Abstract:

Biosensors represent a critical advancement in analytical devices, enabling qualitative, semi-quantitative and quantitative analysis across various fields. Comprising three essential components, receptor, transducer, and detector. Biosensors address various drawbacks associated with conventional analytical methods such as chromatography, spectrophotometry and PCR, while effective, they often experience challenges such as time consumption, the need for pure samples and high costs. In contrast, biosensors offer significant advantages including portability, cost-effectiveness, rapidity and suitability for continuous monitoring. They have widespread applications in industries such as pharmaceuticals, food, diagnostics, environmental monitoring and defense. Projections indicate a significant growth trajectory for the global biosensor market, with an expected value of USD 35 billion by 2025. However, existing biosensors still confront various limitations, including reduced sensitivity, limited detection ranges, false positives, invasiveness, high costs and instability. This study aims to address these challenges by developing biosensors with innovative chemistry, with a focus on enhancing portability, reusability, integration and minimizing health-related expenses. The key goal of this thesis is to explore efficient biosensor fabrication and standardization methodologies. By enhancing specificity, sensitivity and stability for target analytes, these biosensors can be adapted for further standardization and miniaturization, promising a significant advancement in biosensor technology.

The research is focused on carrying out efficient development of biosensors for the detection of Hydroquinone, Lactose, Breast cancer, *Mycobacterium tuberculosis* infection and testing of real samples of SARS-CoV-2 and Triglyceride (TGA) using previously developed biosensors.

An environmental pollutant hydroquinone biosensor was developed and standardized. In the present study, the screen-printed electrode of the biosensor was coated with a solution of polyvinyl alcohol containing carboxyl functionalized multiwalled carbon nanotubes (MWCNTs). A laccase extracted from the fungus *Trametes versicolor* was immobilized on MWCNTs using glutaraldehyde. This strategy showed excellent stability, sensitivity, and selectivity, yielding a rapid response time of only 10 seconds. The optimized linear range for the bioelectrode spans from 50 µM to 1100 µM. The detection limit achieved with the electrode is 5 µM to 1700 µM. The

biosensor detected hydroquinone successfully in real samples, including tap water, sewage water, spiked tap water and spiked sewage water. This biosensor offers a cost-effective detection method and can be used as a portable device **(Chapter 2)**.

Lactose intolerance poses a significant challenge for individuals consuming dairy products, necessitating accurate and efficient methods for lactose detection. Electrochemical biosensors present a promising solution due to their precision, sensitivity, and potential for miniaturization and automation. In this study, we developed an electrochemical biosensor for lactose detection by immobilizing β-galactosidase and galactose oxidase enzymes on multiwalled carbon nanotubes (MWCNTs) functionalized with metal oxide nanoparticles (MONPs). The fabrication process involved coating the electrode with MWCNTs/PVA, layering with TiO_2-CuO/PEI, enzyme immobilization, and crosslinking with glutaraldehyde. Cyclic voltammetry characterization demonstrated enhanced current after each coating step till enzyme immobiliztion. The sensor exhibited linear response to lactose concentration, with a wide detection range (0.05 to 1.5 mM) and low detection limits (0.005 mM to 4 mM). Specificity for lactose was confirmed through selectivity studies and real sample testing with milk demonstrated reliable detection capability. Overall, this study highlights the feasibility and potential of electrochemical biosensors for lactose detection, offering a promising tool for individuals managing lactose intolerance and the dairy industry in producing suitable dairy products for broader consumption. Further optimization and validation of the biosensor could enhance its applicability in various settings **(Chapter 3)**.

Further, efforts were made to develop an electrochemical biosensor for the detection of *Mycobacterium tuberculosis* infection using novel synthetic ssDNA aptamer for PuPE gene. The novel ssDNA aptamer was designed in NCBI website and was covalently bound with modified multiwalled carbon nanotubes via ester linkage. The complex then was drop cast onto electrode chip using polyvinyl alcohol and cross linker glutaraldehyde. The coated chip could successfully detect its complementary ssDNA probe. The fabricated sensor showed linearity between 10^{-8} M and 10^{-16} M. Real samples were detected successfully using this chip **(Chapter 4)**.

DNA-chip based biosensor was tested against the real samples of Covid-19 for its practical application and the biosensor could successfully detect the Covid-19 positive samples. Even though the electrochemical biosensor was constructed earlier in our laboratory, its validation using

real samples was pending (Jinal Thakkar's thesis 2022). The validation of the DNA chip sensor was carried out with 2 cDNA samples of 1 fM & 10 fM concentrations and the CV peak current (Ip) was monitored using the oligonucleotide probe. The change in Ip with respect to the electrode coated with probe, negative control (aptamer free coated electrode) confirms that the hybridization occurred between the DNA sequences on the electrode and those in the reverse transcribed samples. The change in Ip was observed in both the positive samples with error margin of ±6.41 and ±5.28 for 1fM and 10 fM respectively **(Chapter 5)**.

In the present study, a lipase-based triglyceride biosensor designed previously in our laboratory, was used successfully to determine triglyceride content in several real samples and the average recovery of the real sample was found to be 101.05 %, 98.03 % and 95.47 % with coconut oil, groundnut oil and sunflower oil respectively **(Chapter 6)**.

A cyclic voltammetric biosensor for the detection of BRCA1 using novel synthetic ssDNA aptamer. The novel ssDNA aptamer was designed in the NCBI website and was covalently bound with modified multiwalled carbon nanotubes via ester linkage. The complex then was drop cast onto electrode chip using polyvinyl alcohol and cross linker glutaraldehyde. The DNA sensor showed linearity from 10^{-8} M to 10^{-12} M **(Chapter 7)**.

In summary, the thesis successfully showcased the fabrication and standardization of highly efficient biosensors characterized by higher sensitivity and specificity. The study provided convincing evidence of the technology's efficacy through real sample analysis. The findings suggest a promising avenue for the future adaptation of this technology into various commercial applications, by addressing key challenges the advantages of biosensors, such as rapid analysis and cost-effectiveness. Overall, the thesis contributes valuable insights into the advancement of biosensor technology and opens doors for its widespread implementation in real world, potentially revolutionizing various sectors through enhanced analytical capabilities.