Electrochemical Detection of Lactate Produced by Foodborne Presumptive Lactic Acid Bacteria

Ozum Ozoglu (ozoglu@uludag.edu.tr)
Bursa Uludag University: Bursa Uludag Universitesi https://orcid.org/0000-0003-3600-142X
Aytekin Uzunoglu
Necmettin Erbakan University: Necmettin Erbakan Universitesi
Mehmet Altay Unal
Ankara University: Ankara Universitesi
Mehmet Gumustas
Ankara University: Ankara Universitesi
Sibel Aysıl Ozkan
Ankara University Faculty of Pharmacy: Ankara Universitesi Eczacilik Fakultesi
Mihriban Korukluoglu
Bursa Uludag University: Bursa Uludag Universitesi
Evrim Gunes Altuntas
Ankara University: Ankara Universitesi

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Abstract

Background: The detection of lactate is an important indicator of freshness, stability, and storage stability of products as well as degree of fermentation in food industry. In addition, it can be used as a diagnostic tool in patients' healthcare since it is known that the lactate level in blood increases in some pathological conditions including septic shock, tissue hypoxia, and sepsis. Thus, determination of lactate level plays an important role in not only food industry but also health fields. At this point, biosensor has become important to detect lactate due to having many advantages like rapid, cheap and easy to use.

Methods and Results: In the current study, amperometric lactate biosensors based on lactate oxidase immobilization (with nafion 5% wt) were designed and limit of detection, linear range and sensitivity values were determined, which were found as 31 µM, 50-350 µM and 0.04 $\mu A.\mu M^{-1}.cm^{-2}$, respectively. Then, it was used for the measurement of lactic acid produced by 6 different and morphologically identified presumptive lactic acid bacteria (LAB) which were isolated from different naturally fermented cheese samples for this study. Then, the biosensors were used to perform lactate measurements successfully within 3 minutes for each sample even few of them were out of the limit of detection.

Conclusion: Electrochemical biosensors should be alternative and quick solutions for measurement of lactate metabolites instead of using classical methods which are required long working time. Besides, it is the first study to measure lactate produced by foodborne LAB as real sample with a biosensor.

Introduction

Lactate (lactic acid) can be produced naturally by lactic acid bacteria (LAB) and have two enantiomers which are L (+) and D (-). While L lactate is an intermediate in mammalian metabolism, D lactate is produced by microorganisms, algae, and plants, and utilization of D-lactate by humans is limited [1, 2]. Besides, lactate is found in fermentative food products like pickled vegetables such as Korean Kimchi and sauerkraut, fermented dairy products like yogurt and cheese, wine, cured meat, and fish. Additionally, lactate is supplied to foods and beverages as an acidulant or food preservative or to modify the flavor (E270). Apart from these, D (-) enantiomer shows that reduced freshness in vacuum-packed meat products, a racemic mixture of bacterial contamination of tomatoes, tomato paste, and tomato juice, and spoilage of wine. In fruit juice, a higher amount of LAB is referred to as that organoleptic characteristic of the juice has damaged. Thus, detection of both D (-) and L (+) form is an important indicator for the determination of food quality and in other words, at least the total lactic acid amount should be monitored for food safety which is required in terms of sustainable agriculture [1–6].

Lactate is an important metabolite of the anaerobic metabolic pathway [2, 7]. Blood lactate level is a significant parameter for diagnosis of patient conditions during intensive care and surgical operation process, multiple system organ failure and death of septic shock patients, trauma, performance level, fatigue, hydration, and sports medicine. Besides, increasing lactate levels of blood should have come from congestive heart failure, hypoxia, lactic acidosis, and diabetic ketoacidosis [2, 6–8]. At this point,

biosensor has gained importance for measuring or observed of lactate amount by the reason of their reliability, accessibility and quickness [4, 9–11].

Biosensors are analytical devices that are comprised of a biological recognition element (bioreceptor) and a physical transducer. They are classified by their bioreceptors (enzyme, whole-cell, and affinity biomolecule based) and physical transducer (electrochemical, optical, piezoelectric, and thermal). Biosensors, can measure the concentration of biological materials through a signal which is produced from a transducer obtained in proportion to the specificity and the concentration of the bioreceptor [12–14]. Using electrochemical transducers especially amperometric ones on biosensors very common because, having well linear range, high sensitivity, and great accuracy with various bioreceptor [15–18]. They are powerful alternatives instead of common analytical methods which are expensive, need a long time and trained personal like spectrophotometer, HPLC, and chromatography due to the fact that, biosensors are rapid, accurate, sensitive, easy to use, and cheap equipment [12, 19]. Thus, biosensors have been used for traceability and detection of target analytes such as microorganisms, enzymes, nucleic acids, pesticides, etc. in food, health, biotechnology, environment, and military fields [19–23].

Especially enzymatic amperometric types of biosensors have been used commonly for the detection of lactate in food and health sciences [4, 9, 11, 24]. Enzyme-based amperometric biosensor for lactate determination is designed by immobilized lactate dehydrogenase (LDH) or lactate oxidase (LOx) enzymes as bioreceptors. Lactate oxidase is widely used in lactate biosensors due to the fact that, its simple reaction and easy biosensor design configuration. Thus, the detection of lactate concentration is based on the catalytic action of lactate oxidase on the oxidation of lactate to pyruvate that is shown in the following equations [16, 25, 26]:

$$\text{L-lactate} + O_2 \xrightarrow{\text{L-Lactate oxidase}} \text{Pyruvate} + H_2O_2 \quad (1)$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^- \quad (2)$$

Present paper describes the design of an enzyme-based amperometric lactate biosensor for the detection of lactate produced by 6 different and morphologically identified presumptive lactic acid bacteria (LAB). In the first part of the study, LOx-based electrochemical lactate biosensors were constructed by drop-casting a certain amount of enzyme on a mirror-like Pt electrode surface. The electrochemical performance of the sensors was evaluated using cyclic voltammetry (CV) and chronoamperometry (CA) methods. In the second part of the study, the constructed sensors were exposed to 6 different lactic acid environments produced by presumptive LAB, and the level of lactate in those environments was determined. To the best of our knowledge, there is no other study in the literature regarding the detection of lactate production by foodborne LAB by constructing a biosensor.

Material And Methods

Chemicals and Reagents

Lactate oxidase enzyme (LOx) (L0638-100UN Lactate Oxidase from *Pediococcus* sp., CAS:9028-72-2), Phosphate Buffered Saline (PBS) solution (pH 7.4) and lithium lactate were purchased from Sigma Aldrich. Then, the media (M17 and MRS) used for isolation and growth of LAB were obtained from Merck. Besides, the Gram stain Kit used for characterization of LAB was acquired from Norateks Kimya.

Isolation and Morphological Characterization of LAB

LAB strains were isolated from different naturally fermented cheese samples which were provided in Ankara-Turkey. MRS (De Man Rogosa Sharpe) medium and M17 medium were used for the isolation of lactic acid bacteria from the samples. M17 medium was preferred for further analysis due to bacterial growth was slightly better than MRS. The strains were inoculated M17 media concerning pour plate technique and incubated at 30°C at 24-48 hours [27]. To exhibit morphological properties and Gram reaction of the strains, the Gram staining method was applied to the strains by Gram stain Kit [28]. Then, Gram (+) ones were stored at -20°C in an M17 broth medium containing %15 glycerol for further experiments.

Acquirement of Metabolites from LAB

Morphologically identified 6 presumptive LAB isolates (shown in Table 2) were activated at 30°C for 24 hours in M17 broth. Then, supernatants of the active cultures were collected after centrifuging at 6000 rpm for 10 minutes. The supernatants were filtered through a 0.45 µm syringe tip filter (Sartorius, Germany) [29].

Designing Amperometric Lactate Biosensor

Enzyme Immobilisation

Lactate oxidase enzyme (LOx) was used as a bioreceptor and lithium lactate was preferred as substrate. Furthermore, a thin Nafion® layer was created on the surface of the sensors to circumvent any possible interference effect of interfering molecules and enzyme leakage out of the sensor surface. The amount of the polymer layer drop cast on the surface was also experimented to achieve maximum efficiency [11, 30–32].

Preparation of Biosensors

Three-electrode-system was utilized in the electrochemical performance experiments, compromising of Ag / AgCl, Pt electrode and Pt wire as a reference, working and counter electrodes, respectively. Electrochemical sensors were prepared by a drop-casting method. Before the construction of the sensors, the surface of the Pt electrode was polished with alumina solutions (1 µm, 0.3 µM, and 0.05 µm). After that, it was rinsed with deionized water and ethanol mixture to remove residues from the surface. 10 µL PBS solution which contained 2U LOx enzyme was dropped on the surface of the dried electrode and left room temperature to dry for 2 hours. After 2 hours, 8 µL Nafion solution (5 wt%) was dropped on the modified enzyme electrode surface and then dried at room temperature [33, 34].

Performance Analyses

Performance analysis of the biosensor was realized with cyclic voltammetry (CV) and chronoamperometry (CA) methods. CV and CA studies were done with an Ivium Vertex One-100 mA potentiostat and IviumSoft software package. Sensor performance assays were performed at room temperature in PBS solution (0.01 M, 8 mL, pH 7.4) which was in a glass cell with added lactic acid (Lithium lactate). The prepared sensors were kept at 4°C when not in use [34].

Detection of Lactate Produced by Foodborne Lactic acid bacteria

After specified biochemical properties and limit of detection of the biosensor, lactate amounts that were produced by LAB isolates were measured chronoamperometrically in triplicate. The amounts of lactic acids were calculated from the regression equation obtained from the flow-time graphs (as in Figure 3b), y: shows the increase in flow amount, a: sensitivity, and x shows the lactic acid concentration in the solution. This equation allows us to presume the actual lactic acid concentration by measuring the lactate from the diluted solution.

Results

Morphological Characterization of the Bacterial Isolates

According to the Gram stain assay of the bacteria isolates numbered in Table 1, all 6 strains were found as Gram (+) and were morphologically identified as coccus. Metabolites of these 6 isolates were determined in the biosensor carried out within the scope of the study. Also, the ability of these 6 isolates to produce lactate has been confirmed by high performance liquid chromatograph analysis (data not shown) [35].

Discovering Electrochemical Characteristics of the Sensors

Electrochemical characteristics of the sensors were determined by cyclic voltammetry (CV) measurement. The results were shown in Figure 1. According to Figure 1, it is seen that there was an increase in the oxidation peak from 0.4 V by adding 2 mM lactate into the PBS. When the lactic acid amount in PBS increased, the oxidation peak was also increased, which indicates that the enzyme layer immobilized on the electrode surface and working properly. The oxidation reaction of lactate in the presence of LOx is given in Eq. 1. When the hydrogen peroxide (H_2O_2) produced from lactic acid oxidation reaches the electrode surface if there is a suitable applied potential, it gives an oxidation reaction and the electron is released as a result of this reaction. For this reason, the increase in oxidation current seen in Figure 1 was due to the oxidation of hydrogen peroxide on the sensor surface. Considering Equations 1 and 2, there is a correlation between the lactic acid present in the system and the concentration of hydrogen peroxide formed by the reaction and oxidized on the sensor surface. Thus, the amount of lactic acid in the environment can be calculated by determining the amount of hydrogen peroxide and concentration.

The effect of scan rate on the electrochemical behaviour of the sensors was evaluated by conducting the CV experiments at various scan rates. These experiments were carried out in PBS solution in the presence of 2 mM lactic acid and the results are given in Figure 2. As seen in Figure 2a, the increase in the scan

rate resulted in a significant change in the peak current, and the oxidation peaks became more pronounced. The connection of this alteration with the scan rate was shown in Figure 2b. So, peak current demonstrated a linear change with the increasing scan rate (R^2= 0.9998). According to these results, it was deduced that reactions that occurred on the sensor's surface were surface-controlled. In other words, the performances of the prepared sensors show a significant dependence on electrocatalytic surface properties [34, 36].

Activities of Biosensors to Lactic Acid

Activities and analytical performances of prepared biosensors were determined by using the chronoamperometry method (CA) [34, 36]. Lactic acid was added to PBS at certain time intervals and alterations on the current were determined and saved. In electrochemical performance experiments, the applied voltage was fixed at 0.6 V, because the oxidation potential of hydrogen peroxide at the surface of Pt was determined as 0.6 V which is shown in Figure 1 and the peak formation was around this value as seen in Figure 2a. While no lactic acid was present in the system the current of the system seem around zero (Figure 3). When 0.05 mM lactic acid was added to the PBS, it was observed that the measured current increased rapidly and fixed within a short time. The addition of lactic acid was repeated periodically and after each addition, the flow was rapidly increased and fixed. It was shown that demanded sensor performance was obtained, and the prepared lactic acid biosensors are capable of determining the lactic acid healthily and efficiently. Besides, the fast increase of the current in a short period with the addition of lactic acid is an indication of giving rapid results of the prepared sensors. According to this information, the current biosensor provided rapid determination which is an important advantage for biosensors. The calibration curves were obtained according to the results in Figure 3a by performing the same sensor three times independently under specified conditions (Figure 3b).

One of the most significant results which were deduced from the graphs (Figure 3a and 3b) was that the prepared sensors can be used up to 350 µM lactic acid concentration in a proper way. Thus, it was found that the sensor performances increased linearly up to the concentration of 350 µM, yet above this value, an increase in the current was tended to reach saturation and not linear to the concentration.

Slopes of calibration curves give the sensitivity of biosensors. Therefore, the mean sensitivity of the sensors was found as 0.0008 µA/µM when based on the equation in Figure 3b. Then, it was known that the diameter of the Pt electrode used in the study was 1.6 mm, and the obtained sensitivity value was 0.04 $\mu A.\mu M^{-1}.cm^{-2}$.

The limit of detection (LOD) for the biosensors was calculated according to LOD = (s/S)x10 equation. Based on the equation, LOD of the biosensor were found as 31 µM. It was dedicated that the linear working range of the biosensor was 50-350 µM. This determination range could be considered advantageous in some aspects compared to other studies in the literature mentioned in Table 1. Besides, the relative standard deviation (RSD%) was found as 3.9%.

Detection of Lactate Producing by Foodborne Presumptive LAB with the Biosensor

The amount of lactates produced by the presumptive LAB isolates were measured chronamperometrically at room temperature at 0.6 V with the amperometric lactate biosensor developed by determining the optimum working conditions and determination range given at Table 1. The measurements were done for diluted samples and then, lactate amounts were calculated consideration dilution ratios in main samples. Therefore, the results were given as mean value and standard deviation for triplicate measurements.

Table 1
The biosensor measurement results of lactic acids produced by the LAB isolates.

Isolate Number	Food Origin	Lactic Acid Amount (µM)
11	Cheese	280±0.05
12	Cheese	270±0.03
13	Cheese	250±0.06
14	Cheese	330±0.03
23	Cheese	>
24	Cheese	>
*>: Level of lactate amount was measured as higher than the limit of detection range of the biosensor.		

As seen in Table 1, the developing biosensor was successful in measurement amounts of the lactates which were range in limit of detection of the biosensors. These were produced by numbered as 11, 12, 13 and 14 isolates that obtained cheese samples were found 280, 270, 250, 330 µM, respectively. Besides, the amount of lactic acid was found higher than the 250 µM in all samples.

Discussion

According to the results of the Table 1, it is seen that the biosensor developed within the scope of this study has a very high potential in terms of use in the determination of lactate produced by food-borne LAB if the working range is improved. Also, it should be considering that lactate amounts that cannot be determined exactly in the biosensor because of the environmental factors cannot be eliminated, or secondary metabolites in the metabolites may be oxidized on the electrode surface and affect the measurement results (parasite) [32, 37–40]. Nevertheless, using a biosensor for the determination of lactate is an advantageous method, and the success of applying the real samples was shown in many studies like mentioned below.

Canbay and co-workes have developed a microbial biosensor (with 0.1-10 mM determination range) that determines lactic acid and pyruvic acid in their study. They checked the actual sample trials with the

commercial kit (Sigma Aldrich, USA) and determined the amount of L-lactate in kefir, milk, and butter samples. Then, they dedicated that, lactate in the drinks could be determined by using the biosensor [37].

Pereira and Stradiotto (2019) were developed a biosensor that had a modified glassy carbon electrode with MIP (molecularly imprinted polymers)/AuNP(gold nanoparticles)/RGO (graphene oxide) for the determination of lactic acid was obtained by electropolymerization of the ophenylediamine monomer. They indicated that the materials used to modify the electrode allowed to provided low detection and quantification limits and also good selectivity in the presence of molecules with similar chemical groups and sizes. Besides, GCE modification and MIP construction were done in addition to the cyclic voltammetry by electrochemical impedance spectroscopy, scanning electron microscopy, and atomic force microscopy. Then, there were not observed any critical values among the results. Thus, using MIP was an original and an advantage for the study. Also, they have used the sensor for the determination of lactate on sugarcane vinasse [41]. Similarly, the study of Alizadeh and co-workers was shown the benefit of using the MIP. They have developed a novel nano-sized imprinted polymer/multi-walled carbon nanotube (MWCNTs)-based potentiometric sensor for lactic acid determination. Then, the proposed sensor was successfully applied for the determination of lactate in milk and yogurt samples [42].

Table 2
Some examples of lactate biosensors reported in the literature. The biosensor final application, sensitivity and linear range values are shown.

Concept of the Biosensor	Linear Range (μM)	Sensitivity ($\mu A.\mu M^{-1}.cm^{-2}$)	Sample Type	Reference
CNT–LOX on a Glassy Carbon Electrode	1000–4000	-	Skin and Sweat	[33]
CNT–LOX on a Si/ITO substrate	10000-50000			
AuNNs-programmed flexible sensor	1000-25000	-	Sweat	[43]
Carboxylated multiwalled carbon nanotubes/copper nanoparticles/polyaniline modified pencil graphite electrode	1-2500	-	Human Serum Plasma, milk products and wine	[44]
Pt electrode was modified with a composite prepared from rGO, CNTs and AuNPs	50–100.000	35.3	Milk, Blood and Cell Culture Media	[45]
AuNPs-ERGO-PAH/SPE	500-3000	1.08	Yogurt and Wine	[46]
	4000-16000	0.28		
GCE/Chit/LOx/Chit/GA (5 mM $K_3Fe(CN)_6$)	990-5660	1.44×10^{-3} ($C.cm^{-2}.mM^{-1}$)	Not studied.	[47]
GCE/GInk/LOx/PD	740-2440	4.11×10^{-4} ($C.cm^{-2}.mM^{-1}$)		
1 mm carbon working electrode, Pt auxiliary electrode and Ag/ AgCl reference electrode	50-800	3.03	Wine	[48]
Pt working electrode, Ag/AgCl reference electrode and a Pt wire counter electrode (*Lactobacillus delbruecki* sp. based)	100-1000	-	Buttermilk, Kefir and Milk	[37]
MIP/AuNP/RGO/GCE	10^{-4}-10^{-3}	1.9×10^{5} ($\mu A.L.mol^{-1}$)	Sugarcane Vinasse	[41]

*CNT: Carbon nanotube, MWCNTs: multi-walled carbon nanotube, Si/ITO: silicon/indium tin oxide, AuNNs: gold nanopine needles, rGO: reduced graphene oxide, AuNPs: gold nanoparticles, ERGO: electrochemically-reduced graphene oxide, PAH: poly (allylamine hydrochloride), SPE: screen-printed electrode, GCE: glassy carbon electrode, Chit: chitosan, GA: glutaraldehyde, Gink: graphite ink solution, PD: 1,10-Phenan-throline-5,6-dione, MIP: molecularly imprinted polymers

Concept of the Biosensor	Linear Range (µM)	Sensitivity ($\mu A.\mu M^{-1}.cm^{-2}$)	Sample Type	Reference
Nano-MIP/MWCNTs-based membrane electrode	1-100000	-	Milk and Yogurt	[42]
Ag/AgCl, Pt electrode and Pt wire	50-350	0.04	Foodborne Presumptive LAB Metabolite	Present Study

*CNT: Carbon nanotube, MWCNTs: multi-walled carbon nanotube, Si/ITO: silicon/indium tin oxide, AuNNs: gold nanopine needles, rGO: reduced graphene oxide, AuNPs: gold nanoparticles, ERGO: electrochemically-reduced graphene oxide, PAH: poly (allylamine hydrochloride), SPE: screen-printed electrode, GCE: glassy carbon electrode, Chit: chitosan, GA: glutaraldehyde, Gink: graphite ink solution, PD: 1,10-Phenan-throline-5,6-dione, MIP: molecularly imprinted polymers

According to Table 2., Weber and co-workers developed two different biosensors for lactate determination prepared using carbon nanotube lactate oxidase electrodes and used the dripping method that was similar to present study. Furthermore, in that study determination range of the biosensors were found between 1000–4000 and 10000-5000 µM [33]. The fact that, the LOD of the presented paper is much lower than the mentioned study. Thus, even there is very low amount of lactate is presented in a sample, it can also be detected. Similarly, Yu and co-workers developed gold nanostructure-programmed flexible electrochemical biosensor for detection of glucose and lactate in sweat, the limit of detection in sweat was determined as 54 µM which was higher than the current study even using gold nano pin needles [43].

Dagar and Pundir (2017) were constructed an amperometric L-lactate biosensor in which LOx was immobilized on carboxylated multiwalled carbon nanotubes (cMWCNT)/copper nanoparticles (CuNPs)/polyaniline (PANI) hybrid film electrodeposited modified pen graphite electrode(PGE). It was stated that this developed biosensor had a very wide determination range like 1 µM - 2500 µM and it was associated that; the pencil graphite electrode had a quite large surface area [44] however, the preparation difficulties is main disadvantage for this method.

Hashemzadeh and co-workers developed a novel amperometric biosensor, which had a wide linear range 50–100.000 µM of lactate with high electrochemical sensitivity (35.3 $\mu AmM^{-1}.cm^{-2}$) and LOD of 2.3 mM. This was associated with utilizing electrodes modified with a composite prepared from rGO, CNTs, and gold nanoparticles. According to the study, the triple composite components showed synergistic effects on the enzyme loading, electrocatalytic activity, and electron transfer between receptor and electrode surface. Thus, developed nanomaterial that precluded parasites has gained a priority to the study [45]. Similarly, Istrate et al., developed a novel biosensor for L-lactate determination in dairy products and wine samples by using the screen-printed electrode modified with a ternary composite based on gold nanoparticles, electrochemically-reduced graphene oxide, and poly (allylamine hydrochloride). Then, the LDH enzyme was immobilized on the top of the electrode by crosslinking with glutaraldehyde.

Furthermore, in that study linearity and ranges of the biosensor were found between 500-3000, 4000-16000 µM, and sensitivity were found as 1.08, 0.28 $\mu A.mM^{-1}.cm^{-2}$ [46].

A lactate biosensor was fabricated using a chitosan and crosslinker configuration realized solution mediation ($K_3Fe(CN)_6$) in the study of Halpin et al. (2021). Moreover, the linear range of the biosensor was 990-5660 µM, and sensitivity was $1.44\times10^{-3}C.cm^{-2}.mM^{-1}$. Then, they used the heterocyclic quinoid species1,10-phenanthroline-5,6-dione for acting as a proton and electron acceptor concerning $FADH_2$ cofactor regeneration. Besides, graphite ink was formulated and utilized as an underlying conductive layer for the enzyme immobilization and enzymatic polymerization of 1,10-phenanthroline-5,6-dioneata graphite carbon electrode. Therefore, the linear range of the biosensor had become 740-2440 µM and sensitivity was $4.11\times10^{-4}C.cm^{-2}.mM^{-1}$. Thus, the study indicated that the novel surface-confined mediator had gained an advantage over the biosensor for lactate sensing [47]. However, with the higher sensitivity and lower limit of detection values of the biosensor, the limit of range was so high. Therefore, the biosensor developed in presented study could be more favorable for lactate determination, especially in low amounts.

The determination range of biosensors varies according to electrode surface used or the application of nanomaterial membrane or interface to the electrode surface [44, 48–50]. It can be concluded that the sensor, which was designed without using an interface and been surface controlled in this study, was a biosensor with a suitable determination range for use in lactate determination.

Conclusion

In the present study, an electrochemical enzyme-based lactate biosensor with a linear range of 50-350 µM, LOD as 31 µM and a sensitivity of 0.04 $\mu A.\mu M^{-1}.cm^{-2}$ was constructed. The practical applicability of the sensors was evaluated by detecting lactate in metabolite produced by food-borne LAB which is the unique part of the study not to the used metabolite of LAB as substrate (in real sample) before. Apart from these, this has been demonstrated by the current study that the analysis time for the developed biosensor was measured less than 3 minutes for each sample and did not require long pre-treatment. So, the study has shown that amperometric enzyme-based biosensors are the significant alternative for the detection of lactate instead of classical methods. It is showed that improving an interface for the biosensor utilizing a modification of a composite or immobilization of a mediator or enzymes into the catalytic layer to design a interference-free measuring system can be useful by the other studies. The results shared in this study are important part of improving lactate biosensors appealing to the industry and research laboratories.

Declarations

Compliance with Ethical Standards

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Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Statements & Declarations

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Authors' contributions

All authors contributed to the study conception. Evrim Gunes Altuntas, Mehmet Altay Unal, Mehmet Gumustas and Sibel A. Ozkan designed the research. Material preparation, data collection and analysis were performed by Ozum Ozoglu, Aytekin Uzunoglu and Mehmet Gumustas. The first draft of the manuscript was written by Ozum Ozoglu and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

All of the authors declare that they have no conflict of interest.

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Figures

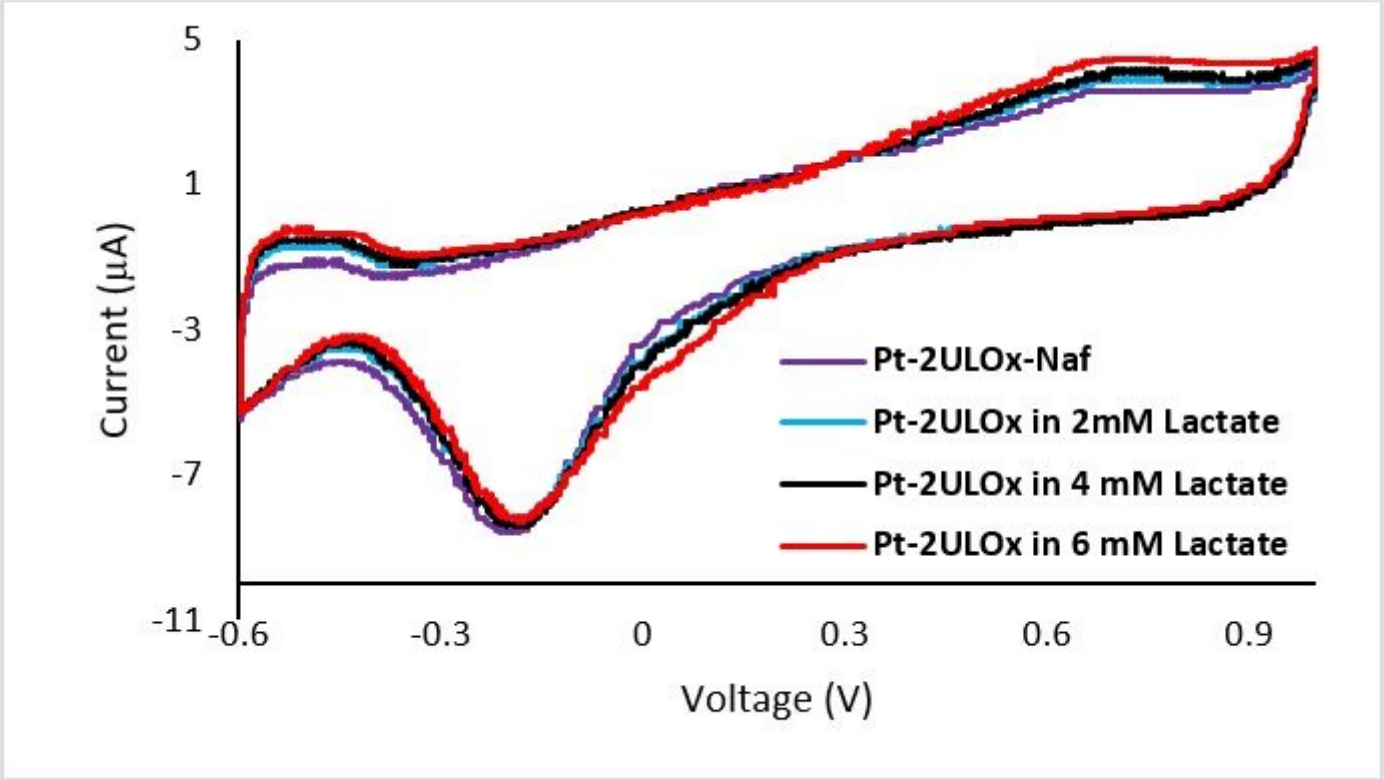

Figure 1

CV curves in lactate concentrations ranging from 2 mM to 6 mM in PBS solution. A potential range of -0.6 V-0.9 V was applied to the working electrode at a scan rate of 100 mV/s.

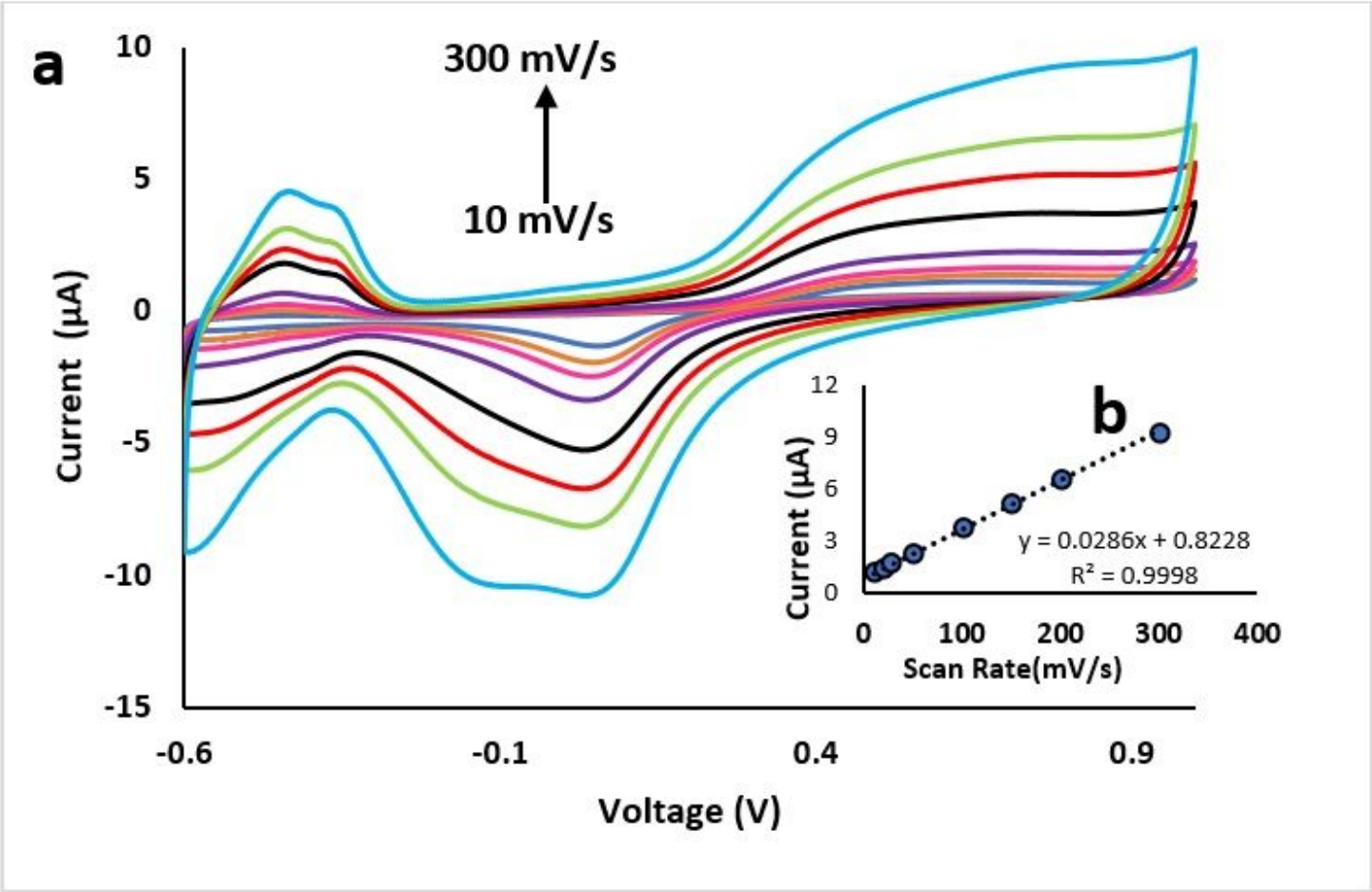

Figure 2

The effect of scan rates changes between 10-300 mV/s on electrochemical properties of the biosensors according to CV results. **a:** Change of current according to -0.6 V-0.9 V voltage potential in CV measurements. **b:** Connection of change in current of the peak with scan rate.

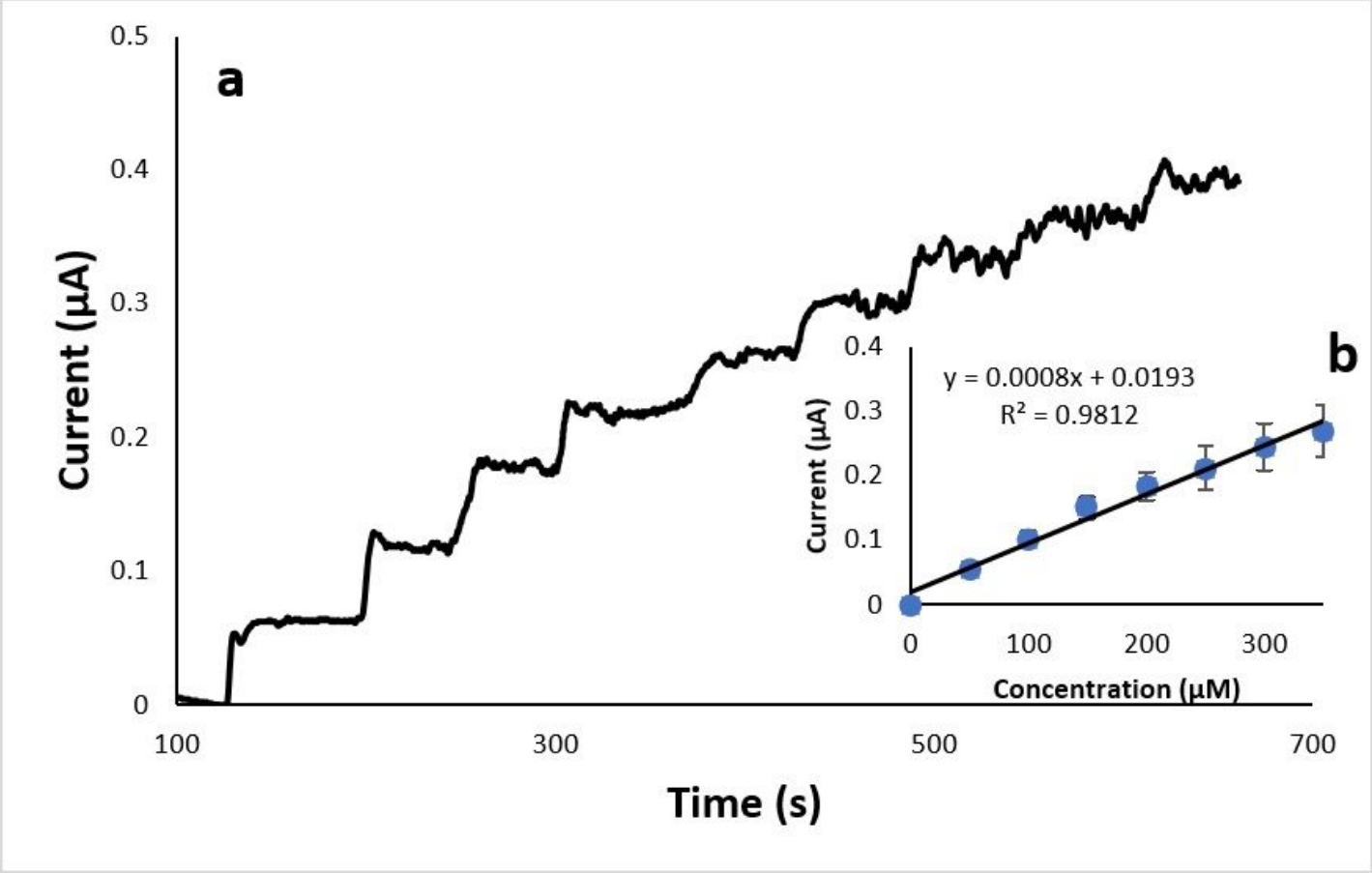

Figure 3

Chronoamperometric response of the sensor upon successive addition of 50 µM lactic acid at 0.6 V(a), The linear calibration curves between the current and the concentration of lactic acid. (b) (n=3)