STUDIES ON APPLICATION OF CONDUCTING POLYMERS TO GENOSENSORS

by

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lymons & Gerosensors.

Certificate

This to certify that the thesis entitled "Studies on Application of Conducting Polymers to Genosensors" being submitted by Kavita Arora to the Indian Institute of Technology, Delhi, for the award of degree of Doctor of Philosophy, has been prepared under our supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The research report and the results presented in this thesis have not been submitted to any other university or Institute for the award of any other degree or diploma.

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Abstract

DNA (deoxyribonucleic acid) the molecule of life coupled with the interesting and "synthetic metal" like properties of conducting polymer has recently gained much interest. Physico-chemical properties of DNA has led to possibility of developing the biosensing electrodes (Genosensor) that can be used to detect the presence of target low molecular geno-toxic analyte (such as pesticides, toxicants etc.) and the complementary target. Therefore, these 'Genosensors electrodes' have profound implications in the field of medical/clinical diagnostics, food quality control and environmental monitoring. In this context, double stranded *calf thymus* deoxyribonucleic acid (CT-dsDNA) physisorbed onto polypyrrole–polyvinyl sulphonate (PPy–PVS) films electrochemicallydeposited onto indium–tin–oxide (ITO) coated glass plates. These CT-dsDNA immobilized PPy–PVS films optimized for various conditions, such as polymerization potential, pH of buffer, DNA concentration and scan rate were characterized using Fourier-transform infrared (FT-IR) spectroscopy, atomic force microscopy (AFM) and cyclic voltammetry (CV) techniques, respectively. The amperometric response studies of these CT-dsDNA/PPy–PVS electrodes were carried out as a function of 2-aminoantharcene (2-AA, 0.01–20 ppm) and o-chlorophenol (OCP, 0.1–30 ppm) concentration, respectively at 25 °C. The observed amperometric current arising due to oxidation of guanine in the CT-dsDNA/PPy–PVS films decreased linearly with the increase in the concentration of 2-AA and OCP. It has been revealed that 10 ppm of 2-AA is sufficient to reduce the observed guanine oxidation peak current by approximately −95±10% as compared to the reported values. A 25 ppm of OCP was capable enough to reduce the guanine oxidation current to zero. These CT-dsDNA/PPy–PVS electrodes were found to have a shelf life of about 4 months when stored at 25°C.

Electrochemical DNA biosensor based on polypyrrole polyvinyl sulfonate (PPy-PVS) coated onto Pt disc electrode (~0.03 cm^2) has been fabricated by immobilizing 25 bases long single stranded probe polydeoxycytidine (dC) using both biotin-avidin binding (indirect immobilization) and carbodiimide coupling (direct immobilization), to detect 25 bases long complementary target sequences polydeoxyguanosine (dG). These DNA immobilized electrodes have been characterized using differential pulse voltammetry (DPV), Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) techniques, respectively. Compared to the indirect method of immobilization, direct immobilization of dC probe results in enhanced detection limit by 2200 times (upto 0.01 fmol) and improved sensitivity by about 6 times. These DNA electrodes have response time of 30 seconds and are stable for about 21 weeks when stored at 25^0C.

Electrochemically prepared polypyrrole-chloride (PPy-Cl) electrode immobilized with single stranded polydeoxycytidine (BdC) probe have been used to detect presence of complementary target polydeoxyguanosine (dG) and the characteristics of. C these electrodes have been compared with BdC-avidin-PPy-PVS electrodes. Compared to Cl^- ions, polyvinyl sulfonate (PVS) ions play significant role towards the improved characteristics such as improved sensitivity, detection limit, lower oxidation potential and concentration of the ionisabe species of the PPy electrode. Besides this, PVS ions contribute to the alignment of the immobilized BdC probe at the positively charged PPy-PVS surface resulting in increased efficiency of hybridization and better signal transduction due to decreased non-specific adsorption of the target dG molecules at the PPy surface and simultaneously eliminating the inevitable need to add PEG and salmon sperm DNA. The presence of the PVS ions on and within the PPy matrix has been affirmed using SEM and FT-IR measurements.

Ultra-sensitive DNA hybridization biosensor based on polyaniline (PANI) electrochemically deposited onto Pt disc electrode has been fabricated using biotin-avidin as indirect coupling agent to immobilize single stranded 5'-biotin end labeled polydeoxycytidine (BdC) probe to detect complementary target polydeoxyguanosine (dG) using both direct electrochemical oxidation of guanine and redox electro-active indicator methylene blue (MB), respectively. Compared to direct electrochemical oxidation of guanine, hybridization detection using MB results in the enhanced detection limit by about 100 times. These DNA immobilized PANI electrodes have hybridization response time of about 60 seconds.

Avidin modified polyaniline (PANI) electrochemically deposited onto Pt disc electrode has been utilized for direct detection of *Escherichia coli* by immobilizing 5' biotin labeled *E. coli* probe (BdE) using differential pulse voltammetric (DPV) technique in presence of methylene blue (MB) as DNA hybridization indicator. Depending on the target sample and the sonication time, this BdE-avidin-PANI bioelectrode can be utilized to electrochemically detect complementary target probe (0.001 fmol), *E. coli* genomic DNA (0.01 ng/μl) and 11 *E. coli* cells/ml in 60 s to 14 minutes (hybridization time) without using PCR and can be used 5-7 times at temperatures, 30-45°C.

Contents

CONTENTS

CHAPTER III: Materials and Methods 68