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Biomimetic oxidation of benzo[*a*]pyrene to a quinone metabolite as a cysteine-oxidation mediator on MWCNT-modified electrode surface



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Sivakumar Nisha^a, Annamalai Senthil Kumar^{a, b, *}

^a Nano and Bioelectrochemistry Research Laboratory, Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore, 632 014, India

^b Carbon Dioxide Research and Green Technology Centre, Vellore Institute of Technology, Vellore, 632 014, India

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ABSTRACT

Bay-region containing polyaromatic hydrocarbons (PAHs) like Benzo(a)pyrene (BaP), which comprise several strained benzenoid rings, are representative organic compounds for the carcinogenic and mutagenic activities in physiological system. In general, cytochrome c, peroxidase and certain soil-bacteria oxidize these compounds to respective hydroxylated metabolites like BaP-7,8-diol (BaP-2OH), that can interact with DNA, RNA and protein, and in turn makes the cellular system dysfunctional. The structure activity relationship of these compounds is still unclear and therefore, it is necessary for a new analytical approach to delineate the intricate mechanism behind the oxidation of the PAHs. Herein, we report, a simple electrochemical approach of surface-confined oxidation of BaP on multiwalled carbon nanotube (MWCNT) modified glassy carbon electrode (GCE/MWCNT) in physiological condition (pH 7 phosphate buffer solution). It has been found that MWCNT-surface adsorbed BaP (electro-inactive compound) gets electro-oxidized to highly redox active BaP-2OH compound at high positive potential, 1.2 V vs Ag/AgCl, in which, the water molecule was oxidized to molecular oxygen via hydroxyl radical intermediate. From the collective electrochemical and physicochemical studies using Raman, FTIR, GC-MS and 1,2-dihydroxy redox active probe, cysteine (CySH), it has been observed that the hydroxyl radical species produced on the surface has assisted the BaP oxidation to BaP-7,8-diol product, which is similar to the biocatalyzed oxidation of BaP observed in the physiological system. The BaP-7,8-diol surface confined MWCNT modified GCE showed a well-defined and stable redox peak at an apparent standard electrode potential, $E^{0} = 0$ V vs Ag/AgCl. The redox process is found to be proton-coupled electron-transfer in nature. As an independent study, selective electrocatalytic oxidation of CySH has been demonstrated as an application. © 2020 Elsevier Ltd. All rights reserved.

1. Introduction

Addressing the environmental pollution and its related issues is a continued research interest [1]. Polyaromatic hydrocarbons (PAHs), a class of organic compounds that contain several fused benzenoid rings, are formed by incomplete combustion of fossil fuels, petroleum products and other anthropogenic activities. These compounds are responsible for the major pollution problems of the ecosystem [2–4]. Amongst, a few hundred known PAHs, most of the compounds are found everywhere (soil, water and air) in our environment. For instance, in water medium, PAHs are present either in solution or as a desorbed particulate system [3]. In 1971, the World Health Organization (WHO) set the acceptable level of PAH in potable water as 200 ng L^{-1} [5]. In general, PAHs are referred to be carcinogenic and mutagenic chemicals [6,7]. Formation of reactive PAH-metabolites (hydroxylated benzene products) and their binding activity on DNA, RNA and protein has been referred as key factor for the PAH carcinogenicity [8-10]. Indeed, because of the different structure activity relationship, not all the PAHs have the same toxicity [11]. In 1975, based on the experimental studies of structure and activity relationship, benzo(a)pyrene (BaP)-7,8-diol-9,10-epoxide metabolite obtained from BaP has been identified for the first time as an ultimate carcinogen of the PAHs family [12]. Following this observation, some other BaP related compounds such as Benzo(a)anthracene (BaA), Benz(c)phenanthrene, Chrysene, Benzo(g)chrysene etc., which contain a bay-region, ie., a space



^{*} Corresponding author. Nano and Bioelectrochemistry Research Laboratory, Department of Chemistry, School of Advanced Sciences, Vellore Institute of Technology, Vellore, 632 014, India.

E-mail addresses: askumarchem@yahoo.com, askumar@vit.ac.in (A. Senthil Kumar).

between the aromatic rings of the fused benzene molecules and has a stressed/strained carbon position (Scheme 1A), were also identified as carcinogenic and mutagenic for the physiological system [12–14]. In connection with this concept, in 1985, Leher et al. reported a "Bay-region theory" and showed a direct correlation between experimental study on mutagenicity and a quantum chemical parameter that estimates chemical reactivity. $\Delta E deloc/\beta$ [15]. In general, cytochrome P450 [16–20] and peroxidase [21–23] based proteins that exist in liver and certain bacterial in soil [24-26] oxidize the PAH to respective hydroxylated product effectively. Some of the BaP metabolites identified under the enzymatic reaction by high-performance liquid chromatography (HPLC) are: BaP-3-OH, BaP-9-OH, a mixture of BaP-1,6-quinones, BaP-3,6-quinones and BaP-6,12-quinones, BaP-4,5-epoxide, BaP-7,8-dihydrodiol, BaP-4,5-dihydrodiol, BaP-9,10-dihydrodiol [27]. For better understanding the structure-activity relationship, biomimicking studies and in-situ identification of the PAH-metabolites by new analytical approach is highly needed. Electrochemical techniques are well-suited for this purpose, since, most of the BaPmetabolites contain phenol and polyphenols as functional groups which are electroactive in nature. Meanwhile, in 1970, Jeftie and Adams have pointed out that there is a parallelism between the electrochemical oxidation and metabolic process of BaP while studying the BaP electrochemical reaction using Platinum as a working electrode in non-aqueous media [28]. Herein, we report a new electrochemical route for biomimicking oxidation of a "bayregion" containing PAH, BaP in physiological solution and in-situ trapping of its intermediate (metabolite) using multiwalled carbon nanotube modified glassy carbon electrode (GCE/MWCNT) as a working electrode system in pH 7 phosphate buffer solution (PBS) (Scheme 1).

For the chemical oxidation of BaP, there are some reagents and catalytic systems reported in the literature. Representative examples are: ascorbic acid + ferrous sulphate + ethylene diamine tetra acetic acid (EDTA) mixture in aqueous-acetone solution [29]: 1.4napthaquinone coupled with nicotinamide adenine dinucleotidereduced form (NADH) as enzyme-free catalytic system in pH 7.5 phosphate buffer solution [30], catalyst like TiO₂, CuO, Fe₂O₃ at high operation temperature, 350 °C [31], Fenton reagent ($Fe^{2+} + H_2O_2$) in acidic condition [32], sunlight and ozone based reactions [33] and Ce incorporated mesoporous material based high valent oxidation species [34] in cyclohexanone medium. Similarly, there are some electrochemical oxidation systems reported for conversion of BaP to several hydroxylated [28] and polymeric [35] products using following working electrodes: (i) Pt in non-aqueous solvent [28,36], (ii) Boron doped-diamond in DMSO+0.1 M LiClO₄ [37] and (ii) glassy carbon electrode in acetonitrile-water medium [38]. Except 1,4-napthaquinone case [30], all other chemical and electrochemical oxidation reactions were conducted in nonphysiological system (either in acid or organic medium) [29,31-37] and thus the oxidation product/s obtained are not comparable with the metabolites of respective PAHs. Note that BaP-7,8-diol is one of the specific metabolites of BaP obtained by biochemical reactions only [7,16,29-37]. Meanwhile, in order to identify the key metabolites of BaP (BaP-7,8-diol), Mi et al. developed a bioelectrode that comprises of cytochrome P540 protein immobilized nitrogen-doped graphene nano-composite modified glassy carbon electrode system [38]. In that study, a dilute solution

Eo' = 0 V vs Ag/AgCl



Scheme 1. Illustration for the (A) adsorption and (B) surface-confined electrochemical oxidation of BaP on MWCNT modified GCE surface. (C) Selective electrocatalytic oxidation function of GCE/MWCNT@BaP-Oxid. Note; BaP-20H= BaP-7,8-diol.

of BaP (10 ng/µL of DMSO) air-saturated pH 7.4 PBS was subjected to electrochemical oxidation/reduction reaction. In the present work, GCE/MWCNT has been used as a biomimicking system for enzymefree oxidation of BaP in neutral pH solution. Recently, our group utilized the GCE/MWCNT as a working electrode to analyse the oxidation reaction pathway of triamterene (Trim) drug [39]. The "on surface" electro-oxidized product formed was found to be similar to that of the product obtained from the pharmacokinetic reaction [39]. A hydroxylated BaP molecule, BaP-7,8-diol obtained in this work was characterized using GC-MS. The identified product (surface-confined) was found to be similar to the compound obtained from the BaP-metabolic (liver) reaction. As an independent study, electrocatalytic oxidation of cysteine (CySH) in neutral pH was performed using BaP-7,8-diol metabolite trapped MWCNT modified GCE. Overall, scope and objective of this work are; (i) to explore MWCNT modified electrode for biomimicking electrochemical oxidation reaction of the carcinogenic "bay-region" PAHs in neutral pH and (ii) to elucidate the intricate reaction mechanism.

2. Experimental section

2.1. Reagents

Benzo(*a*)pyrene (99% purity, GC-MS response in Scheme S1A), MWCNT (>90% carbon basis, outer diameter: 10–15 nm; inner diameter: 2–6 nm; length 0.1–10 µm; 4% of Fe and Ni impurities), and single walled carbon nanotube (SWCNT; 60–70% pure-on carbon basis, 0.7–1.1 nm of diameter size, 5.2 wt% of metal impurities (Co, Fe, Ni)), graphitized mesoporous carbon (GMC; 99.95% purity, 50 nm of pore size), graphitized carbon nanofibre (CNF; 99.9 wt% -on the carbon basis, 100 nm \times 20–200 µm) and graphite nanopowder (GNP; 400 nm of size, 98% purity) were purchased from Sigma Aldrich (USA). All other are American Chemical Society certified chemicals. *Caution!* Benzo(*a*)pyrene and pyrene are carcinogenic and toxic compounds. Care must be taken while handling these chemicals.

2.2. Instrumentation

Electrochemical measurements were all carried out using CHI760D-electrochemical workstation, USA. The three-electrode system is consisted of GCE-working electrode (3 mm diameter, 0.0707 cm²), Ag/AgCl-reference and 2 mm diameter Pt-disc auxiliary electrode. The Bioanalytical system (BAS, USA) polishing kit was used to polish the GCE surface. A JASCO 4100 Spectrophotometer instrument was used for FTIR analysis (by KBr method). For Raman spectroscopic analysis, Agiltron Peakseeker Pro Spectrometer (USA; laser source = 532 nm) instruments were used. Mass spectrophotometer analysis was carried out using a Agilent 7890B Gas Chromatograph instrument. The instrument was set at initial and final temperatures, 50 °C and 250 °C respectively with increment rate of 50 °C/min. For sample preparation, the organic moiety containing chemically modified electrode is sonicated with 500 µL of ethanol and filtered the extract with micro-syringe. Prior to the sample analysis, the crude sample extract was subjected to thinlayer chromatography (TLC) and the specific band obtained for the product was isolated using ethanol as a solvent.

2.3. Preparation of the chemically modified electrodes

In first, the GCE surface was cleaned mechanically and electrochemically by polishing with 0.5 μ m Al₂O₃ powder-water suspension, washed with double distilled water followed by performing CV experiment for 20 continuous cycles in a potential window, -0.2 to 1.2 V vs Ag/AgCl at scan rate (v) = 50 mVs⁻¹ in pH 7 PBS. The GCE/ MWCNT modified electrode was prepared by drop-casting of 5 μ L of 2 mg MWCNT/500 μ L ethanolic suspension on the cleaned GCE surface and drying the electrode in air for 3 \pm 1 min at room temperature (25 \pm 2 °C). In further, the GCE/MWCNT@BaP_{ads}, where ads = adsorbed, was prepared by following two step procedure. Step-1: 5 μ L of 1 mg Benzo(*a*)Pyrene –500 μ L ethanol solution was drop casted on the GCE/MWCNT surface, i.e., GCE/MWCNT@BaP_{ads} followed by drying the electrode in air at room temperature for 3 \pm 1 min. Step-2: The modified electrode was either potential cycled in a window, –0.4 to 1.2 V vs Ag/AgCl at v = 50 mV s⁻¹ for ten continuous cycles or subjected to potentiostatic polarization experiment at an applied potential = 1.2 V vs Ag/AgCl in pH 7 PBS for 300 s (Scheme 1A-D). Overall, ~15 min is required to prepare the GCE/MWCNT@BaP-Oxid modified electrode (electro-oxidized form of the modified electrode).

The surface coverage value of the electroactive BaP_{ads} on the modified electrode, $\Gamma_{\text{BaP-Oxid}}$ (mol cm⁻²), was elucidated using a CV graph obtained for the surface-confined redox peak at $v = 50 \text{ mV s}^{-1}$ and calculated using the equation; $\Gamma_{\text{BaP-Oxid}} = Q/nFA_{\text{e}}$, wherein, Q = charge under the redox peak, n = total number of electrons (n = 2), F=Faraday constant and $A_{\text{e}} =$ geometrical surface area (0.0707 cm²).

3. Results and discussion

3.1. Electrochemical oxidation of adsorbed-BaP on GCE/MWCNT surface

In first, BaP adsorbed glassy carbon electrode, GCE/BaPads as a control sample was examined for the electrochemical reaction in pH 7 PBS medium. As can be seen in Fig. 1, curve a, there is no Faraday current signal noticed on the above case highlighting that PAH is non-amenable for any electrochemical oxidation/reduction reaction on conventional electrode due to its rigid aromatic structure. Interestingly, when the same electrochemical reaction is performed on GCE/MWCNT as an underlying electrode, ie., GCE/ MWCNT@BaPads, a well-defined growth of redox peak current up to first ten cycles after that nearly plateau in the response was noticed (Fig. 1, curve b). After the electrochemical experiment, the modified electrode was washed with pH 7 PBS and CV was performed. The results showed that the redox peak was retained without any alteration in the peak current and peak potential signals (Fig. 1B). A relative standard deviation (RSD) of the peak current signals measured at 1st cycle and 20th CV cycle is 3.2% indicating the good stability of the chemically modified electrode. Calculated apparent standard electrode potential (E°') and surface excess ($\Gamma_{\text{BaP-Oxid}}/\text{mol}$ cm^{-2} , assuming n = 2, BaP-Oxid: Oxidized form of BaP) values are 0 V vs Ag/AgCl and 62×10^{-9} mol cm⁻² respectively in pH 7 PBS. A minor post-peak appearance was noticed at $E^{0} = 0.1$ V vs Ag/AgCl (A1'/C1'), which could be due to electrochemical behaviour of BaPads at energetically different MWCNT site similar to our previous electrochemical studies with catechol on MWCNT and preanodized GCE surfaces [40,41]. Effect of scan rate (ν) on the redox peak showed a regular increase in the peak currents, both anodic (i_{pa}) and cathodic (i_{pc}) , against increase in the v (Fig. 1C). Plot of i_{pa} and i_{pc} versus v yielded a straight line starting from origin indicating the adsorption controlled-electron-transfer mechanism of the modified electrode (Inset Fig. 1C) [42].

Fig. 2A shows the effect of solution pH on the redox peak response. Typical plot of E^{0} , vs pH was shown in Fig. 2B. A slope of $-60 \pm 2 \text{ mV pH}^{-1}$ was obtained ascribing. Nernstian type proton-coupled electron-transfer behaviour of the redox couple with equal number of proton/electron exchange. Hereafter, the electrochemically oxidized BaP is tentatively designated as BaP-Oxid. At this



Fig. 1. (A) Cyclic voltammetry responses of GCE/BaP_{ads} (a) and GCE/MWCNT@BaP_{ads} (b) systems and (B) stability response of GCE/MWCNT@BaP-Oxid (curve a) in pH 7 PBS at $v = 50 \text{ mV s}^{-1}$. Fig. 1B, curves b and c are control CV responses of GCE and GCE/MWCNT. (C) Effect of CV scan rate of GCE/MWCNT@BaP-Oxid in pH 7 PBS and its i_{pa} and i_{pc} vs scan rate plot as inset. Note: BaP-Oxid = BaP-7,8-diol.



Fig. 2. (A) Effect of solution pH on the CV response of GCE/MWCNT@BaP-Oxid at v = 50 mV s⁻¹. Two continuous CV was performed with each system. (B) Plot of E^o vs pH.

stage, it is difficult to propose the defined structure of the BaP-Oxid formed on the surface. Moreover, since, the quantity of the BaP-Oxid is very small (~nanogram) and it is in composite nature, it is practically difficult to identify the precise structure of the BaP-Oxid using conventional characterization method like nuclear magnetic resonance (NMR), which requires about milligram amount of the pure sample. In order to overcome this problem, several critically designed control experiments, physicochemical characterization by Raman and IR spectroscopic techniques, GC-Mass analysis and redox selective organic compound oxidation reaction (CySH) were performed and from the collective results possible structure of the BaP-Oxid was derived.

In order to understand the electrochemical reaction mechanism for the BaP oxidation, GCE/MWCNT@BaP_{ads} (freshly prepared modified electrodes) was subjected to cyclic voltammetry potential segment analysis discreetly at different potential windows in pH 7 PBS (Fig. 3). In the first batch experiments (case-1), starting cathodic potentials were varied as -0.4 (e), -0.5 (d), -0.6 (c), -0.7 (b) and -0.8 (a) V vs Ag/AgCl with a fixed positive potential as 1.2 V vs Ag/AgCl (Fig. 3A). In all these experiments, qualitatively similar redox peak was appeared A1/C1). Indeed, a maximum peak current was obtained at a window, -0.4/-0.5 to 1.2 V vs Ag/AgCl (Fig. 3A, curve d and e). In addition, the redox peak current decreased against increase in the cathodic potentials (positive side). In a second batch experiments (case-II), starting potential was fixed uniformly as -0.4 V and varied the anodic sweeping potentials as (e) 0.6 V, (d) 0.7 V, (c) 0.8 V, (b) 1.0 V and (a) 1.2 V vs Ag/AgCl. As can be seen in Fig. 3B, appearance of A1/C1 redox peak was noticed only when the GCE/MWCNT@BaPads was swept to an anodic potential 1.2 V vs Ag/AgCl (Fig. 2B, curve e). In order to substantiate the experimental observation, GCE/MWCNT@BaPads (fresh electrodes) was subjected to potentiostatic polarization technique at different fixed applied potentials (E_{app} , 0.6, 0.8 1.0 and 1.2 V) for a fixed time, 300 s and then the modified electrode was subjected to CV for two cycles in a window, -0.4 to 1.2 V (case-III). Compared with previous case (Fig. 2A and B, potential dynamic technique), the potentiostatic



Fig. 3. Effect of potential scan direction on the CV responses of GCE/MWCNT@BaP_{ads} in pH 7 PBS at $\nu = 50$ mV s⁻¹. Freshly prepared electrodes were used for each scan. (A) E-cycling experiments with varying cathodic potentials, (e.) -0.4, (d.) -0.5, (c.) -0.6, (b.) -0.7 and (a) -0.8 V vs Ag/AgCl and a fixed anodic potential, +1.2 V. (B) E-cycling experiments with a fixed cathodic potential, -0.4 V and varying anodic potentials, (e.) 0.6, (d.) 0.7, (c.) 0.8, (b.) 1.0 and (a) 1.2 V vs Ag/AgCl. 10th cycle CV responses were displayed in the figures. (C) Effect of applied potential (E_{app}) on the potentiostatic preparation of GCE/MWCNT@BaP-Oxid in pH 7 PBS. Inset Fig. 3C is representative CV responses (2nd cycle) of test samples prepared at two different E_{app} .

experiment used is different from the optimal condition (potentialstatic technique). The later approach is widely used in bulk electroorganic synthesis reaction. Fig. 3C, a plot of i_{pa} vs E_{app} showing a steep increment in the redox peak current signal at $E_{app} > 1$ V vs Ag/ AgCl (a maximum i_{pa} at $E_{app} = 1.2$ V) is parallel to the case-II observation. These results indicate a specific role of the high anodic potential, 1.2 V vs Ag/AgCl for the surface confined BaP oxidation reaction. It is likely that at the high oxidation potential, water molecule gets electrochemically oxidized to molecular oxygen via hydroxyl radical intermediate which can initiate formation of surface adsorbed BaP radical cation species and then to hydroxylation of BaP as redox active BaP-Oxid compound (Scheme 1C) [43]. In the literature, there has been significant number of studies on advanced anodic oxidation of organic molecules like benzene, phenols, synthetic dyes and aliphatic acids etc at an applied potential ~2 V vs Ag/AgCl on solid oxide electrodes like PbO₂, IrO₂, BDD, oriented pyrolytic graphite in acid solution. The general mechanism involes the following steps [43]:

$$\equiv S[] + H_2 O \rightarrow \equiv S[\bullet OH] + H^+ + e^{-1}$$
(1)

$$\equiv S[\bullet OH] + R \rightarrow \equiv S[] + RO + H^+ + e^-$$
(2)

$$\equiv S[\bullet OH] + H_2O \rightarrow \equiv S[] + O_2 + 3H^+ + 3e-$$
(3)

Wherein, \equiv S[] represents the surface sites for adsorption of •OH species and R and RO belong to targeted organic compound and hydroxylated product/s respectively. The intermediate-hydroxyl radical species formed on the reaction phase and/or on the surface (known as physisorbed hydrogen radical species) have been identified using electron-spin resonance and UV-Vis spectroscopic techniques using suitable radical trapping agents like 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) and salicylic acid or N,N-dimethyl-pnitrosoaniline (RNO) respectively [43-46]. Note that in the case of BaP, one electron-oxidation to respective BaP-cationic radical species formation as an intermediate step has been referred as a key in the overall oxidation reaction [28,47,48]. In fact, Kim and Ley have pointed-out that the reactive oxygen species (ROS) generated in biological system oxidize the BaP to hydroxylated metabolites and in turn to DNA and RNA interaction and carcinogenic activity [49]. Based on the obtained results and information, it is proposed that at high anodic potential, BaP is oxidized to hydroxylated product (BaP-xOH, wherein, x=1-4) and it is responsible for the redox response noticed in this work. The redox peak current decrement noticed at high negative potentials (Fig. 3A, curves a-e) could be due to quenching of ROS intermediates.

The effect of carbon matrix like mesoporous carbon, graphene oxide, carbon nanofiber and bulk graphite on the surface-confined electrochemical oxidation reaction of BaP was investigated and the results are presented in Fig. 4A–D. BaP adsorbed carbon material modified electrodes were prepared similar to the GCE/ MWCNT@BaPads case and subjected to ten continuous CV-potential cycling experiments in pH 7 PB at a window, -0.4 to 1.2 V vs Ag/ AgCl. Fig. 4A–D are 10th cycle CV responses of various carbon material modified electrode systems. Based on the surface excess value, $\Gamma_{BaP-Oxid}$ values (Fig. 4E), the order of BaP-Oxid formed is arranged as MWCNT > GMC > CNF > GO > GNP. Note that, in addition to A1/C1, a new redox peak at $E^{o'} = -0.3$ V vs Ag/AgCl was noticed with GNP and GO matrixes, which may be due to the formation of multi-hydroxyl functional groups of BaP, similar to the conversion of pyrene unit of BaP to pyrene-4OH product discussed in the previous section (Fig. 6C) [50]. The conclusions derived from the observations are: (i) multiwalled carbon nanotube is the best carbon material system for BaP adsorption and surface-confined electrochemical oxidation and (ii) oxygen functional group containing carbon (GO) can retard the BaP oxidation reaction, which may be due to some electrostatic repulsion reaction between the charged oxygen functional groups (-COOH, -OH and >C=O) of BaP-Oxid and MWCNT [50,51].

3.2. Physicochemical characterization of MWCNT@BaP-Oxid

Fig. 5A is a comparative Raman spectroscopic response of

MWCNT and MWCNT@BaP-Oxid systems. Specific signals corresponding to the A1g mode of the disorder and structural defective carbon lattice (sp³ site, D band) and normal phonon mode of E2g symmetry from sp²-graphitic carbon atom vibrations (G band) at 1360 and 1580 cm^{-1} were noticed [52]. Since the bands are qualitatively similar, the intensity ratio of the D and G bands, I_D/I_G was considered for the quantitative analysis. Calculated I_D/I_C ratio for the samples are 0.20 and 0.15 respectively. A slight decrease in the I_D/I_G ratio with the GCE/MWCNT@BaP-Oxid case indicates the enriched adsorption of aromatic π -electron system, BaP on the MWCNT surface. Comparative FTIR response of MWCNT, MWCNT (electrochemically treated), BaP, MWCNT@BaPads and MWCNT@BaP-Oxid samples was shown in Fig. 5B. FTIR response of MWCNT@BaP-Oxid is found to be closely matching with vibrational signals of BaP and MWCNT in the finger print region, 1500-500 cm⁻¹, but with a slight shift in the wave numbers. A characteristic IR signal at 3513 cm^{-1} due to the hydroxyl functional group was specifically noticed with the MWCNT@BaP-Oxid sample attributing the existence of -OH functional group on BaP-Oxid. Indeed, it is not clear about the position of the hydroxyl groups on the BaP–20H. In order to confirm the position, couple of control experiments were carried out. In case-1: pyrene molecule, which is an integral part of the structure of BaP but with less of one benzene ring (4 nos), was subjected to surface-confined electrochemical oxidation on GCE/MWCNT as in Fig. 6A. As reported previously, a redox peak at $E^{0} = -0.2$ V vs Ag/AgCl, which is due to a hydroxylated Pyrene derivative (MWCNT@Pyrene-Oxid, Pyrene-



Fig. 4. (A–D) CV responses of BaP-Oxid modified carbon nanomaterial in pH 7 PBS at a scan rate = 50 mV s⁻¹. (E) Plot of $\Gamma_{BaP-Oxid}$ vs Carbon@BaP-Oxid. GNP = Graphite nanoparticle; GO = Graphene oxide; GMC = Graphitized mesoporous carbon; CNF= Carbon nanofiber.



B.FTIR



Fig. 5. Comparative (A) Raman and (B) FTIR spectroscopic responses of MWCNT@BaP-Oxid sample along with its controls. E-treated: Electrochemical treated the sample (GCE/MWCNT) at -0.4 to 1.2 V vs Ag/AgCl for 10 cycles.



Fig. 6. (A) Continuous CV response of GCE/MWCNT@Pyrene_{ads} in pH7 PBS at $\nu = 50$ mV s⁻¹. (B) CV response of GCE/MWCNT@Pyrene-Oxid without (a) and with 500 μ M of CySH in pH 7 PBS at $\nu = 10$ mV s⁻¹. Curve B(c) is a CV response of GCE/MWCNT with 500 μ M of CySH. (C) Illustration for the adsorption (a) and electrochemical oxidation of GCE/MWCNT@Pyrene-Oxid (b) and its nil electrocatalytic response with CySH.



Fig. 7. (A) CV response of GCE/MWCNT@BaP-7,8-diol without (a) and with 500 μ M of CySH in pH 7 PBS at ν = 10 mV s⁻¹. Curve c is a CV response of GCE/MWCNT with 500 μ M of CySH. Note: BaP-Oxid = BaP-2OH=BaP-7,8-diol.

Oxid = Pyrene-4OH [50], was obtained. Note that the redox peak is distinctly different from the E^o value of the BaP–2OH (E^o = 0 V vs Ag/AgCl). In case-II; a specific organic redox probe, CySH which get oxidized at 1,2-dihydroxy benzene derivative [38], was subjected electrocatalysis with GCE/MWCNT@BaP–2OH and GCE/MWCNT@Pyrene-Oxid in pH 7 PBS (Fig. 6B). A selective electrochemical oxidation of CySH was noticed only at GCE/MWCNT@BaP–2OH (Fig. 7A), while the GCE/MWCNT@Pyrene-Oxid was found to be inactive to CySH (Fig. 7B and C), confirming existence of 1,2 dihydroxy benzene derivative like structure of BaP–2OH, i.e., BaP-7,8-diol (Scheme 1). This point onwards the BaP-Oxid is redesignated as BaP-7,8-diol.

For validation of the product, the MWCNT@BaP-Oxid and BaP was subjected to GC-MS analysis (positive mode) after being extracted with ethanol solvent in comparison with precursor-BaP as a control sample. A *m*/*z* signal at 284.30 *m*/*z* corresponding to

a molecular structure of two hydroxyl group functionalized BaP molecule, BaP–2OH (Mol.wt. 284.30) was obtained (data not shown). This result may support the BaP–2OH molecule is the structure of the BaP-Oxid sample. As an independent study, electrocatalytic/amperometric i-t sensing of CySH was performed using GCE/MWCNT@BaP-7,8-OH in neutral pH solution (pH 7 PBS).

As can be seen in Fig. 8A and B, continuous spikes of 25 μ M of CySH resulted in systematic increase in the peak current signal and it is not interfered by addition of other biochemicals such as homocysteine, H₂O₂, citric acid and hydrazine (Fig. 8C). Constructed calibration plot is linear in a window, 25–700 μ M CySH. A noisy response noticed in this work is due to porous and pseudocapacitor like nature of the modified electrode.

Overall, the new findings of this work are: (i) A simple and smart method for the electrochemical oxidation of the carcinogenic "bayregion" containing PAHs in physiological solution unlike the BaP oxidation by non-aqueous and elevated temperature based oxidation procedures. (ii) Biomimicking functions of GCE/MWCNT with natural protein and peroxidase on the oxidation of BaP to BaP-7,8diol molecule. (iii) Simple electrochemical conversion of carcinogenic bay-region PAH pollutant, BaP to electroactive product, BaP-7,8-diol (metabolite). (iv) A scope for room temperature electrochemical reaction based decomposition of BaP in the environmental samples.

4. Conclusions

Electrochemical oxidation of a bay-region containing polyaromatic hydrocarbon, benze(a) pyrene was performed as a surfaceconfined system on multiwalled carbon nanotube modified glassy carbon electrode in pH 7 phosphate buffer solution. A well-defined and highly redox active surface-confined peak which has protoncoupled electron-transfer behaviour was observed. The electrochemical oxidation reaction was found to occur at potential 1.2 V vs Ag/AgCl, where, there is a marked oxidation of water to molecular oxygen involved. It has been proposed that a hydroxyl radical like ROS species formed in the intermediate step has assisted the BaPelectrochemical oxidation reaction. The electro-oxidized BaP chemically modified electrode (MWCNT@BaP-Oxid) was characterized by Raman, IR and GC-MS (as an ethanolic extract) techniques along with several critically designed control experiments based on 1,2-dihydroxy benzene molecule selective electrochemical oxidation probe using cysteine. From the collective



Fig. 8. (A) Amperometric i-t responses of GCE/MWCNT and GCE/MWCNT@BaP-7,8-OH (B) for continuous sensing 25 μM spikes of CySH. (C) for sensing of other interferences, Cysteine (CySH), homocysteine (Homo CySH), H₂O₂, citric acid (CA) in a stirred pH7 PBS at an applied potential of 0 V vs Ag/AgCl. Note: BaP-7,8-diol = BaP-2OH=BaP-Oxid.

results, it has been revealed that BaP oxidizes to BaP-7,8-diol derivative which is similar to the cytochrome c and peroxidase enzymes assisted biochemical oxidation reaction. The results obtained in this work provide a new platform for the effective and room temperature based decomposition of the carcinogenic pollutants to useful electro-active compounds.

Intellectual property

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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Declaration of competing interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

CRediT authorship contribution statement

Sivakumar Nisha: Validation, Formal analysis, Investigation, Resources, Data curation, Visualization. **Annamalai Senthil Kumar:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

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