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Use of Super-Structural Conducting Polymer as Functional Immobilization Matrix in Biosensor Design

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The evolution of electrochemical biosensors reflects a simplification and enhancement of the transduction pathway. The use of novel conducting polymers in the preparation of sensor platforms has become increasingly studied and imparts many advantages. The sensitivity and overall performance of enzymatic biosensors has improved tremendously as a result of incorporating functional group containing conducting polymers into their fabrication. Hereby, an efficient surface design was investigated by modifying the graphite rod electrode surfaces with conducting polymer displaying functional groups for the immobilization of biomolecules. A model enzyme, glucose oxidase, was efficiently immobilized to the modified surfaces via covalent binding. The biosensor was characterized in terms of its storage and operational stability and kinetic parameters. The designed sensor platform revealed excellent stability and promising kinetic parameters without carbon nanotube or graphene additive. Finally, the sensor platform was tested on beverages for glucose detection.
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Electrochemical biosensor platforms designed by using conducting polymers (CP) have received great interest because these platforms are inexpensive and easy to design, and provide a direct electrical signal for the presence of biological analytes with high selectivity and sensitivity.[1–10] Conducting polymers are simplifying sensor designs as they can be used as both transducers and sensing elements of the biological recognition process at the same time.[11] Enzymatic electrochemical biosensors designed by conducting polymer are based on the detection of an electric signal produced by an electroactive species, either produced or consumed by an enzymatic reaction. These enzymatic electrochemical biosensors consist of a biorecognition layer of enzymes attached to a conducting polymer modified working electrode (transducer). As a result different conducting polymers can be oxidized/reduced at different potentials; electrochemical detection also provides further selectivity.[12–14]

As enzymes have high catalytic activity and provide outstanding selectivity for their targeted substrate, they are ideal biorecognition molecules.[15] However, enzymes are the shortest-lived component of these biosensors, and they slowly lose their activity, thus determining the biosensor's lifespan. Most enzyme-based electrochemical biosensors are used in many point-of-care and clinical applications for a broad range of analytes and they do not need highly-priced advanced instrumental devices for their use. Other biorecognition elements are antibodies, cells and microorganisms.[16–19] Immunosensors are biosensors in which immunoactive substances are used as biospecific recognition elements and are based on the complex formation of the antibody with the appropriate antigen. An immunosensor uses antibodies, antibody fragments or antigens to monitor binding events in bioelectrochemical reactions. Usually in bioelectrochemistry, the investigated reaction can produce a measurable current (amperometric), a measurable potential or charge accumulation (potentiometric), or measurably changing the conductive properties of a medium (conductometric) between electrodes. Other types of electrochemical detection techniques are available in the literature such as impedimetric, which measures impedance (both reactance and resistance), and field-effect, which uses transistor technology to measure current as a result of a potentiometric effect at a gate electrode.[13]

Recently, conducting polymers have founded upward demand in various application areas like medical diagnosis, since they are very attractive as suitable matrices for biomolecules.[20–22] Biosensor platforms can be designed by binding via covalent bonds or non-covalent interactions of biorecognition molecules to conductive polymers. Since non-covalent interactions such as π-stacking are influenced by environmental and structural factors such as pH, temperature, mechanical effects etc., they do not provide a good immobilization possibility for the biorecognition molecules to the conducting polymers, however, covalent interactions are a more successful approach.

In this work, 5-amino-N^1,N^3- bis (2,5-di(thiophen-2-yl)-1H-pyrrol-1-yl) isophthalamide (BTP) was synthesized to obtain double-sided, amine substituted thienyl-pyrrole derivative. By doing so, we obtained a superstructure that provides three-dimensional electrical conductivity and has much better optical, electrical properties and stability.

We believe that the presence of a bifunctional amide group in the polymer structure increases interchain interaction with extensive hydrogen bonding and this provides structural rigidity and forces it to planarity.[23] Thus, the optical and electrical properties of the polymer have reached the desired levels as the planar structure allows p-orbital overlapping in greatest extent. P(BTP) was synthesized electrochemically on graphite electrode surface to design sensitive, effective, and stable biosensor platform. The superstructural property of the conducting polymer chains can increase the sensitivity and stability of the platform and also free amine groups can provide covalent binding to the biorecognition molecules. To assess the potential usage in biosensor applications, a model enzyme, glucose oxidase (GOx) was efficiently immobilized to the novel P(BTP) modified surfaces via covalent binding. GOx immobilized biosensor platform was characterized in terms of its storage and operational stability and kinetic parameters. The sensor platform revealed excellent stability and promising kinetic parameters without carbon nanotube or graphene additive. Finally, the sensor platform was tested on beverages for glucose detection.

Experimental

Chemicals.—The experimental studies were carried on with high purity chemicals. D-Glucose, glucose oxidase (GOx, from Aspergillus niger, 200 U/mg) and Glucose (GO) Assay Kit for spectroscopic determination of the glucose were purchased from Sigma Aldrich.

Equipments.—Cyclic voltammetric and amperometric measurements were carried out by Ivium potentiostat/galvanostat electrochemical measurement unit with three electrode systems, respectively. The surface morphologies of the polymer film were evaluated using Zeiss Evo LS10 scanning electron microscope. Three electrode cell geometry was used in all electrochemical experiments. Graphite electrode

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