Synergistic electrocatalytic Action Of Ploy (P-Toluene Sulphonic Acid) And Gold Nanoparticles Based Glassy Carbon Electrode for The Simultaneous Voltammetric Determination Of Xanthine And Hypoxanthine

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DOI: 10.3578/jecsi.68.3&4.2019.106

Abstract:

Xanthine (XA) and hypoxanthine (HX) are the purine bases which are the important intermediates in the purine metabolic pathway. They are mainly found in body tissues and fluids. Hence their abnormal levels are indicative of many clinical conditions such as xanthinuria, hyperuricemia, gout and renal failure. Thus their determination is important from the clinical diagnostic point of view. Electrochemical sensors offer rapid, reliable, less expensive, sensitive and selective simultaneous determination. In view of this, attempt has been made to develop a poly (para toluene sulfonic acid)/gold nanoparticles modified glassy carbon electrode (*p*PTSA /AuNPs /GCE) as a sensor for the simultaneous determination of XA and HX.

The experimental parameters for the simultaneous determination of XA and HX on *p*PTSA /AuNPs / GCE has been optimised. The lowest potential for the electro-oxidation of XA and HX was obtained in 0.1 M NaOH. The increase in peak current with concentration was linear for individual as well as simultaneous determination of these analytes. For simultaneous determination, peak current changes with concentration in the range 6-700 μM for XA and 40-600 μM for HX. The mechanistic and kinetic parameters were determined. The sensor was successfully applied for determination of XA and HX in artificial urine and serum.

Keywords: xanthine, hypoxanthine, poly (para toluene sulfonic acid), simultaneous determination, gold nanoparticles.

1. Introduction

Purines are heterocyclic aromatic bi cyclic compounds which are widely distributed in nature. Xanthines (XA) and hypoxanthines (HX) are intermediates in the purine metabolism and are found mainly in body tissues and fluids [1]. In purine metabolism, the biotransformation of hypoxanthine to xanthine takes place by the catalytic action of xanthine oxidase.

$$\text{Hypoxanthine} + O_2 \xrightarrow{\text{XOD}} \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + H_2O_2$$

Any change in the concentration of them are indicative of many clinical conditions including, xanthinuria, hyperurecemia, gout and renal failure [2]. Hence the determination of these purines is significant in clinical analysis. Also, in food industry, meat or fish freshness [2] can be evaluated by knowing the levels of xanthine and

hypoxanthine. So, development of an effective method for the simultaneous determination of xanthine and hypoxanthine is necessary.

Electrochemical sensors based on chemically modified electrodes offer a simple, economically viable and selective method for the determination of the electro-active purines [3]. In this article, we report a sensor for simultaneous determination of XA and HX, based on a synergistic effect of gold nanoparticles and poly (para toluene sulfonic acid) modified glassy carbon electrode.

2. Experimental

2.1 Chemicals and Instrumentation

All the chemicals used in the present study were of analytical grade and used without further purification. All chemicals used in the present study were of analytical grade. XA and HX were purchased from s. d. fine-chem limited and research-lab fine chem industries respectively. The monomer para toluene sulfonic acid was obtained from s. d. Fine Chemicals, India and gold chloride from Sigma Aldrich.

Electrochemical experiments were performed on CHI6023D electrochemical analyser (CH Instruments Inc. USA) integrated to a desk top computer. Voltammetric three electrode set up comprising of Ag/AgCl reference electrode, Platinum wire auxiliary electrode and glassy carbon working electrode was employed for the measurements.

2.2 Preparation of modified electrode

The p-TSA polymer film was deposited on a polished and mechanically cleaned GCE by cycling the potential between -2.0 V to +2.5 V in 1.0×10^{-3} M solution of the monomer in 0.1 M NaCl at a scan rate of 0.1 Vs^{-1} for 15 cycles [4]. After this step the electrode was washed with double distilled water and electrodeposition was carried out by potential sweeping between 0 to 1.3 V in $5.0 10^{-2}$ M H_2SO_4 solution containing $1.0 10^{-3}$ M gold chloride solution for 15 cycles at a scan rate of 0.1 Vs^{-1} and then dried in air [5]. Prior to use electro activation was carried out by sweeping the potential between -0.8 V to +1.0 V in PBS pH 7 until a stable voltammogram is obtained.

2.3 Analytical Procedure

10 mM stock solution of XA and HX was prepared in 0.1 M NaOH. Solutions of different concentrations were prepared by appropriate dilution of the stock solution with Millipore water. 10 mL of 0.1 M supporting electrolyte, containing a required concentration of XA and HX was taken in the voltammetric cell provided with a three-electrode set up. Square wave voltammograms (SWV) were recorded in the potential window 0.0V to 1.0V with pulse amplitude 0.015 V and pulse frequency 15 Hz at a pulse increment of 0.004 V using *p(*PTSA)/AuNPs/ GCE or GCE.

3. Results and Discussion

3.1 Optimisation of experimental conditions

Among the various supporting electrolytes studied (phosphate buffer, acetate buffer, citrate buffer, sulfuric acid, nitric acid, sodium chloride, potassium chloride, NaOH and potassium hydroxide) the lowest peak potentials were obtained with 0.1 M NaOH for both XA and HX. Hence all electro-oxidations were studied using 0.1 M NaOH as supporting electrolyte. The thickness of the modifier film was optimized and found to be 15 cycles for both p-TSA polymerisation and AuNPs deposition for both XA and HX. Hence all the studies were performed in the electrode fabricated by 15 cycles of p-TSA polymerisation and AuNPs electrodeposition.

3.2 Voltammetric behaviour of purines on the AuNPs/p(PTSA)/GCE

On the *p(*PTSA)/AuNPs/ GCE, well defined and well separated peaks were obtained for the oxidation of HA and HX, using 0.1 M NaOH as supporting electrolyte. Compared to the bare GCE, the oxidation of the purines on *p(*PTSA)/GCE took place at a lower potential with an enhanced peak current as can be seen from Fig. 1. After AuNPs deposition on *p(*PTSA)/GCE, further enhancement in peak current was observed (Fig. 1).

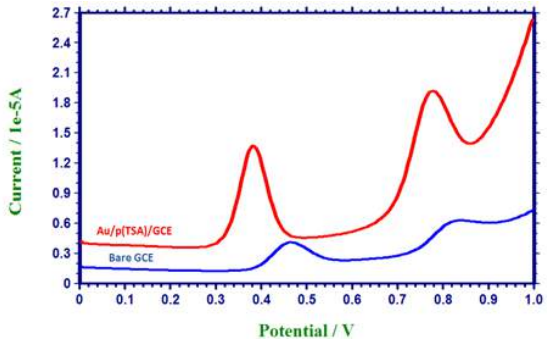
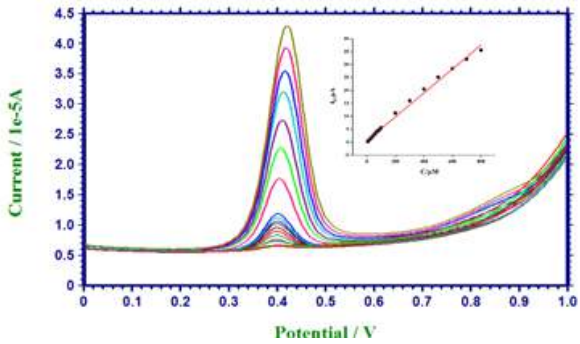


Figure 1: Overlay of SWV for the oxidation of 100 µM concentrations of XA and HX on (a) bare GCE and (b) *p*PTSA/AuNPs/ GCE in 0.1 M NaOH

Compared to bare electrode, a higher oxidation peak current was obtained for XA and HX and appears at lower potential on *p*PTSA/AuNPs/ GCE. For XA, the peak current varied linearly with concentration (Fig 2a) in the range 6 µM to 800 µM. For HX, the oxidation peak at 0.784 V, showed a linear increase of oxidation current with concentration (Fig 2b) in the range 30 µM to 500 µM.



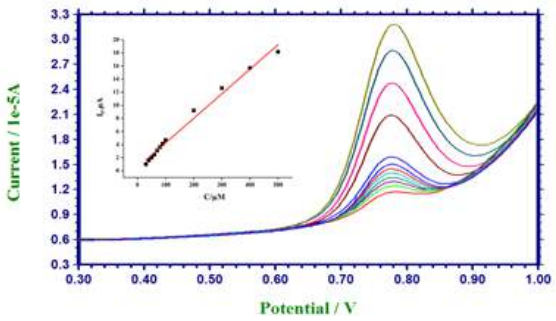


Figure 2: Overlay of SWV for the oxidation of a) 6-800 µM concentrations of XA b) 30-500 µM concentrations of HX

Simultaneous determination of XA and HX was also carried out using the *p*PTSA/AuNPs/ GCE. Overlay of square wave voltammograms obtained for the simultaneous determination of 6-600 µM and 40-600 µM concentrations of XA and HX is shown in Fig.3.

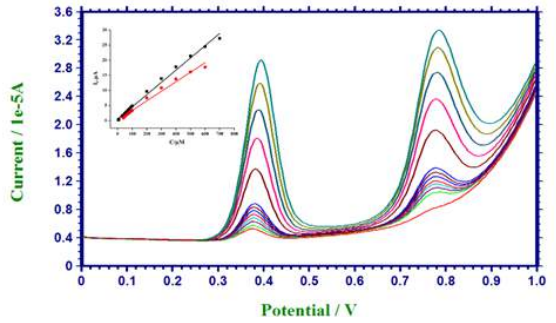


Figure 3: Overlay of SWV for the simultaneous oxidation of 6-600 µM and 40-600 µM concentrations of XA and HX on *p*PTSA/AuNPs/ GCE in 0.1 M NaOH

Table 1: Determination of XA and HX in spiked synthetic urine and blood serum

Analyte	Urine				Blood serum			
	Added (µM)	Found (µM)	Recovery	RSD* %	Added (µM)	Found (µM)	Recovery	RSD* %
XA	80 - 500	80 - 482	100 - 96	2.6	80 - 500	78 - 495	98 - 99	1.7
HX	80 - 500	82 - 482	103 – 96	3.4	80 - 300	81 - 292	100 - 97	2.5

**for 7 measurements*

3.3 Application study

The determination of XA and HX was also carried out in spiked synthetic urine and blood serum [6-7]. The results obtained are presented in Table 1 and the good recoveries obtained pointing to the applicability of the sensor in real sample analysis.

4. Conclusions

A *p*PTSA/AuNPs/ GCE has been fabricated and developed as a voltammetric sensor for the simultaneous as well as individual determination of XA and HX. The experimental conditions for optimal sensor performance has been determined and the sensor calibrated for individual as well as simultaneous determination of the analytes. The utility of the sensor for the determination of the purines in real samples has been demonstrated.

Acknowledgement

The author Manna Rachel Mathew would like to thank the University Grants Commission, government of India for financial assistance in the form of junior research fellowship.

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