Voltammetric resolution of Adenosine at Pretreated/Graphite pencil electrode: A Cyclic Voltammetric Study

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ABSTRACT

The surface of graphite pencil electrode (GPE) was modified by electrochemical pretreatment method and has been employed for the cyclic voltammetric determination of adenosine in the presence of adenine in 0.2M phosphate buffer solution (pH 7.4) with scan rate 0.05 Vs^{-1}. The electrochemical oxidation of adenosine and adenine was investigated by cyclic voltammetry (CV) method at pretreated/graphite pencil electrode (PGPE). The PGPE showed excellent electrocatalytic activity towards adenosine and adenine determination. The parameters such as effect of scan rate, concentration, and interference study were investigated at PGPE. The electrode phenomenon was found to be discussed at PGPE. Furthermore, the modified electrode exhibited good limit of detection for adenosine and adenine. Hence, the PGPE shows good electrocatalytic properties and can be applied for the determination of adenosine and adenine individually and simultaneously.

Keywords: Pretreated/Graphite pencil electrode, Adenosine, Adenine, Simultaneous.

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1. INTRODUCTION

Nowadays, carbon based electrodes are frequently used for electrochemical determination of purine nucleosides [1]. It is known that electrochemical determination of drugs and biological compounds is often difficult with bare electrodes due to their poor responses and high over-potentials. Modification of electrode surfaces using different approaches is often performed to overcome these limitations. The fabrication of modified electrode is one of the key parameter for the electrocatalytic activity of a carbon based electrode. There are several methods for the fabrication of the electrode which improves catalytic activity of an electrode. For carbon electrodes, a typical procedure is the electrochemical treatment which relies on the electrochemical oxidation or reduction at certain conditions [2]. Carbon electrodes, graphite pencil receive increasing interest as electrode material due to favorable electrochemical performances, low cost and disposability. Among them, the development of reagentless biosensor plays an important role in the research field. Electrochemical pretreatment method is one which increases the rate of the electron transfer at the electrode surface in order to improve the kinetics of electrode processes. There are few literature were reported on pretreated carbon based electrode for electrochemical determination of organic compounds [3-6]. Present study describes the electrochemical pretreatment of graphite pencil electrode for electrochemical determinations of adenosine and adenine.

Purine nucleosides are well-known for their metabolic and biological effects in human system. Their detection and determination has become increasingly important in the field of biomedical research owing to their importance in normal cellular functions. The importance of such a determination stems from the fact that changes in the concentration of these nucleosides in body fluids have been used to indicate the presence of various diseases [7, 8] and hence, their analysis is highly desirable. Adenosine modulates physiological functions in heart and brain, and regulates oxygen supply during cell stress [9] and has also been suggested to be useful in the regulation of renal function [10]. The amounts of adenosine in the urine and plasma samples can also be the marker of some diseases such as carcinoma or liver diseases [11]. So it is important to determine adenosine concentration in different body fluids samples. Different analytical methods have been proposed for the determination of adenosine and most of them are based on the chromatography, which is sensitive and accurate with some pretreatment procedures [12, 13]. The electrooxidation of adenosine on the common working electrode showed high oxidation potential and limited sensitivity. So the chemically modified electrodes were used to overcome the disadvantages and improve the sensitivity [14, 15]. On the other hand, adenine is a vital constituent of deoxyribonucleic acids. It is very important in storing genetic information. Measuring the levels of adenine is important in bioscience and clinical diagnosis, because their amount of concentration can act as important indicators for the diagnosis of various illnesses [16-21].

In view of the importance of adenosine and adenine in human physiology, attempts have been made to determine them simultaneously in body fluids, since both occur together in biological system, by various techniques. In the present research, the development of reagentless biosensor was a great challenge for researchers. we have used the GPE which was activated in 0.1 N sulfuric acid (H_2SO_4) under potential between 0.6 to 2.0V at scan rate of 0.1 Vs^{-1} for a 10 cycles in an unstirred solution by using

cyclic voltammetric technique. This PGPE has shown very convincing results for the improvement of the adenosine and adenine signals at 0.2M phosphate buffer solution at Physiological pH.

2. EXPERIMENTAL

25mM Adenosine (Himedia) and 25mM Adenine (Himedia) stock solutions were prepared in 0.1M NaOH. 0.2 M phosphate buffer solution (PBS) (Merck) of pH 7.4 is prepared by mixing the appropriate quantity of 0.2 M aqueous sodium dihydrogen phosphate monohydrate and 0.2 M aqueous disodium hydrogen phosphate. NaOH and acetic acid were used for increasing and decreasing the pH of the buffer. Chemicals mentioned above were all of analytical grade used without further purification. In the preparation of solutions, double distilled water was used.

Cyclic voltammetric (CV) experiments were performed with a model CH 660C from CH Instruments, USA equipped with a personal computer was used for electrochemical measurement. The electrode system enclosed the working electrodes were bare graphite pencil electrode (BGPE) and PGPE, platinum wire as counter electrode and saturated calomel as reference electrode.

The GPE was pretreated by applying a linearly varying potential between 0.6 and 2.0 V at scan rate 0.1 Vs^{-1} for a 10 cycles in an unstirred solution of 0.1 N H_2SO_4 [4, 6, 23-25]. Pretreatment was achieved by increasing active sites on the surface with a concomitant increase in the surface hydrophilicity using CV technique [4, 26]. After surface pretreatment, the electrode was thoroughly washed with distilled water and it was applied for further analysis.

3. RESULTS AND DISCUSSION

3.1. Effect of multiple cycles in the preparation of PGPE

From the obtained experimental results, the pretreatment of the sulphuric acid affects the electrocatalytic property of the electrode. The pretreatment was controlled by applying the multiple cycles. The GPE was modified by applying different multiple cycles (from 5 to 15 multiple cycles) and the corresponding electrocatalytic activity towards adenosine (1×10^{-3} M) was examined. From the Table 1, it can be noticed that, the maximum active surface area obtained at 10 multiple cycles towards the determination of adenosine and have better sensitivity towards the detection of adenosine. The surface area available for reaction of species in solution can be calculated by the Randles-Sevcik equation (1) [4].

$$Ip= 2.69\times10^{5}\, n^{3/2} A\, D^{1/2}\, C_0 \upsilon^{1/2} \qquad (1)$$

Where, I_p is the peak current in A, C_0 is the concentration of the electroactive species (mol cm^{-3}), n is the number of electrons involved, D is the diffusion coefficient in cm^2S^{-1}, υ is the scan rate (Vs^{-1}) and A is the electroactive surface area (cm^2).

Table.1. Variation of surface area of PGPE at different multiple cycles

Number of multiple cycles in 0.1N H_2SO_4 at Scan rate 0.1Vs^{-1}	Active surface area exposed for adenosine in cm^2
5	0.0137
10	0.0156
15	0.0147

3.2. Electrochemical characterization of PGPE using standard potassium ferricyanide system.

The freshly prepared 1mM potassium ferricyanide and 1 M potassium chloride solutions were placed in the electrochemical cell. The fig. 1 shows the cyclic voltammograms recorded for the 1 mM potassium ferricyanide at both BGPE (dashed line) and PGPE (solid line) at scan rate of 0.05 Vs^{-1}. The low redox current signals were observed at BGPE. The anodic and cathodic peak potentials were located at 0.202 V and 0.261 V. The redox peak potentials difference (ΔEp) was 0.059 V. However, the PGPE showed significant improvement in the redox peak current signals. The anodic and cathodic peak potentials were found at 0.218 V and 0.258 V. The ΔEp was 0.040 V. This minimization in over potential and enhancement in sensitivity shows the good electrocatalytic property of PGPE.

3.3. Electrocatalytic response of adenosine at PGPE

Fig.2 shows the cyclic voltammograms of 1×10^{-3} M adenosine at BGPE (solid line) and PGPE (dotted line) in 0.2 M PBS of *p*H 7.4 at 0.05 V/s Scan rate. As can be seen, the oxidation peak potential (Epa) at about 1.286 V was observed at BGPE. The strong current improvement and irreversible peak was observed at PGPE. The Epa was observed around at 1.271V respectively. This is the clear evidence that sulfuric acid pretreatment at GPE has better electrocatalytic activity by exposing large active surface area for electrochemical oxidation of adenosine. Hence, the PGPE shows excellent electrocatalytic activity by reducing over potential and also by improving the current signals. Further investigations were made into electrode transfer characteristics of adenosine at PGPE by varying the scan rate (Fig.3A). The graph showed that the anodic peak current (Ipa) of adenosine was linearly proportional to the scan rate (ν) in the range from 0.05 – 0.5 Vs^{-1} (Fig.3B) and the correlation coefficient was found to be 0.9997, which illustrate that the adsorption controlled electrode process.

The electrocatalytic oxidation of adenosine was carried out by varying the concentration from 2×10^{-4} to 3.5×10^{-3} M as shown in Fig.4 there is an linear increase in the Ipa by increasing the concentration of adenosine. The correlation coefficient for Ipa versus (vs) concentration of adenosine was found to be r^2 = 0.987 and limit of detection (LOD) was 7.1×10^{-4}M obtained, calculated by using formula (2) [4].

$$LOD = 3S/M \qquad (2)$$

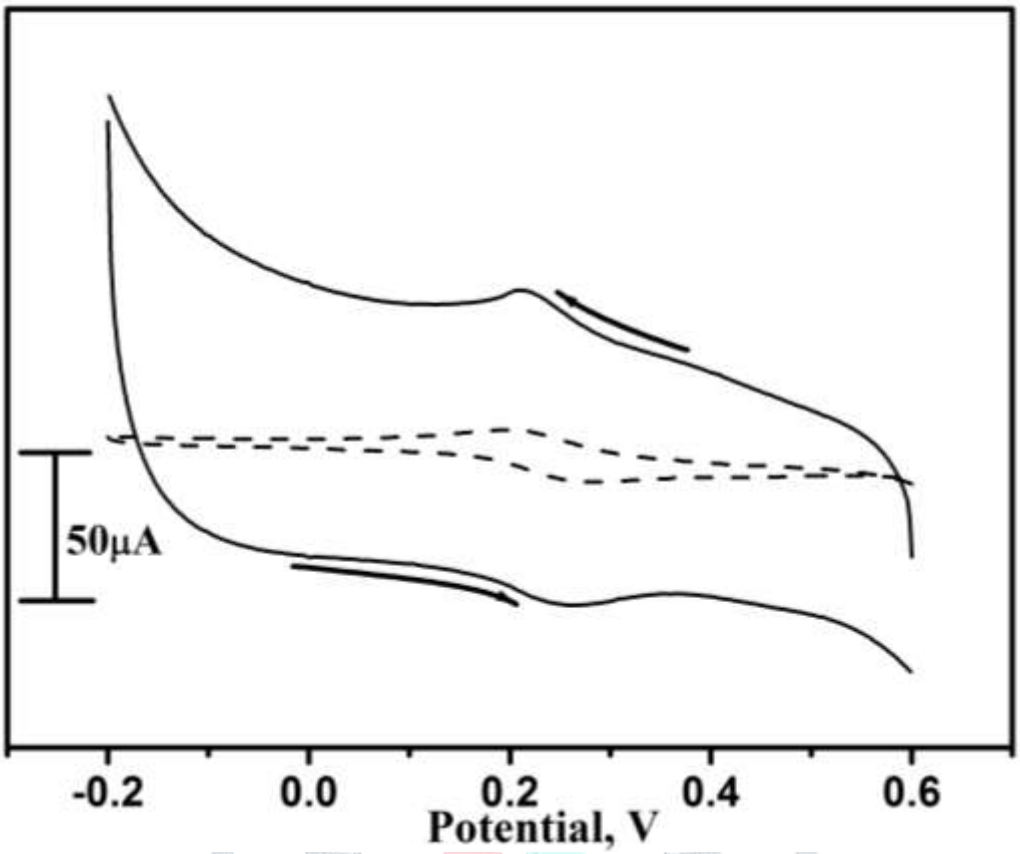

Fig.1- Cyclic voltammograms of 1.0 mM potassium ferricyanide in 1M KCl solution at BGPE (dashed line) and PGPE (solid line) at scan rate of 0.05 Vs^{-1}.

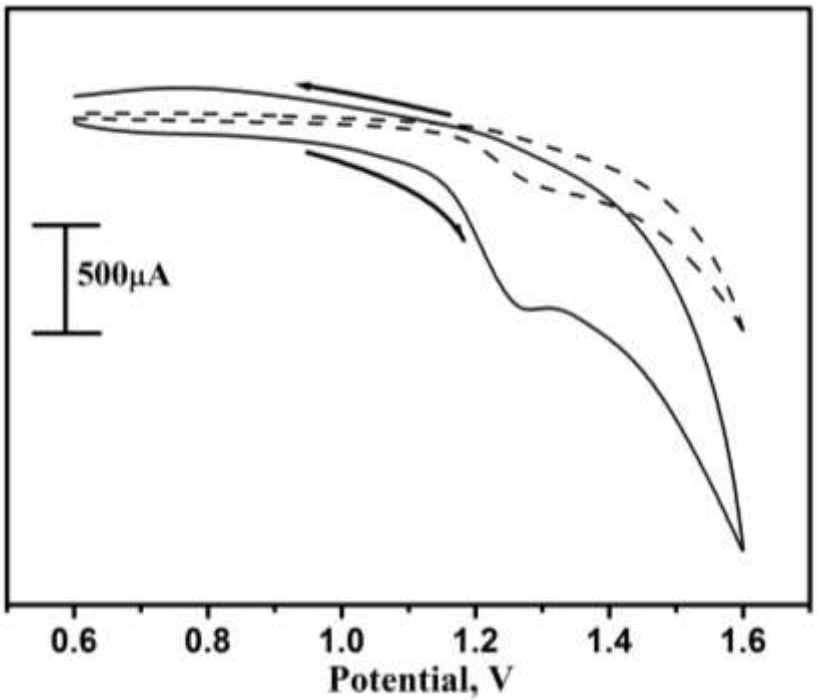

Fig.2- Cyclic voltammograms of 1×10^{-3} M adenosine in 0.2M PBS of pH 7.4 at the BGPE (dashed line) and at the PGPE (solid line) are measured at the scan rate of 0.05 Vs^{-1}.

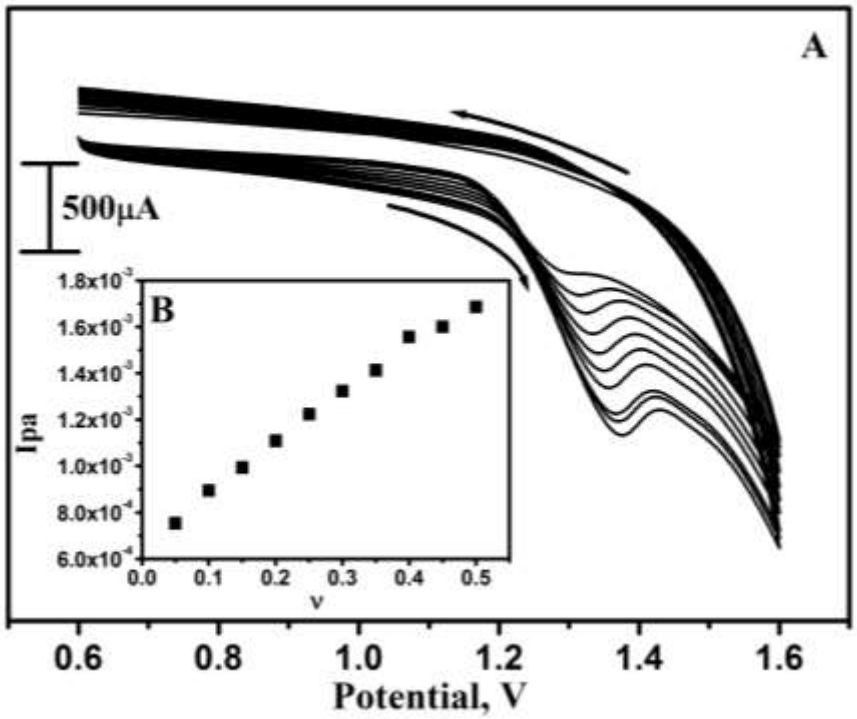

Fig.3- (A) Cyclic voltammograms of 1×10^{-3} M adenosine in 0.2M PBS of pH 7.4 at the PGPE are measured at different scan rate from 0.05 to 0.5 Vs^{-1}. (B) Graph of Ipa vs ν.

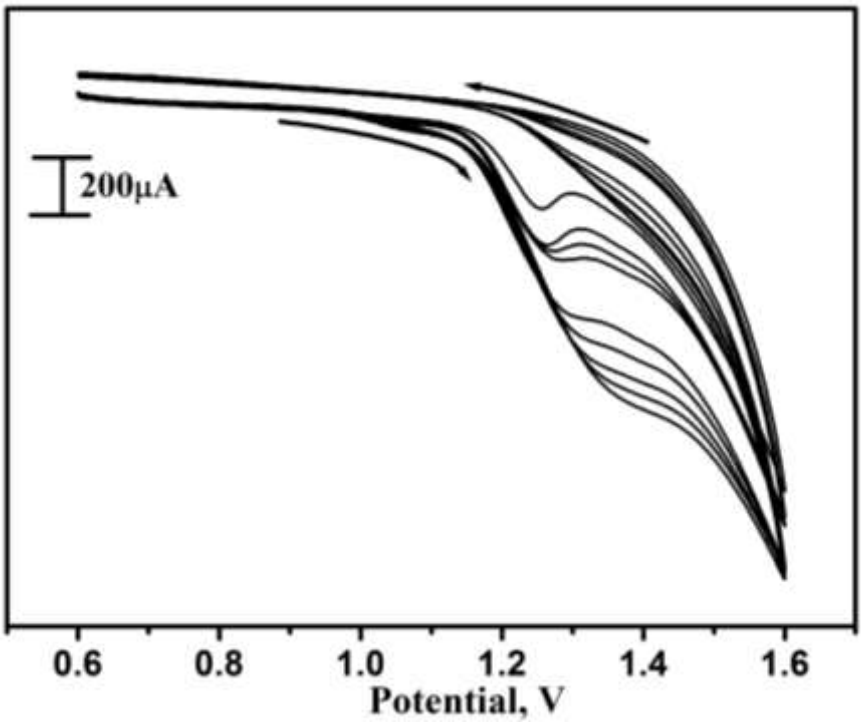

Fig.4- Cyclic voltammograms of adenosine from 2×10^{-4} to 3.5×10^{-3} M in 0.2M PBS of pH 7.4 at the PGPE at scan rate 0.05 Vs^{-1}.

3.4. Electrocatalytic response of Adenine at PGPE

Fig.5 shows the cyclic voltammogram of 1×10^{-3} M adenine at BGPE (dashed line) and PGPE (solid line) in 0.2 M PBS of *p*H 7.4 at 0.05 Vs^{-1} scan rate. As can be seen, the Epa at about 1.086 V was observed at BGPE. The strong improvement in the peak current signal and irreversible redox peak was observed at PGPE. The Epa was observed around at 1.012 V respectively. Hence, the PGPE shows excellent electrocatalytic activity by reducing over potential and also by improving the current signals. Further investigations were made into electrode transfer characteristics of adenine at PGPE by varying the scan rate from 0.05 to 0.4 V/s (Fig.6A) and the graph showed good linearity for Ipa against $\nu^{1/2}$ (Fig.6B). The correlation coefficient (r^2) was found to be 0.9959, which illustrate the diffusion controlled electrode process.

The electrocatalytic oxidation of adenine was carried out by varying the concentration from 2×10^{-4} to 2×10^{-3} M as shown in Fig.7 there is a linear increase in the Ipa by increasing the concentration of adenine. The correlation coefficient for Ipa vs concentration of adenine was found to be $r^2 = 0.998$ and LOD was 4.5×10^{-4}M obtained, calculated by using formula (2) [4].

3.5. Effect of pH

The effect of pH on the determination of 1×10^{-3} M adenosine in 0.2 M PBS solution at the PGPE was investigated in the range of 5.8–7.8. The Epa shifted to negative side and decreasing the Ipa with increasing the pH from 5.8-7.8 as shown in Fig.8A which proved that protons took part in the electrode reaction. Graphs of E_{pa} vs the pH of the solution was shown in Fig. 8B. A linear regression equations obtained was Epa (V) = 0.05484 pH + 0.8736 (n = 6, r^2 = 0.9874) for the PGPE. This result confirms that the equal number of protons and electrons were involved in the electrochemical oxidation of adenosine [27] and for further electrochemical studies physiological pH.7.4 was selected for adenosine.

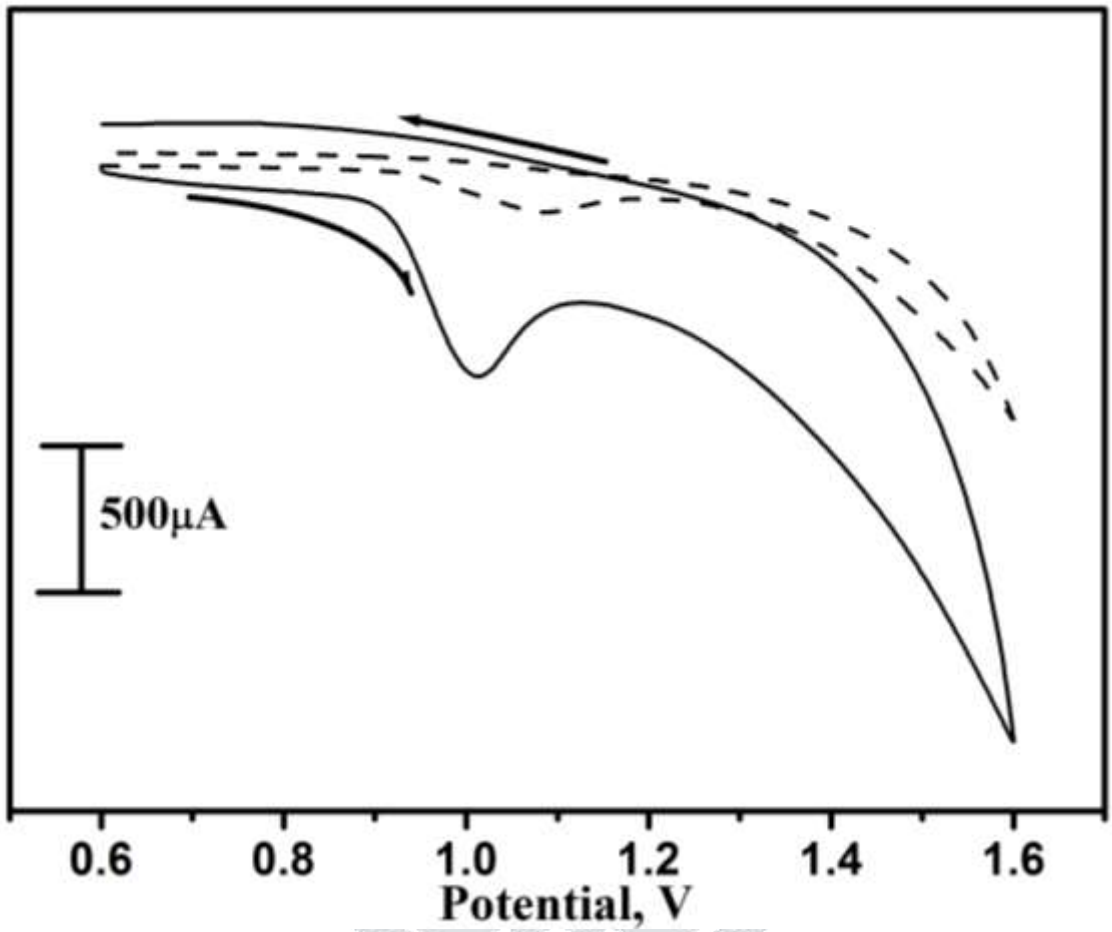

Fig.5- Cyclic voltammograms of 1×10^{-3} M adenine in 0.2M PBS of pH 7.4 at the BGPE (dashed line) and at the PGPE (solid line) are measured at the scan rate of 0.05 Vs^{-1}.

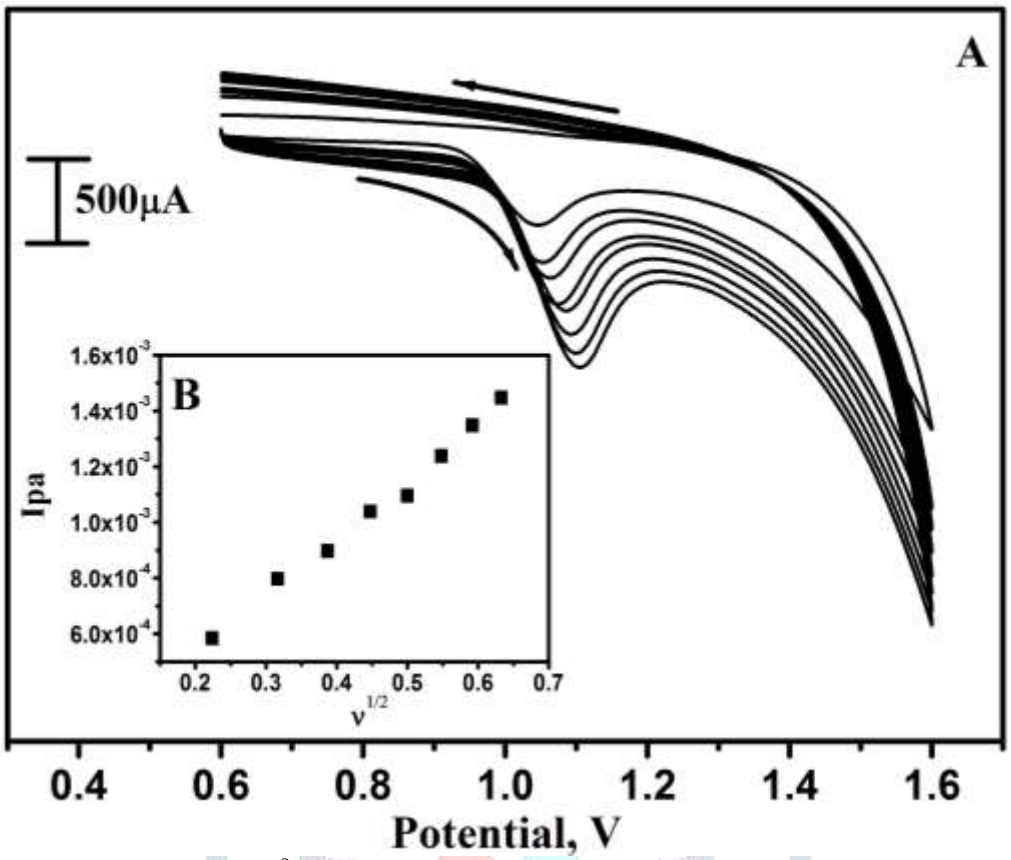

Fig.6- (A) Cyclic voltammograms of 1×10^{-3} M adenine in 0.2M PBS of pH 7.4 at the PGPE are measured at different scan rate from 0.1 to 0.4 Vs^{-1}. (B) Graph of the Ipa vs $\nu^{1/2}$.

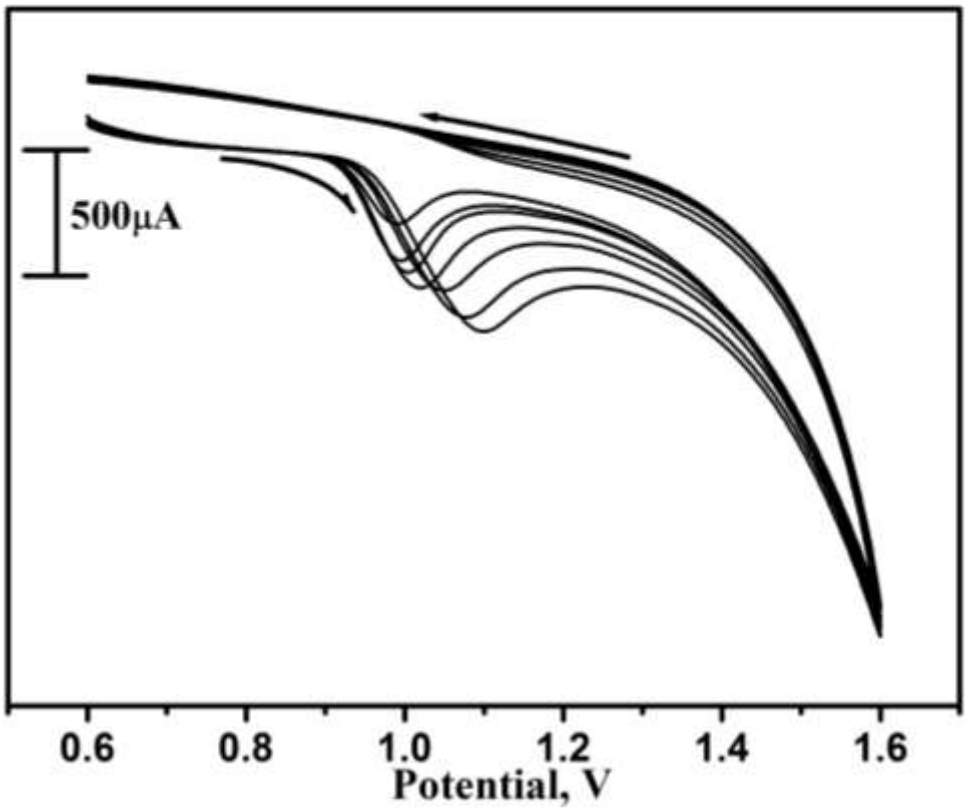

Fig.7- Cyclic voltammograms of adenine from 2×10^{-4} to 2×10^{-3} M in 0.2M PBS of pH 7.4 at the PGPE at scan rate 0.05 Vs^{-1}.

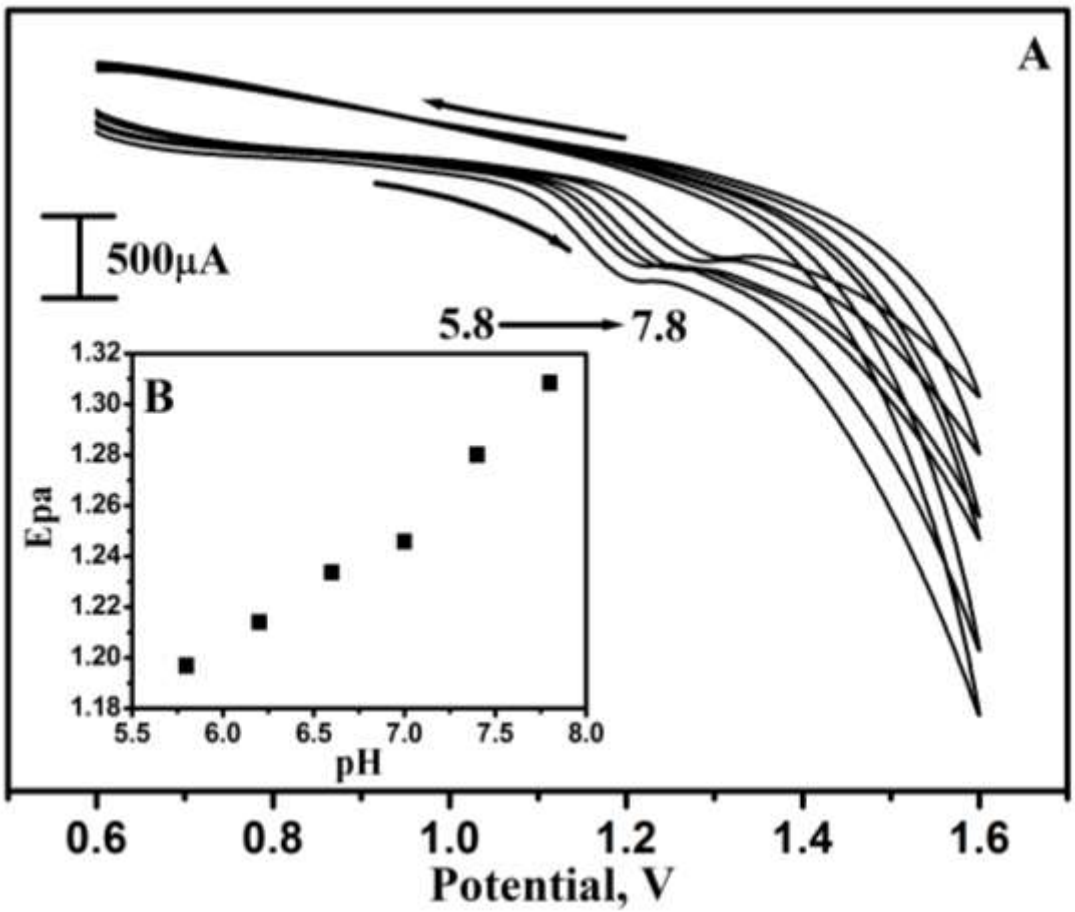

Fig.8- (A) Cyclic voltammogram of 1×10^{-3} M adenosine at PGPE in 0.2M PBS of pH from 5.8 to 7.8 at scan rate of 0.05 Vs^{-1}. (B) Graph of of E_{pa} vs the pH of the solution at PGPE.

3.6. Simultaneous determination of Adenosine and Adenine

In addition, PGPE was introduced for analysis of 5×10^{-4}M adenosine and 5×10^{-4} adenine mixture at pH 7.4. As shown in Fig. 9, the cyclic voltammogram at BGPE (dashed line) appear the peaks at around 1.11 V and 1.27 was observed with poor sensitivity. However, two well distinguished anodic peaks were obtained at PGPE (solid line) with great enhancement in current signals. The anodic peak potentials were located at 1.06 V and 1.29 V for adenine and adenosine respectively. The difference of anodic peak potential between adenosine and adenine was 0.23 V at PGPE by CV technique and these were more enough to identify the adenosine and adenine individually.

4. CONCLUSION

PGPE detect the adenosine and adenine solution mixture at physiological pH electrochemically. The electrode process was adsorption controlled for adenosine and the diffusion controlled for adenine at PGPE. The prepared modified GPE exhibit low detection limit 7.1×10^{-4}M for adenosine and 4.5×10^{-4}M for adenine. pH studies suggest that the equal number of protons and electrons were involved in the electrochemical oxidation of adenosine. The simultaneous studies reveal that the difference of anodic peak potential between adenosine and adenine was 0.23 V, is more enough to identify the adenosine and adenine individually and selectively. This approach can readily be applied to the development of electrochemical sensors for adenosine and related purine nucleosides.

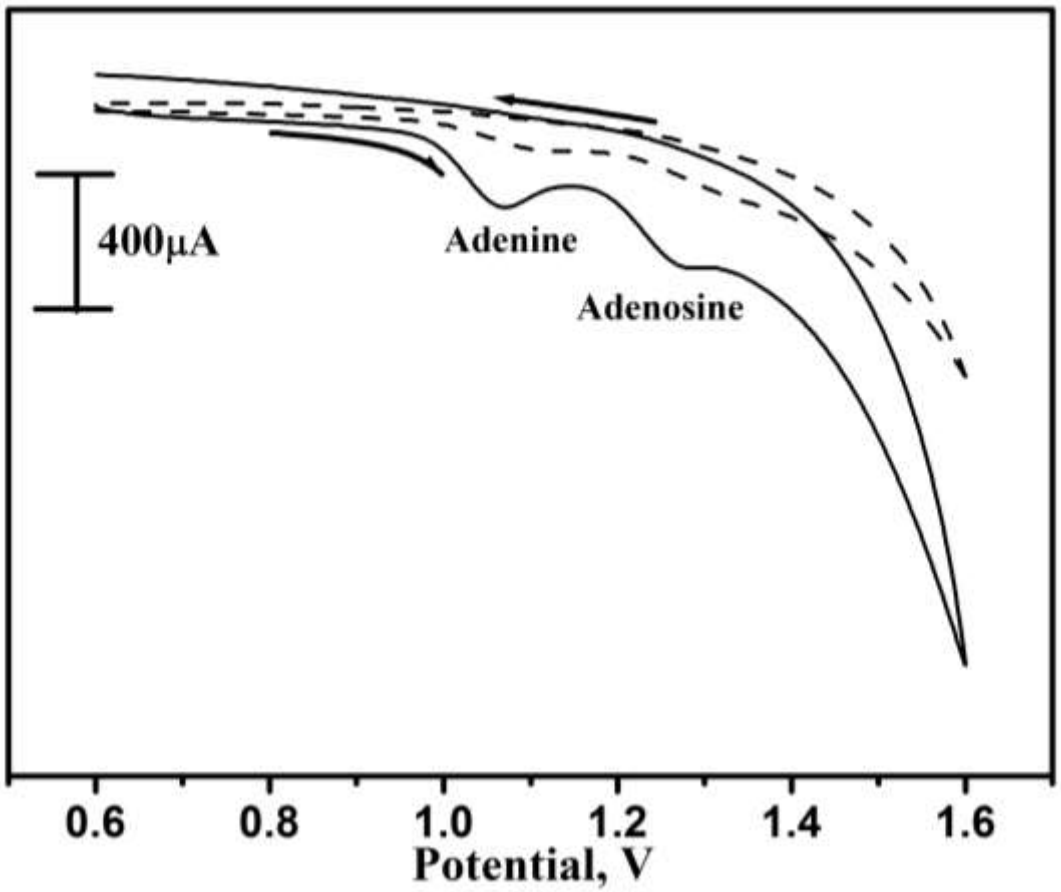

Fig.9- Simultaneous determination of 5×10^{-4} M adenine and 5×10^{-4} M adenosine at BGPE (dashed line) and at PGPE (solid line) in PBS of pH 7.4 by CV at scan rate 0.05 Vs^{-1}.

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