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Development of Enzyme-Immobilized Nanostructured Biosensors for Rapid Detection of Pesticide Residues in Agricultural Products Data

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Abstract--- Due to the high demand for safety in agricultural products, there is a requirement for rapid, sensitive and reliable analytical techniques that can be used to detect pesticide residues in food samples. Traditional pesticide detection systems require the use of costly and complicated equipment, sample preparation steps that take a long period of time and special laboratories; thus, they are not suitable for use in rapid monitoring. In this research project, the application of enzyme-immobilized nanostructure biosensors for rapid detection of pesticide residues in agriculture products will be studied. The uniqueness of this technique will be realized through the combination of enzyme immobilization techniques and nanostructure material technology in order to enhance biosensors sensitivity, catalytic efficiency and performance. Acetylcholinesterase (AChE) serves as the main recognition enzyme due to its sensitivity to organophosphate and carbamate insecticides. The biosensors prepared are characterized using electrochemical techniques, calibration curve method, response time determination, selectivity studies, enzyme stability tests, and pesticide recovery studies. Comparative characterization of the biosensor includes detection limit, sensitivity, linear range of detection, response time, reproducibility, and stability of the working biosensor. This study analyzes the dependence of the pesticide detection capability on the composition of the nanomaterials used and the immobilization of the enzyme. It is believed that the proposed biosensor will show high sensitivity, fast response, and increased stability of the enzyme. Food samples such as fruits and vegetables are analyzed for practical application of the pesticide residues. The expected results reveal the feasibility of the biosensors based on nanostructured enzymes.

Keywords--- Enzyme Immobilization, Nanostructured Biosensors, Pesticide Detection, Acetylcholinesterase, Nanocomposites, Electrochemical Detection, Agricultural Products, Food Safety.

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I. Introduction

With the increasing use of pesticides in modern farming practices, there have been raised many issues related to food safety, environmental pollution, and exposure of humans to toxic chemicals (Sun et al., 2023). The extensive use of pesticides is meant to protect plants from harmful insects, weeds, and agricultural pests. If pesticides are used improperly and excessively or if there is inadequate withdrawal period, then there is a possibility that the pesticides will stay on agricultural products in the form of pesticide residues. Thus, the continuous testing of pesticide residues is of crucial importance to maintain food quality and safety standards (Mohammadi et al., 2023; Guo et al., 2013). Traditional methods for testing of pesticides include gas chromatography, liquid chromatography, and mass spectrometry. These methods offer reliable results but are quite costly and complicated.

Biosensors offer viable solutions as a result of their combination of the use of bio-receptors and physiochemical transducers, which enables them to detect particular chemical compounds. Biosensors based on enzymes find application in detecting pesticides because there are particular pesticides that inhibit the catalytic action of particular enzymes (Sonowal et al., 2025; Bhavaniramya et al., 2019). Biosensors based on acetylcholinesterase are capable of detecting organophosphate and carbamate pesticides by measuring any alteration in the enzymatic activity (Mansoori et al., 2023). Immobilization of acetylcholinesterase on

nanocomposite materials consisting of silver, reduced graphene oxide, and chitosan has been found applicable in the detection of carbaryl pesticides (Patel et al., 2019; Mashuni et al., 2022).

Nevertheless, there are some limitations with traditional enzyme biosensors such as instability of the enzymes, lower activity of the enzymes, poor reusability of the biosensors, and variable sensor output. This can be overcome through enzyme immobilization, which is based on immobilizing the biological components on appropriate support media without affecting their properties. Nanostructures such as graphene derivatives, metal nanoparticles, carbon nanotubes, and metal oxide nanoparticles offer a high surface area and appropriate electrical and chemical properties. Using these together with the process of enzyme immobilization may enhance electron transfer, loading of the enzymes, signal production, and sensor performance.

The current study will explore the design of biosensors using immobilized enzymes in nanostructures for the fast detection of pesticide residues in agriculture. The different nanostructures will be examined in terms of enzyme binding and signals generated. The performance of the immobilized enzyme systems will be characterized electrochemically and by studying the responses to pesticides, sensitivity and stability. Nanotechnology-based biosensors provide better chances of sensitive pesticide residue detection in agriculture and environment (Gheorghe et al., 2017; Naghavi et al., 2026; Hashwan et al., 2020). The main aim is to create an enzyme-based sensor which will ensure stability, sensitivity, speed of response and reliability. The feasibility of the developed method for agricultural samples, such as fruit and vegetable products, will be explored in the investigation.

Problem Statement

Conventional methods for pesticide analysis usually require costly analysis equipment, dedicated laboratory conditions, and complicated sample preparations. These methods enable accurate analysis of pesticide residues but pose challenges due to the complicated process involved. Enzyme biosensors present an interesting option for detecting pesticides, but the practical realization of enzyme biosensors presents difficulties due to enzyme instability, decreased catalytic activity, non-reusability, and insufficient sensitivity. Moreover, the current substrate material used in biosensors provides insufficient enzyme immobilization, inadequate electron transport capability, and non-uniform response. Lack of optimal sensing platform that would incorporate efficient enzyme immobilization and use of conductive nanostructured materials for rapid pesticide detection prevents their widespread use. It means that the detailed study should be performed in order to find out the connection between nanomaterial composition, enzyme immobilization efficacy, and sensing performance. This study aims to overcome the mentioned problems through the development of nanostructured enzyme-immobilized biosensors.

Section Overview

The structure of this research work includes sections on the design and analysis of the enzyme immobilized nanostructured biosensors. In the introductory part, the research motivation and objectives are presented together with an analysis of existing techniques for the detection of pesticides. The literature review section includes the discussion of past developments related to biosensors based on enzymes, nanostructured sensing materials, and enzyme immobilization techniques. The research gap section outlines the problems that remain unresolved. In the methodology section, the materials used in the study, the preparation of nanostructures, enzyme immobilization, sensor development, and sensor evaluation are described.

II. Literature Review

Immobilized enzymes biosensors are used in pesticide analysis due to their ability to provide biological recognition and conversion of such recognition into a quantitative signal either by electrochemical or optical methods. Enzymes react with target molecules, which provides the identification of contamination through catalytic activity change. Acetylcholinesterase (AChE) is commonly used since organophosphate and carbamate pesticides inhibit its activity. Under optimal conditions, inhibition can be correlated with pesticide concentrations. Research on AChE-based biosensors for pesticide detection in agricultural and environmental samples was conducted.

Biosensors development involves enzyme immobilization. Such immobilization can be done by physical adsorption, covalent binding, encapsulation, and cross-linking. These methods are used for retention of catalytic activity, and increasing the stability and reuse of enzymes. However, conditions should be optimized, as incorrect chemical reactions and high enzyme concentrations can reduce enzyme activity.

Significant properties of nanostructured materials include the ratio of surface area to volume, electrical conductivity, and functionalization (Andreescu et al., 2008). Studies focused on graphene oxide, reduced graphene oxide, carbon nanotubes, silver and gold nanoparticles, and metal oxides. These materials allow enzymes to attach, exchange electrons, and provide better signals. Nanocomposites consisting of graphene derivatives, metallic nanoparticles, and biocompatible polymers may help to improve sensor efficiency.

Electrochemical techniques, including cyclic voltammetry, differential pulse voltammetry, and electrochemical impedance spectroscopy, analyze electron exchange, electrode properties, and sensing behavior. Calibration analysis allows one to define the correlation between pesticide concentration and signal changes. Sensitivity, detection limit, linear range, response time, and reproducibility are critical performance characteristics.

Real sample analysis is needed because there are many interferents in agricultural products, including pigments, organic acids, and other substances. Extraction, filtration, dilution, and matrix analysis help to create conditions for reliable testing. Evaluation of recovery studies and comparison with existing analytical techniques allow estimating practical suitability.

However, different immobilization techniques, compositions of nanomaterials, electrode setups, and experimental conditions make it difficult to compare results. Modern research focuses on the combination of enzyme stabilization, nanomaterials with nanostructured substrate, and electrochemical signal amplification.

Research Gap

Although a lot of progress has been made in developing enzyme biosensors for pesticides, many issues have not yet been addressed in existing studies. Most of the current research focuses separately on enzyme immobilization, nanomaterial conductivity, and pesticide detection. Further investigation is needed into the effects of nanostructure properties together with enzyme immobilization on enzyme activity and biosensor performance. Incorrect enzyme immobilization may lead to decreased catalytic activity and even the loss of enzymes. Agricultural sample matrix interference is one of the factors affecting the accuracy of pesticide determination. Differences in electrode preparation, pesticide concentration, and other experiment conditions prevent fair comparison between different biosensor systems. Not much work has been done to establish a common experimental protocol for testing enzyme activity retention, detection sensitivity, response time, selectivity, stability, and recovery efficiency in real samples. This research attempts to overcome all these limitations through comparative nanostructure synthesis and enzyme immobilization.

III. Methodology

The described methodology will include a systematic experimental study on designing and comparing enzyme-immobilized nanostructured biosensors. This methodology will include the steps of selecting nanostructures, preparing sensing substrates, immobilizing enzymes, fabricating sensors and testing their ability to detect pesticides.

Material Selection and Preparation

Appropriate nanostructures, which include graphene oxide, reduced graphene oxide, carbon nanotubes, silver nanoparticles, and gold nanoparticles, will be selected depending on such parameters as their surface, electrical conductivity, biocompatibility and suitability for immobilizing enzymes. Acetylcholinesterase will be selected as a biological recognition element for detecting pesticides. Other necessary chemicals, buffers, electrode substrates and pesticide standards will be selected depending on experimental needs.

Nanostructured Sensing Substrate Preparation

Nanostructured sensing substrates will be prepared using appropriate methods of deposition, dispersion and functionalization. Selected nanomaterials will be dispersed in appropriate solvents or buffer-compatible solutions. In some cases, conductive nanomaterials and biocompatible polymer coatings can be mixed to enhance the quality of electrode surfaces and enzyme immobilization. These nanocomposites will be deposited on appropriate working electrodes.

Enzyme Immobilization

The enzyme acetylcholinesterase is immobilized on the prepared nanostructured surfaces through proper immobilization techniques such as physical adsorption, covalent binding, and polymer entrapping. Immobilization conditions such as enzyme concentration, pH, temperature, incubation period, and the

composition of supporting material are adjusted to ensure enzyme activity. Prepared electrodes are rinsed properly to remove excess enzymes and kept in the right conditions before further analysis.

Biosensor Fabrication

The enzyme-coated nanostructured electrode is placed in the electrochemical sensing setup having working, reference, and counter electrodes. The prepared biosensor is linked to the electrochemical measuring device. Various sensor setups are created to study the effect of the composition of nanomaterial and enzyme immobilization conditions on the detection ability of the sensors.

Pesticide Detection and Electrochemical Characterization

The biosensors are characterized with the following methods:

- Cyclic voltammetry (CV) method: Assesses electrochemical behavior and the nature of electron transfer.
- Differential pulse voltammetry (DPV) method: Determines the difference in electrochemical behavior with respect to inhibition caused by pesticides.
- Electrochemical impedance spectroscopy (EIS): Determines the charge transfer resistance at the interface.
- Enzymatic activity measurement: Determines the enzymatic activity retained after immobilization.
- Response time measurement: Determines the period taken to achieve stable output for pesticide detection.
- Stability measurement: Determines enzyme activity and response stability during storage and multiple uses.
- Selectivity measurement: Determines the effect of interfering species and different types of pesticides.

Pesticide Residue Detection

Various concentration solutions of pesticides are used to correlate pesticide exposure with biosensor signal changes. Organophosphate and carbamate pesticides are taken as target analytes. Biosensor signal changes are used to estimate the inhibition of the activity of acetylcholinesterase. Calibration graphs are plotted to find out the detection range, sensitivity, and limit of detection.

Agricultural Sample Preparation

The agricultural products such as fruits and vegetables are chosen to evaluate the biosensor performance on the real samples. Sample extraction method is optimized by suitable solvents or buffer. Filtration or dilution of the extracts can be done as required prior to the analysis with the biosensor. Known amounts of pesticides can be added to some chosen samples for recovery studies. Comparison of the results with reference values provides the reliability of the analysis.

Performance Evaluation

Biosensor performance is evaluated by sensitivity, limit of detection, response time, selectivity, reproducibility, recovery percent, and stability. Comparison gives the appropriate nanostructured design of the biosensor which shows the good balance between the enzyme activity and signal response.

The percent of the retained activity of the enzyme after the immobilization can be calculated in equation (1):

$$A_r = \frac{A_i}{A_f} \times 100 \quad (1)$$

Where:

- A_r = retained enzyme activity (%)
- A_i = enzyme activity after immobilization
- A_f = enzyme activity before immobilization

The percentage of pesticide inhibition can be calculated in equation (2):

$$I = \frac{S_0 - S_p}{S_0} \times 100 \quad (2)$$

Where:

- I = enzyme inhibition (%)
- S_0 = initial biosensor response without pesticide
- S_p = biosensor response after pesticide exposure

The percentage recovery is calculated in equation (3):

$$R = \frac{C_m}{C_a} \times 100 \quad (3)$$

Where:

- R = pesticide recovery (%)
- C_m = measured pesticide concentration
- C_a = added pesticide concentration

Comparative Performance Analysis

The biosensor systems developed are evaluated in terms of their enzyme activity preservation, sensitivity, detection limit, response time, linear detection range, selectivity, recovery, and stability. The optimized biosensor system is selected by taking into account all these parameters as opposed to optimizing only one of the parameters.

Proposed System Architecture

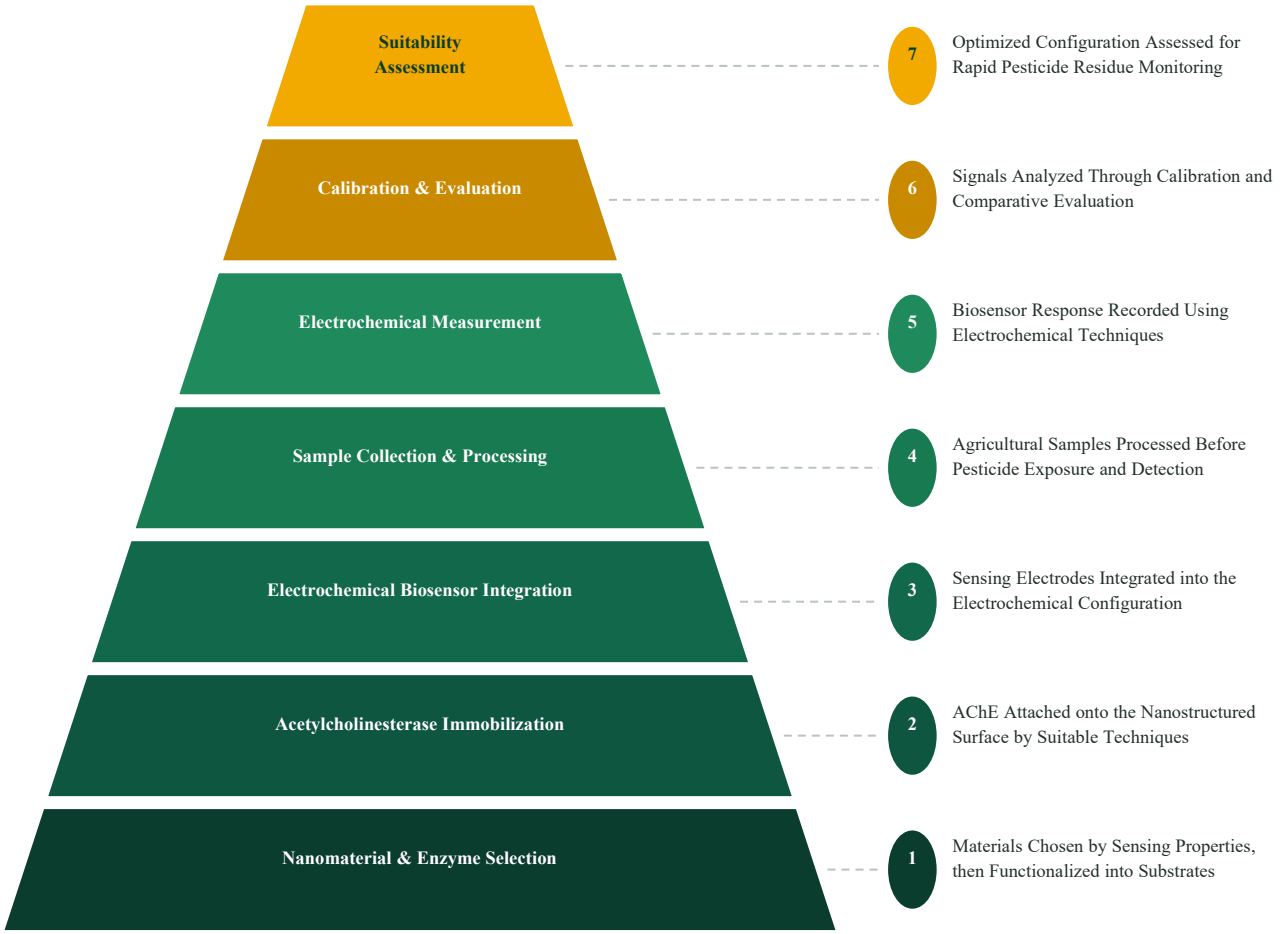


Figure 1: Proposed Pyramid System Architecture of Enzyme-Immobilized Nanostructured Biosensor for Pesticide Residue Detection

The architecture proposed above represents the complete experimental design for the fabrication of the enzyme-immobilized nanostructured biosensors for detection of pesticides residues. First, the suitable nanomaterials and biological recognition enzyme are selected depending on their sensing capabilities and

compatibility. The suitable nanomaterials are subjected to certain processes to make them suitable sensing substrates. After that, acetylcholinesterase is attached to the nanostructured substrate using suitable attachment methods. The sensing electrodes fabricated are used in the design of the electrochemical biosensors. Agricultural samples are collected and processed before carrying out the detection process. The electrochemical signals generated by the biosensor are captured and analyzed using calibration and comparison procedures.

In figure 1, the system architecture design of the proposed approach is presented as a seven-layer pyramid that starts with the choice of nanomaterial and enzymes and culminates in suitability analysis. Acetylcholinesterase is attached to the nanostructure electrodes, which are incorporated into the electrochemical biosensor and then tested on agricultural samples.

Experimental Parameters and Evaluation Criteria

The following list contains the parameters that can be used for systematic testing of experiments.

Table 1 shows the list of essential parameters along with their measurement techniques used to test the fabricated enzyme immobilized nanostructured biosensors. Enzyme activity retention and detection sensitivity can be used as the performance parameters, whereas the detection limit can be used as a parameter that determines the minimum detectable concentration of pesticides through the sensor system. The response time and selectivity indicate the analytical efficiency and reliability of detection.

Table 1: Experimental Parameters and Characterization Techniques

Parameter	Evaluation Technique	Research Objective
Enzyme Activity	Enzyme Activity Assay	Determine Retained Catalytic Activity
Surface Morphology	SEM Analysis	Examine Nanostructure Distribution
Functional Groups	FTIR Analysis	Identify Surface Chemical Characteristics
Electrochemical Response	CV and DPV	Evaluate Sensing Signal Characteristics
Charge Transfer	EIS Analysis	Examine Electrode Interface Properties
Detection Limit	Calibration Analysis	Determine Minimum Detectable Concentration
Response Time	Time-Dependent Measurements	Evaluate Detection Speed
Selectivity	Interference Testing	Examine Detection Reliability
Operational Stability	Repeated Measurements	Determine Sensor Durability
Recovery	Agricultural Sample Analysis	Evaluate Practical Applicability

IV. Proposed Biosensor Development and Performance Evaluation

Development of Enzyme-Immobilized Nanostructured Biosensors

The biosensor development with immobilized enzymes is achieved by selection of appropriate biological receptors and combination of these with nanostructured materials that can perform certain functions. Acetylcholinesterase is identified as the most important enzyme since its catalytic process will be affected by organophosphate and carbamate pesticides. The chosen nanomaterials are then characterized with respect to their morphology, electrical conductivity, chemical stability, and ability to interact with enzymes. Nanomaterials with different structures are made using reduced graphene oxide, silver and gold nanoparticles, as well as carbon nanotubes.

The nanomaterials are then incorporated into the surface of the electrodes in order to optimize the electrochemical detection surface. The quantity and distribution of nanomaterials are varied in order to determine their effect on surface properties and signal generation. Excessive incorporation of nanomaterials could negatively affect the uniformity of surface and interaction with enzymes. Thus, optimization is required to find the proper balance.

Immobilization of enzymes can be achieved via physical adsorption, covalent bonding, or entrapment into polymers. During immobilization, the goal is to retain the structural functionality of the enzyme while making the immobilization more stable and minimizing the loss of the enzyme when repeatedly used. Supporting materials such as chitosan and other suitable biocompatible polymers could be used in immobilization. The resulting electrode biosensors are analysed for enzyme activity, surface properties, electrochemical performance, and operational stability.

The developed biosensor identifies pesticide residues through the enzyme inhibition mechanism. In normal conditions, the acetylcholinesterase would catalyse the hydrolysis reaction of acetylcholine or

electrochemically sensitive substrate. In the presence of organophosphate and carbamate pesticides, the catalytic activity of the immobilized enzyme becomes inhibited, and this leads to changes in the electrochemical response of the sensor. The extent of signal change is then analysed using the calibration equation to estimate the concentration of the pesticides.

Pesticide Detection Performance and Agricultural Product Analysis

The electrochemical response of the obtained biosensors with regard to pesticide detection is assessed through the analysis of the sensor response at various concentrations of pesticides. The data on the current response due to enzyme inhibition can be received using cyclic voltammetry and differential pulse voltammetry techniques. The calibration curves allow us to find the dependence between the pesticide concentration and the signal change.

Additionally, the biosensors are tested for operational stability through multiple measurements. The activity retention of the enzyme at storage and repeated exposure to pesticide solutions is also investigated. The selectivity test helps to investigate the impact of the interference and different pesticide substances on the sensing response.

In terms of agricultural product analyses, the improved biosensor can be used on samples extracted from fruits and vegetables. The samples will undergo extraction, filtration, and dilution prior to electrochemical measurements. The obtained pesticide levels in the biosensor will then be analysed using recovery tests, and whenever possible, by comparing with other analytical techniques. Through such testing, the developed biosensor's applicability can be determined.

The proposed sensing platform can be employed for screening of pesticides in commonly used agricultural products. The possible advantages of the sensing platform include decreased analysis time, ease of use, and potential of developing portable sensors. However, the validation of the biosensor with regard to regulatory requirements and quantification of the pesticides should be performed.

V. Performance Analysis and Visualization

Sensitivity Comparison

The comparative bar chart is chosen as a method of demonstrating the sensitivity of various enzyme-embedded nanomaterials-based biosensors. It will be used in order to understand the effect of the material used and the enzyme embedding techniques on the sensitivity of pesticide detection. The comparison will involve the following biosensors: unmodified enzyme electrode, enzyme-chitosan electrode, graphene-based enzyme electrode, and metal nanoparticles-based nanocomposite electrode.

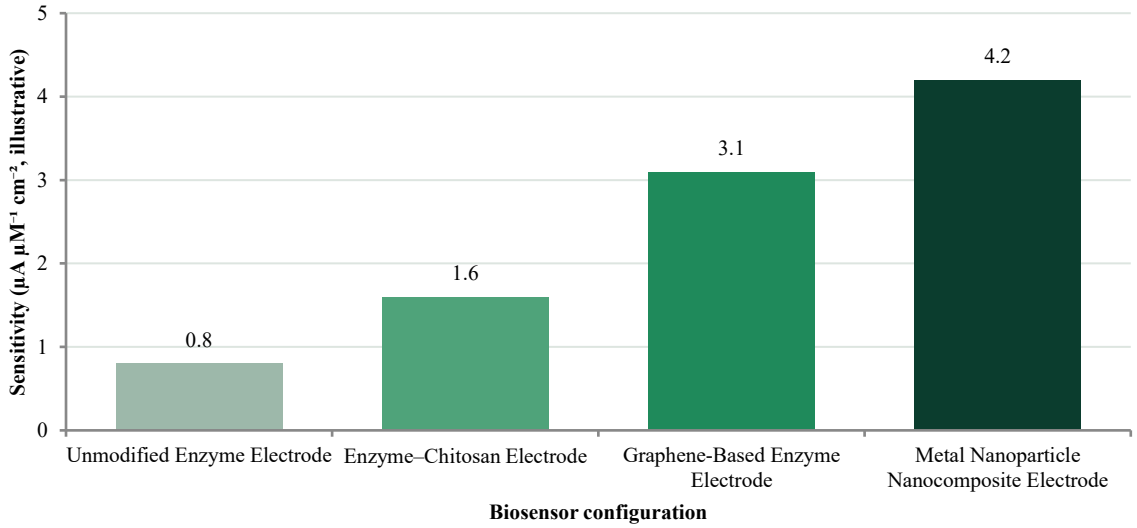


Figure 2: Comparative Sensitivity of Enzyme-Immobilized Nanostructured Biosensor Configurations for Pesticide Residue Detection

Figure 2 is Comparison of the sensitivity of four types of enzyme-immobilized biosensor design. Nanostructured electrodes should demonstrate higher electrochemical sensitivity due to their increased surface area and better charge transfer. Just sensitivity is not the sole parameter to determine superiority of one biosensor to another. Enzyme immobilization, selectivity, response time, and stability play an important role too.

VI. Results and Discussion

This research will investigate the interrelation between nanostructured material composition, enzyme immobilization and pesticide detection. Sensitivity of different configurations of electrodes (classical electrodes and electrodes with enzymes immobilized on nanomaterials) will be compared. The addition of nanomaterials will positively affect the quality of the electrode surfaces, electron transfers and electrochemical processes. But it should be studied how the enzyme activity will be affected by the presence of nanomaterials for finding the optimal combination of materials. Enzyme immobilization is the process that increases enzyme immobilization, maintains enzyme activity and allows the functioning of sensors many times. It depends on the method of enzyme immobilization, enzyme concentration and other factors. It is anticipated that higher levels of pesticides will cause more enzyme inhibition in the detection range, leading to a signal change. Detection limit, sensitivity, linearity, response time, and recovery percentage have been selected as important parameters. Experiments performed for agricultural samples based on the use of extracts from fruits and vegetables give information about the effect of the matrix and the ability of detection. Selectivity and reproducibility tests also indicate the reliability of the sensing in different test conditions. The best performing biosensor is determined with respect to sensitivity of detection, enzyme inhibition, stability, and response time. These are the anticipated results of the evaluation of the biosensor, not the results of the experiment.

Challenges in Enzyme-Immobilized Biosensor Development

There are a number of issues associated with the creation of enzyme-immobilized nanostructured biosensors that impact their detection ability and applications in agriculture. Instability of enzymes due to changes in temperature, pH, and surrounding environment may cause a decrease in their catalytic activity. The other issue that should be taken into account is the enzyme immobilization efficiency, since improper attachment procedures can cause loss of enzymes and affect catalytic activity. Also, nanomaterials' suitability needs to be considered for maintaining good interaction between biological and sensing materials. Sensing interface optimization and efficient electrochemical signal generation are needed to achieve high detection sensitivity at low pesticide levels. The problem with interference from other agricultural compounds, such as pigments and organic acids, also has to be solved. Multiple measurements and long-term storage can have an effect on the stability of biosensors. Finally, there can be inconsistencies in electrode fabrication, distribution of nanomaterials, and enzyme loading.

Future Recommendations

Future studies need to be concerned with optimization of nanomaterial components and enzyme immobilization parameters for improvement of catalytic activity, sensitivity, and stability of the sensor system. Nanocomposites based on graphene analogues, metal nanoparticles, and biocompatible polymers can be considered for enhancement of properties of electrode surface and electron transfer. Experiments on long-term stability will provide information on activity changes of enzymes and sensors in course of prolonged storage and operation. Other systems based on other enzymes and enzyme immobilization techniques can be investigated for the purpose of improvement of pesticide identification efficiency. Electrochemical biosensors that could be used for rapid detection of pesticides in agricultural conditions have to be developed in further studies. The study of multiple pesticide detection ability and effect of interfering components on the sensor responses can be considered. Validation of the sensor using various kinds of fruits and vegetables and comparison with existing chromatographic techniques will prove reliability of the method. Moreover, economic aspects, manufacturability of the proposed device have to be analysed to evaluate commercial prospects of the sensor system.

VII. Conclusion

This study examines the development and evaluation of performances of enzyme-immobilized nanostructure-based biosensors for quick detection of pesticide residues in agricultural products, focusing on enhanced detection sensitivity, enzyme stability, and electrochemical performance. The main purpose is to

develop efficient sensing devices that could detect pesticide contamination while minimizing the complexity in operations of current methods. What makes the current study novel is the use of enzyme immobilization methods together with the application of functional nanostructures in order to improve biological recognition ability, electrochemical performance, and pesticide detection performance of sensing devices. This study will focus on selection of appropriate nanomaterials, fabrication of functional sensing substrates, immobilization of acetylcholinesterase, production of electrochemical biosensors, and evaluation of their analytical performances. The comparative analysis analyses the interconnection between nanomaterial structure, efficiency of enzyme immobilization and pesticide inhibition response. The agricultural samples analysis, including fruits and vegetables is performed to evaluate the capability of the detection using pesticide recovery tests and comparison with conventional methods of pesticide detection. The expected result will be the optimal biosensor based on nanostructure and enzyme immobilization providing a good compromise between sensitivity of detection, reaction time, activity of enzyme and stability. The proposed approach allows incorporating modern biosensing technologies into the agriculture for quality control and pesticide residues detection. The future research should be focused on enzyme stability, simultaneous detection of several pesticides, portable biosensor fabrication, use of real samples and commercialization of the biosensor.

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