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Carbon nanotube ensembled hybrid nanocomposite electrode for direct electrochemical detection of epinephrine in pharmaceutical tablets and urine

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ABSTRACT

An efficient electrochemical sensor for selective detection of the neurotransmitter, epinephrine (Epn), has been fabricated with the aid of a functionalized multiwall carbon nanotube–chitosan biopolymer nanocomposite (*Chit-fCNT*) electrode. Multiwall carbon nanotubes (CNT) were successfully functionalized with the aid of nitric acid and confirmed by the Raman spectral data. Functionalized carbon nanotubes (*fCNT*) were dispersed in chitosan solution and the resulting bio-nanocomposite was used for the fabrication of sensor surface by drop and cast method. Electrochemical characteristics of the fabricated sensor were understood using cyclic, differential pulse voltammetry (CV, DPV) and electrochemical impedance analysis for the detection of Epn in phosphate buffer (pH 7.4). CV and impedance analysis revealed that the *Chit-fCNT* modified electrode enhances the electrodic reaction of Epn and facilitated the electron transfer more readily compared to that of bare electrode. Applying DPV for the detection of Epn, achieved 30 nM as the lowest detection limit in the determination range of 0.05–10 μM and the analytical time as low as 10 s. Selective determination of Epn against the coexistence of a number of biological electroactive interferents and reproducible results for the determination of Epn were demonstrated. The present biosensor has been found efficient for successful direct determination of Epn from pharmaceutical adrenaline formulations and urine samples.

1. Introduction

Epinephrine (Epn), the neurotransmitter, is present in human serum at about nM levels. Epn was first isolated in 1901 and synthesized in 1904 [1]. Epn is crucial for the successful performance of cardiovascular and central nervous systems. Several physiological phenomena are correlative to Epn levels in biological fluids and its measurement is a key factor in the medical diagnosis of diabetes, Parkinson's disease and cerebral malaises [2]. Epn is the most prescribed common emergency healthcare medicine. Recent literature reports reveal that World Anti-Doping Agency (WADA) has banned Epn usage during competitive games [3].

A simple, highly selective and rapid detection of Epn in both physiological fluids and pharmaceutical samples is highly necessitated. Quantitative analysis of Epn is essential in the development of physiological investigations, pharmacological research and life science. Detection and quantification of Epn has been reported by various methods such as absorbance, fluorescence, capillary electrophoresis [4], electrochemiluminescence [5], HPLC [6] and chromatography coupled with mass spectrometry [7–9]. However, these methods suffer from either tedious procedure or low sensitivity. Therefore, a simple and convenient method needs to be developed for Epn detection.

Electrochemical techniques can be employed for the successful quantification of Epn as it can undergo oxidation very easily. But, the electrochemical detection of Epn at bare electrodes involves various basic difficulties, mainly *high overpotential* and *sluggish kinetics of electrodic processes*, which result in insignificant electrochemical responses. Another major problem is the coexistence of Epn with other electroactive biological compounds such as ascorbic or uric acid which would also undergo oxidation at unmodified electrodes at almost the same potentials. So, it is necessary to overcome the electrodic reaction of these interferents to achieve a selective determination of Epn [10].

Selective and rapid detection of Epn despite the coexistence of various potential biological interferents is an important target of electroanalytical research. To overcome these drawbacks, extensive research investigations were dedicated by scientists to develop new surface modified electrodes which eliminate possible interventions from the potential interferents [11]. Wide range of molecular recognition elements were reported in developing highly selective recognition matrices for neurotransmitters. Biomolecules (antibodies, nucleic acids, aptamers and dendrimers), self-assembled monolayers, mesoporous

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materials, sol-gel derivatives, molecular imprinted polymers and conducting polymers have been investigated as recognition matrices [12]. Nanoparticles of noble metals, other metals and non-metals [13–16] can be used in the fabrication of inorganic recognition matrices. Further, the combination of metal nanoparticles with other functional materials steers to the advanced hybrid nanomaterials which possess unique and distinct characteristics. Hybrid core-shell nanostructures [17], metal nanoparticle-hydrogel composites [18], nanoparticle-polymeric hybrid scaffolds [19–21], metal nanoparticle-biomolecule bioconjugates [22,23], anisotropic Janus particles [24] and carbon dots [25] can offer advantages in the fabrication of recognition matrix.

Unique characteristic properties of carbon nanotubes (CNT) offer wide range of applications in innumerable fields. High electrical conductivity and long nanowire-like structure of CNT have attracted for the fabrication of highly sensitive electrochemical sensors. CNT could reduce electrode fouling and thus could promote improved reuse of CNT based electrodes. But, the prime drawback of CNT is their low solubility in majority of the solvents. Several strategies have been proposed to enhance the CNT dispersion in polymer electrolytes, ionic liquids, biopolymer matrices and intercalation with polymers and composite matrices [9,26].

Chitosan (Chit) is a β-linked polysaccharide with remarkable biocompatibility and biodegradability and is extracted from the natural abundant chitin by partial deacetylation. Chit comprises unique characteristic properties such as excellent film forming, adherence, stability and numerous reactive amino and hydroxyl groups for functionalization [27]. In this work, a stable and uniform CNT–Chit nanocomposite film was achieved using Chit.

In the present study, we fabricated an electrochemical biosensor for Epn employing *Chit-fCNT* nanocomposite for the modification of electrode surface. Functionalization of CNT was confirmed using Raman spectroscopy. Morphological characteristics of the nanocomposite film were explored using scanning electron microscopic (SEM) analysis. Electrocatalytic activity and selectivity of the *Chit-fCNT* nanocomposite modified electrode towards the detection of Epn were investigated with cyclic, differential pulse voltammetry (CV, DPV) and electrochemical impedance spectroscopy (EIS) techniques. Feasibility for the direct determination of Epn selectively from pharmaceutical adrenaline injections and artificial urine samples was established.

2. Experimental

2.1. Materials

Epinephrine, ascorbic acid, uric acid, serotonin and chitosan (low molecular weight biopolymer of ~60 kDa from crab shells and ~85% deacetylated) were bought from Sigma (USA). Multiwall carbon nanotubes of 20–50 nm diameter and 2–5 μm length were received from Sisco Research Laboratories Pvt. Ltd. (India). Chemicals used for the preparation of buffers and artificial urine samples were analytical grade with a minimum of 99% purity. Pharmaceutical samples of epinephrine (Vasocon injection, 1 mg mL^{-1} adrenaline bitartrate) were received from the dispensary of our Institute. All the aqueous buffer and electrolyte solutions were prepared with double-distilled deionized water of high resistance (18 MΩ) which passed through a 0.2 μm Whatman filter. Phosphate buffer solution (PBS, 50 mM) was prepared by adding appropriate quantities of K_2HPO_4 and KH_2PO_4 as explained in the Sigma-Aldrich product information, and the anticipated pH was acquired by the addition of 0.1 M NaOH. Artificial urine samples of pH 7.4 were prepared as mentioned in the reported literature [28].

2.2. Functionalization of carbon nanotubes

Multiwall carbon nanotubes (CNT) were treated with nitric acid to bring in functional carboxyl groups according to the procedure [29] with minor modifications as follows. 120 mg of CNT were introduced to 10 mL aq. 3 N nitric acid, then maintained at ~70 °C for 24 h under constant stirring. Nitric acid oxidizes and introduces carboxyl groups at the sidewall defects and edges of the nanotubes. After the treatment, the black solid compound was centrifuged, separated, and then cleaned with water several times so that the supernatant becomes neutral. Functionalized CNT (*fCNT*) was dried at 80 °C for 12 h.

2.3. Electrode modification by fCNT hybrid nanocomposite

At first, fresh surface of glassy carbon electrodes (GCE, 3 mm diameter) was obtained by polishing with alumina slurries of 3 μm and moving down to 0.05 μm, and the electrodes were then cleaned in 1:2 v/v dil.HNO_3, ethanol and water by ultrasonication for 3 min each. Chitosan was dispersed in aq. 1% (v/v) acetic acid solution at 1% (w/v) concentration. Functionalized CNT was dispersed into the chitosan solution at 4 mg mL^{-1} concentration by using ultrasonication for ~10 min. Freshly polished GCE surface was modified using 8 μL of the dispersion of *fCNT* by drop-cast method and the resultant electrode is denoted as *GCE/Chit-fCNT*.

2.4. Analytical methods

Electrochemical measurements (CV, DPV) were performed in a two-compartment cell using a CHI-619D analyzer (CH Instruments, USA) at room temperature with bare or modified GCE as working electrode, a Pt spiral wire as counter electrode and an Ag/AgCl (3 N KCl) electrode as reference electrode. EIS response was investigated using Zahner-IM6e workstation (Germany) in 10 mHz–100 KHz frequency range with 10 mV excitation amplitude and 64 as sine wave count. Electrolyte contents were deaerated with nitrogen gas for 15 min prior to each electrochemical analysis and continuously maintained the flow of nitrogen over the electrolyte surface.

SEM analysis was carried out using SEM-TESCAN, VEGA 3 LMU model for analyzing morphology of *Chit-fCNT* nanocomposite. Sample was prepared by the dispersion of 4 mg mL^{-1} *fCNT* in 1% (w/v) Chit solution. A small volume (~5 μL) of the dispersion was casted on to the carbon tape supported on SEM sample stub. The sample was sputtered for 60 s with gold using a mini sputter coater (QUORUM Technologies, SC7620). Raman vibrational spectra of MWCNT samples were recorded with LabRAM (HR800 model, France) spectrometer; a He-Ne (633 nm) laser of 20 mW power has been employed with 5× objective and the samples were dispersed in 4:1 (v/v) acetonitrile:methanol mixture.

3. Results and discussion

3.1. Raman spectra of CNT and fCNT

Pristine- and functionalized-CNT were characterized by Raman spectroscopy. Both pristine CNT and *fCNT* exhibit four characteristic peaks namely D, G, D′ and D* bands in their Raman spectral data (Fig. 1). The characteristic peak at ~1330 cm^{-1} is relevant to the defect derived D band which represents the defect sites or disordered graphitic structure of CNT. The peak at ~1580 cm^{-1} is assigned as graphitic structure derived G band which represents the >C=C< bond nature in the graphitic plane of CNT. Raman vibrational peak at ~1610 cm^{-1} represents D′ band which indicates the defective sites in the manufacture of CNT. The vibrational peak at ~2660 cm^{-1} (D*) is the overtone of D band originated from a double-resonance process [30–32]. Relatively smaller D band indicates high purity of CNT whereas larger D band is likely due to the influence of terminal groups or defect sites. Quality of CNT could be decided based on the ratio of these peak intensities, in other words I_D/I_G values would be considered in determining the purity of CNT. When compared the I_D/I_G values of *fCNT* (1.4594) with that of pristine CNT (1.1824), the increment in I_D/I_G value of *fCNT* suggests that the formation of some sp^3 carbons by oxidation in the case of