



The Use of CeO₂-TiO₂ Nanocomposites as Enzyme Immobilization Platforms in Electrochemical Sensors

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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 CeO₂-TiO₂ 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, CeO₂-TiO₂ nanoparticles were prepared via a low-temperature co-precipitation method and characterized using X-ray Diffraction (XRD), N₂-adsorption, and Transmission Electron Microscopy (TEM) methods. The XRD results confirmed the successful synthesis of CeO₂-TiO₂ 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 N₂ adsorption results revealed a large surface area of 78.6 cm² 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 oxidase 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 ± 0.008 μA μM⁻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 to be 5.9 μM. The results indicated that the use of CeO₂-TiO₂ 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.

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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 as 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). CeO₂ 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 CeO₂ nanoparticles without undergoing any significant volume change. CeO₂ nanoparticles can bind and release oxygen atoms and shift between Ce⁺⁴ and Ce⁺³ oxidation states reversibly under reductive and oxidative environments (24). CeO₂ and CeO₂-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 CeO₂ nanocubes for the detection of xanthine and hypoxanthine. Likewise, Co-doped CeO₂ 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 CeO₂ nanostructures enhanced the electrochemical performance significantly. In our previous works, we demonstrated that the modification of CeO₂ 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 TiO₂-modified CeO₂ nanoparticles. There is no report showing the use of TiO₂-modified CeO₂ 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 N₂-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

TiCl₄, (99.0 %) and NH₄OH (28-30%) were purchased from Fluka and Science Lab, respectively. Ce(NO₃)₃·xH₂O purchased from Aldrich was used as the CeO₂ precursor. Lactate oxidase (from *pediococcus* 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 Ce⁴⁺ and 25 mL of Ti⁴⁺ solutions were mixed in a beaker under magnetic stirring to obtain a homogeneous precursor solution. Then, the reducing agent, NH₄OH, was added to the solution dropwise and the resulting mixture was left for stirring for 1 h at room temperature. Subsequently, the solution containing CeO₂-TiO₂ 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 °C 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 K α was used as the radiation source, and the scan was conducted between the 2 θ 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 N₂ 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 Al₂O₃ 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, 4 μl of CeO₂-TiO₂ 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/CeO₂-TiO₂/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/AgCl 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 CeO₂-TiO₂ 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 CeO₂ fluorite structure (JCPDS 34-0394). The XRD result revealed no additional peak related to TiO₂ phase. This indicated that Ti⁴⁺ ions introduced into the CeO₂ 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.

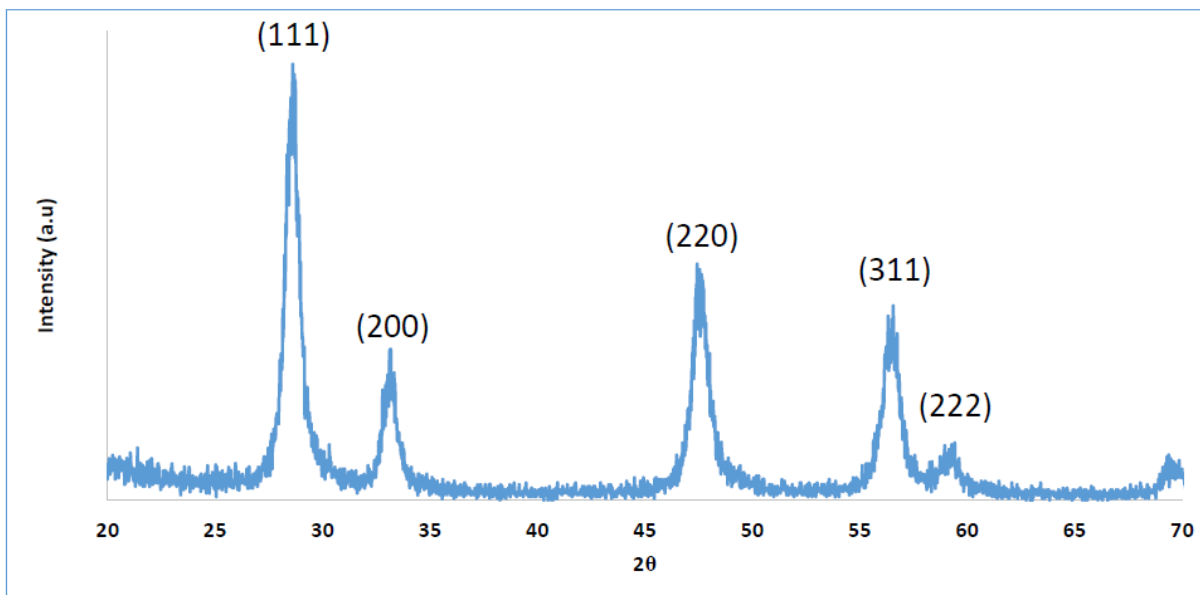


Figure 1: XRD pattern obtained from CeO₂-TiO₂ nanoparticles.

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 $78.6 \text{ m}^2 \text{ g}^{-1}$ was obtained from the N₂-adsorption method. If these results are compared with those obtained from pristine CeO₂ ($55.7 \text{ m}^2 \text{ g}^{-1}$), which was reported in our previous study (24), it was seen that the modification of CeO₂ lattice with Ti⁴⁺ ions yielded a larger surface area.

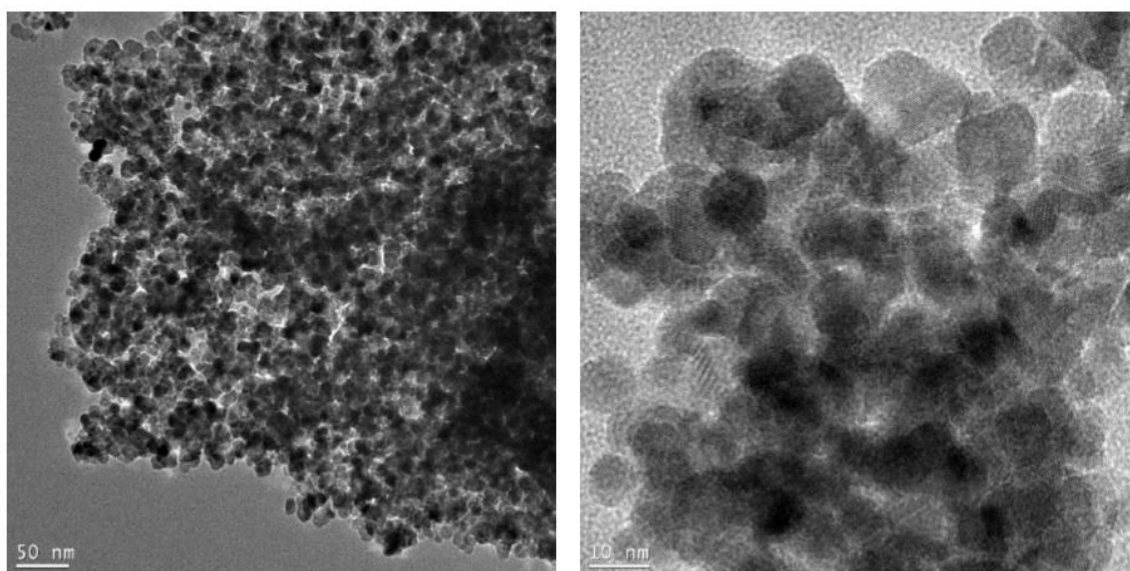
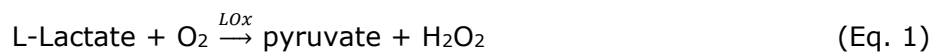


Figure 2: TEM images of CeO₂-TiO₂ nanoparticles taken at different magnifications.

Electrochemical Performance of the Constructed Electrochemical Lactate Biosensors

The electrochemical property of Pt/CeO₂-TiO₂/LOx/Nf biosensors was evaluated using cyclic voltammetry (CV) conducted in 0.01 M PBS. The CV curves of bare Pt and Pt/CeO₂-TiO₂/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 H₂O₂ on the surface of the electrode. H₂O₂ oxidized on the electrode surface was produced from the enzymatic lactate reaction based on Equation 1 (29, 30). In addition, the oxidation reaction of H₂O₂ 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.



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/CeO₂-TiO₂/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 H₂O₂ 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 H₂O₂ 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.

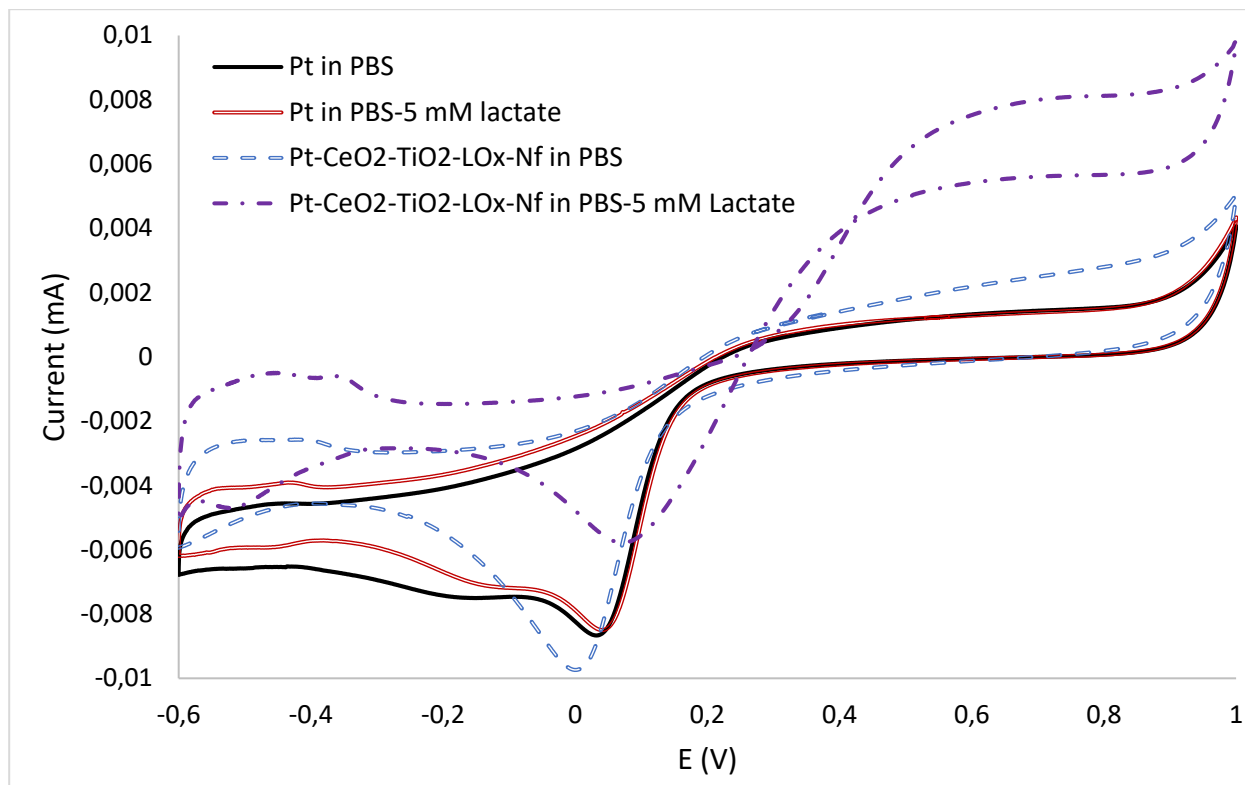


Figure 3: The CV curves of bare Pt and Pt/CeO₂-TiO₂/LOx/Nf in the absence and presence of 5 mM lactate (10 mV s⁻¹, 0.01 M PBS, pH: 7.4).

The chronoamperometry results of Pt/CeO₂-TiO₂/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 ($< 5\text{s}$), 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 $0.085 \pm 0.008 \mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$ ($n=5$). 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.

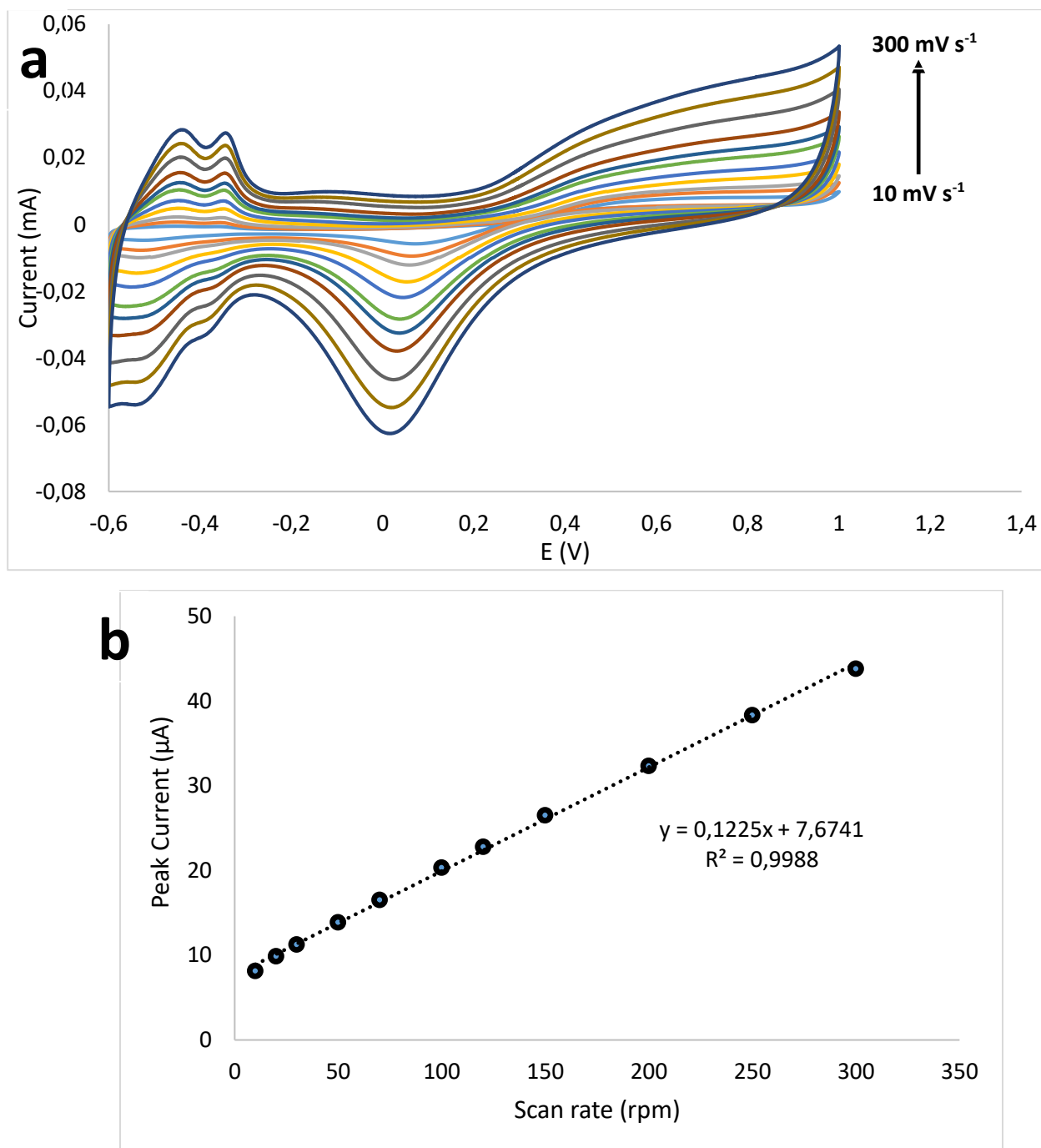


Figure 4: a) Effect of scan rate on the electrochemical performance of Pt/CeO₂-TiO₂/LOx/Nf biosensors, b) peak current vs. scan rate.

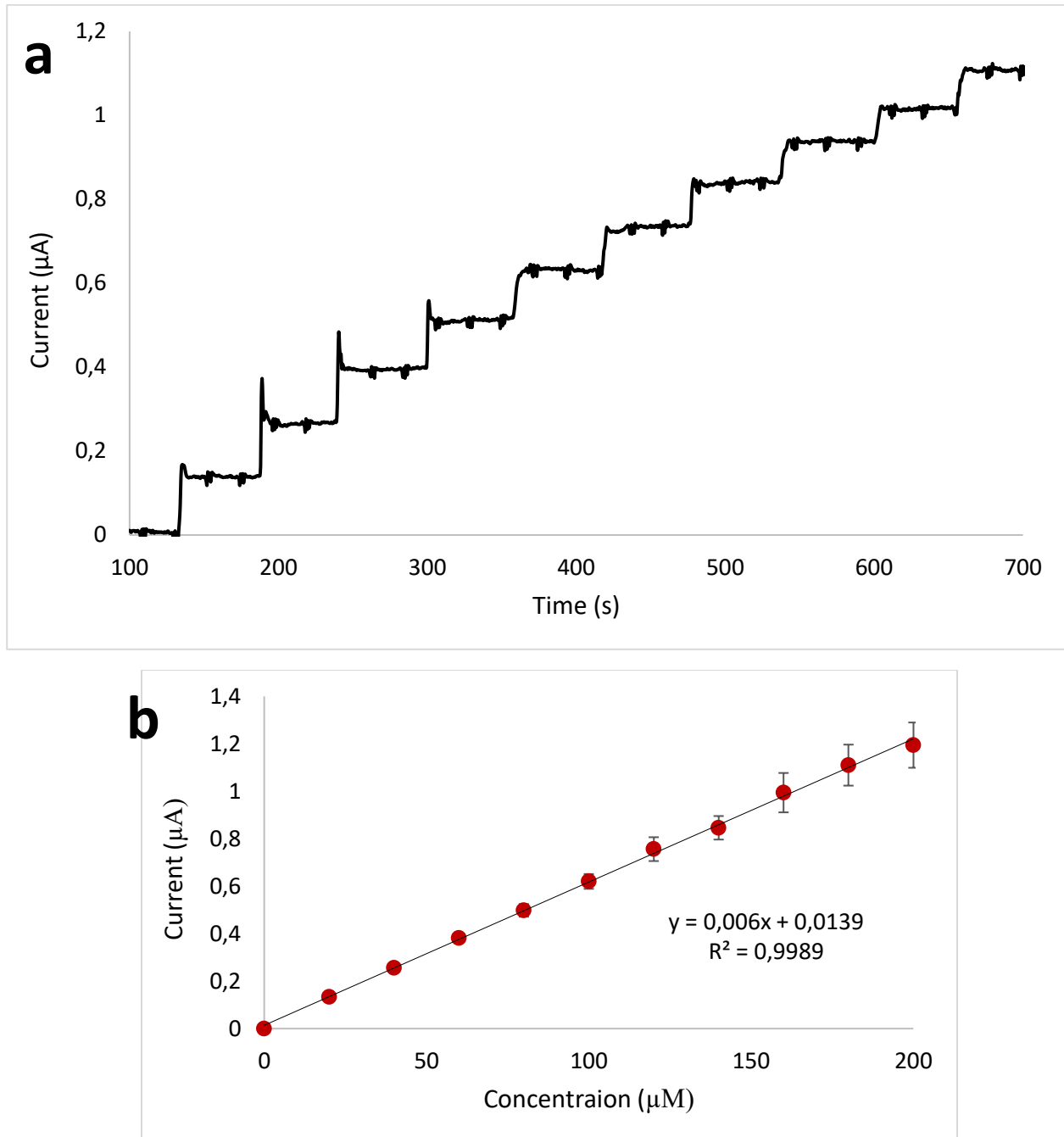


Figure 5: a) Amperometric response of a Pt/CeO₂-TiO₂/LOx/Nf biosensor to the successive addition of 20 μM lactate into the PBS solution at the working potential of 0.6 V, b) the corresponding current vs. concentration graph (n=5).

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/CeO₂-TiO₂/LOx/Nf biosensors was calculated by the formula given in Equation 3 (10). The limit of detection of Pt/CeO₂-TiO₂/LOx/Nf biosensors was found to be 5.9 μM .

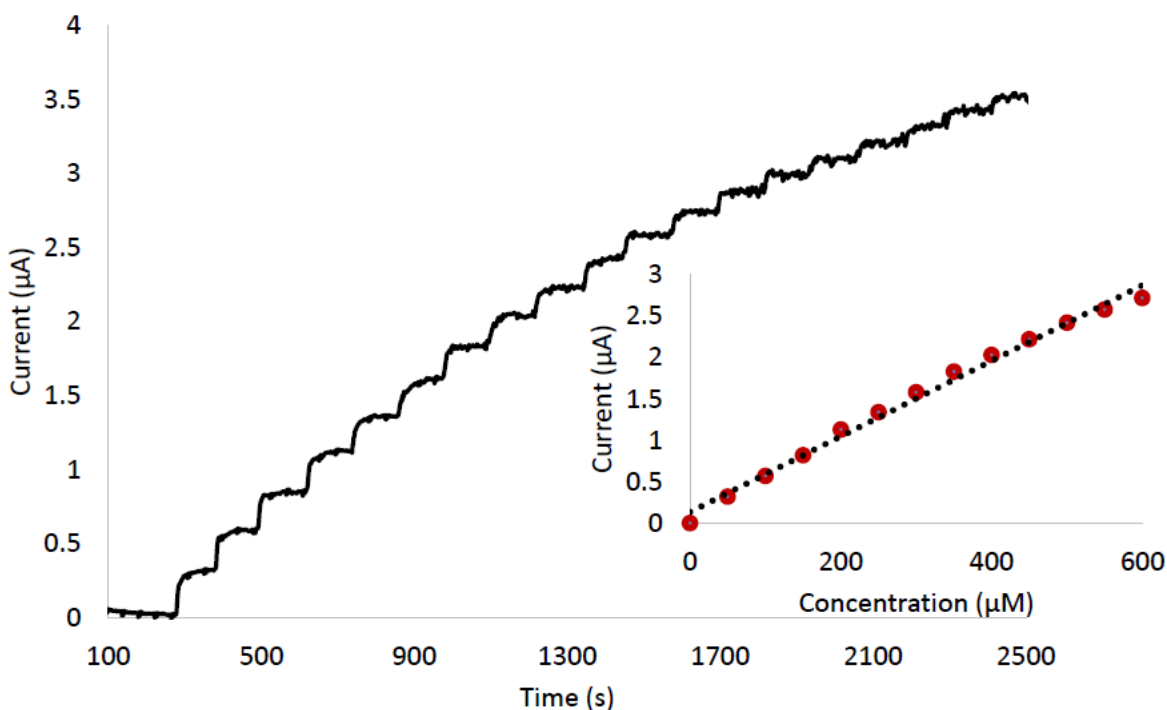


Figure 6: Amperometric response of Pt/CeO₂-TiO₂/LOx/Nf to the successive addition of 50 μM of lactate at the working potential of 0.6 V.

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

The analytical performance of the designed Pt/CeO₂-TiO₂/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 CeO₂-TiO₂ 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/CeO₂-TiO₂/LOx/Nf biosensors with those recently published.

Sensor	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-2}$)	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 CeO₂-TiO₂ mixed metal oxide nanoparticles. In the first part of the study, CeO₂-TiO₂ nanoparticles were synthesized successfully. The introduction of TiO₂ into the CeO₂ lattice formed a mixed metal oxide system, which was confirmed by the absence of any additional XRD peaks associated with the TiO₂ 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 m² g⁻¹, which can be attributed to the formation solid solution. Using the nanoparticles and LOx, Pt/CeO₂-TiO₂/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 $\mu\text{A } \mu\text{M}^{-1} \text{cm}^{-2}$ (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 CeO₂-TiO₂ mixed metal oxide nanoparticles are promising materials which can be successfully used to develop enzymatic electrochemical sensors with enhanced performance.

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