Electrochemical determination of Uric Acid in alkaline media on a Glassy Carbon electrode modified with poly (p-toluene sulfonic acid)

Jesny S.[a,b], Shalini Menon [b] and K. Girish Kumar [b*]
[a] Department of Applied Chemistry, Cochin University of Science and Technology, Kochi-22, Kerala, India.
*giri@cusat.ac.in, salini.smn@gmail.com
[b]Department of Chemistry, Sree Narayana College, Cherthala, Kerala, India. jesnysmail@gmail.com

Abstract

The determination of uric acid (UA) is of significance in clinical analysis, as its abnormal concentration in urine is indicative of gout, kidney stones etc. In the work presented, the electrochemical determination of uric acid is achieved on a poly (para toluene sulfonic acid) (p-PTSA) modified glassy carbon electrode (GCE). The experimental conditions for the sensor fabrication were optimised and the application of the fabricated sensor for the determination of UA in synthetic urine was studied.

Keywords: electrochemical determination, electro-oxidation, poly (p-toluene sulfonic acid) uric acid

1. Introduction

The heterocyclic purine derivative UA, is the principal oxidation product of purine metabolism in human beings. It is excreted through urine in a typical range of 250 mg/dl to 720 mg/dl and in serum it normally exists in the range 4.1 mg/dl - 8.8 mg/dl [1]. The concentration level of UA in body fluids serves as a biomarker for conditions like gout, hyperuricemia, Lesch-Nyan disease or cardiovascular abnormalities [2]. In this context there is a need for sensitive techniques for the determination of UA. The solubility of UA in water is very low but is highly soluble in NaOH. For this reason, here we describe the determination of UA in alkaline medium. Electrochemical methods are fast techniques which provide convenient and inexpensive determinations using miniature instruments [3]. Chemically modified electrodes with conducting polymers electropolymerised on to the electrode surface can accelerate electronic transmission through the electrode surface thereby offering high sensitivity and selectivity. In addition, electrochemical polymerisation ensures controllable and homogeneous formation of the conducting polymer which strongly adheres to the electrode surface [4].

In the work presented here, we describe a *p*-PTSA modified GCE (*p*-PTSA/GCE) being used for the determination of UA in alkaline medium.

EXPERIMENTAL

All the reagents used in the present study were of analytical grade and were used without any further purification. All the solutions were made in Millipore water.

CHI600C electrochemical analyser (CH Instruments Inc. USA) integrated to a desk top computer was used to perform the electrochemical experiments. A three electrode set up with Ag/AgCl reference electrode, Platinum wire auxiliary electrode and the GCE (3mm diameter) or *p*-PTSA/GCE as the working electrode was employed for the measurements. Metrohm pH meter was used to measure the pH of the solutions.

Preparation of *p*-PTSA/GCE

The GCE was mechanically polished with alumina slurries of powder size 0.05 µm to a mirror finish and ultra-sonicated successively in methanol, 50% nitric acid, acetone and water for 5 minutes each so as to remove any adsorbed alumina. The electropolymerisation of PTSA on GCE was performed by a method reported elsewhere [5]. The cleaned GCE was then

immersed in a 1 mM solution of PTSA in 0.1 M NaCl and potential scanned between -2.0 to +2.5 V for 28 segments at $0.1Vs^{-1}$. The modified electrode was washed with Millipore water and dried in air. Prior to voltammetric measurement, the electrode was activated by scanning the potential from -1.0 V to +1.0 V in 0.1 M NaOH solution.

RESULTS AND DISCUSSION

Square wave voltammetric response

A comparison of square wave voltammograms (SWV) of the bare and modified electrodes recorded in a solution of 0.1 M NaOH containing 300 µM of UA (Figure 1) shows that the electro oxidation of UA is catalysed by the PTSA film on GCE. In the bare GCE, oxidation peak for UA appear at 28 mV against Ag/AgCl electrode with peak current 0.743 µA, whereas on *p*-PTSA modified electrode the same appears at -108 mV with peak current 9.52 µA respectively.

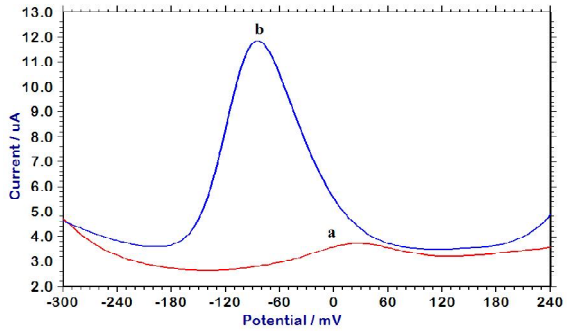


Figure 1. SWV response of 300 µM UA on **a**) bare GCE and **b**) *p*-PTSA/ GCE in 0.1 M NaOH.

Optimisation of Experimental conditions

The choice of supporting electrolyte is an experimental parameter that can influence the electro-oxidation process. The electrochemical oxidation of UA was studied in 0.1 M concentration of various supporting electrolytes such as sulfuric acid, hydrochloric acid, acetate buffer, citrate buffer, sodium chloride, potassium chloride, phosphate buffer, NaOH and potassium hydroxide was studied. The lowest oxidation potential was obtained for 0.1 M NaOH and hence it was selected as the supporting electrolyte.

The thickness of polymer film, best suited for the determination of UA was optimised by varying the number of cycles of polymerisation. The best result in 0.1 M NaOH was obtained when the number of cycles of polymerisation was 14 and hence further studies were conducted after the polymerising PTSA over GCE in 14 cycles.

Mechanistic study by varying scan rate

The variation of the oxidation peak parameters with scan rate (υ) in linear sweep voltammetric (LSV) mode was used to study the mechanism of electro-oxidation. The oxidation peak of UA showed a positive shift with increasing scan rate pointing to the irreversibility of oxidation process (Figure 2).

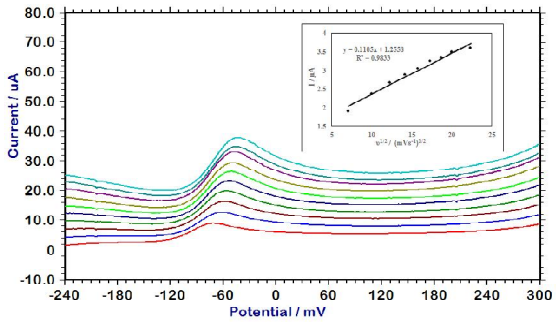


Figure 2. Overlay of LSV of 100 µM UA at scan rates from 50 mVs^{-1} to 500 mVs^{-1}. Inset shows the variation of peak current as a function of square root of scan rate.

The oxidation peak current was found to vary linearly with square root of scan rate in the range 50 -500 mVs^{-1} according to the following equation, Ip (µA) = + 1.25 + 0.11 $\upsilon^{1/2}(mV^{1/2}s^{-1/2})$ [$R^2 = 0.9833$], indicative of a diffusion controlled process [6].

Determination of UA

Square wave voltammetry was used for the determination of UA. The lower LOD for the assay was calculated using the following equation [7].

$$LOD = 3S/b \qquad 6$$

where S is the standard deviation of the lowest response and b is the slope of the calibration curve.

Table 1. Determination of UA in synthetic urine sample

Sample	Uric Acid		Recovery %	RSD %
	Added (µM)	Found (µM)		
Synthetic Urine	20	21.5	102.3	2.46
	40	41.0	102.5	
	50	50.2	100.2	
	70	69.9	99.8	
	100	96.4	96.4	

The change in peak current with concentration (Figure 3) for the electro oxidation of UA was found to be linear in the range 10 µM to 100 µM (Ip (µA) = - 1.14 + 0.09 C (µM) [R^2 = 0.9974]) and in the range 90 µM – 1500 µM (Ip (µA) = +6.34 + 0.02C (µM) [R^2 = 0.9941]) The LOD was calculated to be 3.31 µM.

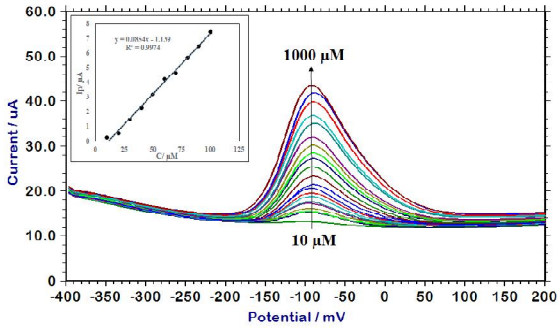


Figure 3. Overlay of SWV of 10 µM to 1000 µM UA at the *p*-PTSA/GCE. Inset shows the calibration curve from 10 µM -100 µM.

Application

The fabricated sensor was utilised for the determination of UA in synthetic urine. The results tabulated in the following table shows that the method gives good recovery values with a relative standard deviation (RSD) of 2.46 %. This indicates that the sensor can be used for the determination of UA in real samples.

CONCLUSIONS

An electrochemical sensor was developed for the determination of UA by electropolymerising PTSA on the surface of a GCE. The experimental conditions optimal for the working of the sensor were determined. The sensor gave best response in basic medium and the sensor was calibrated for determination of UA in basic medium. The developed sensor was successfully applied for the determination of UA in synthetic urine.

ACKNOWLEDGEMENTS

Authors K. Girish Kumar and Jesny S., would like to express their gratitude to University Grants Commission (UGC), Govt. of India for financial assistance in the form of 'One Time Research Grant' and Teacher Fellowship respectively. Shalini Menon would like to acknowledge Kerala State Council for Science, Technology and Environment (KSCSTE) for research fellowship.

REFERENCES

[1]. L. M. Niu, K. Q. Lian, H. M. Shi, Y. B. Wu, W. J. Kang, S. Y. Bi, *Sensors and Actuators B* 178 (2013) 10.

[2]. X. Liu, L. Zhang, S. Wei, S. Chen, X. Ou, Q. Lu, *Biosensors and Bioelectronics* 57 (2014) 232.

[3] D. Kato, N. Sekioka, A. Ueda , R Kurita, S. Hirono, K. Suzuki, O Niwa *Angew. Chem.Int. Ed.* 47 (2008) 6681.

[4] S. Hou, N. Zheng, H. Feng, X. Li *Analytical Biochemistry* 381 (2008) 179.

[5] S. Issac and K. Girish Kumar, *Anal. Methods* 2 (2010) 1484.

[6] A. Thomas, A.E. Vikraman, D. Thomas, K. Girish Kumar, *Food Anal. Methods* 8 (2015) 2028.

[7] M. U. A. Prathap, R. Srivastava, B. Satpathi, *Electrochim. Acta.* 114 (2013) 285.