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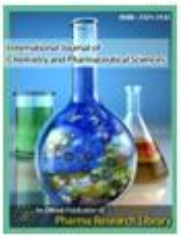

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Amperometric sensor of Cytosine by heterocyclic polymer fabricated glassy carbon electrode

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A B S T R A C T

The electrochemical response for one of the important nucleobases, cytosine (Cyt) is investigated by differential pulse voltammetry (DPV) using an electropolymerized film of 3-amino-5-mercapto-1,2,4-triazole modified glassy carbon (p-AMTa) electrode. Unlike the bare glassy carbon (GC) electrode, p-AMTa electrode shows a well resolved and stable voltammetric signal for Cyt at +1.3 V. In this work, we have achieved a detection of 400 nM of Cyt by amperometry method. Further, the amperometric current response is increased linearly with increasing Cyt concentration in the range of 5.0×10^{-8} to 1.0×10^{-4} M and a detection limit is found to be 2.22×10^{-10} M (S/N = 3). The anti-interference ability of the electrode was also tested with higher concentrations of important interferences.

Keywords: Cytosine, Biosensor, Amperometry, Polymer, Glassy carbon electrode

A R T I C L E I N F O

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1. Introduction

It is well known that adenine, guanine, thymine and cytosine (Cyt) are the nucleobases which exist in both human and animal tissues. Cyt is one of the nucleobases and it was found in the human DNA. The elevated concentration of Cyt in DNA leads to various diseases related to gene mutation. For example, Cyt engage in recreation in DNA binding discrimination of the murine DNA [1], Cyt discrimination in T4 DNA ligase-based single nucleotide polymorphism analysis [2], DNA glycosylases on spontaneous mutation [3] and oxidative DNA damage and disease [4]. Hence, the determination of Cyt is very essential to avoid the various mutations and diseases. Even though many methods are available to find the nucleobases, the electrochemical method has several advantages such as less expensive, more convenient and highly selective and sensitive over other methods. Hence, we wish to attempt the electrochemical determination of one of the important nucleobases of Cyt. Previously, we had reported various important biomolecules by an electropolymerized film of 3-amino-5-mercapto-1,2,4-triazole on glassy carbon (p-AMTa) electrode [5-14]. Those results encouraged us to focus Cyt determination by the same electrode. The sensitive determination of Cyt was demonstrated by differential pulse voltammetry and amperometry methods.

2. Materials and Methods

Chemicals used:

Cytosine (Cyt) and 3-amino-5-mercapto-1,2,4-triazole (AMTa), were purchased from Aldrich and were used as received. 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. All other chemicals used in this investigation were of analytical grade.

Instruments used:

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, NaCl saturated Ag/AgCl as a reference electrode and a Pt wire as a counter electrode. All the electrochemical measurements were carried out with CHI model Electrochemical Workstation. For differential pulse voltammetric (DPV) measurements, pulse width of 0.06 s, amplitude of 0.05 V, sample period of 0.02 s and pulse period of 0.20 s were used. All the electrochemical measurements were carried out under nitrogen atmosphere.

Preparation of p-AMTa film modified GC electrode:

The glassy carbon (GC) working electrode was polished with alumina slurry and further the electrode was rinsed thoroughly with water. The electropolymerization of AMTa on GC electrode by 15 successive potential cycles 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 [15].

3. Results and Discussion

Morphological studies: The electrode surface was investigated by atomic force microscopy. It shows uniformly deposited film with spherical like structure. The sphere shape particles were found to be 20-70 nm in size.

Differential Pulse Voltammogram response of Cyt at modified glassy carbon electrode: The DPV technique was used to optimize the electrocatalytic activity of p-AMTa electrode towards Cyt with respect to deposition cycles and pH. We observed that the p-AMTa film deposited by 15 cycles on GC electrode showed higher electrocatalytic activity towards Cyt than more than 15 cycles. Further, we have performed the oxidation of Cyt at different pH using p-AMTa electrode. Since we have obtained the higher oxidation current for Cyt at pH 7.2 and 15 deposition cycles in 0.2 M PB solution, we have chosen the same condition for further investigations. Moreover, pH 7.2 is a physiological pH and hence we can apply this benefit to real sample applications.

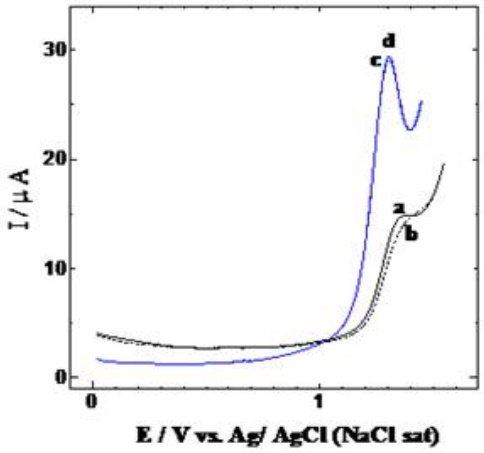

Figure 1: DPVs obtained for 0.5 mM Cyt at bare GC electrode and p-AMTa electrode 1st cycles (a, c) and 8th cycles (b, d), respectively in 0.2 M PB solution (pH 7.2).

Figure 1 shows the differential pulse voltammograms (DPVs) obtained for 0.5 mM Cyt at bare GC and p-AMTa electrodes in 0.2 M PB solution (pH 7.2). Bare GC electrode show a broad voltammetric signal of Cyt at 1.36 V (curve a) and further that peak also lost it shape. As you can see in the 8th cycle of the bare electrode (curve b) is indicating the shape. On the other hand, a clear oxidation peak was observed at 1.3 V for Cyt at p-AMTa electrode in the first cycle (curve c) which is 0.6 V less positive potential side than bare GC electrode. For the stability purpose, we have recorded DPV at different cycles. Surprisingly, trifling change was observed in the 8th cycle at p-AMTa electrode (curve d).

Type of the oxidation process:

The effect of scan rate (v) for Cyt was studied at p-AMTa electrode to understand the oxidation process involving in the reaction. The cyclic voltammograms obtained for Cyt at scan rates from 50 to 1000 mV s^{-1} at regular interval in 0.2 M PB solution (pH 7.2) are shown in Figure 2A. A very good linearity was noticed while we plotting the current against square root of scan rate with a correlation coefficient of 0.9997 (Figure.2B), indicating that the oxidation of Cyt was diffusion controlled process.

Amperometric determination of Cyt:

The core objective of this study is the sensitive amperometric investigation of Cyt at p-AMTa electrode. Figure 3A shows the amperometric *i-t* curve for Cyt at p-AMTa electrode in a homogenously stirred 0.2 M PB solution (pH 7.2) by applying the potential of +1.35 V. The electrode shows the current response for each addition of 400 nM Cyt in every 50 s. For the each addition, the current response was increases and the steady state current was attained within 3 sec for every 400 nM Cyt with a sample interval of 50 s. The dependence of current response with respect to concentration of Cyt was linear from 400 nM to 5200 nM at modified electrode with a correlation coefficient of 0.9964 (Figure 3B). We have observed 0.38 µA current response for each addition of 400 nM of Cyt by amperometry.

Further, we have also attempted the detection of Cyt in a broad range of concentrations. The amperometric current of Cyt was increased linearly with increasing concentration from 5.0×10^{-8} to 1×10^{-4} M at p-AMTa electrode (Figure 4A) with a correlation coefficient of 0.9902 (Figure 4B) by applying a potential of +1.35 V. The detection limit was calculated to be 2.22×10^{-10} M by S/N = 3 for Cyt at p-AMTa electrode.

Anti-interference ability of the p-AMTa electrode by amperometry: The anti-interference ability of the p-AMTa electrode was studied for Cyt detection with the exist of various common ions such as Mg^{2+}, Ca^{2+}, Cl^-, Na^+, NH_4^+, F^-, NO_3^- SO_4^{2-} and some physiological interferents such as oxalate and urea by interval of 50 s. There was no change in the amperometric current response observed for 400 nM Cyt in the presence of 40 µM of $CaCl_2$, $MgSO_4$, NaF, NH_4Cl, $NaNO_3$, oxalate and urea indicating that the modified electrode is highly selective towards Cyt even in the in the presence of 100-fold excess of other interferents.

Stability and reproducibility of the p-AMTa electrode:

To find out the stability of the p-AMTa electrode, the amperometry detection was obtained for 400 nM Cyt in 0.2 M PB solution were recorded for every 10 min interval. It was found that current increases of each addition were remained same with a relative standard deviation of 8.20% for 5 times repetitive measurements indicating that this electrode has a good reproducibility. Further, DPVs obtained for 0.5 mM Cyt 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 7.80% for 10 times repetitive measurements indicating that this electrode has a good reproducibility.

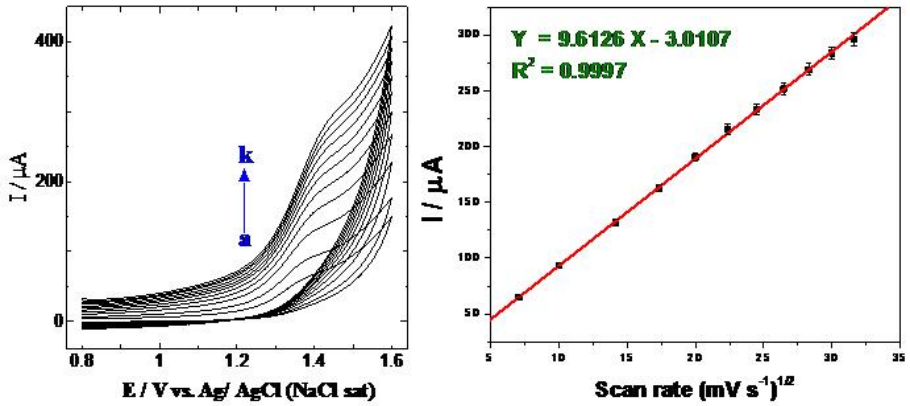

Figure 2 (A): CVs for 0.5 mM Cyt at p-AMTa electrode in PB solution (pH 7.2) at scan rates of (a) 50 (b) 100 (c) 200 (d) 300 (e) 400 (f) 500 (g) 600 (h) 700 (i) 800 (j) 900 (k) 1000 mV s^{-1}. **(B)** Plot of the anodic peak current vs. square root of scan rate.

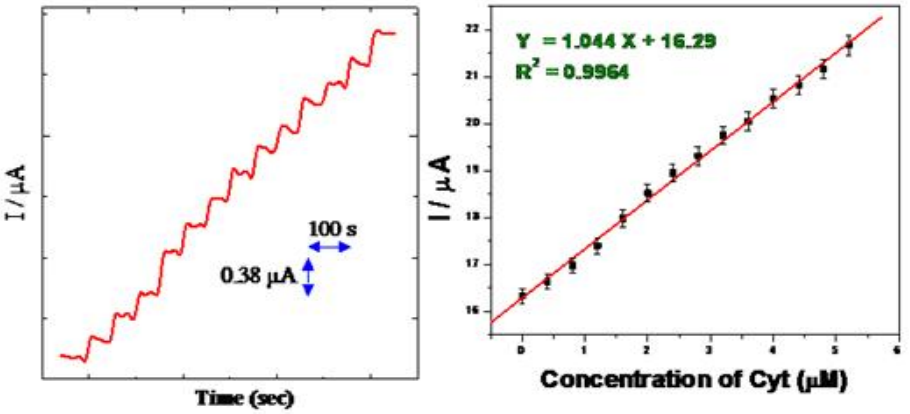

Figure 3: (A) Amperometric i-t curve for Cyt determination at p-AMTa electrode in 0.2 M PB solution (pH 7.2). Each addition increases the concentration of 400 nM of Cyt at a regular interval of 50 s. E_{app} = +1.35 V. **(B)** Plot for concentration of Cyt vs. current.

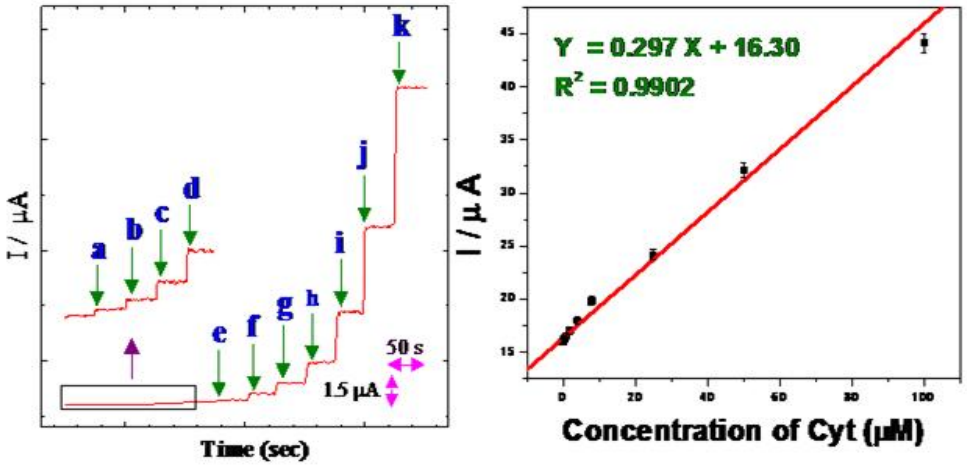

Figure 4: (A) Amperometric i-t curve for Cyt determination at p-AMTa electrode in 0.2 M PB solution (pH 7.2). Each addition increases the concentrations of (a) 0.05 (b) 0.1 (c) 0.2 (d) 0.4 (e) 0.8 (f) 2 (g) 4 (h) 8 (i) 25 (j) 50 (k) 100 µM at a regular interval of 50 s. E_{app} = +1.35 V. **(B)** Plot for concentration of Cyt vs. current.

4. Conclusions

In this paper, the voltammetric and amperometric responses of Cyt were analyzed on the surface of p-AMTa electrode. It showed a clear and stable voltammetric signal for Cyt at +1.3 V when compared to bare GC electrode. The diffusion controlled Cyt oxidation process was confirmed from the liner increment of current against square root of scan rate at the p-AMTa electrode. By amperometric method, the detection of 400 nM of Cyt was achieved successfully. Further, the current response was increased linearly with increasing Cyt concentration from 5.0×10^{-8} to 1.0×10^{-4} M. The detection limit was found to be 2.22×10^{-10} M (S/N = 3). Finally, the anti-interference ability of the electrode also analyzed in the presence of higher concentrations of other physiological interferences. Hence, we can use this electrode for the determination of important Cyt in real samples.

5. Acknowledgment

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