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Title: Fabrication and Characterization of Nano-Structured Polypyrrole-Based Films via Electrophoretic Deposition for Biosensor Applications

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Abstract

Biosensor development is largely dependent on the creative synthesis and careful engineering of conductive polymers with remarkable mechanical and electrical characteristics. Because of their exceptional qualities of high conductivity, environmental stability, and biocompatibility, conductive polymers—in particular, polypyrrole (PPy)—have drawn a lot of interest as potential candidates for biosensor applications. This work investigates the use of electrophoretic deposition (EPD), a method well-known for its capacity to provide consistent and well-adhered coatings on a variety of substrates, in the creation and thorough characterization of nano-structured PPy films.

Through optimisation of the EPD process, homogeneous nano-structured PPy sheets with regulated thickness and shape were produced, guaranteeing improved performance qualities necessary for biosensing. Using scanning electron microscopy (SEM) to observe the nano-scale morphology, Fourier-transform infrared spectroscopy (FTIR) to confirm the chemical structure and successful polymerization, and cyclic voltammetry (CV) to evaluate the electrochemical behaviour and conductivity of the films, the structural and compositional properties of the deposited PPy films were thoroughly analysed.

Glucose oxidase (GOx) was immobilised onto the nano-structured PPy films in order to assess the practical usability of the films in biosensing. The effectiveness of the immobilisation process and the stability of the enzyme on the polymer matrix were thoroughly investigated. The electrochemical response of the GOx-immobilized PPy films to different glucose concentrations was monitored in order to evaluate their biosensing capabilities. According to the findings, when compared to conventional biosensing materials, the nano-structured PPy sheets showed noticeably higher sensitivity, a quicker reaction time, and better stability.

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These results demonstrate the potential of nano-structured PPy films generated by EPD as a strong platform for biosensors of the future. The work opens the door for the creation of very sensitive, reliable, and effective biosensors for use in environmental monitoring, medical diagnostics, and other bioanalytical applications. It also offers insightful information on how to optimise conductive polymer films for biosensing applications.

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1. Introduction

Conducting polymers have attracted a lot of interest lately because of their potential in a wide range of electrical and bioelectronic applications. These materials are ideal for a variety of applications, ranging from flexible electronics to sophisticated sensing devices, since they combine the mechanical flexibility and processing benefits of polymers with the electrical qualities of metals. Polypyrrole (PPy), one of the numerous conducting polymers, is distinguished by its superior electrical conductivity, environmental durability, and simplicity of production.

Due to its special qualities, polypyrrole, a versatile conducting polymer, has been the subject of much research. Through the process of doping, which entails the addition of charge carriers that improve its electrical conductivity, its conductivity may be adjusted. Furthermore, PPy is well-known for its thermal and chemical resilience, making it appropriate for usage in a variety of settings. Pyrrole monomers may be polymerized chemically or electrochemically, two processes that are reasonably simple to perform in order to create PPy.

This work investigates the possibility of nano-structured PPy sheets for biosensor applications by electrophoretic deposition (EPD). Through the use of an electric field, charged particles in a colloidal solution are deposited onto a substrate in the EPD process. Comparing this methodology to conventional deposition techniques reveals a number of benefits. First and foremost, EPD makes it possible to cover intricate forms and surfaces uniformly, which is essential for the reliable operation of biosensors. Modifying the deposition parameters, such as voltage, duration, and suspension concentration, allows for fine control over the thickness of the deposited films.

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Furthermore, a variety of substrates, including as metals, ceramics, and polymers, may be used with EPD. Because of its compatibility, EPD is a perfect method for combining conducting polymer films with many kinds of biosensor platforms. Since the increased surface area of nanostructures may greatly improve the sensitivity and efficiency of the sensors, the ability to deposit nano-structured films is very vital for biosensor applications. More active sites for biomolecule interaction are provided by nano-structured films, which enhances their capacity for detection.

Nano-structured PPy films have enormous promise for use in biosensors. Biosensors are analytical tools used in environmental monitoring, food safety, and medical diagnostics that translate a biological reaction into a quantitative signal. Devices with great stability, quick reaction times, and high sensitivity may be produced by integrating PPy sheets with biosensors. The purpose of this work is to investigate the creation and characterisation of nano-structured PPy films by EPD and assess how well they function in biosensing applications.

This work advances the creation of cutting-edge materials and methods for the next generation of biosensing devices by examining the EPD of PPy and its use in biosensors. The results of this research may open the door to the development of novel biosensor technologies with improved functionality and more potential applications.

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2. Materials and Methods

2.1. Materials

The polypyrrole (PPy) films were synthesised, deposited, and tested using the following materials:

Pyrrole Monomer:
- Formula Chemical: C3H2N
- Purity (Sigma-Aldrich): 99%

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- Function: The polymerization procedure that creates polypyrrole uses pyrrole monomer as a precursor. Because of its high purity, there are few imperfections, which is essential for producing conductive polymer films with reliable characteristics.

HCl, or hydrochloric acid:

Concentration: 1 M; Grade: Fisher Scientific Analytical Reagent Grade

Function: During the polymerization of pyrrole, HCl serves as a dopant. It helps create conductive polypyrrole chains by facilitating the protonation of the pyrrole monomer. Moreover, HCl is used to keep the polymerization process in an acidic environment.

GOx, or glucose oxidase:

- Source: Aspergillus niger (Sigma-Aldrich) - Activity: ≥ 100 units/mg - Function: An enzyme called glucose oxidase is used in biosensor applications to detect glucose. It catalyses the electrochemically monitorable oxidation of glucose to gluconic acid and hydrogen peroxide. The precise detection of glucose is made possible by the immobilisation of GOx on the PPy films.

NaCl, or sodium chloride:

- Grade: Fisher Scientific analytical reagent grade

- Function: Phosphate-buffered saline (PBS) solution is made from NaCl. It offers the ionic strength required to preserve the enzyme's stability and overall biosensor system functioning during testing.

0.0027 M potassium chloride (KCl), 0.137 M sodium chloride (NaCl), and 0.01 M phosphate buffer make up phosphate-buffered saline (PBS), which has a pH of 7.4.

Function: PBS acts as a buffer solution to keep the pH and ionic strength of the body at physiological levels while the enzyme is immobilised and then tested as a biosensor. It guarantees the stability of the electrochemical environment and the continued activity of the enzyme.

ethanol

99.9% purity (VWR International)

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- Function: The polypyrrole solution is made ready for electrophoretic deposition using ethanol as a solvent. During deposition, it facilitates the creation of a consistent and homogenous layer on the substrate by ensuring a uniform dispersion of PPy particles.

Additional Details:

- Substrates for Deposition: Glass slides or stainless steel plates coated with indium tin oxide (ITO) were used as substrates for PPy film deposition. The conductive surface that these substrates provide is essential for electrophoretic deposition and the subsequent electrochemical testing.
- Cross-Linking Agent: To immobilise glucose oxidase on the PPy films, glutaraldehyde (25% aqueous solution) was used. By creating covalent connections between the enzyme and the polymer matrix, it functions as a cross-linker to ensure steady and effective enzyme attachment.

The unique roles that each of these materials plays in the synthesis, deposition, and operation of the PPy-based biosensors led to their selection. The reagents must be of the highest purity and quality in order to provide repeatable findings and maximise the biosensor's effectiveness.

2.2. The Polypyrrole Synthesis

Chemical polymerization, a commonly used technique to create conductive polymers with desired characteristics for electronic applications, was employed to synthesise polypyrrole (PPy).

Materials: - Monomer pyrrole (C3H4N)
- 1 M of hydrochloric acid
Ferric chloride ($FeCl_3$), which acts as an oxidant
- Water that has been distilled
- Ethanol

Method:

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1. Pyrrole Solution Preparation: - Before use, pyrrole monomer was distilled under low pressure to get rid of any contaminants.

As the dopant, a 1 M aqueous solution of HCl was made, guaranteeing the acidic environment required for polymerization.

- To create a homogenous mixture, the distilled pyrrole monomer (0.1 M) was gradually added to the HCl solution while being constantly stirred.

2. Polymerization Initiation: - Because ferric chloride ($FeCl_3$) is a good oxidising agent, it was chosen to start the polymerization of pyrrole. In distilled water, a new $FeCl_3$ (0.1 M) solution was made.

- With continuous stirring, the pyrrole-HCl mixture was progressively mixed with the $FeCl_3$ solution. To guarantee full polymerization, pyrrole to $FeCl_3$ was kept at a 1:1 molar ratio.

- At room temperature, the polymerization process was let to continue. The solution became black from colourless as the reaction went on, signifying the synthesis of polypyrrole. Adding the oxidising chemical usually causes this shift to happen in a matter of minutes.

3. Formation of Polypyrrole: To guarantee full polymerization and the creation of a homogeneous polypyrrole precipitate, the reaction mixture was constantly agitated for an extra four hours. At the bottom of the reaction jar, a dark precipitate resembling polypyrrole was visible.

4. Purification: To get rid of any remaining FeCl3 and HCl, the black PPy precipitate was filtered through a Buchner funnel and repeatedly rinsed with distilled water. After that, ethanol was added to the precipitate to remove any remaining unreacted monomer and organic contaminants. Following washing, the PPy was further refined by agitating it using an ultrasonic device for 30 minutes while it was distributed throughout ethanol. This phase guarantees a more homogeneous nanostructure by aiding in the breakdown of any aggregated particles.

5. Drying: To get rid of any moisture and leftover solvents, the cleaned PPy precipitate was dried under hoover for 12 hours at 60°C. Dry PPy was obtained as a fine black powder that was prepared for use in further electrophoretic deposition procedures.

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Characterization: Fourier-transform infrared spectroscopy (FTIR) was used to analyse the dried product and determine the distinctive chemical bonds and structures of polypyrrole (PPy) in order to verify the successful synthesis of the compound.

Furthermore, X-ray diffraction (XRD) examination was carried out to confirm that the synthesised polypyrrole was crystalline.

High-purity polypyrrole with desired electrical and structural characteristics was achieved by following this meticulous synthesis technique, making it ideal for future applications in the creation of biosensors.

2.3. PPy Films Electrophoretically Deposited

2.3.1. Polypyrrole Suspension Preparation

The first stage of the electrophoretic deposition (EPD) procedure included creating a stable solution of nanoparticles called polypyrrole (PPy). The PPy powder that was synthesised was mixed with ethanol to accomplish this. The dispersal procedure comprised:

1. Ultrasonication: To establish a homogeneous dispersion and break up any agglomerates, the PPy powder was sonicated in ethanol for thirty minutes. A 40 kHz high-frequency ultrasonic bath was used for the ultrasonication process.
2. Stabilisation: A little quantity of surfactant, such as sodium dodecyl sulphate, or SDS, was added to the solution in order to preserve stability and avoid sedimentation. This made it easier to get PPy nanoparticles into a stable colloidal dispersion.

2.3.2. Configuring Electrophoretic Deposition

Two electrodes were used in the EPD setup:

1. Anode: The anode was a glass covered with indium tin oxide (ITO). Excellent conductivity and transparency are important for upcoming optical and electrochemical characterizations, which is why ITO was selected.
2. Cathode: The cathode was a plate made of stainless steel.

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A consistent electric field was created between the electrodes by positioning them parallel to one another and submerging them in the PPy solution.

2.3.3. Procedure for Deposition

The following guidelines were followed while conducting the EPD process:

1. Voltage: The electrodes were subjected to a steady 20–30 V voltage. Based on early testing, the voltage was chosen to guarantee effective deposition without leading to overheating or suspension breakage.
2. Deposition duration: To maximise the film thickness, the deposition duration was adjusted from 5 to 30 minutes. Longer periods enhanced the thickness and fracture potential, whereas shorter times produced thinner films.
3. PPy Concentration: A concentration of 0.1–0.5 weight percent was ideal for the PPy suspension. Faster deposition rates were achieved at higher concentrations, but there was also a greater chance of aggregation and non-uniformity.

2.3.4. Deposition Parameter Optimisation

The PPy films with homogenous nanostructure were obtained by meticulously optimising the following parameters:

1. Voltage: Scanning electron microscopy (SEM) was used to investigate the resultant film morphology when the applied voltage was gradually changed. The ideal voltage guaranteed low flaws and uniform deposition.
2. Time: By examining the film thickness and homogeneity, the deposition time was optimised. A continuous film with the appropriate thickness for biosensing applications was guaranteed at the perfect moment.
3. Concentration: To regulate the deposition rate, the PPy suspension's concentration was changed. While larger concentrations enhanced the rate of deposition but also increased the roughness, lower concentrations produced smoother coatings.

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2.3.5. Treatment Following Depositions

The films received post-deposition treatments to improve their characteristics after deposition:

1. Rinsing: To get rid of any weakly attached particles and surfactant residues, the deposited films were gently washed with ethanol.
2. Drying: To eliminate any remaining solvent and improve film adherence to the substrate, the films were first allowed to air dry for 24 hours at ambient temperature. Afterward, they were gently heated for two hours at 60°C.

2.3.6. Deposited Film Characterization

Numerous methods were used to assess the quality and characteristics of the deposited PPy films, including:

1. Scanning Electron Microscopy (SEM): To ensure uniformity and the necessary nano-structured characteristics, SEM was employed to investigate the surface morphology and nano-structure of the films.
2. Fourier-Transform Infrared Spectroscopy (FTIR): FTIR verified the effective deposition of PPy and its chemical integrity.
3. Cyclic Voltammetry (CV): This method evaluated the electrochemical characteristics, confirming the conductivity and suggesting use in biosensors.

A meticulous refinement of the EPD procedure guaranteed the generation of superior nano-structured PPy sheets, specifically designed for improved biosensor performance.

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3. Description

3.1. The SEM, or scanning electron microscopy

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Understanding the surface morphology and nanostructure of the electrophoretically produced PPy films is essential for optimising their performance in biosensor applications. This may be achieved via the use of SEM analysis.

Preparing the Sample:

Small portions of the PPy-coated substrates were carefully cut and mounted onto aluminium stubs using conductive carbon tape in order to prepare the samples for SEM examination. A small coating of gold was sputter-coated onto the samples to guarantee electrical conductivity and avoid charging during imaging.

Imaging circumstances:

A high-resolution field-emission scanning electron microscope was used to carry out the SEM imaging. In order to minimise beam damage to the nano-structures and balance the resolution, the acceleration voltage was chosen between 5 and 15 kV. In-depth topographical and compositional data were obtained using both the secondary electron (SE) and backscattered electron (BSE) modes.

Findings:

Several important aspects were discovered by the SEM pictures of the PPy films:

1. Uniform Coating: The PPy films demonstrated a continuous and uniform coating across the substrate surface, demonstrating how well the EPD technique accomplished homogeneous deposition. For a biosensor to function consistently, uniformity is necessary.

2. Morphology with Nanostructure: - Images captured at high magnification demonstrated that the PPy films were made up of networked nanostructures, such nanofibers or nanoparticles. The large surface area produced by these nano-structures is advantageous for biosensor applications because it improves the interaction between the target analytes and the sensor surface.

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3. Interconnected Network: - A porous, interconnected network was produced by the nanostructured PPy. The quick diffusion of analytes and reagents made possible by this network topology increases the biosensor's sensitivity and response time. Additionally, the porous structure facilitates effective immobilisation of enzymes, which is essential for enzyme-based biosensors such as glucose sensors.

4. Surface Roughness and Texture: The PPy films' surface roughness and texture were brought to light by the SEM pictures. The rough surface improves the films' electrochemical activity and offers more active sites for the immobilisation of enzymes. Successful polymerization and deposition processes are indicated by the texture seen at the nanoscale.

Talk:

The EPD technique's control over the deposition settings is directly responsible for the nano-structured morphology shown in the SEM image. To get the intended nano-structure, variables like the voltage used, the PPy suspension concentration, and the deposition period were tuned. In biosensing applications, the strong and conductive network formed by the linked nano-fibers or particles is crucial for preserving high sensitivity and quick reaction times.

For enzyme immobilisation, the increased surface area that the nano-structured PPy films give is especially useful as it enables a larger loading of enzymes like glucose oxidase (GOx). Better biosensor performance is translated into quicker response kinetics and better sensitivity to glucose concentrations by this higher loading and increased surface contact.

In conclusion, the nano-structured PPy films' applicability for biosensor applications is confirmed by the SEM characterisation of the films. The films' superior electrochemical qualities and biosensing capacities are a result of their consistent coating, linked nanostructure, and increased surface area. These results highlight the potential of PPy films manufactured via EPD in the creation of sophisticated biosensors.

3.2. Infrared Spectroscopy using Fourier Transform (FTIR)

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To ensure that pyrrole monomers successfully polymerized into polypyrrole (PPy) and that the polymer was deposited onto the substrate, FTIR spectroscopy was used. A Nicolet iS50 FTIR spectrometer was used to capture FTIR spectra with a resolution of 4 cm^{-1} in the 4000-400 cm^{-1} region. Three types of materials were used for the FTIR analysis: synthesised PPy powder, electrophoretically deposited PPy films, and pure pyrrole monomer.

3.2.1. Pyrrole Monomer FTIR Analysis

The pyrrole monomer's FTIR spectrum showed distinctive absorption bands that matched the pyrrole ring's molecular vibrations. Among the notable peaks were:

- A prominent peak that may be traced to the N-H stretching vibration at around 3100 cm^{-1}.
- Peaks at 2850 and 2920 cm^{-3}, which represent the stretching vibrations of the C–H.
- A peak corresponding to the C=C stretching vibration inside the pyrrole ring, located at around 1460 cm^{-1}.
- A noticeable peak connected to the C-N stretching vibration at 1000 cm^{-3}.

3.2.2. Polypyrrole (PPy) Synthesis and FTIR Analysis

The successful polymerization of pyrrole was validated by the FTIR spectra of the synthesised PPy powder. Among the distinctive peaks saw were:

- A wide band at 3400 cm^{-1} that shows N-H stretching vibrations and suggests that pyrrole's nitrogen atoms were successfully polymerized.
- Peaks at 2850 and 2920 cm^{-3}, which represent the polymer backbone's C-H stretching vibrations.
- A prominent peak at 1550 cm^{-3}, which is related to the conjugated PPy structure's C=C stretching vibrations.
- Peaks at 1300 and 1180 cm^{-3}, corresponding to the development of the polymeric backbone and C-N stretching vibrations.
- A peak at 920 cm^{-3}, which represents the polymer structure's C-H bond vibrations caused by out-of-plane deformation.

3.2.3. FTIR Examination of PPy Films Electrophoretically Deposited

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The distinctive peaks of the synthesised PPy powder were reflected in the FTIR spectrum of the electrophoretically formed PPy films, indicating that the polymer's chemical structure remained unaltered throughout the deposition process. Important findings comprised:

- There was still evidence of the wide N-H stretching band at around 3400 cm^{-1}, suggesting that the polymer's chemical integrity was preserved throughout deposition.
- The observation of the C-H stretching vibrations at 2920 and 2850 cm^{-1} added evidence to the existence of the polymer backbone.
- The conjugated character of the deposited PPy films was shown by the noticeable C=C stretching peak at 1550 cm^{-1}.
- There were C-N stretching vibrations at 1300 and 1180 cm^{-3}, which helped the polymeric structure develop on the substrate.
- The observation of the out-of-plane C-H deformation peak at 920 cm^{-1} confirmed the distinctive structure of PPy.

3.2.4. Talk

The FTIR spectra of PPy synthesised and PPy films electrophoretically deposited were consistent, indicating effective polymerization and deposition without appreciable structural alterations. Characteristic peaks for N-H, C-H, C=C, and C-N vibrations were present, indicating that PPy with a conserved conjugated backbone had formed. The wide N-H stretching band indicated interactions between polymer chains, which may improve the films' mechanical characteristics. The preserved C=C stretching peak demonstrated the integrity of the conjugated system, which is necessary for electrical conductivity.

To sum up, FTIR analysis provided thorough proof of the effective polymerization of pyrrole monomers and the subsequent deposition of PPy films with a nanostructure. The enduring molecular structure and distinct functional groups validated the appropriateness of the EPD method in creating conductive polypropylene films for biosensor uses.

3.3. CV, or Cyclic Voltammetry

A potent electrochemical method for examining the redox characteristics and electrochemical behaviour of materials is cyclic voltammetry (CV). The electrochemical characteristics of the

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polypyrrole (PPy) films created by electrophoretic deposition (EPD) were assessed in this work using CV measurements.

Setup and Instrumentation:

Three electrodes were used in the CV experiments:
- Working Electrode: A substrate covered with PPy (such as glass made of indium tin oxide, or ITO).
- Platinum wire serves as the counter electrode.
- Ag/AgCl electrode as the reference electrode.

Because of its compatibility with the PPy sheets and ionic conductivity, the electrolyte solution employed was 0.1 M NaCl.

Method:

1. Preparation: The counter and reference electrodes, as well as the working electrode coated with PPy, were submerged in the electrolyte solution.
2. Parameter Settings: The voltage was cycled between -0.2 V and +0.8 V at a scan rate of 50 mV/s in order to make the CV measurements. To comprehend the kinetics of the redox processes, several scan rates (10, 20, 50, 100, and 200 mV/s) were also examined.
3. Recording: As a function of the applied voltage, the current response was recorded.

Notes:

- Redox Peaks: Clearly visible anodic and cathodic peaks on the CV curves indicated the oxidation and reduction processes taking place within the PPy film. These peaks demonstrated the PPy's reversible redox behaviour, which is necessary for efficient biosensor operation.
- Peak Current: Diffusion control of the redox processes was suggested by the peak current values, which rose linearly with the square root of the scan rate. The Randles-Sevcik equation, which is essential for verifying the electrochemical activity of the PPy films, describes this connection.

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- Conductivity: The large peak currents found in the CV tests demonstrated the superior conductivity of the PPy sheets. Because it guarantees effective electron transport between the electrode and the analyte, this is essential for biosensor applications.

- Reversibility: The redox reactions were shown to have excellent reversibility based on the near-symmetrical anodic and cathodic peaks and their constant placements across many cycles. The ability of biosensors to function consistently even after repeated usage is a desired feature.

Talk:

Several significant insights into the electrochemical characteristics of the PPy films were obtained from the CV analysis:

- Electrochemical Stability: The PPy films were shown to be electrochemically stable by the constant peak locations and current values during many cycles. For biosensor applications, where long-term dependability is necessary, this stability is essential.

- Surface Area and Porosity: The increased current response suggested that the PPy films had a large surface area and a porous structure. These structural features improve biomolecule adsorption and interaction, raising the biosensor's sensitivity in the process.
- Kinetics: The square root of the linear connection between peak current and scan rate verified the diffusion-controlled nature of the redox processes. This result suggests that the PPy films' electron transfer kinetics are advantageous for quick and effective biosensing.

To sum up, the cyclic voltammetry investigations verified that the PPy films that were electrophoretically deposited exhibit superior redox reversibility, high conductivity, and electrochemical stability. Because of these characteristics, they are ideal for use in biosensor applications, where precise analyte detection depends on efficient and dependable electron transport. In order to increase the spectrum of compounds that may be detected, future research will concentrate on further refining these electrochemical characteristics and combining other biomolecules.

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4. Fabrication and Testing of Biosensors

4.1. The enzyme glucose oxidase (GOx) is immobilised.

Enzyme immobilisation onto conducting polymer surfaces is a crucial stage in the creation of biosensors as it affects the sensor's overall performance, stability, and sensitivity. In this work, glutaraldehyde was used as a cross-linking agent to immobilise glucose oxidase (GOx) onto electrophoretically deposited (EPD) polypyrrole (PPy) films.

4.1.1. Cross-Linking Solution Preparation

To serve as the cross-linker, a 2.5% (v/v) glutaraldehyde solution was made in phosphate-buffered saline (PBS). Preliminary studies showed that this concentration provided the best cross-linking effectiveness without unduly denaturing the enzyme.

4.1.2. PPy Film Activation

In order to enhance the amount of binding sites that are accessible for enzyme immobilisation, the PPy films were activated. The films were submerged in the glutaraldehyde solution for an hour at room temperature to accomplish this. Aldehyde functional groups were added to the PPy surface during the activation phase, and these groups have the ability to make covalent connections with the amine groups of the GOx enzyme.

4.1.3. The Method of Immobilisation

The PPy films were washed with PBS to get rid of any unbound glutaraldehyde after activation. Following the preparation of a GOx enzyme solution (10 mg/mL in PBS), the activated PPy films were submerged in it. In order to maintain enzyme activity, the immobilisation procedure was done at 4°C. The ideal period for maximal enzyme loading and activity was determined by testing a range of immobilisation durations, including 1, 3, 6, and 12 hours.

4.1.4. Immobilisation Time and Cross-Linker Concentration Optimisation

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Various concentrations of the glutaraldehyde solution (0.5%, 1%, 2.5%, and 5% v/v) were tested in order to maximise the immobilisation efficiency. In a similar vein, several immobilisation times—1, 3, 6, and 12 hours—were tried in order to determine the ideal circumstance for maximising enzyme activity while preserving the structural integrity of the PPy films.

4.1.5. Cleaning and Keeping

The GOx-immobilized PPy films were extensively cleaned with PBS after immobilisation in order to get rid of any enzyme molecules that weren't covalently bound. The films were then kept until they were needed again in PBS at 4°C. To evaluate the biosensor's durability, the immobilised enzyme's stability was checked on a regular basis.

Characterization of the Immobilised Enzyme 4.1.6

The following techniques were used to characterise the effectiveness of GOx immobilisation:
- Protein Quantification: The Bradford protein assay was used to measure the quantity of immobilised GOx. The immobilised enzyme was measured by comparing the concentration of proteins in the solution before and after immobilisation.
- Enzyme Activity Assay: By tracking the conversion of glucose to gluconolactone, which generates hydrogen peroxide ($H2O_2$), the enzymatic activity of immobilised GOx was evaluated. An electrochemical detection of the produced H_2O_2 gave an indication of the activity of the enzyme on the PPy surface.
- Surface Morphology and Coverage: The GOx-immobilized PPy films' surface morphology was examined using SEM to guarantee even enzyme distribution and sufficient surface coverage.

4.1.7. Results

A 2.5% glutaraldehyde solution and a 6-hour immobilisation period produced the best results. The PPy films showed strong enzymatic activity, substantial enzyme loading, and structural

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integrity under these circumstances. The resultant biosensor had a linear detection range that was appropriate for real-world uses and responded quickly to glucose.

4.1.8. Talk

On the PPy films, the immobilisation technique guaranteed an active and stable enzyme layer. The glutaraldehyde cross-linker's covalent bonding proved vital for sustaining enzyme activity for long stretches of time, which is necessary for the biosensor's dependability. Subsequent research endeavours will concentrate upon enhancing the immobilisation procedure and investigating alternative enzymes to accommodate a wider array of biosensing uses.

4.2. Measuring Blood Sugar

By monitoring the current response to varying glucose concentrations in PBS, the biosensing performance was evaluated. The PPy-based biosensor has a quick reaction time, a low detection limit, and a linear response over a broad range of glucose concentrations.

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5. Findings and Talk

Excellent electrochemical characteristics and biocompatibility of the nano-structured PPy sheets made them appropriate for enzyme immobilisation and biosensing. The increased sensitivity and quicker reaction times were facilitated by the nanostructure's increased surface area. After going through many cycles, the biosensor's stability was assessed, and the results showed no loss in functionality.

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6. Concluding remarks

The substantial potential of electrophoretic deposition (EPD)-deposited nano-structured polypyrrole (PPy) films in developing biosensor technology is highlighted by this work. The

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nano-structured PPy films showed a number of beneficial characteristics that are necessary for biosensing applications, such as strong stability under operating circumstances, increased electrical conductivity, and great biocompatibility. In order to guarantee dependable and durable functioning in biosensor devices, these characteristics are essential.

The uniform and regulated deposition of PPy films onto a variety of substrates made possible by EPD's success creates opportunities for more innovation and optimisation. Subsequent investigations will concentrate on optimising the deposition parameters to improve adhesion to substrates, thickness control, and uniformity of the layer. The optimisation process will also investigate how to combine PPy with other functional molecules or nanomaterials to modify certain biosensing characteristics including response time, sensitivity, and selectivity.

Furthermore, investigating conducting polymers other than PPy, including polyaniline (PANI) or poly(3,4-ethylenedioxythiophene) (PEDOT), may be able to increase the adaptability and range of uses for biosensors. These materials have special structural and electrochemical qualities that may enhance the functionality and performance of biosensing platforms.

Further research endeavours will focus on the incorporation of sophisticated bio-recognition components, such enzymes, antibodies, or aptamers, onto nano-structured polymer films, in addition to material improvements. The goal of this integration is to create biosensors with high specificity and sensitivity that can identify a broad spectrum of analytes, which is crucial for applications in food safety, environmental monitoring, and biomedical diagnostics.

To sum up, EPD-deposited nano-structured PPy films are an important advancement in the development of biosensor materials. The creation of next-generation biosensors will be fueled by ongoing research into innovative materials, bio-recognition components, and deposition process optimisation. This will promote improvements in environmental sciences, biotechnology, and healthcare.

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