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Fabrication of lactate biosensor based on lactate dehydrogenase immobilized on cerium oxide nanoparticles

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ABSTRACT

An electrochemical biosensor was developed to determine lactate that plays an important role in clinical diagnosis, fermentation and food quality analysis. Abnormal concentration of lactate has been related to diseases such as hypoxia, acute heart disorders, lactic acidosis, muscle fatigue and meningitis. Also, lactate concentration in blood helps to evaluate the athletic performance in sports. The main aim of the work is to fabricate NADH/LDH/Nano-CeO_2/GCE bio-electrode for sensing lactate in human blood samples. Toward this, CeO_2 nanoparticles were synthesized by a hydroxide mediated approach using cerium nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$) and NaOH as precursors. X-ray diffraction (XRD) and Field Emission Scanning Electron Microscopy (FE-SEM) studies were carried out to determine the structural and morphological characteristics of CeO_2 nanoparticles. XRD pattern indicated the formation of highly crystalline CeO_2 nanoparticles with face centered cubic structure. The FE-SEM studies revealed the formation of nanospherical particles of size 29.73 ± 2.59 nm. The working electrode was fabricated by immobilizing nicotinamide adenine dinucleotide (NADH) and lactate dehydrogenase (LDH) on GCE surface with CeO_2 nanoparticles as an interface. Electrochemical studies were carried out through cyclic voltammetry using a three electrode system with NADH/LDH/NanoCeO_2/GCE as a working electrode, Ag/AgCl saturated with 0.1 M KCl as a reference electrode and Pt wire as a counter electrode. From the amperometric study, the linearity was found to be in the range of 0.2–2 mM with the response time of less than 4 s.

1. Introduction

Biosensors are widely used as an analytical tool [1] for the measurement and identification of various biomolecules, chemicals and other target substances. The sensing device brings bioreceptor and transducer together and transforms the bio-signal to a computable response [2]. Biomolecules act as indicators of human health, food quality and the measurable quantities of the human activity products. Among various biomolecules that serve as biomarkers for a plethora of health-related symptoms, lactate molecules have great importance. Determination of lactate concentration is very important in a variety of fields such as clinical diagnosis [3], medicine validation [4], and food quality analysis [3,5].

Many researchers have developed lactate biosensors due to its clinical importance in the diagnosis of acute heart diseases [6], hypoxia [6,7], muscle fatigue [7,8], lactic acidosis [8,9], heart failure [6], meningitis, cystic fibrosis [9], etc. Lactate also causes muscle pain in athletes those who are engaged in strenuous physical activity for a prolonged period of time. In the case of mitochondrial disorders, too much of lactate is formed because its ability to burn foods using aerobic respiration is impaired [9,10]. In fermentation industry, lactic acid is produced by the fermentation of lactose due to the microbial activity of lactic acid bacteria [11]. Lactate concentration depends on the total amount of bacteria and it determines the food product quality and preservation.

Many techniques have been used to measure lactate concentration such as high performance liquid chromatography (HPLC) [12], ultraviolet spectrophotometry, and refractive index detection [13,14]. However, these techniques have some limitations such as requirement of sample preparation, longer time period for sample analysis, involvement of tedious processes and higher cost. But, electrochemical sensors are well known for their accuracy, rapidness and cost-effectiveness; they were used extensively to detect proteins [15], biological toxins, target agents/molecules in food [16], environment [17] and clinical diagnosis [18]. Thus, in this work, electrochemical method is considered in the development of a rapid and accurate method for the measurement of lactate molecule.

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The most commonly used lactate sensor work is based on the specific recognition of lactate using lactate dehydrogenase (LDH). LDH enzyme is found in various types of cells in the human body. Some of the organs that are rich in LDH enzyme are kidney, liver, heart, red blood cells and muscle [19]. LDH enzyme catalyzes the reversible reaction between lactate and pyruvic acids in the presence of NADH coenzyme [20].

Though a specific number of matrices have been used for lactate sensor fabrication like enzyme attached between two membrane layers [21], tetraethylorthosilicate (TEOS)-derived sol–gel films [22], LDH and pyruvate oxidase on a Clark-type oxygen electrode [23], electron mediators such as ferricyanide and also with the modified carbon nanotubes [24], conducting copolymer bound with enzyme[25], silica sol–gel modified gold electrode [26], engineered chitosan network composite [27], conducting polymer/multiwall carbon nanotube composite film [28], and cross-linked polymeric matrixes [10], sensitivity, selectivity and rapid current variation through enhanced electron transfer rate need more attention.

In this context, nano-interface can be used to make biosensors more sensitive, selective, and higher signal-to-noise ratio [29]. Among the nanomaterials [30,31], metal oxides have been used effectively by researchers [32,33] because of their higher surface reaction rate, catalytic efficiency and strong adsorption ability in addition to high surface-to-volume ratio [34]. Among these metal oxides, cerium oxide (CeO_2) has gained much importance due to its interesting properties like high isoelectric point (IEP), biocompatibility, chemical stability, high oxygen storage capacity, etc. [35]. Owing to its high IEP (9.2), CeO_2 can immobilize desired enzymes and biomolecules with low isoelectric point through electrostatic interactions. Moreover, the high isoelectric point and conductivity property of cerium oxide nanoparticles help researchers to develop third generation electrochemical biosensor based on the direct electron transfer of redox enzymes [36,37]. Hence, in this work, LDH was immobilized on glassy carbon electrode (GCE) surface with CeO_2 as a nano-interface for the estimation of lactate was developed.

2. Materials and methods

2.1. Materials and reagents

Ascorbic acid, glucose, cerium nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$), lactate dehydrogenase from rabbit muscle L-LDH (130 U mg^{-1}), β-nicotinamide adenine dinucleotide (β-NADH) and Lithium L-Lactate were purchased from Sigma-Aldrich, USA. All other chemicals such as sodium hydroxide, ethanol, monobasic sodium phosphate and dibasic sodium phosphate salts were procured from Merck India Ltd., India. All the reagents and solutions were prepared using double distilled water.

2.2. Preparation of solutions

Phosphate Buffered Saline (PBS) 0.1 M and pH 7.4 solution were prepared by adjusting the proportionate value of monobasic sodium phosphate solution and dibasic sodium phosphate solution and then 0.9% NaCl was added to the solution. A stock solution of LDH (1 mg mL^{-1}), NADH (5 mM) and Lithium L-Lactate (0.1 M) were freshly prepared with 0.1 M PBS buffer of pH 7.4.

2.3. Preparation of CeO_2 nanoparticles

CeO_2 nanoparticles were synthesized by hydroxide mediated approach. Initially, 2 g of cerium nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$) was dissolved in 25 mL (0.1 M) of double distilled water. Then, a 25 mL (0.3 M) solution of sodium hydroxide was added drop-wise to this solution with a constant stirring of about 2–3 h at room temperature. Finally, a pale yellowish white precipitate was obtained. The solution was centrifuged at 8000 rpm for 20 min. Then, the pellet was washed several times with double distilled water and dried at 423 K for an hour. The obtained yellowish particles were dried at 523 K for 3 h to obtain powdered CeO_2 nanoparticles. Then, similar experiments were carried out by optimizing the volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$): NaOH (1:1, 1:2 and 1:3). Then, the above procedure was repeated and desired quantity of cerium oxide particles was obtained.

2.4. Immobilization of NADH and LDH onto Nano-CeO_2/GCE

GCE was sonicated with ethanol and double distilled water before linking ceria nanoparticles. Then, a thin layer of carbon paste was applied over it and the cerium oxide nanoparticles were embedded over it. The electrode designated as Nano-CeO_2/GCE was air-dried and used for further experiments.

A fresh solution of lactate dehydrogenase (LDH) (1 mg mL^{-1}) was prepared in 0.1 M PBS and 10 μL solution of freshly prepared LDH was uniformly spread onto Nano-CeO_2/GCE. And also, 10 μL of NADH (65 μg mL^{-1}) was added uniformly onto LDH/Nano-CeO_2/GCE bio-electrode. The modified bio-electrode was kept undisturbed at room temperature for a period of 2–3 h. Finally, this bio-electrode was washed with 0.1 M PBS solution to remove any unbound biomolecules from the electrode surface. The proposed mechanism for the preparation of the Nano-CeO_2 electrode and the immobilization of LDH and NADH is shown in Fig. 1. The difference in isoelectric point between CeO_2 (IEP ~ 9.2) [37] and LDH (IEP ~ 8.1) [38,39] facilitated higher immobilization of LDH on CeO_2 nanoparticles.

2.5. Characterization techniques

The structural characterization of the prepared CeO_2 nanoparticles was carried out using X-Ray Diffractometer (XRD) (D8 Focus, Bruker, Germany) with Cu Kα radiation of wavelength 1.5418 Å. The surface morphology of the particles was observed through Field Emission Scanning Electron Microscopy (FE–SEM, JSM6701F, JEOL, Japan). Electrochemical experiments were performed on a CHI600 series electrochemical analyzer (CH Instruments, USA) with a conventional three electrode system. GCE with modified CeO_2 nanoparticles (NADH/LDH/Nano-CeO_2/GCE) acts as a working electrode. The Ag/AgCl (saturated with 0.1 M KCl) was used as a reference electrode and Pt wire acts as a counter electrode. Electrochemical experiments were carried out using 0.1 M PBS.

3. Results and discussion

3.1. Structural characterization

Fig. 2(a–c) shows the result of X-ray diffraction studies of cerium oxide (CeO_2) nanoparticles prepared by hydroxide mediated approach. The crystal structure and orientation of the CeO_2 nanoparticles synthesized with different ratios of precursor solution concentrations of cerium nitrate hexahydrate ($Ce(NO_3)_3{\cdot}6H_2O$) and NaOH (1:1, 1:2 and 1:3) were investigated using X-ray diffractometer. The XRD patterns showed diffraction peaks corresponding to (111), (200), (220), (311) and (222) planes which indicated that the CeO_2 nanoparticles were polycrystalline with cubic fluorite structure (JCPDS No. 34-0394). Peak broadening confirmed the typical characteristic of nanodimensional crystallites. The crystallite size of the CeO_2 nanoparticles with different NaOH

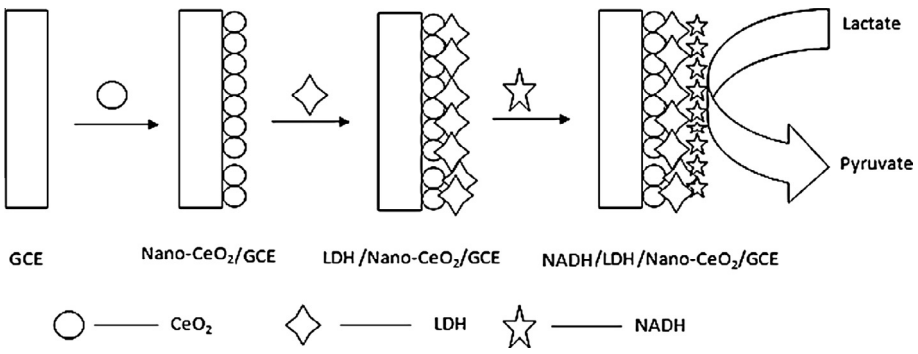

Fig. 1. Fabrication of NADH/LDH/Nano-CeO_2/GCE bio-electrode.

concentrations obtained from Debye–Sherrer equation [37] was found to be in the range of 25–40 nm.

3.2. Surface and morphological characterization using FE-SEM

FE-SEM images of CeO_2 nanoparticles with varying volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$): NaOH (a) 1:1; (b) 1:2 and (c) 1:3 are shown in Fig. 3(a–c). The FE-SEM images showed aggregated spherical morphology of ceria nanoparticles with the mean size of 29 ± 1.76 nm, 30.34 ± 2.43 nm and 29.86 ± 2.64 nm for the varying volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$): NaOH, 1:1, 1:2 and 1:3 respectively. The size distribution of CeO_2 nanoparticles was estimated statistically by determining the size of more than 150 CeO_2 nanoparticles in selected FE-SEM image. Fig. S1 shows the histogram showing particle size distribution of CeO_2 particles with varying volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$): NaOH (a) 1:1; (b) 1:2; (c) 1:3. In all the above three cases, the ceria nanoparticle size distributions were found be positively skewed. This positively skewed size distribution showed that most of the ceria nanoparticles in the sample were around 29 nm for the varying volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$): NaOH, 1:1, 1:2 and 1:3. It also showed that as the concentration of NaOH increases, agglomeration of ceria nanoparticle also increases. The mean size of ceria observed from FE-SEM was in good agreement with the estimated value from XRD.

3.3. Electrochemical studies

3.3.1. Cyclic voltammetry studies

Generally, electrochemical reaction for lactate detection requires LDH, NADH and lactate.

$$\text{L-lactate} + NAD^+ \leftrightarrows \text{Pyruvate} + NADH + H^+$$

$$NADH \leftrightarrows NAD^+ + H^+ + 2e^+$$

In the above reaction, lactate gets converted into pyruvate in the presence of NAD^+ through LDH enzyme. Cyclic voltammograms were recorded in the presence of 0.2 mM of lactate at a scan rate of 0.1 Vs^{-1} in 0.1 M PBS (pH 7.4, 0.9% NaCl) using different electrodes such as bare GCE, Nano-CeO_2/GCE, LDH/Nano-CeO_2/GCE and NADH/LDH/Nano-CeO_2/GCE which are shown in Fig. 4(a). The unmodified glassy carbon electrode (i) has no response, but the addition of CeO_2 nanoparticles (ii) showed reversible cyclic voltammetric current even in the absence of NADH and LDH which might be due to the bio-mimicking nature CeO_2 nanoparticles. After the addition of bio-mimicking CeO_2 nanoparticles, both the LDH/Nano-CeO_2/GCE (iii) and the NADH/LDH/Nano-CeO_2/GCE (iv) bio-electrode showed drastic improvement in the cyclic voltammetric current response with a pronounced increase in the magnitude of anodic (I_{pa}) and cathodic (I_{pc}) peak current especially in the case of NADH/LDH/Nano-CeO_2/GCE bio-electrode. In order to study the

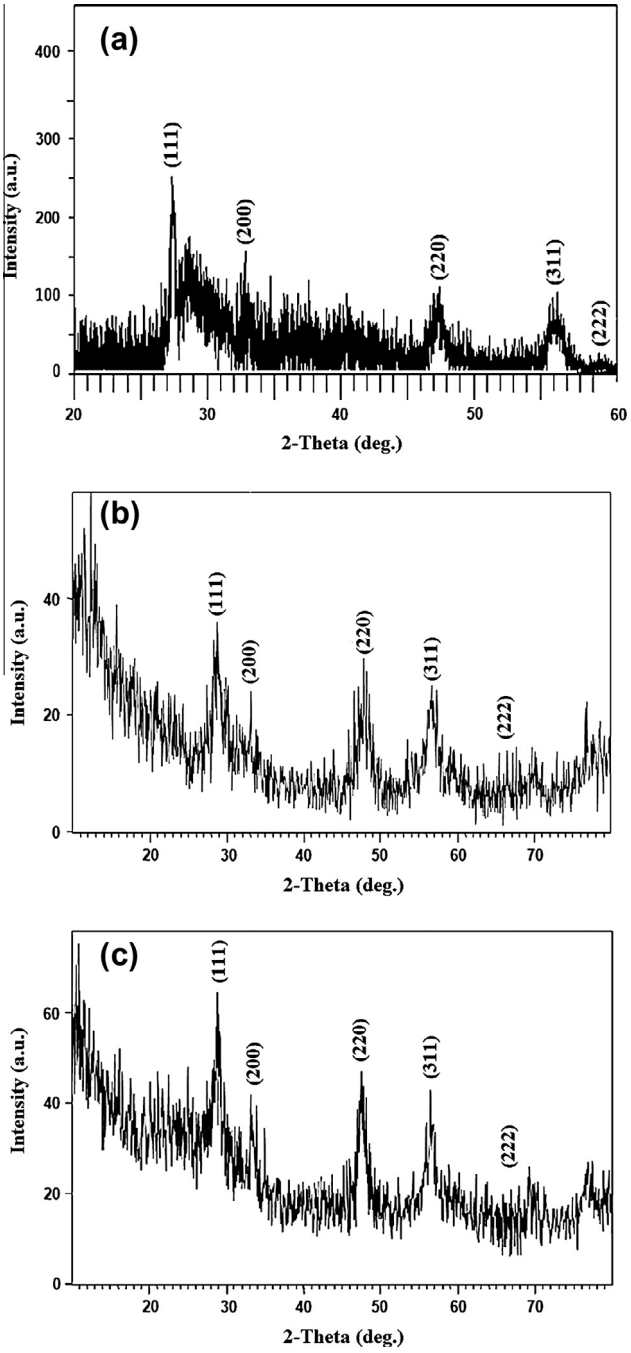

Fig. 2. XRD pattern of CeO_2 particles with varying volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$):NaOH) (a) 1:1; (b) 1:2; and (c)1:3.

impact of NADH on the cathodic and anodic process, ratio of I_{pc}–I_{pa} for different modified GCE electrodes was compared. The ratio of I_{pc}–I_{pa} for Nano-CeO_2/GCE (1.604), LDH/Nano-CeO_2/GCE (1.978) and NADH/LDH/Nano-CeO_2/GCE (2.151) was found to be greater than 1, indicating a large number of electrons got transferred in cathode than in anode. From this, it can be concluded that addition of NADH has the greatest impact on the cathodic process when compared to its influence on anodic process, where the oxidation of lactate to pyruvate occurs. Reversible cyclic voltammetric wave current observed for all CeO_2 modified GCE bio-electrodes studied showed that the CeO_2 nanoparticles had good electron transfer properties. In order to examine the electron transfer properties of CeO_2 nanoparticles in different modified electrodes, the separations between cathodic and anodic peak potential (ΔE_p) for Nano-CeO_2/GCE, LDH/Nano-CeO_2/GCE and NADH/LDH/Nano-CeO_2/GCE bio-electrode were compared. Peak separation of about 194 mV was noticed for Nano-CeO_2/GCE electrode, whereas the peak separation for LDH/Nano-CeO_2/GCE and NADH/LDH/Nano-CeO_2/GCE bio-electrode was about 224 mV and 270 mV respectively. This indicated that Nano-CeO_2/GCE electrode showed fast electron transfer than other modified bio-electrodes. Even though CeO_2 in Nano-CeO_2/GCE electrode possessed enzyme mimicking and fast electron transfer property than in other modified bio-electrodes, only the LDH enzyme modified GCE electrode can react specifically to lactate. Based on this aspect, further electrochemical characterization was performed using NADH/LDH/Nano-CeO_2/GCE bio-electrode.

Fig. 4(b) shows the cyclic voltammogram of NADH/LDH/Nano-CeO_2/GCE bio-electrode at different scan rates (0.01–0.1 Vs^{-1}) in 0.1 M PBS. In the case of cathodic process, as the scan rate varied from 0.01 to 0.1 Vs^{-1}, the cathodic peak current increased in a linear fashion (I_{pc} = 1353 μA [v] + 41.25) with a regression coefficient of 0.99 (Fig. S2(a)), indicating a surface controlled process. But in the case of anodic process, as the scan rate varied from 0.01 to 0.1 Vs^{-1}, the anodic peak current decreased in a linear fashion (I_{pa} = −448.97 μA [$v^{1/2}$]–7.58) with a regression coefficient of 0.99 (Fig. S2(b)), indicating a diffusion controlled process. This also showed that the cathodic reaction occurred was only due to the surface confined molecule, whereas the anodic reaction occurred was only due to the diffused molecule.

Fig. 4(a) Cyclic voltammogram of (i) bare GCE, (ii) Nano-CeO_2/GCE, (iii) LDH/Nano-CeO_2/GCE and (iv) NADH/LDH/Nano-CeO_2/GCE bio-electrode in presence of 0.2 mM of lactate in 0.1 M PBS, pH 7.4; (b) NADH/LDH/Nano-CeO_2/GCE bio-electrode at different scan rates and (c) Concentration profile of lactate in NADH/LDH/Nano-CeO_2/GCE bio-electrode.

The electron transfer rate constant (k_s) of bare GCE, Nano-CeO_2/GCE, LDH/Nano-CeO_2/GCE and NADH/LDH/Nano-CeO_2/GCE were found to be 0.099, 0.417, 0.6057 and 0.731 s^{-1} respectively. It showed that the electron transfer between CeO_2 and LDH was very fast in NADH/LDH/Nano-CeO_2/GCE electrode when compared to that of electron transfer in bare GCE, Nano-CeO_2/GCE and LDH/Nano-CeO_2/GCE electrode. Also, surface coverage (I^*) of adsorbed lactate on various modified GCE was calculated using the following equation.

$$I_p = \frac{n^2F^2I^*AV}{4RT} \quad (1)$$

where I^* is the surface coverage (mol cm^{-2}), n is the number of electrons transferred, A is the area of GCE electrode (cm^2), I_p is the peak current (μA), v is the scan rate (Vs^{-1}), R is the gas constant, F is the Faraday constant and T is the absolute temperature. It was observed that surface coverage of adsorbed lactate on bare GCE, Nano-CeO_2/GCE, LDH/Nano-CeO_2/GCE and NADH/LDH/Nano-CeO_2/GCE was found to be 5.08×10^{-10}, 1.42×10^{-9}, 9.27×10^{-9} and 3.86×10^{-8} mol cm^{-2} respectively. The adsorbed lactate on NADH/LDH/Nano-CeO_2/GCE was in two orders of magnitude higher than that of bare GCE indicating more adsorption of lactate on to the redox center of LDH.

The electrochemical response of the NADH/LDH/Nano-CeO_2/GCE bio-electrode was investigated as a function of lactate concentration and is shown in Fig. 4(c). It was performed by adding varying amounts of 0.2 mM lactate solution in 0.1 M PBS at a scan rate of 0.1 Vs^{-1}. The magnitude of current response got enhanced for

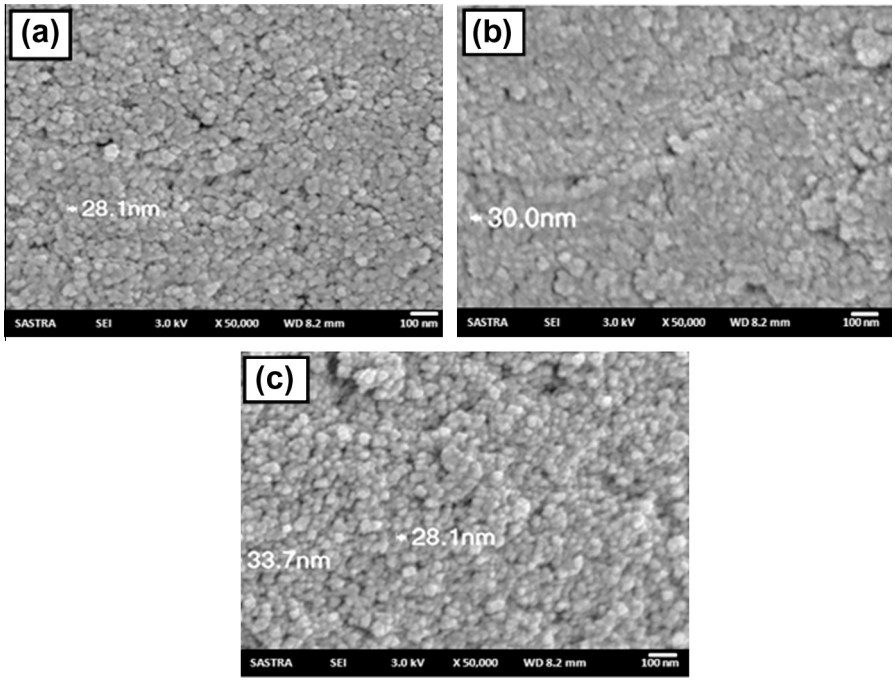

Fig. 3. FE-SEM images of CeO_2 particles with varying volume ratio of ($Ce(NO_3)_3{\cdot}6H_2O$):NaOH (a) 1:1; (b) 1:2; and (c)1:3.

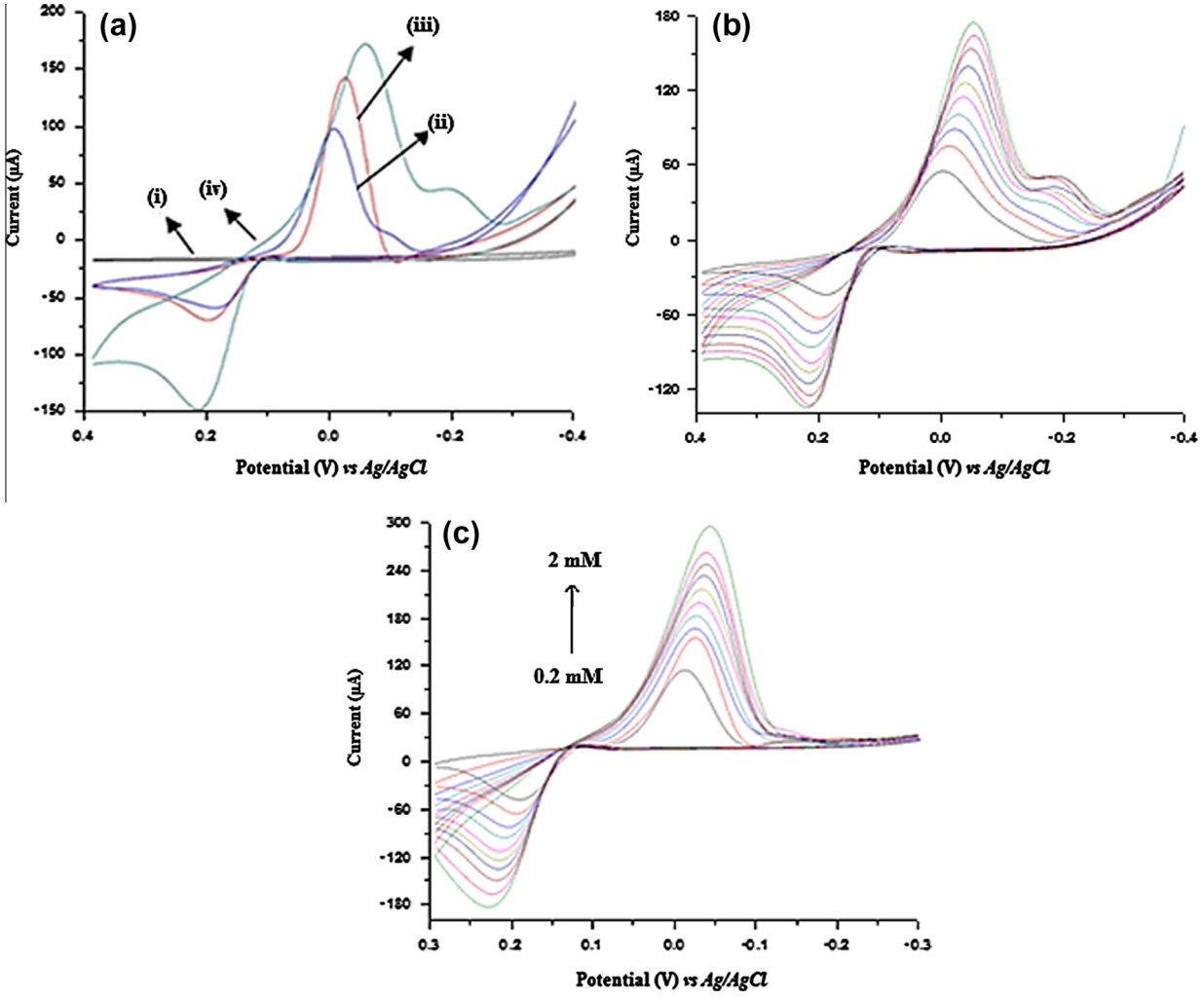

Fig. 4. (a) Cyclic voltammogram of (i) bare GCE, (ii) Nano-CeO_2/GCE, (iii) LDH/Nano-CeO_2/GCE; and (iv) NADH/LDH/Nano-CeO_2/GCE bio-electrode in presence of 0.2 mM of lactate in 0.1 M PBS, pH 7.4; (b) NADH/LDH/Nano-CeO_2/GCE bio-electrode at different scan rates and (c) concentration profile of lactate in NADH/LDH/Nano-CeO_2/GCE bio-electrode.

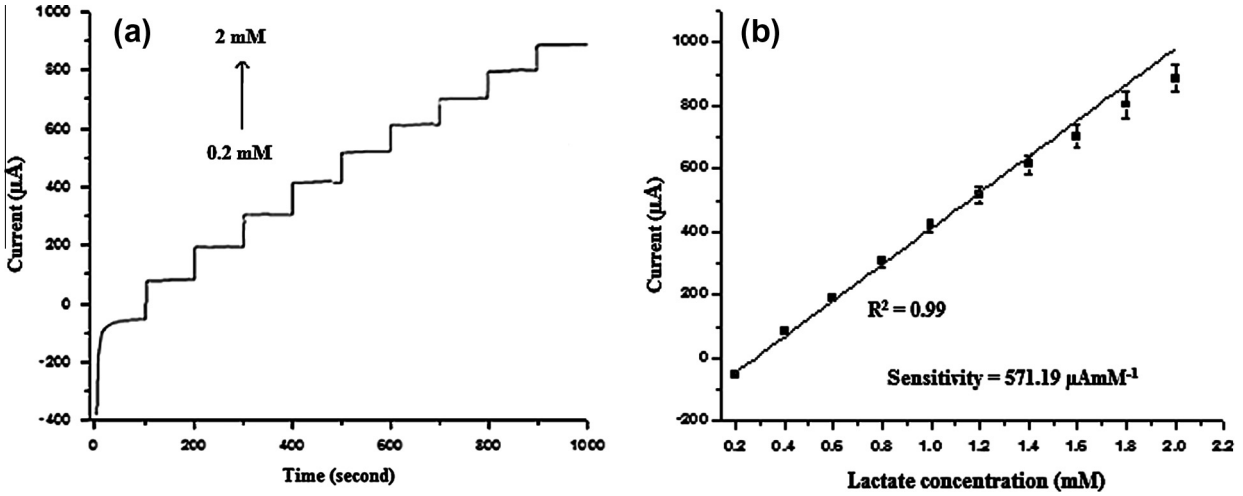

Fig. 5. (a) Amperometric *i*–*t* curve using NADH/LDH/Nano-CeO_2/GCE in 0.1 M PBS (pH 7.4) with varying concentration of 0.2 mM lactate at 0.3 V and (b) calibration plot for NADH/LDH/Nano-CeO_2/GCE bio-electrode.

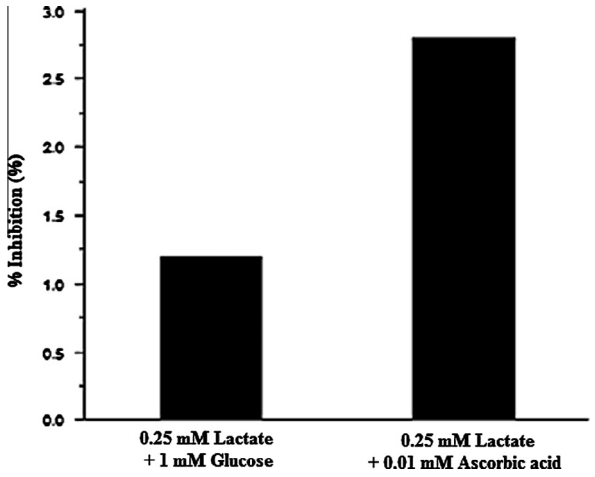

Fig. 6. Effect of interferent molecules on NADH/LDH/Nano-CeO_2/GCE.

each successive addition of 0.2 mM concentration of lactate. Further, it showed significant cathodic peak to peak separation indicating that the fabrication and LDH immobilization on GCE electrode surface were not damaged.

3.3.2. Amperometric detection of lactate

Amperometric studies were recorded continuously for a period of 1000 s through conventional three electrode system using NADH/LDH/Nano-CeO_2/GCE bio-electrode in 0.1 M PBS solution.

Fig. 5(a) shows the typical current–time (i–t) curve at an applied potential of 0.3 V with the addition of varying amount of 0.2 M lactate solution in 0.1 M PBS buffer. A step-wise increase in current was observed for each successive addition of 0.2 mM concentration of lactate. NADH/LDH/Nano-CeO_2/GCE bio-electrode showed a steady increase in current with respect to the concentration of lactate. A similar trend was reported by Mohammed Rahman [38] for the detection of lactate by using Lox/TGA/Au device. Fig. 5(b) shows the calibration plot for the amperometric current response with respect to the concentration of lactate. A good sensitivity of 571.19 μA mM^{-1} was observed throughout the linear range (0.2–2 mM) with R^2 of 0.99. The calculated linear regression equation is I = 517.34 μA [S]–121.783, where I is the NADH/LDH/Nano-CeO_2/GCE bio-electrode response and [S] is the lactate concentration. Limit of detection (LOD) and limit of quantification (LOQ) of lactate that can be detected by NADH/LDH/Nano-CeO_2/GCE bio-electrode were estimated to be 50 and 150 μM using the following equation.

$$\mathrm{LOD} = 3.3 \times (\mathrm{SD}/S)$$
$$\mathrm{LOQ} = 10 \times (\mathrm{SD}/S) \quad (2)$$

where SD is the standard deviation in the absence of lactate and S is the slope of the calibrated curve in the presence of lactate. Levenberg–Marquardt fitting between net current and lactate concentration helps in the estimation of maximum net current (I_{max}) which was found to be 1383 μA mM^{-1}, indicating high LDH loading and activity. The estimated apparent Michaelis–Menten constant (K_M^{app} = 1.536 mM) showed that there exists a good affinity between LDH and lactate. From the Hill plot, the degree of cooperativity (n) between LDH and lactate was observed to be positive as it exhibits as 2.02, which was greater than 1 and it showed that lactate binding on to the redox center of LDH promotes the affinity of further incoming lactate molecules. The biosensor efficiency of lactate detecting biosensor was calculated using the following equation

$$\text{Biosensor efficiency} = \frac{I_{max}/K_M^{app}}{\text{Slope of NADH/LDH/Nano} - CeO_2/\text{GCE}} \quad (3)$$

The developed lactate biosensor showed a high efficiency of 1.576, which indicated that the developed biosensor had the higher ability to convert lactate to pyruvate and thereby showed an increase in sensitivity.

The NADH/LDH/Nano-CeO_2/GCE bio-electrode showed a response time of <4 s and it exhibited a shelf-life of 89% up to 12 days. Rapid response time was obtained due to the nanoceria interface which favored the fast electron transfer between the LDH enzyme and the GCE electrode. A similar linear increase in current with respect to lactate concentration was also reported by Rahman et al. [28], for sensing lactate using conducting polymer/multiwalled carbon nanotube composite film.

3.3.3. Accuracy

Accuracy of NADH/LDH/Nano-CeO_2/GCE toward lactate was calculated by using 0.2, 0.4 and 0.6 mM of lactate in 0.1 M PBS (pH 7.4). For 0.2, 0.4 and 0.6 mM of lactate, mean lactate detected ± relative error for three trials was found to be 0.166 ± 0.17, 0.384 ± 0.04 and 0.550 ± 0.083 mM respectively.

3.3.4. Precision

Reproducibility and repeatability of NADH/LDH/Nano-CeO_2/GCE with 0.2 mM lactate in 0.1 M PBS (pH 7.4) for inter-assay and intra-assay were performed for five times and the results were analyzed using RSD. The RSD of intra-assay and inter-assay were found to be 2.8% and 4.7% respectively. These indicated that the fabrication technique and embedding between LDH and Nano-CeO_2 were good.

3.3.5. Interference study

Interference studies were carried out using ascorbic acid and glucose because of its potential interferents in lactate sensing

Table 1
Characteristics of some lactate biosensors with CeO_2 nanoparticle interface based biosensor.

Nano-interface	Enzyme	K_M^{app}	Sensitivity	LOD	Linear range	References
Fe_3O_4	GOD/LDH	4.785 mM	–	–	–	[40]
PPY and PVS	LDH		–	0.1 μM	0.5–6 mM	[41]
Polyaniline	LDH	4.5 mM	–		1–3 mM	[41]
O-phenylenediamine	LDH/GOD		–	<50 μM	0.05–1 mM	[42]
pTTCA and MWCNT	LDH		0.0106 μA μM^{-1}	–	0.005–0.09 mM	[43]
Polyvinylpyrrolidone	LDH	13.7 mM	0.0626 μA mM^{-1}	110 μM	2 mM	[44]
Silica	LDH	–	–	0.8 μM	0.002–0.03 mM	[26]
Diaphorase	LDH	–	–	–	0.2–8 mM	[45]
Polyaniline	LOD/LDH	1.9 mM	38.5 μA mM^{-1}	10 μM	0.1–1 mM	[46]
Graphite	LDH	–	–	–	0.5–20 mM	[47]
Polysulfone/CNT	LDH	–	–	0.37 μM	0.001–0.02 mM	[48]
CeO_2	LDH	1.536 mM	571.19 μA mM^{-1}	50 μM	0.2–2.0 mM	Present work

[39]. Percentage inhibition of NADH/LDH/Nano-CeO_2/GCE bio-electrode was performed in the presence of 0.01 mM ascorbic acid (2.3 μg mL^{-1}) and 1 mM glucose (0.37 mg mL^{-1}) and it was calculated using the following equation.

$$I\% = \frac{I_0 - I_i}{I_0} \times 100 \quad (4)$$

where I_i and I_o are the obtained current in the presence and absence of inhibitor. In the presence of ascorbic acid and glucose, the variation in the magnitude of cathodic peak current ($I\% < 2.75$) was found to be almost nil (Fig. 6). This showed that the developed bio-electrode has the ability to overcome potential interferents.

Table 1 provides a comparison of the characteristics of some lactate biosensors with our CeO_2 nano-interface based biosensor. From this comparison, it was evident that the developed biosensor with high sensitivity and low K_M^{app} can sense the micromolar concentration of lactate with good affinity and better current response.

4. Conclusion

The lactate sensing performance of an electrochemical biosensor with nano-interface was successfully investigated by fabricating NADH/LDH/Nano-CeO_2/GCE bio-electrode. The cyclic voltammetric response of NADH/LDH/Nano-CeO_2/GCE showed fast electron transfer between LDH enzyme and GCE working electrode. The developed biosensor exhibited high sensitivity (571.19 μA mM^{-1}) and rapid response time (<4 s) with a linearity of 0.2–2 mM. Owing to its high response, the fabricated ceria nano-interface based bio-electrode can be used as a sensitive element in handheld miniaturize system for the detection of lactate in blood samples.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2013.08.009.

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