Electrochemical Characterization of Purines Over Multi-walled Carbon Nanotubes Modified Graphite Electrode

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***Abstract*— Electroactive purine nucleobases, guanine and adenine, purine nucleosides, guanosine and adenosine were immobilized over multi-walled carbon nanotubes modified graphite electrode. The electrooxidation properties of purines were evaluated using differential pulse voltammetry. Mixtures of purine nucleobases and nucleosides in various concentrations were prepared and electrochemically immobilized over the working electrodes using positive potential difference. The anodic current at around 0.7 and 1.0 V were used as analytical signal for guanine and adenine oxidation respectively. The influence of immobilization time, MWCNT concentration and purine concentration were evaluated and electrochemical mechanisms have been discussed. Special emphasis was given to study the stable recognition layer in a redox couple (0.1M NaCl containing 10/10mM $K_3Fe(CN)_6/K_4Fe(CN)_6$) using electrochemical impedance spectroscopy and cyclic voltammetry. Further, electrochemical interaction of immobilized purine structures over benzene substituted derivatives were studied using DPV in 0.1M phosphate buffer, and CV and EIS in 0.1M NaCl containing 10/10mM $K_3Fe(CN)_6/K_4Fe(CN)_6$. The standardized and calibrated purine immobilized electrode could be potentially used as purine based biosensor for the electrochemical detection of benzene substituted organic compounds**.

Keywords- Benzene substituted organic compounds, Cyclic Voltammetry, Differential Pulse Voltammetry, Electrochemical Impedance Spectroscopy, Multi-walled carbon nanotubes, Purine based biosensor.

I. INTRODUCTION

Purine nucleobases, guanine and adenine are one of the basics of nucleic acid structure of DNA of living organisms. M. E. A. Down and his co-workers [1] reported the optical and electrochemical properties of purine bases in nucleic acids. Since then interest has been generated among the electrochemist to explore the electrochemical characteristics of purine bases and its applications as a rapid, cheap and selective biosensor. Purine nucleobases and DNA strands have been reported to be immobilized over noble metals and carbon based electrodes. Initially, wide research has been performed in electrochemical immobilization of graphite and glassy carbon. In the later stage, focus were on the immobilization of purine bases over the modified electrode using metal complexes [2], PANI [3], MWCNT [4] etc. to obtain better efficiency. Significant enhancement in the oxidation signals of purine bases over MWCNT coated carbon based electrodes have been reported due to its unique electrical property and high surface area.

In order to use the immobilized purine nucleobases analytically as a biosensor, it is necessary to study the electrical processes that occur at the surface of the sensor. Out of the various techniques available, voltammetry and electrochemical impedance spectroscopy are the promising technique to study the immobilized purine bases. The electrochemical oxidation of guanine and adenine at the electrode surface can be studied using differential pulse voltammetry. The film forming abilities of purine nucleobases at the electrode surface can be studied from the diffusion of the redox ions from the electrolyte to the electrode surface using EIS and CV. In this report, the immobilized purine bases over MWCNT modified graphite electrode were studied using DPV, EIS and CV and further its interactions with benzene substituted organic compounds were analyzed.

II. EXPERIMENTAL

A. Reagents

Guanine and adenine were purchased from Sisco Research Laboratories, Maharashtra, India. Graphite rods were purchased from HomeScience Tools, Montana, USA. The procured rods were cut into 5 equal sizes and rubbed over micro alumina slurry for several minutes until a smooth surface of diameter 0.636 cm was obtained.

In order to favor the electrical connection, conducting wire of equal length were pasted at the side of the sliced graphite rods using silver paste. It was then coated with teflon leaving the bottom surface for it to act as sensor after modifications. MWCNT were obtained from Applied Science Innovations PVT. Ltd, Maharastra, India. Double distilled water was used throughout the experiment. All the other chemicals were from Sisco Research Laborotaries and were used without any further purification. DPV measurements were carried in 0.1M phosphate buffer at pH 7. CV and EIS measurements were carried out in 0.1M NaCl solution containing 10/10mM $K_3Fe(CN)_6/K_4Fe(CN)_6$. Stock solutions of guanine and adenine were prepared by dissolving its appropriate amount in 0.1M HCl and later diluting it with water. Solutions of benzene substituted organic compounds were prepared immediately before each experiment.

B. Apparatus

All the electrochemical measurements were recorded using the instrument SP-300 from Biologic Science Instrument, France, running on EC-Lab Software (Version 10.18) and with standard calomel electrode as reference electrode, platinum wire as counter electrode and graphite electrode (surface area = 0.318 cm^2) as working electrode. All the electrochemical measurements were conducted in a 20ml cell containing 15ml of the supporting electrolyte.

C. Preparation of Modified Electrodes

Prior to surface modification, graphite electrode was cleaned by polishing with 0.05µm alumina cloth for 1 minute and sonicated in water for 30s. MWCNT were sonicated with concentrated nitric acid for 30 minutes. The modified electrode was prepared by casting desired quantity of MWCNT paste over graphite electrode and allowed it for dryness at room temperature. The resulting electrodes were named as MWCNT/G. These electrodes can be reused by rubbing it over 0.05µm alumina cloth until a smooth polished surface is obtained.

D. Immobilization of Purine Bases

The electrode surface was pretreated by applying a potential of +1.5V for 30s in 0.1M phosphate buffer (pH 5) to remove electrochemical impurities. Purine based biosensor was developed by immobilizing purine nucleobases and nucleosides separately, at fixed potential (+0.3V versus calomel/platinum electrode for 180s). During immobilization step, the electrode was immersed in 0.1M Phosphate buffer (pH 7) containing desired quantity of purines nucleobases and nucleosides. After immobilization, the electrode was washed with distilled water to remove the unbounded purines. The resulting electrodes were named as PN/MWCNT/G and PS/MWCNT/G for purine nucleobases and nucleosides respectively.

E. Electrochemical Measurements

The electrochemical properties of modified electrode were studied by cyclic voltammetry (potential from 0 to +1.5V at scan rate of 50mV/s) in 0.1M phosphate buffer solution. 10/10mM solution $K_3Fe(CN)_6/K_4Fe(CN)_6$ was used as redox probe to study the interface properties of the electrode immobilized with purine bases. 0.1M phosphate buffer solution (pH 7) was used as supporting electrolyte. Electrochemical impedance measurements were performed at open circuit potentials in 10mM $K_4Fe(CN)_6$ + $K_3Fe(CN)_6$ +0.1M NaCl (pH 7) solutions. The electron transfer resistance was obtained through the non-linear regression analysis of the semicircle portion on the Nyquist plot (Zim vs. Zre). CV measurements were performed within the potential range from 0.7 V to -0.7 V at scan rate of 50mV/s in the same electrolyte solution containing redox couple. Three replicate measurements were carried out for each experiment to maintain concordance.

F. Electrochemical Determination of Aromatic Compounds

DPV and EIS of the purine bases immobilized over modified electrodes were obtained by following the procedure as described above. Then the electrode was immersed in the solution containing various aromatic compounds for 5 minutes for the purine bases in the electrode to react with aromatic compounds. DPV and EIS measurements before and after the interaction with benzene substituted organic compounds were carried out. The relative percentage of survived purines after the analyte's interaction was calculated from the change of signals obtained at electrode with and without purine bases related to the difference of signals corresponding to original purine bases as follows:

$$\Delta I_{surv\ PN\ (rel)}\% = [(I_{surv\ purines} - I_{MWCNT})/(I_{DNA} - I_{MWCNT})]*100 \quad (1)$$

$$\Delta R_{ct(rel)}\% = [(R_{ct(surv\ PN)} - R_{ct(MWCNT)})/(R_{ct(PN)} - R_{ct(MWCNT)})]* 100 \quad (2)$$

Where I is the anodic peak current measured during DPV measurement in 0.1M phosphate buffer at the modified electrode without purines and R_{ct} is the electron transfer resistance at EIS measured at the peak potential obtained for 10/10mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1M NaCl solutions at the modified electrodes without purine bases. The indexes used, characterize the chemical modifiers of graphite electrode [5].

III. RESULTS AND DISCUSSION

A. Effect of immobilization time, immobilization concentration and MWCNT concentrations

The amount of purine nucleobases adsorbed over the working electrode is directly proportional to its sensitivity. The increase in quantity of MWCNT provided a greater surface area for the purine bases to immobilize over the electrode surface. This enhances the direct electrochemical response of purine bases and is in consistent with reported work [6]. Hence it is necessary to optimize the minimum quantity of MWCNT needed to immobilize the known maximum concentration of purine bases for a particular electrode surface area. The responses were recorded for MWCNT in 60mg/l of purine nucleobases mixtures for 180s in 0.1M phosphate buffer (pH 7). Saturation peak was obtained for 1.25mg/ml of MWCNT concentration. Immobilization step was performed by applying a potential of +0.3V in 0.1M phosphate buffer (pH 5) for varying time upto 300s. As the immobilization time increases, the corresponding sensor signals for nucleobases increased as expected. Longer the immobilization time, greater the quantity of purine bases adsorbed and hence larger the DPV response. It was found that after a certain immobilization time (180s), the peak current almost remained to be stable, as the purine bases occupied the entire working electrode surface area leaving no space for the remaining purines in the buffer to get adsorbed. This is consistent with the earlier findings [7]. The amount of purine concentration immobilized over working electrode containing 1.25mg/ml MWCNT was varied from 10 to 100 mg/l for 180s at a potential difference of +0.3V. The oxidation peak for purine nucleobases almost remained stable for the immobilization concentration from 60 to 100 mg/l. It was noticed that the guanine and guanosine oxidation peak area is greater than adenine and adenosine oxidation peak area as the working electrode surface is first occupied by guanine and guanosine respectively [8, 9]. However, it can be noted that the peak potential of purine nucleosides are less when compared to purine nucleobases. The oxidation peaks of purine nucleosides are wider when compared to purine nucleobases due to the presence of ribose structure in purine nucleosides.

B. Electrochemical oxidation of Purine Nucleobases and Nucleosides

The electrochemical oxidation of purine nucleobases and nucleosides at the modified graphite electrode followed a two step mechanism. This involves a total loss of four electrons and the loss of first two electrons is the rate determining oxidation reaction. The oxidation of guanine and guanosine resulted in 8-oxo-guanine and 8-oxo-guanosine respectively and the oxidation of adenine and adenosine resulted in 2,8-dioxy-adenine and 2,8-dioxy-adenosine [10].

C. Electron transfer characteristics at the electrode surface

In order to study the interfacial electron transfer properties of the modified electrode immobilized with purine nucleobases, EIS and CV were performed using the electroactive ferrocyanide/ferricyanide redox couple in 0.1M NaCl solution. Nyquist plot of the working electrodes displays a semicircle at high frequencies and it is linear at low frequencies. MWCNT coated graphite electrode shows a small semicircle diameter indicating excellent conductivity of MWCNT. However, on the addition of purine bases, the electron transfer resistance increases but not greater than the electron transfer resistance of bare graphite electrode (Fig. 1). For bare graphite, a short linear part of low frequencies are observed resulting from the diffusion of limiting step of the electrochemical process. The impedence data were simulated using the Randles equivalent circuit consisting of a parallel combination of the capacitance (C_{dl}) and the charge transfer resistance (R_{ct}) redox reactions in series with the supporting electrolyte resistance (R_{sol}).

The increase or decrease in Rct reflecting the increase or decrease in the diameter of the semicircle is directly associated with the blockage behavior of the electrode surface for the charge transfer to the redox couple in the supporting electrolyte [5]. For bare graphite, the value of R_{ct} is 70.38Ω and it reflects the semicircle part with greater diameter. As MWCNT is introduced to the graphite electrode, the diameter of the semicircle portion decreases, decreasing the Rct value till 13.39 Ω. As purine bases are introduced to the modified electrode, the charge transfer resistance of the redox probe increases. The electron transfer resistance of purine nucleosides (25.14Ω) is slightly greatly than purine nucleobases (21.6Ω). This could be due to the presence of ribose in the nucleosides resulting in decrease of electron transfer from the redox couple to the electrode.

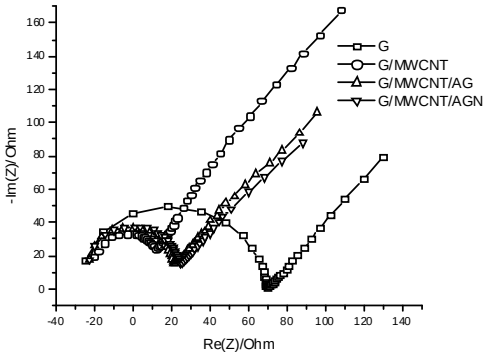

Fig. 1: Nyquist plot for modified graphite electrodes in 0.1M NaCl.

TABLE 1: PARAMETERS OF THE EQUIVALENT CIRCUIT SIMULATING THE COMPLEX IMPEDANCE SPECTRA OF THE ELECTRODES IN THE PRESENCE OF 0.1M NACL SOLUTION. R_{SOL}- RESISTANCE OF THE SUPPORTING ELECTROLYTE, R_{CT}- CHARGE TRANSFER RESISTANCE, C_{DL}-CAPACITANCE.

Working Electrode	R_{sol}, Ω	R_{ct}, Ω	C_{dl}, µF
G/G	-24.53	70.38	0.77
MWCNT/G	-20.01	13.39	1873.6
PN/MWCNT/G	-22.30	21.60	277.2
PS/MWCNT/G	-21.75	25.14	27.4

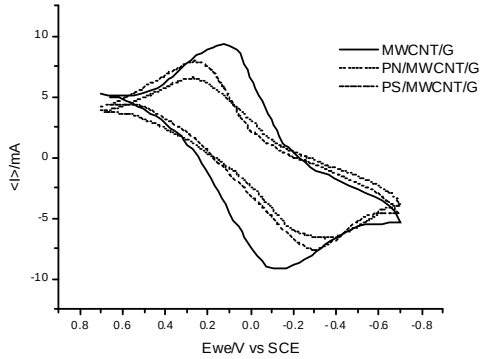

Fig. 2: CV of the redox couple for the purine immobilized MWCNT modified graphite electrode

TABLE 2: CV PARAMETERS OF MODIFIED ELECTRODE IN 10MM $K_4FE(CN)_6$ + $K_3FE(CN)_6$ +0.1M NACL (PH 7) ELECTROLYTE. ΔEP- ANODIC TO CATHODIC PEAK POTENTIAL DIFFERENCE, IA/IC- ANODIC TO CATHODIC PEAK CURRENT RATIO

Modified electrode	ΔEp, V	Ia/Ic
MWCNT/G	0.236	1.01
PN/MWCNT/G	0.571	1.04
PS/MWCNT/G	0.589	1.05

To confirm EIS, CV was performed in the same supporting electrolyte. The mechanism of purine bases detection using $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ resides in the barrier effect of the purine bases towards the redox couple [11], resulting in the reduction in redox couple signal (Fig. 2) after the addition of purine bases to the modified electrode. The addition of MWCNT to the graphite electrode has increased the electrochemical reversibility of the redox couple. The addition of purine bases increases the peak potential difference and decreased the cathodic current. The ΔEp of purine nucleobases immobilized electrode is slightly lesser than the the ΔEp of purine nucleosides. This is due to the presence of ribose sugar which significantly block the redox ion transfer. This is in concordance with the result obtained for DPV in 0.1M phosphate buffer. Hence the presence of other blockage compound places a major role in the transfer of electrons to the redox couple. In the case of MWCNT modified graphite electrode, the anodic to cathodic peak current was 1.01 confirming the reversibility of the redox probe. The presence of purine nucleobases and nucleosides, increases the peak potential value and decreased the cathodic current value and hence increasing Ia/Ic ratio. The peak potential difference and anodic to cathodic peak current ratio for various modified electrodes are summaried in Table 2.

D. Electrochemical determination of benzene substituted derivatives

Purine nucleobases and nucleosides were attacked separately by exposing the modified electrodes to benzene substituted organic compounds. Biosensors of equivalent mixtures of purine nucleobases and nucleosides were prepared separately using concentration of 60 mg/l and were placed in contact with the analytes. Changes in purine oxidation and electron transfer resistance of the redox probe after the sensor exposure to 100ng/l of the analyte is reprensented in Fig. 3. As expected the survived purine were almost similar. This shows that the changes in the oxidation of the purine at the electrode surface is directly associated with the film forming abilities and electron transfer characteristics of the redox probe.

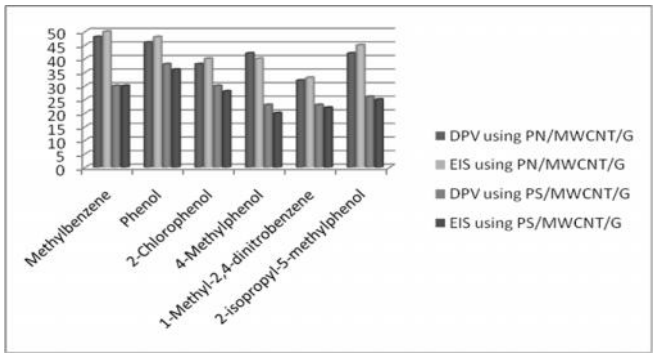

Fig. 3: Relative percentage of the charge transfer resistance and current value obtained for the purine immobilized modified graphite for 100ng/l of benzene substituted organic compound concentration.

IV. CONCLUSIONS

MWCNT paste over the graphite electrode has ensured a good detection window for the voltammetric and impedimetric evaluation of the presence of purine nucleobases and nucleosides. This is based on the oxidation profile obtained from DPV, the increase in the charge transfer resistance measured in EIS and the decrease in cathodic current due to the decrease of the voltammetric current of the negatively charge redox probe ($[Fe(CN_6)]^3$-) for the purine immobilized graphite modified electrode. It was demonstrated that the proposed EIS and DPV procedures can be used for the detection of benzene substituted organic compounds.

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