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An ultrasensitive amperometric determination of lactate by lactate dehydrogenase nanoparticles immobilized onto Au electrode

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Running Title: *LDH nanoparticles based lactate biosensor*

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Abstract

The nanoparticles (NPs) of commercial lactate dehydrogenase (LDH) from rabbit muscle were prepared, characterized and immobilized covalently onto Au electrode to construct an improved amperometric lactate biosensor . The biosensor showed optimum response within 2.5s at an applied potential of 0.10V, pH 7.0 and 35^0C.The biosensor had a wider working range linear (0.01μM to 55 mM) with a higher sensitivity (3.45 ±0.02 μA cm^{-2} mM^{-1}) and a lower detection limit (0.01μM) compared to earlier biosensors. The analytical recovery of added lactate in sera was 98.61% and within and between batches coefficients of variations(CVs) were 1.38% and 1.03%, respectively. A good correlation coefficient (R^2 = 0.99) was observed between sera lactate values as measured by the standard enzymatic colorimetric method and the present biosensor. The biosensor measured lactic acid in the sera of apparently healthy subjects and persons suffering from cardiogenic shocks. There was a 10% loss in the initial activity of LDHNPs/Au electrode after its regular use over a period of 210 days, while being stored dry at 4^oC.

Keywords: Lactate; LDH nanoparticles; Lactate Biosensor

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Introduction

Lactate is a dynamic metabolite assuming the vital part in muscle glycogen generation [1]. It is generated from pyruvic acid under anaerobic conditions in various tissues such as skeletal muscles, brain, blood cells and kidney [2]. The normal blood lactic acid concentration is 4.5 to 19.8mg/dL (0.5-2.2 mmol/L) [3]. It is well known that tissue hypoxia in cardiac shock patients is directly proportional to the increased level of blood lactate. Hence, lactate has been considered as an important biomarker of cardiogenic shock [4]. Additionally, blood lactate level is also vital in the differential finding of endotoxic shock, respiratory failure, liver disease, systemic disorders, renal failure, and tissue hypoxia [5]. A large number of methods are available for monitoring blood lactate level such as colorimetric method [6], spectrophotometric and titrimetric method [7], fluid chromatography method [8], cyclic voltammetry method and proton nuclear resonance method [9]. However, these methods have certain drawbacks, as these are tedious and time consuming and require skilled personnel and expensive instrument set up. Biosensing methods specifically amperometric biosensors overcome these drawbacks[9]. In fabrication of lactate biosensors, two types of enzymes have been used: lactate oxidase (LOx) and lactate dehydrogenase (LDH). The biosensors based on LDH have shown superior performance than those employing LOx in terms of cost of enzyme, its stability, and high catalytic activity. In LDH based biosensors, the enzyme has been immobilized onto different nanocomposites, for detection of lactate in biological materials [10, 11]. However, direct immobilization of enzymes onto nanocomposites brings about their denaturation, prompting loss of their activity and stability. The issue was overcome by aggregating the enzyme molecules into their nanoparticles and immobilizing them directly/covalently onto electrode. Thus, use of enzyme nanoparticles (ENPs) rather than native enzyme has improved the analytic effectiveness of biosensors [12]. Due to their unique electronic, optical, mechanical, electrical, thermal and catalytic (ability to facilitate electron transfer) properties, beside increased surface area, ENPs have shown great promises in improving enzyme electrodes. Au electrode offers numerous attractive features, such as good conductor of electricity, low cost, low background current and easy availability, which makes it a better option than glassy-carbon and Pt electrodes. [13]. We describe herein the simplified fabrication of an ultrasensitive, rapid and cost effective lactate biosensor based on nanoparticles (NPs) of lactate dehydrogenase (LDH) for detection of blood lactate level in patients suffering from cardiogenic shock.

Materials and Methods

Materials

Lactate dehydrogenase (LDH) from rabbit muscle (25U/mg), TRIS-buffer, NAD+, L-lactic acid, glycine, uric acid, glucose, ascorbic acid, acrylamide, potassium chloride, silica gel from SRL, Mumbai, sulphuric acid and hydrochloric acid from Qualigens Fine Chemicals, Mumbai were used. Gold (Au) wire of 2mm diameter was purchased from the local jewelers shop. All other chemicals were of analytical reagent (AR) grade. Double distilled water (DW) (ohmic resistance: 1.8×10^{-5} for every ohm) was used throughout the study. Sera samples of apparently healthy subjects and persons suffering from cardiogenic shocks were collected from local PGIMS hospital.

Instruments used

Potentiostat/Galvanostat (Make: Autolab, model: AUT83785, made by Eco Chemie, The Netherland) with GPES (General purpose electrochemical software) software was used. Scanning electron microscopy (SEM) (Zeiss EV040, USA), UV Spectrophotometer (Make: Shimadzu, Japan, Model 1700), X-Ray diffractometer (XRD), (Make: 122 Rigaku, D/Max2550, Tokyo, Japan), Fourier Infra-red spectrometer (FTIR) and spectronic-20 (Thermo Scientific, USA), were utilized. The electrochemical impedance spectra (EIS) was recorded by FRA software in 5mM $K_3Fe(CN)_6/K_4Fe(CN)_6$, 25ml solution at 0.1 V between frequency range, 0.01 Hz–10 kHz.

Assay of LDH

The assay of LDH was based on decrease of absorbance of NADH at 340 nm, due to reduction of NAD^+. The reaction mixture consisting 2.8ml 0.2MTris-HCl buffer, pH 7.3 (Reaction buffer), 0.1ml 6.6mM NAD+, 0.1ml 30mM lactic acid was incubated in UV spectrophotometer at room temperature (25^0C) for 4-5 min to accomplish temperature equilibration and set up a blank rate. The enzyme (1mg/ml) was added to the reaction mixture and ΔA340/min was recorded from standard curve . The unit activity of the enzyme was calculated using the equation:

$$Units/mg = \frac{\Delta A340/min}{6.22 \times mg\ enzyme/ml\ reaction\ mixture}$$

One unit of LDH is defined as the amount of enzyme required to reduce one micromole of NAD^+ per min per ml under the standard assay conditions [14].

The protein content of dissolved LDH was determined by Lowry method using bovine serum albumin (BSA) as standard protein [15].

Preparation of LDHNPs

The LDHNPs were prepared by desolvation method using ethanol [16, 17]. To 2ml LDH solution (1mg/ml), 6ml of desolvating agent (absolute ethanol) was added drop wise at the rate of 0.1-0.2 ml/min under constant stirring at a speed of 500 rpm. The process was followed by the addition of 1ml, 2.5% glutaraldehyde solution in the mixture under the identical stirring conditions at 4°C for 24 h to ensure complete crosslinking of respective ENPs. The glutaraldehyde provided intermolecular crosslinking of enzyme molecules via Schiff base. The synthesized ENPs were thiol functionalized by adding 0.2 ml of cysteamine solution (0.02g/ml) to each suspension under constant stirring for 5-6 h. ENPs were precipitated from mixture by centrifugation ENPs mixture at 1200×g for 10 min at 4°C. The process was followed by dispersion of ENPs in 2 ml 0.1M phosphate buffer, pH 7.3 and sonication for 5 min. It was assumed that alpha-NH_2 group of cysteamine reacts with excess unreacted –CHO groups of glutaraldehyde cross-linked ENPs to form Schiff base. Thus, the glutaraldehyde cross-linked ENPs get functioned with –NH_2 groups. These functionalized ENPs were stored at 4°C, until use.

Characterization of LDHNPs

LDHNPs were characterized by taking their images in a transmission electron microscope (TEM) and recording their FTIR (4000-500 cm^{-1}) & UV –visible spectra (200-600nm).

Preparation of LDHNPs modified Au electrode

LDHNPs were immobilized onto the surface of Au electrode (0.2x2.5cm, diameter x height; area=3.14cm^2) to prepare a ENPs-based working electrode. The potential pretreatment was provided to the Au electrode by allowing its scanning over the potential of 0 to 0.5 V range in freshly prepared 0.2 M H_2SO_4 until the cyclic voltammogram feature of the clean Au electrode

was developed. The Au electrode was further cleaned by piranha solution (3ml H_2SO_4: 1ml H_2O_2) and treated with absolute alcohol to functionalize with –OH group. After that, the Au electrode was kept in the LDHNPs suspension under mild stirring at 4 °C for 12h for co-immobilization of cysteamine functioned LDHNPs onto Au electrode. This immobilization could be possible by covalent bonding between –SH group of ENPs and –OH group of Au electrode. The LDHNPs/Au electrode was washed with 0.1M sodium phosphate buffer (SPB, pH 7.0) and stored in a SPB at 4 °C, when not in use. The characterization of LDHNPs/Au electrode was carried out by SEM and EIS before and after immobilization of ENPs.

$$\mathbf{Au_n\text{-}AuO + 2(LDHNPs)\text{-}SH \xrightarrow{\text{Dehydration}} Au_n(Au\text{-}S\text{-}LDHNPs) + H_2O}$$

Polycrystalline -AuE **Functionalized LDHNPs** **LDH-E (LDH-electrode)**

Construction of lactate biosensor and its response measurements

An amperometric lactate biosensor was fabricated using LDHNPs/Au electrode as working electrode, Ag/AgCl as reference electrode and Pt wire as an auxiliary electrode connected through galvanostat potentiostat. The electrochemical optimizations, characterization and measurements of modified LDHNPs/Au electrode were carried out using cyclic voltammetry and chronoamperometry. All electrochemical procedures were established in 0.1M phosphate buffer saline containing 0.1mM (0.1ml) lactate and optimized concentration of NAD^+ at an optimized scan rate. The current response was measured between 1s and 10 s at an interval of 2.5 s.

Optimization of lactate biosensor

The kinetic properties such as NAD^+ concentration, applied potential, scan rate, pH, incubation temperature, response time and effect of lactate concentration of LDHNPs/Au electrode were studied electrochemically in order to optimize the operational conditions of the biosensor. The concentrations of NAD^+ were varied from 0.1 to 8 mM to get the meaningful response at optimum concentrations. The effect of applied potential was studied between -0.8 to 0.2V. Similarly, effect of scan rate was studied between 5mV/s to 20mV/s. To get the optimum pH, the pH was varied in the range pH 4.0 to 9.0 at an interval of pH 0.5 using the suitable buffers

(strength- 0.1M) such as: pH 4.0 to 5.0 sodium acetate buffers, pH 5.5 to 7.5 sodium phosphate buffer for pH 8.0. Likewise to get optimum temperature and incubation time the master mix (buffers, lactate, and NAD^+) was incubated at different temperature (5–70ºC) and time duration (0-10s). The effect of lactate concentration on biosensor response was determined by varying the concentration of lactate in the range 0.001mM-65 mM. The limit of detection (LOD) was calculated using the equation: LOD = 3xσ/slope, where σ=standard deviation.

Evaluation of lactate biosensor

The biosensor was evaluated by analyzing its analytical performance in terms of electroactive surface area, electron transfer coefficient, linearity, limit of detection, analytical recovery, coefficient of variation and correlation coefficient. The electroactive surface area of electrode was calculated by Randles –Servcik equation ($I=2.69\times10^5AD^{1/2}n^{3/2}v^{1/2}C$), where I is the maximum current (A), A stands for electroactive surface area of electrode (cm^2), D represents the diffusion coefficient (cm^2/s), *n* is number of electrons transferred in the redox event (usually 1), *C* shows concentration (mol/cm^3) and *v* is scan rate (V/s). Heterogeneous electron transfer coefficient (k_s) was calculated by Laviron formula i.e $E_p= E_0+ (RT/\alpha nF) [\ln (RTk_s/\alpha nF) - \ln\upsilon]$ where E_p is cathodic peak potential, E_0 is reference potential, n is number of electrons, T is temperature coefficient, R is gas constant, F and α stands for faraday constant and electron transfer constant. The average concentration (Γ) of electroactive surfaces onto surface of electrode was calculated by using the formula: Peak current (I_p) = $n^2F^2A\Gamma\upsilon/4RT$. The analytical recovery and coefficient of variation were calculated by the equations:

$$\%\, Recovery = \frac{Observed\ response\ of\ added\ lactate\ \times 100}{Ideal\ response\ of\ added\ lactate}$$

$$Coefficient\ of\ variation = \frac{Standard\ deviation \times 100}{Mean\ of\ samples}$$

The effect of various interferrants found in the blood such as glucose, citric acid, glutamic acid and ascorbic acid were studied at their physiological concentrations into the blood.

Application of lactate biosensor

Blood samples (1 ml each) of apparently healthy people's and patients suffering from cardiogenic shocks in different age groups (n=20 each) were collected from local Pt. BDS Post Graduate Institute of Medical Sciences (PGIMS) Rohtak Hospital. The samples were centrifuged at 5000×g for 5 min to get their supernatants (sera) and kept at 4°C until use. The quantity of lactate in sera was measured by the present biosensor in the same fashion as elucidated above for its response measurement, under optimized operational conditions except that serum was used instead of lactate. The current (mA) was recorded and the quantity of lactate in serum was interpolated from the standard curve between lactate concentrations and current (in mA) under optimal operational conditions (Fig. 1a).

Reusability and storage stability of biosensor

The storage stability and long term stability of the LDHNPs/AuE was studied over a period of 210 days during its regular use, while being stored at 4°C.

Results and discussion

Assay of LDH

The activity of LDH and its protein content in solution were 0.59 μmol min^{-1} ml^{-1} and 0.78mg/ml respectively.

Characterization of LDHNPs

The size of LDHNPs, as measured by TEM, was between 5nm to 100 nm (Fig. 1b) with an average size of 20 nm, which enhanced the surface area of the enzyme, as compared to the native enzyme. FTIR spectra of LDHNPs showed transmittance at 3299, 1928, 1536, and 930. The transmittance at 3299 cm^{-1} divulged the presence of free -NH_2 group inflicted by cysteamine dihydrochloride. The transmittance at 1928 cm^{-1} showed the presence of amide I and amide II linkage and 1536-930 cm^{-1} peaks represent C=N stretching. The covalent linkage (thiolate bond) between polycrystalline Au and –SH group of LDHNPs functionalized by cysteamine, enhanced storage stability, detection limit and sensitivity (Fig. 1c). UV and visible spectra exhibited strong absorbance peak at 250 nm, revealing a high degree of absorption of aromatic amino acids of peptide chain/free enzyme following their NPs formation (Fig. 1d).

Characterization of working electrode (LDHNPs/AuE) at different stages of its construction

By scanning electron microscopy (SEM)

Figure 2a showed the SEM images of bare Au electrode and LDHNPs modified Au electrode. Bare electrode depicted the smooth and flat morphology, whereas LDHNPs/Au electrode revealed globular structural morphology. The globular morphology was due to nanoparticles of LDH, which were directly immobilized onto Au electrode.

By electrochemical impedance spectroscopy (EIS)

The EIS provides the change in impedance of electrode before and after its modification with immobilization of LDHNPs. The semicircle diameter at higher frequencies contributed to the electron transfer resistance (R_{CT}), which directed the electron transfer kinetics of the redox probe at the surface of electrode, while at lower frequencies linear part corresponded to Warburg diffusion process [17]. In Fig. 2b, the Nyquist plot presented EIS of bare Au electrode and LDHNPs/Au electrode in 5mM ($K_3Fe(CN)_6/K_4Fe(CN)_6$) respectively. The R_{CT} value for the LDHNPs/Au electrode was obtained as 200 Ω, which was higher compared to bare Au electrode (R_{CT}=100 Ω). The increased R_{CT} value of LDHNPs/Au electrode was due to high surface area of enzyme nanoparticles. This increase in R_{CT} could be assigned to the reason that most biological molecules, including enzyme nanoparticles, have poor electrical conductivity at low frequencies (<10 kHz; applied voltage: 0.1V) and create obstacle to the transfer of electrons.

Voltammetric response of the LDHNPs/Au electrode

The amperometric response of electrodes bare Au, LDHNPs/Au without and with substrate was recorded (Fig 3a). The bare Au and LDHNPs/AuE without substrate showed no oxidation and reduction peak, while the LDHNPs/AuE in presence of lactate exhibited excellent oxidation and reduction peaks, thus also revealed the successful immobilization of LDHNPS onto AuE. The current increases linearly with increase in scan rate from 5 mV/s to 20 mV/s (Fig. 3b) indicating that the electron transfer kinetics was diffusion controlled which was significant for electrochemical applications. After 20 mV there was major shift in anodic and cathodic peaks therefore all electrochemical studies were done at this scan rate.

Current response measurement of LDHNPs/AuE

The current response of the LDHNPs/Au electrode was analyzed as a function of lactate concentration (0.1mM) by measuring CV at 20 mV/s scan rate in 0.1M PBS of pH 7.0 containing 5 mM [Fe(CN)6]3−/4− (the electrical conductivity of PBS = 25Ω). At a lactate concentration of 30 mM, the current response of the Au electrode with native LDH was 0.11 mA, while the same electrode with mixture of LDHNPs was 1.98 mA.

Optimization of lactate biosensor

An amperometric lactate biosensor was constructed by decorating LDHNPs onto Au electrode. The optimum concentration of NAD^+ was determined by investigating the response of biosensor at different NAD^+ concentrations when applied for 0.1mM lactate. The maximum current was predicted at 6.6 mM concentration of NAD^+. Thus, 6.6 mM NAD^+concentration was used for further work. The biosensor expressed the optimum current at pH 7.0 (Fig. 4a), incubation temperature 30ºC (Fig. 4b) in 0.1 M sodium phosphate buffer with in 2.5s (Fig. 4c). While at other lower and higher pH and temperature there was gradual shift in the oxidation and reduction peaks with decline in the current range. Hence, the succeeding electrochemical studies for lactate detection were performed in 0.1 M sodium phosphate buffer of pH 7.0 and temperature 30°C at optimal potential of 0.1 V. The response of current for LDHNPs increases linearly with increase in lactate concentration. At high concentration of lactate, a plateau current is perceived, depicting the feature of Michaelis–Menten kinetic process. The apparent Michaelis–Menten constant (Km), provided the evidence of enzyme substrate kinetics, which can be attained through the Lineweaver–Burk equation

$$\frac{I}{Iss} = \frac{I}{Imax} + \frac{Km}{ImaxC}$$

Here, I_{ss} indicated steady-state current after addition of substrate, C represented the bulk concentration of the substrate, and I_{max} showed the maximum current sustained under saturated substrate conditions. The LDHNPs/Au electrode exhibited a V_{max} of 2.76±0.12 mA cm^{-2} with app. K_m of 0.2±0.1 mM and showed high sensitivity of 3.45 ±0.02 µA cm^{-2} mM^{-1}at a potential of 0.1V. The small Km value means that the immobilized ENPs at Au electrode possess a high affinity to lactate.

Evaluation of lactate biosensor

The LDHNPs/Au electrode exhibited an excellent electroactive surface area of 3.97 cm^2 as compared to native enzyme LDH bound Au which had 3.34 cm^2. Hence, the synergistic effect of enzyme nanoparticles was significant in improvement of electroactive surface area. The heterogeneous electron transfer constant (k_s) for native enzyme electrode and enzyme nanoparticles electrode was calculated as 0.9 s^{-1} and 2.4 s^{-1} respectively. In case of LDHNPs modified electrode, high k_s value was attributed to the large surface area and high biocompatibility of enzyme nanoparticles. The average surface concentration (Γ) of immobilized LDHNPs was 3.29 $\times 10^{-8}$ mol cm^{-2} which was higher than the concentration of native LDH i.e 1.13×10^{-8} mol cm^{-2}. This higher concentration is due to porous and biocompatible nature of Au electrode which can amplify the number of enzyme nanoparticles and further enhanced the specificity of biosensor. The biosensor offered a wide linear response between current (mA) and lactate concentration ranges of 0.01μM-55mM, under optimized operational conditions (Fig. 4d), which was better than earlier reported biosensors those have in the ranges 0.1-10 μM [18], 0.055-10 μM [19], 5-90 μM [20], 0-0.3 μM [21], 0.005-0.5 μM [22], 0.2-2 μM [23], 0.005-10 μM [24], 0.000014-0.0055 μM [25], 5-50 μM [26], 0.001-0.12 μM [27]. The limit of detection (LOD) of the present biosensor was 0.01μM, which was lower than already reported biosensors those have 100 μM [18], 550 μM [19], 1000 μM [20], 0.3 μM [21], 5 μM [22], 50 μM [23], 0.004 μM [24], 50 μM [25], 0.1 μM [26], 0.87 μM [27]. The analytical recoveries of added lactate in sera at concentration of 0.5 mM and 1.0 mM were 98.61 and 98.83% respectively (Table 1). In addition, within and between batch coefficients of variation for working electrode were detected as 1.38% and 1.03%, respectively (Table 2). The reproducibility of ENPs electrode was checked by constructing three electrodes and then measuring their current responses at different lactate concentrations by cyclic voltammetry. The relative standard deviation (RSD) for the current response was 2.57%.

Correlation

The lactate level detected by the present biosensor was also compared with the standard enzymic colorimetric kit method. A good correlation coefficient (R^2 = 0.99) was obtained between the present method and standard enzymic colorimetric kit method (Fig.5). The correlation coefficient of the present biosensor was better than earlier biosensors [18-26].

Application of lactate biosensor

The quantity of sera lactate in apparently healthy persons as detected by the current biosensor was in the range 0.4±0.01-2.2±0.02 mM (n=20), which was in normal established range. The sera lactate level in persons suffering from cardiogenic shocks was in the range 12±0.02-30±0.03 mM (n=20), which was notably higher than the levels in apparently healthy persons (Table 3).

Interference study

Interfering components like glucose, citric acid, glutamic acid, and ascorbic acid had only 4.2%, 2.7%, 3.8% and 1.5% interference respectively on lactate biosensor response at their physiological concentrations, under the standard assay conditions. The interference less than 5% was considered as practically no interference.

Storage stability of the LDHNPs/AuE

The storage stability of LDHNPs/Au electrode was investigated by measuring its response at lactate concentration (0.01 mM) in 0.1 M PBS for 7 weeks. The biosensor showed no change in redox peaks, current and potential during 5 weeks. However, it showed a drop in the value of the current without any change in redox peaks and potential during the last 3 weeks. Overall, biosensor lost 10% of its initial activity during 7 weeks of its regular uses, while being stored at 4°C.This storage stability is better than those of earlier biosensors, due to use of enzyme nanoparticles, which were immobilized directly onto Au electrode (Table 4).

Conclusion

The use of NPs of LDH in place of its native/free molecules in construction of a lactate biosensor has resulted into its improved analytical performance in terms of rapid response, (2.5s), lower detection limit (0.01μM), wider working range (0.01μM to 55 mM) and prolonged storage stability (210 days) compared to earlier biosensors. Further, it has simplified the fabrication process of a nano-composite based enzyme electrode. Thus enzyme nanoparticles modified Au electrodes could also be used to improve the analytical performance of other biosensors [28]. The future research could also be focused on the development of the miniaturized lactate biosensing device, which can be used at bedside of the patients for real time monitoring of lactate [29-31].

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Table 1

Lactate added (mM)	Lactate found (mM)	% Recovery
-	0.80	-
0.5	1.282	98.61±0.1
1.0	1.779	98.87±0.8

Table 2

Lactate(mM)	Mean	CV (%)
Within assay (5)	49.4	1.38
40		
43		
45		
49		
50		
Between assay (5)	47.4	1.03
38		
42		
43		
46		
48		

Table 3

Age group	Healthy				Diseased			
	Male	Number of samples (n)	Female	Number of samples (n)	Male	Number of samples (n)	Female	Number of samples (n)
1-10	-	-	0.8±.05	3	-	-	20.5±.02	3
11-20	1.36±0.3	3	10.3±0.3	3	-	-	18.3±.02	3
21-30	1.26±.02	3	1.76±0.4	3	19±.04	3	17±.02	3
31-40	1.0±.03	3	1.7±.03	3	22.2±.05	3	20±.05	3
41-50	-	-	0.6±.04	3	21.2±.06	5	-	-

51-60	1.7±.04	3	-	-	23±.05	3	19.6±0.4	4

Table 4

Enzyme used	Support of immobilization	Methods for immobilization	Type of biosensor	Working potenti	Optimum pH	Optimum temp.	Response time (s)	Linearity (mM)	Detecti-on limit	Storage stabili	Sensitivity (µA	Reproducibility RSD %	Application in	References
LDH	MWCNT/MB	Covalent Binding	Amperometric	NR	7.0	32	7	0.1-10	100	21	3.46	NR	Blood	18
LDH	MBRS/SPCE	Covalent Binding	Amperometric	NR	6.5	37	NR	0.055-10	550	NR	NR	4.28	Serum	19
LDH	pTTCA/MWCNT/Au	Covalent Binding	Amperometric	NR	7.0	35	15	5-90	1000	25	10.6	NR	Commercial	20
LDH	Fe3O4/MWCNTs/GC	Covalent Binding	Amperometric	0.65	7.5	37	NR	0-0.3	0.3	NR		4.5	Human Seru	21
LDH	Fe3O4/MWCNT/LDH/NAD$^+$	Covalent Binding	Amperometric	0.65	7.5	37	NR	0.005–0.5	5	NR	7.67	4.7	Human Seru	21
LDH	NADH/LDH/NanoCeO2/GCE	Electrodeposition	Amperometric	0.3	7.4	35	4	0.2-2	50	12	571.19	2.8	Clinical Diag	22

LDH	ZnO/LDH/Au	Covalent Binding	Amperometric	NR	7	32	1	0.00000	0.004	7	571.19	NR	Blood	23
LDH	NB/MSA /CDTe/QDS	Adsorption	Fluorescence based	NR	7.4	22	900	0.005-10	50	NR	NR	10	Cancer Cells	24
LDH	LDH/ DP/ TTH	Adsorption	Amperometric	0.15	7.3	35	NR	0.000014-0.005	NR	9	0.044	4.7	Beer Samples	25
LDH	LDH/GrONPs /PGE	Covalent Binding	Amperometric	0.7	7.3	35	5	5-50	0.1	60	NR	5.04	Wine, Beer, Curd, Milk	26
LDH	LDH-MB-polysulfone-composite film/SPE	Adsorption	Amperometric	NR	7.3	35	30	0.001-0.12	0.87	NR	80	2	Blood	27
LDHN	LDHNPs/Au	Covalent	Amperometri	0.10	7.0	30	16	0.01-55	.01	80	3.45	8.38	Cardiac	Present

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Figure: 5 Correlation between serum lactate values as measured by standard enzo kit method (x axis) and the present biosensor based on LDHNPs/Au electrode(y axis). (LDHNPs-lactate dehydrogenase nanoparticles)

Highlights

- Prepared and characterized lactate dehydrogenase nanoparticles (LDHNPs).
- Constructed an improved amperometric lactate biosensor employing LDHNPs onto Au electrode.
- The limit of detection and sensitivity of biosensor was 0.01μM and 3.45 ±0.02 μA cm^{-2} mM^{-1}.
- Biosensor was evaluated and applied for determination of serum lactate.
- LDHNPs/Au electrode lost only 10% of its initial activity after 210 days storage at 4°C.