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Multi-Walled Carbon Nanotubes based Purine Electrodes for Electrochemical Detection of Benzene and its Derivatives using Differential Pulse Voltammetry

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Abstract

Multi-walled carbon nanotubes (MWCNT) based purine electrodes, prepared by immobilizing purine nucleosides over MWCNT coated graphite electrode were used for electrochemical detection of Benzene and its mono-, di- and poly-substituted derivatives. The film forming abilities of MWCNT coated graphite electrodes were studied using Electrochemical Impedance Spectroscopy (EIS) and Cyclic Voltammetry (CV) by placing the electrodes in electrolyte containing redox couple. Differential Pulse Voltammetry (DPV) was performed to identify the relative change in the oxidation peak of the purine bases after its interaction with analyte. Under optimized conditions, calibration curves were obtained for the modified electrodes over various analytes using DPV and the detection limit was found to be 10 ng/ml of benzene and 30 ng/ml for benzene derivatives. The proposed purine based MWCNT biosensor exhibited stability, high reproducibility, selectivity and regeneration, making it a potential tool for electrochemical detection of Benzene and its derivatives in water based samples.

Keywords: *Purine bases biosensor, Differential Pulse Voltammetry, Electrochemical Impedance Spectroscopy, Cyclic Voltammetry, Benzene, Multi-walled Carbon Nanotubes.*

1. Introduction

Benzene, a known carcinogen is found in the industrial effluents involved in the production of polymers, dyes and drugs, which are known to penetrate into soil and contaminate ground water [1]. Benzene derivatives have also been consistently used in a wide range of industries and its effluents are known to contaminate ground water [2]. Hence, it is necessary to monitor its presence for the effective control of the contaminants in the environment. Nucleic acid based biosensor appears to be an interesting analytical tool for the identification of carcinogens, drugs, mutagens and pollutants with its binding affinity for purine bases [3]. Guanine and adenine, electroactive compounds [4] were found to oxidize over carbon electrodes [5] and since then, studies were reported on purine and nuclic acid electrodes for the evaluation of organic pollutants, DNA hybridization, micro-organism and drugs[6]. Benzene and its substituted derivatives were found to interact with guanine [7] and adenine [8] forming adducts. The formation of these adducts were electrochemically monitored using carbon electrodes from the change in the oxidation signal.

Recently various approaches have been made on increasing the performance of electrode response due to the immobilization of biomolecules over the electrode. Different strategies to modify electrode surface have been proposed. The usefulness of the electrode to detect various analytes has been widely discussed [9, 10]. MWCNT, with a greater surface to volume ratio is found to immobilize large amount of biomolecules. However, one of the major problems associated with the MWCNT is its low solubility in usual solvents [9]. Dispersion of MWCNT in solvent followed by immobilization of the sensing material is an interesting approach to prepare electrochemical sensors. In this report, electrochemical immobilization of purine bases over graphite electrode coated with MWCNT has been performed to develop a stable biorecognition layers for the voltammetric determination of benzene and its derivatives. Special focus was given to the experimental conditions such as concentration of MWCNT, immobilization time, immobilization concentration. The interactions of analytes with the biosensor were evaluated from the change in electrochemical response before and after its interaction with benzene and its substituted derivatives (mono-, di- and poly) using DPV.

2. Experimental

2.1 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 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 derivatives were prepared immediately before each experiment.

2.2 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.

2.3 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.

2.4 Immobilization of Purine Bases

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 guanine and adenine nucleosides 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 guanine and adenine. After immobilization step, the electrode was washed with water to remove unbounded purines and preserved at 4°C for further use.

2.5 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 0.7 and 1.0 V were used as analytical signal for guanine and adenine oxidation respectively.

2.6 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:

$S_G = 1 - (GPA_s/GPA_b)$

$S_A = 1 - (APA_s/APA_b)$

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 [11]

3. Results and Analysis

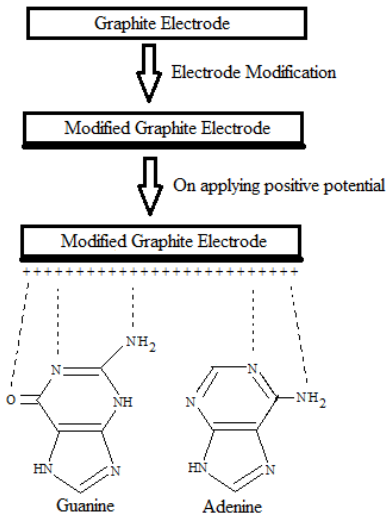

Figure 1: Schematic representation of biosensor preparation

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 [12]. 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 [13].

3.1 Effect of MWCNT concentration

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 consistant with reported work [14]. 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 the 30µl of MWCNT at different concentrations varying from 0.25 to 2mg dispersed in 1ml of 1% acetic acid solution. The reponses were recorded for MWCNT in 60mg/l of purine mixtures for 180s in 0.1M phosphate buffer (pH 7). Saturation peak was obtained for 1.25mg/ml of MWCNT concentration.

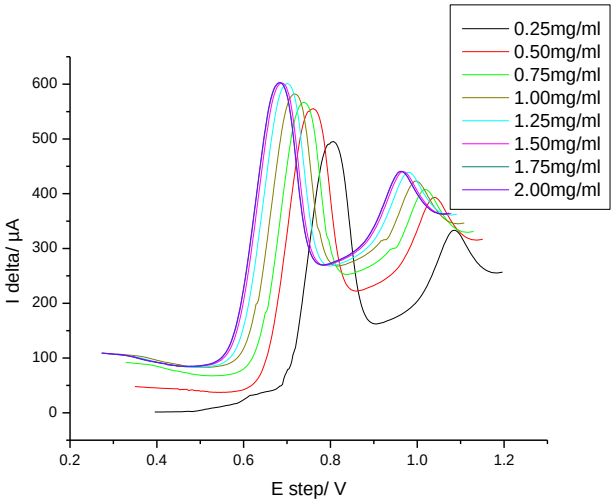

Figure 2: DPV response at different concentrations of MWCNT paste

3.2 Effect of immobilization time

The amount of purine bases 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 guanine and adenine bases 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 [14].

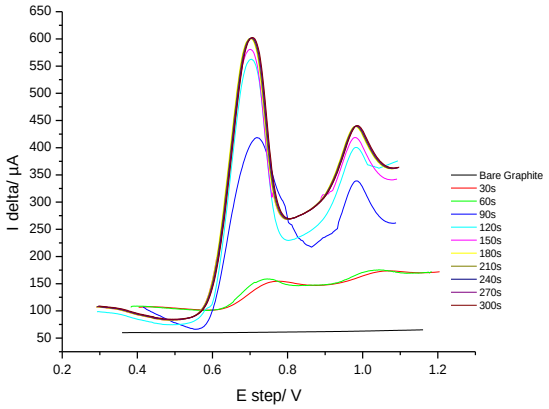

Figure 3: DPV response of 60mg/l of purine bases varying from 30 to 300s for the graphite electrode

3.3 Effect of immobilization concentration

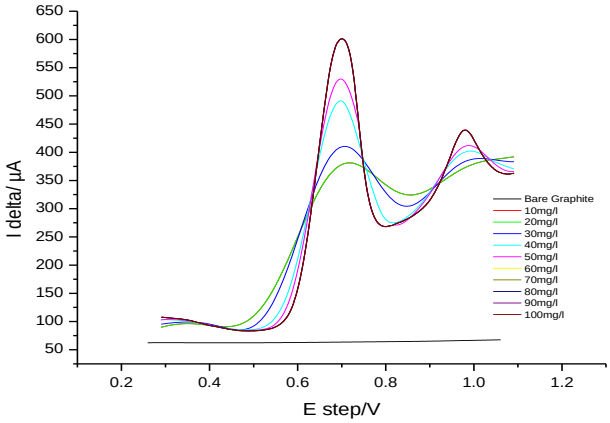

Figure 4: DPV response of 10 to 100mg/l of purine bases over working electrode

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 guanine and adenine almost remained stable for the immobilization concentration from 60 to 100 mg/l (Figure 4). It can be noticed in all the figures that the guanine oxidation peak area is greater than adenine peak area as the working electrode surface is first occupied by guanine [15,16].

3.4 Electron transfer characteristics of the working electrode

In order to study the interfacial electron transfer properties of the modified electrode immobilized with purine bases, 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 [17]. 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 [18]. 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).

It was found that the Rsol shows a negative resistance in the Nyquist plot. Numerous examples of negative resistance have been reported and in all the cases, the condition, Re[Zω)] ω→0<0, is associated with a passivation event in which the steady state current decreases with increasing voltage[19]. In other words, the electrochemical adsorbtion of a blockage intermediate from the electrolyte over the working electrode is a passive event ultimately reperesented as a negative resistance. However, the charge transfer resistance is at the positive portion representing the active transport of the redox ions.

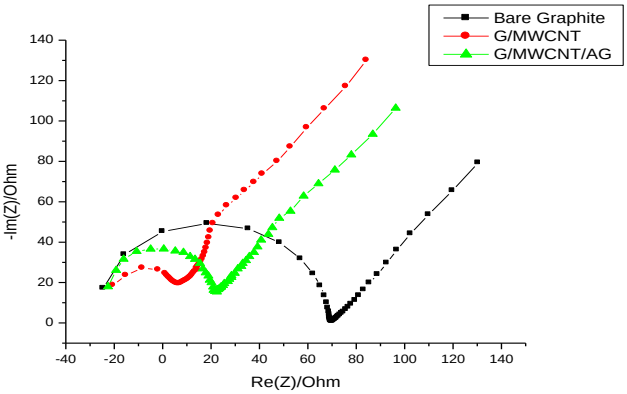

Figure 5: Nyquist plot of working electrodes in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ ions

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 [20]. For bare graphite, the value of Rct is 70.3±0.5 Ohm 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 ohm. The diameter of the semicircle still decreases, decreasing the Rct value till 6.0±0.5 ohm 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 [21].

To confirm EIS, CV was performed in the same supporting electrolyte. The mechanism of purine detection using $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ resides in the barrier effect of the purine bases towards the redox couple [22], resulting in the reduction in redox couple

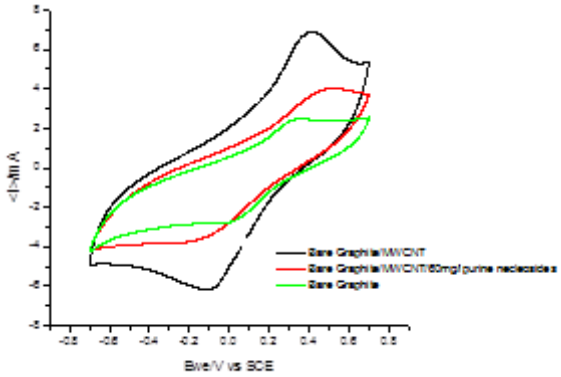

Figure 6: CV of the working electrodes in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ ions

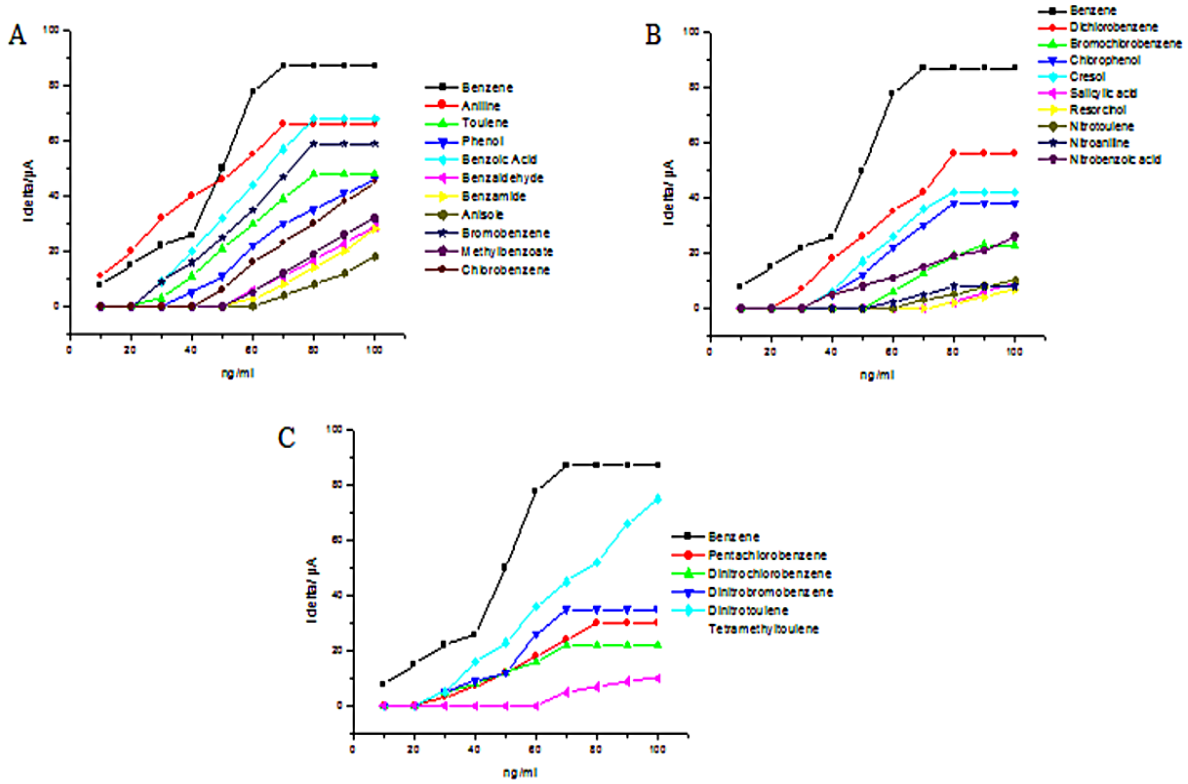

Figure 7: The calibration curves obtained for the biosensor for A. benzene and mono-substituted derivatives, B. benzene and di-substituted derivatives and C. benzene and poly-substituted derivatives.

signal (Figure:6) after the addition of purine bases to the modified electrode.

3.5 Electrochemical Determination of Benzene and its Derivatives

Purine bases were attacked by exposing the modified electrodes to benzene and its derivatives. Biosensors of equivalent mixtures of adenine and guanine were prepared 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 displays the calibration curves obtained from the average relative portion of survived guanine and adenine.

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. The challenge in the making of biosensor lies in increasing this saturation level. This could happen if large quantity of purine bases 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-

Table1: Regression values of linear plots of benzene and its derivatives obtained for biosensor

Analyte	Sensor Range (ng/ml)	R value	SD
Benzene	40-60	0.99902	1.63299
Aniline	10-70	0.99699	1.63663
Toulene	30-80	0.99976	0.41975
Phenol	70-100	0.99931	0.31623
Benzoic acid	20-80	0.99974	0.56484
Benzaldehyde	50-100	0.99941	0.36515
Benzamide	60-100	0.99638	0.96609
Anisole	60-100	0.95289	0.7303
Bromobenzene	40-80	0.99795	1.26491
Methyl Benzoate	50-100	0.99893	0.63621
Chlorobenzene	40-100	0.99895	0.82375
Dichlorobenzene	20-70	0.99676	1.73823
Bromochlorobenzene	50-90	0.99614	0.94868
Chlorophenol	50-80	0.99838	0.7746
Cresol	50-80	0.99519	1.32288
Salicylic acid	80-100	0.99662	0.40825
Resorcinol	80-100	0.9934	0.40825
Nitrotoulene	70-100	0.99655	0.31623
Nitroaniline	60-80	1	0
Nitrobenzoic acid	40-100	0.99709	0.62678
Pentachlorobenzene	30-80	0.99744	0.82231
Dinitrochlorobenzene	20-70	0.9955	0.83666
Dinitrobromobenzene	20-50	0.99381	0.70711
Dinitrotoulene	20-100	0.9972	2.11063
Tetramethyl toulene	70-100	0.98978	0.3878

substituted derivatives 30 to 70 ng/ml for poly-substituted derivatives. Fitting of the linear regression data is most common and simple method used for the calibration of sensing device [23]. Table 1 data can be used as backbone for the detection of these compounds within the specified linear range.

4. Conclusions

Reported studies indicate that benzene strongly acts on nucleic acids forming it's adduct. However, invitro reaction conditions are different from the electrochemical reaction conditions. Concentration dependent increase in purine nucleoside oxidation peak was observed to be more intense for mono-substituted benzene derivatives than di- and poly- substituted derivatives. A mere linear behavior was observed for aniline (10-70ng/ml), toluene (30-80ng/ml), benzoic acid (20-80ng/ml), di-chlorobenzene (20-70ng/ml), pentachlorobenzene (30-80ng/ml) and dinitrotoulene (20-100), which are known to be highly reactive.

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