Characterization of Purine Based Biosensor for Electrochemical Determination of Benzene and its Derivatives

Baby Sakthi Gayathri, and Kamaraj Palanisamy

***Abstract*—**The purine nucleosides, guanine and adenine and purine nucleotides, guanosine and adenosine, the electroactive compounds get oxidized over carbon electrodes. All the four compounds were known to react with benzene and its derivatives forming its adducts. The formation of these adducts were electrochemically monitored using Multi-walled carbon nanotube coated graphite electrodes from the change in oxidation signals. Mixtures of purine nucleosides and nucleotides in various concentrations were prepared and electrochemically immobilized over the working electrodes using positive potential difference. Electrolyte containing redox couple was used to study the film forming abilities of the working electrode using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). The standardized working electrode was exposed to various concentrations of benzene and its moni-, di- and poly- substituted derivatives. Differential Pulse Voltammetry (DPV) was performed to study the oxidation signal of guanine and adenine before and after its interaction with analyte. The anodic current at around 0.6 and 1.0 V was used as analytical signal for guanine and adenine oxidation respectively. Caliberation curves were obtained for the working electrodes in various analytes usinf DPV and the detection limit was found to be 10ng/ml for benzene and 30ng/ml for benzene derivatives.

***Keywords*—**Benzene, Cyclic Voltammetry, Differential Pulse Voltammetry, Electrocemical Impedance Spectroscopy, Purine Based Biosensor.

I. Introduction

Down and his co-workers [1] reported the optical and electrochemical properties of purine bases in nucleic acids. Nucleic acids are polymeric macromolecule, forming basic functional unit behind each living cell's activity. There are five nucleic acid bases namely guanine, adenine, cystein, thymine and Uracil present universally in living organisms. Out of these five bases, guanine and adenine are known purines bases. Purine is a heterocyclic compound consisting of a pyramidine ring fused to an imidazole ring. Guanine is formed when an amino group and an oxygen atom added to 2nd and 6th carbon atom of the pyramidine ring in purines, named guanine nucleosides. Adenine is formed when an amino group is added to the 6th carbon atom of the pyramidine ring in purine. When purine nucleoside is attached to ribose ring via β-N9-glycosidic bond, it forms purine nucleotides, guanosine and adenosine [2].

Benzene and few of its substituted derivatives were known carcinogen and few of its substituted derivatives were known to have pharmaceutical applications. In both the scenario, benzene and its substituted derivatives were known to react with purine nucleobases [3, 4]. The electrochemical behavior of purines and its property to react with benzene and its derivatives have been exploited for its application in electrochemical determination of benzene and its derivatives using purine based biosensor.

II. Experimental

A. Reagents

Guanine, adenine, guanosine and adenosine 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 powder for several minutes until a smooth surface of diameter 0.636cm was obtained. In order to make electrical contact, conducting wires 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 of (30±15) nm diameter and length of several microns were obtained from Applied Science Innovation Pvt. Ltd, Maharashtra, India. MWCNTs were oxidized using concentrated nitric acid by sonicating it for 30 minutes, in order to remove impurities. After which, the suspension was washed several times with water to remove trace amount of nitric acid in the nanotubes. Mono sodium phosphate and di-sodium phosphate were obtained from Merck, NJ, USA. Double distilled water was used throughout the experiment. All other chemicals were obtained from Sisco Research Laborotaties and were used without any further purification.

DPV measurements were carried out in 0.1M Phosphate buffer. CV and EIS measurements were made 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 in appropriate amount in 0.1M HCl and later diluting it with water to desired concentration. Solutions of benzene and its

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derivatives were prepared immediately before each experiment.

B. Apparatus

All the electrochemical measurements were recorded using BioLogic Science SP-300 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 = 0.318cm^2) as working electrode. Calomel electrode used in this experiment has 0.241V (electrode surface area= 0.001cm^2) as offset potential against normal hydrogen electrode. All the potentials were measured with reference to reference electrode. All the electrochemical measurements were made using 20ml cell containing 15ml of supporting electrolyte.

C. Preparation of Modified Electrodes

Prior to surface modification, graphite electrode was cleaned by polishing with 0.05µm alumina powder for 1 minute and sonicated in water for 30s. 1.25gm of oxidized MWCNTs was dispersed in 1ml of 1% V/V acetic acid solution by sonication for 30 minutes. The modified electrode was prepared by casting desired quantity of MWCNT paste over graphite electrode. The resulting electrode was named as MWCNT/G, which can be stored at 4°C for further use. These electrodes can be reused by rubbing it over 0.05µm alumina powder until a smooth polished surface is obtained.

D. Immobilization of purine nucleobases

The electrode 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 nucleosides and purine nucleotides separately 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 purine nucleobases. After immobilization step, the electrode was washed with water to remove unbounded purines and preserved at 4°C for further use.

E. Voltammetric Measurements

The electrochemical properties of modified electrode were studied by cyclic voltammetry (CV conditions: Potential from -0.7 to +0.7V at scan rate of 50mV/s) and electrochemical Impedence spectroscopy (EIS conditions: Frequency scan range from 0.1Hz to 1MHz and sinusoidal potential amplitude at 10mV in 51 frequency steps). 10/10mM solution of $K_3Fe(CN)_6/K_4Fe(CN)_6$ in 0.1M NaCl solution was used as redox probe to study the interfacial properties of the modified electrode immobilized with purine bases.

Electrochemical detection of Benzene and its derivatives were determined from the change in the oxidation peak obtained from guanine and adenine before and after its reaction with these compounds using Differential Pulse Voltammetry (DPV conditions: potential increase of 0.04V, pulse amplitude of 0.05V, pulse width of 0.017s and pulse period 0.2s). The anodic current at around 0.6 and 1.0 V were used as analytical signal for guanine and adenine oxidation respectively.

F. Electrochemical determination of Benzene and its derivatives

DPV of the purine bases immobilized over modified electrodes were obtained by following the procedure as described above. In order to study the electrochemical damage of the purine nucleosides by benzene and its derivatives, the electrode was immersed in the solution containing various concentrations of the analytes for 5 minutes, for the purine bases in the electrode to react with them. DPV after the interaction with the analytes was carried out. Survived purine base after the interaction was calculated as follows:

$$S_G = 1 - (GPA_s/GPA_b) \quad (1)$$

$$S_A = 1 - (APA_s/APA_b) \quad (2)$$

Where, S_G and S_A is the survived guanine and adenine base respectively. GPA_s and APA_s are the guanine and adenine peak area after the interaction with the analyte. GPA_b and APA_b are the guanine and adenine peak area after the interaction with the buffer solution [5].

III. Results and Discussion

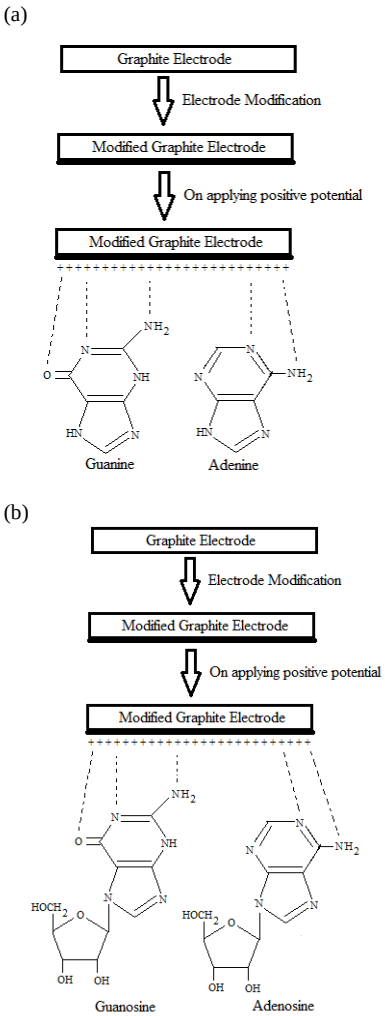


Fig. 1 Schematic representation of (a) Purine nucleoside based biosensor and (b) Purine nucleotide based biosensor preparation

Purine nucleoside based biosensors with the layers of guanine and adenine immobilized over MWCNT at the graphite surface have been investigated. Amount and concentration of MWCNT were optimized from the obtained electrochemical responses.

Figure 1 displays the schematic representation of the preparation of purine based biosensor. Electrochemical pretreatment was performed by anodization at 1.5V for 30s (versus standard calomel/platinum reference electrode) in order to electrochemically activate the working electrode and to remove electrochemical impurities at the electrode surface. However the responses of the electrochemically activated working electrode depend on the experimental parameters such as the potential limits, redox reaction time, composition, concentration and pH of the supporting electrolyte. This pretreatment procedure was found to improve the hydrophilic character of the electrode surface [6].

A. Effect of MWCNT concentration

The oxidation peaks of purine nucleotides are wider when compared to purine nucleosides. Moreover the oxidation reaction of guanine and adenine initiates at 0.55±0.02V and 0.85±0.02V respectively and for guanosine and adenosine it initiates at 0.50±0.02V and 0.80±0.02V respectively for 60mg/l of purine nucleobases in modified electrode containing 1.25mg/ml MWCNT. This could be due to the presence of ribose structure which initiates the oxidation. However, it can be noted that the peak potential of purine nucleotides are less when compared to purine nucleosides (Fig. 2).

The increase in the 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 [7]. Hence it is necessary to optimize the minimum quantity of MWCNT needed to immobilize the known minimal concentration of guanine and adenine for a particular electrode surface area. Figure 2 displays the DPV response of MWCNT at different concentrations (0.25 to 2mg dispersed in 1ml of 1% acetic acid solution). 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.

A. Effect of immobilization time

The amount of purine nucleobases adsorbed over the working electrode is directly proportional to the sensitivity of aromatic compounds. 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. Figure 3 shows the DPV response of 60mg/l mixed concentration of purine bases varying from 30 to 300s for the working electrode containing 1.25mg/ml of MWCNT paste in 0.1M phosphate buffer, pH 7. 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].

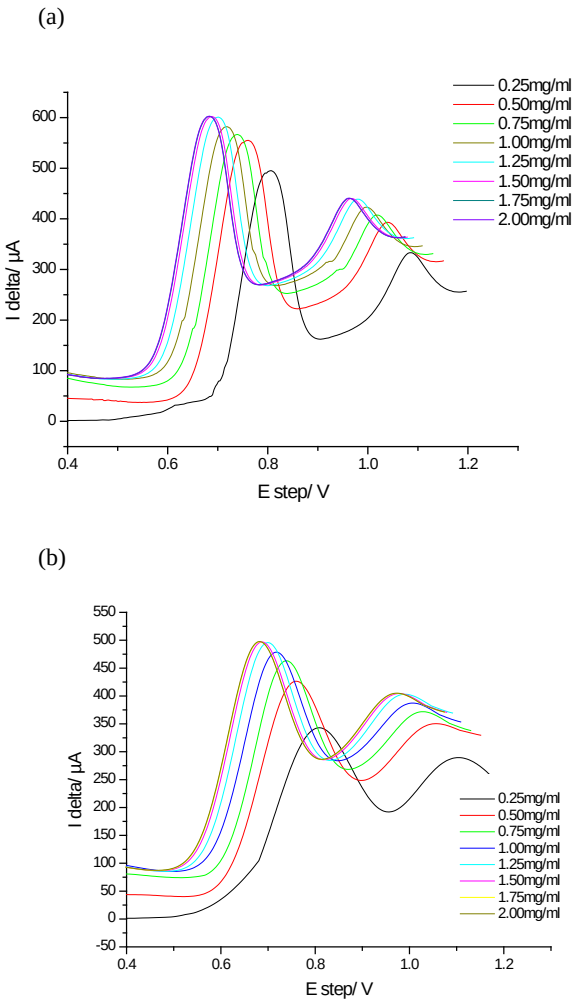
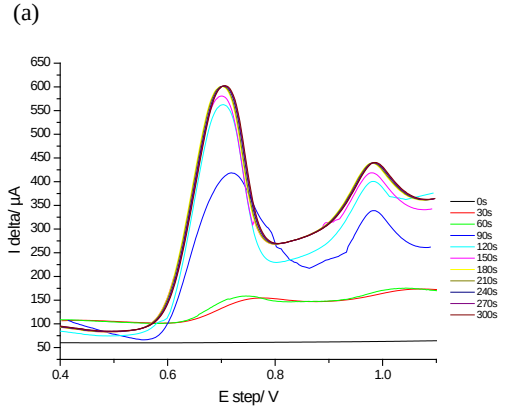


Fig. 2 DPV response of (a) Purine nucleosides and (b) Purine nucleotides at different concentrations of MWCNT paste



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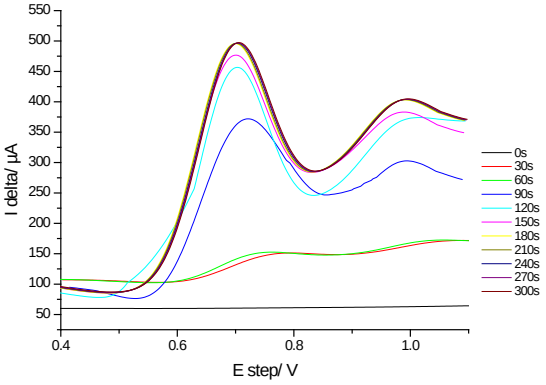
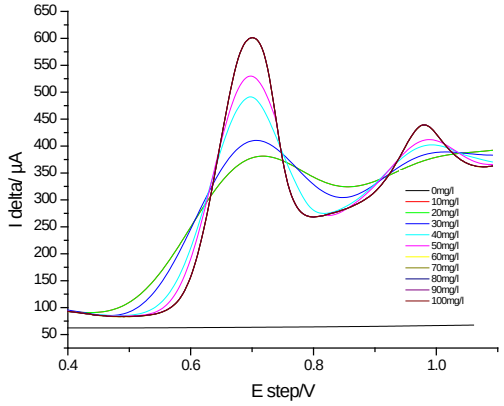


Fig. 3 DPV response of (a) 60mg/l of purine nucleosides and (b) purine nucleotides varying from 30 to 300s for the graphite electrode

B. Effect of immobilization concentration

(a)



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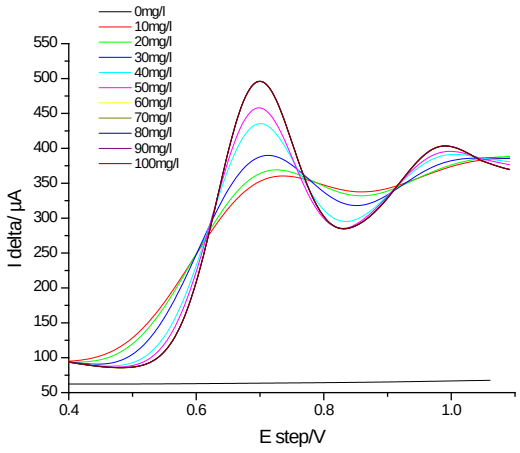


Fig. 4 DPV response of 10 to 100 mg/l of (a) Purine Nucleosides and (b) Purine Nucleotides over working electrode

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 (Fig. 4). It can be noticed in all the figures 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].

C. Electron transfer characteristics of working electrode

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. The semicircle portion and the linear portion of the Nyquist plot represent electron transfer- limited process and diffusion limited process respectively. 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.

Nyquist plot (dependence of an imaginary part of the impedence Z" vs a real part of the impedence Z') of the modified electrodes represent a semicircle at high frequencies illustrating an electron transfer limiting process. For bare graphite, a short linear part of low frequencies are observed resulting from the diffusion of limiting step of the electrochemical process is obtained. It is important to consider the fact that this part of the spectrum represents the properties of the electrolyte solution and the diffusion of the redox couple in the supporting electrolyte and thus not affected by the modification of the electrode surface [10]. The impedence data were simulated using the Randles equivalent circuit consisting of a parallel combination of the capacitance (C) and the charge transfer resistance (Rct) redox reactions in series with the supporting electrolyte resistance (Rsol).

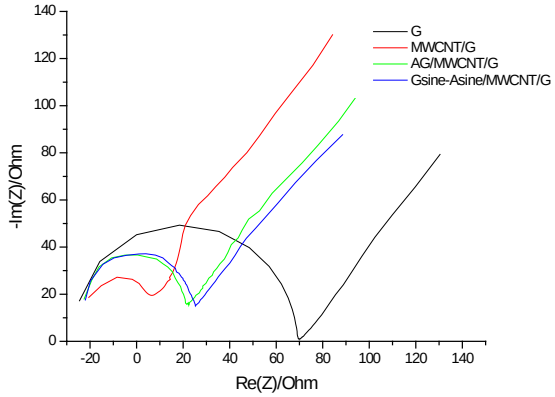


Fig. 5 Nyquist plot of working electrodes in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ ions and

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 [11]. For bare graphite, the value of Rct is 70.3±0.5 Ω and it reflects the semicircle part with greater diameter. As the purine bases are introduced to the graphite surface, the diameter of the semicircle decreases and hence decreasing the Rct value till 22.4±0.5 Ω. The diameter of the semicircle still decreases, decreasing the Rct value till 6.0±0.5 Ω with the introduction of MWCNT. MWCNT immobilized on the graphite surface plays an important role similar to an electron conducting tunnel making electron transfer to the electrode surface easier. The increase in the Rct value for MWCNT electrode containing purine nucleosides is due to the formation of highly organized layer of the purine bases over the modified electrode, resulting in the blockage of electron transfer to the redox couple, in other words, restricting the redox species to penetrate the MWCNT layer [12]. The electron transfer resistance of purine nucleotides (25.1±0.5Ω) is slightly greater than purine nucleotides (22.4±0.5Ω). This could be due to the presence of ribose in the nucleotides resulting in decrease of electron transfer from the redox couple to the electrode.

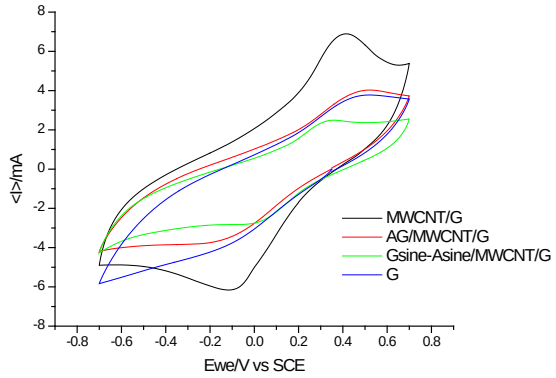


Fig. 6 CV of the working electrodes in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ ions

To confirm EIS, CV was performed in the same supporting electrolyte. The mechanism of purine nucleobases detection using $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ resides in the barrier effect of the purine bases towards the redox couple [13], resulting in the reduction in redox couple signal (Fig. 6) after the addition of purine bases to the modified electrode.

E.Electrochemical determination of Benzene and its derivatives

Purine nucleobases were attacked by exposing the modified electrodes to benzene and its derivatives. Biosensors of equivalent mixtures of purine nucleosides and nucleotides were prepared separately using concentration of 60 mg/l and were placed in contact with the analytes. Survived purine bases were calculated from the DPV peaks before and after the exposure. Figure 7 and 8 displays the calibration curves obtained from the average relative portion of survived nucleosides and nucleotides respectively.

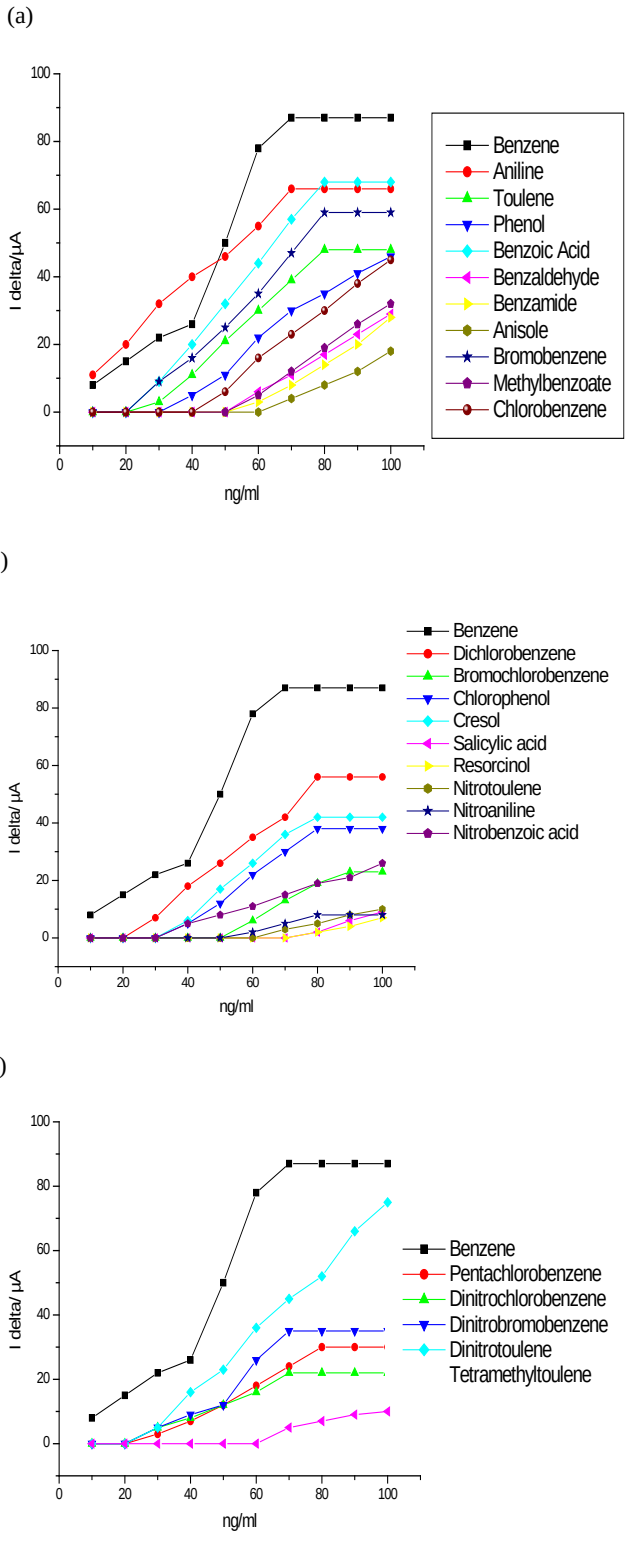
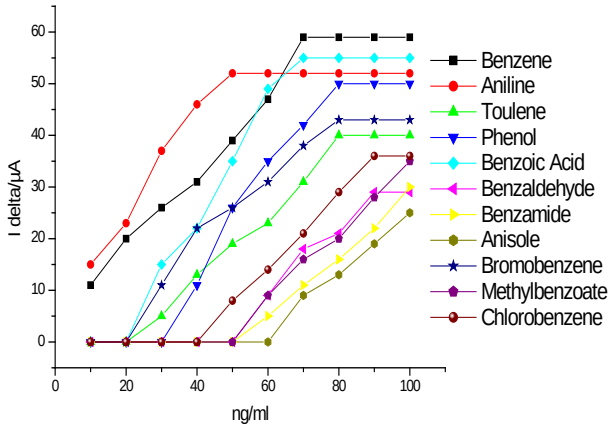


Fig. 7 The calibration curves obtained for the purine nucleosides based biosensor for (a) benzene and mono-substituted derivatives, (b) benzene and di-substituted derivatives and (c) benzene and poly-substituted derivatives.

(a)



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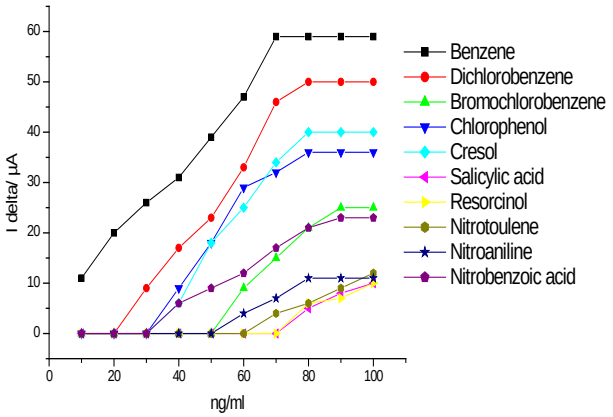

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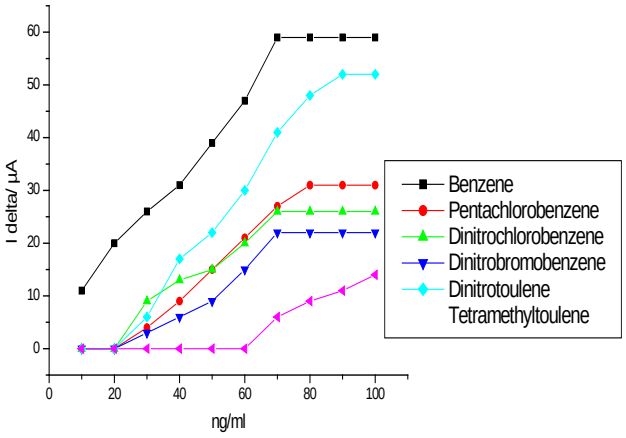

Fig. 8 The calibration curves obtained for the purine nucleotides based biosensor for (a) benzene and mono-substituted derivatives, (b) benzene and di-substituted derivatives and (c) benzene and poly-substituted derivatives.

Calibration plot for benzene shows a sudden increase from 40 to 50 ng/ml. This could be due to the significant electrochemical reaction of benzene between these concentrations. It could be noted that beyond the concentration of 70ng/ml, the survived purine response almost remained stable representing the saturation level for purine nucleoside based biosensor. The reaction behavior and hence the calibration plot for nucleosides and nucleotides based biosensor differ significantly. However the detection limit is not less than 30ng/ml for most of the benzene substituted derivates for both the types of biosensor. The challenge in the making of biosensor lies in increasing this saturation level. This could happen if large quantity of purine nucleobases were available for benzene to react with. For the derivatives of benzene, the detection limit and the saturation level varies depending on the analyte's reaction with the sensing material. However, a nearly linear behavior was observed for most of its derivatives in the approximate concentration ranges 40 to 80ng/ml for mono-substituted derivatives, 50 to 80ng/ml for di-substituted derivatives 30 to 70 ng/ml for poly-substituted derivatives. Fitting of these linear regression data is most common and simple method used for the calibration of sensing device [14].

IV. Conclusions

Reported studies indicate that benzene strongly acts on nucleic acids forming it's adduct. Purine nucleosides and nucleotides based biosensor were prepared and standardized separately. Calibration curves were developed for the analyte's reaction with the biosensor. The obtained data can be used in the fabrication of devices for effective sensing of benzene and its derivatives in aqueous medium. It should be noted that in-vitro reaction conditions are different from the electrochemical reaction conditions.

Acknowledgements

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