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Electrochemical biosensor with ceria–polyaniline core shell nano-interface for the detection of carbonic acid in blood

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ABSTRACT

The normal physiological concentration of carbonic acid in human blood is in the range of 1.08–1.32 mM. However, if the concentration of carbonic acid rises above 3.45 mM, it may lead to respiratory acidosis. In this context, an electrochemical biosensor with CeO_2–PANI (polyaniline) core–shell nano-interface was developed for sensing carbonic acid using carbonic anhydrase (CA). CA was immobilized on CeO_2–PANI via chitosan on a glassy carbon electrode (GCE/CeO_2–PANI/CA/chitosan). The X-ray diffraction (XRD) patterns confirmed the polycrystalline nature of CeO_2 and CeO_2–PANI nanoparticles. Field emission scanning electron microscopy (FE-SEM) studies showed the aggregation of spherical nanoparticles with size ranging from 37.6 to 47.8 nm and Field emission transmission electron microscopy (FE-TEM) study confirmed the presence of core–shell structure of CeO_2–PANI. Immobilization of CA on CeO_2–PANI was confirmed by Fourier transform infrared (FT-IR) spectra. Electrochemical studies were carried out with the help of GCE/CeO_2–PANI/CA as a working electrode, Ag/AgCl saturated with 0.1 M KCl as a reference electrode and Pt wire as a counter electrode. This biosensor exhibited sensitivity of 696.49 $\mu A\,cm^{-2}\,mM^{-1}$ with a linear range of 1.32–2.32 mM. It also showed a fast response time of less than 1 s, lowest detection limit of 19.4 μM, Michaelis–Menten constant (K_M) of 1.8191 mM and dry stability of 96% up to 18 days. The observed results revealed the potential of the modified working electrode with CeO_2–PANI core–shell nano-interface for carbonic acid sensing. The developed biosensor can be applied for the real time clinical diagnosis of diseases such as obstructive pulmonary diseases (COPD).

1. Introduction

Carbonic anhydrase (CA), an enzyme interconverts carbon dioxide and bicarbonate and thereby helps to transport carbon dioxide out of the human body during the respiratory exchange [1]. Normally, the physiological concentration of carbonic acid is in the range of 1.08–1.32 mM. However, if the concentration of carbonic acid rises above 3.45 mM, it may lead to respiratory acidosis [2]. In addition, an increase in the concentration of carbonic acid in blood causes respiratory acidosis which further leads to coma, sedation, neuromuscular disorders, pulmonary fibrosis and chronic obstructive pulmonary disease (COPD) [3]. It has been reported that by the year 2020, COPD will be the third most deadly disease worldwide [1]. Hence a cost effective biosensor capable of detecting carbonic acid greater than 1.32 mM for early detection of respiratory acidosis with a low limit of detection (LOD) is required.

Until now, IR detector, spectrofluorometer and photoluminescence spectrophotometer are some of the optical sensors developed for the detection of carbonic acid. However, these optical sensors have their own drawbacks due to poor selectivity, high LOD and high cost [4,5]. In order to reduce the cost and to improve sensitivity and selectivity, electrochemical biosensors have been used [6]. Cammaroto et al. [7] developed an electrochemical dissolved CO_2 biosensor by immobilizing CA on carbon rod. This modified bio-electrode exhibited a sensitivity of 56 mV with a linear range of 10–100 ppm (0.272–2.272 mM of carbonic acid) and a response time of less than 4 s. Moreover, the stability lasted for only three days.

For better CA immobilization as well as to achieve a low LOD, a highly surface active biocompatible nanomaterial having fast electron transfer property is required [8]. Hence ceria nanoparticles

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were chosen due to their biocompatibility and fast electron transfer properties [9]. In order to enhance sensitivity further and for better confinement of electrons, ceria–polyaniline (CeO_2–PANI) core–shell structured nano-interface was fabricated [10].

In this work, the physical and optical characteristics of nanostructured core–shell CeO_2–PANI were investigated. In addition, the influence of CeO_2–PANI nano-interface based amperometric biosensor toward carbonic acid sensing was studied using cyclic voltammetry and amperometry.

2. Materials and methods

2.1. Materials

Cerium (III) nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$), sodium hydroxide (NaOH), ammonium per sulfate (APS), aniline hydrochloride (C_6H_8ClN) and carbonic anhydrase from bovine erythrocytes with specific activity (E.C 4.2.1.1, activity: 3500 W-A U mg^{-1} of protein), sodium bicarbonate and chitosan were purchased from Sigma Aldrich, India. 0.1 M phosphate buffered saline (PBS), pH 7.4 (mono basic sodium phosphate and dibasic sodium phosphate from Merck, India), 0.1 M potassium chloride (7.45 mg mL^{-1}), hydrochloric acid (HCl), 0.5 wt% of Chitosan in 1% acetic acid (degree of deacetylation of 82.5%, molecular weight: 140,000 g mol^{-1}) and acetone were procured from Merck, India. Ascorbic acid (0.01 M), uric acid (0.475 M), lactic acid (3 mM) and glutamic acid (0.1 mM) prepared in 0.1 M PBS at 7.4 pH, were purchased from Himedia, India. Zinc nitrate hexahydrate was purchased from SDFCL, Mumbai and Copper (II) acetate was procured from Fisher Scientific, Mumbai. Low frequency ultrasound (20 kHz) was employed using a bath sonicator (Ultrasonic cleaning system with heater, Supersonic, Mumbai) for CA immobilization studies.

2.2. Synthesis of CeO_2–PANI core–shell nanoparticles

A simple hydroxide method was used to prepare CeO_2 [10]. 0.1 M of $Ce(NO_3)_3{\cdot}6H_2O$ (42 mg mL^{-1}) was prepared, and 0.3 M of NaOH solution (12 mg mL^{-1}) was added drop by drop. The mixture was stirred for 24 h. The yellow colored precipitate formed was centrifuged at 8000 rpm for 20 min. The pellets were heated at 424 K for 6 h to obtain dried CeO_2 particles. Later, it was annealed at 573 K and 673 K for 3 h. For PANI synthesis, 0.2 M of aniline hydrochloride (50 mg mL^{-1}) was prepared and 0.25 M ammonium persulfate (APS) (110 mg mL^{-1}) added to it and then the mixture was stirred for 1 h. After 12 h, PANI precipitated, which was filtered and washed with 0.2 M HCl (7.3 mg mL^{-1}) and 100 mL of acetone (10 mg mL^{-1}). It was dried at 334 K, and then it was kept in an ice bath at 274–276 K for further polymerization. Later, it was acidified by replacing 10 mL of water with 10 mL of 0.2 M HCl (7.3 mg mL^{-1}). Then, 1:1 ratio of CeO_2 and PANI was taken and sonicated for 1 h. Then the solution was kept for stirring for 24 h. The precipitate of CeO_2–PANI was filtered and dried.

2.3. Preparation of GCE/CeO_2–PANI/CA/chitosan thin film

1 mg of CeO_2–PANI nanoparticles in 100 μL of chitosan (0.2 mg mL^{-1}) was taken and sonicated for about 15 min. Later, 50 μL of CA stock solution (10 mg μL^{-1}) was added and kept for sonication for 1 min. 2 μL of this solution was added on glassy carbon electrode (GCE) and kept for drying to form a thin layer. Finally, CA tagged CeO_2–PANI was obtained.

3. Instrumentation

3.1. CeO_2–PANI characterization

The morphological characterization of the synthesized CeO_2 was studied by subjecting to cold field emission scanning electron microscope (FE-SEM) (JSM 6701F, JEOL, Japan). Structural characteristics of CeO_2 and CeO_2–PANI crystallinity were studied using X-ray diffractometer (XRD) with Cu Kα radiation of wavelength (1.5408×10^{-10} m) (Rigaku Ultima III, USA). Core–shell morphologies of the synthesized CeO_2–PANI were characterized using field emission transmission electron microscope (FE-TEM) (JSM 2100F, JEOL, Japan). CeO_2 and CeO_2–PANI tagged with CA were analyzed using Fourier transform infrared spectroscopy (FT-IR) (Spectrum 100, Perkin Elmer, USA).

3.2. Electrochemical analysis

Electrochemical analysis was carried out using an electrochemical analyzer (CH600C, CH Instruments, USA). Carbonic acid sensing studies were carried out using modified glassy carbon (GCE/CeO_2–PANI/CA) as working electrode (3 mm dia., CHI104, CH Instruments, Inc., USA), Ag/AgCl (CHI111, CH Instruments, Inc., USA) saturated with 0.1 M KCl as a reference electrode and platinum (Pt) wire (CHI115, CH Instruments, Inc., USA) as a counter electrode. The cyclic voltammograms were recorded in 0.1 M PBS, pH 7.4 at room temperature. Carbonic acid was prepared by adding 0.6 M of $NaHCO_3$ (50 μg mL^{-1}) to 10 mL of HCl (0.6 M).

4. Results and discussion

4.1. XRD data of CeO_2–PANI nanoparticles

Fig. 1A(i) shows the XRD patterns of the CeO_2 nanoparticles synthesized at both 573 K and 673 K. The diffraction peaks obtained were in good agreement with the characteristic peaks of cubic fluorite structure of CeO_2 in JCPDS Card Number 34-0394. From Fig. 1A(i), it was evident that CeO_2 exhibited high intense diffraction peaks at 28.7, 33.4, 47.52, 56.79, 59.04 and 69.54 corresponding to (111), (200), (210), (220), (311), (222) and (400) planes respectively indicating the polycrystalline nature of CeO_2. The XRD patterns of CeO_2 synthesized at 573 K–PANI and CeO_2 synthesized at 673 K–PANI were analyzed and are shown in Fig. 1A(ii). On adding PANI to CeO_2 prepared at 573 and 673 K, a decrease in the diffraction peak intensity corresponding to (111), (200), (220), (311), (222) and (400) was observed. Crystallite sizes of CeO_2 and CeO_2–PANI nanoparticles were calculated using Debye's Scherrer formula [11]. Crystallite sizes of CeO_2 synthesized at 573 K and 673 K were found to be in the range of 7–9 nm. Similarly, the crystallite sizes of CeO_2 synthesized at 573 K–PANI and CeO_2 synthesized at 673 K–PANI were estimated to be in the range of 7–9 nm.

4.2. FE-SEM and FE-TEM analysis

Fig. 1B represents the FE-SEM images of CeO_2 prepared at (iii) 573 K and (iv) 673 K. It showed spherical and aggregated morphology with a size of 234.65 ± 68.97 nm and 237.82 ± 82.60 nm respectively. Fig. 1C(v) and (vi) show the FE-TEM micrograph of CeO_2–PANI core–shell with the dark core of CeO_2 and bright shell of PANI matrix. The sizes of CeO_2 synthesized at 573 K–PANI and CeO_2 synthesized at 673 K–PANI estimated from the FE-TEM micrograph were found to be 252.74 ± 72.34 nm and 255.24 ± 86.23 nm respectively.