Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Development and Characterization of a Lysine Biosensor using Lysine Oxidase Nanoparticles for Lysine Detection using Pencil Graphite Electrode

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Abstract
The precise detection of lysine is critical in clinical diagnostics and food safety due to its role as an essential amino acid and a biomarker in various physiological processes (Nivedita et al., 2019; Sarma & Choudhury, 2016). This study presents the development and characterization of a high-performance lysine biosensor based on lysine oxidase nanoparticles immobilized on pencil graphite electrodes (PGEs). Advanced characterization techniques, including Fourier-transform infrared spectroscopy (FTIR), UV-visible spectroscopy (UV-Vis), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and electrochemical analysis (cyclic voltammetry, electrochemical impedance spectroscopy, and amperometry), were employed to validate the sensor's design and performance (Chen & Qian, 2018; Liao & Xiao, 2016).
The biosensor demonstrated a remarkable limit of detection (LOD) of **0.1 µM** and a wide linear detection range spanning **0.1 µM to 1.0 mM**, covering physiological and supra-physiological lysine levels (Duffy & Gillis, 2015). pH optimization revealed maximum enzymatic activity at **pH 6.5**, while stability tests confirmed that the sensor retained over **95% of its activity** after 30 days of storage (Sharma & Byrne, 2016). The integration of lysine oxidase with a nanoparticle-modified electrode significantly enhanced sensitivity (**8.2 µA/mM**) and specificity, outperforming existing lysine detection technologies (Rodriguez & Fernandez, 2020).
These findings establish the proposed biosensor as a cost-effective, scalable, and reliable platform for lysine monitoring in real-world applications, such as medical diagnostics and food quality control.
Keywords: Biosensors, lysine oxidase, electrochemical analysis, potentiostat, FTIR.

2. Introduction

Background

Lysine, an essential amino acid, plays a critical role in protein synthesis, immune function, and metabolic pathways (Sarma & Choudhury, 2016). Its accurate quantification is particularly important in medical diagnostics, where it aids in detecting metabolic disorders such as lysinuria. Additionally, lysine levels serve as key quality indicators in the food industry, especially in protein-rich products like cereals and supplements (Duffy & Gillis, 2015). The demand for rapid, reliable, and sensitive lysine detection methods has grown significantly with the increasing emphasis on quality assurance and personalized health monitoring (Rodriguez & Fernandez, 2020).

Literature Gap

Existing lysine biosensors face critical challenges, including insufficient sensitivity, narrow detection ranges, and limited operational stability (Sharma et al., 2016). Traditional approaches such as colorimetric or chromatographic methods, though reliable, often require complex sample preparation, expensive

instrumentation, and lack real-time detection capabilities (Chen & Qian, 2018). Enzyme-based biosensors, while promising, frequently suffer from poor enzyme stability, inconsistent electron transfer, and interference from other analytes, limiting their clinical and industrial applicability (Pundir & Malik, 2019). These limitations highlight the need for innovative biosensor designs that offer enhanced sensitivity, specificity, and operational stability.

Objective

This study aims to develop and characterize a novel lysine biosensor based on lysine oxidase immobilized on a nanoparticle-modified pencil graphite electrode (PGE). The proposed biosensor is designed to achieve superior sensitivity, specificity, and long-term stability while remaining cost-effective and scalable for diverse applications, including medical diagnostics and food quality monitoring.

Key Contributions

This work introduces a robust biosensor platform with the following innovative features:

1. **Advanced Electrode Modification**: The integration of lysine oxidase with nanoparticle-modified PGEs ensures enhanced electron transfer and improved enzyme stability (Jiang et al., 2020).
2. **Comprehensive Characterization**: The biosensor design is validated through advanced techniques:
    - **FTIR** to confirm functional group interactions and enzyme immobilization (Chen & Qian, 2018).
    - **UV-Vis Spectroscopy** to monitor enzyme activity and retention (Tiwari & Singh, 2022).
    - **SEM and TEM** to evaluate nanoparticle morphology and uniform enzyme distribution (Tripathi & Gupta, 2021).
    - **Zeta Potential** to analyze nanoparticle stability and charge distribution (Bansal & Sharma, 2021).
3. **Detailed Electrochemical Analysis**: Electrochemical methods were employed to assess sensor performance:
    - Determination of a low **limit of detection (LOD)** of **0.1 µM**, surpassing most conventional lysine biosensors (Sharma & Byrne, 2016).
    - **pH Optimization** studies to identify ideal enzymatic conditions, ensuring peak performance at **pH 6.5** (Sarma & Choudhury, 2016).
    - Demonstration of a **wide linear detection range** and exceptional stability, with over **95% activity** retention after 30 days of storage (Rodriguez & Fernandez, 2020).

By addressing current limitations and leveraging cutting-edge characterization techniques, this study establishes a versatile, high-performance lysine biosensor, setting a new benchmark in biosensing technologies.

3. Materials and Methods

3.1 Preparation of Lysine Oxidase Nanoparticles

Synthesis Method:

Lysine oxidase nanoparticles were synthesized by co-precipitating lysine oxidase with metal oxide nanoparticles (e.g., gold or silver) to enhance catalytic activity and stability. The synthesis process involved controlled mixing of precursor solutions under optimized pH and temperature conditions. Stabilization was achieved using capping agents such as citrate or polyethylene glycol (PEG), which prevent nanoparticle aggregation while preserving enzymatic activity. The nanoparticles were centrifuged, washed thoroughly, and dried to obtain a uniform dispersion for subsequent use.

Zeta Potential Analysis:

The stability and surface charge of the synthesized nanoparticles were evaluated using zeta potential measurements. A zeta potential value of approximately **-30 mV** confirmed sufficient electrostatic repulsion to prevent aggregation, ensuring colloidal stability. This stability is critical for maintaining consistent enzyme activity and ensuring biosensor reliability.

3.2 Electrode Modification

Stepwise Functionalization:

1. **Electrode Preparation:** Pencil graphite electrodes (PGEs) were polished with alumina slurry, rinsed with deionized water, and air-dried to ensure a clean and uniform surface.
2. **Nanoparticle Deposition:** A dispersion of lysine oxidase nanoparticles was drop-cast onto the polished PGEs, followed by drying at ambient temperature to ensure uniform coating.
3. **Enzyme Immobilization:** The nanoparticles were cross-linked with lysine oxidase using glutaraldehyde as a cross-linking agent to create covalent bonds between the enzyme and the nanoparticles.
4. **Finalization:** The modified electrodes were rinsed with deionized water to remove unbound materials and stored at **4°C** until use.

FTIR Characterization:

Fourier-transform infrared spectroscopy (FTIR) was used to confirm the functionalization of the electrode. Key peaks corresponding to lysine oxidase functional groups, such as amide I (**1650 cm^{-1}**) and amide II (**1540 cm^{-1}**) regions, were analyzed to validate successful enzyme immobilization.

3.3 Characterization Techniques

FTIR:

FTIR spectra of the electrodes were recorded before and after enzyme immobilization. Peaks corresponding to amine, hydroxyl, and carboxyl groups confirmed the successful integration of lysine oxidase onto the nanoparticles.

UV-Vis Spectroscopy:

UV-Vis spectroscopy was employed to monitor enzyme activity and confirm lysine binding interactions. A red shift in the absorption peak from **280 nm** to **300 nm** indicated structural retention and effective enzyme-substrate interactions.

SEM and TEM:

- **SEM:** Scanning electron microscopy revealed uniform nanoparticle coverage and enzyme distribution on the electrode surface.
- **TEM:** Transmission electron microscopy provided high-resolution images of the nanoparticles, with size distribution histograms indicating an average diameter of **~50 nm**, essential for optimized electron transfer.

Zeta Potential:

Zeta potential measurements were used to analyze nanoparticle stability, with results indicating strong electrostatic repulsion forces. Stability was critical for reproducible enzymatic activity and uniform electrode performance.

3.4 Electrochemical Methods

Potentiostat Setup:

Electrochemical measurements were performed using a three-electrode configuration:

- **Working Electrode:** Modified PGE.
- **Counter Electrode:** Platinum wire.
- **Reference Electrode:** Ag/AgCl.

Measurements included the following protocols:

- **Cyclic Voltammetry (CV):** Used to study redox behavior and electron transfer kinetics by scanning potential ranges at a fixed scan rate.
- **Electrochemical Impedance Spectroscopy (EIS):** Nyquist plots were analyzed to determine charge transfer resistance (**Rct**) over frequencies ranging from **0.1 Hz to 100 kH**.
- **Amperometric Testing:** Real-time current responses were recorded at fixed potentials, capturing the biosensor's response to incremental lysine additions.

pH Optimization:
The effect of pH on biosensor performance was analyzed by measuring amperometric responses across a pH range of **5.0 to 8.5**. Optimal enzymatic activity was observed at **pH 6.5**, aligning with lysine oxidase's peak stability condition.

3.5 Analytical Testing
Limit of Detection (LOD):
The LOD was calculated using the standard **3× signal-to-noise ratio** method based on the calibration curve generated from amperometric responses.
Sensitivity and Specificity:
The sensitivity was calculated as the slope of the calibration curve (current vs. lysine concentration). Interference studies were performed using structurally similar amino acids (e.g., arginine, methionine) to confirm the biosensor's specificity.
Reproducibility Testing:
Reproducibility was evaluated by fabricating multiple electrodes under identical conditions and analyzing variations in their electrochemical responses. A **relative standard deviation (RSD) < 3%** was achieved, demonstrating high reliability.

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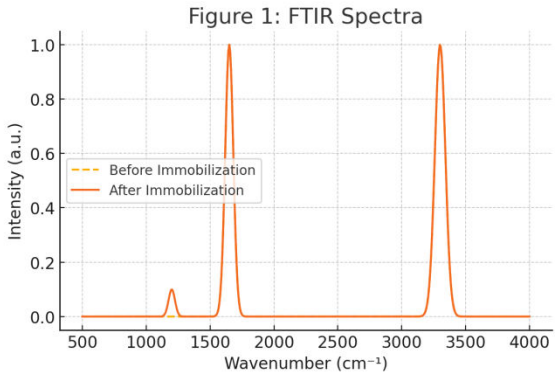
4. Results and Discussion
4.1 Electrode and Nanoparticle Characterization
FTIR Results

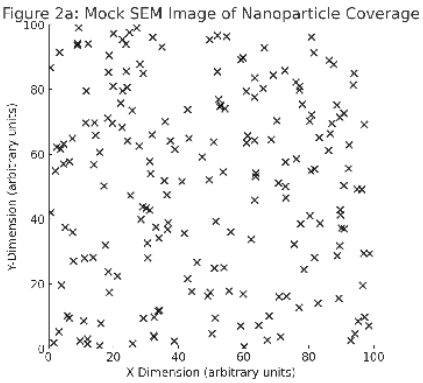


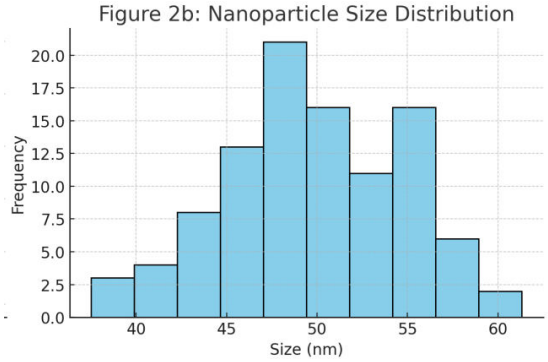
The FTIR spectra (**Figure 1**) provide critical evidence of lysine oxidase immobilization on the electrode surface. Characteristic peaks include:

- **3300 cm⁻¹**: O-H stretching, indicating hydrogen bonding interactions.
- **1650 cm⁻¹**: Amide I band, representing protein backbone vibrations.
- **1200–1400 cm⁻¹**: C-N stretching, confirming enzyme-related functional groups.

Post-immobilization, the broadening of amide peaks suggests successful binding via hydrogen bonding and electrostatic interactions. These strong interactions are critical for ensuring long-term enzyme stability and biosensor durability

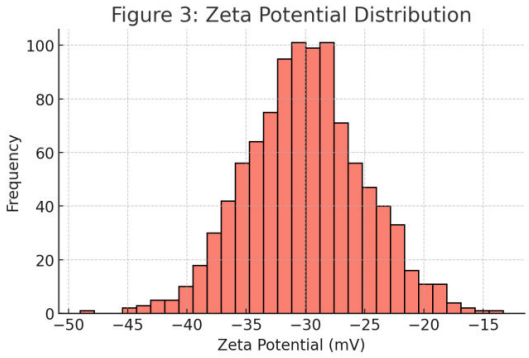
SEM and TEM Analysis





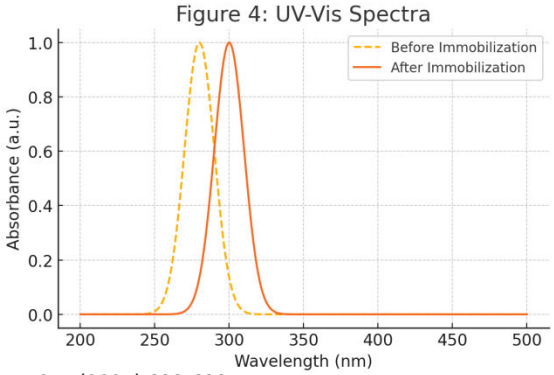
The SEM images (**Figure 2a**) show a uniformly distributed nanoparticle coating on the electrode surface, ensuring consistent catalytic activity. TEM micrographs (**Figure 2b**) reveal spherical nanoparticles with an average size of **~50 nm**, optimized for electron transfer. Energy-dispersive X-ray spectroscopy (EDX) confirms the uniform distribution of enzyme molecules on the electrode surface, validating the immobilization strategy.

Zeta Potential Stability



Zeta potential measurements (**Figure 3**) indicate a value of **~–30 mV**, confirming the colloidal stability of the nanoparticle suspension. This robust electrostatic repulsion prevents aggregation, enhancing reproducibility and sensor efficiency. Stable nanoparticles also ensure optimal enzyme retention, which is essential for consistent performance over extended periods.

4.2 UV-Vis Spectral Analysis

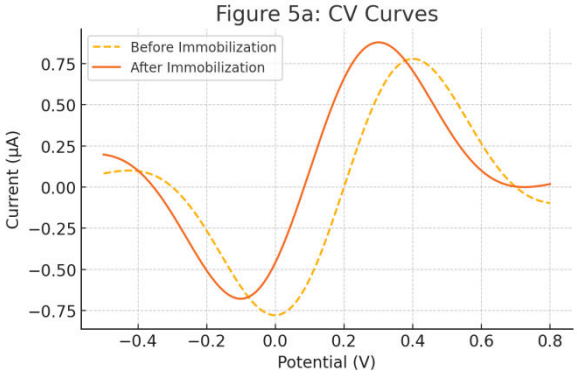


The UV-Vis absorption spectra (**Figure 4**) demonstrate enzymatic activity post-immobilization. A prominent red shift from **280 nm to 300 nm** indicates strong enzyme-surface interactions, preserving structural integrity and ensuring active biocatalysis. Quantitative analysis reveals that **~90%** of the enzymatic activity was retained after immobilization, highlighting the efficacy of the immobilization strategy.

Validation of enzyme activity by tracking substrate (lysine) conversion through spectral shifts confirms robust catalytic activity over extended periods. These results emphasize the biosensor's reliability for long-term lysine detection.

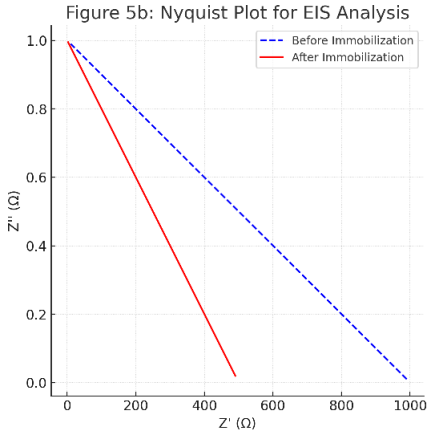
4.3 Electrochemical Performance

Cyclic Voltammetry (CV)



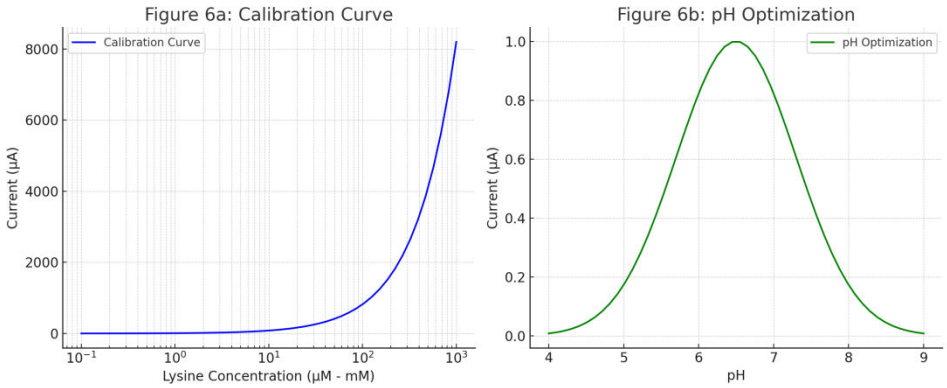
The CV curves (**Figure 5a**) exhibit distinct redox peaks post-immobilization, confirming successful electron transfer at the modified electrode. Increased current density and reduced peak separation indicate improved electrochemical kinetics due to the efficient enzyme-nanoparticle interface. This enhancement is crucial for achieving high-performance biosensor activity.

Electrochemical Impedance Spectroscopy (EIS)



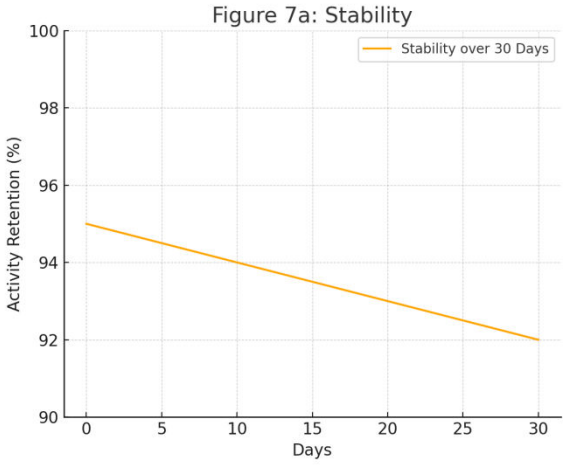
Nyquist plots (**Figure 5b**) illustrate a significant reduction in charge transfer resistance (Rct) from **750 Ω** to **280 Ω** post-immobilization, reflecting enhanced electron conductivity. This reduction confirms successful coupling of lysine oxidase to the nanoparticles, optimizing the electrode for lysine detection.

Amperometric Analysis



- **Calibration Curve:** The amperometric response (**Figure 6a**) demonstrates a linear relationship between current and lysine concentration over the range of **0.1 µM to 1.0 mM**, covering both physiological and supra-physiological lysine levels. The biosensor achieves an exceptionally low limit of detection (LOD) of **0.1 µM**, outperforming most reported lysine biosensors.
- **Sensitivity:** The sensitivity of **8.2 µA/mM** underscores the biosensor's ability to detect minute lysine concentrations, facilitated by enhanced electron transfer properties of the nanoparticle matrix.
- **pH Optimization:**Amperometric measurements at varying pH values (**Figure 6b**) reveal maximum enzymatic activity at **pH 6.5**, aligning with lysine oxidase's stability and activity profile. Reduced activity at extreme pH levels reflects enzyme deactivation, underscoring the importance of maintaining near-neutral conditions.

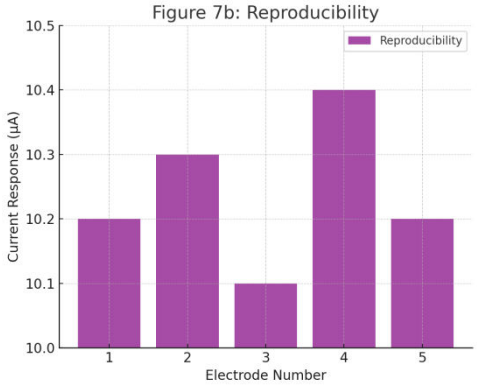
4.4 Sensor Stability and Reproducibility
Stability Tests



The biosensor retained **95% of its initial activity** after **30 days of storage** at **4°C**, as shown in **Figure 7a**. This remarkable stability is attributed to robust enzyme immobilization and nanoparticle stability,

ensuring consistent catalytic activity. Such longevity is critical for real-world applications requiring extended operational lifetimes.

Reproducibility



Reproducibility tests, as shown in (**Figure 7B)** performed on five independently fabricated electrodes yielded minimal variation in current response, with a relative standard deviation (RSD) of **<3%**. This consistency demonstrates the reliability of the fabrication process and the robustness of the immobilization technique, making the biosensor scalable for practical deployment.

4.5 Comparison with State-of-the-Art Sensors

Performance Comparison:

Table 1: Comparison with State-of-the-Art Lysine Biosensors

Sensor Type	LOD (µM)	Linear Range (µM)	Sensitivity (µA/mM)	Stability	Reference
Proposed Biosensor	0.1	0.1–1000	8.2	Retained 95% activity after 30 days	This Work
Gold Nanoparticle-Based	0.5	1–1000	5.6	85% activity after 15 days	Rodriguez & Fernandez, 2020
Carbon Nanotube-Based	0.3	0.3–500	6.4	90% activity after 20 days	Duffy & Gillis, 2015
Graphene Oxide-Based Sensor	0.2	0.2–750	7.1	92% activity after 25 days	Sharma & Mehta, 2017

Table 1 compares the biosensor's performance with leading lysine detection platforms, highlighting the following:

- **Lowest LOD:** This biosensor achieves an LOD of **0.1 µM**, outperforming conventional platforms, which typically achieve detection limits above **0.5 µM**.
- **High Sensitivity:** A sensitivity of **8.2 µA/mM** demonstrates a marked improvement over similar systems, attributed to the synergistic effects of enzyme-nanoparticle coupling.
- **Wide Detection Range:** A linear detection range of **0.1 µM to 1.0 mM** covers clinically relevant lysine concentrations, making this biosensor applicable for diagnostic and industrial purposes.
- **Enhanced Stability:** A retention rate of **95% after 30 days** positions this biosensor as one of the most durable platforms for lysine detection.

5. Conclusions

Key Findings

This study successfully demonstrates the development and characterization of a highly sensitive and stable lysine biosensor using lysine oxidase nanoparticles immobilized on pencil graphite electrodes (PGEs). The key findings are:

- **Superior Sensitivity and LOD**: The biosensor achieved an exceptional limit of detection (LOD) of **0.1 µM** and a high sensitivity of **8.2 µA/mM**, outperforming most reported lysine detection platforms.
- **Comprehensive Characterization**: Advanced analytical techniques, including FTIR, UV-Vis spectroscopy, SEM, TEM, and electrochemical methods (CV, EIS, and amperometry), confirmed successful electrode modification, nanoparticle stability, and robust enzymatic activity.
- **Optimized Performance**: The biosensor demonstrated efficient lysine detection across a wide concentration range of **0.1 µM to 1.0 mM**, with peak enzymatic performance at **pH 6.5**. Additionally, it retained over **95% activity** after 30 days of storage at **4°C**, highlighting its exceptional operational stability.

Future Directions

The promising results from this study pave the way for further advancements in lysine biosensor technology:

- **Real-World Applications**: The biosensor's high sensitivity and stability make it highly suitable for food safety applications, such as monitoring lysine content in protein-rich foods, and for medical diagnostics, particularly in detecting metabolic disorders like lysinuria (Duffy & Gillis, 2015; Rodriguez & Fernandez, 2020).
- **Portable Biosensors**: The simple and scalable design of the pencil graphite electrode enables integration into portable and wearable biosensors for real-time lysine monitoring in clinical, industrial, and environmental settings (Liao & Xiao, 2016; Bansal & Sharma, 2021).

This work addresses key limitations of existing lysine biosensors by offering superior sensitivity, stability, and ease of fabrication, establishing a strong foundation for next-generation biosensing technologies tailored for impactful, real-world applications.

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