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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.

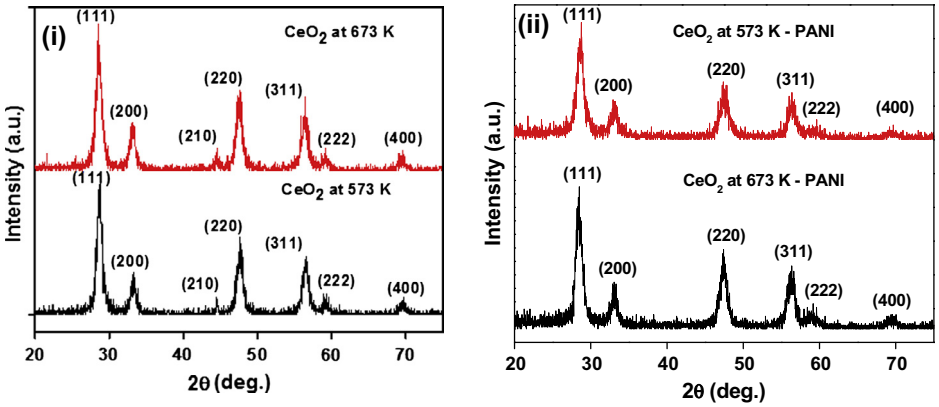

Fig. 1A. The XRD pattern of CeO_2 prepared at (i) 573 and 673 K and (ii) CeO_2 at 573 K–PANI and CeO_2 at 673 K–PANI.

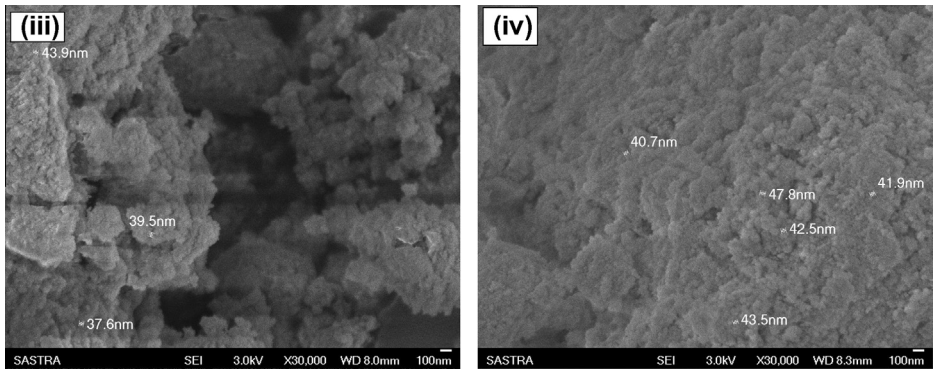

Fig. 1B. The FE-SEM image of CeO_2 prepared at (iii) 573 K and (iv) 673 K.

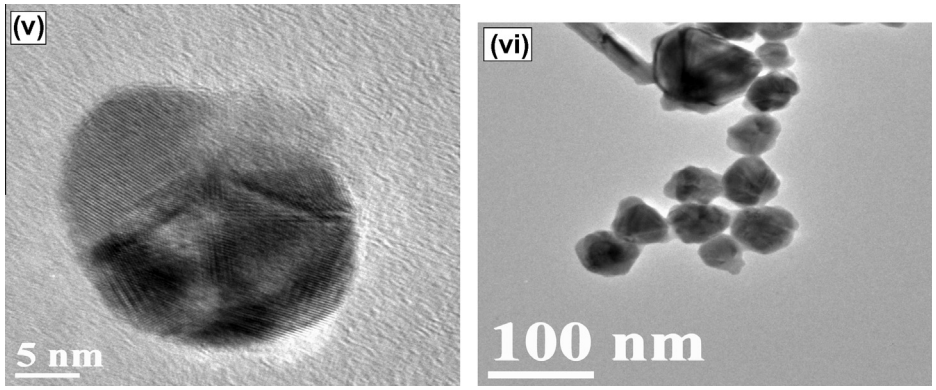

Fig. 1C. The FE-TEM micrograph of (v) CeO_2 at 573 K–PANI and (vi) CeO_2 at 673 K–PANI core–shell nanoparticles.

4.3. FT- IR spectra analysis

The FT-IR spectra of CeO_2 prepared at 573 K and 673 K are shown in Fig. 1D(vii). The absorption band observed at 3409 cm^{-1} corresponds to the O—H stretching of residual water, the band at 1618 cm^{-1} corresponds to the scissor bending mode of water associated with CeO_2, the bands at 1471, 1379 and 507 cm^{-1} correspond to the Ce—O stretching [12] and the band at 828 cm^{-1} corresponds to the Ce—O metal oxygen bond [13]. The FT-IR spectra of CeO_2 synthesized at 573 K–PANI and CeO_2 synthesized at 673 K–PANI in the presence and absence of CA are represented in Fig. 1D(viii). The absorption band at 2874 cm^{-1} may be attributed to the —CH_2— groups from chitosan [14]. The absorption band at 1587 cm^{-1} represents the primary amine N—H vibrations, the band at 1494 cm^{-1} corresponds to the C=C stretch, the band at 1393 cm^{-1} corresponds to the aromatic primary amines, the band at 1310 cm^{-1} corresponds to the C—H stretch in plane bending, the band at 1236 cm^{-1} corresponds to the C—H stretch in aromatic ring and the band at1125 cm^{-1} corresponds to the secondary amine C—H stretch. CeO_2–PANI tagged with CA was confirmed from FT-IR absorption bands observed at 3033 cm^{-1} (=CH stretching), 1456 cm^{-1} (aromatic ring in CA) and 1541 cm^{-1} (N—H peak) [14].

4.4. Electrochemical studies

Electrochemical studies were carried out in the absence of carbonic acid in 0.1 M PBS (pH 7.4) by measuring current 5 times from the bare GCE working electrode. It was observed that instrument

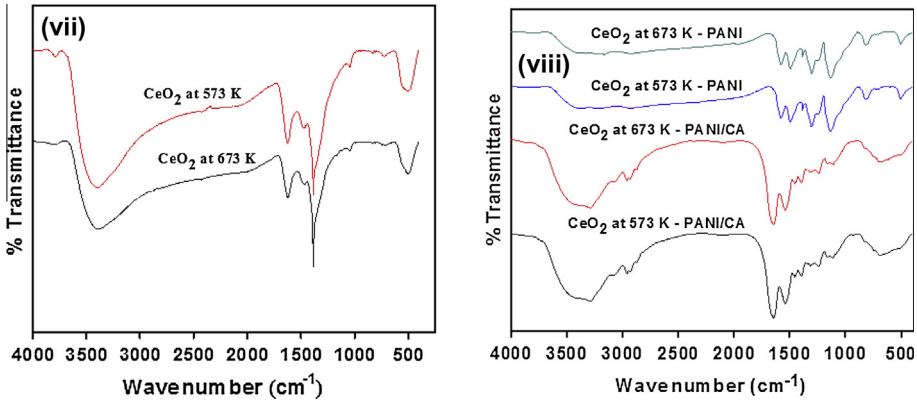

Fig. 1D. The FT-IR spectra of (vii) CeO_2 prepared at 573 K and 673 K and (viii) CeO_2 at 573 K–PANI and CeO_2 at 673 K–PANI in the presence and in the absence of CA.

noise was low which was equal to 73 nA with a relative standard deviation (RSD) of 0.048.

In order to optimize the experimental conditions, control experiments were carried out using bare GCE, GCE/CA, GCE/CeO_2/CA, GCE/CeO_2–PANI/CA with both CeO_2 synthesized at 573 and 673 K. Fig. 2 shows that the cyclic voltammograms were recorded in the presence of 1.32 mM of carbonic acid in 0.1 M PBS (pH 7.4) at a scan rate of 0.1 V s^{-1}. It is evident that GCE/CeO_2 at 573 K–PANI/CA in the presence of 1.32 mM carbonic acid showed higher cathodic peak current (I_{pc}) of 8.23 μA, which was 1.23 times greater than that of GCE/CeO_2 at 673 K–PANI/CA (I_{pc} = 6.65 μA).

Effect of scan rate on GCE/CeO_2 at 573 K–PANI/CA was studied by varying scan rates ranging from 0.03 to 0.06 V s^{-1} in 0.1 M PBS at pH 7.4 containing 1.32 mM of carbonic acid and is shown in Fig. 3A(i). A linear increase in the cathodic sigmoidal wave current (I_W) with an increase in scan rate was observed with a regression coefficient (R^2) of 0.99 indicating that the rate of reaction depends only on the surface controlled electrochemical process. It also inferred that the reaction occurred only due to the surface confined molecules.

In order to determine the efficiency of CeO_2–PANI nano-interface in transferring the electron between CA and working electrode, electron transfer rate constant ($K_s = I_{pc}/Q$, where K_s is the electron transfer rate constant, I_{pc} is the cathodic peak current and Q is the amount of charge consumed) for GCE/CeO_2 at 573 K–PANI/CA and GCE/CeO_2 at 673 K–PANI/CA were calculated. The K_s of GCE/CeO_2 at 573 K–PANI/CA (1.8348 s^{-1}) was 1.29 times

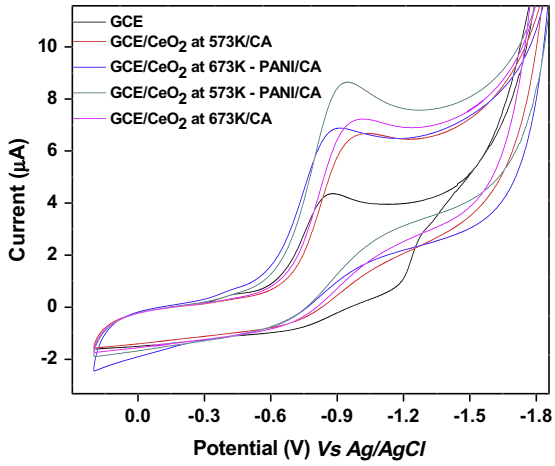

Fig. 2. Cyclic voltammograms of modified electrode in 0.1 M PBS (pH 7.4) in the presence of 1.32 mM carbonic acid.

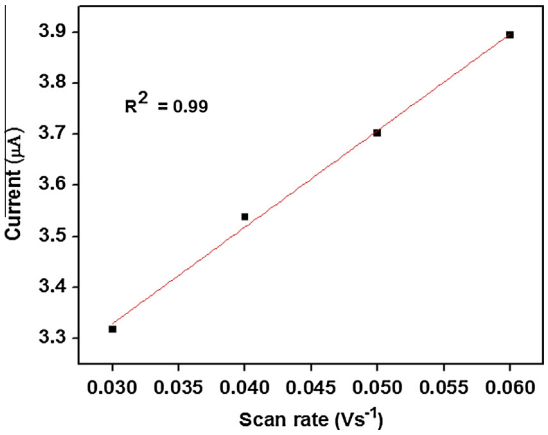

Fig. 3A. (i) The effect of scan rate on GCE/CeO_2 at 573 K–PANI/CA in 0.1 M PBS (pH 7.4).

higher than that of K_s of GCE/CeO_2 at 673 K–PANI/CA (1.4223 s^{-1}). This high value of K_s represented fast electron transfer between CA and GCE/CeO_2 at 573 K–PANI.

Surface coverage (Γ) of carbonic acid over GCE/CeO_2 at 573 K–PANI/CA and GCE/CeO_2 at 673 K–PANI/CA was calculated using the Eq. (1)

$$\Gamma = \frac{Q}{nFA} \quad (1)$$

where Γ is the surface coverage (mol cm^{-2}), n is the number of electrons transferred, F is the Faraday constant (96485.33 coulomb mol^{-1}), Q is the charge in coulomb and A is the area of GCE electrode (cm^2). It was found that GCE/CeO_2 at 573 K–PANI/CA (6.413×10^{-9} mol cm^{-2}) showed nearly 1.176×10^2 times of higher Γ of carbonic acid than on GCE (5.450×10^{-11} mol cm^{-2}) and 5.425 times of higher Γ of carbonic acid than on GCE/CeO_2 at 673 K–PANI/CA (1.182×10^{-9} mol cm^{-2}). High surface coverage of carbonic acid over GCE/CeO_2 at 573 K–PANI/CA proved more adsorptions of carbonic acid took place on modified working electrode.

4.5. Voltammetric detection of carbonic acid based on modified GCE/CeO_2 at 573 K–PANI/CA

Fig. 3B(ii) shows the effect of increasing carbonic acid concentration (1.32–2.32 mM) on the cyclic voltammograms of GCE/CeO_2 at 573 K–PANI/CA for successive additions of 166.6 μM carbonic acid to 0.1 M PBS at 7.4 pH. Significant I_{pc} separations with

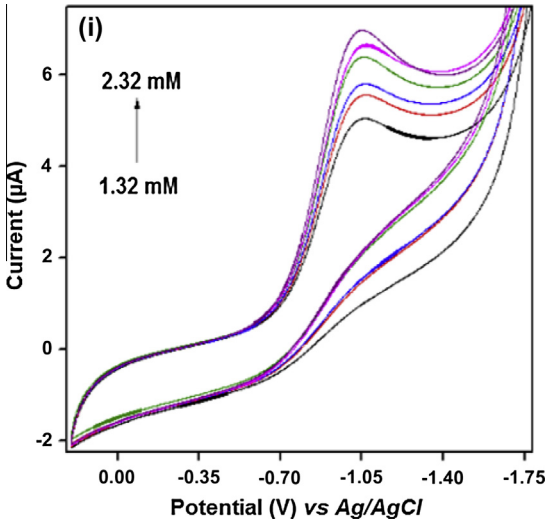

Fig. 3B. (ii) The effect of increasing carbonic acid concentration (1.32–2.32 mM) on the cyclic voltammograms of GCE/CeO_2 at 573 K–PANI/CA for successive additions of 166.6 μM carbonic acid to 0.1 M PBS at 7.4 pH.

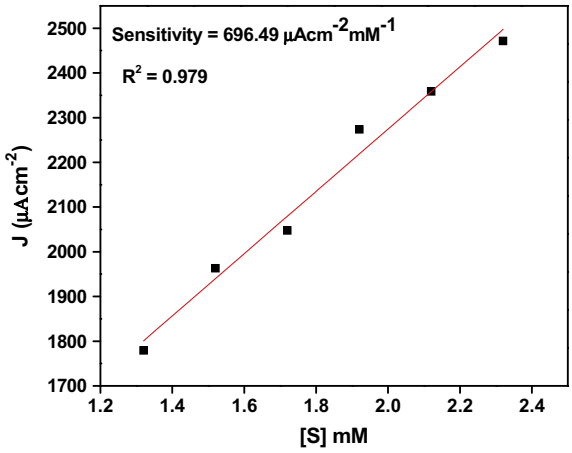

Fig. 3C. (iii) Estimation of linear range and sensitivity of GCE/CeO_2 at 573 K–PANI/CA electrode.

well resolved peaks and the absence of the shift in cathodic peak potential (E_{pc}) at different concentrations of carbonic acid were observed. This indicated that the fabrication of GCE/CeO_2 at 573 K–PANI/CA and CA immobilization at the electrode surface was good. Furthermore, the reduction potential (E_{pc}) is −0.98 V matched the standard electrode potential of −0.96 V (Vs Ag/AgCl) for Zn^{2+} ion, which is presented in the active site of CA suggesting that CA retained its bio-activity even after embedding on CeO_2 at 573 K–PANI.

Fig. 3C(iii) shows the estimation of linear range and sensitivity of GCE/CeO_2 at 573 K–PANI/CA electrode. An extended linear range (1.32–2.32 mM) with an R^2 of 0.979 was observed along with good sensitivity of 696.49 μA cm^{-2} mM^{-1}. Levenberg–Marquardt fitting between current density (J) and carbonic acid concentration helped to determine the maximum current density (J_{max} = 2839.963 - μA cm^{-2}). This supported high CA loading as well as high enzyme activity. The degree of co-operability (n) between carbonic acid and CA was determined from Hill plot (Fig. 3D(iv)) and was found to be positive (n = 4.015). It confirmed the binding of carbonic acid to active sites of CA in-turn enhanced the affinity of further incoming carbonic acid molecules. Another characteristic parameter evaluated was the Michaelis–Menten constant (K_M), which was

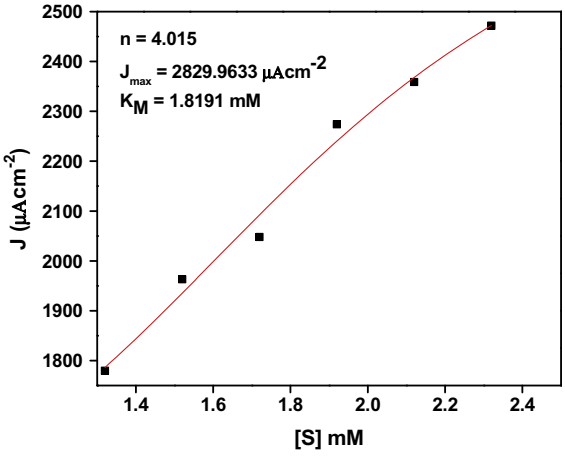

Fig. 3D. (iv) Estimation of Michaelis–Menten constant (K_M) and maximum current density (J_{max}).

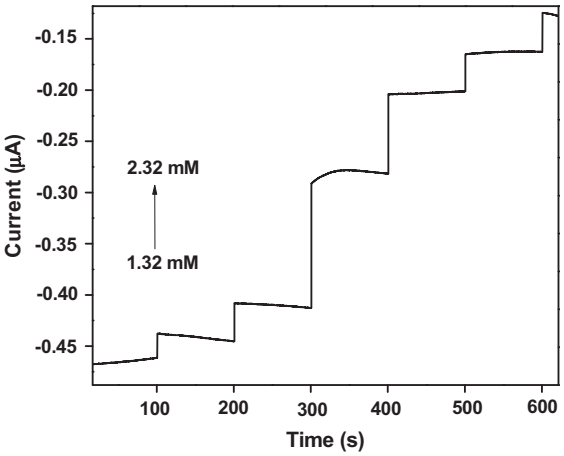

Fig. 3E. (v) Amperometric response for successive additions of 166.6 μM carbonic acid to 0.1 M PBS (pH 7.4) on modified GCE/CeO_2 at 573 K–PANI/CA at −0.1 V.

1.819 mM indicating that there exists a good affinity between carbonic acid and CA. Limit of detection (LOD) and limit of quantification (LOQ) were calculated using Eq. (2).

$$\mathrm{LOD} = \frac{3s_b}{S} \text{ and } \mathrm{LOD} = \frac{10s_b}{S} \quad (2)$$

where s_b is the standard deviation of the blank signal and S is sensitivity. LOD and LOQ of GCE/CeO_2 at 573 K–PANI/CA was estimated to be 19.4 μM and 98 μM respectively.

4.6. Amperometric studies

Fig. 3E(v) shows the amperometric current–time response of GCE/CeO_2 at 573 K–PANI/CA for successive additions of 166.6 μM

Table 1
Comparison of characteristics of the developed carbonic acid biosensor with existing biosensors.

Stability (days)	Linear range	LOD	Response time	References
3 days	–	–	4 s	[7]
2 days	1.4–4.6 μM	0.375 μM	10 s	[15]
–	87.8–2700 μM	39.3 μM	3.6 min	[16]
–	670 mg L^{-1}	1 mg L^{-1}	<15 min	[17]
18 days	1.32–2.32 mM	19.4 μM	<1 s	Present work

Table 2
Recovery characteristics of carbonic acid biosensor.

Blood sample (20 μL of prepared stock solution)	Carbonic acid added (mM)	Mean carbonic acid detected[a] (mM)	Recovery (%)	RSD (%) ($n = 3$)
1	1.5	1.53	102	0.02
2	1.6	1.62	101	0.01
3	1.8	1.82	101	0.01
4	1.9	2.0	105	0.03

[a] Average of three determinations.

carbonic acid to 0.1 M PBS (pH 7.4) with increasing concentration of carbonic acid ranging from 1.32 to 2.32 mM. Amperometric studies were carried out using a potential of −0.1 V as this lower potential helped for prolonged enzyme stability. Amperometric response showed an increase in current with an increase in carbonic acid concentration. The response time of this biosensor was estimated to be <1 s, which was considered to be very fast when compared to previous works, which reported a response time of less than 4 s [7] while Nikolelis and Siontorou [15] reported a response time of less than 10 s (Table 1).

4.7. Repeatability and reproducibility

Reproducibility and repeatability for inter-assay and intra-assay were determined from RSD and Analysis of variance (ANOVA) at 1.32 mM of carbonic acid in 0.1 M PBS at 7.4 pH using GCE ($n = 8$). The RSD of intra-assay and inter-assay was found to be 0.184 and 0.083 respectively. At 0.05 level ($p > 0.05$), the mean current ($n = 8$) for GCE/CeO_2 at 573 K–PANI/CA was not significantly different, indicating good embedding between CA and CeO_2 at 573 K–PANI. To see any significant mean difference between the reproduced measured current ($n = 8$), Two-way ANOVA was performed. It was found that the mean difference between the reproduced measured current ($n = 8$) was not significantly different at 0.05 level ($p > 0.05$). This result confirmed the good fabrication technique.

4.8. Interference studies

Interference studies were conducted to test the specificity of the developed biosensors. Ascorbic acid (0.01 M), uric acid (0.475 M), lactic acid (3 mM) and glutamic acid (0.1 mM) were the common interfering agents used in the interference studies because they are metabolic acids and have effect in metabolic acidosis. For interference studies, interferents were taken to a concentration higher than the normal physiological concentration in human blood as acidosis occurs only beyond the normal ranges. Percentage inhibition was calculated using Eq. (3).

$$I\% = \frac{I_0 - I_i}{I_0} \times 100 \tag{3}$$

where I_i is the obtained current in the presence of inhibitor and I_0 is the obtained current in the absence of inhibitor. Usually interference contribution higher than 5% is not accepted. Even though the concentration of interferents was higher than the physiological concentration, the percentage inhibition was <5% ($I_{\text{ascorbic acid}}\% = 3.2$, $I_{\text{uric acid}}\% = 2.6$, $I_{\text{lactic acid}}\% = 1.5$ and $I_{\text{glutamic acid}}\% = 2.1$) which, confirmed that the developed sensor could overcome the potential interferences. Moreover, the inhibition of carbonic anhydrase immobilized in GCE/CeO_2 at 573 K–PANI was also performed by adding 100 μM of Ce^{3+}, Zn^{2+} and Cu^{2+} individually to the PBS (0.1 M, 7.4 pH) containing 1.32 mM of carbonic acid. Ce^{3+} and Cu^{2+} metal ions showed an inhibition of 0.12% and 0.25% respectively. On the other hand, experiments performed on Zn^{2+} enhanced the I_{pc} to almost 2.63%. In addition to the Zn^{2+} ion presented in the active sites of CA, the added Zn^{2+} ion was also involved in catalytic reaction and hence the I_{pc} enhancement.

The stability of GCE/CeO_2 at 573 K–PANI/CA was evaluated using the Eq. (4)

$$\%\text{Stability} = 100 - \left[\frac{I_n - I_0}{I_0} \times 100\right] \tag{4}$$

where I_0 is the obtained current in the first day and I_n is the obtained current in the 'n' day. The dry stability of the developed biosensor was checked for 18 days at room temperature and was observed to be 96%.

4.9. Application

The applicability of developed bisensor was tested using human blood sample. To test the recovery of developed biosensor, 2 μL of human blood sample was collected and it was diluted in 30 mL of PBS (0.1 M, 7.4 pH). From the stock solution, 20 μL of product was transferred to the known carbonic acid concentrations of 1.5, 1.6, 1.8 and 1.9 mM and detection was done in triplicates. Recoveries of carbonic acid from human blood were ranged from 101% to 105% with an RSD of <0.03% (Table 2) which was within the acceptable limit. Recovery studies within the range of 101–105% indicated that the developed bio-electrode had the ability to overcome potential interferents.

5. Conclusion

An electrochemical biosensor with ceria nano-interface was developed to detect carbonic acid in human blood. CA was immobilized on the modified GCE for carrying out cyclic voltammetry and amperometry studies. The developed bioelectrode showed fast electron transfer with increased sensitivity of 696.49 μA cm^{-2} - mM^{-1} and a quick response time of <1 s. The estimated response time (<1 s) was found to be better than the existing biosensors. This biosensor can detect abnormal concentrations of carbonic acid in human blood samples and hence can be employed for the determination of acidosis. It may also be examined for COPD diagnosis.

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