Voltammetric determination of pyrazinamide at graphene-zinc oxide nanocomposite modified carbon paste electrode employing differential pulse voltammetry

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ABSTRACT

Pyrazinamide (PZN) also called as Pyrazinecarboxamide is a widely used drug for the treatment of Tuberculosis. In the present work, we proposed a sensitive method for determination of PZN using Graphene–Zinc oxide-Carbon paste electrode (GNS-ZnO-CPE). The surface characterization of electrode materials was done by using X-Ray diffraction, Scanning electron microscopy, Energy dispersive X-ray spectroscopy and transmission electron microscopy. The electrocatalytic response of PZN at GNS-ZnO-CPE was measured using cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS). In addition, electrochemical impedance studies revealed that the smaller R_{ct} value was observed at GNS-ZnO-CPE as compared to that of CPE, which authenticates its good conductivity. Under the optimized conditions, I_p (μA) was proportional to PZN concentration in the range of 1.5×10^{-7} to 4.0×10^{-4} M with a detection limit of 4.31×10^{-8} M. The proposed electrochemical sensor showed excellent recovery in pharmaceutical formulations, urine and blood serum samples which revealed the promising practicality of sensor for PZN detection.

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1. Introduction

Pyrazinamide (PZN) also called as pyrazinecarboxamide is a first-line oral drug used to treat tuberculosis (TB). The drug is mainly used for treatment of tuberculosis caused by *mychobacterium tuberculosis* [1–3]. Generally, PZN is used in combination with other anti-TB drugs viz; isoniazide, rifampicin, and ethambutol hydrochloride. It has been used for treatment of TB since 1952 and the exact mechanism of action is not well known. PZN is converted to pyrazinoic acid, the active form of the drug, by the pyrazinamidase enzyme (PZase) produced by *mychobacterium tuberculosis*. It is assumed that pyrazinoic acid has ability to lower the pH to an acidic medium that the organism will not tolerate [4,5]. The use of PZN results in shortening the duration of therapy from nine to six months. However, continuous dosage of PZN leads to various side effects. The most serious side effect is hepatotoxicity. The other side effects include nausea, vomiting, exanthema, anorexia, sideroblastic, liver injury, anemia, skin rash, urticaria, pruritus, dysuria, intestinal nephritis, malaise, dark urine,

rarely porphyria and fever. Therefore, development of sensitive and selective method for determination of PZN in body fluids is of tremendous importance.

Literature survey reveals several analytical methods for determination of PZN such as chromatographic methods [6–8], UV–vis method [9] and capillary electrophoresis [10]. However, most of these methods are lengthy, overpriced, complicated, require expert knowledge and often need the pretreatment step that make them unsuitable for routine analysis. To overcome the drawbacks associated with the aforementioned methods electrochemical methods such as cyclic voltammetry (CV), differential pulse voltammetry (DPV) and stripping methods are used extensively due to their sensitivity, selectivity, simplicity, low cost and relatively short analysis time. To the best of our knowledge, few electrochemical methods are reported for determination of PZN [11–16].

Over the past two decades, chemically modified electrodes (CMEs) have attracted broad interest in sensor development due to low background current, wide range of potential window, easy surface renewal, lower detection limit, low sensitivity to dissolved oxygen and low cost. Due to such advantages CMEs have been used in various analyses. In recent years, our group has reported chemically modified electrodes for determination of various organic [17–23] as well as inorganic [24,25] species. Carbon has been widely used as an electrode material since many years with or without

modification. The wide applications of carbon electrodes started when carbon paste electrode (CPE) was invented by Adams in 1958 [26]. Graphene (GNS), a two-dimensional honeycomb lattice structure of graphite is extensively used for development of sensor as well as supercapacitor due to its large surface area, very high electrical and thermal conductivity, great mechanical strength and low manufacturing cost [27–31]. These properties make graphene as a suitable modifier for modification of different electrodes. Metal oxide nanoparticles have been widely explored in the field of electrochemical sensors as they offer many advantages such as high abundance, low cost, easy synthetic procedures, high electrical conductivity, etc. Due to such advantages, many metal oxides such as TiO_2, Fe_3O_4, MnO_2, ZnO, Cu_2O, Co_3O_4, etc. have been widely used for the electrochemical detection of various species. Among all these metal oxide nanoparticles, ZnO is widely used because of its stable chemical and physical characters, easy route of synthesis, high surface activity and stability. In addition to this, ZnO is biochemically stable material, which makes it ideal for direct use in biomedical applications without the need of surface passivation. ZnO has a large excitonic energy, low-cost synthesis, biocompatibility, good electrochemical activities, non-toxicity, high-electron communication features and high mechanical strength. Nanosize zinc oxide (ZnO) is a thermally stable semiconductor with a wide band gap (3.37 eV) and it is used widely in the field of chemical sensors, gas sensors, solar cells, catalyst, electrical and optical devices etc [32–36]. The ZnO has been widely used in sensor applications due to its high surface to volume ratio, which results in greater interaction of an analyte with the sensing part of device. Graphene sheets suffer from agglomeration and restacking due to van der Waals interaction which leads to great loss of effective surface area of electrode. But when graphene is used along with ZnO nanoparticles, it weakens the interactions between graphene sheets preventing agglomeration, which leads to increase in surface area of the electrode. The conductivity of ZnO is enhanced when it is used along with GNS [37]. Therefore, synergistic effect of GNS and ZnO leads to a highly sensitive sensor for determination of PZN.

In the present study, we propose a simple method for fabrication of graphene-Zinc oxide carbon paste electrode (GNS-ZnO-CPE) for sensitive and selective determination of PZN. The graphene nanosheets have been synthesized by modified Hummers method and zinc oxide nanoparticles have been synthesized by precipitation method. To the best of our knowledge there is no report on the electrochemical determination of PZN using GNS-ZnO-CPE electrode. The GNS-ZnO-CPE electrode has been used for determination of PZN in pharmaceutical formulations, urine and blood serum samples. The nanocomposite based electrode displayed excellent recovery results in synthetic and biological samples indicating its good practicality. The surface characterization of the synthesized materials has been carried out by X-ray diffraction (XRD), Scanning electron microscopy (SEM), Energy dispersive X-ray spectroscopy (EDX) and Transmission electron microscopy (TEM). Electrochemical characterization of the electrode material is carried out by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS).

2. Experimental

2.1. Materials and reagents

Pure pyrazinecarboxamide was acquired from Sigma-Aldrich, USA. Natural graphite (99.5%, ≤50 µm) was provided by SD fine, India. Zinc chloride (≥98%), hydrazine hydrate (50–60%), potassium permanganate (≥99.0%) and sodium nitrate (≥99%) were obtained from Sigma-Aldrich, USA. Mineral oil (IR spectroscopic grade) as a binder was purchased from Sigma-Aldrich, USA. The

double distilled water was used throughout analysis. All reagents used were of analytical reagent grade and used as received without any further purification. A stock solution of 8.0×10^{-3} M PZN was prepared in double distilled water and kept under refrigeration at 5 °C. The supporting electrolyte used throughout analysis was Britton Robinson-buffer (B.R.) of pH 7.0 (0.04 M). The working standard solutions of various concentrations were prepared by diluting appropriate quantity of stock solution to desired volume by using 0.04 M Britton-Robinson (B.R.) universal buffer (0.04 M boric acid, 0.04 M acetic acid and 0.04 M phosphoric acid) (pH 7.0) .The pharmaceutical formulations were obtained from local pharmacy store and used as received. The tablets containing PZN (*Pyzina:* 500 mg/Tab and *PZA-CIBA:* 1000 mg/Tab) were crushed and diluted to appropriate quantity with B.R. (pH 7.0) buffer. The blood serum and urine samples were obtained from local pathology laboratory Mumbai, India.

2.2. Apparatus

All voltammetric, cyclic voltammetry (CV) and differential pulse voltammetry (DPV), and electrochemical impedance spectroscopy (EIS) measurements were performed using an Autolab PGSTATE 30 equipped with USB electrochemical interface using GPES software, version 4.9.005 and frequency response analyzer, software version 2.0 respectively. The voltammetric measurements were carried out with a conventional three electrode system employing, GNS-ZnO-CPE as working electrode, Ag/AgCl (sat. KCl) as reference electrode and platinum wire as counter electrode. Scanning electron microscopy (SEM) was carried out on FEI Quanta-200 with an operating voltage of 15 kV. The TEM analysis was carried out on PHILIPS-CM 200 electron microscope with operating voltages of 20–200 kV and resolution of 2.4 A°. X-ray diffraction (XRD) analysis was carried out on X-ray diffractometer (Shimadzu 7000S, Shimadzu Analytical, Japan) equipped with CuKα radiation (λ = 0.154 nm). A METTLER balance (Toledo AB 204) was used to weigh solid materials. The pH measurements were carried out by using ELICO LI 120 pH meter, calibrated with standard buffers of specific pH. The working standard solutions are stable at room temperature and there was no change in composition with time. All experiments were carried out at room temperature of 25 ± 1 °C.

2.3. Synthesis of graphene sheets

Graphite oxide was synthesized from natural graphite by modified Hummers method [38]. Briefly, 2.0 g of graphite powder and 1.0 g $NaNO_3$ were mixed, and then put into 96.0 mL Conc. H_2SO_4 in an ice bath. Under vigorous stirring, 6.0 g $KMnO_4$ was added gradually. The temperature of the mixture was maintained below 20 °C. The ice bath was removed and mixture was stirred in a water bath for 2 h. To the brownish color pasty liquid, 150 mL of water was added. To maintain temperature below 50 °C water was added continuously till total volume of water was 240 mL. To the above mixture, 5 mL of 30% H_2O_2 was added and it was observed that the solution color transformed into brilliant yellow along with bubbling. The mixture was stirred for 2 h; it was filtered and washed with 10% HCl aqueous solution, water, and ethanol. The product obtained was dried under vacuum at 60 °C. For the synthesis of reduce graphene oxide, 100 mg of graphite oxide was dispersed in 100 mL of water and sonicated for 1 h. In this step conversion of graphite oxide to graphene oxide (brown dispersion) took place. To the above dispersion 2.0 g of hydrazine hydrate in 5 mL water was added and the mixture was refluxed at 100 °C for 24 h under magnetic stirring. Finally, the mixture was filtered, washed thoroughly with water and dried at 60 °C for 12 h.