Biosensors and Bioelectronics 86 (2016) 777–790

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

# Determination of lactic acid with special emphasis on biosensing methods: A review

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ARTICLE INFO

*Article history:*
Received 11 May 2016
Received in revised form
16 July 2016
Accepted 22 July 2016
Available online 25 July 2016

*Keywords:*
Lactic acid
Lactate determination
Lactate biosensor
Nanomaterials
Biological materials

ABSTRACT

Lactic acid (2-Hydroxypropanoic acid) is generated from pyruvic acid under anaerobic condition in skeletal muscles, brain, red blood cells, and kidney. Lactate in normal human subjects get cleared very quickly at a rate of 320 mmol/L/hr, mostly by liver metabolism and re-conversion of lactate back to pyruvate. Measurement of lactate level in serum is required for the differential diagnosis and medical management of hyperlactatemia, cardiac arrest and resuscitation, sepsis, reduced renal excretion, hypoxia induced cancer, decreased extra hepatic metabolism, intestinal infarction and lactic acidosis. Determination of lactate is also important in dairy products and beverages to access their quality. Among the various methods available for detection of lactate, most are complicated, nonspecific, less sensitive and require time-consuming sample pretreatment, expensive instrumental set-up and trained persons to operate, specifically for chromatographic methods. Biosensing methods overcome these drawbacks, as these are simple, fast, specific and highly sensitive. Lactate biosensors reported so far, work optimally within 3–180 s, between pH, 5.5–8.5 and temperature 22 °C to 37 °C and lactate concentration ranging from 10 to 2000 μM. These biosensors have been employed to measure lactate level in embryonic cell culture, beverages, urine, and serum samples and reused upto 200-times within a period of 7–216 days. This review presents the principles, merits and demerits of various analytical methods for lactate determination with special emphasis on lactate biosensors. The future perspective for improvement of analytic performance of lactate biosensors are discussed.



**Contents**



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http://dx.doi.org/10.1016/j.bios.2016.07.076
0956-5663/

## 1. Introduction

Lactic acid is the final product of glycolysis in many organisms under anaerobic conditions and considered as the dead end waste product (Gladden, 2004; Kristensen et al., 2005). Lactate is an active metabolite playing important role in muscle glycogen production (Cori and Cori, 1933). Lactate production occurs in all tissues, namely skeletal muscle, brain, red blood cells, and kidney under anaerobic conditions (Leverve et al.,1999). Lactate in normal human subjects clears very quickly at a rate of 320 mmol/L/h, mostly by liver metabolism and re-conversion of lactate back to pyruvate. This action keeps "basal" levels of lactate below one mmol/L in both arterial and venous blood (Bakker et al., 2013). In a normal steady state with adequate tissue resources and oxygenation, more cellular energy can be extracted aerobically by means of the citric acid cycle and the electron transport chain. In this case, cells convert pyruvate to acetyl CoA through oxidative decarboxylation (Brooks, 2002, Barrie et al., 2006). In humans, lactate exists in the levorotatory isoform. Tissue sources of lactate production include erythrocytes, perivenous hepatocytes, skeletal myocytes and skin. Basal lactate production is 0.8 mmol $kg^{-1}$ $h^{-1}$ (1300 mmol $day^{-1}$). The normal range of lactic acid in blood is 4.5–19.8 mg/dL (0.5–2.2 mmol/L) (Kompanje et al., 2007). Many variables measured in critically ill patients have been used to estimate severity of disease, prognosticate morbidity and mortality, evaluate costs of treatment, and finally indicate specific treatment and monitor the adequacy of treatment and its timing. Blood lactate concentrations $>5$ mmol $L^{-1}$ in patients with severe acidosis pH $<7.35$ or base deficit $>6.0$ carries a mortality of 80% (Cohen and Woods, 1983).

Determination of serum lactate is of great importance for the differential diagnosis and medical management of hyperlactatemia, cardiac arrest and resuscitation, sepsis, reduced renal excretion, chronic disease, decreased extra hepatic metabolism, intestinal infarction and lactate acidosis (Huckabee 1961; Liu et al., 2012). Blood lactate is used as fitness indicator, as it increases to 12 mM and 25 mM during exercise and extensive work respectively.. Lactic acid level is monitored in fermentation of dairy products to indicate their freshness, stability and quality. Lactate has been applied, as a food preservative. Lactate is monitored in lactic acid fermentation in wine industry, where malic acid is converted into lactic acid, which leads to lowering of overall acidity and tartness of wine. The blood lactate level is also used as a sign of stress in marine products/ fish cultivation industry (Rassaei et al., 2013).

The present review deals with different methods for determination of lactate with special emphasis on biosensing methods and also applications of nanomaterials for construction of improved lactate biosensors. Finally, it concludes with an outlook of the challenges and future opportunities of lactate biosensors.

## 2. Basic analytic methods of lactate determination

A variety of analytic methods are available for quantification of lactate such as colorimetric method (Barker et al., 1941), enzymatic spectrophotometric method (Marti et al., 1997), enzymic colourimetric method (Suman et al., 2005, White et al., 2009) enzymatic microassay (Lin et al., 1999), Enzymic fluorometric method (Olsen and Petersen,1971), voltammetric method (Schmitt et al., 2012), proton nuclear magnetic resonance method (Nishijima et al., 1997) and chromatographic methods (Yang et al., 1997). The principle of these methods are as follow:

### 2.1. Colourimetric method

The method was developed by Barker and Summerson (1941), in which lactic acid is converted into acetaldehyde by treatment with conc. $H_2SO_4$ and then acetaldehyde is determined by its color reaction with p-hydroxyphenyl in presence of cupric ions . The resulting blue color absorbs at 560 nm in a colourimeter in accordance to lactic acid concentration.

### 2.2. Enzymic spectrophotometric method

In this enzymic method, lactate is oxidized into pyruvic acid by lactate dehydrogenase(LDH) using $NAD^+$ as co substrate/co-enzyme, which is reduced to $NADH+H^+$. The reduced NADH absorbs at 340 nm, which is directly proportional to lactate concentration (Marbach and Weil, 1967).

### 2.3. Enzymic colourimetric method

The method is based on measurement of $H_2O_2$ generated from aerobic oxidation of lactate by lactate oxidase(LOx) using a color reaction consisting of 4-aminophenazone and phenol and peroxidase as chromogen. The resulting dye (pink colored) absorbs at 520 nm, which is directly proportional to the amount of lactate (Suman et al., 2005).

### 2.4. Enzymic micro assay method

The biochemical reactions involved in the method are same as those in enzymic colourimetric method except that the absorbance change is quantified by a microlitre plate reader and optimum assay conditions are developed like enzyme concentration, reaction time, optimum pH, sample volume (Lin et al., 1999).

### 2.5. Enzymic fluorometric method

The method is based on LDH catalyzed oxidation of lactate with concomitant generation of NADH from $NAD^+$. The changes in the concentration of NADH are measured by its native fluorescence. This fluorescence emission is directly proportional to lactate concentration and, is measured in fluorescence meter (Olsen and Petersen, 1971).

### 2.6. Voltammetrc method

In voltammetric method, a time dependent potential is applied to an electrochemical cell and the resulting current is measured as a function of that potential. The plot of current vs. applied

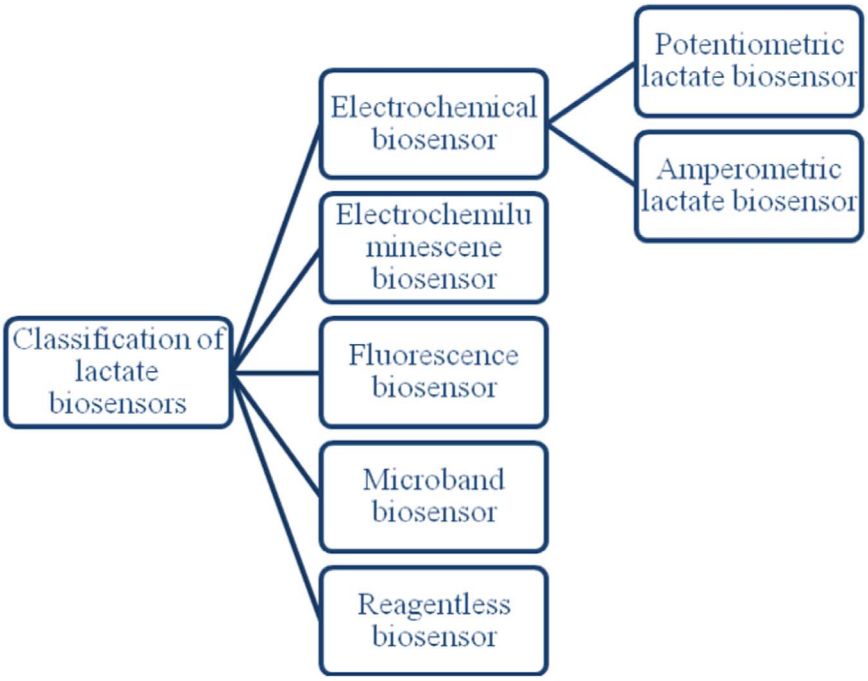



**Fig. 1.** Classification of lactate biosensors.

potential is called voltammogram. It provides the quantitative and qualitative information about the species involved in the oxidation and reduction method (Schmitt et al. 2012).

### 2.7. Proton nuclear magnetic resonance method

Proton nuclear magnetic resonance (H'NMR) spectroscopy method (an imaging method) is a method that makes use of proton double quantum nuclear magnetic resonance (NMR) to construct images based on selected metabolites such as lactate (Hurd and Freeman, 1989).

### 2.8. Chromatographic methods

#### 2.8.1. HPLC method

In HPLC, lactate is eluted through a strong cation exchange column (Aminox HPx87-H column), with dilute $H_2SO_4$ and monitored in UV spectrophotometer at 280 nm. The use of mobile phase at different pH allows the separation of lactate from other organic acids (Schneider et al., 1987).

#### 2.8.2. GC-MS method

In this method, lactic acid is converted into a volatile derivative, trimethyl silyl trifluoroacetamide (MSTFA) using silylating agent, N-methyl-N-trimethyl silyltrifluoroacetamide(TMS-Cl) followed by passing through a fused silica capillary column coated with HP-S cross linked 5%phenylmethyl silican using highly pure ethylacetate (Suh et al., 2003).

The merits and demerits of the above methods are summarized as follow:

**Merits**: Colourimetric method and voltammetric methods are simple and cheaper, while enzymatic methods and **c**hromatographic methods are specific, sensitive and reliable & accurate. Proton nuclear magnetic resonance method is fast, noninvasive and reproducible at moderate magnetic strength.

**Demerits**: Colourimetric method is not reliable, specific and sensitive, while enzymatic methods are costlier, cumbersome, and require time consuming sample preparation, expensive apparatus & trained person to operate, while chiral separation is not possible by simple chromatographic methods. Advanced chromatographic methods are sensitive but have low resolution. Further, these are too cumbersome for small volume samples, due to the multiple steps necessary for sample cleanup and derivatization. Proton nuclear magnetic resonance method does not provide absolute lactate concentration.

## 3. Biosensing methods/Biosensors

A biosensor is an integrated receptor- transducer device, which is capable of providing selective quantitative or semi-quantitative determination. According to recent IUPAC recommendations, "A biosensor is a self-contained integrated device, which is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is in direct spatial contact with a transducer element" (Turner et al., 1987). The biosensors are widely used in diagnostic, medical, quality control, agriculture industry, veterinary & dairy sciences, bacterial and viral diagnosis, drug production, control of industrial waste water, mining, military science and defense (Sadana et al., 2006, Schurig, 2001). The amperometric biosensors combine the advantage of the electrochemical techniques with high substrate specificity of the enzyme, quick response time and ease of procedure. Recently, analytic performance of biosensors has been improved using nanomaterials. Nanostructure of metal oxides is known to have unique ability to promote faster electron transfer kinetics between electrode and the active site of the desired enzyme (Wang, 2005; Jianrong et al., 2004). In recent years, with the development of nanotechnology, a lot of novel nanomaterials have been fabricated and their novel properties are being gradually discovered and their applications have also advanced/improved biosensors (Dzyadevych et al., 2008; Lata et al., 2012). During the last decades great attention has been paid to various biosensors for detection of lactate level in different biological materials. (Fig. 1).

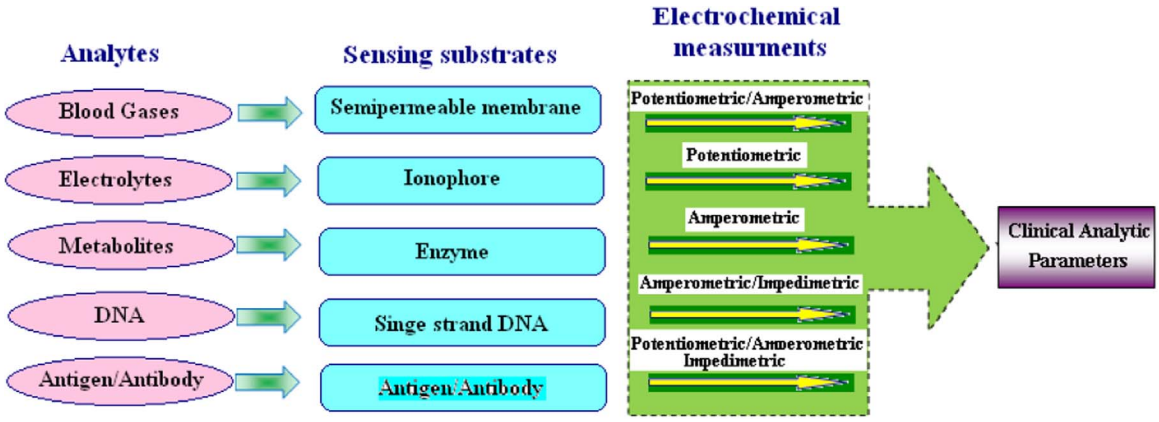



**Fig. 2.** Structure of various types of electrochemical biosensors (Turner et al., 1987).

*3.1.* ***Classification of lactate biosensor*** *The lactate biosensors can be classified as follow*

*3.1.1. Electrochemical biosensors*

Electrochemical biosensors are one of the most sensitive devices. Various strategies were developed to amplify the electrochemical signals. Electrochemical sensors are based upon potentiometric, amperometric, or conductivity measurements (Grieshaber et al., 2008). The different principles always require a specific design of the electrochemical cell. The structure of various types of electrochemical biosensors is given in Fig. 2. Their operating and measurement principles are summarized in the next sections according to their types.

**Merits:** Electrochemical biosensors are amenable to miniaturization and have compatible.

instrumental sensitivity and even operate in turbid media.

**Demerits:** Poor coupling of biochemical recognition materials and electrochemical transducers, affect selectivity, sensitivity, dynamic range and stability of electrochemical biosensors.

These electrochemical lactate biosensors can be classified further as follow:

*3.1.1.1. Potentiometric lactate biosensor:.* Potentiometric biosensors measure the accumulation of a charge potential at the working electrode compared to the reference electrode in an electrochemical cell, when zero or no significant current flows between them. Electrochemical Field Effect Transistor (ElecFET) devices are of interest for the detection of different molecules in solution. ElecFET are electrochemical microsensors in liquid phase and, based on two elements: (i) a pH-sensitive chemical field effect transistor (pH-ChemFET) and (ii) a metallic microelectrode deposited around the sensitive gate. The co-existence of these two elements combines (i) potentiometric and (ii) amperometric detection effects at the microscale. Design, fabrication and experimental validation of ElecFETs based on silicon and polymer microtechnologies, were reported. The ElecFET concept was extended to the detection of glucose and lactate in the concentration range 1–30 mM and 1–6 mM respectively. The sensitivities were between 2–6 mV/mM and 8–20 mV/mM respectively (Diallo et al., 2013).

Nanostructuring surfaces in order to improve the quality of determinations, in terms of detection limit and signal-to-noise ratio, had received a great attention during the past years. Based on a nanostructured (ND) $Si_4N_3$ surface, a potentiometric bionanosensor for determination of lactate has been reported. The potentiometric sensor employed an electrolyte–membrane–insulator–semiconductor (EMIS). The surface was first modified by a polyacrylic acid (PAA) layer, deposited by plasma enhanced chemical vapor deposition (PECVD), which was covalently linked to -$NH_2$ groups of the lactate dehydrogenase. Secondly, the nanostructures were formed on the surface by colloidal lithography. The sensitivity of the biosensor in aqueous media was 49.7 mV per decade. The detection limit (LOD) for determination of lactate was $2 \times 10^{-7}$ M, with a linear range up to $10^{-5}$ M. The intra- and inter-electrode standard deviations were about 2.4% and 11%, respectively. Thus nanostructuring led to signal amplification (Lupu et al., 2007).

**Merits:** These biosensors have the advantage of relative simplicity, due to its well established gas-sensing electrode technologies.

**Demerits:** These biosensors suffer from interference by endogenous $NH_4^+$ in blood but more significantly in urine specimens, low detection limits in biofluids and poor stability of the enzyme.

*3.1.1.2. Amperometric lactate biosensors:.* Amperometry is a method of electrochemical analysis in which the signal of interest is current that is linearly dependent upon the concentration of the analyte. Amperometry is a specific electrochemical technique taking advantage of the fact that certain electroactive species are oxidized or reduced (redox reactions) at inert metal electrodes driven at a constant applied potential (Ding et al., 2008, Perra et al., 2006). As certain electroactive species are oxidized or reduced (redox reactions) at inert metal electrodes, electrons are transferred from the analyte to the working electrode or to the analyte from the electrode. Amperometric biosensors also have the advantages of being more sensitive, rapid, inexpensive and disposable as compared to conductometric and potentiometric biosensors (Chaubey and Malhotra, 2002; Dzyadevyeh et al., 2002).

Amperometric lactate biosensors can be classified further depending upon the type of lactate utilizing enzymes i.e. Lactate oxidase(LOx) and lactate dehydrogenase(LDH).

a. **LOx based amperometric biosensors:**
   In these lactate biosensors, LOx was preferred due to its simple reaction and easy configuration of biosensor. LOx catalyzes the aerobic oxidation of lactate to pyruvate. Enzyme can be re-oxidised in the presence of dissolved $O_2$, releasing $H_2O_2$. This last product is oxidized at the electrode surface restoring the former concentration of $O_2$ and giving a current proportional to the amount of dissolved lactate (Taurino et al., 2013). Chemical and electrochemical reactions are as follows:

$$\text{L-lactate} + \text{LOx} \rightarrow \text{pyruvate} + \text{LOx}_{red}$$

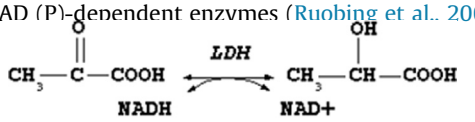
$$\text{LOx}_{red} + \text{O}_2 \rightarrow \text{LOx}_{ox} + \text{H}_2\text{O}_2$$

$H_2O_2 \rightarrow O_2 + 2\,h^+ + 2e^-$

A number of LOx based biosensors have been reported, in which LOx has been immobilized onto various supports/matrices e.g. polyamide/collagen membrane (Marquette and Blum, 1999), diethyl amino ethyl (DEAE-dextran)/ carbon electrode (CE) (Gavalas and Chaniotakis, 2000), polypyrrole (PPYox)/Platinum (Pt) (Palmisano et al., 2000a, 2000b), prussian blue/ Nafion membrane (PB/NFmembrane) (Garjonyte et al., 2001), horse radish peroxidase (HRP)/ ferrocene/ carbon paste electrode HRP/FcH/CPE) (Zaydan et al., 2004), polyaniline(PANI)-fluroaniline/indium tin oxide(ITO) plate (Suman et al., 2005), carbon nanotubes (CCNT)/Si/ITO (Weber et al., 2006), prussian blue/ Nafion membrane (PB/NFmembrane) (Lowinsohn and Bertotti, 2007), silicon sol-gel/ multiwall carbon nanotubes/ glassy carbon (Sisol/c-MWCNT/GC) (Huanga et al., 2007), cMWCNT/ platinum nanoparticles/ glassy carbon electrode (MWCNT/PtNPs/GCE) (Huang et al., 2008), albumin-mucin hydrogel/polycarbonate membrane (Romero et al., 2008), microband/CoPC/SCPE (Rawson et al., 2009), laponite/ chitosan hydrogel/ glassy carbon electrode(Laponite/CHIT hydrogel/GC) (Zanini et al., 2011), three dimensional matrix/ dithiobis-N-succinimidyl propionate/gold electrode (3DOM/DTSP/AuE)(Gamero et al., 2012), mesoporous silica/ Nafion membrane/ cobalt phthalocyanine / screen printed carbon electrode (FSM8.0/NaF/CoPC/SPCE) (Shimomura et al., 2012), platinum nanoparticles-carbon nanofiber/ poly(diallyldimethylammonium chloride)/ screen printed carbon electrode (PtNP-CNF/PDDA/SPCE) (Ardisana et al., 2014), HRP/ organic field effect transistor(HRP/OFET) (Minmani et al., 2015), diamond nanoparticles/gold (DNPs/Au) (Briones et al., 2015), diamond nanoparticles(DNPs)/sol-gel matrix/gold electrode (DNPs/Sol-Gel/Au) (Briones et al., 2016), dimethylferrocene modified linear poly (ethylenimine) ($FcMe_2$-LPEI) hydrogel/ GCE (Hickey et al.,2016), polypyrrole 3 dimensional matrix ($PPY_{3DOM}$)/ HRP /Au (Gomez et. al., 2016), PB/SPCE (Jiang et al., 2016) and albumin/Au (Shkotova et al., 2016).

b. **LDH based amperometric biosensors**

LDH catalyzes the conversion of pyruvate to lactate and back, as it converts NADH to $NAD^+$ and back. LDH exists in four distinct enzyme forms. Each one acts on either D-lactate (D-lactate dehydrogenase (cytochrome)) or L-lactate (L-lactate dehydrogenase (cytochrome)). Two are cytochrome c-dependent enzymes and two are NAD (P)-dependent enzymes (Ruobing et al., 2000).




In LDH based biosensors, LDH has been immobilized onto different supports such as diapherase/microstructured membrane/Au (Tap et al., 2000), polyacrylicacid (PAA)/ nanostructured silicon nitrite surface ($NDSi_4N_3$) (Lupu et al., 2007), flavocytochrome $b_2$ ($FCb_2$)/CE (Smutok et al., 2005), Meldola's Blue (MB)/MWCNT (Pereira et al., 2007), Meldola's Blue- reinecke Salt (MBRS)/SPCE (Piano et al., 2010), poly-5,2′ − 5′,2″-terthiophene-3′carboxylic acid (pTTCA)/cMWCNT/AuE (Rahman et al., 2012), Iron nanoparticles ($Fe_3O_4$NPs)/cMWCNT/$NAD^+$/GCE (Teymourian et al., 2013), $NAD^+$/SPCE (Yoon and Kim, 2013), cytochrome C oxidoreductase (Cyto C)/graphite (Smutok et al., 2014), Nano ZnO/CHIT/Au (Nesakumar et al., 2014), reduced graphene oxide (RGO)/AuNPs/SPCE (Azzouzi et al., 2015), diaphorase(DP)/tetrathiafulvalene (TTH)(vargas et al., 2016), NB (Nile blue)/ mercapto- succnic acid (MSA)/ cadmium telleitum (CDTe)/ quantum dots (QDS) (Zhang et al., 2016), GrONPs/PGE (Batra et al., 2016).

**Merits:** Amperometric biosensors have good operational stability, long storage /lifetime, relatively short response time and high sensitivity. In addition, other interferences (e.g. ammonia) are not a problem and nearly all biosensors achieve the required analytical range.

**Demerits:** Poor coupling of biochemical recognition materials and electrochemical transducers, affect selectivity, sensitivity, dynamic range and stability of electrochemical biosensors.

*3.1.2. Electrochemiluminescence lactate biosensor*

Chemiluminescence transducers rely on the emission of light, as release of energy in a chemical reaction occurs due to an oxidation reaction in the presence of $O_2$ or $H_2O_2$ and without any excitation source. An electrochemiluminescent (ECL)-based lactate biosensor was developed with luminol as signaling species. Lactate was oxidized under the catalysis of immobilized LDH and pyruvate oxidase (PyOD) with $NAD^+$ as coenzyme. As a result, the reactions yield $H_2O_2$, which enhances the electrochemiluminescence of luminol, which sense the lactate concentration. This biosensor showed excellent response for lactate. A detection limit of $8.9 \times 10^{-12}$ mol/L was observed with the relative standard deviation (RSD) of 4.13% ($C_{LA}$ $1.34 \times 10^{-10}$ mol/L, $n=6$) and an average recovery of 101.3% for real sweat samples. The biosensor was practicable for detecting the lactate in sweat samples of athletes during the exercitation, for evaluation of exercise degree (Cai et al., 2010).

The electrochemiluminescence of luminol was also employed for glucose and lactate flow injection analysis. The electrochemiluminescence of luminol was generated using a GC electrode polarized at +425 mV vs. a Pt-pseudo reference electrode. After optimization of the reaction conditions and physicochemical parameters influencing the sensor response, the measurement of $H_2O_2$ was performed in the range 1.5 pmol to 30 nmol. Glucose oxidase or LOx, were immobilized on polyamide and collagen membranes. With collagen as the enzymatic support, the detection limits for glucose and lactate were 60 pmol and 30 pmol respectively, whereas with the enzymatic polyamide membranes, the corresponding values were 150 pmol and 60 pmol respectively. Good agreements were obtained between this method and reference methods. The coefficient of variation (CV) for 53 repeated measurements performed over a 10 h period was 4.8%, while for blood lactate analysis, 80 assays performed over a 15 h period, gave a CV of 6.7% (Marquette and Blum, 1999).

**Merits:**

Chemiluminescence methods offer advantages of simple instrumentation, low detection limits and wide dynamic range.

**Demerits:**

The method lacks selectivity and depend on the environmental conditions such as temperature and pH.

*3.1.3. Fluorescence based lactate biosensor*

Selective and sensitive determination of extracellular lactate is of fundamental significance for studying the metabolic alterations in tumor progression. The rational design and synthesis of a quantum-dot-hydrogel-based fluorescent probe for biosensing and bioimaging the extracellular lactate has been reported. By surface engineering, the destabilized Nile-Blue- functionalized quantum dot sol was not only self-assemble forming quantum dot hydrogel but also monitored lactate in the presence of $NAD^+$ and LDH through fluorescence resonance energy transfer. Notably, the surface engineered quantum dot hydrogel showed high selectivity toward lactate over common metal ions, amino acids and other small molecules that widely coexist in biological system. Moreover, the destabilized Nile-Blue-functionalized quantum dots could encapsulate isolated cancer cells, when self-assembled into a hydrogel and thus specifically detected and imaged the extracellular lactate metabolism. By virtue of these properties, the

functionalized quantum dot hydrogel was further successfully applied to monitor the effect of metabolic agents.

**Merits:** This method has opened up a facile way for exploration of spatial variation of metabolites with high sensitivity and selectivity. Benefiting from mild hydrogel formation and sol–gel switching, as well as porosity and biocompatibility, the Nile-Blue-functionalized CdTeQD hydrogel encapsulated individual cells and imaged their extracellular lactate metabolism.

**Demerits:** Disadvantages of fluorescence detection include the rapid photo bleaching of fluorescent organic dyes conjugated to the biomolecules of interest and the potential loss of biological activity of biomolecules upon chemical conjugation with a fluorescent dye.

### 3.1.4. *Microband lactate biosensor*

The screen-printed carbon microband electrodes(SPCEs) fabricated from water-based ink readily detected $H_2O_2$. The same ink, with the addition of lactate oxidase, was used to construct microband biosensors to measure lactate. These microband devices were fabricated by a simple cutting procedure using conventional sized SPCEs containing the electrocatalyst cobalt phthalocyanine (CoPC). These devices were characterized with $H_2O_2$ using several electrochemical techniques. Cyclic voltammograms were sigmoidal; a current density value of 4.2 mA $cm^{-2}$ was obtained. A scan rate study revealed that the mass transport mechanism was a mixture of radial and planar diffusion. However, a further amperometric study under quiescent and hydrodynamic conditions indicated that radial diffusion predominated. A chronoamperometric study indicated that steady-state currents were obtained with these devices for a variety of $H_2O_2$ concentrations and that the currents were proportional to the analyte concentration. Lactate microband biosensors were then fabricated by incorporating lactate oxidase into the water-based formulation prior to printing and then cutting as described. Voltammograms demonstrated that lactate oxidase did not compromise the integrity of the electrode for $H_2O_2$ detection. A potential of +400 mV was selected for a calibration study, which showed that lactate could be measured over a dynamic range of 1–10 mM, that was linear up to 6 mM; The limit of detection was 289 μM. This provided a platform for monitoring cell metabolism in-vitro by measuring lactate electrochemically via a microband biosensor (Rawson et al., 2009).

### 3.1.5. *Reagentless lactate biosensor*

A reagentless lactate biosensor was constructed based on an electro-polymerized copolymer film poly (5-hydroxy-1,4-naphthoquinone-*co*-5-hydroxy-3-acetic acid-1,4-naphthoquinone). The quinone group, as part of the polymer backbone, was electroactive and very stable in neutral aqueous medium. It therefore acted as an immobilized mediator for the enzyme recycling, at a working potential much lower than those commonly reported in the

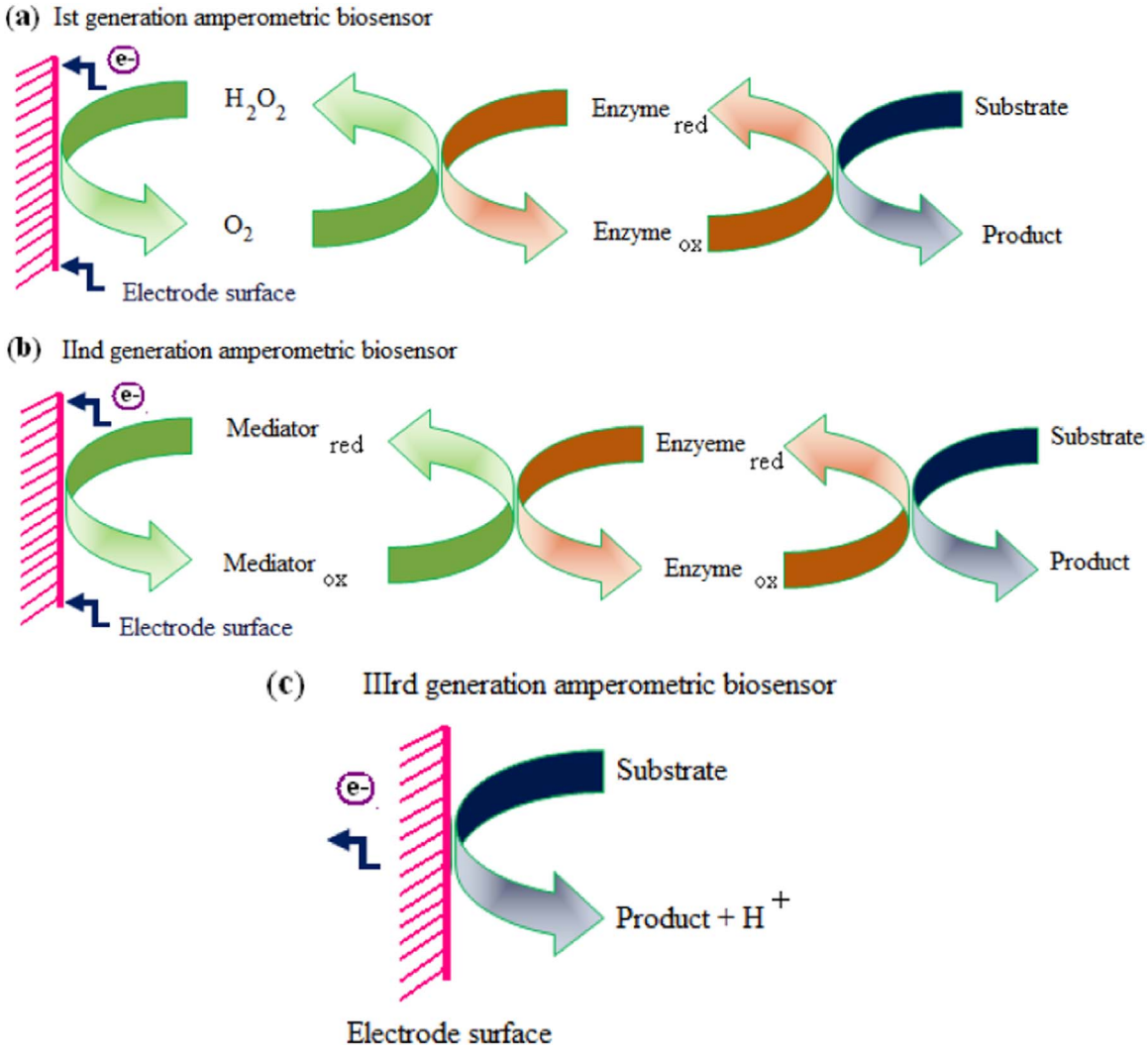



**Fig. 3.** Mechanism of electron transfer in various types of amperometric biosensing systems.

literature for other mediators. Experimental conditions for amperometric measurements (temperature, pH) were studied, especially the interference between quinone and molecular oxygen to investigate the enzyme/quinone recycling kinetic. Some well-known interferents were shown to have no measurable effect on the amperometric curves (Haccoun et al., 2010).

### 3.2. Classification of lactate biosensor on the basis of biological recognition transducer

#### 3.2.1. LOx based biological recognition transducer

These can be classified further based on type of electrode:

*3.2.1.1. Screen Printed Electrode (SPE):.* These are further divided on the basis of matrix used.

a) **Platinum nanoparticle/ carbon nanofiber/ PDDA matrix:**
A lactate biosensor based on PtNPs/ carbon nanofiber/ PDDA matrix was developed using screen-printed carbon electrodes (SPCE) and lactate oxidase (LOx). The sensitivity of the optimized lactate biosensor was 36.8 (mA/M $cm^2$) with a linear range of 25–1500 μM. The limit of detection was 11 μM ($S/N=3$). The immobilization of LOx on top of these PtNp-CNF-PDDA/SPCEs resulted into amperometric biosensors with high operational stability. Through this model, lactate in sweat and blood samples was measured in a sport test using LOx/PtNp-CNF-PDDA/SPCEs and commercial biosensors respectively. This disposable biosensor exhibited good reproducibility, selectivity, storage stability, sensitivity and low cost (Ardisana et al., 2014). (Fig. 3)

b) **Cobalt phthalocyanine/mesoporous silica/nafion layer matrix:**
A novel amperometric lactate biosensor based on cobalt phthalocyanine/mesoporous silica/nafion layer matrix/SPCE was designed. In this biosensor, the sampling unit attached to the SPCE required only a small sample volume of 100 μL for each measurement. The measurement of l-lactate was based on the signal produced by $H_2O_2$, the product of the enzymatic reaction. The sensor showed an optimum response at a pH of 7.4 and an applied potential of +450 mV. The determination range and the response time for L-lactate were 18.3 μM to 1.5 mM and approximately 90 s, respectively. In addition, the sensor exhibited high selectivity for L-lactate and was quite stable in storage, showing no noticeable change in its initial response after being stored for over 9 months. The method provided a simple, cost-effective, high-performance biosensor for L-lactate. Nafion layer on the electrode surface acted as a barrier to interferents (Shimomura et al., 2012). The scheme of biosensor is shown in Fig. 4

**Principle of operation:**

c) **Cobalt phthalocyanine/microband:**
The study demonstrated that screen-printed carbon microband electrodes fabricated from water-based ink can readily detect $H_2O_2$ and that the same ink, with the addition of lactate oxidase, can be used to construct microband biosensors to measure lactate. These microband devices were fabricated by a simple cutting procedure using conventional sized screen-printed carbon electrodes (SPCEs) containing the electrocatalyst cobalt phthalocyanine (CoPC). In this cyclic voltammograms were found to be sigmoidal; a current density value of 4.2 mA $cm^{-2}$ was obtained. A scan rate study revealed that the mass transport mechanism was a mixture of radial and planar diffusion. However, a further amperometric study under quiescent and hydrodynamic conditions indicated that radial diffusion predominated. A chronoamperometric study indicated that steady-state currents were obtained with these devices for a variety of $H_2O_2$ concentrations and that the currents were proportional to the analyte concentration. Lactate microband biosensors were then fabricated by incorporating lactate oxidase into the water-based formulation prior to printing and then cutting as described. Voltammograms demonstrated that LOx did not compromise the integrity of the electrode for $H_2O_2$ detection. A potential of +400 mV was selected for a calibration study, which showed

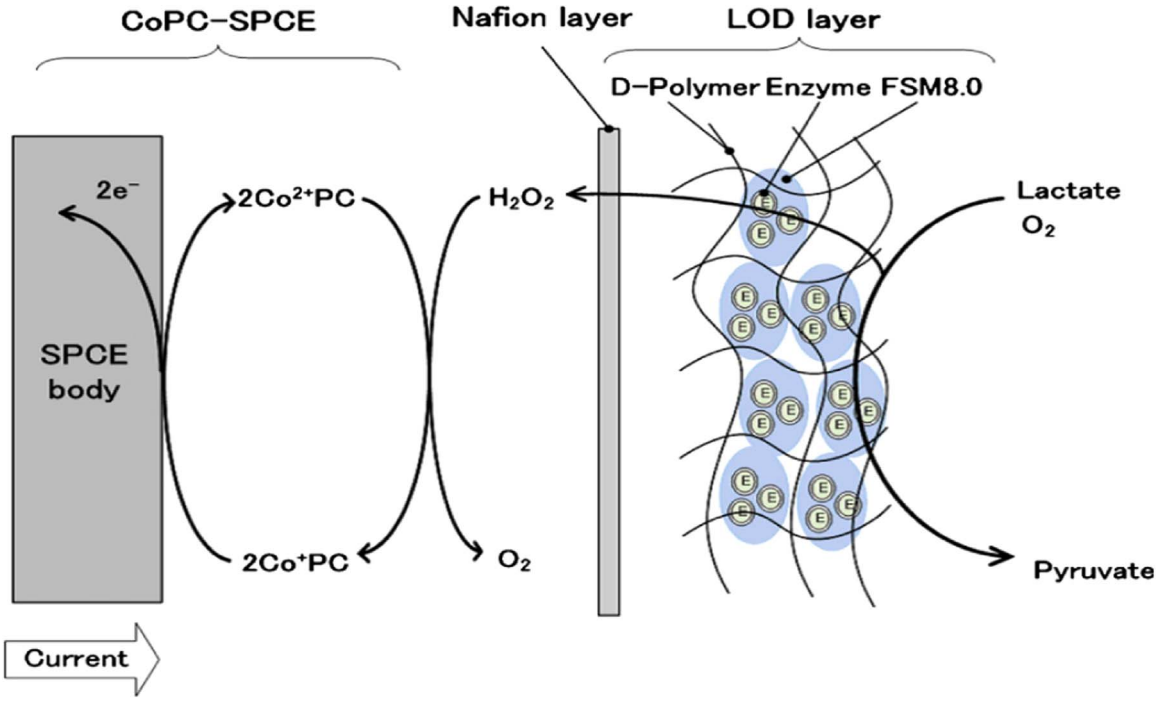



**Fig. 4.** Principle of operation for (LOD-FSM8.0/Naf/CoPC-SPCE) working electrode where enzymatic generation of $H_2O_2$ is oxidized on the CoPC-SPCE surface resulting in a flow of electrons to the working electrode. (Shimomura et al., 2012).

**Table 1**
Comparision of analytic characteristics of various lactate biosensors.

| Enzyme used | Support of immobilization | Methods for immobilization | Type of biosensor | Working potential (V) | Optimum pH | Optimum temp. (°C) | Response time (s) | Linearity (μM) | Detection limit (LOD) μM | Storage stability (Days) | Sensitivity (μA mp/ $mM/cm^2$ | Reproducibility RSD % | Application in | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LOx | PAA/$Si_4N_3$ (ND) Surface | Covalent | Potentiometric | 0.497 | 7.5 | 37 | NR | 0–10 | 0.2 | NR | NR | NR | Blood | Lupu, et al., 2007 |
| LOx | Zno/Au | Crosslinking | Potentiometric | 0.413 | 7.3 | 35 | 7 | 0–100 | 100 | NR | NR | NR | Drugs, Food and other biological samples | Ibupoto et al.2012 |
| LDH | LDH-MB-poly-sulfon e- compo-site film/SPE | Adsorption | Amperometric | NR | 7.3 | 35 | 30 | 1–120 | 0.87 | NR | 80 | 2 | Blood | Simon et al. 2007 |
| LDH | MWCT/MB | Covalent Binding | Amperometric | NR | 7.0 | 32 | 7 | 100–10,000 | 100 | 21 | 3.46 | NR | Blood | Pereira et al. 2007 |
| LDH | MBRS/SPCE | Covalent Binding | Amperometric | NR | 6.5 | 37 | NR | 55–10,000 | 550 | NR | NR | 4.28 | Serum | Piano et al. 2010 |
| LDH | pTTCA/M WCNT/ Au | Covalent Binding | Amperometric | NR | 7.0 | 35 | 15 | 5000 −90,000 | 1000 | 25 | 10.6 | NR | Commercial milk and blood samples | Rahman et al. 2012 |
| LDH | Fe3O4/MWCNTs/ GC | Covalent Binding | Amperometric | 0.65 | 7.5 | 37 | NR | 0–300 | 0.3 | NR |  | 4.5 | Human Serum Samples | Teymourian et al.,2012 |
| LDH | Fe3O4/MWCNT/ LDH/$NAD^+$ | Covalent Binding | Amperometric | 0.65 | 7.5 | 37 | NR | 50–500 | 5 | NR | 7.67 | 4.7 | Human Serum Samples | Teymourian et al.,2012 |
| LOx | AuR-LOx | Covalent Binding | Amperometric | 0.73 | 7 | 35 | NR | 0–1300 | 29.82 | NR | 1.32 | 4.2 | Medicine and Food Industry | Gamero et al., 2012 |
| LOx | AuR-DTSP-LOx | Covalent Binding | Amperometric | 0.73 | 7 | 35 | NR | 0–1200 | 21.50 | NR | 1.49 | 3.9 | Medicine and Food Industry | Gamero et al., 2012 |
| LOx | $Au_{3DOM}$-LOx | Covalent Binding | Amperometric | 0.73 | 7 | 35 | NR | 0–600 | 16.22 | NR | 0.79 | 3.9 | Medicine and Food Industry | Gamero et al., 2012 |
| LOx | $Au_{3DOM}$-DTSP-LOx | Covalent Binding | Amperometric | 0.73 | 7 | 35 | NR | 0–1300 | 3.93 | NR | 1.63 | 3.5 | Medicine and Food Industry | Gamero et al., 2012 |
| LDH | NADH/LDH/Nano-CeO2/GCE | Electrodeposition | Amperometric | 0.3 | 7.4 | 35 | 4 | 200–2000 | 50 | 12 | 571.19 | 2.8 | Clinical Diagnosis, Food quality analysis | Noel Nesakumar 2013 |
| LDH | MB/Sol- Gel/SPE | Sol-Gel entrapment | Amperometric | NR | 8.0 | 37 | 10 | 125–24800 | 260 | NR | NR | NR | Blood | Kwon et al. 2013 |
| LOx | LODP/HRP/Au | Crosslinking | Conductometric | NR | 7 | 37 | 180 | 0–210 | 0.05 | 20 | 1.16 | 1–8 | Dairy Products | Boisse et al., 2013 |
| LDH | ZnO/LDH/Au | Covalent Binding | Amperometric | NR | 7 | 32 | 1 | 0.2–0.8 | 0.004 | 7 | 571.19 | NR | Blood | Nesakumar et al., 2013 |
| LOx | LOx/PtNp-CNF-PDDA /SPCEs | Covalent Binding | Amperometric | 0.5 | 7 | 35 | 60 | 25–1500 | 11 | 192 | 0.0368 | 5.3 | Sweat | Pedro J. et all. 2014 |
| LOx | Cell debris/Os-mium complex/ Graphite electrode | Covalent Binding | Amperometric | NR | 7.8 | 24 | 5 | NR | 3.1 | 10 | 460 | NR | Food Technology | . Smutoket al, 2014 |
| LOx | GCE/ nanoZnOMWCNTs | Covalent Binding | Amperometric | NR | 8.5 | 35 | NR | 10,000 to 10,000,000 | 0.004 | NR | NR | 7.4 | Human Plasma | Haghighi et al., 2014 |
| LOx | (PtNps/GCNF–SPCEs) | Covalent Binding | Amperometric | 0.3 | 7 | 20 | 60 | 10–2000 | 6.9 | 216 | 413 | 4.9 | Wines and Ciders | Oscar A. Loaiza2015 |
| LDH in Lactobacillus delbruecki | PPy/Pt | Electropolymerisation | Amperometric | 0.2 | 5.5 | 35 | 3 | 100–1000 | 12 | 25 | – | 3.04 | Milk, Buffer, Kefir | Canbay et al. 2015 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LDH | RGo-AuNPs | Sol-Gel Entrapment | Amperometric | 0.48 | 7.5 | 35 | 8 | 10–5000 | 0.13 | 25 | 154 | 1.67 | Tumor Biomarker | Sawsen et al 2015 |
| LOx | DNPs/Au | Covalent Binding | Amperometric | 0.2 | 7 | 35 | 5 | 50–700 | 15 | 15 | 4.0 | 6 | Blood and Sera | Briones et al. 2015 |
| LOx | ($FcMe_2$-LPEI) Hydrogel GCE | Crosslinking | Amperometric | 0.3 | 7.5 | 37 | 5 | 0–5000 | 3 | 21 | 400 | – | Beverages/ Soft drinks | Hickey2016 |
| LOx | DNPs/Sol-Gel matrix/Au | Sol-Gel Entrapment | Amperometric | 0.27 | 7 | 35 | NR | 53–1600 | 16 | 15 | 2.6 | 7 | Wine Samples | Briones et al. 2016 |
| LDH | LDH/ DP/ TTH | Adsorption | Amperometric | 0.15 | 7.3 | 35 | NR | 1.4–55 | NR | 9 | 0.044 | 4.7 | Beer Samples | Vargas et al., 2016 |
| LOx | $PPY_{3DOM}$/HRP/ LOx/Au | Entrapment | Amperometric | 0.075 | 7.3 | 30 | 100 | 1–100 | 0.52 | 40 | 13.5 | 4 | Monitoring of monolactic fermentation in wine | Gomez et. al., 2016 |
| LOx | PB/SPCE | Adsorption | Amperometric | 0.45 | 6.5 | 45 | NR | 20–150 | NR | NR | 4.54 | NR | Blood | Jiang et al., 2016 |
| LOx | Albumin/Au | Entrapment | Amperometric | 1 | 7.2 | 35 | 5 | 5–800 | 5 | NR | NR | NR | Wine | Shkotova et al., 2016 |
| LOx | Lox/Peroxidase ALP/Al/PCM | Adsorption | Chemiluminescence based | NR | 8.8 | 35 | 5 | 50–400 | 9.2 | 22 | NR | 5.5 | Yogurt | Claver et al. 2008 |
| LDH | NB/MSA /CDTe/ QDS | Adsorption | Fluorescence based | | 7.4 | 22 | 900 s | 50–10,000 | 50 | NR | NR | 10 | Cancer Cells | Zhang et al. 2016 |

ABBREVIATIONS USED: AuNPS= Gold Nanoparticles; ALP=*Arthromyces ramosus*, *PCM*= polyion complex membrane; CHIT= Chitosan; CNT= Carbon Nanotubes; DNPs= Diamond Nanoparticles; DP= Diaphorase; $FcMe_2$-LPEI= Dimethylferrocene modified linear poly (ethylenimine); GCE= Glassy carbon electrode; LDH= Lactate dehydrogenase; LOx= Lactate Oxidase ; MBRS= Meldola's Blue –Reinecke salt; MPTS=3-(mercaptopropyl)-trimethoxysilane; MWCNT= Multiwalled carbon nanotubes; NAD= Nicotinamide adenine dinucleotide; NR= Not Reported ; PPY= Polypyrrole; Pt= Platinum; Pttca= Poly-5,2′−5′−2″-terthiophene-3′-carboxylic acid; SPCE= Screen printed carbon electrode; TTH= Tetrathiafulvalene; ZnO= Zinc Oxide; NB= Nile blue; CDTe= cadmium telleitum; QDS= Quantum dots; MSA=mercapto- succnic acid, PAA: Polyacrylic acid, $NDSi_4N_3$(Nanostructured silicon nitrite)

that lactate could be measured over a dynamic range of 1–10 mM, which was linear up to 6 mM, with a lower limit of detection of 289 μM. This provided a platform for monitoring cell metabolism in-vitro by measuring lactate electrochemically via a microband biosensor (Rawson et al., 2009).

d) **CHIT/CNT/SPCE**

L-lactate is an essential metabolite present in embryonic cell culture. Changes of this important metabolite during the growth of human embryo reflect the quality and viability of the embryo. Ibanez et al., (2016) designed a sensitive, stable, and easily manufactured electrochemical biosensor for the detection of lactate with in embryonic cell cultures media. Screen-printed disposable electrodes were used as electro-chemical sensing platforms for the miniaturization of the lactate biosensor. A CHIT/cMWCNT composite was employed as matrix for immobilization of LOx This novel electrochemical lactate biosensor measured lactate in model (buffer) solutions and exhibited a linearity in the concentration range, 30.4–243.9 mM in phosphate buffer solution, with a corresponding detection limit (basedon3-sigma) of 22.6 mM and a sensitivity of $3417 \pm 131$ μA $M^{-1}$ The novel biosensor exhibited a high reproducibility, with a relative standard deviation of less than 3.8% and an enzymatic response over 82% after 5 months when stored at 4 °C. Furthermore, HPLC was utilized to independently valid at the electrochemical lactate biosensor for determination of lactate in a commercial embryonic cell culture medium, there was excellent agreement between the two analytical protocols.

**Merits:** Lactate biosensor based on screen-printed electrode, was simple to fabricate and use. Activated electrode containing cobalt-phthalocyanine lowered operating potential of the biosensor. Mesoporous silica and nafion layers ensured excellent stability and high selectivity. Only a small sample volume is required for the measurement. High-performance L-lactate biosensor was obtained with a simple and cost effective way.

*3.2.1.2. Microporous gold electrode:.* A general bioanalytical platform for biosensor applications was developed based on three-dimensional ordered macroporous (3DOM) gold film modified electrodes using LOx as a case study, within the framework of developing approaches of broad applicability. The electrode was electrochemically fabricated with an inverted opal template, making the surface area of the 3DOM gold electrode up to 18 times higher than that of bare flat gold electrodes. These new electrochemical transducers were characterized by using field emission scanning electron microscopy (FE-SEM), atomic force microscopy (AFM) and the x-ray diffraction (XRD). The biosensor was developed by immobilization of LOx, on a 3DOM gold electrode modified with a self-assembled monolayer of dithiobis-N-succinimidyl propionate (DTSP). The resulting lactate oxidase electrode was characterized by electrochemical impedance spectroscopy (EIS). The 3DOM gold electrode not only provided a good biocompatible microenvironment but also promoted the increase of conductivity and stability. Thus, the developed LOx bioanalytical platforms showed higher mediated bioelectrocatalytic activity compared to others previously described based on polycrystalline gold transducers. The response to varying lactate concentrations was obtained in the presence of hydroxymethylferrocene as redox mediator in solution. Under these conditions, the bioanalytical platform response for DTSP covalently bound enzyme was improved with respect to that obtained in absence of DTSP (Gamero et al., 2012).

*3.2.1.3. Glassy carbon electrode*

a) **VBT–VBA/laponite matrix:**
An amperometric electrode for lactate was developed by immobilizing LOx on GC electrode with a hydrogel film composed of laponite and different amounts of a novel bioinspired polycation obtained by copolymerization of 4-vinylbenzyl thymine (VBT) and 4-vinylbenzyl triethylammonium chloride (VBA) in a molar ratio 1:4, respectively. The electrochemical behavior of the redox couple probe $[Fe(CN)_6]^{3-/4-}$ of these VBT–VBA bioelectrodes was compared with that observed for a bioelectrode containing the classical polycation polydiallyldimethylammonium chloride (PDDA). The best response was obtained for a bioelectrode containing a VBT–VBA/laponite mass ratio double than the cationic exchange capacity of the clay, demonstrating that under this condition, the polycation induces an optimal microenvironment in the interlamellar space of the clay, both for the position and the functionality of LOx. The VBT–VBA bioelectrode displayed a very high sensitivity $(7.2 \pm 0.2) \times 10^2$ μA $mM^{-1}$ $cm^{-2}$, a short time response ( < 5 s), a wide linear response range (e.g. 0.01–1.0 mM of L-lactate) and an excellent stability over a storage period of 60 days, when sensing L-lactate. The analytical response of the bioelectrode was tested in real food samples, e.g. milk, white wine, and beer, as well as during milk fermentation at 37 °C. No effect of molecular interferences in the food matrices was detected, and the quantification of L-lactate was in complete agreement with standard assays reported values. Results indicated that polycations containing the multifunctional green monomer VBT showed high potential for their use in hydrogel film formation producing more responsive and stable electrochemical biosensors (Zanini et al., 2013).

b) **laponite–chitosan hydrogel:**
Zanini et al., 2011, presented a lactate biosensor based on the immobilization of LOx on a GC electrode modified with laponite/chitosan hydrogels for the quantification of L-lactate in alcoholic beverages and dairy products . Ferrocene–methanol (FcMe) was used as artificial mediator. The purpose of this work was to determine the best hydrogel composition from the analytical point of view. The characterization of the hydrogels was carried out by CV, amperometry and EIS. According to permeabilities and charge transfer resistances for ferrocyanide (used as molecular probe) as well as the enzymatic behavior of the enzyme for L-lactate, the best laponite/chitosan mass ratio found was 25/50. The distinct features of the bioelectrode are its long stability, its ability to reject or minimize most interferents including ascorbic acid, and its excellent analytical response, which allowed the reduction of the enzyme content below 0.5 U, for a sensitivity of $(0.326 \pm 0.003)$ A $cm^{-2}$ $M^{-1}$, with a time response lower than 5 s and a detection limit of $(3.8 \pm 0.2) \times 10^{-6}$ M.

c) **Sol-gel film with MWCNTs**

A new type of amperometric L-lactate biosensor based on silica sol-gel and MWCNTs organic–inorganic hybrid composite material was developed. The sol-gel film was used to immobilize LOx on the surface of GCE. MWCNTs were used to increase the current response and improve the performance of biosensor. The sol-gel film fabrication process parameters such as $H_2O$: TEOS and pH were optimized, Effects of some experimental variables such as applied potential, temperature, and pH on the current response of the biosensor were investigated. Analytical characteristics and dynamic parameters of the biosensors with and without MWCNTs in the hybrid film were compared, and the results showed that analytical performance of the biosensor was improved greatly after introduction of the MWCNTs. Sensitivity, linear range, limit of detection ($S/N=3$) were 2.097 μA $mM^{-1}$, 0.3–1.5 mM, $0.8 \times 10^{-3}$ mM for the biosensor without MWCNTs and 6.031 μA $mM^{-1}$, 0.2–2.0 mM,

$0.3 \times 10^{-3}$ mM for the biosensor with MWCNTs, respectively. This method was used to determine the L-lactate concentration in real human blood samples (Huanga et al., 2007).

i) **Silica sol–gel film on MWCNT/PtNPs**

Ptnanowire (Ptnano) were used in combination with MWCNTs for fabricating sensitivity-enhanced electrochemical L-lactate biosensor. The composite film of MWCNTs and Ptnano was dispersed on the surface of the GCE. LOx was immobilized on MWCNTs/Ptnano/GCE surface by adsorption. The resulting LOx/MWCNTs/Ptnano electrode was covered by a thin layer of sol–gel to avoid the loss of LOx in determination and to improve the anti-interferent ability. Moreover, the sol–gel microenvironment contributes to both intensified stability and permselectivity. The cyclic voltammetry results indicated that MWCNTs/Ptnano catalyst displayed a higher performance than MWCNTs. Under the optimized conditions of applied potential 0.5 V, pH 6.4, room temperature, the proposed biosensor showed a large determination range (0.2–2.0 mM), a short response time (within 5 s), a high sensitivity (6.36 μA $mM^{-1}$) and good stability (90% remains after 4 weeks). The fabricated biosensor had practically good selectivity against interferences. (Huang et al., 2008).

*3.2.1.4. Pt electrode.* These can be classified further on the basis of type of membrane used.

(1) **Albumin mucin hydrogel polycarbonate membrane:**
An amperometric biosensor was constructed by immobilizing LOx in an albumin and mucin composed hydrogel. The enzyme was then cross-linked with glutaraldehyde to the polymeric matrix and entrapped between two polycarbonate membranes. The $H_2O_2$ produced by the oxidation of lactate by LOx was detected on a Pt electrode operated at 0.65 V versus Ag/AgCl. The performance of the biosensor was evaluated in matrices with different amounts of albumin, mucin and glutaraldehyde. The response time of the sensor to 10 μM lactate required 90 s to give a 100% steady-state response of 0.079 μA. Linear behavior was obtained for 0.7 μM to 1.5 mM. The detection limit, as calculated from the signal to noise ratio was 0.7 μM. Only 0.1 U of enzyme was necessary to get a biosensor with a relatively high current flow and an excellent stability over a storage period of 30 days. High reproducibility in the response was obtained when several biosensors were prepared with the same composition (Romero et al., 2008).

(2) **Polypyrrole(ox) anti interface membrane:**

An interference and cross-talk free dual electrode amperometric biosensor integrated with a microdialysis sampling system was fabricated for simultaneous monitoring of glucose and lactate by flow injection analysis. The biosensor was based on a conventional thin layer flow-through cell equipped with a Pt dual electrode (parallel configuration). Each Pt disk was modified by a composite bilayer consisting of an electrosynthesised overoxidized polypyrrole (PPyox) anti-interference membrane covered by an enzyme entrapping gel, obtained by glutaraldehyde co-cross-linking of glucose oxidase or lactate oxidase with bovine serum albumin. The advantages of covalent immobilization techniques were coupled with the excellent interference-rejection capabilities of PPyox. Ascorbate, cysteine, urate and paracetamol produced lactate or glucose bias in the low micromolar range, their responses were, however, completely suppressed, when the sample was injected through the microdialysis unit. Under these operational conditions the flow injection responses for glucose and lactate were linear up to 100 and 20 mM with typical sensitivities of 9.9 ($\pm$0.1) and 7.2 ($\pm$0.1) nA/mM, respectively. The shelf-lifetime of the biosensor was at least 2 months. The potential of the described biosensor was demonstrated by the simultaneous determination of lactate and glucose in untreated tomato juice samples; results were in good agreement with those of a reference method (Palmisano et al., 2000a, 2000b).

*3.2.1.5. Gold electrode:.* The modified gold electrode was used with undoped diamond nanoparticles (DNPs) and its applicability to the fabrication of electrochemical biosensing platforms. DNPs were immobilized onto a gold electrode by direct adsorption and the electrochemical behavior of the resulting DNPs/Au platform was studied. The CV study of work showed four well-defined peaks corresponding to the DNPs oxidation/reduction at the underlying gold electrode, which demonstrate that, although undoped. DNPs have an insulating character, they show electrochemical activity as a consequence of the presence of different functionalities with unsaturated bonding on their surface. The glucose biosensor showed a good electrocatalytic response in the presence of (hydroxymethyl)ferrocene as redox mediator. Once the suitability of the prototype system to determine glucose was verified, in a second step, a similar biosensor, but employing the enzyme lactate oxidase (LOx/DNPs/Au) was designed. A linear concentration range from 0.05 mM to 0.7 mM, a sensitivity of 4.0 μA/mM and a detection limit of 15 μM were obtained (Briones et al., 2015).

**b) Working electrode:**

The diamond nanoparticles (DNPs) in a sol–gel matrix derived from (3-mercaptopropyl)-trimethoxysilane (MPTS) were used to improve electron transfer in a LOx based electrochemical biosensing platform. The platform was based on gold electrodes (Au) modified with the sol-gel matrix (Au/MPTS) in which diamond nanoparticles (Au/MPTS/DNPs) and lactate oxidase (Au/MPTS/DNPs/LOx) have been included. The response of Au/MPTS/DNPs/LOx towards lactate was obtained. A linear concentration range, from 0.053 mM to 1.6 mM, a sensitivity of 2.6 mA $mM^{-1}$ and a detection limit of 16 mM were obtained. These analytical properties presenting also as advantages that DNPs are inexpensive, environment-friendly and easy-handled nanomaterials (Briones et al., 2016).

*3.2.1.6. ITO Plate based electrode:.* These can be classified further depending on the composite/hybrid film electrodeposited on the type of electrode.

a. **PANI fluroaniline:**
An amperometric enzyme electrode based on polyaniline-co-fluoroaniline film/ITO was developed for determination of lactate in serum. To prepare this electrode, commercial lactate oxidase from Pediococcus species has been immobilized through glutaraldehyde coupling onto polyaniline-*co*-fluoroaniline film deposited on an Indium tin oxide (ITO) coated glass plate. The method was based on generation of electrons from $H_2O_2$, which was formed from lactic acid by immobilized lactate oxidase. The concentration of lactic acid was directly proportional to the current measured. The enzyme electrode showed optimum response when operated at 42 °C in 0.05M, sodium phosphate buffer pH 6.5 for 50 s. The biosensor exhibited a good performance with a linear response range from 0.1 to 5.5 mM/l. The minimum detection limit of the method was 0.1 mM/l and sensitivity of the sensor is 1.18 μA/mM/l lactate. This electrode was employed for determination of lactate in serum. The serum values in healthy and diseased persons were in the range 0.51–2.9 and 5.0–15.0 mM, respectively. The analytical recovery of added lactic acid was 71%. Within batch and between batch CV were $<4$ and $<5$%, respectively. Among the various serum substances tested only 8-hydroxyquinoline, urea, ammonium molybdate and uric

acid caused 64%, 38%, 34% and 31% inhibition, respectively. The electrode was used 150 times over 26 days without any considerable loss of activity, when stored at 4 °C (Suman et al., 2005).

b. **CCNT/Si**

Carbon nanotubes (CNTs) were functionalized and employed in an electrochemical cell to serve as a biosensor to specifically detect either lactate or pH in an electrolyte solution of artificial sweat. They were functionalized with the carboxyl group (-COOH) to detect pH and lactate. The functionalized CNT–COOH electrode displayed a linear response to pH 1–10, with a negative voltage shift corresponding to an increase in pH. Two types of lactate sensors were fabricated, both of which exhibited an increase in current corresponding to an increase in lactate concentration. The functionalized CNT–LOx on a glassy carbon(GC) electrode displayed an amperometric response in the range of 1–4 mM lactate. The CNT–LOx on a silicon/indium tin oxide (Si/ITO) substrate displayed an amperometric response in the range of 0.01–0.05 M lactate (Weber et al., 2006).

*3.2.1.7. Carbon electrode:.* These can be classified further depending on the composite /hybrid film electrodeposited on the type of matrix.

a) **DEAE-Dextran:**
The lifetime and the reproducibility of novel enzyme stabilizing techniques based on polyelectrolyte agents such as diethylaminoethyl-dextran have been studied. Glucose oxidase(GOx) and LOx used as the model enzymes, were stabilized with the cationic polyelectrolyte, and the resulting enzyme–polyelectrolyte complexes were physically adsorbed into a highly porous and conductive carbon electrode for the construction of the biosensors. The amounts of diethylaminoethyl-dextran and enzyme were optimized with respect to sensor's sensitivity and stability. Optimum results were obtained using soaking solutions of 2500 U/ml GOx and 1.0 w/v DEAE-dextran. Additionally, experiments with LOx sensors constructed using 200 U/ml LOx and 0.5% w/v dextran solution showed improved operational, and storage stability. The sensor-to-sensor reproducibility was good, the relative standard deviation being less than 5.0% (Gavalas and Chaniotakis, 2000).

b) **Horseradish peroxidase-ferrocene:**
The development and characteristics of a bioreactor employing bacteria (Streptococcus thermophilus) encapsulated in Ca-alginate beads coupled with an L-lactate biosensor are reported. The biosensor comprised a carbon paste electrode modified with horseradish peroxidase(HRP), LOx, and FcH (ferrocene) as redox mediator. The measurement of L-lactate is based on the signal produced by $H_2O_2$, the product of the enzymatic oxidation of L-lactate by LOx. The detection of $H_2O_2$ is performed at the electrode surface via HRP/FcH at low operating potential ($-100$ mV vs Ag/AgCl). Bronopol (2-bromo-2-nitro propane-1,3-diol) was chosen to simulate the effect of an inhibitory agent of milk fermentation. The results indicated that the evaluation of the amount of L-lactate amount produced through the bioreactor could be used as a measure of inhibition of lactic acid production in milk samples (Zaydan et al., 2004).

c) **Prussian Blue (PB)**

A lactate microbiosensor based on Prussian Blue (PB)-modified carbon fiber electrodes (CFEs) was fabricated to detect enzyme-generated hydrogen peroxide at a low applied potential ($\sim$0 V against SCE). The potential use of this approach included pharmacological, physiological and electrophysiological stimulation, showing that this microbiosensor design was well suited to exploring the role of lactate in brain extracellular fluid (Salazar et al., 2012).

*3.2.2. LDH based biological recognition transducer*

These can be classified further based on type of electrode:

*3.2.2.1. Glassy carbon electrode based biosensor:.* These can be classified further depending on the composition of matrix.

(1) **$Fe_3O_4$NPs/MWCNTs:**
$Fe_3O_4$ magnetic nanoparticles were in situ loaded on the surface of multiwalled carbon nanotubes (MWCNTs) by a simple co-precipitation procedure. The resulting $Fe_3O_4$/MWCNTs nanocomposite brought new capabilities for electrochemical sensing by combining the advantages of $Fe_3O_4$ magnetic nanoparticles and MWCNTs. $Fe_3O_4$ had redox properties similar to those of frequently used mediators used for electron transfer between NADH and electrode. The cyclic voltammetric results indicated the ability of $Fe_3O_4$/MWCNTs modified GC electrode to catalyze the oxidation of NADH at a very low potential (0.0 mV vs. Ag/AgCl) and subsequently, a substantial decrease in the over potential by about 650 mV compared with the bare GC electrode. The catalytic oxidation current allowed the stable and selective amperometric detection of NADH at an applied potential of 0.0 mV (Ag/AgCl) with a detection limit of 0.3 μM and linear response up to 300 μM. This modified electrode could be used as an efficient transducer in the design of biosensors based on coupled dehydrogenase enzymes. LDH and $NAD^+$ were subsequently immobilized onto the $Fe_3O_4$/MWCNTs nanocomposite film by covalent bond formation between the amine groups of enzyme or $NAD^+$ and the carboxylic acid groups of the $Fe_3O_4$/MWCNT film. Differential pulse voltammetric detection of lactate on $Fe_3O_4$/MWCNT/LDH/$NAD^+$ modified GC electrode gives linear responses over the concentration range of 50–500 μM with the detection limit of 5 μM and sensitivity of 7.67 μA $mM^{-1}$. Furthermore, the sensor was applied for quantitative analysis of lactate in human serum samples (Teymourian et al., 2012).

(2) **NADH/LDH/Nano-$CeO_2$**

An electrochemical biosensor based on NADH/LDH/Nano-$CeO_2$/GCE was developed to determine lactate. The biosensor had linearity in the range of 0.2–2 mM, with a response time of less than 4 s. The biosensor showed high accuracy and specificity (Nesakumar et al., 2013).

*3.2.2.2. Screen printed carbon electrode based biosensors:.* These can be classified further depending on the basis of matrix.

a) **MBRS-SPCE:**
A lactate biosensor was developed based on a screen printed carbon electrode, modified with Meldola's Blue-Reinecke Salt (MBRS-SPCE), coated with the LDH from porcine heart and NAD+. A cellulose acetate (CA) layer was deposited on the top of the device to act as a perm selective membrane. The biosensor was incorporated into a commercially available, thin-layer, amperometric flow cell operated at a potential of only +0.05 V vs. Ag/AgCl. The mobile phase consisted of 0.2 M phosphate buffer pH 10 containing 0.1 M potassium chloride solution; a flow rate of 0.8 ml $min^{-1}$ was used throughout the investigation. The biosensor response was linear over the range 0.55–10 mM lactate. The detection limit being 0.55 mM. The precision of the system was determined by carrying out 10 repeat injections of 10 mM lactic acid standard; the calculated

coefficient of variation was 4.28%. The biosensor system was applied to the direct measurement of lactate in serum without pre-treatment, which allowed high throughput-analysis, at low cost, for this clinically important analyte (Piano et al., 2010).

b) **RGO-AuNPs**

A novel amperometric biosensor based on gold nanoparticles anchored on reduced graphene oxide (RGO-AuNPs) and LDH was developed for the sensing of L-lactate. The RGO-AuNPs modified screen printed electrodes were tested for NADH detection showing a wide dynamic range and a low detection limit. Next, the biosensor was constructed by incorporating both enzyme and RGO-AuNPs in a sol gel matrix derived from tetrametoxysilane and methyltrimetoxysilane. The biosensor linearly responded to L-lactate in the range of 10 μM–5 mM and showed a good specific sensitivity of 154 μA/mM $cm^2$ with a detection limit of 0.13 μM. This was accompanied by good reproducibility and operational stability. Tests on artificial serum proved that L-lactate can be determined practically without interferences from commonly interfering compounds such as urate, paracetamol and L-ascorbate. LDH/RGO-AuNPs/SPCE based biosensor thus performed as electrochemical device for the detection of L-lactate as a viable early cancer bio-marker (Azzouzi et al., 2015).

**Merits:** This type of composite electrodes gave rise to a three-dimensional rigid biocomponents reservoir, whose surface could be easily regenerated by polishing.

**Demerits:** The stability of the working electrode was not high due to the physical inclusion of enzyme, which allows the leaching of enzyme.

*3.2.2.3. Gold electrode based biosensors:.* These can be classified further depending on the basis of matrix.

(1) **pTTCA/MWCNT:**
An amperometric lactate biosensor was fabricated based on a conducting polymer, poly-5,20 50,20 0-terthiophene- 30-carboxylic acid (pTTCA), and MWCNT composite on a gold electrode. LDH and $NAD^+$ were subsequently immobilized onto the pTTCA/MWCNT composite film. The detection signal was amplified by the pTTCA/MWNT assembly, onto which a sufficient amount of enzyme was immobilized and stabilized by the covalent bond formation between the amine groups of enzyme and the -COOH groups of the pTTCA/MWNT film. Analytical performances and dynamic ranges of the sensor were determined, and the results showed that the sensitivity, stability, and reproducibility of the sensor improved significantly using pTTCA/MWNT composite film. The calibration plot was linear ($r^2=0.9995$) over the range of 5–90 mM. The sensitivity was approximately 0.0106 lA/lM, with a detection limit of 1 mM, based on a signal/ noise ratio of 3. The sensor measured L-lactate concentration in commercial milk and human serum samples (Rahman et al., 2009).

(2) **Meldola's blue (MB)**

MWCNT was evaluated as transducer, stabilizer and immobilization matrix for the construction of an amperometric biosensor based on LDH and MB. The amperometric response was based on the electrocatalytical properties of MB to oxidize NADH, which was generated in the enzymatic reaction of lactate with $NAD^+$ under catalysis of LDH. The employed materials were promising as electrochemical mediators and enzyme stabilizers. The enzyme was immobilized onto the MWCNT adsorbed with MB by cross-linking with glutaraldehyde. The dependence on the biosensor response for lactate was investigated in terms of pH, supporting electrolyte, LDH and $NAD^+$ amounts and applied potential. The amperometric response for lactate using this biosensor showed excellent sensitivity (3.46 $Acm^{-2}$ $mmol\,L^{-1}$), operational stability (around 96.5% of the activity was maintained after 300 determinations) and wide linear response range (0.10–10 mmol/L). These favorable characteristics allowed its application for direct measurements of lactate in blood samples. Good agreement was found between the results obtained by the developed biosensor indicating that it should be highly selective for the lactate. Moreover, the biosensor also presented excellent stability in operational conditions (Pereira et al., 2007).

*3.2.2.4. Carbon electrode:.* A carbon electrode biosensor was constructed employing carbon-based thick-film electrodes using $NAD^+$'dependent dehydrogenases by screen printing. Cyclic voltammetric and amperometric investigations of the thick-film electrode showed significantly different electrocatalytic properties toward NADH compared to conventional polished electrodes. Electrochemical oxidation of NADH was attained at the low potential of +350 mV vs. Ag/AgCl at the thick-film carbon electrode, and this could be attributed to increased electrocatalytic sites due to the rough and jagged electrode surface and the formation of a microelectrode array-like structure at the electrode surface (Yoon et al., 1996).

*3.2.2.5. Graphite film:.* Two strategies were investigated for the development of lactate biosensors based on sol–gel matrixes and polysulfone composite films, both containing L-LDH. Firstly, reagentless disposable screen-printed electrodes (SPE's) with MB and the cofactor $NAD^+$ inside a sol–gel matrix were prepared. These showed relatively low sensitivities (260 μA/M). Secondly, mediator-modified-polysulfone–graphite composite films deposited over both cylindrical epoxy–graphite and SPE's. These electrodes showed enhanced performance characteristics: improved sensitivity (80 mA/M), detection limit (0.87 μM) and reproducibility (2%). Reagentless electrodes, incorporating $NAD^+$ in the polysulfone film, had a decreased sensitivity, although better than that achieved by the sol–gel electrodes. While sol–gel electrodes showed a linear range between $1.25\times10^{-4}$ and $2.48\times10^{-3}$ M, the epoxy–graphite composite electrodes based on polysulfone composite films allowed the detection of lactate at a linear range of lower concentrations from $1\times10^{-6}$ to $1.2\times10^{-5}$ M. Finally, the performance of the LDH-MB-polysulfone-composite film-based SPE's in a flow system was studied. Short response times were obtained ($t<30$ s). Furthermore, repeatability and reproducibility values were notably improved, especially when working with electrodes covered with a polyamide layer prepared with *N*-(2-aminoethyl)-piperazine (Simón et al., 2007).

*3.2.2.6. Solid binding matrix based transducer:.* Solid binding matrix (SBM) based composite transducers were used for development of series of multibiosensor systems applicable in various fields. Two hybrid three-channel multibiosensors for simultaneous amperometric operation in food quality control, i.e. glucose/fructose/ethanol multibiosensor, based on glucose oxidase/fructose dehydrogenase/alcohol dehydrogenase surface-modified enzyme electrodes and L-lactate/L-malate/sulfite multibiosensor, based on L-lactate dehydrogenase/L-malate dehydrogenase/sulfite oxidase surface-modified enzyme electrodes were reported. Different parameters were studied in order to optimize the response of the multibiosensor systems. The multibiosensor showed a good sensitivity, linear range and storage stability. The multibiosensors were used for the determination of glucose, fructose, ethanol, L-lactate, L-malate and sulfite in samples of wine, resulting in a good agreement with data certified by the supplier. Comparison of various designs, surface-modified, bulk-modified and thick-cover,

of SBM based biosensors is studied on the example of fructose biosensor (Miertuša et al., 2000).

*3.2.2.7. Pencil graphite electrode based transducer:*. An amperometric lactate biosensor was developed based on immobilization of lactate dehydrogenase (LDH) onto graphene oxide nanoparticles (GrONPs) decorated pencil graphite electrode (PGE). The biosensor exhibited optimum response within 5 s at pH 7.3 (0.1 M sodium phosphate buffer) and 35 °C, when operated at 0.7 V. The biosensor showed excellent sensitivity, (detection limit as low as 0.1 μM), fast response time (5 s) and wider linear range (5–50 mM). Analytical recovery of added lactic acid (0.5 mM and 1.0 mM in sera was between 95.81 and 97.87% and within batch and between batch coefficients of variation were 5.04% and 5.40% respectively. A good correlation was obtained between serum lactate values measured by standard colorimetric method and the present biosensor ($R^2$=0.99). The biosensor was employed for determination of lactate in sera of apparently healthy subjects and persons suffering from lactate acidosis & other biological materials (milk, curd, yogurt, beer, white & red wine). The working electrode lost 25% of its initial activity after 60 days of its regular uses, when stored dry at 4 °C (Batra et al., 2016).

Table 1 summarizes the analytical characteristics of various lactate biosensors.

## 4. Conclusion and future perspective

In conclusion, (i) Biosensing methods are comparatively better than traditional colorimetric, enzymic colorimetric and spectrophotometric, chromatographic methods including proton nuclear magnetic resonance for determination of lactic acid, as these are very simple, sensitive, selective, disposable, work with complete automation and provide rapid results. (ii) Lactate biosensors are most promising device for clinical analysis of several metabolic disorders related to hypoxia and assessment of food quality. (iii) Although a variety of nanomaterials have been employed to improve the analytic performance of lactate biosensors during the past one decade, there is no report on use of nanoparticles of enzymes (LDH/LOx) in lactate biosensors. (iv) Enzyme nanoparticles are the aggregates of enzyme molecules in the nanoscale which show their exceptional electronic, mechanical, optical and thermal properties besides increased surface area (Pundir, 2015). (v) The future research could be focused on the use of enzyme nanoparticles in fabrication of lactate biosensors. (vi) There is further need to develop more simple, accurate, reliable and cheap lactate biosensors. (vii) Attempts could be made to integrate the laboratory model with chip designing companies to develop a fully automatic chip system for a portable device which can be used at the bedside of the patient.

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