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An electrochemical biosensor with nanointerface for lactate detection based on lactate dehydrogenase immobilized on zinc oxide nanorods

Noel Nesakumar [a,b], Kavitha Thandavan [a,c], Swaminathan Sethuraman [a], Uma Maheswari Krishnan [a,c], John Bosco Balaguru Rayappan [a,b,*]

[a] Centre for Nanotechnology & Advanced Biomaterials (CeNTAB), SASTRA University, Thanjavur 613 401, Tamil Nadu, India
[b] School of Electrical & Electronics Engineering, SASTRA University, Thanjavur 613 401, Tamil Nadu, India
[c] School of Chemical & Biotechnology, SASTRA University, Thanjavur 613 401, Tamil Nadu, India

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ABSTRACT

Hepatic immaturity is observed particularly in children whose age is under three, when the lactate concentration is greater than the normal level in blood. An electrochemical lactate biosensor was developed by immobilizing lactate dehydrogenase (LDH) on to ZnO nanorods at pH 7.4 via chitosan. Growth of polycrystalline ZnO nanorods towards (101) plane was confirmed using XRD. The FE-SEM study revealed the formation of ZnO nanorods with an aspect ratio of 3.24. Immobilization of LDH on ZnO nanorods was confirmed using FTIR spectra and surface coverage. Electrochemical studies were carried out through cyclic voltammetry and amperometry using three electrode system with Au/NanoZnO/LDH as working electrode, Ag/AgCl in 0.1 M KCl as reference electrode and Pt wire as counter electrode. The sensitivity of the biosensor was found to be 1.832 μA μmol^{-1} L exhibiting linearity 0.2–0.8 μmol L^{-1} with the detection and quantification limits of 4.73 and 15.75 nmol L^{-1} respectively. The response time of Au/NanoZnO/LDH bioelectrode was found to be <1 s. Prediction band for net current was framed to enhance specificity. Michaelis–Menten constant (K_M^{app}) and maximum rate (I_{max}) values for immobilized LDH were found to be 0.38 μmol L^{-1} and 2.798 μA respectively. Repeatability and reproducibility of LDH biosensor were also reported.

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1. Introduction

Lactate, a biochemical compound produced from pyruvate, is studied in various fields such as food preservation, biosensor and microbial fermentation [1–4]. Sulfites, benzoates, propionates, nitrites and nitrates are the commonly used preservatives for food products [5–7]. It has been reported that these chemicals induce adverse effects in human such as stomach cancer and acidity [4–7]. Due to these adverse effects, lactate is used as a preservative for food products related to meat, poultry and fish [1–4]. The enhanced shelf life of food products is due to its antibacterial activity [1–4]. In 1990, Friedman [8] observed hepatic immaturity in children under the age of three when they were fed with food containing lactate. Hence it is important to accurately quantify lactate in food products before feeding it to the children under the age of three.

High performance liquid chromatography (HPLC) [9], UV–Vis spectrophotometry [10] and refractive index detection [11] are some of the methods used to measure lactate concentration. Although HPLC is popular among these methods, it is lengthy and time consuming. UV–Vis spectrophotometry and refractive index methods also have drawbacks arising from the nonselective nature of absorbance. The absorbance of interfering molecules in the same wavelength often obscures the information about the lactate concentration. Electrochemical biosensors reported recently have the ability for rapid determination of lactate concentration in the blood sample owing to their high sensitivity and specificity [12]. One of the biggest requirements of an electrochemical sensor is to develop a bioelectrode with nanointerface by patterning the electrode surface with tailored catalytic properties [13,14]. The chemical signal produced by the reaction between the biomolecule and the bioreceptor is effectively transduced into an electrical signal through electrochemical reaction at the surface of nanointerface. Biological compounds such as enzymes are mostly preferred as receptors rather than chemicals owing to their high specificity and sensitivity [1,15]. The electrochemical biosensors typically use metal oxide semiconductor and composite nanoparticles as interface materials for improving sensitivity and specificity. The high aspect ratio of nanoparticles in particular is promising for optoelectronics and sensor applications [16–19]. These uni-dimensional nanointerfaces, which facilitate direct electron

* Corresponding author at: Centre for Nanotechnology & Advanced Biomaterials (CeNTAB), SASTRA University, Thanjavur 613 401, Tamil Nadu, India. Fax: +91 4362 264120.
E-mail address: rjbosco@ece.sastra.edu (J.B.B. Rayappan).

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amplification possess high sensitivity and can achieve good enzyme immobilization [20]. Recently ZnO nanorods have become more popular among researchers for biomolecule sensing due to their high surface area, biocompatibility, chemical stability and electronic amplification property [21,22].

Lactate dehydrogenase (LDH) [23–25], lactate oxidase [26–28] and horseradish peroxidase are the commonly used enzymes in the development of lactate sensor [20]. In the present work, LDH is used for the determination of lactic acid. The isoelectric point of LDH and ZnO are 8.5 and 9.5 respectively. This difference in isoelectric point facilitates easy binding of LDH on to ZnO nanorods through electrostatic interaction [29,30]. Recently, Ibupoto et al. [20] developed a lactic acid biosensor by immobilizing lactate oxidase enzyme with a cross linker gluteraldehyde onto the ZnO nanorods. This Au/NanoZnO/LOD bio-electrode was shown to have a detection limit (LOD) of 1×10^{-4} mmol L^{-1} with a response time and sensitivity of 10 s and 41.33 ± 1.58 mV $decade^{-1}$ respectively presenting linearity over 0.1–1 mmol L^{-1}. The stability of this LDH biosensor was found to be more than three weeks [20]. In this work, physical and electrochemical results of lactate detection using Au/NanoZnO/LDH bioelectrode with nanointerface have been reported.

2. Materials and methods

2.1. Materials and reagents

Zinc acetate, Urea, L-LDH from rabbit muscle (130 U mg^{-1}), β-nicotinamide adenine dinucleotide with molecular weight 663.43 of g M^{-1} (>96% enzymatic) and Lithium L-lactate (Molecular weight: 96.01 g M^{-1}) were purchased from Sigma–Aldrich, USA and methanol was purchased from Fisher Scientific, Mumbai. All other chemicals such as chitosan, sodium hydroxide (NaOH), monobasic sodium phosphate and dibasic sodium phosphate were procured from Merck India Ltd., India. The working electrode Au (2 mm diameter, CH 101) was purchased from CH instruments. All the reagents and solutions were prepared with doubly deionized water (Millipore, USA).

2.2. Synthesis of ZnO nanorods

In order to synthesizes ZnO nanorods, 0.1 M of $Zn(CH_3COO)_2 \cdot 2H_2O$ was dissolved in 50 mL methanol under constant stirring. To this stock solution, 0.3 M of NaOH was added in methanol and kept under constant stirring to obtain a pH of 9.5. The solution was transferred into hot air oven and maintained at 373 K for 6 h. It was then cooled naturally to room temperature. The white product obtained was washed with methanol, filtered and dried in air and subjected to characterization techniques.

2.3. Fabrication of bioelectrode with nanointerface

Fig. 1 shows the schematic of LDH immobilized on Au/NanoZnO electrode for developing lactate sensor. For the preparation of bio-electrode based on Au electrode with ZnO and LDH enzyme, 1 mg of ZnO nanorods was weighed using electronic balance (BT 224s, Sartorius) and added to the mixture of 1 μL of chitosan and 100 μL of phosphate buffered Saline (PBS), (7.4 pH). The above mixture was sonicated for one hour. After the sonication was completed, 10 μL of LDH [1 mg mL^{-1}] was added and sonicated for another two minutes for dispersing the LDH uniformly. Thus, Au/NanoZnO/LDH electrode was obtained by using 3 μL of this solution for coating on the working electrode made of Au and drying at room temperature.

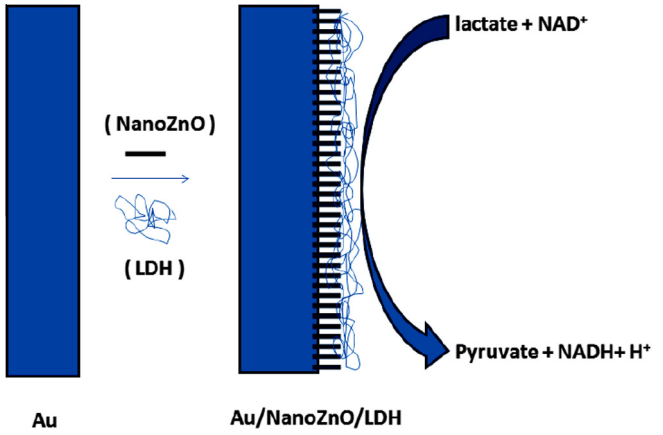

Fig. 1. The schematic of LDH immobilized on Au/NanoZnO electrode for developing lactate biosensor.

2.4. Characterization techniques

The structural characteristic of ZnO nanorods was studied using X-ray Diffractometer (XRD) (D8 Focus, Bruker, Germany). Scanning electron micrograph of ZnO nanorods was obtained using Field Emission Scanning Electron Microscopy (FE-SEM) (JSM 6701F, JEOL, Japan). Fourier Transform Infrared (FTIR) spectrum of the immobilized working electrode was recorded using FTIR spectrometer (Spectrum 100, Perkin Elmer, USA).

2.5. Electrochemical measurements

All electrochemical measurements were performed using an electrochemical analyzer (CHI600C, CH Instruments, USA). A conventional three electrode system comprising of the fabricated bioelectrode as working electrode, Pt as auxiliary electrode and Ag/AgCl, 0.1 M KCl as a reference electrode was employed for all electrochemical measurements. All experiments were performed at room temperature. 0.5 μL of lactic acid [9.6 mg mL^{-1}] was diluted with 0.5 mL of 0.10 M oxygen saturated PBS, (pH 7.4). 10 μL of this solution was transferred to the electrochemical cell containing 5 mL of oxygen saturated PBS (pH 7.4) and 10 μL of NADH [65 μg mL^{-1}]. Electrochemical detection of the analytical signal was performed at different concentrations of lactic acid varying from 0.2 to 1.6 μmol L^{-1}. The interference effects of lactate biosensor were evaluated by injecting urea at higher concentration than normal values in fertigation because nitrogen content in urea helps in plant growth.

3. Results and discussion

3.1. Characterization of ZnO nanorods

Fig. 2A shows the XRD pattern of ZnO nanorods synthesized via solvothermal method. The characteristic diffraction peak positions (100), (002), (101), (102), (110), (103), (200), (112), (201), (004) and (202) confirmed the polycrystalline nature of ZnO with hexagonal wurtzite structure and were found to be in agreement with Joint Committee for Powder Diffraction Standards [JCPDS 36-1451]. Well-defined peaks with high intensity were observed in the XRD spectra revealed the presence of a large quantity of ZnO nanorods. Relatively larger peak corresponding to (101) indicates that the growth of ZnO nanorods was preferentially towards (101) plane. Crystallite size was calculated using Debye's Scherrer equation [31] and the average crystallite size of the samples was found to be 31 nm.

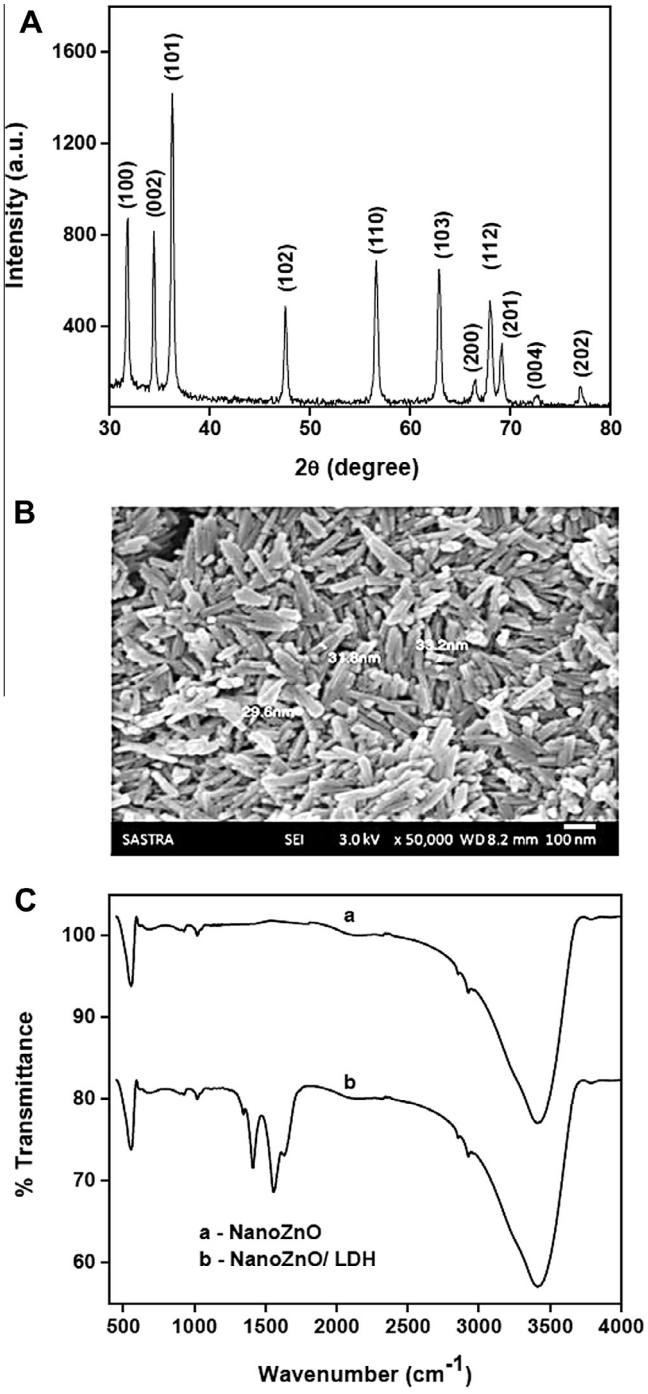

Fig. 2. (A) XRD pattern of ZnO nanorods, (B) FE-SEM image of ZnO nanorods and (C) FT-IR spectra of ZnO nanorods (a) in the absence of LDH and (b) in the presence of LDH.

Fig. 2B shows the surface morphology of ZnO nanorods prepared by solvothermal method. The pH of the precursor solution for making ZnO nanorods was maintained at optimum value of 9.5 for getting uniform defect-free ZnO nanorods. At this higher pH of 9.5, the effective charge repulsion produced by the increased negative charges experienced by ZnO molecules, directs ZnO formation towards one dimension [32]. Thus, narrow-sized ZnO nanorods were observed by optimum choice of pH. Generally, the aspect ratio of nanorods of width in the range of 1–100 nm will be between 1 and 20 [33]. From the FE-SEM image, the aspect ratio for the ZnO nanostructures used in this study was found to be 3.24, which confirmed the formation of ZnO nanorods. Fig. S1 shows the histogram showing particle size distribution of ZnO nanorods. The size distribution of ZnO nanorods were calculated by investigating the size of more than 260 ZnO nanorods in selected FE-SEM image. The mean width of the ZnO nanorods was approximately in 30.63 ± 1.515 nm.

Fig. 2C (a) and (b) represents the FTIR spectra of ZnO nanorods in absence and in presence of LDH respectively. The peak at 550 cm^{-1} in both the graphs corresponds to the vibration band on Zn–O bond [34]. Further, the additional peaks at 1121.04 cm^{-1} (C–N stretching), 1415 cm^{-1} (aromatic C–C stretching), 1559 cm^{-1} (N–H stretching), 825 cm^{-1} (aromatic C–H stretching) and 2926 cm^{-1} (aromatic C–H bending) in the Fig. 2C. (b) arose due to the coupling of LDH on to ZnO nanorods.

3.2. Cyclic voltammetry studies

To detect lactate with high sensitivity and low limit of detection (LOD), biomolecules such as LDH, NADH, lactate and a nanointerface for high LDH immobilization are needed.

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

$$NADH \rightarrow NAD^+ + H^+ + 2e^-$$

Electrochemical reaction for lactate detection involves the conversion of lactate to pyruvate in the presence of NAD^+. Cyclic voltammograms observed for bare Au, Au/LDH, Au/NanoZnO and Au/NanoZnO/LDH electrode after the addition of 0.2 μmol L^{-1} of lactate at a scan rate of 0.1 V s^{-1} in oxygen saturated PBS (0.1 M, pH 7.4) containing 10 μL of NADH are shown in Fig. 3A. Since the PBS was saturated with oxygen, at electrolyte/modified working electrode (Au/NanoZnO, Au/NanoZnO/LDH and Au/LDH bioelectrode) interface, the electrons from the NADH to O_2 produced NAD^+ and H_2O [35].

$$NADH + H^+ \rightarrow NAD^+ + 2H^+ + 2e^-$$

$$\frac{1}{2}O_2 + 2H^+ + 2e^- \leftrightarrow H_2O$$

Generally, the standard reduction potential (E^0) of Ag/AgCl is 0.2223 V. And, the E^0 of $\frac{1}{2}O_2 : H_2O$ is 0.82 V (Vs SHE). If the electrochemical reaction is performed with Ag/AgCl as reference electrode, the E^0 will shift to 0.6 V. Au/LDH, Au/NanoZnO, Au/NanoZnO/LDH bio-electrode showed reversible cyclic voltammetric current with the E^0 of 0.69 V, 0.65 V and 0.65 V respectively. This confirmed the occurrence of redox reaction of $\frac{1}{2}O_2 : H_2O$ in the cathodic and anodic process. It also showed that the LDH biomolecule retains its catalytic activity even after the adsorption on to the ZnO nanorods. Even in the absence of ZnO nanorods and LDH, the unmodified Au working electrode showed an irreversible cyclic voltammetric current at an oxidation potential (E_{pa}) of 0.84 V. This value is matches with the E_{pa} of Au/LDH (0.87 V), Au/NanoZnO (0.84 V) and Au/NanoZnO/LDH (0.84 V) bio-electrode confirmed negligible oxidation of H_2O to O_2 in the anodic process. The unmodified bare Au electrode has no current response in the cathodic process. After the addition of LDH on to the bare Au electrode, a reversible cyclic voltammetric current with a small increase in cathodic current response (I_{pc}) was observed. Similarly, in the absence of LDH enzyme, Au/NanoZnO showed reversible cyclic voltammetric current with the E^0 of 0.65 V. This value is close to the E^0 of 0.69 V for Au/LDH indicating bio-mimicking activity of ZnO nanorods. In order to prove the bio-mimicking property of ZnO nanorods, cyclic voltammograms for both electrodes in the presence and absence of 0.2 μmol L^{-1} of lactate was reported [Fig. S2]. In the presence of lactate, Au/NanoZnO and Au/LDH

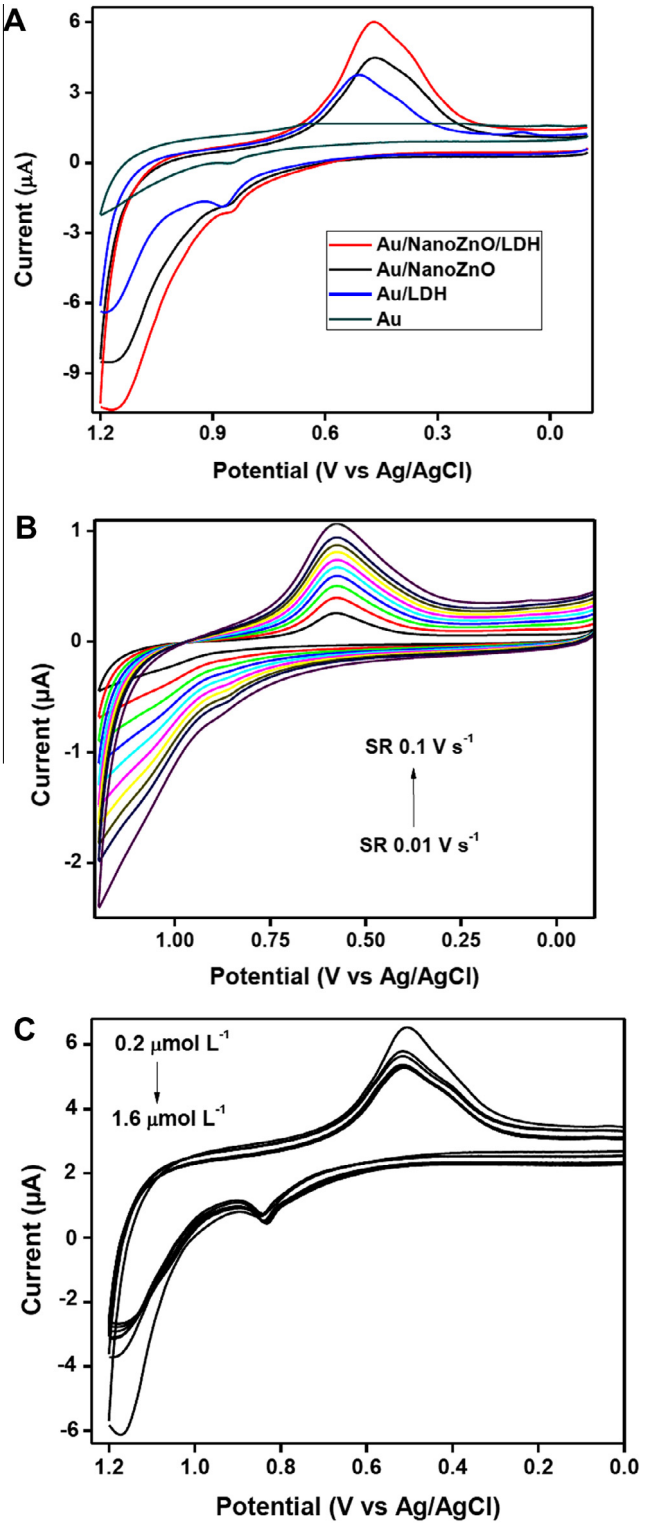

Fig. 3. (A) Cyclic voltammogram observed for bare Au, Au/LDH, Au/NanoZnO and Au/NanoZnO/LDH electrode after the addition of 0.2 μmol L^{-1} lactate in oxygen saturated PBS (0.1 M, pH 7.4), B) Au/NanoZnO/LDH bioelectrode at different scan rates (0.01–0.1 V s^{-1}) with 0.2 μmol L^{-1} of lactate in oxygen saturated PBS (0.1 M, pH 7.4) and C) cyclic voltammograms of Au/NanoZnO/LDH bioelectrode for varying concentrations of lactate (0.2–1.6 μmol L^{-1}) in oxygen saturated PBS (0.1 M, pH 7.4).

modified working electrodes showed pronounced increase in the magnitude of cathodic peak current than in the absence of lactate. Moreover, ZnO nanorods also showed better cathodic current response than Au/LDH bio-electrode even in the absence of LDH. Thus the LDH-mimicking nature of ZnO nanorods was proved. After the addition of LDH on to the Au/NanoZnO electrode, pronounced increase in the magnitude of I_{pc} was observed. In order to study the impact of ZnO nanorods on the cathodic process, full width at half maximum of peak height (FWHM) and the ratio of I_{pc} in the presence of 0.2 μmol L^{-1} of lactate to the I_{pc} in the absence of 0.2 μmol L^{-1} of lactate for Au/NanoZnO and Au/NanoZnO/LDH electrode (Fig. S2) were studied. The ratio of I_{pc} in the presence and the absence of 0.2 μmol L^{-1} of lactate for Au/NanoZnO and Au/NanoZnO/LDH electrode were found to be 2.34 and 3.18 respectively. This showed that addition of ZnO nanorods have a strong influence on the cathodic process. In addition, FWHM observed for Au/LDH (200 mV), Au/NanoZnO (188 mV) and Au/NanoZnO/LDH (161 mV) also confirmed that ZnO nanorods have a greatest impact in transferring electrons between LDH and Au electrode during the cathodic reaction for lactate detection.

Fig. 3B shows the cyclic voltammogram of Au/NanoZnO/LDH bio-electrode at different scan rates (0.01–0.1 V s^{-1}) in oxygen saturated PBS (0.1 M, pH 7.4). In the case of Au/NanoZnO/LDH bioelectrode, as the scan rate (not $v^{1/2}$) varied from 0.01 to 0.1 V s^{-1}, the cathodic peak current increased linearly (I_{pc} = 8.724 μA [v] + 0.228) with a regression coefficient of 0.98 (Fig. S3A), indicating surface controlled process. This showed that the cathodic reaction occurred was only due to the immobilized biomolecules (not controlled by diffusion). It also indicated that the ZnO nanorods can be used as an immobilization matrix for LDH enzyme and there by offering sufficient availability to electrons to shuttle between LDH and Au electrode. In the case of Au/NanoZnO electrode the I_{pc} increased linearly (I_{pc} = 7.857 μA [v] + 0.074; R^2 = 0.99) with the increasing scan rate (not $v^{1/2}$) varied from 0.01 to 0.1 V s^{-1} (Fig. S3B). This showed that the electron transfer between ZnO nanorods and Au working electrode was a surface controlled electrochemical process (not controlled by diffusion) i.e., the peak current observed in the cathodic process was from the immobilized system.

The electron transfer rate constant (k_s) was calculated according to the formula, $k_s = I_p/Q$, where I_p is the cathodic peak current and Q is the charge consumed. The k_s of Au/NanoZnO and Au/NanoZnO/LDH were found to be 0.537 ± 0.027 s^{-1} and 0.687 ± 0.043 s^{-1} respectively. It revealed that the electron transfer between ZnO nanorods and LDH was very rapid in the case of Au/NanoZnO/LDH bioelectrode than in Au/NanoZnO electrode. The surface coverage (I^*) of amount of lactate adsorbed on various modified Au electrode was evaluated using the following equation:

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

where I_p is the peak current (μA), A is the area of Au electrode (cm^2), I^* is the surface coverage (mol cm^{-2}), v is the scan rate (V s^{-1}), T is the absolute temperature, n is the number of electrons transferred, F is the Faraday constant and R is the gas constant. The surface coverage of amount of lactate adsorbed on Au/NanoZnO/LDH (44.28×10^{-9} mol cm^{-2}) was almost two fold higher than on Au/LDH bioelectrode (14.72×10^{-11} mol cm^{-2}) indicating high adsorption of lactate on to the redox center of LDH.

Fig. 3C shows the cyclic voltammograms of Au/NanoZnO/LDH electrode for varying concentrations of lactate (0.2–1.6 μmol L^{-1}) at a scan rate of 0.1 V s^{-1} in oxygen saturated PBS (0.1 M, pH 7.4) with 10 μl of coenzyme NADH in electrolyte solution. On successive addition of lactate, the number of electrons consumed by the oxygen during electrochemical reaction got increased. This in turn reduced the cyclic voltammetric current response.

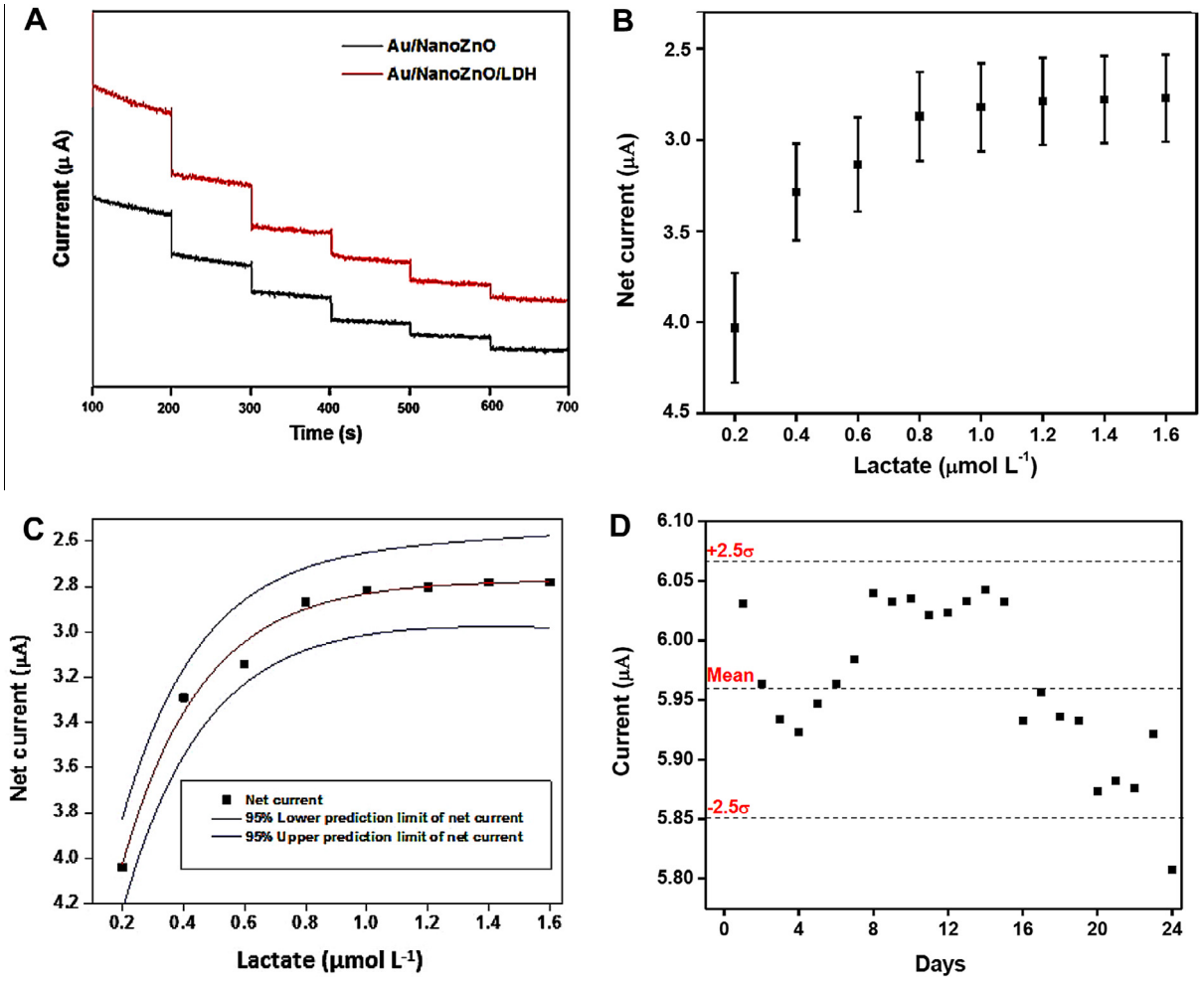

Fig. 4. (A) Amperogram of Au/NanoZnO and Au/NanoZnO/LDH electrode for varying concentrations of lactate (0.2–1.2 μmol L^{-1}) in oxygen saturated PBS (0.1 M, pH 7.4) at 468 mV, (B) calibration curve of the net current response vs. the concentrations of lactate, (C) prediction band for the observed net current and (D) control chart constructed for Au/NanoZnO/LDH biosensor.

3.3. Performance of amperometric LDH biosensor response to the varying concentration of lactate

Fig. 4A shows the amperogram of Au/NanoZnO and Au/NanoZnO/LDH electrode for varying concentrations of lactate (0.2–1.2 μmol L^{-1}) in oxygen saturated PBS (0.1 M, pH 7.4) at 468 mV. In the case of Au/NanoZnO and Au/NanoZnO/LDH electrode, a decrease in the amperometric current response was observed for an every successive addition of 0.2 μmol L^{-1} of lactate. This was due to the consumption of number of electrons by the oxygen in 0.1 M PBS during enzymatic rate of reaction [36]. The similar trend was also observed in cyclic voltammetric response to the varying concentration of lactate [36]. Au/NanoZnO/LDH bioelectrode showed a better sensitivity of 1.832 μA μmol^{-1} L while Au/NanoZnO electrode showed a linear response of 1.125 μA μmol^{-1} L over a lactate concentration range of 0.2–0.8 μmol L^{-1}. This showed that the ZnO nanorods are highly sensitive towards lactate even in the absence as well as in the presence of LDH. Au/NanoZnO/LDH bioelectrode showed a rapid response time of <1 s. The estimated linear regression equation was $I_{net} = -1.832\ \mu A\ [S] + 4.252$ with R^2 of 0.89, where I_{net} is the Au/NanoZnO/LDH bio-electrode net current response and [S] is the lactate concentration. A calibration curve for the Au/NanoZnO/LDH bioelectrode net current response vs. the concentration of lactate was plotted and is shown in Fig. 4B. The minimum signal required to detect the lowest concentration of lactate, LOD was found to be 4.73 nmol L^{-1} with a quantification limit (LOQ ≈ 3.33 × LOD) of 15.75 nmol L^{-1}. From the Hill plot, the degree of co-operativity was found to be greater than one (1.702) indicating positive co-operativity. The apparent Michaelis–Menten constant (K_M^{app}) and maximum net current (I_{max}) obtained from Hanes-Woolf plot were found to be $0.38 \pm 1.2 \times 10^{-3}$ μmol L^{-1} and 2.798 μA respectively. This lower K_M^{app} indicated that LDH and lactate were tightly bound and formed the LDH-lactate complex quickly. From the dose response analysis, the concentration of lactate (EC 50) at which Au/NanoZnO/LDH bio-electrode exhibited 50% of current response was found to be 0.41 μmol L^{-1}. This EC 50 value was in good correspond with K_M^{app} showing that there exists a good affinity between lactate and LDH.

3.4. Precision, accuracy and biosensor efficiency

Au/NanoZnO/LDH biosensor showed good repeatability and reproducibility (using five different electrodes) with a relative standard deviation of 2.42% and 3.87% for a lactate concentration of 1.2 μmol L^{-1} ($n = 6$). Accuracy of Au/NanoZnO/LDH towards lactate was calculated by adding 0.25, 0.5 and 0.75 μmol L^{-1} of lactate in oxygen saturated PBS (0.1 M, pH 7.4). For 0.25, 0.5 and 0.75 μmol L^{-1} of lactate, the predicted lactate concentration ± relative error for four trials were found to be 0.256 ± 0.025,

Table 1
Comparative study of existing lactate biosensors with the present ZnO nanorods based lactate biosensor.

Interface	Enzyme	K_M^{app}	Sensitivity	LOD	Linear range	Stability	References
Fe_3O_4	GOD/LDH	4.785 mM	–	–	–	–	[38]
PPY & PVS	LDH	–	–	10^2 nM	0.5–6 mM	14 days	[23]
Polyaniline	LDH	4.5 mM	–	–	1–3 mM	14 days	[23]
O-phenylene diamine	LDH/GOD	–	–	$<50 \times 10^3$ nM	50 μM to 1 mM	–	[39]
pTTCA & MWCNT	LDH	–	0.0106 μA μM^{-1}	–	5–90 μM	–	[40]
Polyvinylpyrrolidone	LDH	13.7 mM	62.6 nA mM^{-1}	1.1×10^5 nM	2 mM	40 days	[41]
Silica	LDH	–	–	800 nM	2–30 μM	–	[42]
Diaphorase	LDH	–	–	–	0.2–8 mM	–	[43]
Polyaniline	LOD/LDH	1.9 mM	38.5 μA mM^{-1}	10^4 nM	0.1–1 mM	21 days	[44]
Graphite	LDH	–	–	–	0.5–20 mM	–	[45]
Polysulfone/CNT	LDH	–	–	370 nM	1–20 μM	–	[46]
ZnO nanorods	LDH	3.8×10^{-4} mM	1.832 μA μM^{-1}	4.73 nM	0.2–0.8 μM	23 days	Present work

0.051 ± 0.017 and 0.762 ± 0.016 μmol L^{-1} respectively. The biosensor efficiency of Au/NanoZnO/LDH biosensor was estimated using the following equation:

$$\text{Biosensor efficiency} = \frac{I_{max}/K_M^{app}}{\text{Slope of Au/NanoZnO/LDH bio-electrode}} \times 100 \quad (2)$$

The Au/NanoZnO/LDH biosensor exhibited a biosensor efficiency of 4.02% which showed that the Au/NanoZnO/LDH biosensor had the higher ability to convert lactate to pyruvate and thereby exhibited an increase in sensitivity.

3.5. Response study of lactate biosensor from percentage inhibition

Urea was chosen as an interferent because it supplies nitrogen for the development of stems and leaves in plants [37]. Interference studies on lactate detecting biosensor were studied from percentage inhibition by injecting urea at higher concentration (83 mmol L^{-1}) than higher value (<49 mmol L^{-1} used in fertigation) to 0.2 μmol L^{-1} of lactate. The percentage inhibition can be calculated from the following equation:

$$I\% = \left[\frac{I_o - I_i}{I_o}\right] \times 100 \quad (3)$$

where I_i is the obtained current in the presence of urea and I_0 in the absence of urea. Nearly 50% of LDH active sites were inhibited. This maximal inhibition might results in change in I_{max} and K_M^{app}. Hence a prediction band (Fig. 4C) was developed for the observed net current at 95% confidence interval, so that there was a 95% chance of observed current fall within this prediction band when the same experiment is repeated.

3.6. Life time

Fig. 4D shows the control chart for Au/NanoZnO/LDH biosensor. In the control chart, mean value ($n = 3$) represented the average value of the current density evaluated on the first day whereas the upper and lower limits represent mean current response ± 2.5 standard deviation of the current response value. On the 24th day of the stability study, the measured current response exceeds the outer limit (mean current response − 2.5 standard deviation). This showed that the life time of Au/NanoZnO/LDH biosensor was 23 days.

3.7. Comparative study of existing lactate biosensor with novel ZnO based lactate biosensor

Table 1 compares characteristics of some lactate biosensor with our ZnO nanorods based lactate biosensor. From the Table 1, it was found that only ZnO based lactate sensor showed low K_M^{app} and LOD. These results showed that ZnO nanorods based lactate biosensor can sense nanomolar concentrations of lactate with better affinity.

4. Conclusion

Au/NanoZnO/LDH bioelectrode was fabricated by tagging LDH on ZnO nanorods. The one dimensional ZnO nanorods exhibit high surface area and act as an immobilization matrix for LDH. This lactate detecting biosensor was shown to have a LOD of 4.73 nmol L^{-1}, LOQ of 15.75 nmol L^{-1}, response time of <1 s, sensitivity of 1.832 μA μmol^{-1} L and a linearity of 0.2–0.8 μmol L^{-1}. This electrode was stable up to 23 days under dry condition at room temperature. Prediction band was developed to improve the specificity of lactate biosensor. Moreover lactate biosensor showed good reproducibility, repeatability and accuracy. These results suggest that the lactate biosensor can be used for sensing lactate in food products.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2013.09.052.

References

[1] L.D. Mello, L.T. Kubota, Food Chem. 77 (2002) 237.
[2] F. Palmisano, R. Rizzi, D. Centonze, P.G. Zambonin, Biosens. Bioelectron. 15 (2000) 531.
[3] M.J.F. Villamil, A.J.M. Ordieres, P.T. Blanco, Anal. Chim. Acta 345 (1997) 37.
[4] F. Palmisano, M. Quinto, R. Rizzi, P.G. Zambonin, The Analyst 126 (2001) 866.
[5] M.H. Ward, W.H. Pan, Y.J. Cheng, F.H. Li, L.A. Brinton, C.J. Chen, P.H. Levine, C.S. Yang, A. Hildesheim, Int. J. Cancer 86 (2000) 603.
[6] S.C. Larsson, L. Bergkvist, A. Wolk, Int. J. Cancer 119 (2006) 915.
[7] B. Timbo, K.M. Koehler, C. Wolyniak, K.C. Klontz, J. Food Prot. 67 (2004) 1806.
[8] M.J. Friedman, G.Q. Sharieff, Pediatric Clinics North Am 53 (2006) 257.

[9] B. Bleiberg, J.J. Steinberg, S.D. Katz, J. Wexler, T. LeJemtel, J. Chromatogr. B 568 (1991) 301.
[10] R. White, D. Yaeger, S. Stavrianeas, Int. J. Exerc. Sci. 2 (2009) 83.
[11] S.J. Niven, J.D. Beal, P.H. Brooks, Anim. Feed Sci. Technol. 117 (2004) 339.
[12] M. Stoytcheva, R. Zlatev, Z. Velkova, B. Valdez, M. Ovalle, Portugaliae Electrochim. Acta 27 (2009) 353.
[13] N. German, A. Ramanaviciene, J. Voronovic, A. Ramanavicius, Microchim. Acta 168 (2010) 221.
[14] J.J. Gooding, M.H.L. Lai, Y.I. Goon, Adv. Electrochem. Sci. Eng. 11 (2009) 1.
[15] S. Vaddiraju, I. Tomazos, D.J. Burgess, F.C. Jain, F. Papadimitrakopoulos, Biosens. Bioelectron. 25 (2010) 1553.
[16] J. Wang, Anal. Lett. 130 (2005) 21.
[17] Chopra, N. Gavalas, V.G. Bachas, L.G. Hinds, B.J. Bachas, Anal. Lett. 40 (2007) 2067.
[18] Kerman, K. Saito, M. Amamura, S.Y. Takmura, Y. Tamiya, Trends Anal. Chem. 27 (2008) 585.
[19] J. Chow, D.C. Johannes, M.S. Lee, W.K. Clark, R.L. Zauscher, S. Chilkoti, Mater. Today 8 (2005) 30.
[20] Z.H. Ibupoto, S.M.U.A. Shah, K. Khun, M. Willander, Sensors 12 (2012) 2456.
[21] Z. Zhao, W. Lei, X. Zhang, B. Wang, H. Jiang, Sensors 10 (2010) 1216.
[22] S.K. Arya, S. Saha, J.E. Ramirez-Vick, V. Gupta, S. Bhansali, S.P. Singh, Anal. Chim. Acta 737 (2012) 1.
[23] A. Chaubey, M. Gerard, R. Singhal, V.S. Singh, B.D. Malhotra, Electrochim Acta 46 (2000) 723.
[24] Zayats, M. Kharitonov, A.B. Katz, E. Buckmann, A.F. Wilner, Biosens. Bioelectron. 15 (2000) 671.
[25] Leonida, M.D. Starczynowski, D.T. Waldman, R. Aurian-Blajeni, Anal. Bioanal. Technol. 376 (2003) 832.
[26] J. Spohn, U. Narasaiah, D. Gorton, Electroanalysis 8 (1996) 507.
[27] Garjonyte, R.Y. Yigzaw, Meskys, R. Malinauskas, A. Gorton, Sens. Actuators B 87 (2001) 33.
[28] J. Hirano, K. Yamato, H. Kurimoto, K. Ohwa, Biosens. Bioelectron. 17 (2002) 315.
[29] J. Ali, S.M.U. Nur, O. Willander, M. Danielsson, IEEE Trans. Nanotechnol. 8 (2009) 678.
[30] J. Ali, S.M.U. Nur, O. Willander, M. Danielsson, Sens. Actuators B 145 (2010) 869.
[31] V. Prasad, C. D'Souza, D. Yadav, A.J. Shaikh, N. Vigneshwaran, Spectrochem. Acta Part A 65 (2006) 173.
[32] N. Nesakumar, J.B.B. Rayappan, B.G. Jeyaprakasah, U.M. Krishnan, J. Appl. Sci. 12 (2012) 1758.
[33] J.C. Murphy, R.N. Jana, Adv. Mater. 14 (2002) 80.
[34] L. Wu, Y. Wu, W. Lu, Physica E 28 (2005) 76.
[35] Mary K. Campbell, Shawn O. Farrell, Biochemistry, seventh ed., Brooks/Cole, USA, 2011.
[36] B. Unnikrishnan, S. Palanisamy, S.M. Chen, Biosens. Bioelectron. 39 (2013) 70.
[37] G. Bi, C.F. Scagel, R. Harkess, Hort. Sci. 43 (2008) 472–477.
[38] O.J. Sohn, C.K. Kim, I.J. Rhee, Biotechnol. Bioprocess Eng. 13 (2008) 716.
[39] Tuan Vo-Dinh, Brian Cullum, J. Fresenius, Anal. Chem. 366 (2000) 540.
[40] E.I. Afaf, Ansary, M. Layla Faddah, Nanotechnol. Sci. Appl. 03 (2010) 65.
[41] C. Hyun Yoon, Hak-Sung Kim, Anal. Chim. Acta 336 (1996) 57.
[42] June Di, Jiongjia Cheng, Xu Quan, Huie Zheng, Jingyue. Zhuang, Yongbo Sun, Keyu Wang, Xiangyin Mo, Shuping Bi, Biosens. Bioelectron. 23 (2007) 682.
[43] Franco Mazzei, Alfredo Azzoni, Barbara Cavaleiri, Francesco Botre, Claudio Botre, Food Chem. 55 (1996) 413.
[44] A. Chaubey, K.K. Pande, V.S. Singh, B.D. Malhotra, Anal. Chim. Acta 407 (2000) 97.
[45] J. Wang, Q. Chen, Electroanalysis 6 (1994) 850.
[46] S. Perez, S. Sanchez, Esteve. Fabregas, Electroanalysis 24 (2012) 967.