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Ag ion irradiated based sensor for the electrochemical determination of epinephrine and 5-hydroxytryptamine in human biological fluids

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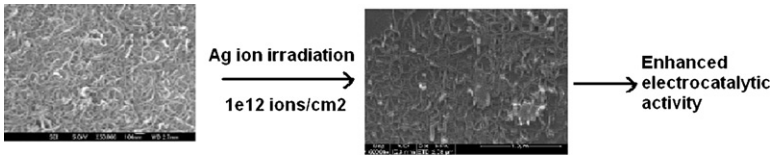
HIGHLIGHTS

- Ag ions irradiation enhances the electrocatalytic activity of carbon nano tubes.
- The low fluence of irradiation caused the ordering of carbon nano tubes.
- Simultaneous determination of epinephrine and 5-hydroxytryptamine has been carried out.
- The determination of the neurotransmitters in human blood and urine is reported.

GRAPHICAL ABSTRACT



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ABSTRACT

A promising and highly sensitive voltammetric method has been developed for the first time for the determination of epinephrine (EP) and 5-hydroxytryptamine (5-HT) using 120 MeV Ag ion irradiated multi-walled carbon nano tube (MWCNT) based sensor. The MWCNT were irradiated at various fluences of 1e12, 3e12 and 1e13 ions cm^{-2} using palletron accelerator. The simultaneous determination of EP and 5-HT has been carried out in phosphate buffer solution of pH 7.20 using square wave voltammetry and cyclic voltammetry. Experimental results suggested that irradiation of MWCNT by Ag ions enhanced the electrocatalytic activity due to increase in effective surface area and insertion of Ag ions, leading to a remarkable enhancement in peak currents and shift of peak potentials to less positive values as compared to the unirradiated MWCNT (pristine). The developed sensor exhibited a linear relationship between peak current and concentration of EP and 5-HT in the range 0.1–105 μM with detection limit ($3\sigma/b$) of 2 nM and 0.75 nM, respectively. The practical utility of irradiation based MWCNT sensor has been demonstrated for the determination of EP and 5-HT in human urine and blood samples.

1. Introduction

Neurotransmitters are the endogenous primary chemical messengers released by the pre-synaptic nerve cells relay, amplify and transmit the signals to post-synaptic nerve cells and play a vital role in neuronal communication of central nervous system (CNS) [1–3]. Epinephrine (1-(3,4-dihyroxyphenyl)-2-methylaminoethanol, EP) is an important catecholamine, which acts as a neurotransmitter in mammalian central nervous system for transporting the information between biological cells [4]. EP is secreted by medulla of adrenal gland in the situation of high psychological pressure or low blood sugar level and is used as drug to treat myocardial infarction, hypertension, bronchial asthma, severe allergic reaction, cardiac reaction and sepsis [5,6]. EP, also known as adrenaline, is a hormone and plays an important role during mental stress and stimulates series of actions of sympathetic nervous system called as "fight or flight response". EP concentration in blood affects regulation of blood pressure and heart rate, lipolysis, immune system and glycogen metabolism [7,8]. These important functions also make EP a potent doping agent, hence, it is banned by the World Anti Doping Agency (WADA) during competitive games [9]. Therefore, determination of this catecholamine in human body fluids is of

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significant use for nerve physiology, medical diagnosis and especially for patients suffering from Parkinson's disease, phaeochromocytoma and stress [10].

5-Hydroxytryptamine (serotonin, 5-HT) is one of the major neurotransmitters of human brain and is known to play a central role in wide variety of pharmacological, biological and psychopathological functions including depression, eating disorders, alcoholism, obsessive-compulsive disorders and anxiety [11]. 5-HT is found in gastro intestinal tracts (GI) and CNS and acts both as neurotransmitter and a local hormone in peripheral vascular system in gut. A large amount of 5-HT in the body (over 90%) is found in enterochromaffin (EC) cells of GI and stored in blood platelets, whereas, the brain contains only a minor proportion [12]. 5-HT is synthesized from an essential amino acid tryptophan, a protein constituent of normal diet by tryptophan hydroxylase enzyme present in EC cells. 5-HT is implicated in various gastrointestinal disorders including irritable bowel syndrome, inflammatory bowel disease and food hypersensitivity [13]. In addition neuro-degeneration of 5-HT has been found to associate with late-onset neurological diseases, including Parkinson's disease and Alzheimer's disease and possibly to normal aging of brain [14,15]. Therefore, quantitative investigation of 5-HT in human urine and plasma is essential because of its coexistence in biological systems and regulating several physiological functions. Although, various techniques have been implemented for the determination of EP and 5-HT such as high performance liquid chromatography, spectro-photometric technique, capillary electrophoresis and flow injection analysis [16–18], however, these techniques require expensive instruments and time consuming pretreatment and derivatization processes which results in low recoveries and tedious procedure. Electrochemical techniques based on various approaches have been developed to overcome these difficulties [19–21]. The determination of EP has been reported at carbon paste and variety of other electrodes [22–24]. However, simultaneous determination of EP and 5-HT by electrochemical methods remained a challenge due to the interference of other biomolecules like ascorbic acid, uric acid and dopamine present in biosystems. These interferents oxidize at a potential close to that of EP and 5-HT, resulting in an overlapping voltammetric response.

Therefore, the aim of the present investigation is to develop a sensitive voltammetric sensor for the simultaneous determination of EP and 5-HT in human fluids. A new approach based on irradiation of multi-walled carbon nano tube (MWCNT) by swift heavy Ag ions has been used to improve catalytic activity and conductivity. Carbon nano tubes are one of the most exciting materials these days due to their unique electronic, chemical and electronic properties and possess sp^2 carbon units with several nanometers in diameter and many microns in length [25]. The irradiation of carbon nano tubes by energetic Ag ions produce ion tracks (columnar defects) leading to the formation of amorphous carbon (*a-C*) inside the material which makes it more conductive than unirradiated MWCNT [25–27]. The sputtering of carbon atoms is also observed which produce the vacancies on the side walls and interistial atoms between the shells providing the rough surface that leads to an increase in surface area of nano tubes thin film [28]. The size and hybridization of carbon system is also tailored by the ion beam treatment that makes ion beam irradiation a promising field of research [29–31]. In the present studies Ag ions of high energy (~120 MeV) at different fluence 1e12, 3e12, 1e13 ions cm^{-2} were used for the irradiation of MWCNT. After optimizing the experimental parameters, the irradiated sensor has been employed for the determination of EP and 5-HT in various human urine samples. The effect of common metabolites present in urine such as ascorbic acid, uric acid and dopamine has also been evaluated.

2. Experimental

2.1. Chemicals and reagents

EP and 5-HT were purchased from Sigma–Aldrich and used as received. Phosphate buffer solutions were prepared according to the method of Christian and Purdy [32] by mixing the standard solutions of H_3PO_4, Na_2HPO_4 and NaH_2PO_4. MWCNT (98%) were purchased from Bucky, USA and used for the modification of indiumtinoxide (ITO) surface. ITO spurted glass sheets of size 10 mm × 20 mm × 1.1 mm were obtained from Geomatec, Japan. Adrenaline bitartrate injections (G.K. Pharamaceuticals; Mfg. Lic. No. 5/SC/P-2006) were obtained from the Institute hospital of Indian Institute of Technology, Roorkee. All the reagents used were of analytical grades and double distilled water was used throughout the experiments.

2.2. Instrumentation

The square wave voltammetry and cyclic voltammetry experiments were performed using a computerized BAS (Bioanalytical Systems, West Lafayette, USA) CV-50W voltammetric analyzer. A conventional three-electrode cell assembly consisting of Ag/AgCl electrode (3 M NaCl) (Model MF-2052 RB-5B) as reference electrode, platinum wire as counter electrode and irradiated MWCNT as working electrode was used for the electrochemical measurements. The pH of the buffer solutions was measured using Century India Ltd. Digital pH meter (Model CP-901). MWCNT modified ITO was irradiated using 15 UD Pelletron Accelerator at Inter University Accelerator Centre, New Delhi, India. Raman spectra of un-irradiated (pristine) and 120 MeV Ag ions-irradiated MWCNT modified ITO were recorded using Renishaw in-via Raman microscope with Ar ion laser excitation at 514 nm at room temperature. Field emission scanning electron microscopy (FE-SEM, JEOL-JSM 7400) instrument was used to study the surface morphology of pristine and irradiated electrode.

2.3. Irradiation of MWCNT

Suspension of MWCNT (0.5 mg mL^{-1}) was prepared in N,N-dimethylformamide solution and ultrasonic machine was used to achieve well dispersed suspension. A known volume (25 μL) of this suspension was coated on the surface of bare ITO (10 mm × 10 mm) and was allowed to evaporate at room temperature. MWCNT modified ITO was then irradiated using 120 MeV Ag ions at fluences of 1e12, 3e12 and 1e13 ions cm^{-2} with 15 UD Pelletron Accelerator. The vacuum in the chamber during the irradiation was kept $\sim 5 \times 10^{-6}$ Torr. Electromagnetic scanner was used to scan the beam on the full area of thin film. The thickness of the carbon nano tubes layer was found to be bit larger as compared to the range of 120 MeV Ag ions in carbon. The sensor was prepared by putting the ITO between two scotch tapes and connected with copper strip for connections. A 3 mm diameter hole on one side of the tape was made to expose the sensor for making the contact with solution. Time base technique was used to remove adsorbed analyte from the surface of irradiated MWCNT by applying a potential of −200 mV for 60 s after each run.

2.4. Experimental procedure

Stock solutions of EP and 5-HT (1 mM) were prepared by dissolving the required amount of compounds in double-distilled water. A known amount was added to 2.0 mL phosphate buffer and the total volume was made 4.0 mL with double distilled water. Square wave voltammograms were then recorded at the optimized parameters: initial *E*: 0 mV, final *E*: 1600 mV, square wave frequency (*f*): 15 Hz,