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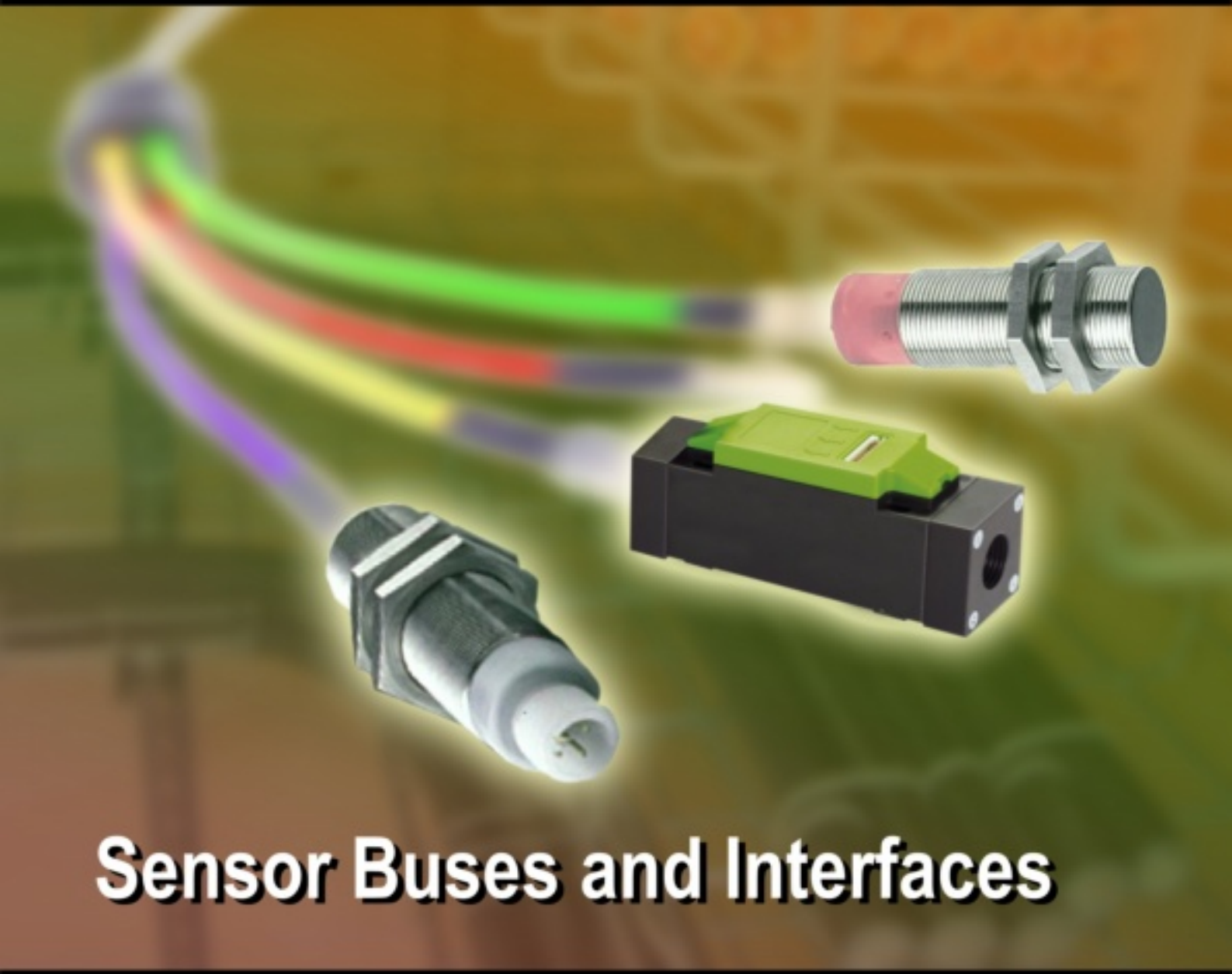

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Lactate Biosensor Based on Cellulose Acetate Membrane Bound Lactate Oxidase

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Abstract: Lactate biosensor was fabricated by immobilizing lactate oxidase in cellulose acetate membrane and by mounting over the sensing part of Pt electrode (working) and connected to Ag/AgCl electrode (reference) along with auxillary electrode through potentiostat. The enzyme electrode was anodically polarized at +400mV to generate electrons from H_2O_2, which was formed from oxidation of serum lactate by immobilized lactate oxidase. The minimum detection limit of the electrode was 0.1mmoles/L and sensitivity of the sensor was 0.008mA/mM/L lactate. Assay coefficients of variation were <2% .A good correlation (r=0.99) was found between lactate values obtained by colorimetric method and lactate biosensor. The self-life of the biosensor was 18 days at 4ºC and enzyme electrode can be re-used 150 times without any significant loss in enzyme activity. *Copyright © 2007 IFSA.*

Keywords: Lactate biosensor, Lactate oxidase, CA membrane, Amperometric biosensor

1. Introduction

Lactate, a metabolite, is formed from pyruvate in muscles and liver due to inadequate supply of oxygen. Normal range of lactate in blood is 0.5-2.5 mM. The determination of lactate in serum is essential in the diagnosis and medical management of various diseases such as tissue hypoxia, circulatory failure and hematologic disorder [1]. The pattern of change or the trend towards an increase of blood lactate is a sensitive indicator of survival [2]. Lactate level of serum indicates oxygenation state of tissues, warning of ischemic condition. Lactate sensor is generally employed in critical cases, during surgical operation and intensive therapy. Lactate sensors find application in sports, medicine and spatial medicine [3].

Recently, amperometric lactate determination has been exploited by constructing enzyme electrodes. Amperometric enzyme electrodes are biosensors that measure the change in concentration of a redox-active reactant or product via an analyte specific enzyme reaction [4]. Biosensors are simple, sensitive, rapid and read the sample directly. The importance of electrochemical biosensors has increased considerably during the past decade, as it combine the specificity of the biological systems with the advantage of electrochemical transduction. More recently, different immobilized enzyme biosensors have been described for lactate monitoring. To develop such biosensors cytochrome b_2 [5], lactate monooxygenase (LMO) [6], lactate oxidase (LOD) [7] have been immobilized on a poly-o-phenylenediamine film [8, 9]. Bi-enzyme electrode using co-immobilized lactate oxidase (LOD) and lactate dehydrogenase (LDH) onto polyaniline (PANI) films [10] and cytochrome b_2 /LDH [11], soybean peroxidase (SBP)/LOD [12], glutamate pyruvate transaminase (GPT)/LDH [13], peroxidase and lactate oxidase (HRP/LOD) [14] have also been developed.

Though lactate dehydrogenase is highly selective in many cases, as oxygen is not involved in the reaction, yet the difficulty in oxidation of coenzyme (NADH or NADPH) poses a problem, as an additional parameter to be optimized. Therefore, LOD was preferred over LDH due to its simple reaction, which involves aerobic oxidation of lactic acid into pyruvate and H_2O_2. The electrochemical biosensor based on LOD operates according to the following reactions:

$$\text{Lactic acid} + O_2 \xrightarrow{\text{Lactate oxidase}} \text{Pyruvate} + H_2O_2$$

$$H_2O_2 \longrightarrow 2H^+ + O_2 + 2e^-$$

The H_2O_2 thus generated, is measured at working electrode poised at 0.4V vs platinum electrode. Few lactate biosensors have been developed by immobilizing lactate oxidase onto poly-o-phenylenediamine [13], polycarbonate and cellulose acetate (CA) membrane [15], poly vinylalcohol (PVA) membrane [16] and nylon net [6]. However, CA membrane was demonstrated a better support for preparation of enzyme electrode based on electrochemical oxidation of H_2O_2 than PVC membrane e.g. oxalate oxidase, urate oxidase and lactate oxidase based enzyme electrode [17]. Wandrup *et al* immobilized LOD onto membrane laminate of polycarbonate and CA membrane and employed it for semiautomatic lactate analyzer [15]. However, they had neither studied kinetic properties of CA membrane bound LOD. Lactate oxidase has also been immobilized onto various conducting polymer [18] for the preparation of biosensor. But all these electrodes suffer from one or other drawbacks such as less storage stability, poor electrical response, less processability. Therefore, the present work was aimed to immobilize LOD onto CA membrane and develop an amperometric lactate biosensor for estimation of serum lactate.

2. Materials and Method

2.1. Chemicals

Lactate oxidase (39 units/mg, from *Pediococcus species*), L(+) lactic acid, glutaraldehyde (25% solution) from Sigma Chemical Co, USA. Cellulose acetate (CA) from Fluka. The interferants such as urea, uric acid, glycine, succinic acid, sodium dithionite, L- ascorbic acid and 8-hydroxyquinoline were used from SISCO Research Laboratory Pvt. Ltd. India. All other chemicals were of analytical reagent grade. Amperometric measurements were conducted with a Potentiostat(Model PC 4/ 750) was from Gamry Instrumentation, USA.

2.2. Preparation of lactic acid solution and reaction cocktail

Lactic acid was used as a substrate for lactate oxidase. Solutions of different concentration of lactic acid ranging from 0.1mM to 6mM were prepared and stored at 4ºC until use. The reaction cocktail was

prepared, as described by Lockridge et al [19] and consisted of 200mM dimethylglutaric acid (DMGA) (2.0ml), horseradish peroxidase (50U/1.0ml), 10 mM lactic acid (1.0ml) and deionized water (3.0ml). The reaction cocktail was stored in amber colored bottle at 4ºC. The reaction cocktail older than a week was discarded.

2.3. Assay of free lactate oxidase

The assay of native/ free LOD was carried out, as described by Lockridge et al [19]. The reaction mixture contained 0.8 ml reaction cocktail, 0.2 ml dimethylaniline (0.2% DMA). The contents were mixed well and equilibrated at 37ºC for 2 min followed by addition of 0.02ml of dissolved enzyme. The contents were mixed, preincubated at 37ºC for 2 min and 2.0ml of dodecylbenzene sulphonic acid (0.25%DBS) was added to the reaction mixture. An absorbance was recorded at 565nm (A_{565}) against control and the content of H_2O_2 generated in the reaction was calculated from standard curve between A_{565} vs. H_2O_2 concentration.

2.4. Preparation of CA membrane

Cellulose acetate membrane was prepared by the method of Reddy [17] with slight modification. A polymer solution was prepared by dissolving 0.2g of cellulose acetate in 10ml acetone. This polymer solution was poured on a glass petri-dish and then rotated manually on a horizontal flat surface to give an even distribution of polymer solution over glass. The dish was covered with its lid for slow, controlled evaporation of solvent and to create a membrane of even thickness.

2.5. Immobilization of lactate oxidase on CA membrane

Commercially available lactate oxidase was co-immobilized with BSA on cellulose acetate membrane through glutaraldehyde coupling by the method of Reddy et al [17] with modification: Lactate oxidase (3mg; 0.85U/mg) and BSA (50 mg) were dissolved in 250µl distilled water. 25µl of enzyme solution and 12.5µl of 2.5% (w/v) of glutaraldehyde in distilled water were mixed rapidly and placed on a 4cm^2 portion of cellulose membrane (inner membrane). A further 4cm^2 portion of cellulose acetate membrane (outer membrane) was then placed on enzyme membrane and two glass slides were used to compress both the membrane under mild finger pressure for approximately 5 min. The resulting laminate was then washed with buffer solution (0.05M sodium phosphate buffer, pH 7.0) to remove excess glutaraldehyde.

2.6. Preparation of enzyme electrode and response measurement

The membrane laminate construction of lactate oxidase was mounted over the sensing part of Pt (working) electrode with the help of paraffin tape. The enzyme electrode was connected to Ag/AgCl (Reference) electrode and an auxillary electrode (Ag) through a potentiostat. The working (enzyme) electrode containing CA membrane bound lactate oxidase , reference (Ag/AgCl) and auxillary electrode were immersed in a beaker containing 0.9ml sodium phosphate buffer (0.05M, pH 6.0) kept in a water bath maintained at 25°C. As soon as the lactate (0.1ml,1mM) was introduced into the buffer, it was converted into pyruvate and H_2O_2 by LOD immobilized onto CA membrane. The electrons thus generated from H_2O_2 were detected at the applied potential utilizing membrane, bound lactate oxidase. The flow of electron i.e. current was measured in mA by potentiostat. The enzyme electrode was washed with sodium phosphate buffer (0.05M, pH 6.0) and stored at 4°C when not in use.

2.7. Standardization of optimal conditions for working of enzyme electrode

2.7.1. Effect of pH

The optimal pH of the biosensor was determined by varying pH 5.5 - 7.5 using 0.05M sodium succinate

buffer (pH 5.5 - 6.0) and 0.05 M sodium phosphate buffer (pH 6.0 -7.5).

2.7.2. Effect of temperature

The optimum temperature for biosensor response was determined by incubating the reaction mixture at different temperature ranging from 20- 40°C.

2.7.3. Response time

The response time was determined at maximum current and was measured upto 2 min at an interval of 10 sec.

2.7.4. Effect of substrate concentration

The effect of substrate concentrations on membrane bound lactate oxoidase was determined at varying concentrations of lactate from 0.01- 3.0 mM in reaction mixture.

2.8. Preparation of standard curve of lactate by enzyme electrode

The assay of CA membrane bound lactate oxidase was carried out under optimal assay conditions at different concentrations of lactate.

2.9. Determination of lactate in serum

Blood samples (1ml each) from the 25 male and 25 female patients suffering from various diseases (tissue hypoxia, circulatory failure and hematological disorder) and apparently healthy persons were collected from the local PGIMS hospital and centrifuged at 5000 rpm for 5min and their supernatant (serum) was collected. Lactate content was determined in these serum samples by LOD/CA biosensor.

2.10. Effect of Interfering substances

The effect of various possible interfering substances found in blood such as urea, uric acid, glycine, succinic acid, sodium dithionite, L-ascorbic acid and 8-hydroxyquinoline were tested at their physiological concentrations.

2.11. Reusability and storage

The enzyme electrode was washed several times in 0.05M sodium phosphate buffer, pH 6.0. and stored at 4°C when not in use.

2.12. Criteria for evaluation of method of serum lactate determination

2.12.1. Linearity and detection limit

In order to check the linearity of the method, the amperometric measurement was made at varying lactate concentrations from 0.01-3.0mM. Minimum detection limit of the method was considered the concentration of lactate at which the current was 0.01mA.

2.12.2. Percent recovery, Precision and Accuracy

To determine the reliability of the method, 0.1 ml lactate (10mM and 50mM) was added to serum samples and the lactate content was determined before and after addition of lactate. The percent recovery

of added lactate was calculated. To work out the reproducibility of the method, the lactate content was determined in five samples repeatedly on the same day (within batch) and then in the same samples after their storage at –20°C for one week (between batch). The within and between day coefficient of variation (CV) was calculated for serum lactate determination.

In order to determine the accuracy of the method, the lactate in serum samples was determined by colorimetric method of Baker and Summerson with modification [20] (x) and by the present method (y). Lactate values obtained by both the methods were correlated using the regression equation.

3. Results and Discussion

Lactate oxidase purified from *Pediococcus species* was co-immobilized with BSA in between two CA membranes through glultaraldehyde. A procedure is described for construction of a lactate biosensor (enzyme electrode) based on this CA membrane bound lactate oxidase. The electrode measures lactate amperiometrically. The optimal working conditions of this present electrode were as follows:

3.1. Optimum pH

An optimal electrode response was observed at pH 6.0, which is lower to that of free enzyme pH-7.5 (data not shown). A decrease in pH optima of lactate oxidase after immobilization on PANI film has also been reported [10].The decrease in optimum pH of LOD after immobilization on CA membrane could be due to loss of $–NH_2$ groups on the surface of enzyme as result of glutaraldehyde coupling.

3.2. Incubation temperature for maximum activity and response time

Enzyme electrode showed maximum response at 25°C, which is lower than that of free enzyme (Table 1). The response time of the enzyme electrode was found about 50 s.

Table 1. A comparison of kinetic parameters of free and immobilized lactate oxidase on cellulose acetate (CA) membrane

Parameter	Free lactate oxidase	Lactate oxidase immobilized on CA membrane
Optimum pH	7.5	6.0
Optimum temperature (°C)	37	25
Thermal stability (%) (at 80°C for 15 min)	57	28
Time for linearity (s)	300	50
K_m for lactate	2.3×10^{-4}M	0.17×10^{-4}M
V_{max} (µmole/min)	0.11	0.04
Stability at 4°C during regular use	40% loss in 60 days	50% loss in 18 days

3.3. Determination of K_m

There was a hyperbolic relationship between lactate concentration and response of enzyme electrode upto 2.5mM. K_m value for lactate as calculated from Lineweaver-Burke plot was 0.29M and V_{max} 0.04μmole/min (Figure 1), which is lower than that for free enzyme (2.8mM). The decrease in K_m of enzyme after immobilization indicates its increased affinity towards its substrate. The change in K_m could be due to change in its conformation after immobilization.

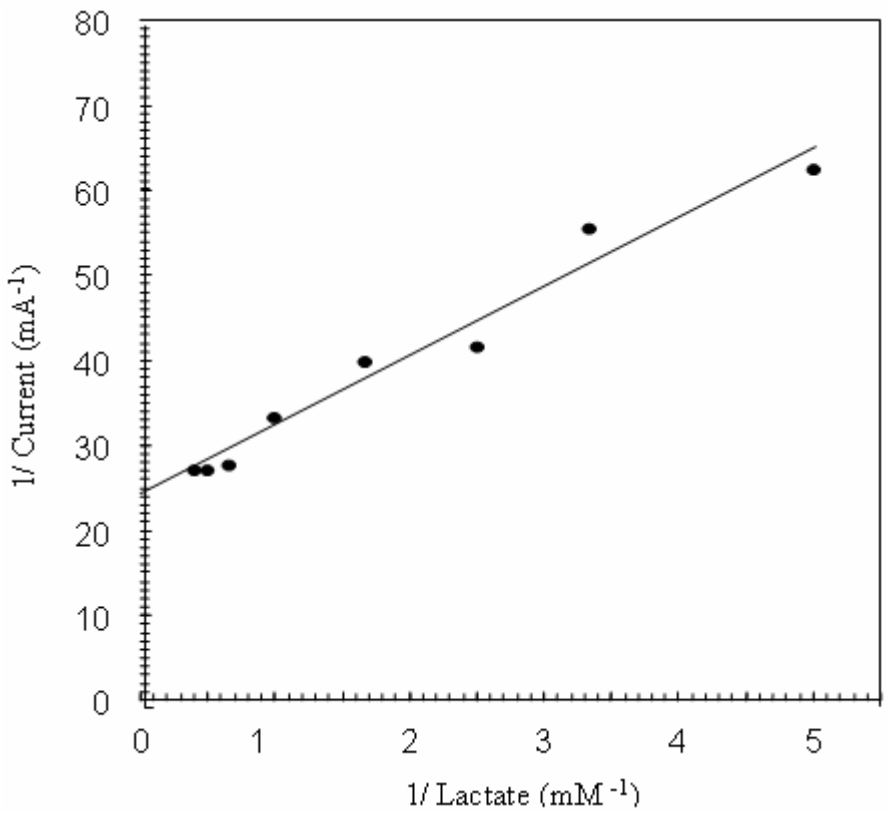

Fig. 1. Lineweaver-Burk plot for LOD/CA electrode at 0.4V.Standard assay conditions were used except for the lactic acid concentrations.

A new method for amperometric determination of serum lactate was developed employing the enzyme electrode under its optimal assay conditions. The method is based on generation of e^- from H_2O_2 produced by oxidation of serum lactate by CA membrane bound lactate oxidase. The flow of e^- i.e. current in mA was measured by potentiostat. The method has the advantage that it provides the instant measurement of lactate.

The following criteria were studied to evaluate the performance of the biosensor:

3.4. Linearity and detection limit

There was a linear relationship between lactic acid concentration ranging from 0.01mM to 0.6mM reaction mixture and current upto 28mA.(Figure 2).The minimum detection limit of the present method is 0.1mM, similar to that by bi-enzyme electrode (0.1mM) [10] but better than that needle type lactate biosensor (1mM) [9].

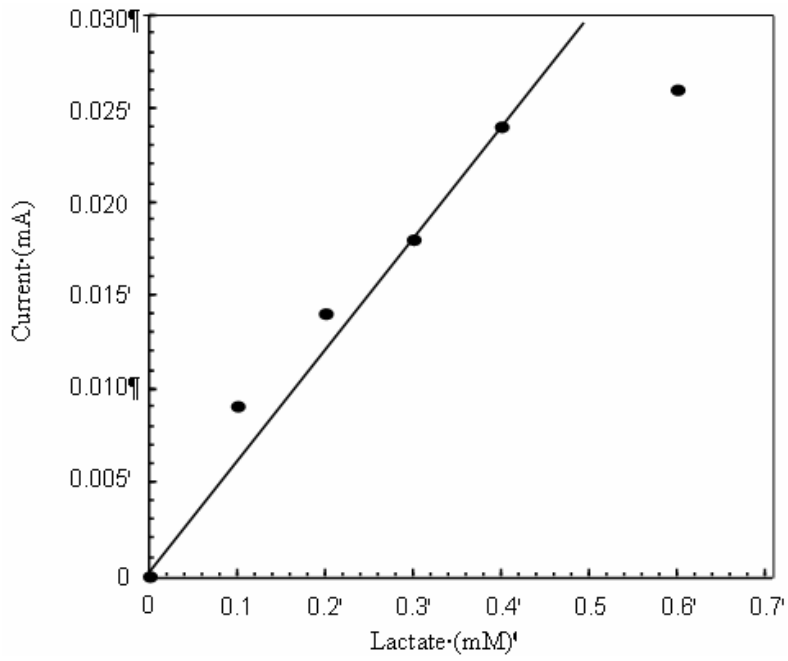

Fig. 2. Standard curve for lactic acid concentrations varying from 0.1mM to 0.6mM for LOD/CA biosensor. Standard assay conditions were used except for the lactic acid concentrations.

3.5. Recovery, Precision and Accuracy

The % recovery of added lactic acid in serum (10mM and 50mM) by the present method was 85.05 ±0.48% and 84.8 ±0.68% (mean + S.D., n=6) respectively. The within and between batch coefficient of variation (CV) for lactate determination in serum by the present method were <2% and <4% respectively, which is comparable to those employing direct amperometry method (4.2 % & 12% for added lactic acid Concentration 3.7mM and 0.5mM) [21].

To check the accuracy of the present method, lactic acid level in 25 serum samples as determined by the present method (y) was compared with those obtained by colorimetric method (x) of Barker and Summerson [20].The serum lactate values obtained by both the methods showed good correlation with r=0.99) (Figure 3), which is comparable with that reported earlier (r = 0.998) [21].

3.6. Interference study

The biosensor response to consecutive addition of different interfering compounds such as urea, uric acid, glycine, succinic acid, sodium dithionite,L- ascorbic acid and 8-hydroxyquinoline were studied at their physiological concentrations. Only 8-hydroxyquiniline and uric acid showed 31% and 20% decrease in biosensor response respectively, which is comparable with that of earlier report [9] and rest had no significant effect.

3.7 Lactate value

Serum lactic acid level in apparently healthy and diseased individuals (suffering from tissue hypoxia, circulatory failure and hematological disorder) as measured by present method ranged from 0.51- 2.9mM and 6.8 –15.2mM (n=25) respectively, which is in the established normal range 0.5-2.5 mM.

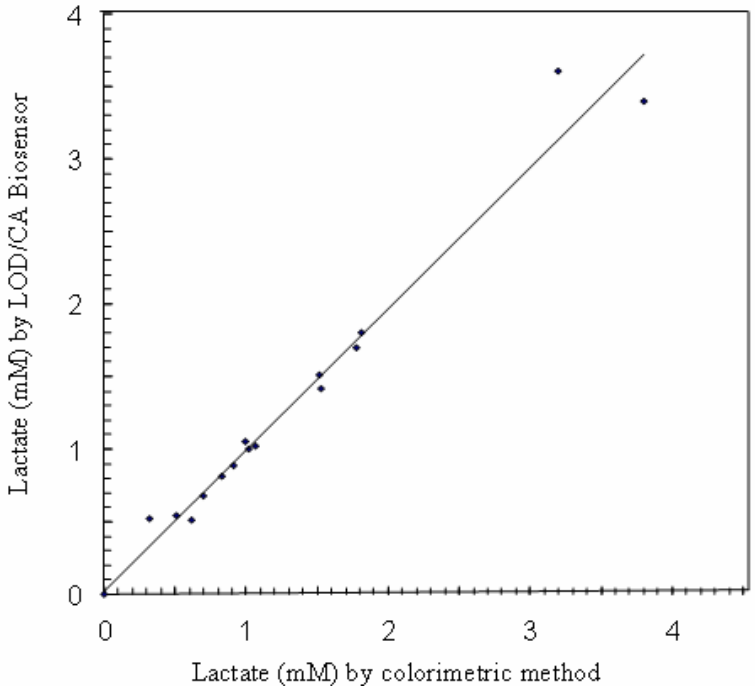

Fig. 3. Correlation between serum lactate values as determined by the modified method of Bakers and Summerson [20](x) and LOD/CA biosensor method (y). Regression eq; y=0.897x+ 0.070, r= 0.99).

3.8. Reusability and storage

The shelf life of biosensor was 18 days when stored in 0.05M, sodium phosphate buffer, pH 6.0 at 4ºC. During this storage period, the biosensor was reused about 150 times, revealing a good reusability of present lactate biosensor (Figure 4).

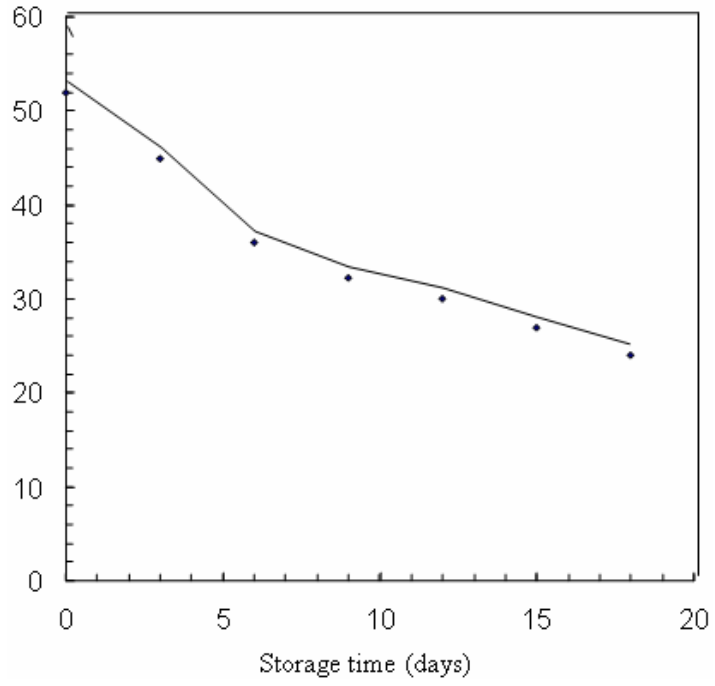

Fig. 4. Effect of storage time on the response of LOD/CA electrode at 0.4V in 0.05M sodium phosphate buffer, pH 6.0 at 4^0C.

4. Conclusions

Lactate biosensor was developed through potentiostat by attaching immobilized lactate oxidase on cellulose acetate membrane to platinum electrode for estimation of lactate in blood sample of patients. The determination of lactate in blood is essential for the diagnosis and medical management of various diseases such as tissue hypoxia, circulatory failure and hematologic disorder. The electrode was used for 150 times with shelf of 18 days without any considerable loss of activity, when stored at 4ºC. The response time of the lactate biosensor was 60 sec. It is fast, simple, sensitive and economical technique for determination of lactate in patients blood samples.

Acknowledgement

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References

[1]. E. P. Marbach, M. H. Weil, Rapid enzymatic measurement of blood lactate and pyruvate, *Clin Chem.,* 13, 1967, pp. 314-325.
[2]. B.N. Cowan, I. I. J. G. Burns, P. Boyle, I. M. Ledingham, The relative prognostic value of lactate and haemodynamic measurement in early shock, *Anaesthes*,39, 1984, pp. 750-755.
[3]. G. Volpe, D. Moscone, G. Palleschi, In vivo continuous monitoring of L-lactate coupling subcutaneous microdialysis and an electrochemical biocell, *Sensors and Actuators: B Chem.,* 24, 1995, pp. 138-141.
[4]. R. Singhal , W. Takashima, K. Kaneto, S. B. Samanta, S. Annapoorni, B. D. Malhotra, Langmuir, Blodgett films of poly(3-dodecyl thiophene) for application to glucose biosensor, *Sensor and Actuators :B Chem.,* 86, 2002, pp. 42-68.
[5]. A. Amine, J. Deni, J. M. Kauffmann, Amperometric biosensor based on carbon paste mixed with enzyme lipid and cytochrome C, *Bioelectrochem. Bioenerg*., 34, 1994, pp.123-128.
[6]. M. Mascini, D. Mosconc, G. Pallcschi, A lactate electrode with lactate oxidase immobilized on nylon net for blood serum serum samples in flow system, *Anal. Chim. Acta*, 157, 1984, pp.45-51.
[7]. D. Pfeiffer, B. Moller, N. Klimes, J. Szeponik , S. Fisher, Amperometric lactate oxidase catheter for real-time lactate monitoring based on thin film technology, *Biosens. Bioelectron*., 12, 1997, pp. 539-50.
[8]. F. Palmisano, D. Centonzc, P. G. Zambonin, An *in situ* electro-synthesized amperometric biosensor based on lactate oxidase immobilized in a poly-O- phenylenediamine film: determination of lactate in serum by flow injection analysis, *Biosens. Bioelect.*, 9, 1994, pp. 471-479.
[9]. Y. Quingling, P. Atanasov, E. Wilkins, Needle type lactate biosensor, *Biosen. Bioelect*., 14, 1999, pp. 203-210.
[10]. A. Chaubay, K. Krishan, M. K. Pandey , V. S. Singh, Signal amplification by substrate recycling on polyaniline /Lactate oxidase/ Lactate dehydrogenase bi-enzyme electrode, *Appl. Biochem. Biotech.,* 96, 2001, pp. 239-248.
[11]. F. Schubert, D. Kirstein, K. L. Schrober, F. W. Schellor, Enzyme electrode with substrate and coenzyme amplification, *Anal. Chim. Acta*, 169, 1985, pp. 391-396.
[12]. G. O. Kenausis, Q. Chen, A. Heller, Electrochemical glucose and lactate sensor based on "wired" thermostable soybean peroxidase operating continuously and stablised at 37^0C, *Anal. Chem.,* 69, 1997, pp. 1054-1060.
[13]. M. J. Lobo-Castannon, A. J. Miranda-Ordiercs, P. Lunon-Blanco, Abienzyme-poly-(o-phenylenediamine) -modified carbon paste electrode for the amperometric detection ofl-lactate, *Anal. Chim. Acta.,* 346, 1997, pp. 165-174.
[14]. Vikash, Harish, D. S. Ahlawat, Novel fabrication of CA membrane bound carbon electrode for bi-enzymatic determination of lactate, *Sensors & Transducers Journal*, 73, 2006, pp. 804-809.
[15]. J. Wandrup, K. Tvede, J. Grinsted, H. Jordening, Stat measurement of L-lactate in whole blood and cerebrospinal fluid assessed, *Clin. Chem.,* 35, 1989, pp. 1740-1743.

[16].K. Hajizadch, H. B. Halsall, W.R. Hoineman, Immobilization of lactate oxidase in a poly(vinylalcohol) matrix on platinized graphite electrodes by chemical cross-linking with isocyanate, *Talanta*, 38, 1991, pp. 37-47.
[17].S. M. Reddy, S. P. J. Higson., J. M. Christie, P. K. Vadgama, Selective membrane for the construction and optimization of an amperometric oxalate enzyme electrode, *Analyst*, 119, 1994, pp. 949-952.
[18].O. Lockridge, V. Massey, P. A. Sullivan, Mechanism of action of the flavoenzyme lactate oxidase, *J. Biol. Chem.*, 247, 1972, pp. 8097-8106.
[19].M. Trojanowicz, O. Goschke, T. Krawxynski, K. Cammann, Biosensors based on oxidases immobilized in various conducting polymers, *Sensors and Actuators: B Chem.*, 28, 1995, pp.191-199.
[20].Barker and Summerson, Enzymatic colorimetric method for lactate determination in biological materials, *J. Biol. Chem.*, 138, 1941, pp. 535-554.
[21].Q. Lining, N. D. Danielson, Determination of lactate or oxalate using injected lactate oxidase and peroxidase by capillary electrophoresis with UV detection, *Electrophoresis*, 24, 2003, pp. 2070-2075.

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- Virtual instruments;
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