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Simultaneous Determination of Adenine and Thymine in Presence of Guanine at Electrochemically Activated Glassy Carbon Electrode

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Here we report simple cyclic voltammetry method for electrochemical activation of Glassy carbon electrode (GCE) by 0.1M sodium bicarbonate ($NaHCO_3$) solution without acidic medium. Under optimized condition, the electrochemically activated glassy carbon electrode (EAGCE) was applied for individual and simultaneous determination of DNA bases adenine (Ade) and Thymine (Thy) in presence of high concentration of Guanine (Gu) for the first time. The surface study was examined using scanning electron microscope (SEM). Linear sweep voltammetry (LSV) method shows the oxidation of Ade, Thy and Gu individually. Moreover the simultaneous determination of Ade and Thy in presence of high concentration of Gu also exhibited using LSV. The EAGCE detects in the range of 2 µM to 230 µM for Ade, 100 µM to 2.3 mM for Thy and 10 µM to 0.2 mM for Gu respectively. The low limit of detection was found as 0.1 µM, 0.11 µM and 1.01 µM for Ade, Thy and Gu respectively. Additionally the proposed sensor exhibits good repeatability, reproducibility and sufficient stability.

Keywords: Electrochemically activated GCE, Adenine, Thymine and Guanine.

1. INTRODUCTION

Glassy carbon electrode (GCE) is one of the most widely used electrodes in electro analytical applications, specifically in biomolecule related determinations such as DNA electroanalysis, bio recognition, bio molecules, etc., with the advantage of wide working window in both the anodic and cathodic directions[1-4]. Mostly, GCE is made up of special type of carbon which is fabricated by pyrolysis of polymer resin, exhibits good electrical conductivity with well-defined surfaces [5]. Nevertheless the Surface treatment of a solid electrode has been used extensively to improve the

electrochemical performance of the electrode. Especially, electrochemical pretreatment is used for cleaning and activating the surface of electrode [6-8]. Various pretreatment (or activation) procedures, depending on the kind of analysis and nature of the redox system, have been adopted to achieve faster electron transfer rates and more reproducible results [9-15]. There are few Reports have demonstrated that the electrochemical activation could be performed in a phosphate buffer or H_2SO_4 solution. In those the surface properties and structures were investigated and indicated that phenolic oxygen and carbonyl oxygen functional groups had been generated [16, 17].

Deoxyribonucleic acid (DNA) is an important substance that plays an important role in the storage of genetic information and protein biosynthesis [18]. Adenine (Ade), guanine (Gu) and thymine (Thy) are the important components found in the DNA. Abnormalities in the ratio or error in the DNA base transcription may be the indication for cancer, aging, and other diseases [19, 20]. So, determination of individual concentrations rations of Ade, Gu and Thy or their ratio in DNA is important for the measurement of nucleic acid concentration itself. Thus, the determination of these analytes is pharmacologically necessary using chemically modified electrodes, which are done by means of electrocatalysis [21]. The previously attempted methods for determination of these analytes have problems like irreversible adsorption of purine bases on the electrode surface and have led to surface fouling [22].Recently; there were reports for the electrochemical detection of adenine, guanine and thymine compounds using activation of GCE. Such as , Simultaneous determination of guanine and adenine in DNA using an electrochemically pretreated glassy carbon electrode [23] and purine and pyrimidine DNA bases based on the recognition properties of azocalix[4]arene [24]. However, selective determination of Ade and Thy in presence of GU with higher selectivity, stability and sensitivity is still one of the challenging tasks for the electrochemists.

All the literature survey clearly exhibits that the pretreatment or activation of GCE is a simple and elegant pathway for the electrode modification process. Also, the activation process modifies the electrode surfaces with specific functional groups (carbonyl, carboxyl, and hydroxyl species). Therefore, in this report, we have utilized the activation process to modify the GCE surface with functional groups and employed for the selective detection of adenine and thymine in presence of guanine using cyclic voltammetry (CV) and linear sweep voltammetry (LSV). In this report, the surface morphology of activated GCE has been examined by using scanning electron microscopy (SEM) and the activation by sodium bicarbonate ($NaHCO_3$) solution. The proposed GCE possesses the capability for the detection and determination of adenine and thymine in presence of guanine in the reasonable linear ranges using CV and LSV techniques, respectively.

2. MATERIALS AND METHODS

Adenine, Guanine and Thymine were bought from sigma Aldrich. Sodium bicarbonate ($NaHCO_3$) was purchased from yakbri chemicals. All the electrochemical experiments were conducted by using 0.05M phosphate buffer solution as the supporting electrolyte, prepared by using Na_2HPO_4 and NaH_2PO_4 and required pH was adjusted by using either NaOH or HCl. All the solutions used in

this work were prepared by doubly distilled (DD) water. Nitrogen N_2 gas was purged 10 minutes through the experiment solution before the experiments.

Cyclic voltammogram work station CHI 1205A was utilized for all electrochemical studies. The three electrode system, Glassy carbon electrode (0.079 cm2) as working electrode, Ag|Agcl in saturated Kcl as reference electrode and Platinum wire as counter electrode were used for electrochemical measurements. The surface morphology study was carried out by Scanning electron microscopy (Hitachi S-3000H, Japan).

3. PREPARATION OF MODIFIED ELECTRODE

The GCE surface was polished with alumina slurry using polishing kit and washed with doubly distilled water further sonicated with ethanol to eliminate the alumina particles followed by washed with DD water and dried in open air. The precleaned GCE was bringing to activation. The GCE surface was activated by electrochemically in 0.1M $NaHCO_3$ by performing 20 consecutive cyclic voltammograms shortly explained in section [4.1]. Resulting electrode was dried and used for further studies.

4. RESULTS AND DISCUSSIONS

4.1 Electrochemically activation of GCE

The GCE activation was carried out by using cyclic voltammetry technique. The well polished GCE was positioned in electrochemical cell containing 0.1M $NaHCO_3$ solution. Fig 1A shows the electrochemical activation of GCE in the potential range of -1.0 to 0.55 V at scan rate of 100 mV/S. During the first cycle, an anodic peak appeared marginally at 0.95 V could be due to the activation of GCE, moreover increases the number of cycles causes to increase the anodic peak current obviously. At the end of the 10^{th} cycle the anodic peak current does not saturated. Further, when the cycle was continued, at end of the 20^{th} cycle the anodic peak attained maximum current thereafter it remained same, confirm that the GCE surface was activated completely. The inset plot shows the number of cycle vs. Ipa, according to the plot the anodic peak current achieved maximum at the end of the 20^{th} cycle; moreover the anodic current was same as the 25^{th} cycle. Hereafter 20 cycles were used in all our experiments for GCE activation, unless otherwise it is specified. The SEM image of activated GCE at 50 μM resolution is shown in fig 1B. It can be seen that the highly porous, highly reactive oxygen containing functional groups and oxidized carbon were formed on the surface of GCE during the activation process [25]. The surface of GCE surface was highly porous and rough. Compared to bare GCE, the EAGCE surface being rough and porous favorable to electro catalytic oxidation.

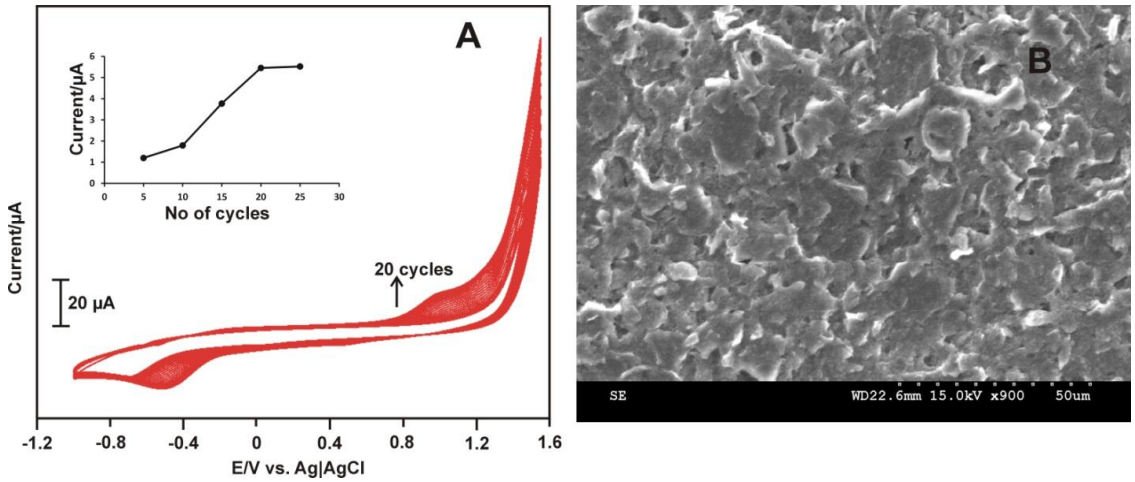

Figure 1. A) Cvs of electrochemical activation of GCE by 20 cycles in 0.1M $NaHCO_3$ solution at the scan rate of 100 mVs^{-1}.Inset: I_{pa} vs number of cycles. **B**) SEM image of activated GCE at 50 µm.

4.2 Effect of scan rate and pH optimization

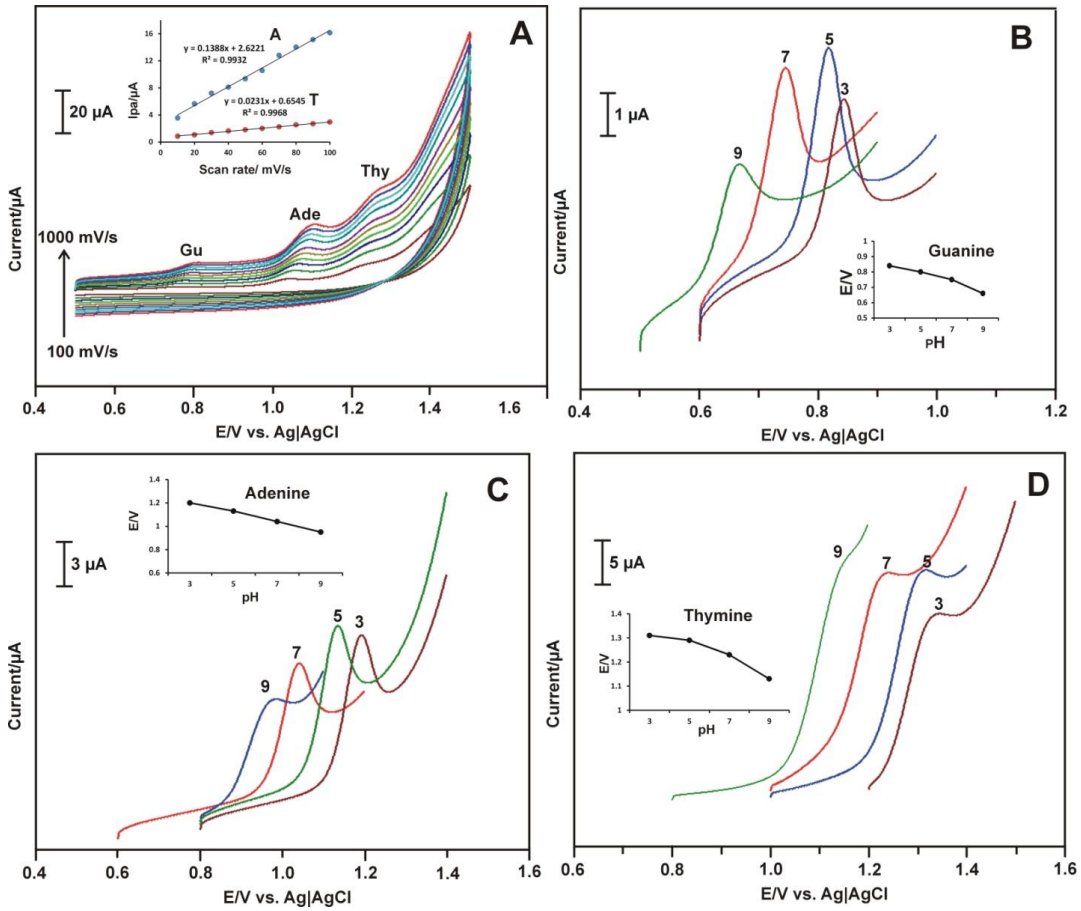

Figure 2. A) Cvs of EAGCE in PBS containing Gu 10 µM, Ade 2 µM and Thy 2 µM at different sacn rate (100 mV/s to 1000 mV/s) . **B), C), D)** LSV of Effect of pH (3, 5, 7, and 9) on the differences of peak potential for Gu 0.1 mM, Ade 10 µM and Thy 10 µM.

In Fig 2A the cyclic voltammogram of EAGCE in the PBS (pH 7) in the presence of 100 μM of Gu, 20 μM of Ade and 200 μM of Thy were recorded at the scan rate of 100 mV/s to 1000 mV/s. The inset plot illustrated as the increase of scan rate, caused to the anodic peak current increased progressively, revealing that diffusion controlled processes moreover this results exhibit the electrode reactions of Gu, Ade and Thy on EAGCE were an adsorption controlled process. The pH optimization studies were carried out using linear sweep voltammetry. Fig 2 B, C and D shows peak potential of the PBS solution pH range 3, 5, 7 and 9 containing Gu, Ade and Thy individually. As can be seen that the anodic peak potential of respective compound was shifted more positive side towards decrease the pH likewise while increase pH value, anodic peak potential of respective compound was move towards negative side. Interestingly, the maximum anodic peak current of respective compound (Gu, Ade, and Thy) at less positive potential was found at pH 7.According to the inset plot (pH vs. Ep) peak potential shifted depends on value of pH. Hereafter, the further electro catalysis has been studied using pH 7.

4.3 Individual determination of Guanine, Adenine and thymine

The individual determination of Gu, Ade and Thy at EAGCE were performed using Linear sweep voltammetry (LSV). Fig 3A shows the different concentration of Gu in the absence of A and T. In fig 3A the oxidation peak current at 0.74 V increased linearly by adding the different concentration range of Gu from 10 μM to 166.6 μM. The limit of detection was found as1.01 μM. The inset linear calibration Plot indicates the concentration of Gu vs. oxidation peak current is linear. The linear regression equation can be stated as I_{pa} (μA) = 0.0183 C (μM) + 0.224, R^2 = 0.9885. Likewise fig 3B shows the various concentration of Ade in the absense of Gu and Thy. The oxidation peak current increases linearly with increasing the concentraion range of Ade from 2 μM to 230.7 μM. The detection limit for Ade was found as 0.103 μM. The inset plot shows the linearity of concentarion vs current. The linear regressin equation is I_{pa} (μA) = 0.0976 C (μM) + 2.4321, R^2 = 0.9722. Similarly the fig 3C shows the LSV of Thy at various increasing concentration. The linear range was 0.1 mM to 2.3 mM. The inset plot also indictes the linearity of concentration vs current.The linear regressin equation is I_{pa} (μA) = 0.0154 C (μM) - 0.2944, R^2 = 0.9984 with low detection limit 0.11 μM. The above results exhibits the EAGCE was capable for determine the Gu, Ade and Thy individualy.Moreover as can be see that in the individual determination of Guanine fig 3A, the minor oxidation peak current was observed at higher concentration of Gu, when compared to peak current of Ade and Thy. Since the EAGCE is capable for determination of Ade and Thy in the presence of high concentration of Gu.

In presence of high concenration of Gu the respective determination of Ade and Thy in their mixtures were performed when the concentration level of one compound remined constant. In fig 4A , the anodic peak current (1.04 V) incresed linearly by adding different concentration of Ade in constant concentration of Thy and presence of high concentration (0.2mM) of Gu. Similaraly in fig 4B the anodic peak current 1.24 V incresed by addition of different concentration of Thy in constant concentration of Ade and high concentraion of Gu.The linear regression equation was found as I_{pa} (μA) = 0.0792 C μM + 5.2335, R^2 = 0.973 for Ade and I_{pa} (μA) = 0.0115 C μM - 0.0197, R^2 = 0.9984 for Thy respectively.

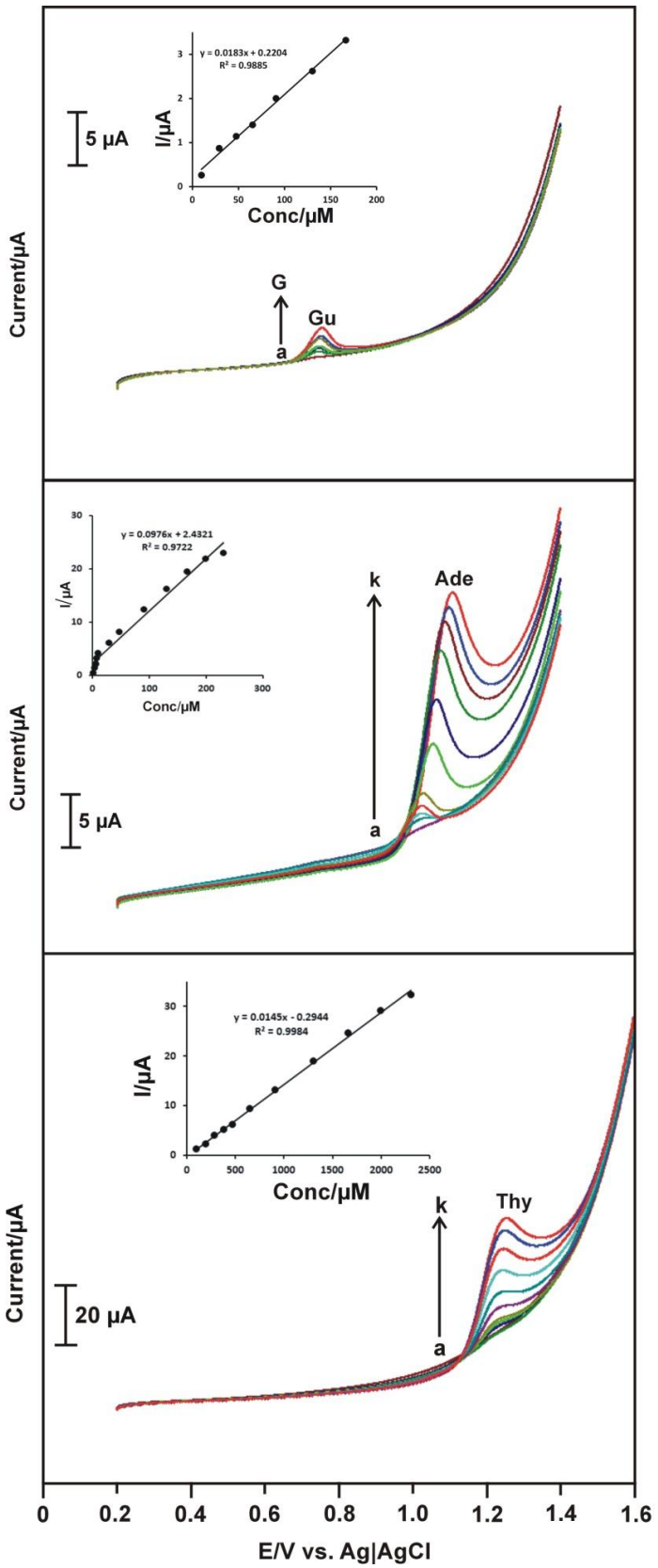

Figure 3. LSV of **A)** EAGCE at different concentration (10 µM to 230 µM) of Guanine in the absence of Ade and Thy. **B)** Various concentration range (2 µM to 230 µM) of Ade in the absence of Gu and Thy**. C)** Various concentration range (100 µM to 2.3 mM) of Thy in absence of Gu and Ade.

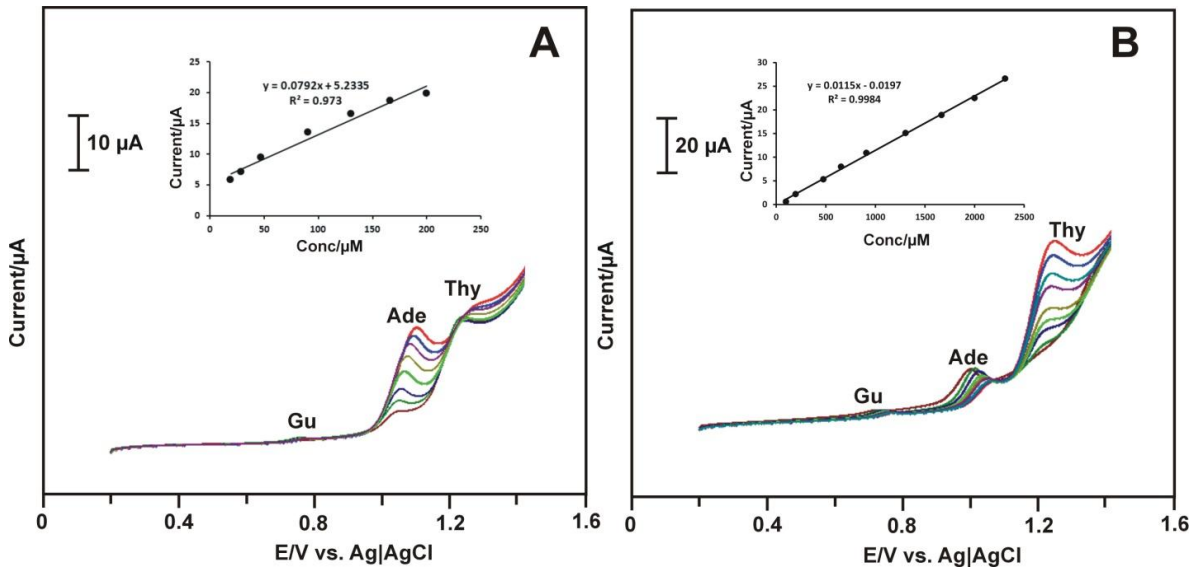

Figure 4. A) LSV of various concentration range (2 µM to 230 µM) of Adenine in presence of 0.2 mM of Gu and Thy. **B)** Various concentration range (200 µM to 2.3 mM) of Thy in presence of 0.2 mM of Gu and 50 µM of Ade.

Table 1. The summary for analytical parameters by individual and binary for the two bases.

Process	Base	Detection limit µM	Linear range µM	Sensitivity µA μM^{-1} cm^2	Regression coefficient R^2
Individual	Adenine	0.103	2 – 230	1.23	0.972
	Thymine	0.115	100 - 2300	0.18	0.9984
Binary	Adenine	0.12	2 – 230	1.003	0.973
	Thymine	0.14	100 - 2300	0.14	0.9984

4.4 Simultaneous determination of Ade and Thy in presence of Gu

We emloyed LSV technique for the simultaneous determination of Ade and Thy. As illustrated in Fig 5A in presesnce of 0.2mM concentration of Gu, followed by addition of various concentration of Ade and Thy simultaneously. Initially 10 µM of Ade and 100 µM of Thy added to electrochemical cell containing 10 ml of PBS (pH 7) with 0.2 mM of G.The oxidation peaks appears at 0.74 V , 1.04 V and 1.24 V respectively. Additionally the oxidation peak current of Ade (1.04) and Thy (1.23) increasing linearly with increse the concentration range from 10 µM to 200 µM of Ade and from 100 µM to 2.0 mM of Thy. Neverthless the oxidation peak current of Gu at 0.74 V does not incresed linearly but diminished slightly due to increase the current density of Ade and Thy.The peak to peak (ΔE_P) seperation between G and A was found as 300 mV. Similarly peak to peak seperation between Gu and Thy was found 0.50 V. The peak seperation (ΔE_P) between Ade and Thy was found as 200 mV.

Similarly the Fig 5B illustrates the cyclic voltammogram for simultaneous determination of Ade and Thy in presence of high concentration of Gu. The EAGCE placed in electrochemical cell

containing 0.1M PBS (pH 7) with different concentration of Ade, Thy, and 0.2 mM of Gu. The cyclic voltammogram peak appears at 0.74 V, 1.04 and 1.24 respectively for Gu, Ade and Thy. Likewise we found well resolved peak to peak separation (ΔE_P) between Ade and Thy. The peak to peak separation of 200 mV was enough for the simultaneous determination of Ade and Thy. Relative to bare GCE the oxidation peak potentials for Ade and Thy was decreased by 100 mV. The increase in peak currents and decrease in peak potential validates the excellent catalytic activity of EAGCE for the simultaneous determination of Ade and Thy in presence of high concentration of Gu.

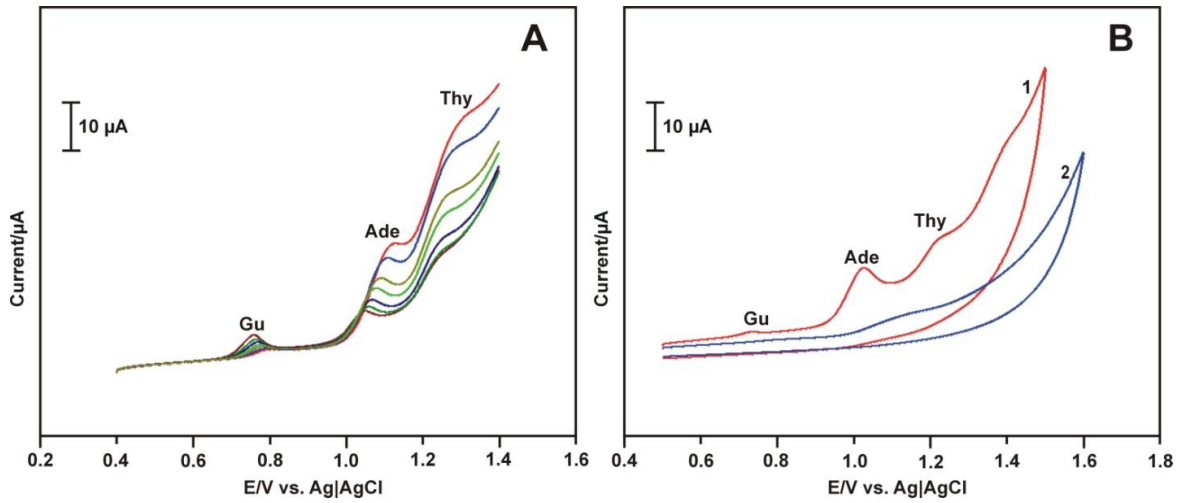

Figure 5. A) LSV of simultaneous addition of (Ade 20 µM to 200) µM and Thy (200 µM to 2 mM). **B)** Cv of EAGCE in PBS contain 100 µM of Ade, 500 µM of Thy and 0.2 mM of Gu Peak 1. Peak 2 Bare GCE at same condition.

4.5 Repeatability, reproducibility and stability

Repeatability, reproducibility and stability studies were evaluated using CV. Four different EAGCE were prepared and kept dried. The anodic peak responses toward fixed concentration Ade, Gu and Thy in 0.1M PBS (pH 7) were tested. The relative standard deviation (R.S.D) value was measured 4.1 %, indicating good repeatability. Similarly the RSD value for four repetitive measurements of Ade, Thy and Gu at an EAGCE are 2.7%, suggesting good reproducibility. Furthermore the stability of the EAGCE was measured everyday using CV. The EAGCE was stored in PBS (pH 7) at 4^o C and its response towards Ade and Thy were monitored. The modified electrode retains 95% of its initial response even after one week, revealing its good stability.

5. CONCLUSION

In this work, a simple and novel electrochemical activation of GCE without acidic condition was prepared and used for the simultaneous determination of DNA bases (Ade & Thy) in presence of high concentration of Gu. The EAGCE was showed excellent electro catalytic activity towards Ade

and Thy moreover sufficient peak to peak separation for the simultaneous determination of Ade and Thy. Finally the EAGCE exhibits high sensitivity with low limit of detection and remarkable linear range.

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