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Zinc Oxide nanoparticles based lactate oxidase biosensor for lactate detection in soy milk sample.

Pallavi Verma[1], Manvender Singh[1], Dr. Vikas Dhull[1*]
Department of Biotechnology, University Institute of Engineering and Technology, Maharshi Dayanand University, Rohtak, Haryana, India
[*]Corresponding Author
Dr. Vikas Dhull, Assistant Professor , Department of Biotechnology Engineering, University Institute Of Engineering & Technology, Maharshi Dayanand University, Rohtak- 124001, Haryana, INDIA
Email: v.nano87@gmail.com Institutional Email: vikasbtdhull.gf.uiet@mdurohtak.ac.in

Abstract
In the present work, we have synthesized zinc oxide nanoparticles chemically and characterized nanoparticles using various techniques. UV-visible spectroscopy successfully confirmed the optical activity of nanoparticles. The nano size of particles was confirmed using a particle size analyzer, which successfully confirmed that chemically synthesized nanoparticles are in the nano range. Further, the nanoparticles used for fabrication of the working electrode in a three-electrode system biosensor. The working electrode is comprised of nanoparticles and single-walled carbon nanotube paste. The electrode was characterized using scanning electron microscopy, which confirmed surface modification of the electrode. Then the electrode was optimized at various parameters such as optimal working pH, temperature, substrate concentration, cyclic voltammetry, etc. Finally, a biosensor was developed to detect lactate concentration in soy milk samples, which successfully determined lactate concentration.
Keywords: nanoparticle; single-walled carbon nanotube; biosensor; working electrode; lactate.

1. Introduction

Lactate is a biomolecule that is produced during anaerobic respiration and can function as a source of energy in the absence of oxygen in various living organisms. Detection of lactate can be used for illness diagnostics, in bioprocess engineering such as fermentation, and in real-time monitoring of various microorganisms such as bacteria, fungi, etc (D. Wang et al., 2023). L-lactate levels in the blood typically vary from 0.5 - 2.2 mmol/l which can rise to 12–25 mmol/l during vigorous exercise. Lactate detection plays an important role in clinical diagnosis of various diseases such as lactate acidosis, hepatic metabolism, hyperlactatemia, chronic disease etc(Pundir et al., 2016)(Kucherenko et al., 2019)(Rathee et al., 2016).Lactate detection not only plays an important role in the field of medicine but also in the food industry, such as in food preservation processes, fermentation processes in the cheese, bread, and beverage industries, conversion of malic acid into lactic acid in the bioprocess industry, etc(Pundir et al., 2016). Various methods that are used for the detection of lactate in real samples include colorimetic, chromatography (HPLC, size exclusion etc.), spectrophotometric approach, fluorometric method, enzymatic assay(Barker & Summerson, 1941)(Rahman et al., 2009)(Pundir et al., 2016).With the latest developments in the field of science and technology, biosensors are the best approach to detecting the presence of lactate in a given sample(Anand et al., 2022).A biosensor is a device that utilizes a biological component, such as enzymes or antibodies, in combination with a physicochemical transducer to detect, measure, and analyse a specific analyte in a sample. These devices have a wide range of applications in fields such as healthcare, environmental monitoring, and biodefense (Anand et al., 2022)(J. Wang, 2006).

2. Experimentation

2.1. Materials

LOD (L-Lactate Oxidase) taken from Sisco Research Laboratories Pvt. Ltd., Mumbai, India, zinc oxide nanoparticles (chemically synthesized self-made nanoparticles), single-walled carbon nanotubes taken from Sisco Research Laboratories Pvt. Ltd., India , glutaraldehyde, soy milk , and distilled water.

2.2. Methodology

2.2.1. Formation of Zinc Oxide Nanoparticles

Zinc sulphate ($ZnSO_4.6H_2O$) (the main zinc source) and oxalic acid ($H_2C_2O_4 \cdot 2H_2O$) (the precipitating agent) were used as precursors in the lab (for the preparation of zinc oxide nanoparticles. One molar solution of zinc sulphate ($ZnSO_4.6H_2O$) was created using 100 ml of distilled water and subjected to stirring on a magnetic stirrer for 16 minutes. In order to create a white colour suspension, oxalic acid ($H_2C_2O_4 \cdot 2H_2O$) was added to one molar solution of zinc sulphate solution drop wise at room temperature. The powder form of zinc oxide nanoparticles was obtained using a centrifuge (5000 rpm for 20 minutes) and drying at room temperature for a few days. Characterization of zinc oxide nanoparticles was done using UV spectroscopy, particle size analyzer and zeta potential(Mahmood et al., 2022)(Lee et al., 2013)(Gupta et al., 2021)(Mustapha et al., 2020).

2.2.2. Modification of surface of electrode

The surface of the gold electrode was modified using a mixture of 2.5 µL of zinc oxide nanoparticles (ZnO) and single-walled carbon nanotubes in glutaraldehyde and was subjected to a dry heat oven at 35°C for drying. A suspension was created using 6U lactate oxidase enzyme (powder form) and 0.1 mol L^{-1} phosphate buffer with a pH of 7.4. Now pipette out this solution on the surface of the modified gold electrode with the help of glutaraldehyde and then subject it to room temperature for drying. Characterization was done using a scanning electron microscope (Haghighi & Bozorgzadeh, 2011).

2.2.3. Cyclic Voltammetry

Cyclic voltammetry was performed using potentiostat (Autolab 302N potentiostat-galvanostat) which consist of three electrodes working electrode (gold electrode modified with zinc oxide nanoparticles and single-walled carbon nanotubes), reference electrode (Ag/AgCl), and counter electrode (platinum). Performed in the Central Instrumentation Laboratory, Centre for Biotechnology, Maharshi Dayanand University, Rohtak(Wang et al., 2011)(Schmitt et al., 2012).

2.3. Result and Discussions

2.3.1. Characterization of Zinc Oxide Nanoparticles

Fig.1 represents the UV graph of the zinc oxide nanoparticles. The absorbance of ZnO nanoparticles is obtained at 374 nm.

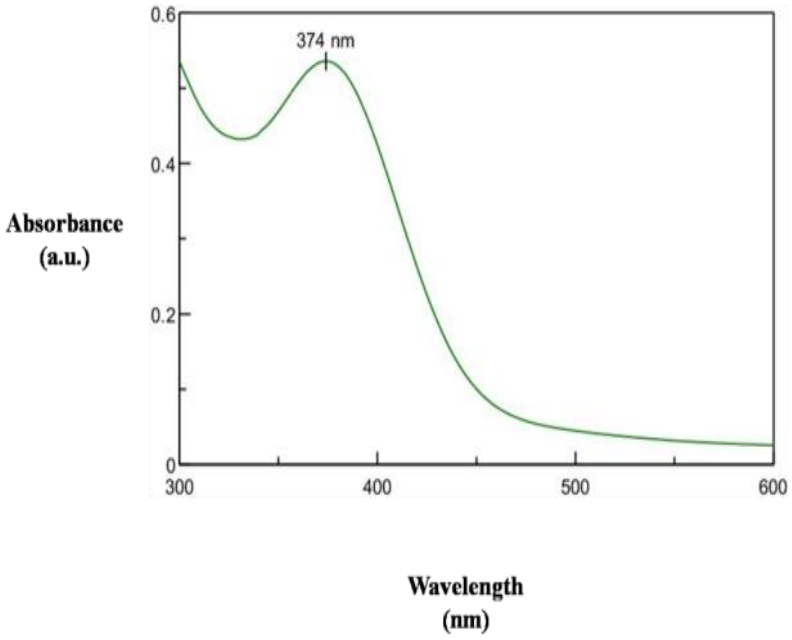

Figure 1: UV graph of ZnO Nanoparticles

Table 1 represents the particle size analysis of the zinc oxide nanoparticles. The z-average of the zinc oxide nanoparticles was obtained at 71.23 d.nm. Fig. 2 represents a graphical analysis of the particle size of the zinc oxide nanoparticles.

Table 1: PSA of Zinc Oxide Nanoparticles

Results		**Size (d.nm.)**		**% Intensity:**	**Standard Deviation (d.nm.)**
Zeta-Av. (d.nm) :	71.23	**Peak 1:**	75.75	92.8	48.13
Pre Delivery Intercept :	0.154	**Peak 2:**	6.922	7.2	1.907
Interception :	0.809	**Peak 3:**	0	0	0

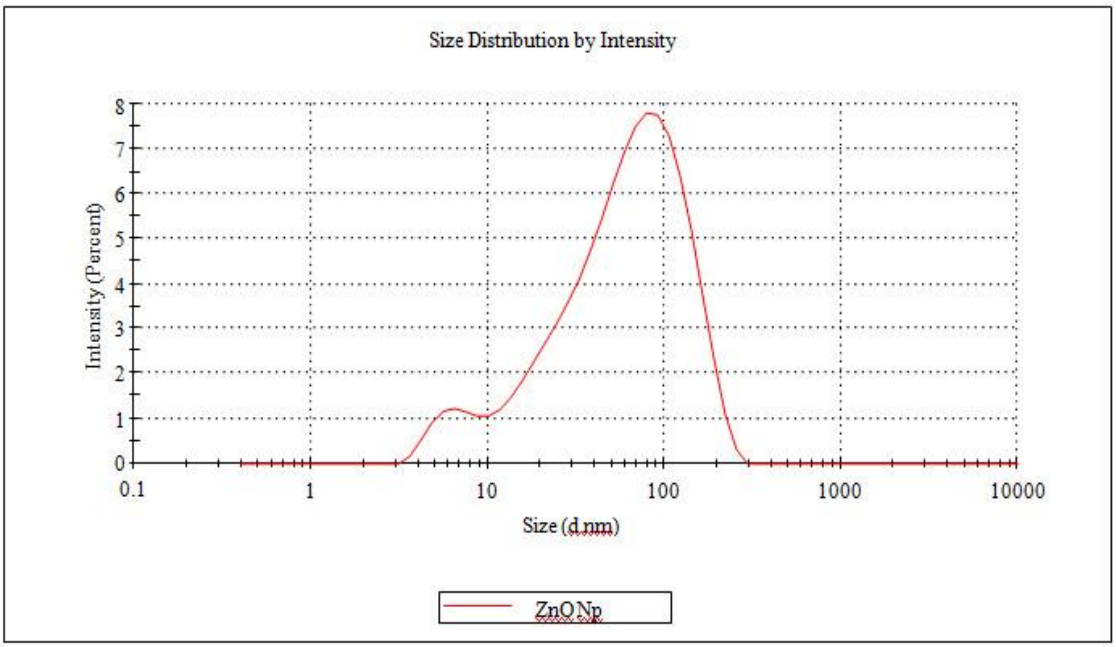

Figure 2: Graphical analysis of particle size Zinc Oxide Nanoparticles

Table 2 represents the zeta potential of the zinc oxide nanoparticles. The zeta potential of the zinc oxide nanoparticles was obtained at -42.1 mv. Fig. 3 represents the graphical analysis of the zeta potential of zinc oxide nanoparticles.

Table 2: Zeta Potential of Zinc Oxide Nanoparticles

Results		**Mean (mV)**		**Area (%)**	**St Dev (mV)**
Zeta Potential (mV) :	-42.1	**Peak 1:**	-52.7	40.6	7.85
Zeta Deviation (mV) :	13	**Peak 2:**	-35.9	39.5	4.37
Conductivity (mS/cm) :	2.51	**Peak 3:**	-26.4	19.3	2.94

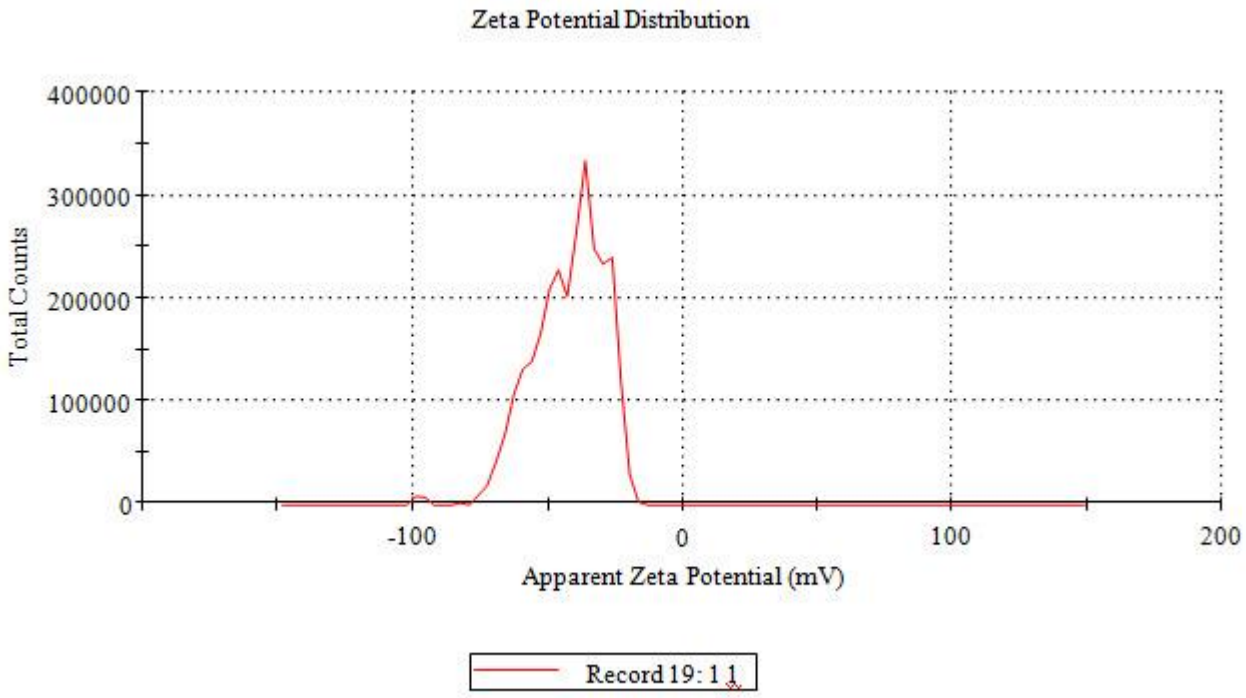

Figure 3:Graphical analysis of Zeta Potential of Zinc Oxide Nanoparticles

2.3.2. Characterization of modified electrodes.

Fig. 4 represents scanning electron microscopy pictures of ZnO nanoparticles on a gold electrode at x100 resolution. Fig 5 represents SWCNTs and ZnO nanoparticles on a gold electrode at x1.5K resolution. Fig. 6 represents Au-ZnO-SWCNTs along with the lactate oxidase enzyme at x250 resolution.

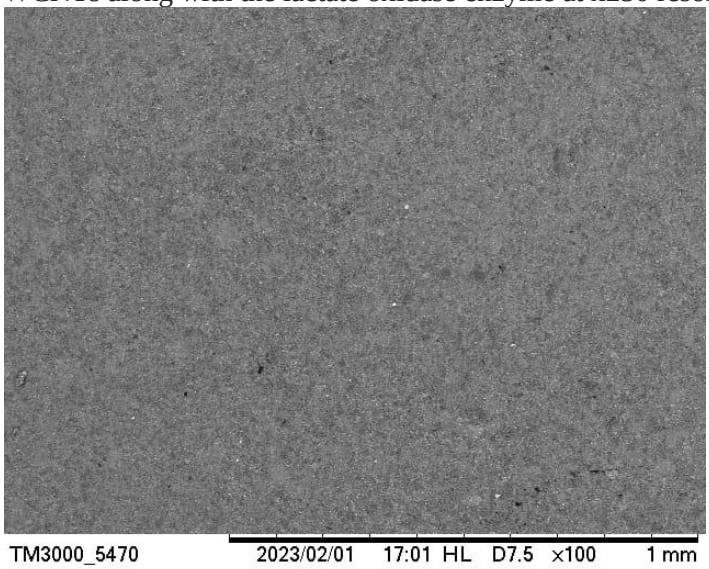

Figure 4: SEM of the ZnO/Au electrode

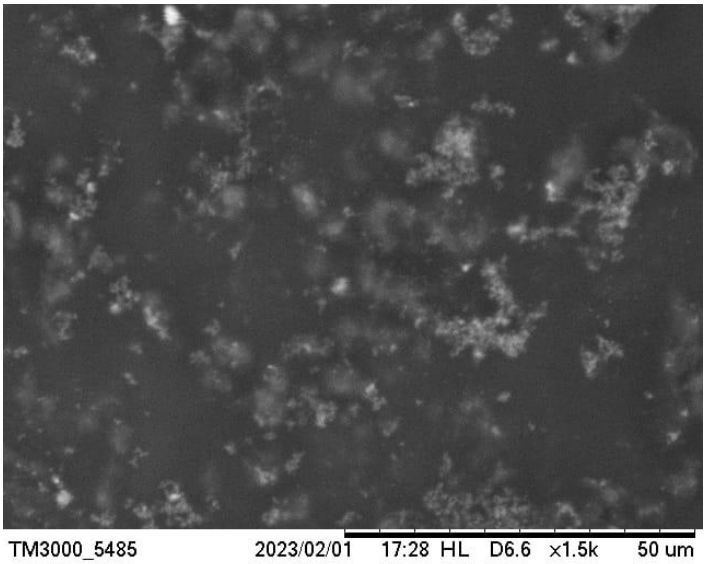

Figure 5: SEM of the ZnO/ SWCNT/Au electrode

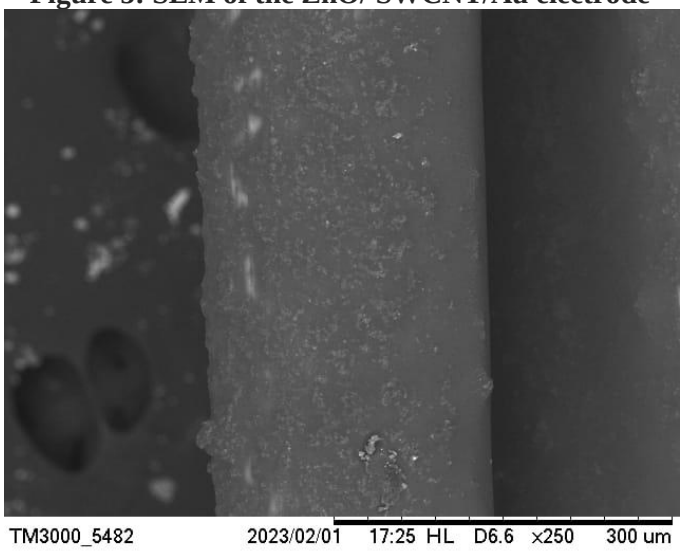

Figure 6: SEM of the ZnO/SWCNT/Au electrode along with LOD enzyme

2.3.3. Cyclic Voltammetry

Fig. 7 represents the cyclic voltammetry of the LOD-based self-made biosensor. Cyclic voltammetry tells us about the oxidation and reduction of H_2O_2 which indirectly determines the presence of lactate in the soy milk sample.

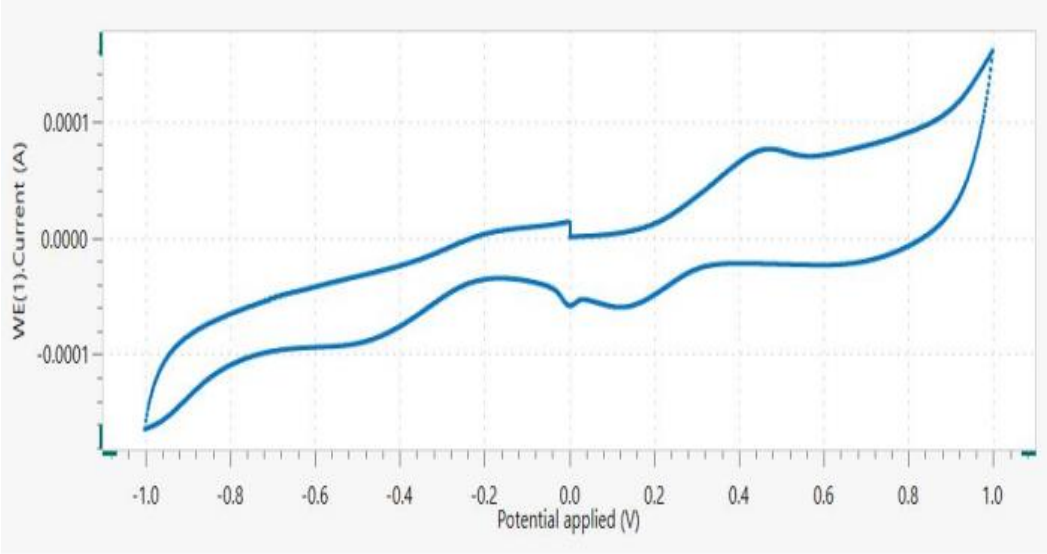

Figure 7 Cyclic Voltagramm.

2.3.4. Effect of Concentration of Lactate Oxidase on modified Electrode

Fig. 8 represents the effect of the concentration of lactate oxidase on a self-made biosensor, which increases as the concentration of lactate oxidase increases.

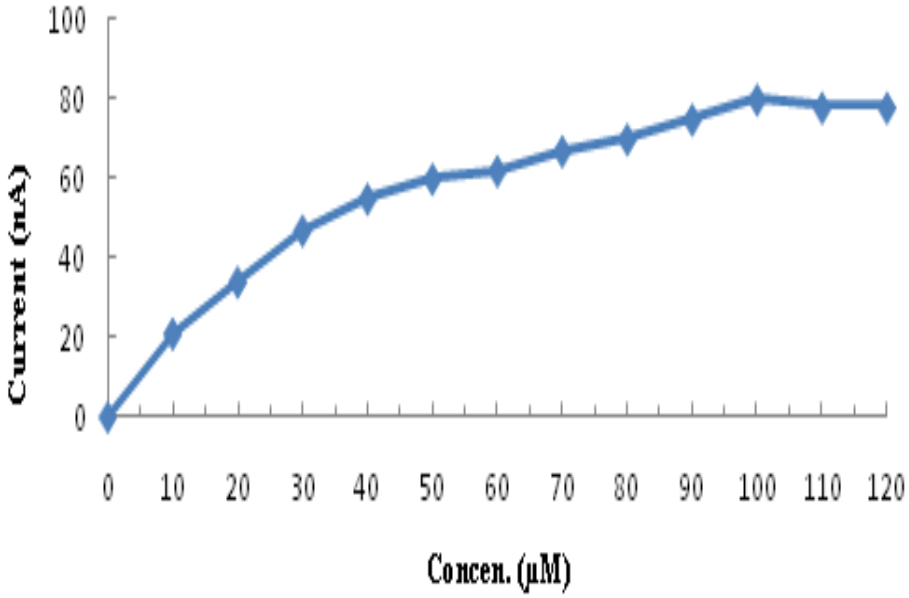

Figure 8: Effect of Lactate Oxidase on biosensor

2.3.5. Effect of Temperature on the modified electrode

Fig.9 represents the effect of temperature on a self-made biosensor. The most suitable temperature for the operation of this biosensor is 35°C.

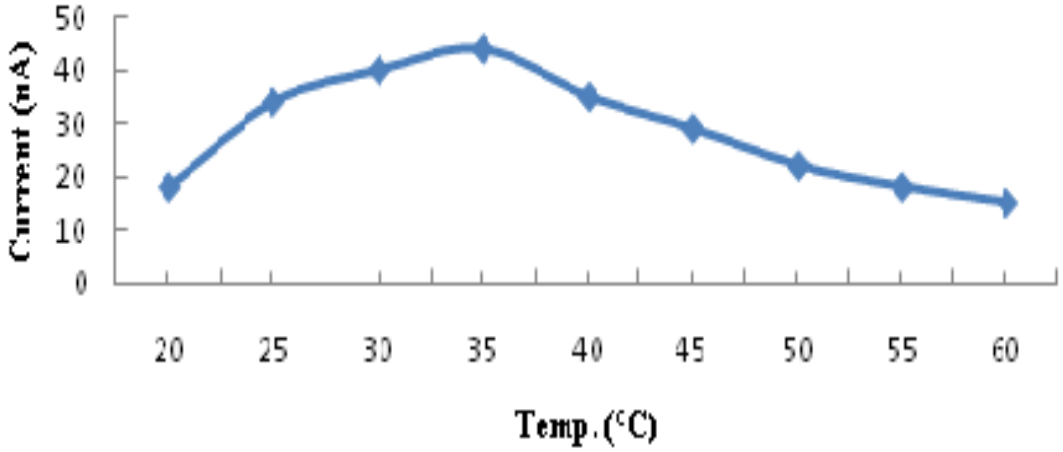

Figure 9: Effect of temperature on biosensor

2.3.6. Effect of pH on modified electrode

Fig. 10 represents the effect of pH on the self-made biosensor. The most suitable pH for the operation of this biosensor is 7.

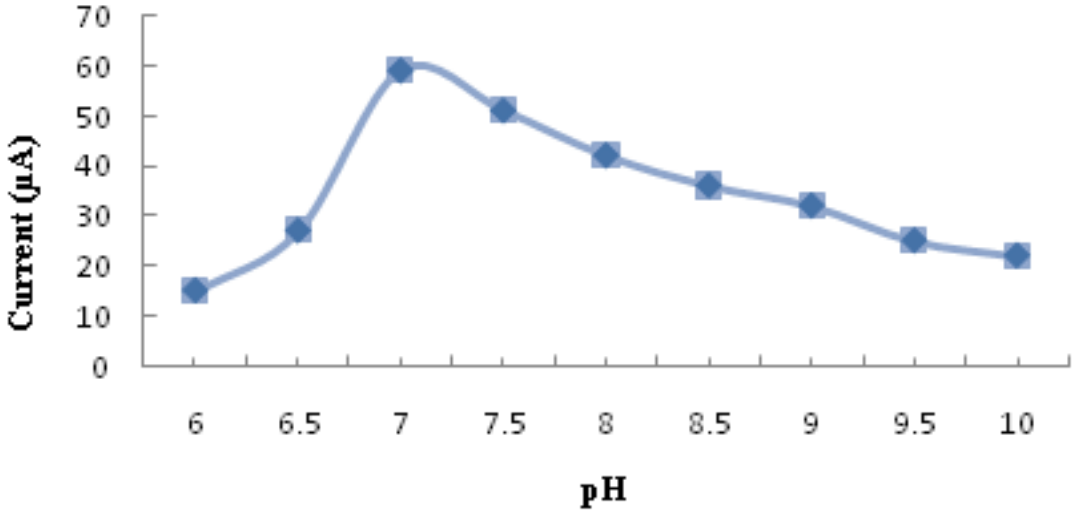

Figure 10: Effect of pH on the Biosensor

2.3.7. Effect of Reaction Time on the modified electrode

Fig. 11 represents the effect of reaction time or response time on a self-made biosensor. The most suitable reaction time is 6 seconds.

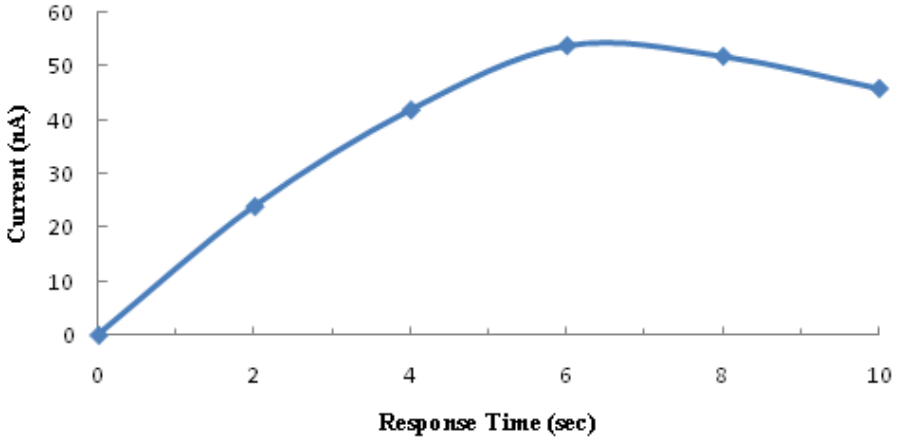

Figure 11: Response Time

2.3.8. Lineweaver Bruk analysis of modified electrode

Fig. 12 represents the lineweaver bruk analysis of the self-made biosensor with the help of Michaelis-Menton-equation:

$$\frac{1}{I} = \frac{Km}{Imax}\frac{1}{[A]} + \frac{1}{Imax}$$

Where, I = current, I max. = maximum current, A = analyte/substrate concentration, and Km = Michaelis-Menton constant

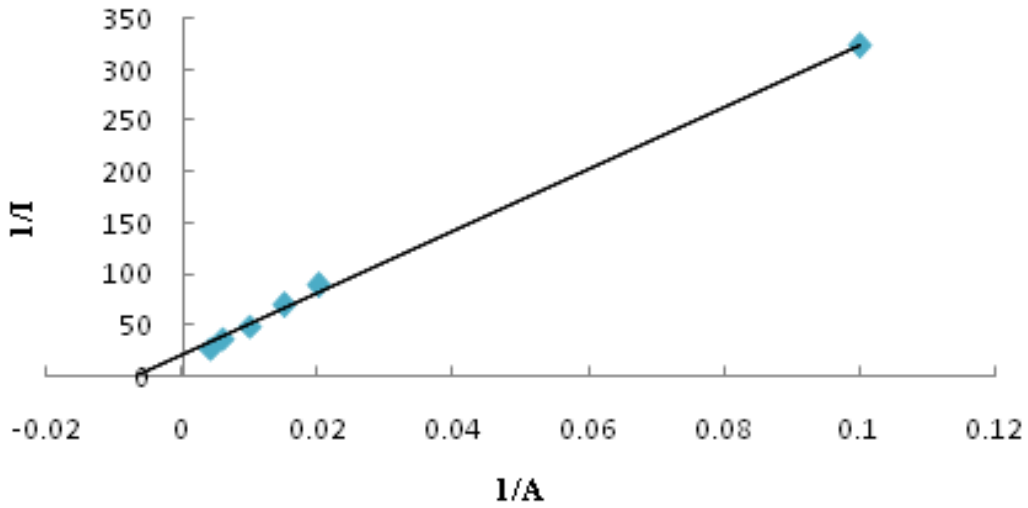

Figure 12: Lineweaver Bruk Analysis

2.3.9. Accuracy, Storage and Stability of the modified electrode

Various food samples (soy milk) were detected using a self-made biosensor and compared with UV spectroscopy to determine their accuracy. The obtained results show low levels of deviation. Table 3 represents the amount of lactate in food samples.

Table 3: Determination of Lactate in food samples

SNo.	UV–Spectrophotometry	Modified electrode	Deviation	Relative Standard Deviation
1	0.30	0.28	-0.02	6.6
2	0.60	0.64	0.04	6.6
3	1.08	1.03	-0.05	4.6

It was found that the response current reduced only by 15% after 3.5 months, which shows the good stability of the biosensor. The biosensor is stored in a refrigerator when not in use. The reason behind the decrease in stability is the immobilization of the enzyme after a few months.

2.4. Conclusion

A stable and reactive biosensor with a sensitivity of 2.834 mA/µm, linearity between 60 µm to 80 µm and a detection limit of 1.058 µm by using zinc oxide nanoparticles and single-walled carbon nanotubes for modification of the gold electrode. The obtained results show low levels of deviation from those obtained using UV spectrophotometry, which represents the good accuracy of this biosensor. Suitable temperature and pH conditions for the operation of this biosensor are 35°C and 7, respectively. This biosensor shows a good responsive time of 6 seconds with a stability of 3.5 months. This approach to lactate determination in soy milk can also be used in various other food samples.

References

[1] D. Wang *et al.*, "Microfluidic assembly for biosensing," *Encycl. Nanomater.*, pp. 79–112, Jan. 2023, doi: 10.1016/B978-0-12-822425-0.00010-5.

[2] C. S. Pundir, V. Narwal, and B. Batra, "Determination of lactic acid with special emphasis on biosensing methods: A review," *Biosens. Bioelectron.*, vol. 86, pp. 777–790, Dec. 2016, doi: 10.1016/J.BIOS.2016.07.076.

[3] I. S. Kucherenko, Y. V. Topolnikova, and O. O. Soldatkin, "Advances in the biosensors for lactate and pyruvate detection for medical applications: A review," *TrAC Trends Anal. Chem.*, vol. 110, pp. 160–172, Jan. 2019, doi: 10.1016/J.TRAC.2018.11.004.

[4] K. Rathee, V. Dhull, R. Dhull, and S. Singh, "Biosensors based on electrochemical lactate detection: A comprehensive review," *Biochem. Biophys. Reports*, vol. 5, pp. 35–54, Mar. 2016,

doi: 10.1016/j.bbrep.2015.11.010.

[5] S. B. Barker and W. H. Summerson, "THE COLORIMETRIC DETERMINATION OF LACTIC ACID IN BIOLOGICAL MATERIAL," *J. Biol. Chem.*, vol. 138, no. 2, pp. 535–554, Apr. 1941, doi: 10.1016/S0021-9258(18)51379-X.

[6] M. M. Rahman, M. J. A. Shiddiky, M. A. Rahman, and Y. B. Shim, "A lactate biosensor based on lactate dehydrogenase/nictotinamide adenine dinucleotide (oxidized form) immobilized on a conducting polymer/multiwall carbon nanotube composite film," *Anal. Biochem.*, vol. 384, no. 1, pp. 159–165, Jan. 2009, doi: 10.1016/J.AB.2008.09.030.

[7] U. Anand *et al.*, "Recent advances in the potential applications of luminescence-based, SPR-based, and carbon-based biosensors," *Appl. Microbiol. Biotechnol. 2022 1068*, vol. 106, no. 8, pp. 2827–2853, Apr. 2022, doi: 10.1007/S00253-022-11901-6.

[8] J. Wang, "Electrochemical biosensors: Towards point-of-care cancer diagnostics," *Biosens. Bioelectron.*, vol. 21, no. 10, pp. 1887–1892, Apr. 2006, doi: 10.1016/J.BIOS.2005.10.027.

[9] N. B. Mahmood, F. R. Saeed, K. R. Gbashi, and U. S. Mahmood, "Synthesis and characterization of zinc oxide nanoparticles via oxalate co-precipitation method," *Mater. Lett. X*, vol. 13, p. 100126, Mar. 2022, doi: 10.1016/J.MLBLUX.2022.100126.

[10] B. W. Lee, J. H. Koo, T. S. Lee, Y. H. Kim, and J. S. Hwang, "Synthesis of ZnO Nanoparticles via Simple Wet-Chemical Routes," *Adv. Mater. Res.*, vol. 699, pp. 133–137, 2013, doi: 10.4028/WWW.SCIENTIFIC.NET/AMR.699.133.

[11] S. Gupta, C. Ravikant, and A. Kaur, "One-pot wet chemical synthesis of reduced graphene oxide-zinc oxide nanocomposites for fast and selective ammonia sensing at room temperature," *Sensors Actuators A Phys.*, vol. 331, p. 112965, Nov. 2021, doi: 10.1016/J.SNA.2021.112965.

[12] S. Mustapha *et al.*, "The role of kaolin and kaolin/ZnO nanoadsorbents in adsorption studies for tannery wastewater treatment," *Sci. Reports 2020 101*, vol. 10, no. 1, pp. 1–22, Aug. 2020, doi: 10.1038/s41598-020-69808-z.

[13] B. Haghighi and S. Bozorgzadeh, "Fabrication of a highly sensitive electrochemiluminescence lactate biosensor using ZnO nanoparticles decorated multiwalled carbon nanotubes," *Talanta*, vol. 85, no. 4, pp. 2189–2193, Sep. 2011, doi: 10.1016/J.TALANTA.2011.07.071.

[14] Y. T. Wang *et al.*, "A novel l-lactate sensor based on enzyme electrode modified with ZnO nanoparticles and multiwall carbon nanotubes," *J. Electroanal. Chem.*, vol. 661, no. 1, pp. 8–12, Oct. 2011, doi: 10.1016/J.JELECHEM.2011.06.024.

[15] R. Schmitt, H. Molitor, T. W.-I. J. E. Sci, and undefined 2012, "Voltammetric method for the determination of lactic acid using a carbon paste electrode modified with cobalt phthalocyanine," *electrochemsci.org*, vol. 7, pp. 10835–10841, 2012, Accessed: Feb. 25, 2023. [Online]. Available: http://www.electrochemsci.org/papers/vol7/71110835.pdf