Electrochemical determination of benzene derivatives using MWCNT modified DNA based biosensor

[1]S Baby Gayathri, [2]P Kamaraj, [3]M Arthanareeswari and [4]S Devikala

Department of Chemistry, SRM University, Kattankulathur 603203, India.
Email: [2]kamaraj97@yahoo.co.in

Abstract: Multi-walled carbon nanotubes (MWCNT) modified DNA based biosensors were prepared by immobilizing ss-DNA over MWCNT coated graphite electrode. 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) and EIS were performed to identify the relative change in the oxidation peak and charge transfer resistance respectively after its interaction with benzene derivatives (aniline, toluene, phenol, anisole and mesitylene). Under optimized conditions, calibration curves were obtained for the modified electrodes over various analytes using DPV and EIS. The proposed ss-DNA based MWCNT biosensor exhibited stability, high reproducibility, selectivity and regeneration, making it a potential tool for electrochemical detection of Benzene derivatives in water based samples.

Key words: Benzene derivatives, Biosensor, CV, DNA, DPV and EIS.

I. INTRODUCTION

Benzene, a common industrial solvent and a component of gasoline, is known to be highly toxic due to its carcinogenic activity. Benzene as such is an insoluble liquid which undergoes several mechanisms inside a living cell to introduce functional group to the benzene ring to make it more soluble. In this process several benzene metabolites are formed in vivo which in turn becomes genotoxic. It should be noted that even the secondary metabolite of benzene becomes genotoxic inside the cell. The term genotoxic refers to the adverse effect that happens in the cell that damages genetic information and finally leading to cell damage. This is initiated by benzene and its metabolites directly reacting with DNA and, benzene and its metabolites reacting with by products of the cell during a mechanism pathway. The metabolism of benzene has been reported to yield glucuronide and sulphate conjugates of phenol, quinol, catecol, L-phenylmercapturic acid, mucoaldehyde and trans, trans-muconic acid by ring sucission [1]. In addition, the metabolic mechanisms of benzene involve the formation of phenol metabolites by peroxidase in bone marrow which again generates reactive quinols [2]. Quinol is oxidized to p-benzoquinone, which binds to vital cellular components or undergoes redox cycling to generate oxygen radicals [3]. Apart from benzene, there are several benzene derivatives that are used in many industries including pharmaceutical industries and human beings are daily exposed it [4].

Aniline is a toxic organic compound, used as a base to make dyes, drugs, explosives, plastics, and photographic and rubber chemicals. The dye, chemical and rubber manufacturing industries were major sources of occupational exposure to aniline. Aniline was the key intermediate in the developing textile dye industry in Europe during the 1800s [5]. Aniline was not carcinogenic in experimental animals [6], but found to produce selective toxicity in spleen by release of free iron, however, the mechanism involved in the free iron release and Aniline-DNA interaction is not known [7]. It should also be noted that 4-aminobiphenyl, 2-naphthylamine and benzidine in aniline dyes were shown to be carcinogenic [6].

Toulene, mono substituted benzene derivative and also an aromatic hydrocarbon is used as industrial feed stock and as a solvent. Toulene was reported genotoxic in terrestrial environment due to greater extent of DNA damage it induces upon interaction [8]. Recent investigations have shown that toluene may induce male reproductive dysfunctions and carcinogenicity. It was found that 8-oxy-7,8-dihydroxy-2'-deoxyguanosine was formed in testes of the male rats induced by toluene via DNA damage induced by toluene metabolites [9].

Phenol, an aromatic organic compound is both a manufactured chemical and natural substance. Phenol, although one of the compounds used during the extraction and purification of DNA from the living cell [10], it is known to interaction with DNA in vivo and cause DNA damage. During the extraction process, phonol can oxidize the nucleobases, especially guanine forming 8-hydroxyguanine (8-OHGua). 8-OHGua has been reported to be a key biomarker relevant to carcinogenesis and cellular oxidative stress, important in tumor promotion.

Anisole is a colourless liquid, used as precursor in perfumes, insect pheromone and pharmaceutical industries [11]. Anisole is relatively non-toxic with lethal dose value of 3700 mg/kg in rats. There is no report on the purine reaction with anisole till date, however, anisole derivatives have been found to react with purine derivatives. On the other hand, nitrite-butylated hydroxylanisole (BHA) was tested by Natake et al [12], for its DNA-damaging and mutagenic activity. The active DNA damaging product in nitrate-BHA system was determined to be 2-tert-butyl-quinone which gave positive rec-assay test (- a test performed for the detection of DNA damaging agents [13] and negative Ames test (- a test performed to assess the mutagenic potential of a chemical compound [14].

The electrochemical determination of all these five commonly used benzene derivatives using single stranded DNA templates have been performed. DNA exists in two forms, single stranded (ss) and double stranded (ds) DNA structures. ss-DNA, by its name refers "having single strand", consists of the purine and pyramidine nucleotides covalently linked via phosphodiester bonds. Single stranded DNA genome is found widely in Parvoviridae (class II viruses). It can also be produced artificially by rapid cooling a heat degenerated ds-DNA [15].

As mentioned earlier, as, and when DNA come across a molecule which damages its structure, a change in oxidation signal of purine bases in the DNA was observed. This property of DNA was used for determination studies, provided the molecule damages DNA. DNA damaging properties of pollutants in the water have been explored for its application in the preparation of biosensor for analysis of waste water samples [16], environmental pollutants [17], aromatic amines [18], phenolic pollutants [19], etc. The DNA biosensor response indicated the binding of one or more molecules present in waster water sample with a promising correlation with the Toxalert response (an indispensable tool for high-throughput toxicity prediction) [17]. Yanyan Qui et al. [20] developed the procedure for the electrochemical detection of bisphenol A radicals through electro-oxidation signals of guanine from DNA damaging property of BPA radicals.

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 [21]. 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. 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 derivatives using DPV and EIS.

II. MATERIALS AND METHODS

2.1 Reagents

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. ss- DNA template of 50bp length were manually designed (5'TCTGAGTCTGTATGGAGTGACATGCTTTCTGG GTGGACTCAAGTTGAAGA3') and purchased from Sigma Aldrich, Mumbai, India.

DPV measurements were carried out in 0.1M Phosphate buffer. CV and EIS measurements were made in 0.1M Nacl solution containing 10/10mM K3Fe(CN)6/K4Fe(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 = 0.318cm2) as working electrode. Calomel

electrode used in this experiment has 0.241V (electrode surface area= 0.001cm2) 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 ss-DNA templates

The electrode was pretreated by applying a potential of +1.5V for 30s in 0.1M phosphate buffer (pH 5) to remove electrochemical impurities. DNA based biosensor was developed by immobilizing ss-DNA templates 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 ss-DNA templates. After immobilization step, the electrode was washed with water to remove unbounded templates 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 K3Fe(CN)6/K4Fe(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 derivatives were determined from the change in the signals obtained before and after the biosensor 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) and EIS. 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 derivatives

The ss-DNA immobilized modified electrode was immersed in the solution containing benzene derivatives for 5 minutes for the purines in the electrode to react with the analyte. DPV and EIS measurements before and after the interaction with benzene derivatives 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 purines. This has been related to the difference of signals corresponding to that of original purines 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 Rct is the electron transfer resistance measured at the peak potential obtained for 10/10mM K3Fe(CN)6/K4Fe(CN)6 in 0.1M NaCl solutions at the modified electrodes without purine bases. The indexes used, characterize the chemical modifiers of graphite electrode [22].

III. RESULTS AND ANALYSIS

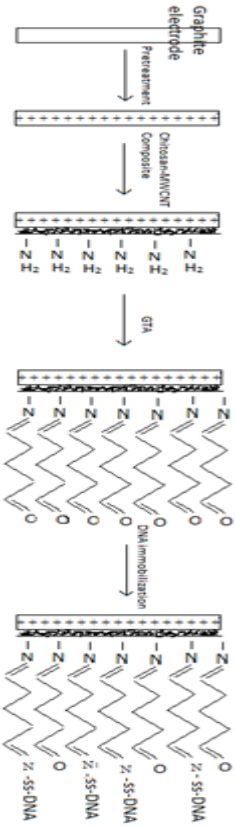

Figure 1: Schematic representation of biosensor preparation

DNA based biosensors with the layers of ss-DNA template 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 DNA based biosensor. Electrochemical pretreatment was performed by anodization and cathodization 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 [23]. 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 [24].

3.1 Effect of MWCNT concentration

The increase in the quantity of MWCNT provided a greater surface area for DNA to immobilize over the electrode surface. This enhances the direct electrochemical response of purine bases and is in consistant with reported work [25, 26]. Hence it is necessary to optimize the minimum quantity of MWCNT needed to immobilize the known minimal concentration of DNA templates 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 70mg/l of ss-DNA template mixtures for 180s in 0.1M phosphate buffer (pH 7). Saturation peak was obtained for 1 mg/ml of MWCNT concentration.

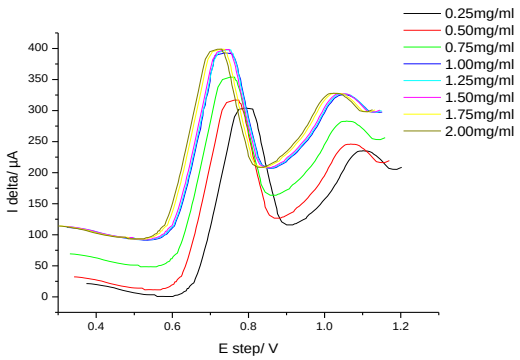

Figure 2: DPV response at different concentrations of MWCNT paste

3.2 Effect of immobilization time

The amount of ss-DNA adsorbed over the working electrode is directly proportional to the sensitivity of benzene derivatives. 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 70mg/l ss-DNA templates varying from 30 to 300s for the working electrode containing 1 mg/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 DNA 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 [25, 26].

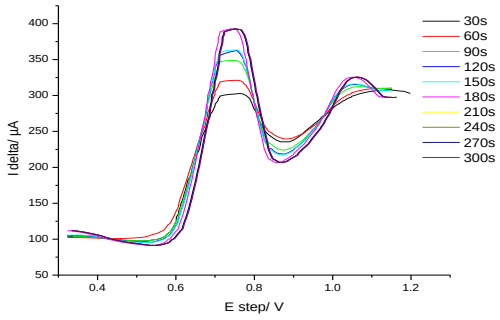

Figure 3: DPV response of 60mg/l of purine bases varying from 30 to 300s for the graphite electrode

3.3 Effect of immobilization concentration

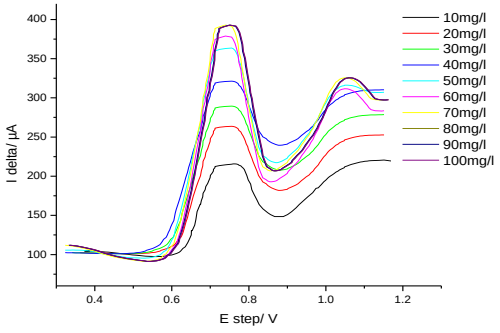

Figure 4: DPV response of 10 to 100mg/l of purine bases over working electrode

The amount of ss-DNA concentration immobilized over working electrode containing 1 mg/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).

3.4 Electron transfer characteristics of the working electrode

In order to study the interfacial electron transfer properties of the modified electrode immobilized with DNA templates, 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 [27]. 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 [28]. 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[29]. 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.

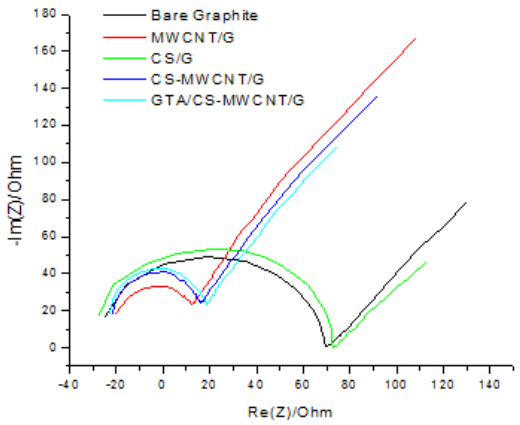

Figure 5: Nyquist plot of working electrodes in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ ions

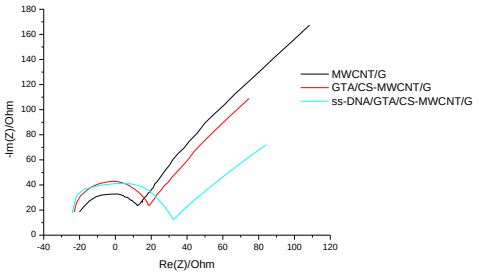

Figure 6: Nyquist plot of DNA based biosensor in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ ions

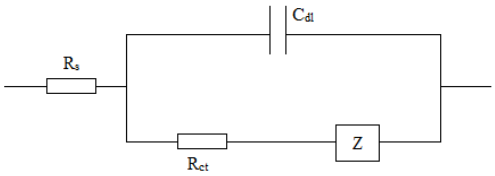

Figure 7: The scheme of equivalent circuit simulating the impedance spectra. Rsol- resistance of the supporting electrolyte, Rct- charge transfer resistance, C-capacitance

Table 1: Parameters of the equivalent circuit simulating the complex impedance spectra of the electrodes in the presence of 0.1M NaCl solution containing 10/10mM K3Fe(CN)6/K4Fe(CN)6. Rsol- resistance of the supporting electrolyte, Rct- charge transfer resistance, Cdl-capacitance.

Working electrode	R_{sol} Ω	R_{ct} Ω	C_{dl} µF
Bare Graphite	-24.53	70.38	0.77
MWCNT/G	-20.01	13.39	1873.6
CS/G	-27.63	72.77	64.39516
CS-MWCNT/G	-22.06	16.28	26138.32
GTA/CS-MWCNT /G	-23.05	18.62	124897
ss-DNA/GTA/CS-MWCNT/G	-24.37	31.89	3552.888

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 [22]. For bare graphite, the value of Rct is 70.3±0.5 Ohm and it reflects the semicircle part with greater diameter. As MWCNTs are to the graphite surface, the diameter of the semicircle decreases and hence decreasing the Rct value till 13.39±0.5 ohm. However, when chitosan, glutaraldehyde and ss-DNA were introduced, the Rct value increases as tabulated in Table 1. 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 ss-DNA is due to the formation of highly organized layer of the ss-DNA templates formed 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 [22].

To confirm EIS, CV was performed in the same supporting electrolyte. The mechanism of DNA detection using [Fe(CN)6]3-/[Fe(CN)6]4- resides in the barrier effect of the DNA towards the redox couple [22], resulting in the reduction in redox couple signal (Figure:8) after the addition of purine bases to the modified electrode.

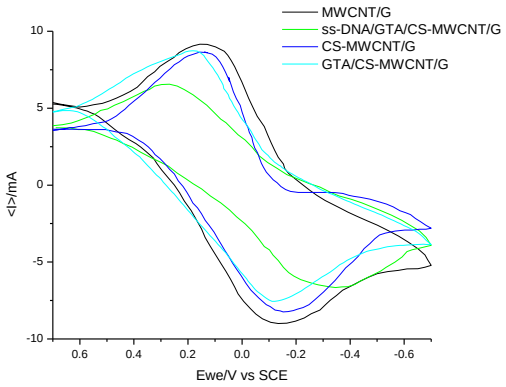

Figure 8: CV of the working electrodes in 0.1M NaCl containing 10/10mM $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$ ions

3.5 Electrochemical Determination of Benzene and its Derivatives

DNA bases were attacked by exposing the modified electrodes to benzene derivatives. Survived purine bases were calculated from the DPV peaks and Rct values obtained from EIS before and after the exposure. Figure 7 displays the calibration curves obtained from the average relative portion of survived DNA bases.

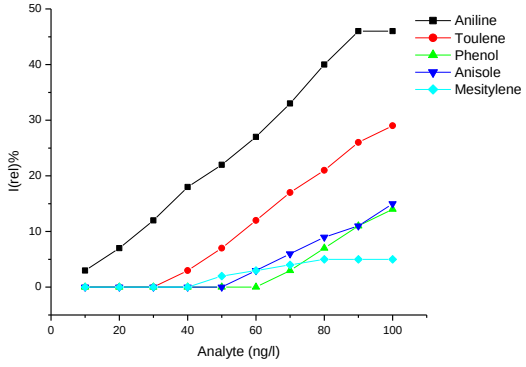

Figure 9: Calibration plot obtained from benzene derivatives using adenine signal of DPV

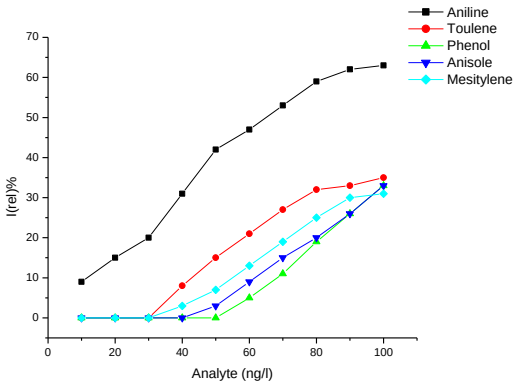

Figure 10: Calibration plot obtained from benzene derivatives using guanine peak signal of DPV

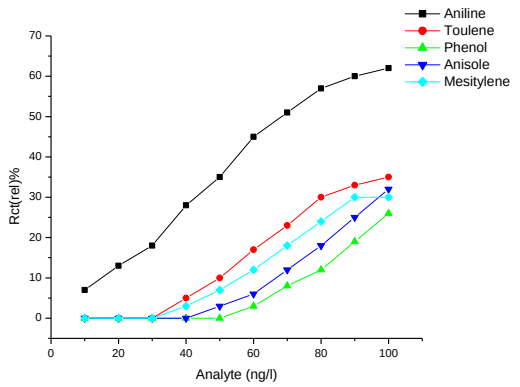

Figure 11: Calibration plot obtained from benzene derivatives using Rct values of EIS

It can noticed that as the analyte concentration increases, the relative percent DNA damage also increases, consistant with the DNA damaging property of benzene derivatives. These values may be used for the design of biosensor for the determination of benzene derivatives present in the sample.

IV. CONCLUSIONS

MWCNT paste over the graphite electrode has ensured a good detection window for the voltammetric and impedimetric evaluation of the presence of ss-DNA templates. 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(CN6)]3-) for the DNA immobilized graphite modified electrode. It should be noted that invitro reaction conditions are different from the electrochemical reaction conditions. It was demonstrated that the proposed EIS and DPV procedures can be used for the detection of benzene derivatives.

V. ACKNOWLEDGEMENTS

The authors gratefully thank University Grants Commision, India, for its financial assistance and SRM University, India, for providing facilities for conducting this part of the experimental work.

VI. REFERENCES

[1] A. Yardley-Jones, D. Anderson and D. V. Parke, The toxicity of benzene and its metabolism and molecular pathology in human risk assessment, Br J Ind Med., Vol. 48(7), pp. 437-44, 1991.

[2] D. Ross, Metabolic basis of benzene toxicity, European Journal of Haematology, Vol. 57(S60), pp. 111-118, 1996.

[3] F. Tattersfield, The relationship between the chemical constitution of organic compounds and their toxicity to insects, The Journal of Agricultural Science, Vol. 17(2), pp. 181-208, 1927

[4] S B Gayathri and P Kamaraj (2014), Genotoxicity of Benzene and Soluble Benzene Substituted Organic Compounds in Mammals- A Review, International Journal of Pharmaceutical Science and Health Care, Vol. 4, No. 4, pp. 24-39.

[5] T. S. S. Dikshith, Safe Use of Chemicals: A Practical Guide, CRC Press, 2008.

[6] E. G. Nicholas and B. Suse, The Chemical Biology of DNA Damage, John Wiley and Sons, 2011

[7] W. Xiaohong, K. Subburaj, V. M. S. Ramanujam, M. K. Firoze, Iron release and oxidative DNA Damage in Splenic toxicity of Aniline, Journal of Toxicology and Environmental Health, Part A: Current Issuses, Vol. 68(8), pp. 657-666, 2007.

[8] Y. Liu, Q. Zhou, X. Xie, D. Lin and L. Dong, Oxidative stree and DNA damage in the earthworm Eisenia fetida induced by toluene, ethylbenzene and xylene, Ecotoxicology, Vol. 19(8), pp. 1551-1559, 2010

[9] N. Nakai, M. Murata, M. Nagahama, T. Hirase, M. Tanaka, T. Fujikawa, N. Nakao, K. Nakashima and S. Kawanishi, Oxidative DNA damage induced by toluene is involved in its male reproductive toxicity, Free Radic Res., Vol. 37(1), pp. 69-76, 2003.

[10] H. G. Claycamp, Phenol sensitization of DNA to subsequent oxidative damage in 8-hydroxyguanine assays, Carcinogenesis, Vol. 13(7), pp. 1289-1292, 1992.

[11] H. Fiege, H. Voges, T. Hamamoto, S. Umemura, T. Iwata, H. Miki, Y. Fujita, H. Buysch, D. Garbe, W. Paulus, "Phenol Derivatives" in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, 2002.

[12] M. Natake, G. Danno, T. Maeda, K. Kawamura and K. Kanazawa, Formation of DNA-damaging and mutagenic activity in the reaction systems containing nitrite and butylated hydroxyanisole, tryptophan, or cystene, J Nutr Sci Vitaminol, Vol. 25(4), pp. 317-32, 1979

[13] G. Mazza, Bacillus subtilis "rec assay" test with isogenic strains, Appl Environ Microbiol., Vol. 43(1), pp. 177-184, 1982.

[14] K. Mortelmans, E. Zeiger, The Ames Salmonella/microsome mutagenicity assay. Mutat. Res., Vol. 455 (1–2), pp. 29-60, 2000

[15] Freifelder D (1987), Jones and Bartlett, pp.834

[16] F Lucarelli, A Kicela, I Palchetti, G Marrazza, M. Mascini (2002), Electrochemical DNA biosensor for analysis of waste water samples, 58, 113-118.

[17] Giacomo Chiti, Giovanna Marrazza, Marco Mascini (2001), Electrochemical DNA biosensor for environmental monitoring, Analytical Chmica Acta, 427, 155-164

[18] Joseph Wang, Gustavo Rivas, Denbai Luo, Xiaohua Cai, Florenda S Valera and Narasaiah Dontha (1996), DNA-modified electrode for the detection of aromatic amines, 68, 4365-4369.

[19] Yanqiong Zheng, Changzhu Yang and Wenhong Pu (2009), Carbon nanotube-based DNA biosensor for monitoring phenolic pollutants, Microchim Acta, vol. 166, pp. 21-26

[20] Yanyan Qui, Hai Fan, Xu Liu, Shiyun Ai (2010), Electrochemical detection of DNA damage induced by in situ generated bisphenol A radicals through electro-oxidation, Microchim Acta, 171: 363-369.

[21] S B Gayathri and P Kamaraj (2014), Development of electrochemical DNA biosensors- A review, Chemical Science Transactions, Vol. 4, In Press.

[22] H. Peng, C. Soeller, N.A. Vigar, V. Caprio and J. Travas-Sejdic (2007), Label-free detection of DNA hybridization based on a novel

functionalized conducting polymer, Biosens. Bioelectron., vol. 22, no. 9-10, pp. 1868-1873

[23] P. Kissinger and W. R. Heineman (1996), Laboratory techniques in Electroanalytic chemistry, Second Edition, Revised and Expanded, CRP Press.

[24] A. H. Kamel, F. T. C. Moreira, C. Delerue-Matos and M. G. F. Sales (2008), Electrochemical determination of antioxidant capacities in flavored waters by guanine and adenine biosensors, Biosensors and Bioelectronics, Vol. 24, no. 1, pp. 591-599

[25] S B Gayathri, P Kamaraj, M Arthanareeswari and S Devi Kala (2014), Electrochemical Determination of Benzene Substituted Derivatives using Carbon Based Purine Electrodes through Electrochemical Impedance Spectroscopy, International Journal of Electrochemical Sciences, vol. 9, no. 11, pp. 6113-6123;

[26] S B Gayathri, P Kamaraj, M Arthanareeswari and S Devi Kala (2014), Electrochemical Characterization of Guanine and Guanosine based biosensors over multiwalled-carbon nanotube modified graphite electrode, Chemical Science Transactions, Vol. 3, no. 4., pp-1446-1454

[27] A. Lasia (1999), Modern Aspects of Electrochemistry, Ed. By B.E. Conway, J. Bockris, and R. E. White, Kluwer, Springer Publishers, vol. 32, no.1.

[28] L.Yang and Y. Li (2005), AFM and Impedance spectroscopy characterization of the immobilization of antibodies on indium-tin oxide electrode through self-assembled monolayer of epoxysilane and their capture of Escherichia coli O157:H7, Biosens. Bioelectron., vol. 20, no. 7, pp. 1407-1416.

[29] D. D. Macdonald (1990), Review of mechanistic analysis by electrochemical impedance spectroscopy, Electrochemical Acta., Vol. 35 no. 10, pp. 1509-1525

◈◈◈