

**STUDIES ON APPLICATION OF CONDUCTING POLYMERS TO  
GENOSENSORS**

**by**

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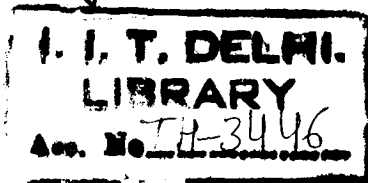
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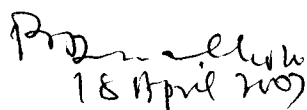
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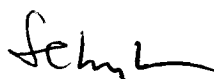
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## Certificate

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 electrochemically deposited 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-aminoanthracene (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  $-95\pm 10\%$  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 ( $\sim 0.03 \text{ cm}^2$ ) 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°C.

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<sup>-</sup> 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 ionisable 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

|                                                                            | Page No   |
|----------------------------------------------------------------------------|-----------|
| CERTIFICATE                                                                |           |
| ACKNOWLEDGEMENTS                                                           |           |
| SUMMARY                                                                    |           |
| List of Figures                                                            | i-iv      |
| List of Tables                                                             | v         |
| List of Schemes                                                            | vi        |
| List of Abbreviations                                                      | vii-x     |
| <b>CHAPTER I: Introduction</b>                                             | <b>1</b>  |
| 1.1 BIOSENSORS                                                             | 3         |
| 1.1.1 Applications of biosensors                                           | 7         |
| 1.1.2 Classification of biosensors on the basis of bio-recognition element | 7         |
| 1.1.2.1 DNA as a candidate for sensing                                     | 8         |
| 1.1.2.2 Genosensor                                                         | 12        |
| 1.1.2.3 Fabrication of Genosensor (DNA biosensor)                          | 14        |
| 1.1.2.4 Immobilization of DNA                                              | 15        |
| 1.1.2.5 Immobilization matrices                                            | 17        |
| 1.1.2.6 Conducting polymers                                                | 17        |
| 1.1.3 Classification of biosensors on the basis of transducers             | 19        |
| 1.1.3.1 Electrochemical Transducers                                        | 20        |
| 1.1.3.2 Optical transducer                                                 | 22        |
| 1.1.3.3 Piezoelectric transducer                                           | 23        |
| 1.1.3.4 Thermal transducer                                                 | 24        |
| 1.2 APPLICATION OF CONDUCTING POLYMERS BASED GENOSENSORS                   | 26        |
| 1.2.1 Detection of Genotoxics                                              | 27        |
| 1.2.2 Detection of DNA hybridization                                       | 27        |
| 1.2.3 Detection of <i>Escherichia coli</i>                                 | 28        |
| 1.3 REFERENCES                                                             |           |
| <b>CHAPTER II: Review of Literature</b>                                    | <b>31</b> |
| 2.1 FABRICATION OF GENOSENSORS                                             | 33        |
| 2.1.1 Carbon based immobilization matrices                                 | 34        |
| 2.1.2 Metal surfaces based immobilization matrices                         | 39        |
| 2.1.3 Insulating polymers based DNA biosensors                             | 45        |
| 2.1.4 Conducting polymer based DNA biosensors                              | 48        |
| 2.1.4.1 Polypyrrole Based Genosensors                                      | 49        |
| 2.1.4.2 Polyaniline based genosensors                                      | 58        |
| 2.1.4.3 Polythiophene based genosensors                                    | 60        |
| 2.1.4.4 Miscellaneous conducting polymers based genosensors                | 62        |

|                                                                                                                                                                                           |           |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| <b>CHAPTER III: Materials and Methods</b>                                                                                                                                                 | <b>68</b> |
| 3.1 REAGENTS AND CHEMICALS                                                                                                                                                                | 69        |
| 3.2 EQUIPMENTS AND INSTRUMENTS                                                                                                                                                            | 71        |
| 3.3 METHODOLOGY                                                                                                                                                                           | 71        |
| 3.3.1 Application of electrochemically prepared polypyrrole-polyvinyl sulphonate films (PPy-PVS) to DNA biosensor for detection of 2-aminoanthracene (2-AA) and o-chlorophenol (OCP)      | 72        |
| 3.3.1.1 Preparation of conducting polypyrrole-polyvinylsulphonate (PPy-PVS) thin films using chronoamperometry onto indium-tin-oxide (ITO) glass Plates.                                  | 73        |
| 3.3.1.2 Immobilization of double stranded calf thymus DNA (CT-dsDNA) probe using physical adsorption                                                                                      | 74        |
| 3.3.1.3 Characterization and optimization of various parameters for CT-dsDNA/PPy-PVS/ITO electrodes                                                                                       | 75        |
| 3.3.1.4 Response studies of CT-dsDNA/PPy-PVS/ITO electrode                                                                                                                                | 76        |
| 3.3.2 Immobilization of single stranded DNA probe onto polypyrrole-polyvinyl sulfonate for application to DNA hybridization biosensor                                                     | 77        |
| 3.3.2.1 Preparation of PPy-PVS thin films using chronopotentiometry onto Pt disc electrodes                                                                                               | 77        |
| 3.3.2.2 Immobilization and characterization of polydeoxycytidine DNA (BdC, probe I) onto polypyrrole-polyvinyl sulphonate (PPy-PVS/Pt) coated onto Pt disc electrode                      | 78        |
| 3.3.2.2 Hybridization detection of complementary sequences using BdC-avidin-PPy-PVS/Pt and dC-PPy-PVS/Pt electrodes.                                                                      | 82        |
| 3.3.3 Role of dopant polyvinyl sulfonate (PVS) ions in conducting polypyrrole (PPy) matrix towards DNA hybridization detection.                                                           | 84        |
| 3.3.3.1 Preparation of polydeoxycytidine DNA (BdC, probe I) immobilized polypyrrole-polyvinyl sulphonate (PPy-PVS) and polypyrrole-chloride (PPy-Cl) films coated onto Pt disc electrode. | 84        |
| 3.3.3.2 Hybridization detection of complementary target probe II (dG) using differential Pulse voltametric (DPV) measurements.                                                            | 87        |
| 3.3.3.3 Use of methylene blue to detect DNA hybridization using BdC-avidin-PPy-PVS/Pt electrodes.                                                                                         | 87        |
| 3.3.4 Ultra-sensitive label-free reusable DNA hybridization biosensor based on polyaniline.                                                                                               | 89        |
| 3.3.4.1 Preparation of polydeoxycytidine DNA (BdC, probe I) immobilized polyaniline (PANI) films coated onto Pt disc electrode                                                            | 90        |
| 3.3.4.2 Response studies of BdC-avidin-PANI bioelectrodes                                                                                                                                 | 91        |
| 3.3.4.2.1 DNA hybridization detection using electrochemical impedance spectroscopy (EIS)                                                                                                  | 91        |
| 3.3.4.2.2 DNA hybridization detection using guanine oxidation                                                                                                                             | 92        |

|                                           |                                                                                                                                                                                                                   |           |
|-------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|
| 3.3.4.2.3                                 | DNA Hybridization detection using methylene blue as redox electro active hybridization indicator                                                                                                                  | 92        |
| 3.3.5                                     | Polyaniline based label-free genosensor for direct detection of <i>Escherichia coli</i>                                                                                                                           | 93        |
| 3.3.5.1                                   | Preparation of <i>E. coli</i> specific probe DNA (BdE or probe V/IX) immobilized polyaniline (PANI) films coated onto Pt disc electrode                                                                           | 93        |
| 3.3.5.2                                   | Differential pulse voltammetric (DPV) studies                                                                                                                                                                     | 94        |
| 3.3.5.3                                   | DNA hybridization detection using methylene blue (MB) as hybridization indicator                                                                                                                                  | 94        |
| 3.3.5.4                                   | Sample preparation using target sample as <i>E. coli</i> genomic DNA and <i>E. coli</i> cells                                                                                                                     | 95        |
| <b>CHAPTER IV: Results and Discussion</b> |                                                                                                                                                                                                                   | <b>97</b> |
| 4.1                                       | Application of electrochemically prepared polypyrrole-polyvinyl sulphonate films (PPy-PVS) and polypyrrole-chloride (PPy-Cl)) to DNA biosensor for detection of 2-aminoanthracene (2-AA) and o-chlorophenol (OCP) | 99        |
| 4.1.1                                     | Electrochemical characterization of polypyrrole-polyvinyl sulphonate (PPy-PVS) and DNA/polypyrrole-polyvinyl sulphonate (CT-dsDNA/PPy-PVS) films coated onto Indium tin oxide coated glass plates                 | 100       |
| 4.1.2                                     | Optimization and characterization of CT-dsDNA immobilized PPy-PVS films                                                                                                                                           | 101       |
| 4.1.2.1                                   | Effect of polymerization potential on CT-dsDNA/Polypyrrole-polyvinyl sulphonate (CT-dsDNA/PPy-PVS) films                                                                                                          | 101       |
| 4.1.2.2                                   | Effect of CT-dsDNA amount onto PPy-PVS films                                                                                                                                                                      | 103       |
| 4.1.2.3                                   | Effect of pH of phosphate buffer onto cyclic voltammograms of CT-dsDNA/PPy-PVS films                                                                                                                              | 104       |
| 4.1.2.4                                   | Effect of scan rate onto cyclic voltammetric response of CT-dsDNA/PPy-PVS films                                                                                                                                   | 104       |
| 4.1.2.5                                   | Fourier transform infrared (FT-IR) spectrum of CT-dsDNA/polypyrrole-polyvinyl sulfonate (CT-dsDNA/PPy-PVS) film                                                                                                   | 105       |
| 4.1.2.6                                   | AFM Studies of CT-dsDNA/polypyrrole-polyvinyl sulfonate (CT-dsDNA/PPy-PVS) film                                                                                                                                   | 106       |
| 4.1.2.7                                   | Stability studies of CT-dsDNA/PPy-PVS films coated onto ITO Electrodes                                                                                                                                            | 107       |
| 4.1.3                                     | Effect of o-chlorophenol (OCP) and 2-aminoanthracene (2-AA) onto DNA (guanine) oxidation current of CT-dsDNA/PPy-PVS films                                                                                        | 108       |
| 4.2                                       | Immobilization of Single Stranded DNA probes onto Polypyrrole-Polyvinyl Sulfonate (PPy-PVS) films for DNA Hybridization Detection of Target Complementary sequences                                               | 113       |

|         |                                                                                                                          |     |
|---------|--------------------------------------------------------------------------------------------------------------------------|-----|
| 4.2.1   | Differential pulse voltammetric (DPV) studies for DNA hybridization detection                                            | 114 |
| 4.2.2   | FT-IR studies of BdC-avidin-PPy-PVS and dC-PPy-PVS electrodes                                                            | 115 |
| 4.2.3   | Scanning electron microscopic studies                                                                                    | 117 |
| 4.2.4   | Response Studies of BdC-avidin-PPY-PVS/Pt and dC-PPy-PVS/Pt electrodes                                                   | 118 |
| 4.3     | Role of dopant polyvinyl sulfonate (PVS) ions in conducting polypyrrole (PPy) matrix towards DNA hybridization detection | 123 |
| 4.3.1   | DNA hybridization detection using PPy-PVS and PPy-Cl as matrix.                                                          | 124 |
| 4.3.2   | Use of methylene blue to detect DNA hybridization using dG-BdC-avidin-PPy-PVS/Pt electrodes                              | 130 |
| 4.4     | Development of Ultrasensitive reusable DNA hybridization biosensor based on polyaniline                                  | 132 |
| 4.4.1   | Electrochemical characterization of PANI, avidin-PANI and BdC-avidin-PANI bioelectrodes                                  | 133 |
| 4.4.2   | Scanning Electron Microscopic (SEM) studies of PANI, avidin-PANI and BdC-avidin-PANI electrodes                          | 135 |
| 4.4.3   | FT-IR characterization of PANI, avidin-PANI and BdC-avidin-PANI bioelectrodes                                            | 136 |
| 4.4.4   | Electrochemical response studies for of BdC-avidin-PANI bioelectrodes                                                    | 137 |
| 4.4.4.1 | DNA Hybridization detection via monitoring guanine oxidation                                                             | 137 |
| 4.4.4.2 | DNA Hybridization detection using methylene blue as redox electro-active hybridization indicator                         | 137 |
| 4.4.4.3 | DNA Hybridization detection using impedance spectroscopy                                                                 | 139 |
| 4.4.5   | Calibration of BdC-avidin-PANI bioelectrodes                                                                             | 140 |
| 4.4.5.1 | DNA hybridization detection by monitoring guanine oxidation                                                              | 140 |
| 4.4.5.2 | DNA hybridization detection by monitoring methylene blue as redox hybridization indicator                                | 141 |
| 4.4.6   | Reusability of BdC-avidin-PANI bioelectrodes                                                                             | 142 |
| 4.5     | Direct detection of <i>Escherichia coli</i> using polyaniline based electrochemical genosensor                           | 146 |
| 4.5.1   | Differential pulse voltammetric studies of BdE-avidin-PANI bioelectrodes                                                 | 147 |
| 4.5.2   | DNA hybridization detection using methylene blue as hybridization indicator                                              | 148 |
| 4.5.3   | Effect of concentration of complementary target probes onto BdE-avidin-PANI bioelectrodes.                               | 149 |
| 4.5.4   | Effect of temperature on DNA hybridization detection using I-BdE-avidin-PANI                                             | 152 |
| 4.5.5   | Optimization of DNA hybridization time to detect complementary target sequence of <i>E. coli</i> genomic DNA             | 154 |
| 4.5.6   | Optimization of DNA hybridization time to detect complementary target sequence of <i>E. coli</i> cells                   | 155 |

|                                                    |                                                                                                                                                 |     |
|----------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 4.5.7                                              | Calibration curves of I-BdE-avidin-PANI bioelectrodes using heat shock treated <i>E. coli</i> genomic DNA and <i>E. coli</i> cells              | 156 |
| 4.5.8                                              | Reusability of I-BdE-avidin-PANI bioelectrode                                                                                                   | 160 |
| <b>CHAPTER V: Conclusions and Future Prospects</b> |                                                                                                                                                 | 162 |
| <b>REFERENCES</b>                                  |                                                                                                                                                 | 166 |
| <b>APPENDICES</b>                                  |                                                                                                                                                 | 179 |
| Appendix I                                         |                                                                                                                                                 | 180 |
|                                                    | Brief introduction to various techniques used for fabrication, characterization and response studies of conducting polymer based genoelectrodes |     |
| Appendix II                                        |                                                                                                                                                 | 190 |
|                                                    | Brief Biodata of Author                                                                                                                         |     |
|                                                    | List of Publications                                                                                                                            |     |
|                                                    | List of Conferences/symposiums                                                                                                                  |     |
|                                                    | Details of Course work at DBEB, IITD                                                                                                            |     |
|                                                    | Date of passing written comprehensive Examination at IITD                                                                                       |     |
|                                                    | Date of passing Oral comprehensive Examination                                                                                                  |     |