# CHAPTER 8

# SUMMARY AND CONCLUSIONS

## 8. Summary and conclusions:

Biosensors, as analytical tools, offer versatility for qualitative and quantitative analyses. Biosensors offers portability, cost-effectiveness, rapidity and capability for continuous online detection, rendering a valuable replacement for traditional methods across various fields including the pharmaceutical, food industry, diagnostics, environmental monitoring, defense and security. However, despite their benefits, existing biosensors encounter several challenges such as reduced sensitivity, limited sensitivity range, instability, cost, false positive signals and invasive nature of detection methods. The research conducted in this thesis was aimed to address some of these issues by exploring efficient fabrication and standardization techniques for biosensors. This work represents a significant step towards improving the effectiveness and applicability of biosensor technology in various industries.

The fabrication, standardization and characterization of a biosensor for hydroquinone pollutant detection, employing the electrochemical technique cyclic voltammetry. The study focuses on utilizing -COOH functionalized carbon nanotubes (COOH-MWCNTs) to immobilize laccase enzyme via crosslinking. Screen-printed electrodes were coated with carboxylic acid-modified multiwalled carbon nanotubes (MWCNTs), followed by laccase doping. The interaction between the carboxylic groups of MWCNTs and the amine group of laccase, forming an amide bond. The biosensor's performance is based on the activity of Laccase enzyme immobilized on the electrode. When an enzyme is exposed to hydroquinone substrate, it forms p-benzoquinone + $2H_2O + 2H^+ + 2e^-$. Current produced due to this reaction was measured by the fabricated electrode. Thus, the sensor was exposed to various concentrations of hydroquinone pollutant and current was measured and compared with the measurement in its absence. The research focuses on optimization and standardization of hydroquinone biosensor. The chapter focuses on optimization and standardization of the regeneration procedure, stability of the sensor and its linearity of the measurements with respect to the concentration of hydroquinone pollutant. At the end of optimization procedures, the biosensor was also tested against real samples proving its practical application aspect. In summary, a SPCE/MWCNTs/GA/LACCASE biosensor was successfully created, employing a novel fabrication approach. The enzyme laccase was immobilized onto the MWCNTs using glutaraldehyde covalent crosslinking. The electrode demonstrated linearity in detecting hydroquinone (HQ) within the range of 50 to 1100 μM, with a detection limit ranging

from 5 to 1700 µM. Specificity tests revealed the effectiveness of the biosensor in distinguishing HQ from various interfering molecules. Furthermore, real sample analysis validated the biosensor's practical application, showing promising reproducibility with a standard deviation of 0.56%. This biosensor holds potential as a valuable tool for hydroquinone detection. Additionally, exploring the immobilization of other enzymes using the same fabrication chemistry could pave the way for developing biosensors for a wide range of molecules.

Lactose intolerance presents a challenge for individuals consuming milk and dairy products, leading to a decrease in their consumption. Consequently, the dairy industry is investing in producing lactose-reduced or lactose-free milk and milk products. Accurate lactose detection methods are crucial. Electrochemical biosensors offer a promising solution due to their precision, sensitivity, and potential for miniaturization and automation. In this study, an electrochemical biosensor was developed using multiwalled carbon nanotubes (MWCNTs) functionalized with metal oxide nanoparticles (MONPs) as a matrix for enzyme immobilization. The enzymes β-galactosidase and galactose oxidase were immobilized on the electrode surface to catalyze the hydrolysis of lactose and subsequent reactions. The fabrication process involved several steps, including coating the electrode with MWCNTs/PVA, layering with $TiO_2$-CuO/PEI, enzyme immobilization and crosslinking with glutaraldehyde. The electrochemical behavior of the lactose biosensor was characterized through cyclic voltammetry, indicating increased current after each coating step till enzyme immobilization. The sensor's performance was evaluated in terms of pH sensitivity, calibration curve for lactose detection, selectivity against interfering substances, and real sample testing using milk. The developed lactose biosensor demonstrated promising performance characteristics, including linear response to lactose concentration, with a good detection range. Its specificity for lactose was confirmed through selectivity studies, and real sample testing with milk demonstrated reliable detection ability. Overall, this study demonstrates the feasibility of using electrochemical biosensors for lactose detection.

The development of an electrochemical DNA chip for the detection of *Mycobacterium tuberculosis* (Mtb) corresponds to a significant advancement in tuberculosis (TB) diagnostics. This innovative biosensor addresses several key limitations of existing diagnostic methods by offering improved sensitivity, rapid point-of-care testing, improved sample collection and processing, and

continuous monitoring and surveillance of TB at the population level. By pointing specific antigens or nucleic acid sequences unique to Mtb, the biosensor can reliably distinguish TB from other respiratory infections, providing prompt and accurate diagnosis crucial for controlling TB transmission and reducing morbidity and mortality rates.

The DNA chip utilizes a novel approach involving the attachment of single stranded DNA (ssDNA) oligonucleotide probes to multi-walled carbon nanotubes (MWCNTs) through strong covalent bonds. This complex is then dispersed in a polyvinyl alcohol (PVA) polymer solution and drop cast onto a screen printed carbon electrode, forming a thin film. The stability of this film is ensured by crosslinking with glutaraldehyde (GA), resulting in a robust platform for the electrochemical detection of the PupE gene, a critical biomarker for Mtb infection.

Experimental results demonstrate the successful immobilization of ssDNA probes on MWCNTs and the fabrication of a stable DNA-modified electrode chip. The biosensor exhibits excellent sensitivity and specificity, with a linear detection range from $1\times10^{-8}$ M to $1\times10^{-16}$ M, and high selectivity towards its complementary target sequence. Moreover, the sensor can be replicated with consistent performance, and its electrode can be regenerated for reuse, enhancing its practical utility and cost effectiveness.

The DNA chip was designed for the electrochemical detection of a nonstructural protein Nsp3 gene of SARS-CoV-2. To create this chip, a single stranded DNA oligonucleotide probe representing the sequence from the gene of the nonstructural protein was covalently attached to functionalized multi-walled carbon nanotubes (MWCNTs). The resulting ssDNA-MWCNT complex was mixed with polyvinyl alcohol (PVA) polymer and applied as a coating on a screen-printed carbon electrode. This film was crosslinked using glutaraldehyde (Jinal Thakkar, Ph.D. thesis 2022). However, Validation of performance of the electrode with real samples remained to be tested. This study was focused on real sample analysis by using previously made DNA-chip and successful detection of real sample was done.

The strong correlation between obesity and elevated plasma triglyceride levels is well-documented among obese individuals. High triglyceride levels pose a significant risk for cardiovascular diseases such as heart disease and stroke by contributing to artery hardening and blockages. Given these risks, physicians often recommend lipid profile testing for obese patients

to monitor their triglyceride levels. Traditionally, lipid profile testing has been conducted using conventional methods, which can be costly and time-consuming. However, there's an opportunity to revolutionize this process by utilizing immobilized lipase on a novel conductive polymer film to develop an electrochemical biosensor for triglyceride detection in bodily fluids.

The biosensor could provide accurate and timely results, facilitating better management of triglyceride levels in obese individuals and ultimately reducing their risk of cardiovascular complications. In the present study, three electrode configuration system with cyclic voltammetry technique is used for detecting real samples of triglycerides. The GCE/GO/MWCNTs/PEI-$TiO_2$/GA/Lipase bioelectrode was tested with real samples using groundnut oil, coconut oil and sunflower oil to evaluate its practical application. The biosensor demonstrated potential as an effective tool for triglyceride detection. Moreover, the fabrication chemistry developed here shows promise for the fabrication of other biosensors targeting various metabolites and compounds using different enzymes. This suggests broader applications beyond triglyceride detection, highlighting the versatility and adaptability of biosensor technology.

Breast cancer is a significant global health concern, BRCA1 gene mutations playing a crucial role in its development. Conventional detection methods have limitations, leading to the necessity for rapid, affordable, and sensitive detection systems. Electrochemical biosensors offer promising solutions due to their sensitivity, selectivity, and cost effectiveness. This study presents a DNA chip-based electrochemical detection method for the BRCA1 gene, utilizing a single stranded DNA probe immobilized on multi-walled carbon nanotubes (MWCNTs) within a polyvinyl alcohol (PVA) matrix on a screen-printed carbon electrode (SPE). The process involves hybridization with complementary target DNA sequences, leading to changes in electrochemical signals detected by cyclic voltammetry. The biosensor demonstrates good detection abilities, with a linear range from $1\times10^{-8}$ M to $1\times10^{-12}$ M, showing promise for early detection of breast cancer. The developed DNA chip-based electrochemical biosensor shows potential for the early detection of breast cancer, particularly associated with BRCA1 gene mutations. The immobilization of ssDNA probes on MWCNTs within a PVA matrix provides stability and sensitivity to the biosensor. The method offers advantages such as rapid detection, affordability and good sensitivity, making it suitable for point-of-care diagnostic applications. Overall, this research contributes to the advancement of biosensing technologies for early cancer detection, emphasizing the importance of innovative approaches in improving healthcare outcomes.

In conclusion, this thesis focused on the development of advanced biosensors designed for detecting hydroquinone pollutant, lactose, *Mycobacterium tuberculosis* and breast cancer, along with conducting real sample analyses for TGA and SARS-CoV-2. Significant time and effort were dedicated to troubleshooting the limitations of current biosensors and conventional methods. The research proposes new fabrication chemistries and standardization protocols for electrochemical biosensors targeting hydroquinone, lactose and tuberculosis. These efforts aim to enhance sensitivity, specificity, and reliability, thereby advancing the field of biosensing technology.

| Sr. No. | Analyte | Type of detector | Fabrication chemistry | Focus of the work | Specific for analyte | Response time | Linear range | Detection limits |
|---|---|---|---|---|---|---|---|---|
| 1 | Hydroquinone pollutant | Electrochemical (Cyclic voltammetry) | SPE-MWCNTs/ GA/Laccase | Standardization | Yes | 10 sec. | 50 µM to 1100 µM | 5 µM to 1700 µM |
| 2 | Lactose | Electrochemical (Cyclic voltammetry) | SPE/MWCNTs/PEI-CuO-$TiO_2$/GA/β-gal + GaOx | New fabrication chemistry and standardization | Yes | 10 sec. | 0.05 mM to 1.5 mM | 0.005 mM to 4 mM |
| 3 | *Mycobacterium tuberculosis* | Electrochemical (Cyclic voltammetry) | SPE/PVA-MWCNTs-ssDNA/GA | Standardization | Yes | 30 min. | $1\times10^{-8}$ M, $1\times10^{-16}$ M | $1\times10^{-10}$ M, $1\times10^{-15}$ M |
| 4 | Breast Cancer | Electrochemical (Cyclic voltammetry) | SPE/PVA-MWCNTs-ssDNA/GA | Standardization | Yes | 30 min. | $1\times10^{-8}$ M, $1\times10^{-12}$ M | $1\times10^{-4}$ M, $1\times10^{-18}$ M |
| 5 | SARS-CoV-2 | Electrochemical (Cyclic voltammetry) | SPE/PVA-MWCNTs-ssDNA/GA | Real sample analysis | Yes | 30 min. | $1\times10^{-10}$ M, $1\times10^{-15}$ M | $1\times10^{-4}$ M, $1\times10^{-14}$ M |
| 6 | TGA (triglycerides) | Electrochemical (Cyclic voltammetry) | GCE/GO/MWCNTs/PEI-$TiO_2$/GA/ Lipase | Real sample analysis | Yes | | 100-500 mg/dL | 100 mg/dL, 500 mg/dL |

**Table 8.1. Summary of the research work carried out in the thesis**