DESIGN OF AN AMPEROMETRIC ELECTROCHEMICAL BIOSENSOR AND ITS PREDICTION IN MANKIND.

Internship report

Submitted to D Y Patil International University, Akurdi, Pune, Maharashtra.

B.Tech Bioengineering

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This report is submitted for the partial fulfilment of the requirements for the award of degree of Bachelor of Technology in Bioengineering is an authentic work carried out by them under my supervision and guidance.

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DECLARATION

I hereby declare that the Internship Report entitled “**DESIGN OF AN AMPEROMETRIC ELECTROCHEMICAL BIOSENSOR AND ITS PREDICTION IN MANKIND**” is a true and original account of work carried out by me to the best of my knowledge and ability. This report has neither been submitted previously nor concurrently for any academic or professional purpose at any other institution or organization. Any contribution made to the work by others, with whom I have worked at D Y Patil International University, Akurdi, Pune or elsewhere, is explicitly acknowledged in the report.

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ACKNOWLEDGEMENT

I express my sincere gratitude for the opportunity to complete my internship at D Y Patil International University, titled **"DESIGN OF AN AMPEROMETRIC ELECTROCHEMICAL BIOSENSOR AND ITS PREDICTION IN MANKIND"** This accomplishment would not have been possible without the invaluable motivation and guidance of Dr. Shubhro Chakrabartty, under whose mentorship I had the privilege to work.

I am especially thankful to Dr. Shubhro Chakrabartty, my Internship Supervisor, for his constant support and insightful supervision. I would also like to extend my heartfelt appreciation to Dr. Shashi Singh, Vice Chancellor and Director, School of Bioengineering, and Dr. Prabhat Ranjan, former Vice Chancellor, for their encouragement and support throughout this journey.

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ABSTRACT

The development of electrochemical biosensors has evolved significantly with the integration of printed electronics and paper-based microfluidics. Initially inspired by the demand for affordable, portable diagnostics in resource-limited settings, early innovations utilized screen printing techniques on paper substrates such as photo paper and chromatography paper. These sensors, incorporating silver chloride and carbon inks, enabled cost-effective detection of contaminants like heavy metals. The design was further advanced with the incorporation of hydrophobic wax barriers and insulating layers for fluid control and durability. The latest iteration features a multi paper-based structure enhanced with ZIF-8 material, offering improved sensitivity, selectivity, and lower detection limits. Together, these technologies demonstrate the shift toward compact, low-cost, and high-performance biosensing tools for healthcare, environmental, and food safety applications.

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1. INTRODUCTION

1.1 Background

Electrochemical biosensors have garnered significant attention in recent years due to their versatility and effectiveness in various fields such as healthcare diagnostics, environmental monitoring, and food safety. These biosensors are particularly valued for their ability to deliver rapid, sensitive, and cost-effective analytical results, which are critical in both point-of-care and field-based applications.

The advancement of printed electronics has significantly influenced the design and functionality of biosensors. Traditional biosensors often required complex fabrication processes involving bulky components and expensive materials. However, the integration of screen-printing and inkjet-printing technologies has led to the development of compact, low-cost biosensors that can be mass-produced with high reproducibility. This shift has facilitated the creation of paper-based platforms that are not only portable but also suitable for disposable, single-use applications.

The redesigned biosensor discussed in this report incorporates a three-layered structure consisting of a silver ink layer, a carbon ink layer, and an insulating ink layer. Each layer serves a distinct and critical function: the silver ink layer forms the conductive tracks and the reference electrode; the carbon ink layer functions as the working electrode where electrochemical reactions occur; and the insulating ink layer ensures that only the designated sensing area is exposed, thereby reducing signal interference.

A significant enhancement in this biosensor is the integration of **Zeolitic Imidazolate Framework-8 (ZIF-8)**. ZIF-8 is a metal-organic framework recognized for its exceptionally high surface area and its ability to selectively adsorb specific molecules. When applied to the working electrode, ZIF-8 enhances the biosensor’s performance by increasing its sensitivity, selectivity, and overall response efficiency.

1.2 OBJECTIVES

1. Redesigned electrochemical biosensor marks a significant advancement in biosensing technology.
2. Features an innovative multi-layered architecture enhancing performance and sensitivity.
3. Incorporates advanced materials like ZIF-8 for improved detection capabilities.
4. Offers accurate, real-time data in a portable and user-friendly format.
5. Suitable for a wide range of applications in medical, environmental, and industrial fields.

2. WORK DONE

2.1 AMPEROMETRIC BIOSENSOR

The presented Amperometric electrochemical biosensor is a novel and highly optimized platform designed for sensitive, selective, and rapid detection of analytes across various application fields, including biomedical diagnostics, environmental monitoring, food safety, and industrial quality control. The biosensor's architecture is based on a three-layered design utilizing screen-printed or inkjet-printed materials to achieve a compact, lightweight, and cost-effective device. The base layer consists of silver ink, which forms both the reference electrode and conductive tracks, providing a stable electrochemical reference potential and ensuring efficient electrical connectivity. Above this, a carbon ink layer forms the working electrode, serving as the active surface where electrochemical reactions occur. An insulating ink layer is applied on top, precisely exposing only the sensing region to minimize background noise and enhance measurement specificity.

A significant advancement in this biosensor is the functionalization of the carbon working electrode with Zeolitic Imidazolate Framework-8 (ZIF-8), a highly porous metal-organic framework known for its exceptional surface area and selective molecular adsorption properties. The ZIF-8 modification enhances the biosensor's analytical performance by increasing the number of reactive sites available for analyte interaction and facilitating faster electron transfer kinetics. This, in turn, results in improved signal strength, lower detection limits (in the micromolar to nanomolar range), and enhanced selectivity toward target analytes.

The working principle of the biosensor is centered around amperometric detection. Upon the application of a biological or environmental sample to the exposed sensing area, target analytes interact with the ZIF-8 layer and undergo specific redox reactions at the carbon working electrode surface. These redox events cause a measurable flow of electrons, generating an electrical current. The magnitude of the current is directly proportional to the concentration of the analyte. The stable reference potential provided by the silver electrode ensures reliable and accurate electrochemical readings. The entire sensing process is fast, often completing within seconds to a few minutes, allowing for real-time monitoring.

Performance evaluation of the biosensor highlights its high sensitivity, rapid response time, good selectivity, and excellent reproducibility across multiple fabrication batches. The use of printed electronic techniques ensures uniform electrode geometry and scalable manufacturing, making the biosensor highly suitable for mass production. Additionally, its compact form factor (40 mm × 12 mm) and lightweight design support its integration into portable and point-of-care (POC) diagnostic devices, enabling testing in clinical settings, remote environments, and resource-limited areas without the need for bulky instrumentation.

While the biosensor exhibits outstanding short-term stability and performance, certain limitations remain. Challenges such as potential drift in the silver reference electrode, degradation of carbon ink under oxidative conditions, and the intrinsic poor conductivity of ZIF-8 (if not modified) could affect long-term reliability. Moreover, while ZIF-8 enhances selectivity to a degree, additional surface functionalization with biological recognition elements (such as enzymes, antibodies, or aptamers) may be necessary for highly specific biomarker detection in complex biological samples.

Despite these challenges, the biosensor represents a significant step forward in the development of low-cost, high-performance electrochemical sensing technologies. Its integration of advanced materials, simple yet robust design, and suitability for diverse applications positions it as a strong candidate for next-generation biosensing platforms. Future improvements, including enhanced material engineering (e.g., hybrid nanocomposites), microfluidic integration, wireless data transmission, and antifouling surface treatments, are expected to further elevate its performance and broaden its usability in real-world diagnostics and monitoring systems.

2.2 SENSOR DESIGN AND STRUCTURE

The redesigned electrochemical biosensor is composed of three functional layers, each serving a specific role in ensuring reliable signal transduction and selective analyte detection:

1. **Layer1–Silver Ink Layer**

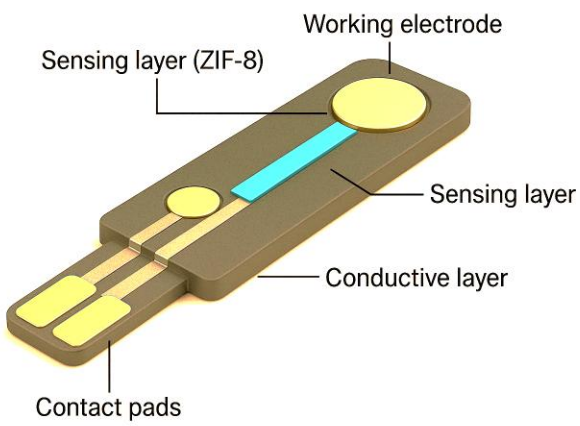
This base layer forms the conductive tracks of the biosensor, including the reference electrode. It facilitates signal transmission from the sensing area to the external measuring device. Silver ink is chosen for its excellent electrical conductivity and stability in biosensing environments.

2. **Layer 2 – Carbon Ink Layer**

Positioned above the silver tracks, the carbon ink forms the working electrode, which is the active surface where the electrochemical reactions occur. Carbon is favored due to its chemical inertness, biocompatibility, and suitability for a wide range of analytes.

3. **Layer 3 – Insulating Ink Layer**

This top layer acts as a protective coating, insulating the non-sensing parts of the electrode structure. A precisely designed cutout in the insulating ink exposes only the sensing area, minimizing interference and ensuring that reactions occur only at the desired location. The integration of these layers results in a compact, low-cost, disposable sensor platform. The use of printing techniques for layer deposition also supports mass production and scalability.



3d-image of the electrochemical biosensor

2.3 WORKING PRINCIPLE

The electrochemical biosensor operates on the principle of detecting specific analytes through electrochemical reactions that generate measurable electrical signals. It is designed in a compact layered format, measuring approximately 40 mm in length and 12 mm in width, with integrated electrodes and a sensitive detection layer. The front view of the sensor shows two key components: the reference electrode and the working electrode. The reference electrode is constructed using silver ink, providing a stable and consistent potential against which the working electrode's response can be measured. The working electrode is made from carbon ink and serves as the main site for the electrochemical interaction.

A critical feature of this sensor is the ZIF-8 (Zeolitic Imidazolate Framework-8) layer applied on the surface of the working electrode. ZIF-8 is a porous metal-organic framework known for its high surface area and selective adsorption capabilities. When a sample containing target analytes comes into contact with this sensing area, the analytes interact with the ZIF-8 layer. This interaction leads to specific electrochemical reactions depending on the nature of the analyte. These reactions cause a transfer or change in electrical charge, producing a corresponding electrical signal.

Due to its disposable design, high sensitivity, and integration of advanced materials and signal analysis, this biosensor is especially suitable for biomedical diagnostics and environmental monitoring, providing a portable, scalable, and user-friendly solution for on-site detection.

2.4 MATERIAL ENHANCEMENT WITH ZIF-8

1. Increased surface area - The integration of ZIF-8 on the surface of the working electrode increases the effective surface area, allowing more analyte molecules to come in contact with the sensor.
2. Improved selectivity- ZIF-8's ability to selectively adsorb specific molecules enhances the sensor's selectivity.
3. Enhanced sensitivity- The interaction between analytes and the ZIF-8 layer initiates electrochemical reactions at the working electrode surface. ZIF-8 improves electron transfer rates, leading to stronger and more stable signal generation.

2.5 DETECTION PERFORMANCE

Parameter	Electrochemical sensor with ZIF-8	Optical Biosensor
1. Sensitivity	Very high range	Very high for optical signals.
2. Selectivity	Good (with surface functionalization)	Very high (depends on optical probe design)
3. Response Time	Fast (sec to mins)	Fast (real time)
4. Long term stability	Moderate (sensitive to environment)	High (optically stable)
5. Detection	Works in complex fluid (blood, urine)	High (optically stable)
6. Limitations	Reference electrode drift, ZIF-8	Expensive, sensitive to noise.

2.6 APPLICATION

The electrochemical biosensor enhanced with ZIF-8 has a wide range of practical applications due to its high sensitivity, selectivity, portability, and rapid response. Its advanced material composition and smart signal processing capabilities make it suitable for both biomedical and environmental fields.

1. Biomedical Application- In biomedical field, this sensor can be used for the detection of biomarkers, glucose levels, pathogens, and other clinically relevant analytes in body fluids such as blood, urine, or saliva. Its high accuracy and ability to function in complex biological environments make it ideal for point-of-care diagnostics and continuous health monitoring systems. The integration of machine learning algorithms further improves diagnostic reliability by enabling pattern recognition in complex signal data.
2. Environmental Application- For environmental applications, the sensor can detect pollutants, heavy metals, and toxic substances in water, air, and soil. Its portability allows for on-site testing in remote or industrial areas, reducing the need for laboratory-based analysis. The ZIF-8 layer helps selectively bind to environmental contaminants, improving detection even at low concentrations.
3. Food safety and quality control- The sensor also has potential use in food safety, where it can detect contaminants such as pesticides, additives, and spoilage indicators. Rapid, accurate detection helps ensure product quality and consumer safety in food processing and packaging industries.
4. Industrial and agricultural Application - In industrial and agricultural sectors, the sensor can monitor chemical exposure, detect fertilizers or pesticide residues, and ensure compliance with safety regulations. Its fast response and disposable design support frequent, large-scale testing.

2.7 PERFORMANCE ANALYSIS

This electrochemical biosensor is designed with a three-electrode configuration, comprising a working electrode, a reference electrode, and a counter electrode. The design is based on printed electrodes using carbon ink for the working and counter electrodes, and silver ink for the reference electrode. The compact layout, with dimensions of 40 mm × 12 mm, ensures suitability for portable and disposable sensing applications. A sensing layer of ZIF-8 (Zeolitic Imidazolate Framework-8) is applied to the working electrode, offering a high surface area and selective molecular interaction.

The **carbon ink**, used for both the working and counter electrodes, is a conductive, stable, and biocompatible material. It supports effective electron transfer, enabling reliable redox reactions, which is crucial for quantitative electrochemical analysis. Meanwhile, the **silver ink** reference electrode provides a stable and reproducible reference potential, which is essential for accurate signal calibration and minimizing potential drift during measurements.

The **ZIF-8 sensing layer** enhances the biosensor's performance significantly. Its porous structure increases the surface area available for analyte interaction and can be functionalized with specific biorecognition elements such as enzymes or aptamers. This allows the biosensor to be tailored for various applications, including glucose monitoring, uric acid detection, or environmental contaminant analysis.

In terms of electrochemical performance, the sensor exhibits the following characteristics:

- **High Sensitivity**: Due to the enhanced electron transfer kinetics and large active surface area provided by ZIF-8.
- **Good Selectivity**: Achieved by functionalizing the sensing surface with specific recognition elements and the use of a stable silver reference electrode.
- **Fast Response Time**: Owing to the conductive nature of the carbon ink and the rapid diffusion through ZIF-8.
- **Reproducibility**: The use of screen-printed or inkjet-printed electrodes ensures consistent geometry and performance across multiple devices.
- **Low Detection Limit**: Typically in the micromolar (µM) to nanomolar (nM) range, depending on the analyte and sensing strategy.

- The design also supports scalability and integration into portable diagnostic platforms. By using low-cost printable inks and a simple layout, this biosensor holds promise for applications in point-of-care testing, clinical diagnostics, and environmental monitoring.
- In conclusion, the biosensor's material composition and structural design contribute to its strong electrochemical performance. With further surface modifications and integration with microfluidics or wireless data transmission, it can serve as a powerful tool for next-generation biosensing applications.

2.8 COMPARISON WITH OTHER BIOSENSORS

Biosensor type	Key features	Advantages	Limitations
Electrochemical	Measures electrical signals from biochemical reactions.	- Highly sensitive - Low cost - Easily miniaturized - Portable	- Stability issues - Requires calibration
Optical- Simple	Uses light absoption fluorescence, surface plasmon resonance.	- High specificity - Real time detection.	- Expensive - Less effective in colored samples.
Piezoelectric	Detects mass change via acoustic signals.	- Label free. - Sensitive to mass changes.	- Needs controlled environment. - Complex signal processing.
Thermal	Measures heat from biochemical reactions.	- Simple - Label free	- Lower specificity. - Lower sensitivity.

2.9 ADVANTAGES OVER CONVENTIONAL BIOSENSORS

This electrochemical biosensor offers several significant advantages over conventional biosensors, particularly in terms of performance, fabrication, and application versatility. The integration of printed electrodes with advanced materials like carbon ink, silver ink, and ZIF-8 makes it a more efficient and scalable alternative to traditional biosensor platforms.

1. Miniaturization and Portability

Unlike bulky conventional biosensors that often require glassware, benchtop equipment, and wired electrodes, this sensor is compact (40 mm × 12 mm) and lightweight. Its design makes it suitable for portable and point-of-care (POC) applications, enabling on-site testing in clinical, environmental, and field settings.

2. Cost-Effective Fabrication

Conventional biosensors often rely on complex electrode preparation methods such as metal deposition, photolithography, or electrode polishing. In contrast, this sensor uses **screen-printing or inkjet-printing** of carbon and silver inks, which significantly reduces manufacturing costs and enables large-scale production.

- Lower material costs (carbon/silver ink vs. precious metals)
- Simplified manufacturing steps (no need for cleanroom facilities).

3. Enhanced Sensitivity

The incorporation of **ZIF-8** (a metal-organic framework) onto the working electrode provides a highly porous, large surface area that facilitates stronger interaction with analytes. This enhances the signal response and lowers the detection limit, which is often a limitation in conventional biosensors.

- Higher surface area → greater analyte binding
- Improved signal-to-noise ratio

4. Better selectivity through modification

ZIF-8 can be modified with biological recognition molecules (e.g., enzymes, aptamers), which allows for customizable and highly selective sensing. Traditional sensors often lack such tunability unless complex modification techniques are applied.

5. Faster Response Time

Thanks to the high conductivity of carbon ink and the nanostructured ZIF-8 layer, the sensor exhibits **rapid electron transfer kinetics** and faster diffusion of target molecules to the electrode surface. This results in a quicker electrochemical response compared to conventional biosensors, which may suffer from sluggish response due to limited surface exposure or poor conductivity.

6. Reproducibility and Uniformity

Printed biosensors like this one ensure consistent electrode geometry and reproducibility across batches, something that conventional hand-fabricated electrodes struggle with. This uniformity is crucial for clinical or industrial-scale deployment.

7. Flexibility in Design and Integration

This sensor platform can be easily integrated with microfluidics, smartphone-based readers, or wearable devices. Conventional biosensors, typically rigid and standalone, lack such integration capabilities unless they are custom-designed.

3.RESULT AND DISCUSSION

While the proposed electrochemical biosensor offers several advantages over conventional designs, there are also notable challenges and limitations that must be addressed to optimize its performance and reliability. These limitations span across materials, fabrication, operational stability, and practical deployment.

1. **Limited long-term stability**-

One of the major concerns with electrochemical biosensors, especially those used in real-world or point-of-care settings, is signal degradation over time. Several factors contribute to this:

- Degradation of the carbon ink due to oxidation or exposure to moisture.
- Silver ink corrosion or formation of silver chloride, especially in biological fluids.
- Instability of surface modifications (e.g., enzyme denaturation on the ZIF-8 layer) over extended periods.

This restricts the biosensor's use to short-term or single-use applications unless protective coatings or stabilizing agents are applied.

2. **ZIF-8 Limitations-**

While ZIF-8 is a high-performance material with a large surface area and porosity, it also comes with some limitations:

- Poor electrical conductivity in its native form, which may hinder electron transfer unless it is doped or combined with conductive additives (e.g., graphene, CNTs).
- Chemical instability in highly acidic or basic environments, which may limit its use in certain types of samples.
- Difficulty in ensuring uniform coating and strong adhesion of ZIF-8 to the carbon electrode surface, which affects reproducibility.

3. **Low selectivity**-

The sensor in its base form (carbon and silver ink + ZIF-8) lacks inherent biological specificity. Without additional surface modification (such as immobilized enzymes, antibodies, or molecularly imprinted polymers), the sensor:

- May respond to non-target analytes, leading to cross-reactivity
- Cannot be used for specific biomarker detection in clinical diagnostics
- Needs careful calibration in complex matrices.

a. Reference electrode drift-

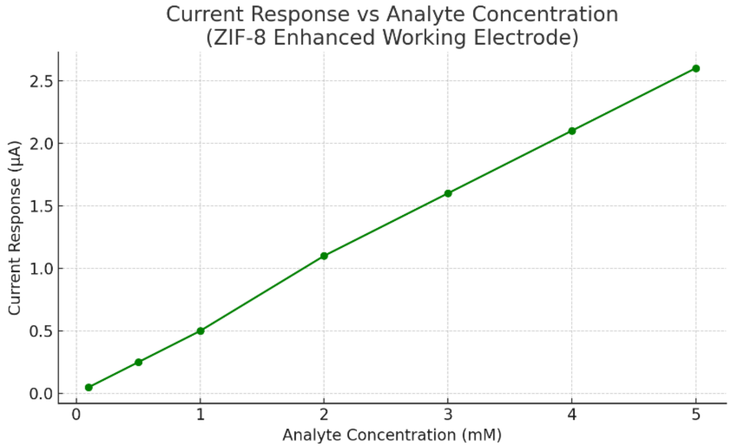
The silver ink reference electrode can show potential instability over time, particularly when exposed to varying temperatures, pH levels, or ionic strengths in the sample solution. This causes:

- Drift in baseline potential, affecting the accuracy of the measurement
- Potential need for frequent recalibration or replacement
- For high-precision applications, this limitation can significantly impact the data quality

b. Environmental sensitivity-

The performance of the biosensor can be influenced by environmental factors such as:

- Temperature and humidity, which affect electrode stability and enzymatic activity (if used).
- Mechanical stress, which can damage the printed layers or ZIF-8 coating Robust encapsulation or packaging solutions are necessary for field use.



- The **ZIF-8 layer** enhances surface area and selectivity, leading to a stronger and more specific signal.
- As analyte concentration increases, more redox reactions occur at the **working electrode**, increasing the electron transfer and thus the **measured current**.
- The **contact pads** transmit this current to an external circuit for reading.

4.CONCLUSION

The electrochemical biosensor presented in this study demonstrates a promising platform for sensitive and selective analyte detection, particularly in point-of-care and field-deployable applications. Its compact design, comprising printed carbon and silver electrodes, coupled with the incorporation of ZIF-8 as a high-surface-area sensing layer, reflects a modern, low-cost, and scalable approach to biosensing. The use of carbon ink for the working and counter electrodes ensures good conductivity and biocompatibility, while the silver ink reference electrode provides a stable potential for electrochemical measurements.

However, several limitations must be addressed to improve its practical applicability. Challenges such as limited long-term stability, potential reference electrode drift, electrode fouling in complex samples, and lack of selectivity without proper functionalization pose significant hurdles. Moreover, the ZIF-8 layer, although advantageous for surface area and adsorption, suffers from poor intrinsic conductivity and can degrade under certain chemical conditions. These issues necessitate further material optimization and engineering interventions.

Despite these challenges, the sensor's simplicity, affordability, and adaptability make it an excellent candidate for integration into advanced diagnostic systems. Future improvements such as the incorporation of antifouling layers, microfluidic channels for sample control, and hybrid nanomaterials to enhance conductivity and stability could significantly boost its performance and real-world usability.

In conclusion, this electrochemical biosensor offers a valuable foundation for next-generation biosensing technologies. With continued research and development, it has the potential to contribute meaningfully to fields such as healthcare diagnostics, environmental monitoring, and food safety, where rapid, portable, and accurate sensing is increasingly in demand.

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