Uzunoglu, JOTCSA. 2017; 4(3): 855-868. **RESEARCH ARTICLE**

JOURNAL OF THE TURKISH CHEMICAL SOCIETY
SECTION: A
CHEMISTRY

The Use of CeO_2-TiO_2 Nanocomposites as Enzyme Immobilization Platforms in Electrochemical Sensors

Aytekin Uzunoglu

Alaca Avni Celik Vocational School, Hitit University, Alaca, Corum, 19600, Turkey

Abstract: The use of metal oxide-based nanoparticles plays a key role in the development of electrochemical sensors with superior properties such as high sensitivity, wide linear range, low limit of detection, and extended storage stability. In this work, we aimed to synthesize CeO_2-TiO_2 mixed metal oxide nanoparticles which were used as substrate materials for the immobilization of bio-recognition element for the construction of enzyme-based electrochemical sensors. For this purpose, in the first part of the study, CeO_2-TiO_2 nanoparticles were prepared via a low-temperature co-precipitation method and characterized using X-ray Diffraction (XRD), N_2-adsorption, and Transmission Electron Microscopy (TEM) methods. The XRD results confirmed the successful synthesis of CeO_2-TiO_2 mixed metal oxide nanoparticles with the average crystallite size of 8.51 nm. The calculated crystallite size value was compatible with that obtained from the TEM images. The N_2 adsorption results revealed a large surface area of 78.6 $cm^2\ g^{-1}$ which is essential for the construction of electrochemical sensors with improved performance. The electrochemical sensors were developed by the deposition of nanoparticles on the surface of a Pt electrode, followed by the immobilization of lactate oxide enzyme. The electrochemical performance of the sensors was evaluated by cyclic voltammetry (CV) and chronoamperometry methods. The constructed sensors showed a sensitivity of $0.085 \pm 0.008\ \mu A\ \mu M^{-1}\ cm^{-2}$ (n=5) with a high reproducibility (RSD % = 1.3) and a wide linear range (0.02-0.6 mM). In addition, the detection limit towards lactate was found be 5.9 μM. The results indicated that the use of CeO_2-TiO_2 nanoparticles used as a modifier on the surface of the Pt electrode enabled the construction of electrochemical lactate sensors with high sensitivity.

Keywords: Electrochemical sensor; biosensor; ceria; titanium dioxide; metal oxide; nanoparticle.

Submitted: July 10, 2017. **Accepted:** August 09, 2017.

Cite this: Uzunoglu A. The Use of CeO2-TiO2 Nanocomposites as Enzyme Immobilization Platforms in Electrochemical Sensors. JOTCSA. 2017;4(3):855–68.

DOI: 10.18596/jotcsa.327686.

***Corresponding author. E-mail:** aytekinuzunoglu@hitit.edu.tr, aytekingyte@gmail.com.

INTRODUCTION

The detection of lactate level in the human blood has gained much attention in the last couple of years since its level can be used an indicator for some pathological conditions including cardiogenic or endotoxic shocks, respiratory failure, liver disease, systemic disorders, renal failure, and tissue hypoxia (1-3). Furthermore, its increased level in the blood may be associated with some medical conditions such as oxygen depletion in tissues, shocks, suffocation, CO intoxication, and liver diseases (4-6). As a result, the detection of lactate with high sensitivity and selectivity in a wide detection range is of keen interest. Lactate level in blood can be detected using various analytical methods such as liquid and gas chromatography, optical analysis, and chemical oxidation (4, 7, 8). These methods, however, pose many drawbacks related to their low sensitivity and selectivity and require expensive devices and chemicals for the analysis (9). Furthermore, these analytical methods are conducted by trained people (8, 9). Therefore, the development of new approaches circumventing the disadvantages above is in high demand. Electrochemical detection of lactate has many advantages over the conventional analytical methods due to enabling the detection of lactate with high sensitivity and selectivity by an inexpensive and fast manner (3, 9, 10). Therefore, the development of electrochemical sensors using novel sensing platforms is considered one of the most promising solutions for the elimination of the disadvantages associated with the use of conventional analytical techniques (4, 11, 12).

After the discovery of the unique physicochemical properties of nanomaterials, they have started to be used as catalysts in various applications to achieve improved performance in different applications ranging from energy and sensing to electronic materials (13-17). By the use of nanoparticles in electrochemical sensors, it became possible to construct electrochemical sensors with enhanced electrochemical performance due to their unique physical, chemical, and catalytic properties (18, 19). Nanoparticles show very high electron transfer rate, strong adsorption capacity, and significant physical surface area compared to their bulk counterparts (20-22). CeO_2 nanoparticles have been widely used in catalysis applications owing to their large surface area and high catalytic property as well as their high oxygen storage capacity (23). The high oxygen storage capacity (OSC) is related to the high reduction and oxidation capability of CeO_2 nanoparticles without undergoing any significant volume change. CeO_2 nanoparticles can bind and release oxygen atoms and shift between Ce^{+4} and Ce^{+3} oxidation states reversibly under reductive and oxidative environments (24). CeO_2 and CeO_2-based nanomaterials have been successfully employed in the electrochemical sensors to achieve enhanced sensor performance (9, 10, 25-28). Ibrahim *et al*. (25), for example, developed electrochemical sensors using boron-doped CeO_2 nanocubes for the detection of xanthine and hypoxanthine. Likewise, Co-doped CeO_2 nanoparticles were used as an electrocatalyst for the detection of the xanthine and hypoxanthine level in meat samples (26). The results showed that the chemical modification of CeO_2 nanostructures enhanced the electrochemical performance significantly. In our previous works, we demonstrated that the modification of CeO_2 lattice with a second metal oxide system altered the physicochemical properties of the nanoparticles, which in turn resulted in an enhanced catalytic performance (9, 10, 24).

In the present work, we report on the construction of electrochemical lactate biosensors by exploiting the high electrocatalytic activity and large surface area of TiO_2-modified CeO_2 nanoparticles. There is no report showing the use of TiO_2-modified CeO_2 nanoparticles as electrocatalysts as well as enzyme immobilization platforms in electrochemical sensors. The mixed metal oxide nanoparticles were synthesized using a facile low-temperature chemical precipitation method. The obtained nanoparticles were characterized using XRD, TEM, and N_2-adsorption methods. The electrochemical performance of the sensors was examined using CV and chronoamperometry techniques to determine the sensitivity, limit of detection (LOD), and linear detection range. The obtained results were compared with recently published works to show the superior performance of our novel sensor design.

MATERIALS AND METHODS

Synthesis of Mixed Metal Oxide Nanoparticles

$TiCl_4$, (99.0 %) and NH_4OH (28-30%) were purchased from Fluka and Science Lab, respectively. $Ce(NO_3)_3xH_2O$ purchased from Aldrich was used as the CeO_2 precursor. Lactate oxidase (from pedioccoccus species) and l-Lactic acid were obtained from Sigma-Aldrich. The chemicals were used as received.

The mixed metal oxide nanoparticles were synthesized using a chemical precipitation method explained in our previous work (24). Briefly, 75 mL of Ce^{4+} and 25 mL of Ti^{4+} solutions were mixed in a beaker under magnetic stirring to obtain a homogeneous precursor solution. Then, the reducing agent, NH_4OH, was added to the solution dropwise and the resulting mixture was left for stirring for 1 h at room temperature. Subsequently, the solution containing CeO_2-TiO_2 precipitates was left for aging overnight undisturbed. To obtain the mixed metal oxide nanoparticles, the suspension was centrifuged and washed multiple times. The obtained slurry was dried at 100 °C overnight. The nanoparticles were calcined at 500 ℃ for 5h under ambient atmosphere.

The XRD data of the samples were recorded using a Rigaku Ultima IV diffractometer at the scan rate of 2° min^{-1}. Cu Kα was used as the radiation source, and the scan was conducted between the 2θ values of 20° and 70°. The average crystalline size of the nanoparticles was calculated using (111) peak. The TEM images of the samples were taken using an FEI-Technai transmission electron microscope operating at 200 kV. The BET surface area of the mixed metal oxide nanoparticles was measured using an Autosorb – 6B surface area analyzer. Before the experiment, the samples were degassed at 300 °C for 3 h under N_2 atmosphere. The XRD and BET experiments were conducted at the Central Laboratory at Middle East Technical University (METU).

Electrochemical Study

The surface of the working electrode (Pt electrode with the diameter of 3 mm, obtained from BASi, West Lafayette, IN) was cleaned with Al_2O_3 slurries with the particles sizes of 1, 0.3, and 0.05 μm until a mirror-like surface was obtained. To modify the surface of the Pt electrode, 4µl of CeO_2-TiO_2 mixed metal oxide-containing suspension (10 mg ml^{-1}) was dropped on the surface and left for drying at room temperature. Then, 2U of lactate oxidase (LOx) solution was drop-casted on the modified surface and left for drying. Subsequently, the surface of the electrode was covered with a thin Nafion layer to prevent both enzyme leakage and possible interference effects of electroactive species. The constructed sensor was named as Pt/CeO_2-TiO_2/LOx/Nf.

The electrochemical performance of the sensors was evaluated using a 3-electrode electrochemical cell. 0.01 M PBS solution with the pH of 7.4 was used as the electrolyte. The experiments were carried out using a Biologic SP-150 potentiostat. Pt wire and Ag/AgCI electrodes were used as counter and reference electrodes, respectively. The term of sensitivity employed in the manuscript defines the surface area-normalized sensitivity values.

RESULTS AND DISCUSSION

Physical Characterization of the Nanoparticles

The XRD pattern of CeO_2-TiO_2 metal oxide system is shown in Figure 1. The peaks appeared at 28.57°, 33.14°, 47.52°, 56.46° and 59.20° are associated with the typical reflection of CeO_2 fluorite structure (JCPDS 34-0394). The XRD result revealed no additional peak related to TiO_2 phase. This indicated that Ti^{4+} ions introduced into the CeO_2 lattice formed a substitutional solid solution which is beneficial for enhanced physicochemical property. The broad XRD peaks indicated the nanoscale crystalline size of the metal oxide system. The lattice parameter of the mixed metal oxide system was calculated as 5.41 Å. The average crystalline size of the mixed metal oxide nanoparticles calculated using Scherer's Equation was found to be 8.51 nm.

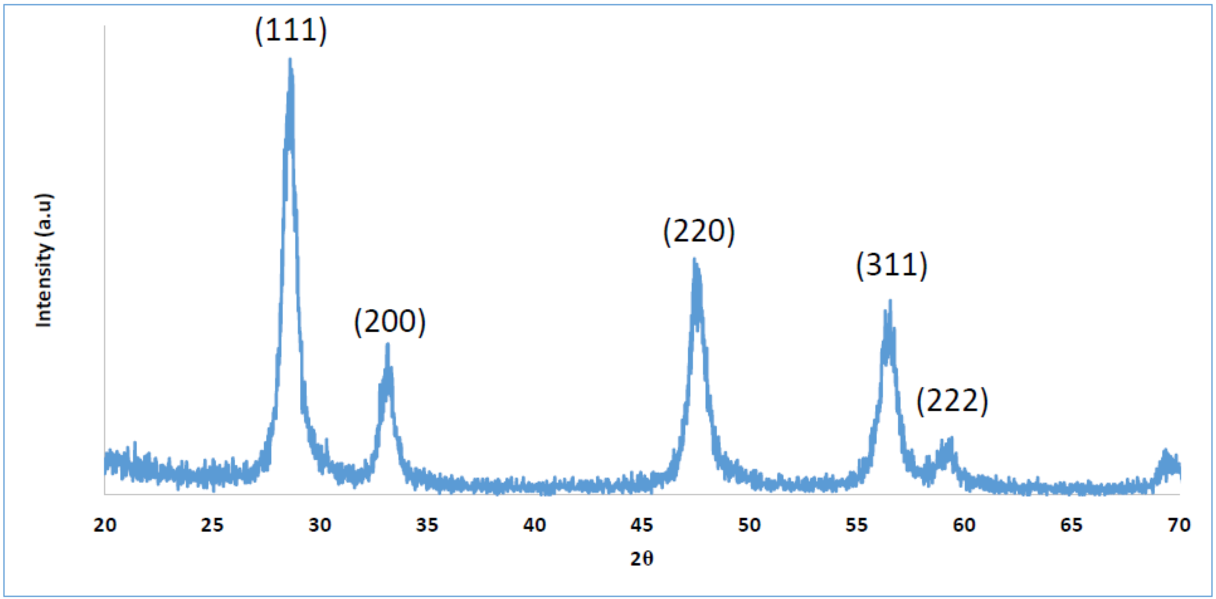

Figure 1: XRD pattern obtained from CeO_2-TiO_2 nanoparticles.

Representative TEM images are shown in Figure 2. As shown in the images, the synthesized nanoparticles had a spherical shape. The average crystallite size calculated from TEM images was found to be 10.2 ± 2.2 nm, which is in good agreement with the XRD result. In accordance with the small crystalline size, a large BET surface area of 78.6 $m^2 g^{-1}$ was obtained from the N_2-adsorption method. If these results are compared with those obtained from pristine CeO_2 (55.7 $m^2 g^{-1}$), which was reported in our previous study (24), it was seen that the modification of CeO_2 lattice with Ti^{4+} ions yielded a larger surface area.

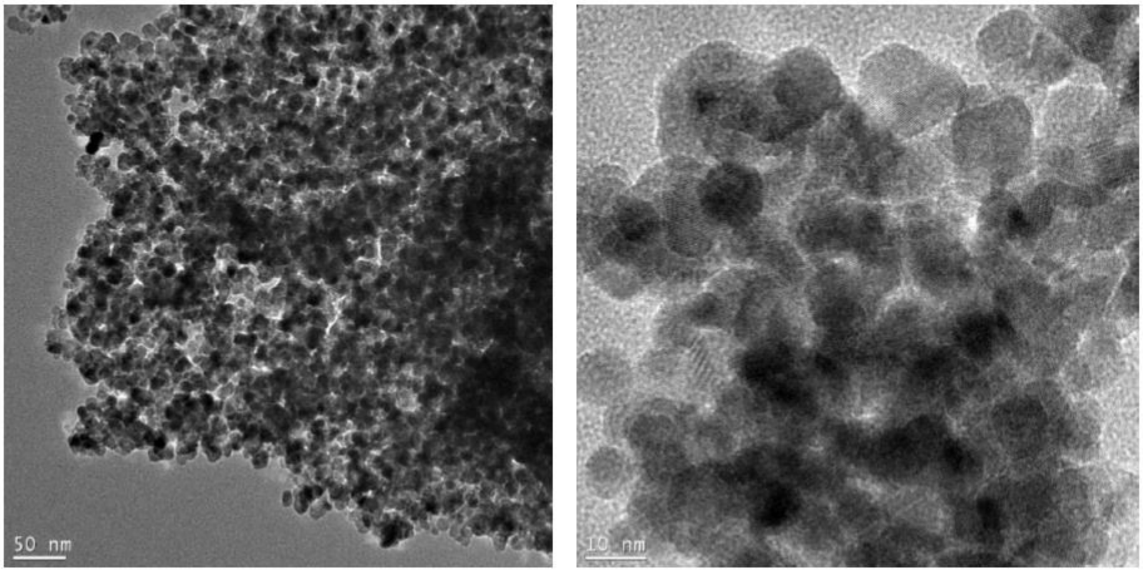

Figure 2: TEM images of CeO2-TiO2 nanoparticles taken at different magnifications.

Electrochemical Performance of the Constructed Electrochemical Lactate Biosensors

The electrochemical property of Pt/CeO_2-TiO_2/LOx/Nf biosensors was evaluated using cyclic voltammetry (CV) conducted in 0.01 M PBS. The CV curves of bare Pt and Pt/CeO_2-TiO_2/LOx/Nf electrodes recorded in the absence and presence of 5 mM lactate are shown in Figure 3. It was observed that bare Pt electrode did not display any change in the CV curve after the addition of 5 mM lactate into the electrolyte, indicating that Pt surface did not have any electrochemical activity towards lactate. On the other hand, when the surface of the electrode was modified with nanoparticles and the enzyme layer, the electrode displayed a significant increase in the oxidation current in the presence of 5 mM lactate. The oxidation started at around 0.3 V was due to the oxidation of H_2O_2 on the surface of the electrode. H_2O_2 oxidized on the electrode surface was produced from the enzymatic lactate reaction based on Equation 1 (29, 30). In addition, the oxidation reaction of H_2O_2 on the electrode surface is shown in Equation 2 (30). As a result, the CV curves confirmed that the constructed sensors showed a significant response to the addition of lactate into the PBS solution.

$$\text{L-Lactate} + O_2 \xrightarrow{LOx} \text{pyruvate} + H_2O_2 \qquad \text{(Eq. 1)}$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \qquad \text{(Eq. 2)}$$

The effect of the scan rate on the electrochemical performance of the sensors was evaluated by conducting the CV experiments at various scan rates between 10 and 300 mV s^{-1}. The scan rate vs. oxidation current graph is shown in Figure 4a. It was found that the oxidation current of Pt/CeO_2-TiO_2/LOx/Nf sensors measured at 0.8 V increased linearly with the increasing scan rate as shown in Figure 4b, demonstrating that the oxidation of H_2O_2 on the surface of the sensor is a surface controlled electrochemical reaction (9).

The performance of the constructed biosensors was evaluated using chronoamperometry method conducted in 0.01 M PBS at the pH of 7.4. The CV results of the sensors were taken into account in order to determine the optimum working potential. As shown in Figure 3, the oxidation of H_2O_2 started at the potential of 0.3 V, and a further increase in the applied voltage yielded higher oxidation current. Although the increase in the applied voltage resulted in a significant increase in the response current up to 0.6 V, further increase in the voltage did not affect the peak current to the same extent due to reaching a plateau at around 0.8 V. Therefore, the optimum working potential for the chronoamperometry experiments was chosen as 0.6 V. It is also noteworthy that another reason behind the selection of 0.6 V was to circumvent the use of high positive potential values where the interferents (i.e. ascorbic acid, uric acid, dopamine, etc.) become highly electroactive.

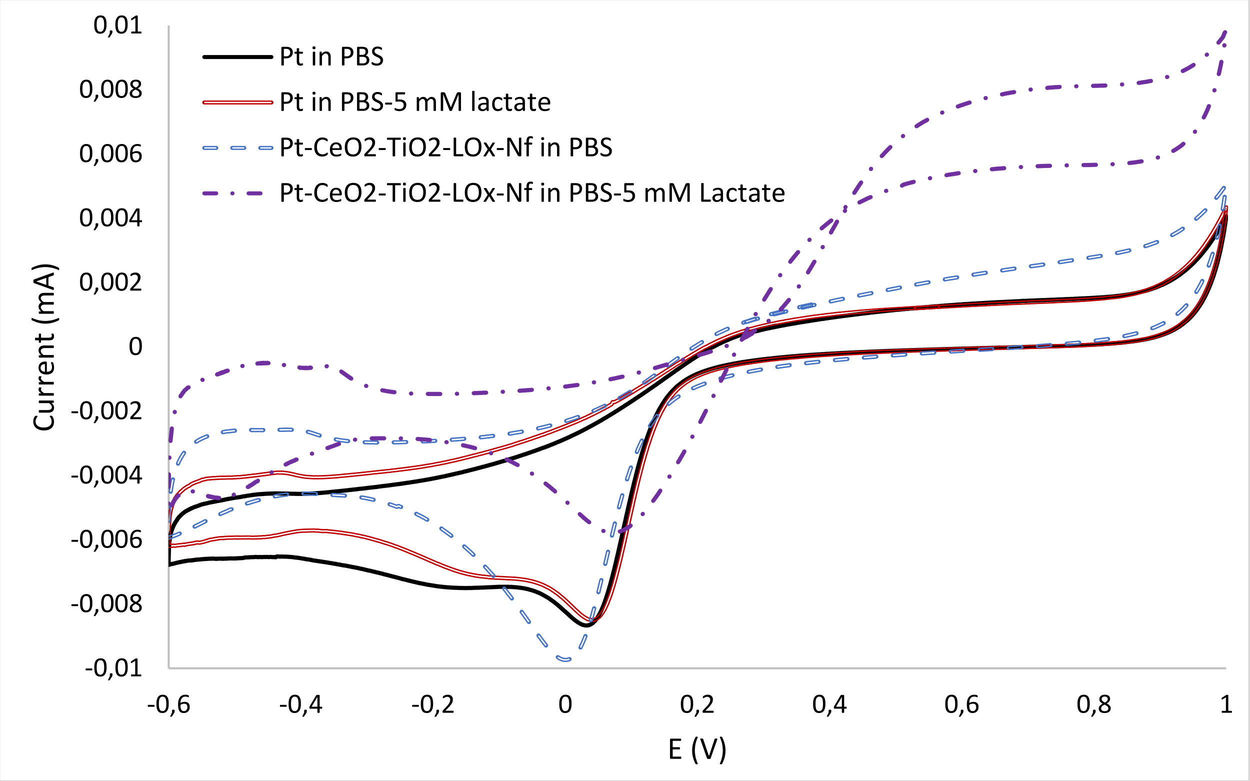

Figure 3: The CV curves of bare Pt and Pt/CeO2-TiO2/LOx/Nf in the absence and presence of 5 mM lactate (10 mV s-1, 0.01 M PBS, pH: 7.4).

The chronoamperometry results of Pt/CeO_2-TiO_2/LOx/Nf-based biosensors are shown in Figure 5. As seen from Figure 5a, when 20 μM of lactate was injected into the electrolyte, a fast increase in the current was observed, indicating the detection of lactate introduced into the PBS solution. The response current reached a constant value in a short amount of time (< 5s), which confirmed the short response time of the constructed sensors. The current-concentration graph obtained from successive addition of 20 μM of lactate is shown in Figure 5b. The sensitivity of the biosensors was found to be 0.085 ± 0.008 μA μM^{-1} cm^{-2} (n=5). In order to demonstrate the reproducibility of the biosensor fabrication process, the RSD % value was calculated using multiple biosensors (n=3). The results indicated an RSD % of 1.3 showing an excellent reproducibility of the fabrication process.

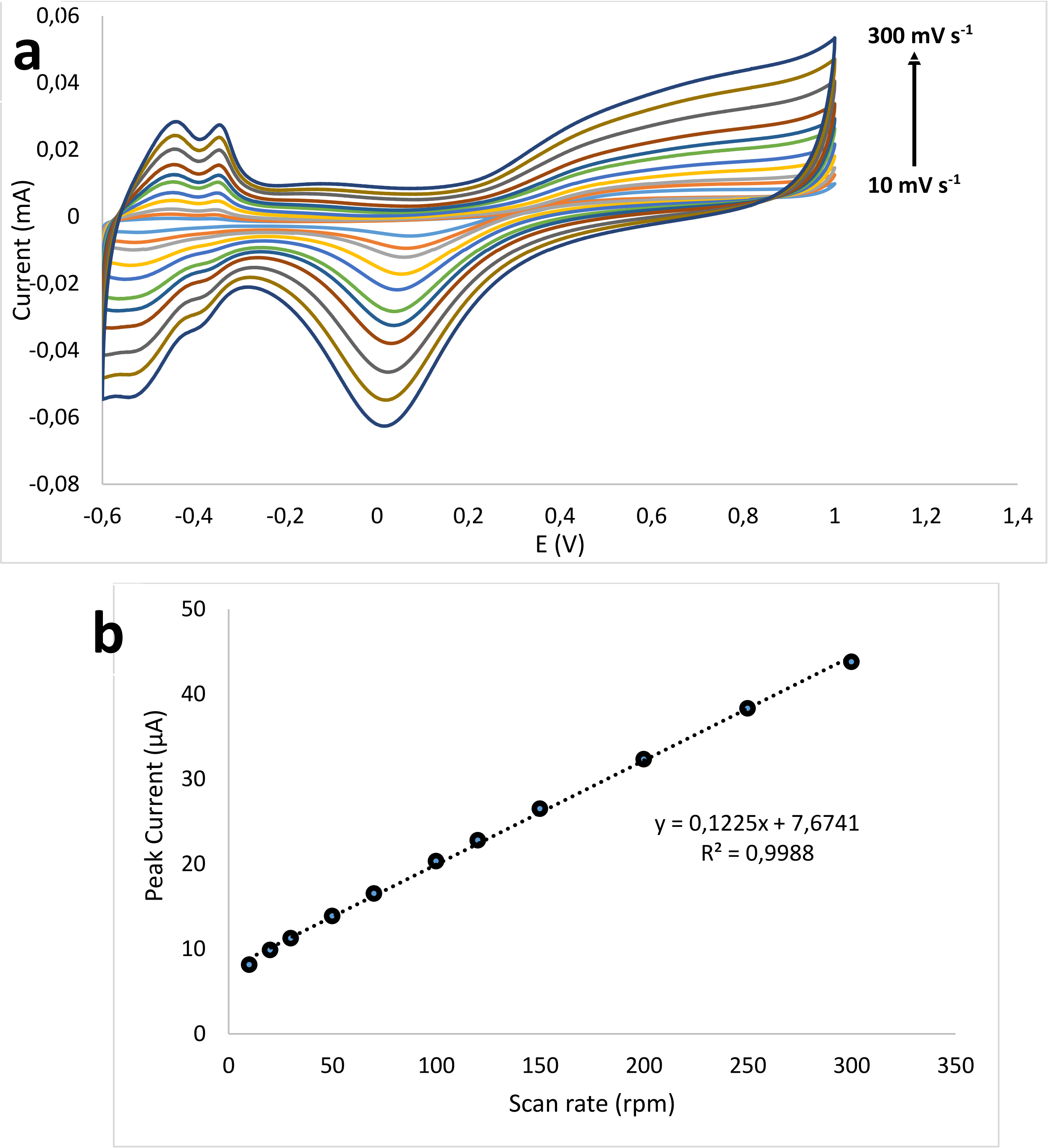

Figure 4: a) Effect of scan rate on the electrochemical performance of Pt/CeO2-TiO2/LOx/Nf biosensors, b) peak current vs. scan rate.

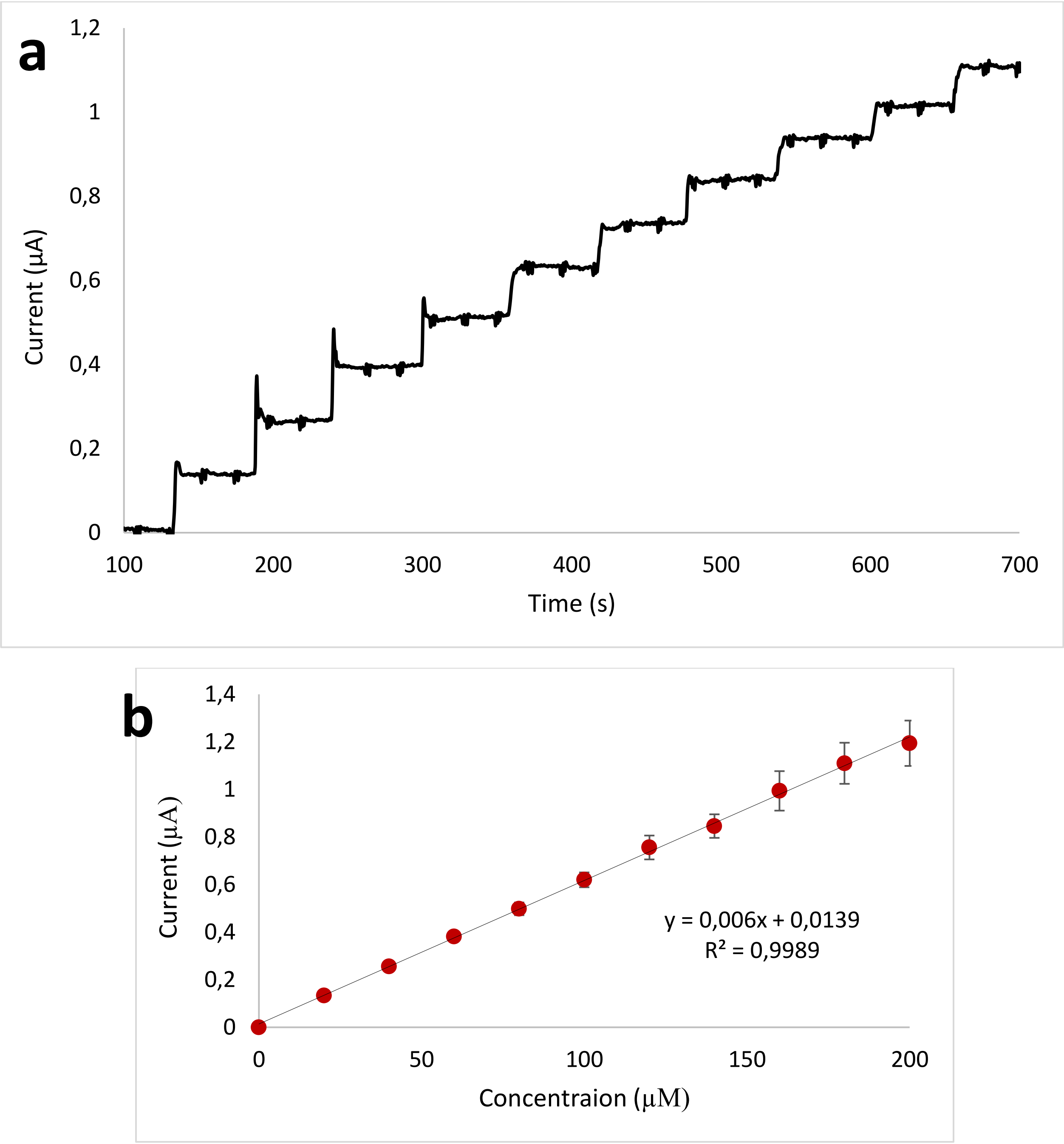

Figure 5: a) Amperometric response of a Pt/CeO2-TiO2/LOx/Nf biosensor to the successive addition of 20 µM lactate into the PBS solution at the working potential of 0.6 V, b) the corresponding current vs. concentration graph (n=5).

The linear range of the biosensors was determined by the successive addition of 50 µM lactate into the PBS solution, and the obtained I-t curve is shown in Figure 6. It was observed that the successive addition of 50 μM lactate produced a linear increase in the response current up to 0.6 mM. The further increase in the concentration, however, resulted in a deviation from linearity of sensor response. When the current-concentration graphs shown in Figure 5b and 6 are taken into account, it was concluded that the constructed biosensors showed a linear range between 20 μM and 600 μM towards the detection of lactate. The lowest lactate concentration which can be detected using Pt/CeO_2-TiO_2/LOx/Nf biosensors was calculated by the formula given in Equation 3 (10). The limit of detection of Pt/CeO_2-TiO_2/LOx/Nf biosensors was found to be 5.9 μM.

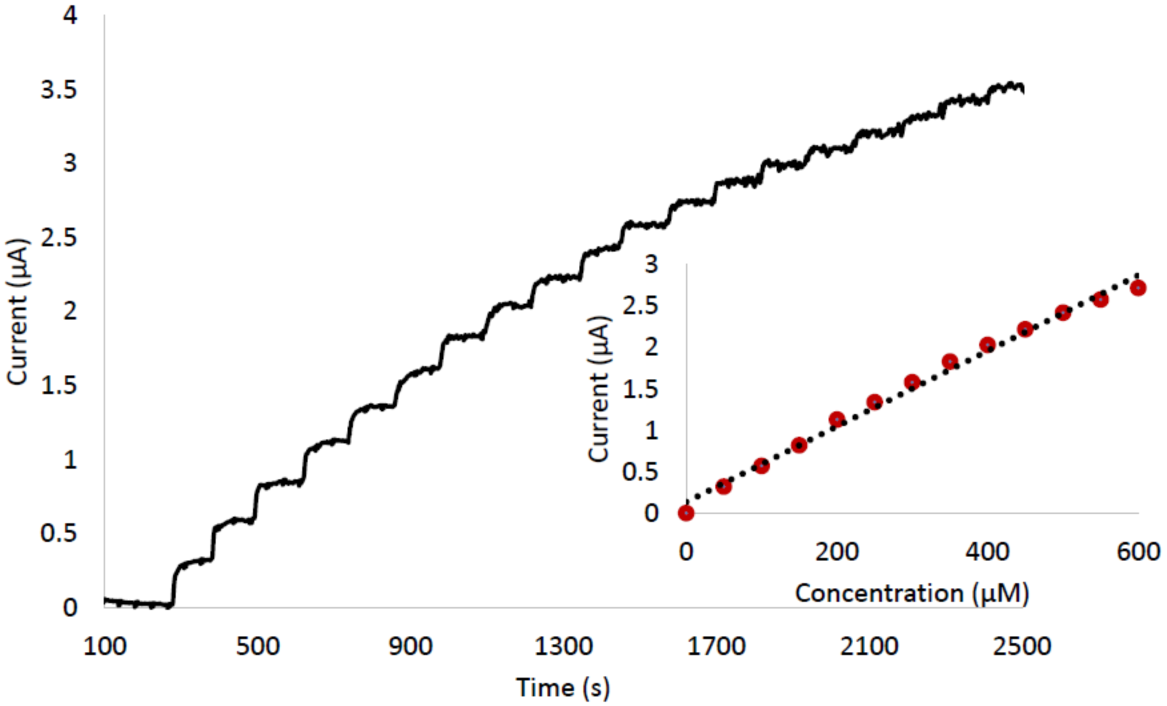

Figure 6: Amperometric response of Pt/CeO2-TiO2/LOx/Nf to the successive addition of 50 μM of lactate at the working potential of 0.6 V.

$$\text{Limit of Detection (LOD)} = 3.3 \times (\text{std}_{\text{background}} / \text{Sensitivity}) \qquad \text{(Eq. 3)}$$

The analytical performance of the designed Pt/CeO_2-TiO_2/LOx/Nf biosensors were compared with already reported lactate biosensors, and the results are listed in Table 1. The table shows that the use of CeO_2-TiO_2 mixed metal oxide nanoparticles with large surface area and high catalytic activity enabled the construction of electrochemical lactate biosensors with higher sensitivity and lower limit of detection compared to some of the other works (31-34). The novel sensor design reported in this work exhibited one of the highest sensitivity values among all. When the linear range of the sensors is compared, it was observed that our novel biosensor design had a similar linear range with others.

Table 1. Comparison of the performance of Pt/CeO_2-TiO_2/LOx/Nf biosensors with those recently published.

Sensor	Sensitivity (μA mM^{-1} cm^{-2})	Linear range (mM)	LOD (μM)	Ref.
LOx/DNPs/Au	4.0	0.05-0.7	15	(31)
LOx/ZnO-NRs/Au/Glass	N/A	0.1-1	0.1	(11)
LOx/DTSP/Au	1.49	up to 1.2	14	(32)
Nafion/LOx/Au/ZnO	24.56	0.01-0.6	6	(33)
LOx/ZnO-NWRs	15.6	0.012-1.2	12	(35)
Nafion/LOx/ CeO_2-CuO/Pt	89.3	0.02- 0.6	3.3	(9)
Pt/CeO_2-TiO_2/LOx/Nf	85.0	0.02- 0.6	5.9	This work

CONCLUSIONS

In this work, we designed novel electrochemical biosensors for the detection of lactate by exploiting the large surface area and high catalytic activity of CeO_2-TiO_2 mixed metal oxide nanoparticles. In the first part of the study, CeO_2-TiO_2 nanoparticles were synthesized successfully. The introduction of TiO_2 into the CeO_2 lattice formed a mixed metal oxide system, which was confirmed by the absence of any additional XRD peaks associated with the TiO_2 phase. The average crystalline size of the nanoparticles was found to be 8.51 nm, also confirmed by TEM images. The synthesized mixed metal oxide system revealed a large surface area of 78.6 m^2 g^{-1}, which can be attributed to the formation solid solution. Using the nanoparticles and LOx, Pt/CeO_2-TiO_2/LOx/Nf biosensors were constructed, and the performance of these sensors was analyzed using CV and chronoamperometry methods. The sensitivity of the sensors was found to be 0.085 ± 0.008 μA μM^{-1} cm^{-2} (n=5) with a high reproducibility (RSD % = 1.3). The sensors showed a linear range of 0.02-0.6 mM and a low LOD (5.9 μM). When the performance of the constructed sensors was compared with the literature, it was seen that one of the highest sensitivity values was obtained from our novel sensor design. The results suggested that CeO_2-TiO_2 mixed metal oxide nanoparticles are promising materials which can be successfully used to develop enzymatic electrochemical sensors with enhanced performance.

ACKNOWLEDGEMENT

The author expresses his gratitude to Prof. Dursun Ali Kose for his valuable supports.

REFERENCES

1. Meakins J, Long CNH. Oxygen consumption, oxygen debt and lactic acid in circulatory failure. Journal of Clinical Investigation. 1927;4(2):273-93.

2. Sayeed MM, Murthy PNA. ADENINE-NUCLEOTIDE AND LACTATE METABOLISM IN THE LUNG IN ENDOTOXIN-SHOCK. Circulatory Shock. 1981;8(6):657-66.

3. Rassaei L, Olthuis W, Tsujimura S, Sudholter EJR, van den Berg A. Lactate biosensors: current status and outlook. Analytical and Bioanalytical Chemistry. 2014;406(1):123-37.

4. Nikolaus N, Strehlitz B. Amperometric lactate biosensors and their application in (sports) medicine, for life quality and wellbeing. Microchimica Acta. 2008;160(1-2):15-55.

5. Taleat Z, Khoshroo A, Mazloum-Ardakani M. Screen-printed electrodes for biosensing: a review (2008-2013). Microchimica Acta. 2014;181(9-10):865-91.

6. Crawford SO, Hoogeveen RC, Brancati FL, Astor BC, Ballantyne CM, Schmidt MI, et al. Association of blood lactate with type 2 diabetes: the Atherosclerosis Risk in Communities Carotid MRI Study. International Journal of Epidemiology. 2010;39(6):1647-55.

7. Brinkert W, Rommes JH, Bakker J. Lactate measurements in critically ill patients with a hand-held analyser. Intensive Care Medicine. 1999;25(9):966-9.

8. Perez S, Sanchez S, Fabregas E. Enzymatic Strategies to Construct L-Lactate Biosensors Based on Polysulfone/Carbon Nanotubes Membranes. Electroanalysis. 2012;24(4):967-74.

9. Uzunoglu A, Stanciu L. Novel CeO2-CuO-decorated enzymatic lactate biosensors operating in low oxygen environments. Analytica Chimica Acta. 2016;909:121-8.

10. Uzunoglu A, Ramirez I, Andreasen E, Stanciu LA. Layer by layer construction of ascorbate interference-free amperometric lactate biosensors with lactate oxidase, ascorbate oxidase, and ceria nanoparticles. Microchimica Acta. 2016;183(5):1667-75.

11. Ibupoto ZH, Shah S, Khun K, Willander M. Electrochemical L-Lactic Acid Sensor Based on Immobilized ZnO Nanorods with Lactate Oxidase. Sensors. 2012;12(3):2456-66.

12. Uzunoglu A, Scherbarth AD, Stanciu L. Bimetallic PdCu/SPCE non-enzymatic hydrogen peroxide sensors2015; 220:[968-76 pp.].

13. Xing L, Yang F, Rasouli S, Qiu Y, Li ZF, Uzunoglu A, et al. Understanding Pt Nanoparticle Anchoring on Graphene Supports through Surface Functionalization. Acs Catalysis. 2016;6(4):2642-53.

14. Xin L, Yang F, Qiu Y, Uzunoglu A, Rockward T, Borup RL, et al. Polybenzimidazole (PBI) Functionalized Nanographene as Highly Stable Catalyst Support for Polymer Electrolyte Membrane Fuel Cells (PEMFCs). Journal of the Electrochemical Society. 2016;163(10):F1228-F36.

15. Yang F, Xin L, Uzunoglu A, Qiu Y, Stanciu L, Ilaysky J, et al. Investigation of the Interaction between Nafion lonomer and Surface Functionalized Carbon Black Using Both Ultrasmall Angle X-ray Scattering and Cryo-TEM. Acs Applied Materials & Interfaces. 2017;9(7):6530-8.

16. Uzunoglu A, Ahsen AS, Dundar F, Ata A, Ozturk O. Structural, electronic, and electrochemical analyses of sputter-coated Pt and Pt-Co/GCE electrodes with ultra-low metal loadings for PEM fuel cell applications. Journal of Applied Electrochemistry. 2017;47(2):139-55.

17. Karimi A, Othman A, Uzunoglu A, Stanciu L, Andreescu S. Graphene based enzymatic bioelectrodes and biofuel cells. Nanoscale. 2015;7(16):6909-23.

18. Albelda JAV, Uzunoglu A, Santos GNC, Stanciu LA. Graphene-titanium dioxide nanocomposite based hypoxanthine sensor for assessment of meat freshness. Biosensors & Bioelectronics. 2017;89:518-24.

19. Bas SZ. Gold nanoparticle functionalized graphene oxide modified platinum electrode for hydrogen peroxide and glucose sensing. Materials Letters. 2015;150:20-3.

20. Uzunoglu A, Siyi SY, Stanciu LA. A Sensitive Electrochemical H2O2 Sensor Based on PdAg-Decorated Reduced Graphene Oxide Nanocomposites. Journal of the Electrochemical Society. 2016;163(7):B379-B84.

21. Wang J. Nanoparticle-Based Electrochemical DNA Detection. Electrochemistry of Nucleic Acids and Proteins: Towards Electrochemical Sensors for Genomics and Proteomics. 2005;1:369-84.

22. Siangproh W, Dungchai W, Rattanarat P, Chailapakul O. Nanoparticle-based electrochemical detection in conventional and miniaturized systems and their bioanalytical applications: A review. Analytica Chimica Acta. 2011;690(1):10-25.

23. Trovarelli A. Catalytic properties of ceria and CeO2-containing materials. Catalysis Reviews-Science and Engineering. 1996;38(4):439-520.

24. Uzunoglu A, Zhang H, Andreescu S, Stanciu S. CeO2–MOx (M: Zr, Ti, Cu) mixed metal oxides with enhanced oxygen storage capacity Journal of Materials Science: Springer; 2015. p. 3750-62.

25. Ibrahim H, Temerk Y. A novel electrochemical sensor based on B doped CeO2 nanocubes modified glassy carbon microspheres paste electrode for individual and simultaneous determination of xanthine and hypoxanthine. Sensors and Actuators B-Chemical. 2016;232:125-37.

26. Lavanya N, Sekar C, Murugan R, Ravi G. An ultrasensitive electrochemical sensor for simultaneous determination of xanthine, hypoxanthine and uric acid based on Co doped CeO2 nanoparticles. Materials Science & Engineering C-Materials for Biological Applications. 2016;65:278-86.

27. Ensafi AA, Noroozi R, Zandi-Atashbar N, Rezaei B. Cerium(IV) oxide decorated on reduced graphene oxide, a selective and sensitive electrochemical sensor for fenitrothion determination. Sensors and Actuators B-Chemical. 2017;245:980-7.

28. Sun LF, Ding YY, Jiang YL, Liu QY. Montmorillonite-loaded ceria nanocomposites with superior peroxidase-like activity for rapid colorimetric detection of H2O2. Sensors and Actuators B-Chemical. 2017;239:848-56.

29. Zanini VIP, Tulli F, Martino DM, de Mishima BL, Borsarelli CD. Improvement of the amperometric response to L-lactate by using a cationic bioinspired thymine polycation in a bioelectrode with immobilized lactate oxidase. Sensors and Actuators B-Chemical. 2013;181:251-8.

30. Anzai J, Takeshita H, Kobayashi Y, Osa T, Hoshi T. Layer-by-layer construction of enzyme multilayers on an electrode for the preparation of glucose and lactate sensors: Elimination of

ascorbate interference by means of an ascorbate oxidase multilayer. Analytical Chemistry. 1998;70(4):811-7.

31. Briones M, Casero E, Petit-Dominguez MD, Ruiz MA, Parra-Alframba AM, Pariente F, et al. Diamond nanoparticles based biosensors for efficient glucose and lactate determination. Biosensors and Bioelectronics. 2015;68:521-8.

32. Gamero M, Pariente F, Lorenzo E, Alonso C. Nanostructured rough gold electrodes for the development of lactate oxidase-based biosensors. Biosensors & Bioelectronics. 2010;25(9):2038-44.

33. Zhao Y, Fang X, Gu Y, Yan X, Kang Z, Zheng X, et al. Gold nanoparticles coated zinc oxide nanorods as the matrix for enhanced L-lactate sensing. Colloids and Surfaces B: Biointerfaces. 2015;126:476-80.

34. Zhao YG, Yan XQ, Kang Z, Fang XF, Zheng X, Zhao LQ, et al. Zinc oxide nanowires-based electrochemical biosensor for L-lactic acid amperometric detection. Journal of Nanoparticle Research. 2014;16(5):9.

35. Zhao Y, Yan X, Kang Z, Fang X, Zheng X, Zhao L, et al. Zinc oxide nanowires-based electrochemical biosensor for L-lactic acid amperometric detection. Journal of Nanoparticle Research. 2014;16:2398.