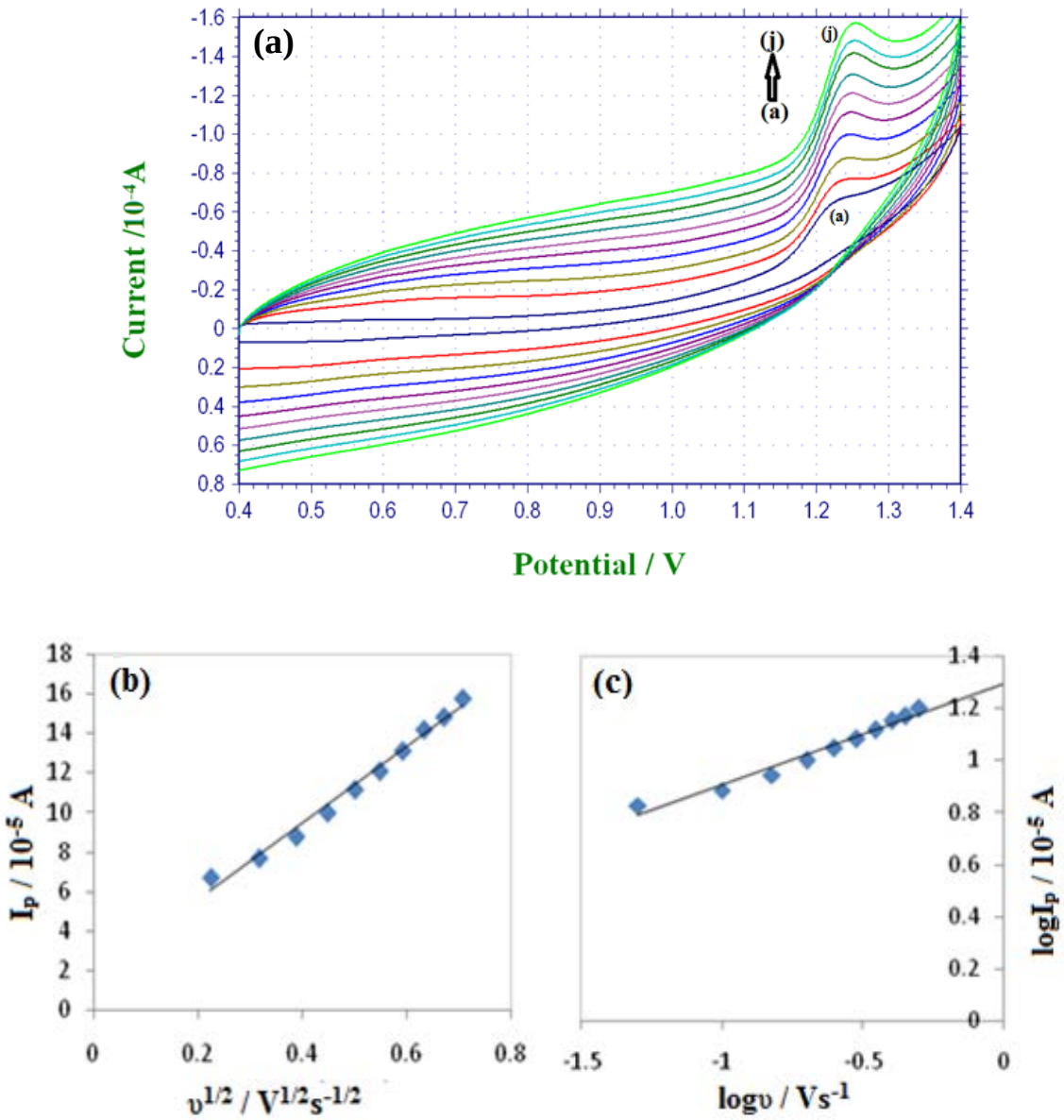
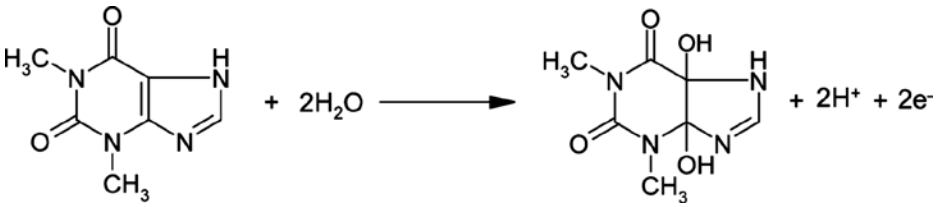


Fig. 4. a) Cyclic voltammograms obtained for 1.0 mM TP in PBS of pH 3 at scan rates of (a) 0.05, (b) 0.1 , (c) 0.15 , (d) 0.2 , (e) 0.25 , (f) 0.3 , (g) 0.35 , (h) 0.4 (i) 0.45 and (j) 0.5 Vs^{-1}; **b)** Dependence of the oxidation peak current on the square root of scan rate; **c)** Dependence of the logarithm of peak current on logarithm of scan rate

3.5. Mechanism

From the earlier literature, TP oxidation is a two-electron two-proton process [9,14]. The plausible mechanism is shown in Scheme 2.



Scheme 2. Plausible electrode reaction mechanism of theophylline

3.6. Calibration curve

In order to develop a voltammetric method for determining the drug, differential pulse voltammetric mode was selected, because, the peaks are sharper and better defined at lower concentration of TP than those obtained by CV, with a lower background current, resulting in improved resolution. According to the results obtained, it was possible to apply this technique to the quantitative analysis of TP. The phosphate buffer solution of pH 3.0 was selected as the supporting electrolyte for the quantification as TP gave maximum peak current at pH 3.0.

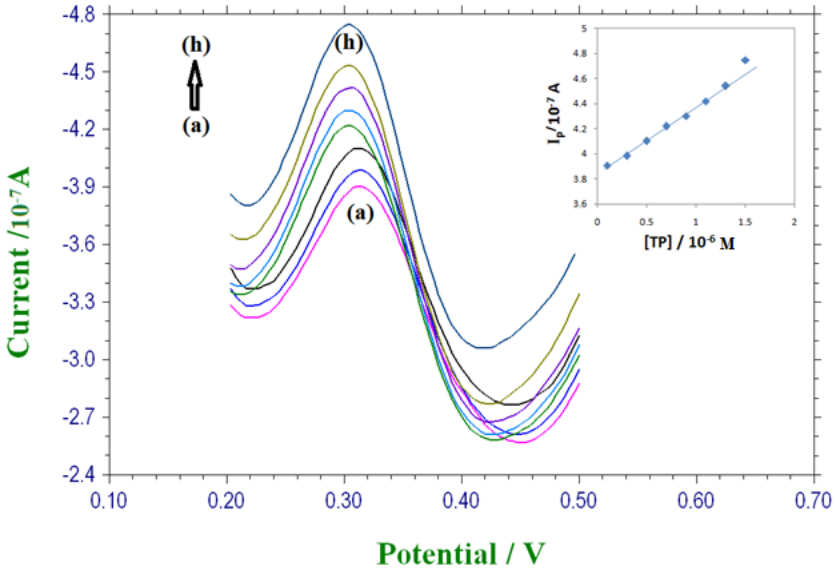


Fig. 5. Differential pulse voltammograms of GPE/CTAB in TP solution at different concentrations of (a) 0.1, (b) 0.3, (c) 0.5, (d) 0.7, (e) 9.0, (f) 1.1 (g) 1.3 and (h) 1.5 µM
Inset: Plot of peak current *vs*. concentration of TP. (Linearity range: 0.1 to 1.3 µM)

Differential pulse voltammograms obtained with increasing amounts of TP showed that the peak current increased linearly with increasing concentration, as shown in Fig. 5. It was found that the plot of i_p versus concentration showed linearity in the range of 1.0×10^{-7} to 1.3×10^{-6} M. The linear equation was i_p (10^{-7}A)=0.115+3.758C (r=0.985, C is in µM). Related statistical data of the calibration curves were obtained from the five different calibration

curves. Limit of detection (LOD) and quantification (LOQ) were calculated based on the peak current using the following equations shown below.

LOD=3 s/m; LOQ=10 s/m.

Where s is the standard deviation of the peak currents of the blank (five replicates), and m is the slope of the calibration curve.

The detection limits reported at different electrodes are tabulated in Table 1. The LOD, LOQ and linear range values were calculated to be 2.63×10^{-9} M, 8.77×10^{-9} M and 1.0×10^{-7} - 1.3×10^{-6} M respectively. Linearity range, sensitivity, selectivity, LOD and LOQ values calculated by the present method are better compared to the reported work [9-16].

Table 1. Comparison of some methods for the determination of TP with the proposed method

Analytical Methods	Linearity range (M)	Detection limit (M)	Ref.
Carbon paste electrode	8.0×10^{-7}-2.0×10^{-4}	1.85×10^{-7}	[9]
Xanthine oxidase electrode	1.0×10^{-6} – 5.0×10^{-5}	2.0×10^{-7}	[10]
Ampherometric enzyme oxidase electrode	-----	2.0×10^{-6}	[11]
Nefion/lead-ruthenium oxide pyrochlore chemically modified electrode	20×10^{-6} – 100×10^{-6}	1.0×10^{-7}	[12]
Multi-wall carbon nanotube modified glassy carbon electrode	3.0×10^{-7} – 1.0×10^{-5}	2.0×10^{-8}	[13]
Multi-wall carbon nanotube paste electrode	2.0×10^{-6} - 1.5×10^{-4}	1.9×10^{-8}	[14]
Phthalocyanine Particles Modified CPE	4.0×10^{-7}-1.0×10^{-4}	1.4×10^{-7}	[15]
Poly (AHNSA)/GCE	1.0×10^{-6}-1.0×10^{-4}	4.7×10^{-8}	[16]
GPE/CTAB	1.0×10^{-7}-1.3×10^{-6}	2.63×10^{-9}	Present work

3.7. Repeatability and reproducibility

In order to study the repeatability of the analysis, five measurements of 1.0 mM TP solution were taken using GPE/CTAB electrode at intervals of one hour. The RSD value of peak current was found to be 1.65%, which indicated that electrode has good repeatability. As to the reproducibility between days, it was similar to that of within day repeatability if all conditions were kept constant.

3.8. Interference

TP was formulated in single as well as multi-component tablets. The oxidation peaks of interferents should not appear where the peak corresponding to TP appears. So in order to

investigate the effect of co-formulated substances such as citric acid, dextrose, glucose, gum acacia, lactic acid, oxalic acid, starch and sucrose on the voltammetric response of TP, this study was made. Differential pulse voltammetric experiments were carried out for 1.0×10^{-5} M TP in the presence of 1.0 mM of each of the interferents. It was observed that 100 folds of citric acid, dextrose, glucose, gum acacia, lactic acid, oxalic acid, starch and sucrose did not interfere with the voltammetric signal of TP. Thus, the procedures were able to assay TP in the presence of interferents and hence it was considered specific.

3.9. Tablet analysis

In order to evaluate the applicability of the proposed method in the pharmaceutical sample analysis, a commercial medicinal sample containing TP i.e., Theo-Asthalin (100 mg per tablet) was used. Differential pulse voltammograms were then recorded under exactly identical conditions that were employed while recording differential-pulse voltammograms for plotting calibration plot. The results were found to be in good agreement with the content marked in the label (Table 2). The detected content was 98.20 mg per tablet with 98.20% recovery. Recovery studies were also carried out after the addition of known amount of the drug to various pre-analyzed formulations of TP. The recovery in the sample was found to be 98.00% with RSD 1.14%.

Table 2. Analysis and recovery studies of TP in tablets

	Theo-Asthalin [(a)]
Labeled claim (mg)	100.0
Amount found (mg)[(a)]	98.2
RSD (%)	1.03
Added (mg)	2.00
Found (mg)[(a)]	1.96
Recovered (%)	98.0
RSD (%)	1.14
Bias (%)	2.00

(a) Mean average of five determinations

3.10. Detection of TP in urine samples

The applicability of the proposed method for the determination of TP in biological fluid of human urine was attempted. Drug-free human urine samples, obtained from healthy volunteers, filtrated through a filter paper and stored frozen until the assay. The developed differential-pulse voltammetric method for the TP determination was applied to urine samples. The recoveries from urine were measured by spiking drug free urine with known

amounts of TP. The urine samples were diluted 100 times with the phosphate buffer solution before analysis without further pretreatments. A quantitative analysis can be carried out by adding the standard solution of TP into the detect system of urine sample. The details of such analysis are shown in Table 3. The calibration graph was used for the determination of spiked TP in urine samples. The detection results of four urine samples obtained are listed in Table 3. The recovery determined was in the range from 98.42% to 99.00% and the R.S.D. was 1.30%. Good recoveries of TP were achieved from these matrices, denoting that application of the proposed method to the analysis of TP in biological fluid could be easily assessed.

Table 3. Determination of TP in urine samples

Added (μM)	Found[(a)] (μM)	Recovery (%)	SD ± RSD (%)	Bias (%)
0.3	0.296	98.66	0.0141±0.62	1.33
0.7	0.689	98.42	0.0122±0.32	1.57
0.9	0.891	99.00	0.0096±0.11	1.00

(a) Mean average of five determinations

4. CONCLUSION

The electrochemical behavior of TP at GPE in the presence of CTAB was studied. The results indicated that, CTAB can adsorb at GPE surface via strong hydrophobic interaction and voltammetric responses of TP which was facilitated in the presence of CTAB. The proposed differential pulse voltammetric procedure can be used successfully to determine TP in tablet and urine samples. This method can be a good alternative for the analytical determination of TP, because it is simple, sensitive, fast, accurate, and inexpensive. Furthermore, the present method could possibly be adopted for pharmacokinetic studies as well as clinical and quality control laboratories.

Acknowledgements

One of the authors (PAM) thanks Department of Science and Technology, New Delhi for the award of INSPIRE fellowship.

REFERENCES

[1] M. R. Majidi, K. Asadpour-Zeynali, and B. Hafezi, Electrochim. Acta 54 (2009) 1119.
[2] C. Umesh, B. E. Kumara Swamy, O. Gilbert, M. Pandurangachar, S. Reddy, S.Sharath Shankar, and B. S. Sherigara, Chin. Chem. Lett. 21 (2010) 1490.
[3] P. A. Mitenko, and R. I. Ogilvie, N Eng. J. Med. 289 (1973) 600.
[4] G. Levy, and R. Koysooko, J. Pediatr. 86 (1975) 789.

[5] M. McKee, and R. J. Haggerty, N Engl. J. Med. 256 (1957) 956.
[6] C. G. Hector, C. O. Alejandro, and M. P. Arsenio, Anal. Chim. Acta 384 (1999) 95.
[7] B. Srdjenovic, V. D. Milic, N. Grujic, R. Injac, and Z. Lepojeric, J. Chromatogr. Sci. 46 (2008) 144.
[8] F. L. Coco, F. Lanuzza, G. Mical, and G. Cappellana, J. Chromatogr. Sci. 45 (2007) 273.
[9] R. N. Hegde, R. R. Hosamani, and S. T. Nandibewoor, Anal. Lett. 42 (2009) 2665.
[10] S. Miroslav, P. Andrea, M. Stanislav, and S. Jozef, Anal. Biochem. 285 (2000) 225.
[11] J. Wang, E. Dempsey, M. Ozsoz, and M. R. Smyth, Analyst 116 (1991) 997.
[12] J. M. Zen, T. Y. Yu, and Y. Shih, Talanta 50 (1999) 635.
[13] H. Z. Yuan, Z. L. Zhang, and D. W. Pang, J. Electroanal. Chem. 581 (2005) 303.
[14] S. J. Malode, N. P. Shetti, and S. T. Nandibewoor, Colloid. Surf. B 97 (2012) 1.
[15] Y. Gong-Jun, W. Kun, X. Jing-Juan, and C. Hong-Yuan, Anal. Lett. 37 (2005) 629.
[16] M. Amare, and S. Admassie, Bull. Chem. Soc. Ethiop. 26 (2012) 73.
[17] P. Manisankar, G. Selvanathan, and C. Vedhi, Talanta 68 (2006) 686.
[18] G. A. Angeles, S. C. Avendano, M. P. Pardave, A. R. Hern´andez, M. R. Romob, and M. T. R. Silva, Electrochim. Acta 53 (2008) 3013.
[19] C. Li, Colloid. Surf. B 55 (2007) 77.
[20] J. Zhao, F. Q. Zhao, Z. Y. Chen, and B. Z. Zheng, Electroanalysis 17 (2005) 1071.
[21] P. P. Xie, X. X. Chen, F. Wang, C. G. Hu, and S. S. Hu, Colloid. Surf. B 48 (2006) 17.
[22] Q. He, X. P. Dang, C. Hu, and S. S. Hu, Colloid. Surf. B 35 (2004) 93.
[23] G. D. Christian, and W. C. Purdy, J. Electroanal. Chem. 3 (1962) 363.
[24] B. Rezaei, and S. Damiri, Sens. Actuators B 134 (2008) 324.
[25] I. Martins, F. C. Cristiani, S. C. Larissa, A. Francisco, M. C. Letícia, and R. Susanne, Talanta 85 (2011) 1.
[26] D. K. Gosser, Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms, VCH, New York (1993).
[27] E. Swatz, and I. S. Krull, Analytical Method: Development and Validation, Marcel Dekker, New York (1997).