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To Analyses of Bioelectrodes Based on NS-Pani for the Estimation of Target Analytes

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ABSTRACT

In addition, the sizes of the nanoscaled CP building blocks and those of some biological macromolecules, including as proteins, enzymes, and nucleic acids, are similar. Consequently, it is expected that any interaction between these biomolecules would cause a major alteration in the electrical characteristics of nano-structured biointerfaces. Improved charge transfer behavior might arise from the incorporation of carbon nanotubes (CNT) into the PANI matrix, activating the enzyme through bioelectrocatalytic processes and enhancing biosensing properties. Efforts have been made to investigate electrophoretically deposited NS-PANI films as platforms for biomolecules immobilization in order to develop reagentless biosensing configurations for the detection of cholesterol and triglycerides. This is because of the significance of the electrophoretic technique in producing nanostructured conducting polymer films and the potential of these conducting nano-materials in providing direct electrical communication between the redox center of an enzyme and the electrode surface. This has involved the electrophoretic synthesis of various PANI nano-structures onto indium-tin-oxide (ITO) coated glass substrates, such as nano-patterned PANI, PANI nanospheres (PANI-NS), and PANI nanotubes (PANI-NT)..

Keyword: Nano-Structured, Polymer Films, Proteins, Enzymes, Bioelectrocatalytic

INTRODUCTION

One of the most fascinating cutting-edge areas in analytical chemistry in recent years is nanotechnology. The most fascinating areas of science and technology have discovered uses for a wide range of nanomaterials, such as semiconducting nanoparticles, carbon nanostructures, nano-structured metal oxides, organic-inorganic nanocomposites, and nano-structured conducting polymers (NCP). Because of the length of polymer chains, one of the most active fields at the moment is polymer nanotechnology. The ability to study individual polymer chains has become rare due to the development of nanotechnology instruments. Conversely, polymers, being a soft material family member, provide remarkable versatility in creating desired nanostructures with appropriate control over their characteristics, potentially resulting in the development of novel technologies and apparatus. The distinctive chemical, physical, and mechanical features of nano-structured conducting polymers, such as nanotubes, nanorods, and nanospheres, may be attributed to their tiny size.

These qualities include a high surface-to-volume ratio, improved surface reactivity, greater processibility, and superior electrochemical behavior. With regard to the construction of next-generation bioelectronic devices, these unique features of NCP provide tremendous opportunities for integrating biological recognition events with electronic signal transactions.10. Polyaniline (PANI) is one of the most promising electroactive materials among the many conducting polymers because of its easy synthesis, low cost, high environmental stability, distinctive redox tunability, and controlled electrical conductivity.

The primary barrier to the full fulfillment of its technical potential, however, is its interact-ability, or infusibility and insolubility. To overcome its processibility issue and enable PANI for new applications, including actuators, energy conversion and storage, light-emitting display devices, microelectronics, chemical sensors, biosensors, and so on, the synthesis of nano-structured PANI (NS-PANI) is a crucial first step. NS-PANI differs from the bulk material in specific physical and chemical properties.

PANI has been identified as a material of interest for sensor and biosensor interfaces because it can be employed as an appropriate matrix for immobilizing biomolecules and functions as an efficient mediator for electron transfer in redox or enzyme processes. According to recent findings, PANI serves as an enzyme amplifier to amplify signals during the recognition process.

REVIEW OF LITERATURE

Mária Omastová et. al. (8) Composite polypyrrole–silver colloids are produced when pyrrole is oxidised with silver nitrate in the presence of appropriate water-soluble polymers. The polydispersity index of the polypyrrole–silver nanoparticles stabilised by poly(N-vinylpyrrolidone) is 0.20, indicating a considerable polydispersity in size, with a typical size of about 350 nm. Comparable outcomes have been seen when poly(vinyl alcohol) is used as a stabiliser. The stabiliser concentration has a negligible impact on particle size. In addition to the already utilised poly(N-vinylpyrrolidone), a number of stabiliser types have been examined in the current investigation. The gemini morphology of polypyrrole and silver colloidal nanoparticles was shown by transmission electron microscopy and optical microscopy, which also validated the size and size-distribution found by dynamic light scattering. An effective method for characterising polypyrrole using UV–vis and FT Raman spectroscopy, including the transition between polypyrrole salt and matching polypyrrole base, is the use of colloidal dispersions. The dispersions were employed to prepare coatings on foils made of polyethylene terephthalate, and the characteristics of the composites made of polypyrrole and silver were contrasted with those obtained from polypyrrole colloids alone.

Mária Omastová et. al.(9) By chemically oxidatively polymerizing pyrrole in an aqueous solution comprising an oxidant, ferric sulphate, and a surfactant, conducting and stable polypyrrole (PPy) was created. The following surfactants were used as additives: cationic surfactant: tetradecyltrimethylammonium bromide; non-ionic surfactants: poly(ethylene oxide) (20) sorbitan monostearate (Tween® 60), poly(ethylene oxide) (10) iso-octylphenyl ether (Triton® X-100), and poly(ethylene oxide) (20) sorbitan monolaurate (Tween® 20). Poly(ethylene oxide) (20) sorbitan monostearate (Tween® 60). Only the anionic surfactants were integrated into PPy in a manner comparable to that of the doping anion, as demonstrated by the results of the elemental analysis and FTIR spectroscopy. Better stability in the direction of deprotonation results from this. Additionally, thermal stability was enhanced as shown by TGA in air. Studies using scanning electron microscopy revealed that the anionic surfactant's presence.

Jaroslav Stejskal, et. al. (10) Among the conducting polymers available today, polypyrrole nanotubes have one of the greatest conductivities at tens S cm−1. As a result, they are better than the typical globular shape, whose conductivity is at most S cm−1. After being treated with alkalis, the conductivity of both forms decreases but does not decrease; the units of S cm−1 and 10−2 S cm−1, respectively, remain high. The salt-base transition in polypyrrole is used as a foundation for discussing the deprotonation, which causes the conductivity to decrease. Only a portion of the conductivity is recovered by reprotonation with acids, and it is not entirely reversible. It is suggested that surfactants and methyl orange have comparable roles in supporting the production of nanotubes. Methyl orange is tightly linked to polypyrrole in its acidic state, as demonstrated by FTIR and Raman spectroscopies. To account for the nanotubes' resistance to deprotonation, a "insertion" process is put forth. The spectra also show that, at pH values close to 14, when the globular form gets destroyed, the molecular structure of nanotubular polypyrrole is maintained. When it comes to applications that need electrical conduction even in neutral or alkaline environments—where other conducting polymers, such polyaniline, lose their useful conductivity—polypyrrole, especially in its nanotubular form, shows promise.

Jitka Škodová et. al. (11) Pyrrole was oxidised using iron(III) chloride in the presence of methyl orange to create polypyrrole nanotubes. Subsequently, they were employed in the reduction of silver ions into nanoparticles. The capacity of polypyrrole to remove silver ions is compared between its nanotubular form and its traditional globular shape. In the trials, bases and polypyrrole salts were both utilised. The resultant composite's 21–31% weight percentage of metallic silver was found using thermogravimetric measurement. The discussion of elemental composition also involves energy-dispersive X-ray spectroscopy. The conductivity of polypyrrole nanotubes in salt form, which was 35.7 S cm−1, was unexpectedly lowered to 20.9 S cm−1 upon the addition of silver. The presence of silver had generally little effect on the conductivity. The temperature dependence of conductivity reveals that the composites maintain the semiconducting character of polypyrrole and their conductivity increased with increasing temperature. The conductivity of the composites surprisingly increased when the samples were placed in vacuo.

Irina Sapurina et. al. (12) Pyrrole was oxidised using iron(III) chloride in the presence of methyl orange to create polypyrrole nanotubes. After that, they served as a substrate for the reductive deposition of particles made of noble metals. Palladium, platinum, rhodium, or ruthenium nanoparticle-adorned polypyrrole nanotubes were examined using electron microscopy, conductivity, energy dispersive X-ray analysis, FTIR, and Raman spectroscopies. The range of a typical metal composition was 15–20 weight percent. The reduction of 4-nitrophenol to 4-aminophenol served as an example of the composites' catalytic activity. Thermogravimetric examination in a nitrogen environment has been conducted subsequent to the carbonisation of composite materials. Up to 830 °C, polypyrrole's nanotubular shape was preserved during carbonisation. With the exception of ruthenium, the nanometer-sized noble-metal nanoparticles fused to clusters during this procedure. Nanotubes of polypyrroles.

OBJECTIVES OF THE STUDY

1. Immobilization of enzymes on NS-PANI-based electrodes created electrophoretically, such as cholesterol oxidase, lipase, and glycerol dehydrogenase.
2. Response analyses of bioelectrodes based on NS-PANI for the estimation of target analytes (triglycerides, cholesterol) .

RESEARCH METHODOLOGY

This work includes research on two topics: 1) Preparing nano-patterned PANI, PANI nanotubes, PANI nanospheres, and PANI-CNT composite, then fabricating their films onto ITO-coated glass plates; and 2) Immobilizing biomolecules (ChOx, LIP, GDH, etc.) onto conducting PANI-based electrodes to investigate their potential uses for triglyceride and cholesterol detection. These bioelectrodes and electrodes that were fabricated have undergone different stages of preparation. These stages have included characterization through the use of X-ray diffraction (XRD), UV-Visible (UV-Vis) spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM), Contact Angle (CA), and electrochemical techniques [Cyclic Voltammetry (CV), Linear Sweep Voltammetry (LSV), and Electrochemical Impedance Spectroscopy (EIS)].

Infrared Fourier Transform Spectroscopy (FT-IR)

One of the most significant analytical methods for differentiating between organic and inorganic materials that has gained widespread recognition is Fourier transform infrared spectroscopy.1-2 In FT-IR, the beam divides into two parts: one beam is used as a reference beam while the other passes through the sample (transmission) or is alternatively reflected. Once a route difference is added, this transmitted or reflected beam recombines with the reference beam. The spectrum of a desired substance being studied as a function of wavelength or wave number of incident radiation is obtained by Fourier transformation of the interference pattern so created. Using time domain measurements of electromagnetic radiation or another kind of radiation, spectra are gathered based on measurements of the temporal coherence of a radiative source in Fourier transform infrared spectroscopy, a characterisation technique. A molecule's atoms move around a mean location rather than maintaining a constant relative position. This type of vibration is known as infrared (IR) active when the dipole moment periodically alternates throughout the oscillation. Only the radiation that the vibrating molecule can interact with in a coherent way—that is, the radiation of its own oscillation frequency—is absorbed by the molecule.3–4. The presence or absence of specific vibrational frequencies in the FT-IR spectra provides important details on the structure of a given molecule. Because each functional group has a unique spectrum of vibrational frequencies and is highly sensitive to the chemical environment, knowing which functional groups are present in a given sample is important for further characterizing those groups.

RESULTS AND DATA ANALYSIS

Due to their much better performance in many applications, as well as their significantly greater dispensability and processibility when compared to granular and colloidal PANI, conducting PANI nanostructures are the subject of extensive study. Because of its remarkably long and incredibly thin macromolecular structure, polyaniline nanotubes and nanofibers have garnered the most attention among the many PANI nanostructures and have been the subject of substantial study in synthesis, characterisation, processing, and application.5–7 With regard to the selective oxidation of sulfides to sulfoxides in water, the catalytic activity of doped and undoped PANI nanostructures has recently been investigated. The results showed that PANI-NT had the best activity and selectivity, which may be related to its increased surface area. The goal of the past ten years has been to create PANI nanocomposites by combining the advantages of each individual component. PANI-CNT composites, which exhibit redox activity at neutral pH, are one of these nanocomposites that should be intriguing to develop.

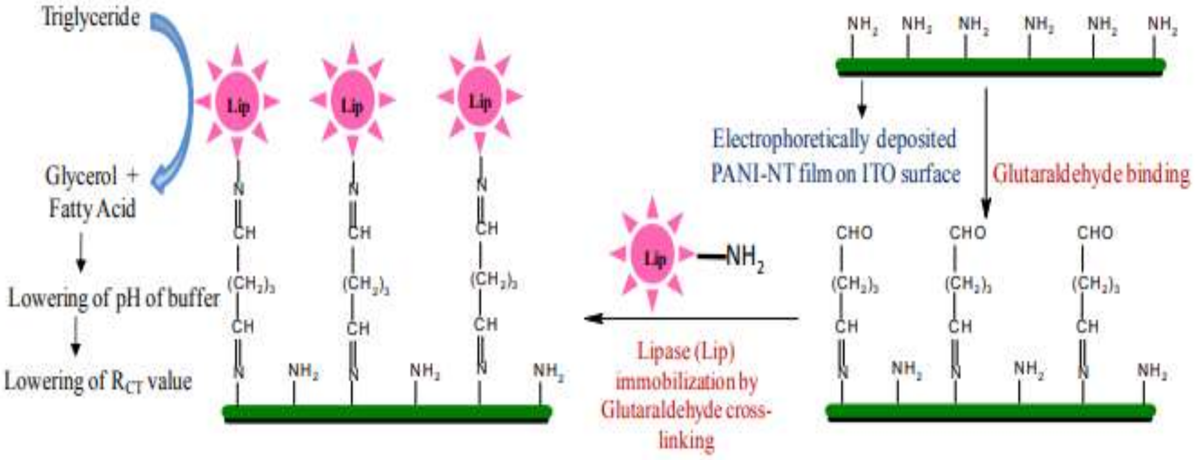
Their bioelectrochemical activation based on their synergistic impact will be investigated.Nine to ten carbon nanotubes (CNTs) are thread-like macromolecules with potential use in the production of durable, quick, and efficient electronics. These almost one-dimensional carbon allotropes have a high mechanical strength, a large surface area, rich electrical characteristics, exceptional chemical and thermal stability, and a very low weight.Eleven CNTs can be utilized to improve the electrochemical reactivity and electron transport rates of biomolecules, accumulate important biomolecules, and reduce the impacts of surface fouling, according to recent studies.

Electrical interaction between redox biomolecules, such as enzymes, and the electrode surface presents a problem for the production of biosensors. The majority of bioelectrocatalytic systems rely on mediated electron transfer between conductive supports and redox enzymes, and an electron mediating system's higher electrical conductivity can facilitate this process. Therefore, it should be fascinating to create highly conductivity PANI-CNT composite systems and research these composites as matrix for bioelectrocatalytic activation of enzymes. It is commonly recognized that the robust contact between the graphitic structures of CNT and the aromatic rings of PANI may significantly improve the charge-transfer interaction between the two components, which can be helpful in the development of third-generation mediator-free biosensors.

Since a high blood triglyceride concentration is linked to an increased risk of atherosclerotic events, it is important to detect and treat high blood triglyceride levels in conditions like diabetes mellitus, nephrosis, liver obstruction, and other disorders involving lipid metabolism of different endocrine disorders.eighteen The color created during the enzymatic reaction provides the basis for existing triglyceride measurement techniques, such as Cayman's triglyceride test.Nineteen However, these techniques are costly, complex, and time-consuming. As a result, there is a greater desire to create an efficient triglyceride biosensor that would enable quick, simple, accurate, and affordable clinical detection. In order to investigate the implications of the superior biosensing characteristics for PANI-NT and PANICNT composite matrices in triglyceride biosensor, efforts have been made to explore the electrophoretically deposited PANI-NT and PANICNT composite film as platform for LIP and GDH immobilization in Chapter IV.

Triglyceride Biosensor Using Electrophoretically Deposited Polyaniline Nanotubes Based Film

Using glutaraldehyde (Glu) as a cross-linker, LIP (Aspergillus oryzae) {1 mgmL-1, freshly produced in phosphate buffer saline (PBS, 50 mM, pH 7.4)} has been covalently immobilized onto PANIT/ITO electrode for triglyceride detection. After that, this electrode is cleaned with PBS and kept in storage at 4°C when not in use. The method of LIP immobilization is depicted in Scheme 5.1. After being produced in ethanol, the various tributyrin concentrations were kept at 4 o C.



Scheme 5.1: Diagram Showing The Electrochemical Process Used In Triglyceride Detection And The Construction Of The Lip/Glu/Pani-Nt/Ito Bioelectrode.

Analysis By Scanning Electron Microscopy (Sem)

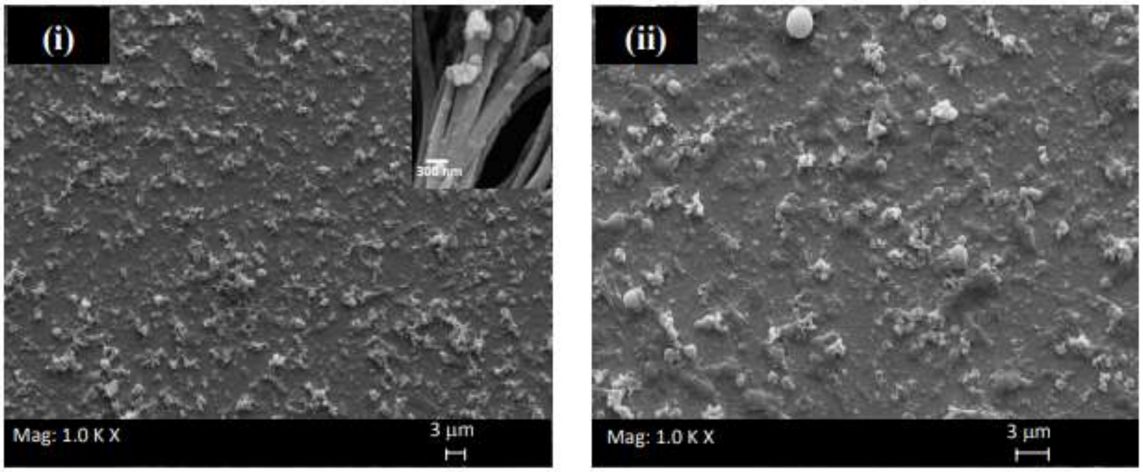


Figure 5.2 Shows The Sem Images Of The Lip/Glu/Pani-Nt/Ito Bioelectrode And The (I) Pani-Nt/Ito Electrode.

The homogeneous distribution of PANI-NT, which gives the electrophoretically formed PANI-NT film its fibrous shape, is readily seen in the SEM picture of PANI-NT/ITO [Figure 5.1 (i)]. A enlarged picture of the PANI-NT film is shown in the inset of Figure 5.1(i), which unmistakably demonstrates the development of PANI-NT with closed ends and a diameter that ranges from 120 to 275 nm. PANI nanotubes form a fibrous network that increases LIP immobilization surface area and increases enzyme loading.

Infrared Spectroscopy Using Fourier Transform

FT-IR spectra of LIP/Glu/PANI-NT/ITO and PANI-NT/ITO In [Figure 5.2], absorption bands are seen at 1561 and 1484 cm−1 (corresponding to C=C stretching deformation of quinoid and benzenoid rings), 1289 cm−1 (assigned to C–N stretching of secondary aromatic amine), 1123 cm−1 (assigned to aromatic C–H in-plane bending), and 807 cm−1 (associated with C–H out-of-plane deformation in the 1,4-disubstituted benzene ring). PANI was doped with CSA as evidenced by the bands seen at 1030 and 504 cm−1, which are attributed to the absorption of the –SO3H group. An amide bond between the lipase molecules immobilized onto the PANI-NT surface is responsible for the emergence of extra absorption bands at 1642, 3050, and 3200 cm-1 in the FT-IR spectra of the LIP/Glu/PANI-NT/ITO bioelectrode.

CONCLUSION

APS was used as an oxidant and CSA as a dopant in the miceller polymerization process to create PANI-NT. Furthermore, XRD, TEM, SEM, and AFM methods have been used to study the observed morphological alteration of CSA doped PANI from its nanotubular form to nanospheres in the presence of ethylene glycol as solvent. This shift in the PANI nanostructure's shape is explained by ethylene glycol's great affinity for hydrogen bonding with the -NH2 moieties of PANI. In addition, homogeneous and consistent PANI-NS films were created by solution casting PANI-EG solution onto ITO coated glass plates. These films were then used to create a ChOx/PANI-NS/ITO bioelectrode, which may be used to measure cholesterol within the physiological range. The ChOx/PANI-NS/ITO bioelectrode's apparent enzyme activity, sensitivity, and app K m have been determined to be 2.1 x 10-3 Ucm-2, 4.43 x 10-5 Absmg1 dL, and 2.5 mM, respectively. The bioelectrode has an ideal temperature range of 25–30 °C, and when stored at 4 °C, it may be reused ten times and have a shelf life of around 12 weeks. Improved thermal stability of the ChOx/PANI-NS/ITO bioelectrode is necessary since it exhibits significant activity within a relatively limited temperature range.

PANI-NS/ITO electrodes clearly offer an effective matrix for immobilizing ChOx, as demonstrated by their substantial enzyme activity, broad detection range, and long shelf life. Nevertheless, the ChOx/PANI-NT/ITO bioelectrode's limited temperature range for response measurement (optimal temperature: 25 °C) restricts its application to biosensor devices. Additionally, there is room to increase the app K m value, or ChOx's affinity for the comparable analyte (cholesterol). There have been attempts to detect cholesterol using electrophoretically deposited CP-based matrices. An improved app K m value was observed in the case of the PANI-CNT matrix, indicating a high affinity of ChOx with that of its corresponding analyte (cholesterol) on this nanocomposite film. These results were compared with those of the PANI-NT and nanostructured PANI used as an immobilization platform for ChOx. A longer shelf life of 12 weeks is achieved by the bioelectrode due to improved thermal stability caused by the integration of CNT inside the PANI matrix. Among the two matrices, PANI-CNT composite has the highest level of sensitivity for the detection of cholesterol.

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