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Simultaneous Detection of DNA Bases on Electrodes Chemically Modified with Graphene-New Fuchsin

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The graphene combined with New Fuchsin (NF) to prepare graphene-NF modified electrode. Graphene obtained high mechanical strength, non-antigenic biopolymer and low toxicity toward mammalian cells. Electrochemical impedance spectroscopy (EIS) applied diffusion coefficient values and some information about the kinetics of electron transfer during the redox reactions. Surface morphology of the modified electrode using scanning electron microscopy (SEM) which revealed that graphene and NF were coated on electrode. Differential pulse voltammetry (DPVs) was used for the determination of analytes. DPVs not only increased the electrocatalytic current linear concentration range, also lowered the overpotential to oxidation the interferences in the measurements. The Graphene exhibited a promising enhanced electrocatalytic activity towards analytes and enhances the loaded and stability. In this paper, the electrochemical oxidation of Cytosine (C), Thymine (T), Guanine (G) and Adenine (A) at same time.

Keywords: Cytosine (C), Thymine (T), Guanine (G), Adenine (A), Graphene and New Fuchsin (NF)

1. INTRODUCTION

DNA is the abbreviation for deoxyribonucleic acid, which is an important biological macromolecule that plays crucial roles in the storage of genetic information and protein biosynthesis

[1-3]. It is the genetic material present in the cells of all living organisms. DNA is the fundamental building block for an individual's entire genetic makeup. DNA is comprised of four building blocks called bases. Guanine (G), adenine (A), thymine (T) and cytosine (C) are purine and pyrimidine bases found in DNA molecular structure, which are involved in cellular energy transduction and signalling mediated by enzymatic oxidation reactions. These are commonly referred to as C, G, T, A. It is the order (sequence) of these building blocks that determines each person's genetic characteristics. Base pairs that match up in the pairing reactions in double helical nucleic acids (A with T in DNA or with U in RNA, and C with G) [4]. All mutations are changes in the sequence of DNA. One basic mutation in mitochondrial DNA has been suggested as a cause for heart and muscle disease. Our knowledge that breast and ovarian cancer is caused by genetic factors in a small percentage of the population is not new information but our discoveries of specific genetic mutations have occurred more recently-over the past two decades. Therefore, abnormal changes of DNA bases in organism suggest the deficiency of the immunity system and may indicate the presence of various diseases including cancer, Alzheimer's disease, epilepsy and HIV infection [5-6]. The detection of DNA bases is of great significance for clinical diagnosis as well as insight into fundamental mechanisms of genetic information [7-8]. Detecting about DNA mutations is important to help us discover ways to identify those most likely to develop the disease and then find appropriate ways to prevent or treat disease.

Several analytical methods have been developed to detect DNA bases [9-10], such as microchip capillary electrophoresis [11-13], flow injection chemiluminescence [14-15], ionpairing liquid chromatography [16-17], laser-induced fluorescence detection [18-19], and micellar electrokinetic chromatography [20-21]. Although these methods exhibit some merits and advantages, expensive instruments, complicated operations, or time-consuming sample pretreatments are usually involved [22]. Electrochemical techniques are promising for the analysis of DNA bases due to their advantages of rapidity, convenience, low cost and ease of miniaturization for small volume samples [23-24]. Among these techniques, DNA biosensor has emerged as a promising alternative for microbial detection due to the specificity of hybridization between the probe and the complementary target sequence [25]. However, the non-specific adsorption in the hybridization system increases the strong demand for development of a sensitive and specific DNA biosensor.

The electrocatalytic oxidation of purine bases has been extensively investigated in literature [26-29]. However, the electrochemical detection of pyrimidine bases was rarely studied. The traditional solid electrodes often suffer from fouling effects due to accumulation of oxidized products on the electrode surface, resulting in rather poor sensitivity and reproducibility [30-31]. The high oxidation potentials cause large background currents in blank solutions, which severely mask the peak currents and greatly influence their sensitive detection [32-33]. The electrochemical oxidation of pyrimidine bases is irreversible. It is difficult to obtain accurate oxidation signals of pyrimidine bases because of their extremely positive oxidation potentials and slow electron transfer kinetics [34-35]. In order to overcome these limitations, modified nanomaterials electrodes with a wide potential window, high electrocatalytic activity and excellent antifouling property are highly required.

Graphene have been used as hosts for biomolecule immobilization to improve the performance of molecule in various biocatalytic processes. They exhibit unique optical, electrochemical and catalytic properties, moreover, their small size allows the miniaturization of final sensors. It is evident

that, when nanostructure of conductive materials are used, the large surface area of these nanomaterials can increase the molecule loading and facilitate reaction kinetics, and thus improving the biocatalytic processes of biosensor. In addition, research efforts have also been made to improve the activity and stability of immobilized biomolecule by using nanostructures. For example, TiO_2 nanoparticles and Au nanoparticles modified electrodes were also used for the electrocatalytic determination of guanine and adenine [36-37]. Recently, various nanobiocatalytic approaches have been gaining more and more attention due to their successful results in biomolecule stabilization. It appears to be reasonable to us to expect that progress in nanostuctured biocatalysts will play a critical role in overcoming the major obstacles in the development of biosensor. Dye molecules for sensor devices exhibits interesting enhancement in the electrocatalytic activity towards the oxidation or reduction of several biochemical and inorganic compounds [38], where some of the groups in the dye will act as a catalyst [39-41]. In this, the word "enhanced electrocatalytic activity" could be explained as both; increase in peak current and lower overpotential [42]. Among these dye molecules, a group of them representing azines such as phenazines, phenothiazines, phenoxazines, etc., have a wide use in bioelectrochemistry as redox indicators and mediators [43]. Using these types of materials for the functionalization of graphene will lead to the construction of efficient electrochemical sensors.

In this work, we have exploited unique properties of New Fuchsin (NF) for modified electrodes. Graphene were simple and would be applicable to enhance the stability. Graphene is important for the development of new types of biosensor. The electrochemical oxidation of Cytosine (C), Thymine (T), Guanine (G) and Adenine (A) at same time.

2. EXPERIMENTAL

2.1. Materials

Graphene, New Fuchsin (NF), Cytosine (C), Thymine (T), Guanine (G) and Adenine (A) were purchased from Sigma-Aldrich (USA). All other chemicals used were of analytical grade and used without further purification pH 7.0 (0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4) Phosphate buffer solutions (PBS) was used as supporting electrolyte. Aqueous solutions were prepared using doubly distilled deionized water and then deaerated by purging with high purity nitrogen gas for about 20 min before performing electrochemical experiments. Also, a continuous flow of nitrogen over the aqueous solution was maintained during measurements. Indium tin oxide (ITO) (7 Ω/cm^{-2}) was purchased from Merck Display Technologies (MDT) Ltd (Taiwan).

2.2. Apparatus

Cyclic voltammetry (CVs) was performed in an analytical system model CHI-1205B and CHI-1205A, Differential pulse voltammetry (DPVs) were CHI-900 and CHI-410 potentiostat. A conventional three-electrode cell assembly consisting of an Ag/AgCl reference electrode and a Pt wire counter electrode were used for the electrochemical measurements. The working electrode was glassy

carbon electrode (GCE; area 0.07 cm^2). In these experiments, all the potentials have been reported versus the Ag/AgCl reference electrode. The morphological characterizations of the films was examined by means of scanning electron microscopy (SEM) (Hitachi S-3000H). Electrochemical impedance spectroscopy (EIS) measurements were performed using an IM6ex Zahner instrument (Kroanch, Germany). All the experiments were carried out at room temperature (≈ 25°C).

3. RESULTS AND DISCUSSIONS

3.1. Electrochemical characterizations of grapene/New Fuchsin (G/NF) modified electrodes

3.1.1 Electropolymerization of NF with graphene

There was an important challenge in the preparation of graphene. Because of its hydrophobic nature, it was difficult to disperse it in any aqueous solution to get a homogeneous mixture. This was done by weighing 100 mg of graphene dissolved in 10 ml of double distilled deionizedwater for 6 h to get uniform dispersion. This functionalization process of graphene was done to get hydrophilic nature for the homogeneous dispersion in water.

The cleaned GCE was coated with 4 μL of graphene and the solvent allowed evaporating at room temperature. The electropolymerization of 0.1 mM New Fuchsin (NF) was done by electrochemical oxidation on the graphene modified GCE in pH 7.0 PBS. It was performed by consecutive CVs over a suitable potential range of -1.2 to 0.9 V; scan rate = 50 mV s^{-1}. The optimization of poly- New Fuchsin growth potential has been determined by various studies with different electropolymerization potentials.

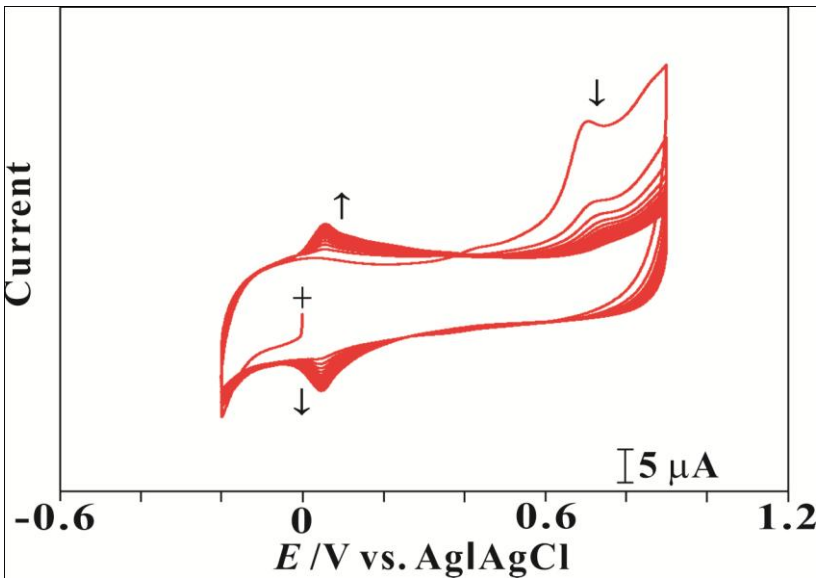

Figure 1. Cyclic voltammetry of graphene (G) modified electrodes was immersed pH 7.0 PBS containing 0.1×10^{-3} M of new fuchsin (NF) at the potential range of -0.2 to 0.9 V, scan rate 50 mV/s for 40 cycles.

Fig. 1 the growth in the CVs current showed that the redox couple occurred at a formal potential of $E^{0'}$ = 0.05 V (vs. Ag|AgCl), indicated that film formation occurred, and that this was enhanced by the graphene on the modified electrode surface. Electrochemical characterizations of monomeric NF showed the formal potential of $E^{0'}$ = 0.7 V (vs. Ag|AgCl). Electropolymerization of NF showed higher peak current in first cycle at o.7 V, the poly-NF peak currents of the film redox couples which have increased with the decrease of monomer NF.

3.1.2 Different pH studies of G/NF modified electrodes

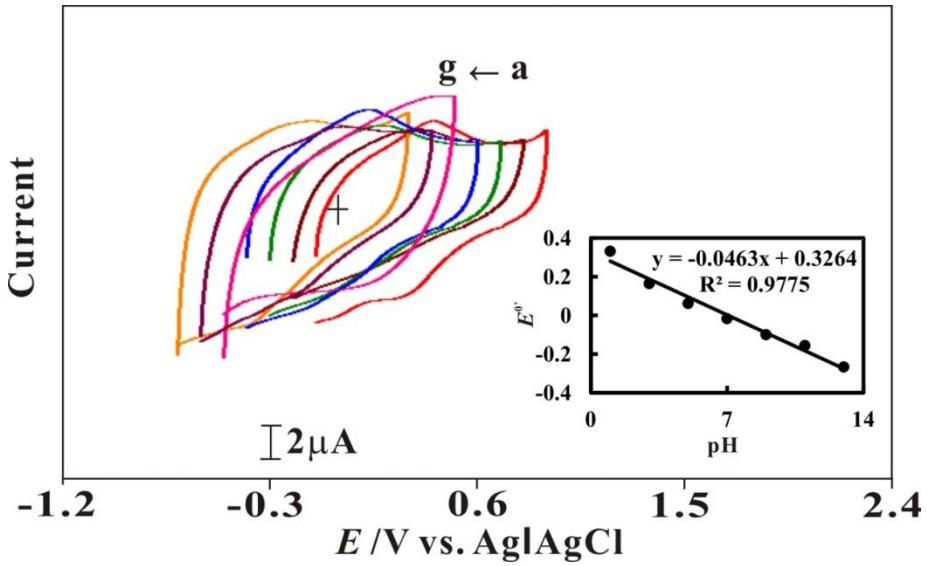

Figure 2. Cyclic voltammetry of G/NF modified electrodes in different pH (a) 1.0, (b) 3.0, (c) 5.0, (d) 7.0, (e) 9.0, (f) 11 and (g) 13, scan rate 100 mV/s. The inset showed the potential of G/NF modified electrodes plotted over a pH range from 1.0 to 13.

Fig. 2 showed the cyclic voltammetric of G/NF modified electrodes obtained in pH 7.0 PBS, then washed with deionized water and was transferred to various pH aqueous buffer solutions. This showed that the film is highly stable in the pH range between 1.0 to 13 (curve a to g). The values of E_{pa} and E_{pc} depends on the pH value of the buffer solution. The inset showed the potential of G/NF modified electrodes plotted over a pH range from 1.0 to 13. The response shows a slope of -46 mV/pH, which is close to that given by the Nernstian equation for equal number of electrons and protons transfer [44-45]. The values of $E^{0'}$, which depend on the pH, also show that the redox couple of the polymeric film includes proton transfer in the reduction and oxidation processes. The chemical composition and possible electropolymerization of a poly(NF) film is analogous to that of polyaniline and its analogues [46-47].

3.1.3 Different scan rate studies of G/NF modified electrodes

Fig. 3 showed that the G/NF film on a glassy carbon electrode had one chemically reversible

redox couple at 0.05 V in the pH 7.0 when cyclic voltammetry PBS was performed at different scan rates (50 to 2500 mVs^{-1}).

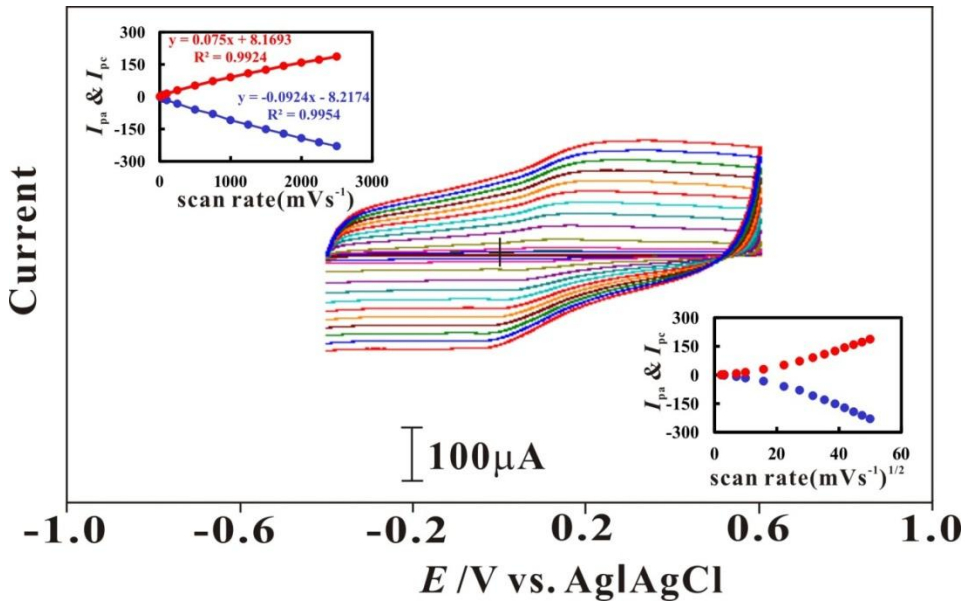

Figure 3. Cyclic voltammetry of G/NF modified electrodes was immersed pH 7.0 PBS at the potential range of -0.4 to 0.6 V, scan rate from 50 mV/s to 2500 mV/s. The inset showed I_{pa} & I_{pc} vs. scan rate and I_{pa} & I_{pc} vs. (scan rate)$^{1/2}$.

The anodic and cathodic peak currents of both the film redox couples which have increased linearly with the increase of scan rates. The inset calibration curve for data in Fig. 3 showed I_{pa} & I_{pc} vs. scan rate and I_{pa} & I_{pc} vs. (scan rate)$^{1/2}$. The ratio of I_{pa}/I_{pc} from the inset has demonstrated that the redox process has not been controlled by diffusion. This behavior perhaps occurs because of a reversible electron transfer process involving the poly(NF) on the graphene layer, with a proton exchange process occurring along with the electron transfer process. However, the ΔE_p of each scan rate reveals that the peak separation of composite redox couple increases as the scan rate is increased.

3.2. Electrochemical impedance spectra (EIS) of analysis

Electrochemical impedance spectra (EIS) was applied to monitor the whole process of the electrode modification. EIS can give useful information of the impedance changes on the electrode surface between each step. The EIS includes a semicircular part and a linear part. The semicircular part at higher frequencies corresponds to the electron transfer limited process and the diameter is equivalent to the electron transfer resistance (R_{ct}). The linear part at lower frequencies corresponds to the diffusion process. During the fabrication, significant differences were observed. Fig. 4 showed the results of EIS for a bare GCE and other three modified electrodes in the presence of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$ in pH 7.0 PBS. R_{ct} of a bare GCE is 1319.1 Ω (curve c). The GCE was modified with NF, R_{ct} value was increased markedly to 3352.9 Ω (curve d). EIS results for the electrode modified

with the G/NF was shown in curve a and R_{ct} was considerably decreased to 105.5 Ω. These results confirmed that the G/NF film was successfully immobilized on the GCE surface. Only graphene R_{ct} was 200.64 Ω (curve b), from these observations, we can conclude that the grapheme were highly conductive and expected as a good platform for sensing applications. The insert displayed the equivalent circuit (Randles model) was used to fit Nyquist diagrams.

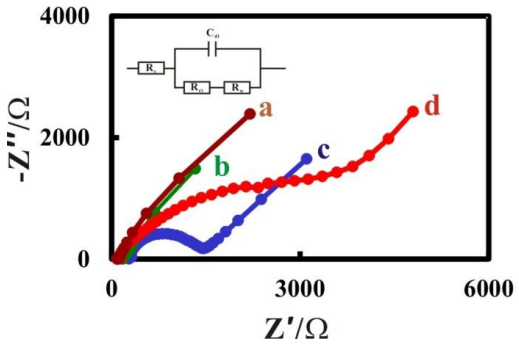

Figure 4. Electrochemical impedance spectroscopy (EIS) for (a) G-NF/GCE, (b) G/GCE, (c) Bare GCE and (d) NF/GCE in the presence pH 7.0 PBS of equimolar 5 mM $[Fe(CN)_6]^{3-/4-}$. The insert displayed the equivalent circuit (Randles model) was used to fit Nyquist diagrams.

3.3. Morphological characterization of G/NF modified electrodes

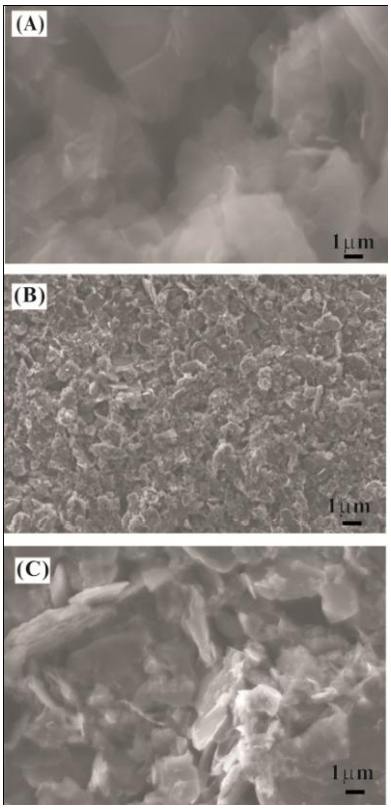

Figure 5. Scanning electron microscope (SEM) of (a) G/ITO, (b) NF/ITO and (c) G-NF/ITO.

Fig. 5 represents the top view SEM images of different films coated on ITO surfaces taken at a resolution. In prior to modification, ITO surfaces were cleaned and ultrasonicated in acetone–water mixture for 15 min and then dried. Graphene and NF have been prepared on the ITO electrode. From Fig. 5, it is significant that there are morphological differences between both the films. The top views of nanostructures Fig. 5 (A) on the ITO electrode surface showed only graphene on this electrode. Graphene showed thin, uniform, chippy contours. Poly-NFshowed in Fig. 5 (B), indicated that electropolymerization of monomer NF to polymer. The G/NF film in Fig. 5 (C) reveals that the NF had covered the entire graphene to form G/NF modified electrode. We can clearly see that the immersed G/NF have been gathered together and resemble like a sliced one, respectively.

3.4. Electrocatalytic reaction of DNA bases at G/NF modified electrode

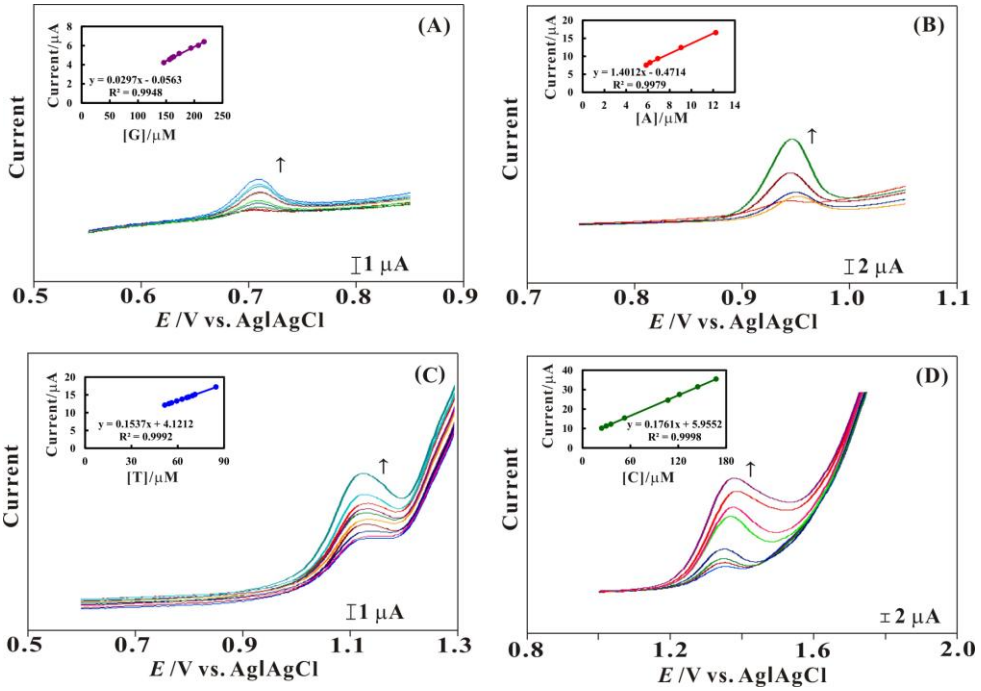

Figure 6. Differential pulse voltammetry (DPVs) of G/NF modified electrodes in different concentration of (A) guanine, (B) adenine, (C) thymine and (D) cytosine. The inset showed different concentration vs. current.

The Differential pulse voltammetry (DPVs) of had been obtained different concentrations of (A) guanine, (B) adenine, (C) thymine and (D) cytosine at G/NF modified electrode in 0.1 M PBS (pH 7.0) the potential range of 0.5 to 1.8 V, as shown in Fig. 6. The DPVs had been recorded at a constant time interval of 2 min with nitrogen purging before the start of each experiment. The electrocatalytic oxidation of guanine at 0.71 V, the sensitivity and correlation coefficient were calculated to be 0.424 μA μM^{-1} cm^{-2} and 0.9948, respectively. From the same results, limit of detection (LOD) was calculated to be 19 μM at a signal-to-noise ratio of 3. On the addition of analytes a new growth in the oxidation peak appeared at E_{pa} = 0.95 V for adenine (Fig. 6B). The sensitivity, correlation coefficient and LOD were calculated to be 20.02 μA μM^{-1} cm^{-2}, R^2= 0.9979 and 0.9 μM. In the above

electrocatalysis experiment, an increase in concentration of thymine simultaneously produces a linear increase in oxidation peak current at 1.11 V with good stability showed in Fig. 6 (C). The sensitivity, correlation coefficient and LOD were calculated to be 2.195 μA μM^{-1} cm^{-2}, R^2=0.9992 and 9.9 μM. From the slopes of linear calibration curves (Fig. 6 D), cytosine of sensitivity, correlation coefficient and LOD were calculated to be 2.16 μA μM^{-1} cm^{-2}, R^2=0.9998 and 9.9 μM, respectively. These results can be observed from the I_{pa} and E_{pa} values, where the increase in peak current and lower overpotential both are considered as the electrocatalysis [48].

3.5. Differential pulse voltammetry (DPV) of the mixture analytes

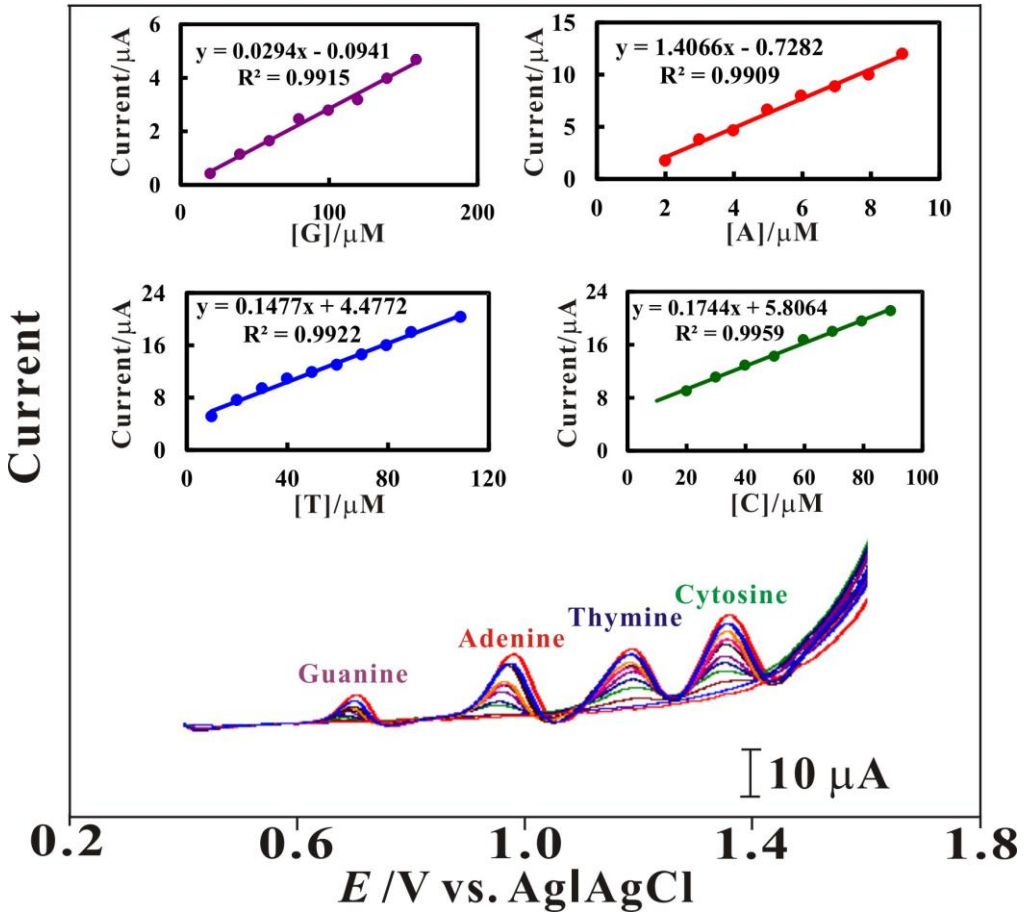

Figure 7. Differential pulse voltammetry (DPVs) of G/NF modified electrodes in different concentration of mixture analyte (guanine, adenine, thymine and cytosine). The inset showed different concentration vs. current

The DPVs have been recorded at a constant time interval of 2 min with nitrogen purging before the start of each experiment. The DPVs have been obtained different concentrations of mixture analyte (guanine, adenine, thymine and cytosine) at G/NF modified electrode, as showed in Fig. 7. Simultaneous change the concentrations of analyte mixture accumulative at G/NF modified electrode. Interestingly, the peak currents for mixture analyte increases linearly with the increase of analyte concentration. They demonstrate the calibration curves for analyte, which are almost linear for a wide

range of concentrations as shown in inset. All these values showed higher efficiency of the G/NF towards the analytes when comparing only graphene and NF. Here also the G/NF modified electrode successfully exhibits four well separated electro oxidation peaks for the detection of guanine, adenine, thymine and cytosine. This peak separation is sufficient enough for the selective determination of mixture. Oxidation peaks of guanine, adenine, thymine and cytosine were 0.71V, 0.99 V, 1.19 V and 1.37 V.

4. CONCLUSIONS

We have demonstrated application of the graphene - New Fuchsin (G-NF) modified electrode for determination of DNA bases. High sensitivity and stability together with very easy preparation G-NF/GCE as promising candidate for constructing simple electrochemical sensor. This feature provides a favorable clinical diagnosis for the electroanalytic oxidation of Cytosine (C), Thymine (T), Guanine (G), Adenine (A) at G-NF/GCE. The SEM results have shown the difference type morphological data. The experimental methods of Differential pulse voltammetry (DPVs) with biosensor integrated into the G-NF/GCE which was presented in this paper, provide an opportunity for qualitative and quantitative characterization, even at physiologically relevant conditions. Therefore, this work establishes and illustrates, in principle and potential, a simple and novel approach for the development of a biosensor.

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