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Discerning Sarcosine Biosensor in the Presence of Elevated Concentrations of Ascorbic Acid and Uric Acid

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A B S T R A C T

The electrochemical response of Sarcosine (Sar) in the presence of elevated concentrations of Ascorbic acid (AA) and Uric acid (UA) is reported by differential pulse voltammetry (DPV) using an electro-polymerized film of 3-amino-5-mercapto-1,2,4-triazole on glassy carbon (p-AMTa) electrode for the first time. Bare glassy carbon (GC) electrode fails to show voltammetric signal for Sar. However, p-AMTa electrode shows a clear voltammetric signal for Sar at 0.96 V. Further, p-AMTa electrode separates the voltammetric signals of AA, UA and Sar in a mixture with the potential differences of 200 mV for AA-UA and 680 mV for UA-Sar. The selective determination of Sar even in the presence of 40-fold high concentrations of AA and UA was successfully demonstrated in this work.

Keywords: Polymer; Sarcosine; Biosensor; Ascorbic acid; Uric acid

A R T I C L E I N F O

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1. Introduction

It is well known that Sarcosine (Sar) is one of the α-amino acids and it is found in both human and animal tissues [1, 2]. The elevated concentration of Sar in human fluids such as blood plasma and urine leads to Sarcosinemia [3, 4].

Further, Sar is also recognized as a marker for prostate cancer [2] and leads to many diseases [5]. Since Ascorbic acid (AA) and Uric acid (UA) exist in human fluids, AA and UA are the major interferents when we determine Sar from human fluids for clinical purposes. AA concentration in normal plasma range is 50-150 µM [6] and UA concentration in normal serum range is 240-520 µM [7]. However, the Sar level is ~76.8 ng ml^{-1} in human serum, ~138.5 ng ml^{-1} in men urine and ~94.8 ng ml^{-1} in women urine was observed [8]. Hence, it is clearly proved that the AA and UA concentrations present in human fluids are much higher than the Sar concentration. Even though various methods were attempted to determine Sar [8-11], no one method was reported for the mixture determination of AA, UA and Sar. Commonly, the electro-chemical method has several advantages such as less expensive, more convenient and highly selective and sensitive over other methods. In general, human fluids pH range is 7 [12]. By consider the above factors, I wish to attempt the selective determination of Sar in the presence of AA and UA at physiological pH. Since I had successfully reported biosensor works by an electro-polymerized film of 3-amino-5-mercapto-1,2,4-triazole on glassy carbon (p-AMTa) electrode [13-21], I targeted the surface to do this sensor work. By this work, the selective determination of Sar was demonstrated even in the presence of high concentrations of AA and UA.

2. Materials and Methods

Chemicals: Sarcosine (Sar), ascorbic acid (AA), uric acid (UA) and 3-amino-5-mercapto-1,2,4-triazole (AMTa), 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.

Instruments: Electrochemical measurements were carried out 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 differential pulse voltammetry (DPV) measurements, pulse width of 0.06 s, amplitude of 0.05 V, sample period of 0.02 s and pulse period of 0.20s were used. All the electrochemical measurements were carried out under nitrogen atmosphere. The tapping mode AFM images were recorded using a Nanoscope (IV) instrument (Vecco).

Preparation of p-AMTa modified GC electrode:

The GC working electrode was polished with alumina slurry and then rinsed thoroughly with water. The electro-polymerization 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 [22].

3. Results and Discussion

Morphology of working electrode surface:

The morphology of the working electrode surface was investigated by AFM. It shows uniformly deposited film with spherical like structure. The diameter of each particle was found to be 20-70 nm [22].

Electrochemical response of Sar at working electrode:

I have optimized the electrocatalytic activity of p-AMTa electrode towards Sar with respect to deposition cycles and pH. I found that the p-AMTa film deposited by 15 cycles on glassy carbon (GC) electrode showed higher electrocatalytic activity towards Sar than the films deposited by more than 15 cycles. Further, I have performed the oxidation of Sar at different pH using p-AMTa electrode. I have obtained the higher oxidation current for Sar at pH 7.2. Thus, p-AMTa film deposited by 15 cycles and 0.2 M PB solution (pH 7.2) were chosen for the determination of Sar. Figure 1 shows the differential pulse voltammograms (DPVs) obtained for 0.5 mM Sar at bare GC and p-AMTa electrodes in 0.2 M PB solution at pH 7.2. Bare GC electrode fails to show the voltammetric signal of Sar (curve a) and hence, this electrode was not suitable to determine Sar. However, a clear oxidation peak was observed at 0.96 V for Sar at p-AMTa electrode in the first cycle (curve b). In the subsequent cycles, the oxidation of Sar was slightly shifted (~10 mV) to more positive potential with decreased peak current (curve c). In the negative scan between the potential window of 1.2 to 0 V, no electrochemical response was observed for Sar at p-AMTa electrode. Hence, the reaction of Sar is an irreversible process at p-AMTa electrode. The p-AMTa electrode does not show any redox peak in the absence of Sar in 0.2 M PB solution at pH 7.2 (curve d).

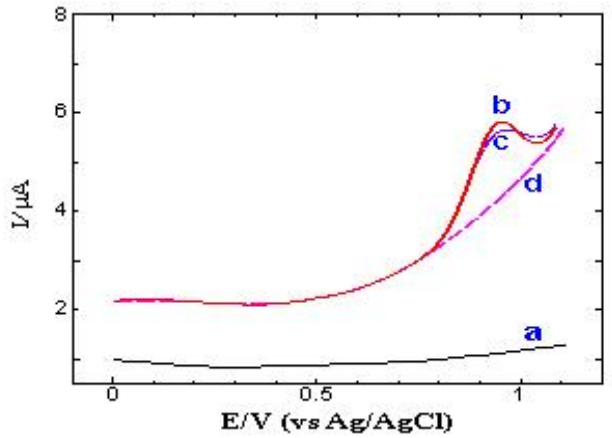

Figure 1: DPVs obtained for 0.5 mM Sar at (a) bare GC electrode and p-AMTa electrode (b) 1st cycle and (c) 4th cycle in 0.2 M PB solution (pH 7.2). DPV obtained for the absence of Sar (d) at p-AMTa electrode in 0.2 M PB solution (pH 7.2).

Electrochemical behavior of AA, UA and Sar in a mixture at working electrode:

Since AA and UA coexist with Sar in human fluids [6-8], it is essential to determine selectively Sar in the presence of AA and UA in a mixture. Figure 2 displays the DPVs obtained for a mixture of 0.5 mM each AA, UA and Sar at bare GC and p-AMTa electrodes in 0.2 M PB solution (pH 7.2). Bare GC electrode shows only one oxidation peak at 0.53 V and absence of voltammetric signal of Sar (curve a). It is well known that AA and UA peaks are merged together

to give a single oxidation peak (0.53 V) at bare GC electrode in 0.2 M PB solution [23].

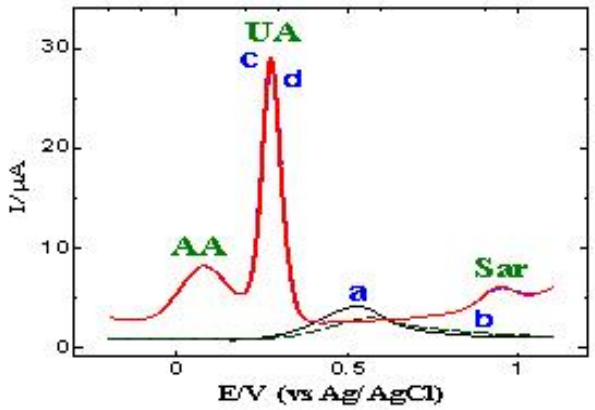

Figure 2: DPVs obtained for 0.5 mM each AA, UA and Sar at bare GC and p-AMTa electrodes 1st cycles (a and c) 4th cycles (b and d) in 0.2 M PB solution (pH 7.2).

In the subsequent cycles, the obtained voltammetric signal also shifted to more positive potential with decreased current (curve b). This imply the understanding of inappropriateness of bare GC electrode towards the mixture determination of AA, UA and. However, p-AMTa electrode clearly separates the oxidation peaks of AA, UA and Sar with a peak separation of 200 mV between AA-UA and 670 mV between UA-Sar (curve c). The oxidation potentials were observed at 0.08 V for AA, 0.28 V for UA and 0.96 V for Sar. In the subsequent cycles, the UA and Sar oxidation potentials were slightly shifted to more positive potential with small decreased peak current (curve d).

The obtained huge oxidation potential difference between UA-Sar was more than enough for the selective determination of Sar in the presence of AA and UA. It is well known that AA [24, 25] and UA [25] exist as anionic forms at pH 7.2. It is expected that the positively charged back bone of the p-AMTa film [20] was electrostatically attract the negatively charged AA (Figure 3A) and UA (Figure 3B). Generally, amino acids exist as zwitterion form around pH ~7.2 [26-28] and hence the positively charged back bone of the p-AMTa film [22] was electrostatically attract the negatively charged carboxyl group of Sar (Figure 3C). Further, the present polymer film contains -NH- group in the heterocyclic ring and it is expected the strong interactions of AA, UA and Sar with -NH- of the p-AMTa film are possible via hydrogen bonding (Figures 3A-3C) [29-31]. These are the possible reasons for the enhanced oxidation peak currents of AA, UA and Sar with huge peak separations between AA-UA and UA-Sar at p-AMTa electrode.

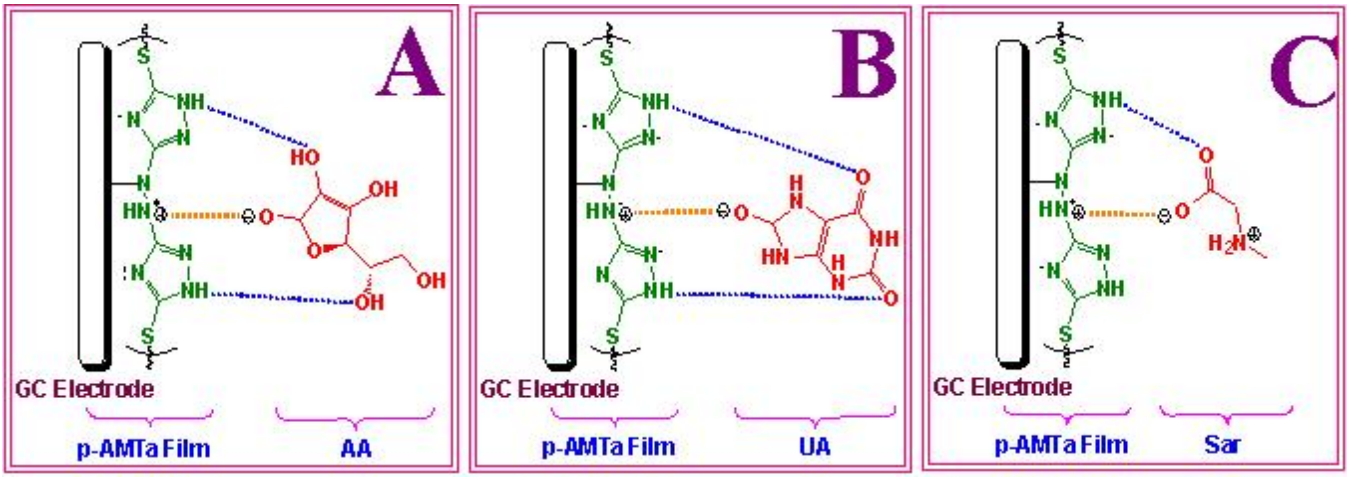

Figure 3: Schematic representation for the possible hydrogen bonding and electrostatic attractions between pAMTa film and AA (A), UA (B) and Sar (C).

Selective determination of Sar in the presence high concentrations of AA and UA:

AA and UA coexist with Sar in human fluids and further their concentrations are much higher than the Sar concentration [6-8]. Hence, the selective determination of Sar in the presence of high concentrations of AA and UA is essential for the clinical point of view. The obtained large peak separation between AA-Sar (870 mV) and UA-Sar (680 mV) was encouraged us to determine Sar selectively using p-AMTa electrode at physiological pH. Figure 4 shows the DPVs obtained for 50 µM Sar in the presence of 2 mM each AA and UA in PB solution at pH 7.2. A clear voltammetric signal was observed for 50 µM Sar even in the presence of 40-fold higher concentrations of each AA and UA (curve a), which revealed that the detection of low concentration of Sar is possible even in the presence of high concentrations of AA and UA. While adding 50 µM Sar increment to 2 mM each AA and UA, the oxidation current of Sar was increased linearly (curves a-f) with a correlation coefficient of 0.9997 (inset of Figure 4). Hence, p-AMTa electrode can be used to determine Sar even in the presence of high concentrations of AA and UA. By this method, we can simply detect Sar concentration in human fluids for bio-sensing application.

Stability and reproducibility tests:

In order to investigate the stability of the p-AMTa electrode, the CVs obtained for 0.5 mM Sar in 0.2 M PB solution were recorded for regular time interval. It was

found that oxidation peak current remained same with a relative standard deviation of 6.95% for 10 times repetitive measurements indicating that this electrode has a good reproducibility. To ascertain the reproducibility of the results, three different GC electrodes were modified with the p-AMTa film and their responses towards a mixture of 0.5 mM each AA, UA and Sar was recorded by 10 repeated measurements. The separation between the voltammetric peaks of AA-UA and UA-Sar was the same at all the three electrodes. The peak current obtained in the 10 repeated measurements of three independent electrodes showed a relative standard deviation of 5.75%, confirming that the results are reproducible. The above results showed that the present modified electrode was very much stable and reproducible towards these analytes.

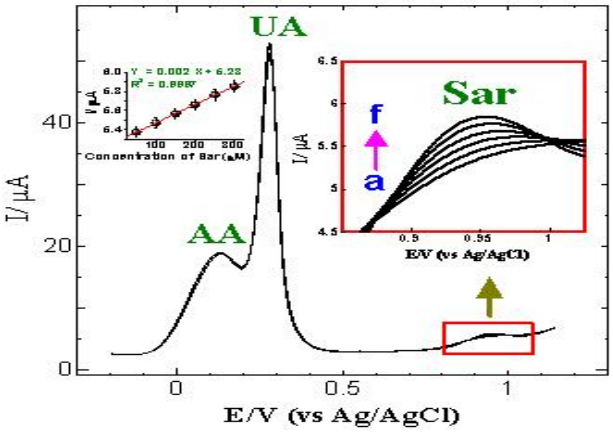

Figure 4: DPVs obtained for the each increment of 50 µM Sar to 2 mM AA and UA (curves a-f) at p-AMTa electrode in 0.2 M PB solution (pH 7.2). Inset: Plot of concentration of Sar vs. current.

4. Conclusion

In this paper, the voltammetric signal of Sar at physiological pH was reported using an electro-polymerized film of AMTa modified electrode for the first time. Bare GC electrode failed to show the voltammetric signal of Sar. However, p-AMTa electrode showed a voltammetric signal for Sar oxidation at 0.96 V in DPV. This is due to the strong attraction between positively charged back bone of the p-AMTa film and negatively charged carboxyl group of Sar and also the formation of hydrogen bonding between heterocyclic -NH- group of the p-AMTa film with Sar. The working electrode successfully resolved the voltammetric signals of AA, UA and Sar with potential differences of 200 mV between AA-UA and 680 mV between UA-Sar. The modified electrode exhibited an excellent sensitivity and selectivity towards Sar even in the presence of 40-fold higher concentrations of AA and UA. By this electrode, we can determine the concentration of Sar in human fluids for imminent medical application.

5. Acknowledgment

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