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Sensitive Amperometric Determination of Sarcosine for Imminent Biosensor Application by Polymer Decorated Electrode

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Abstract—

The sensitive amperometric determination using an electropolymerized film of 3-amino-5-mercapto-1,2,4-triazole on glassy carbon (p-AMTa) electrode at physiological pH for the first time. Using amperometric method, detection of 400 nM of Sar was achieved. Further, the amperometric current response was increased linearly with increasing Sar concentration in the range of 4.0×10^{-7} to 1.0×10^{-4} M and a detection limit was found to be 2.2×10^{-9} M (S/N = 3). The modified electrode was used to determine Sar in the presence of 500-fold excess of interferents such as of $MgSO_4$, $CaCl_2$, NaF, NH_4Cl, $NaNO_3$, glucose, urea and oxalate. The practical application of the present modified electrode was demonstrated in human urine samples.

Keywords— Amperometry; polymer; Sarcosine; Biosensor; Human urine

I. INTRODUCTION

Sarcosine (*N*-methylglycine; Sar) is an α-amino acid and it is naturally found in muscles and other body tissues by the creatine metabolism [1, 2]. It is an important intermediate of one-carbon metabolism [3], a competitive inhibitor of the type I glycine transporter [4, 5] and a N-methyl-D-aspartate receptor coagonist [6]. Sarcosine (Sar) plays an important role for balancing the labile methyl group in human metabolism [7]. It was oxidized by D-amino acid oxidase to yield methylamine and glyoxylic acid [8]. It was associated with prefrontal cortex and spinal mediated anti-neuropathy and analgesia [9]. Sarcosinemia is a rare inborn metabolic disorder of amino acid and it is due to the increased Sar level of blood plasma and urine [10, 11]. Recently, Sar was identified as a potential marker for prostate cancer aggressiveness by significantly higher urinary Sar level and serum prostate specific antigen level elevated from 2 to 10 ng ml^{-1} [2].The Sar dosage (2g day^{-1}) was used in the treatment of Schizophrenia [12]. Hence, the accurate determination of Sar concentration is much essential from the human fluids. But, only limited methods were reported for the determination of Sar which include, HPLC-mass spectroscopy (MS) [13], LC-MS [14, 15] and fluorometry [16]. HPLC based methods are very tedious and more time consuming process while fluorometric method showed poor detection limits. However, electrochemical method has several advantages such as less expensive, more convenient and highly selective and sensitive.

In this work, the sensitive determination of 400 nM was achieved by amperometry using an electropolymerized film of 3-amino-5-mercapto-1,2,4-triazole modified glassy carbon (p-AMTa) electrode. Further, the amperometric current was linearly increases with increasing the Sar concentration from 4.0×10^{-7} to 1.0×10^{-4} M and a detection limit was found to be 2.2×10^{-9} M (S/N = 3). Generally, pH of the human fluids like blood and urine is ~7 [17] and hence I have chosen the physiological pH for the clinical purposes.

II. EXPERIMENTAL

A. Chemicals

3-amino-5-mercapto-1,2,4-triazole (AMTa), sarcosine (Sar), ascorbic acid (AA) and uric acid (UA) were purchased from Aldrich and were used as received. All other chemicals used in this investigation were of analytical grade. pH 7.2 phosphate buffer (PB) solution was prepared using Na_2HPO_4 and NaH_2PO_4. Double distilled water was used to prepare the solutions used in this investigation.

B. Instruments

Electrochemical measurements were performed in a conventional two compartment three electrode cell with a mirror polished 3 mm GC electrode as a working electrode, Pt wire as a counter electrode and a NaCl saturated Ag/AgCl as a reference electrode. All the electrochemical measurements were carried out with CHI model Electrochemical Workstation. For amperometry measurements, +1.2 V was used for applying potential to determine Sar. All the electrochemical measurements were carried out under nitrogen atmosphere. The tapping mode AFM images were recorded using a Nanoscope (IV) instrument (Vecco).

C. Fabrication of p-AMTa modified GC electrode

The GC working electrode was polished with alumina slurry and then rinsed thoroughly with water. The electropolymerization of AMTa on GC electrode by 15 successive potential sweeps between -0.20 V to +1.70 V at a scan rate of 50 mV s^{-1} in 1 mM AMTa containing 0.1 M H_2SO_4 [20].

III. RESULTS AND DISCUSSION

A. Atomic Force Microscopy (AFM) studies of p-AMTa film.

The morphology of the working electrode surface was investigated by AFM. Fig. 1 shows the tapping mode AFM 2-D (Fig. 1A) and 3-D (Fig. 1B) images of p-AMTa film. It shows uniformly deposited film with spherical like structure. The diameter of each particle was found to be 20-70 nm.

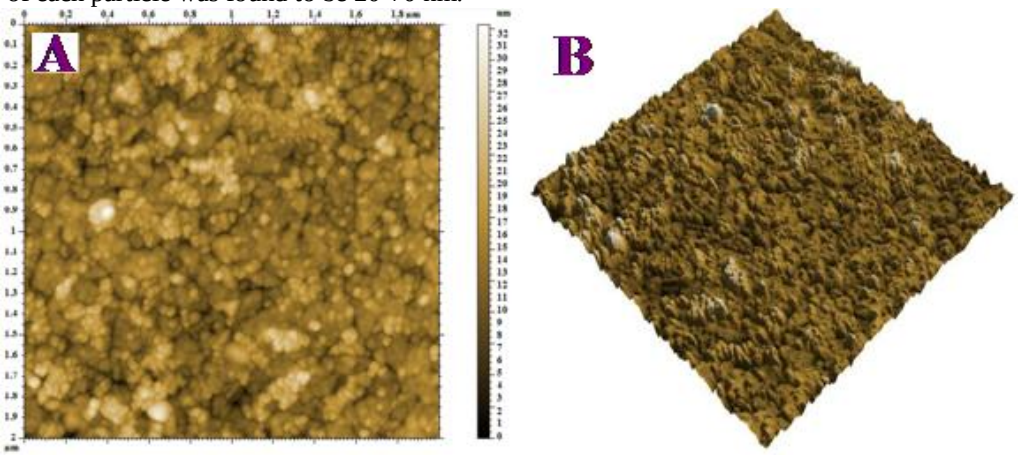

Fig. 1 Tapping mode AFM (A) 2-D and (B) 3-D images of the p-AMTa film.

B. Amperometric determination of Sar

Amperometric method was used to examine the sensitivity of p-AMTa electrode towards the detection of Sar. Fig. 2A shows the amperometric *i-t* curve for Sar at p-AMTa electrode in a homogenously stirred 0.2 M PB solution (pH 7.2) by applying the potential of +1.2 V. The p-AMTa electrode shows the current response for each addition of 400 nM Sar in every 50 s. The current response increases and the steady state current response was attained within 3 sec for further addition of 400 nM Sar in each step with a sample interval of 50 s. The dependence of response current with respect to concentration of Sar was linear from 400 nM to 4000 nM at p-AMTa electrode with a correlation coefficient of 0.9997 (inset of Fig. 2A). The amperometric *i-t* curve for each addition of 400 nM Sar showed linear current increase without noise.

Further, I have also studied the detection of Sar in a wide range of concentrations. The amperometric current was increased linearly with increasing concentration of Sar from 4.0×10^{-7} to 1×10^{-4} M at p-AMTa electrode (Fig. 2B) with a correlation coefficient of 0.9998 (inset of Fig. 2B) by applying a potential of +1.2 V. The detection limit was found to be 2.2×10^{-9} M (S/N = 3) for Sar.

C. Anti-interference ability of the p-AMTa electrode

Fig. 3 shows the anti-interference ability of the p-AMTa electrode was tested towards the detection of Sar from various common ions such as Mg^{2+}, Ca^{2+}, Na^{+}, NH_4^{+} SO_4^{2-}, Cl^{-}, F^{-}, NO_3^{-} and some physiological interferents such as glucose, urea and oxalate by interval of 50 s. No change in the amperometric current response was observed for 400 nM Sar in the presence of 200 µM of $MgSO_4$, $CaCl_2$, NaF, NH_4Cl, $NaNO_3$, glucose, urea and oxalate indicating that the present p-AMTa electrode is highly selective towards Sar in the presence of 500-fold excess of these interferents.

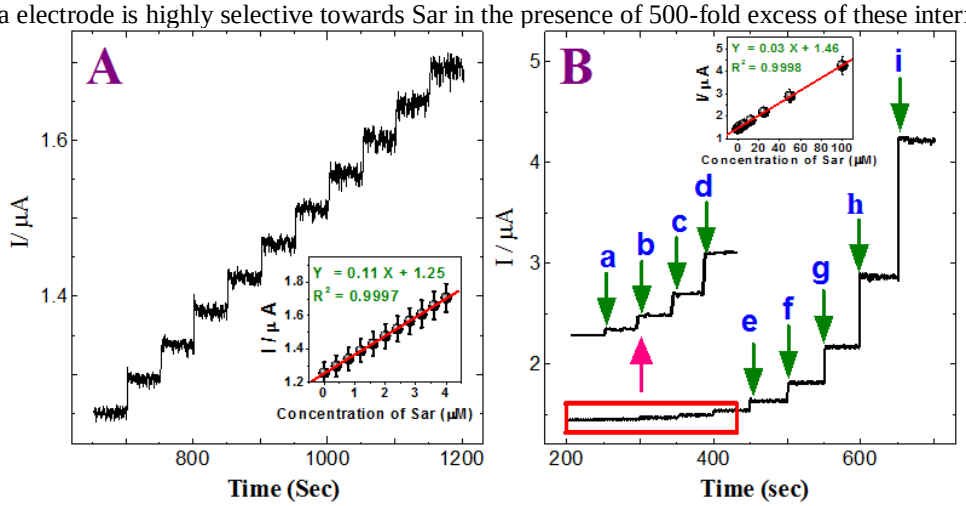

Fig. 2 (A) Amperometric i-t curve for the determination of Sar at p-AMTa electrode in 0.2 M PB solution (pH 7.2). Each addition increases the concentration of 400 nM of Sar at regular interval of 50 s. E_{app} = +1.2 V. **Inset:** Plot of concentration of Sar vs. current. **(B)** Amperometric i-t curve for the increment of (a) 0.4 (b) 0.8 (c) 1.5 (d) 3 (e) 6 (f) 12.5 (g) 25 (h) 50 and (i) 100 µM Sar at p-AMTa electrode in 0.2 M PB solution (pH 7.2) at a regular interval of 50 s. E_{app} = +1.2 V. **Inset:** Plot of concentration of Sar vs. current.

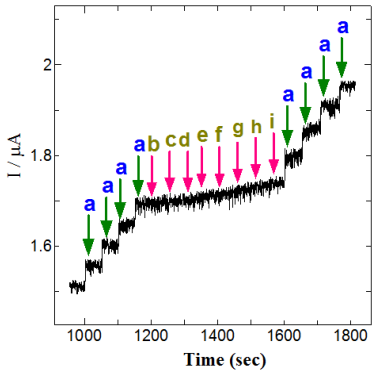

Fig. 3 Amperometric i-t curve for the determination of (a) 400 nM Sar in the presence of various interferents such as 200 µM each (b) $MgSO_4$ (c) $CaCl_2$ (d) NaF (e) NH_4Cl (f) $NaNO_3$ (g) glucose (h) urea and (i) oxalate at pAMTa electrode in 0.2 M PB solution (pH 7.2). Each addition increases the concentration of 400 nM of Sar at regular interval of 50 s. E_{app} = +1.2 V.

D. Stability and reproducibility of the p-AMTa electrode

In order to investigate the stability of the p-AMTa electrode, the amperometry obtained for 0.5 mM Sar in 0.2 M PB solution were recorded for every 5 min interval. It was found that oxidation peak current remained same with a relative standard deviation of 6.90% for 10 times repetitive measurements indicating that this electrode has a good reproducibility.

E. Determination of Sar in human urine samples

The practical application of the p-AMTa electrode was tested by measuring the concentration of Sar in human urine samples. I was collected my urine samples and diluted to 10 times with 0.2 M PB solution (pH 7.2) without any treatment. The recovery experiments were carried out by standard addition method. The clear amperometric response of Sar was obtained for 150 µM of commercial Sar was spiked with urine sample. The obtained recovery of Sar was summarized in Table 1. The proposed method showed the better recoveries for spiked Sar in urine samples, indicating the present system could be applied for the determination of Sar.

Table 1 Determination and recovery test of Sar in human urine.

Urine	Spiked (µM)	Found (µM)	Recoveries (%)
Sample 1	150	149.20	99.47
	200	197.70	98.85
	250	248.02	99.21
Sample 2	150	148.98	99.32
	200	197.58	98.79
	250	246.57	98.63

IV. CONCLUSION

In this paper, the sensitive amperometric determination at physiological pH was reported using an electropolymerized film of AMTa modified electrode in PB solution at physiological pH for the first time. The sensitive detection of 400 nM Sar was achieved at the p-AMTa electrode by amperometry method. The amperometric current response was increased linearly with increasing Sar concentration in the linear range of 4.0×10^{-7}-1×10^{-4} M and the detection limit was found to be 2.2×10^{-9} M (S/N = 3). The practical application of the present method was demonstrated by measuring the concentration of Sar in human urine samples.

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Dr. S. BRILLIANS REVIN had worked as a **Post-doctoral Research Scientist** in the **Chemistry department** (Institute of Bio-Physio Sensors Technology (IBST), **Nanobio Electrochemistry & Sensors Lab**) at **Pusan National University, SOUTH KOREA** in the period of **2012-2014.** He had worked in the lab of ***Prof. Yoon-Bo Shim (Associate Editor*** *in the Journal of '****Electroanalysis****',* ***Wiley Publishing group, Professor at Pusan National University***, ***SOUTH KOREA***). Currently, He is working as an Assistant Professor, Department of Chemistry, Francis Xavier Engineering College, Scad Group of Institutions, Tirunelveli, Tamilnadu, India. He pursued M.Sc degree (Chemistry) from University of Madras (Guindy Campus, Chennai, India) in 2007 and Ph.D (Chemistry) from Gandhigram Rural Institute (Central Government Institute, India) in 2012. **He has published 14 articles in International Journals (ISSN, Annexure-I,** ISI journals**). The average impact factor of his published Journals is 4 and the cumulative impact factor is 55.62.** At the present time, **his published articles have 130 citations, 7 h-index and 6 i-10 index. Moreover, his research articles were cited by many books, research & educational related websites for their studies and research investigations.** He has presented papers in 10 conferences and also attended many Faculty development programs (FDPs) under AICTE. He is working as a reviewer in International Journals and also working as Technical consultant in Dynamic Scientific Research and Development (DSRT) (http://dsrt.in/technical-management). He has handled numerous research instruments which are related to chemistry. Currently, he has two years teaching experience under the Anna University affiliated college (AICTE). **His research areas include synthesis of different size and shape of different metal nanoparticles, nano-composites, graphene and conducting polymers. He has exposure to technologies associated with electrode modification for the designing of biosensors, super capacitors, batteries, corrosions, solar cells and fuels.**

