CHAPTER 6

SUMMARY AND CONCLUSION

6. Summary and conclusion:

Biosensors being analytical devices are useful for qualitative, semi-quantitative and quantitative analysis. Biosensors are portable, cost-effective, rapid, non-tedious and make continuous online detection possible and thus replace the traditional methods. Biosensors are useful in pharma-food industry, diagnostics, environmental monitoring, defense and security. The available biosensors are confronted by several issues such as lower sensitivity, narrow sensitivity range, low stability, high cost false positive signals and invasive methods in detection. The research work carried out in the thesis explored efficient fabrication and standardization of biosensors, while addressing some of the above problems. Focus of the work was to increase specificity, sensitivity and stability for target analytes so that they can be reformed for further miniaturization and commercialization.

Use of immobilized acetylcholine esterase (AChE) for detecting organophosphorus pesticides in water sources and body fluids can bring down the environmental hazard and detection costs dramatically. In the present study, AChE was directly doped on multiwalled carbon nanotube (MWCNT) surface modified with carboxylic groups through amide bond and used for organophosphorus pesticide detection. Amide bond formation between MWCNTs and the enzyme molecules avoid use of any intermediate membranes, cross-linkers or binding materials. This strategy overcomes the hindrance to electron transfer posed by membranes or cross-linkers and increases the sensitivity of detection. MWCNTs carrying carboxyl groups were deposited on glassy carbon electrode and were subsequently immobilized with AChE. The activity of AChE was monitored by cyclic voltammetry after immobilization. Scanning electron microscopy and atomic force microscopy were used to characterize the electrode surface. FT-IR spectra were taken to characterize enzyme-MWCNT complex. Under optimized parameters, the electrode showed linear range between 10 and 50 nM, which is promising for detection of trace amounts of the pesticide. The lower and higher detection limits of the sensor are 0.1 nM and 500 nM respectively. The stability and reusability of the electrode were determined. Finally, successful detection of organophosphorus compounds in real samples established it as reliable for sensor applications.

Significant correlation between obesity and high plasma triglyceride level is commonly observed in obese individuals. High level of triglycerides can harden and block the arteries increasing the risk of heart diseases and strokes. Obese patients are recommended to undergo lipid profile testing by physicians. These tests so far are conducted by conventional methods.

Immobilized lipase onto novel conductive polymer film can be used to make electrochemical biosensor for detecting triglycerides in body fluids. This can bring down the detection costs dramatically. In the present study, three electrode configuration system with cyclic voltammetry technique is used for detecting triglycerides. Glassy carbon electrode is functionalized with a combination of nanoparticles followed by polyethyleneimine, glutaraldehyde and lipase. Lipase is bound through amide bonds. The strategy increased the electrochemical conductance many fold and overcomes the hindrance to lipase posed by membranes as it is oriented on outside of the membrane. Thus, it increases the sensitivity and selectivity of detection. Results of scanning electron microscopy and FT-IR spectra are used for characterizing the electrode surface. Optimized linear range for the bioelectrode is 100-500 mg/dL. Successful detection of triglycerides in real sample is also tested.

Molecular imprinting technique has been explored to develop biomimetic receptor cavities on polyacrylamide polymer gel slab. The study focuses on the possibility of developing optical biosensor using molecularly imprinted gel slab. *Staphylococcus aureus* whole bacterial cells were used as template for imprinting in polyacrylamide gel slabs of 1×1×0.2 cm that can detect/recognize *Staphylococcus aureus*. The *Staphylococcus aureus* cells were entrapped in 2, 4, 6, 8 and 10% of acrylamide gels but only 4, 6 and 8% of acrylamide gels showed *Staphylococcus aureus* entrapment. The entrapped bacteria were removed by subjecting to lysis using lysis solution containing SDS and NaOH mixture. Gram's staining was used to confirm entrapment and subsequent removal of *Staphylococcus aureus* from the polyacrylamide gel. Imprinted polyacrylamide gel slabs were allowed reabsorb bacteria, where only 4% of polyacrylamide gel showed reabsorption over a period of time from 15-180 min. Incubation of the 4% acrylamide with *Staphylococcus aureus* suspension at 75 minutes showed highest reabsorption of the bacteria. Cross-reactivity or cross-reabsorption was checked with other bacteria namely, *Salmonella typhi* and *Escherichia coli* on the imprinted polymer. Motive of the study is to develop synthetic materials with enhanced specificity and selectivity at lowest cost which can bind imprinted proteins of the bacterial surface in a given suspension and help in the recognition of the bacteria.

Since January 2020, SARS-CoV-2 has been declared as the global pandemic and world health emergency by World Health Organization. Development of rapid diagnostic methods to identify infected subjects can limit the extent of spread of the virus and consign the early actions.

Sr. No.	Analyte	Type of detector/method	Fabrication chemistry	Focus of the work	Specific for analyte	Response time	Linear range	Detection limits
1	Organophosphorus pesticide	Electrochemical (Cyclic voltammetry)	GCE/AchE-MWCNTs	Standardization	Yes	10 min.	10-50 nM	0.1 nM, 500 nM
2	Triglyceride	Electrochemical (Cyclic voltammetry)	GCE/GO/MWCNTs/PEI-TiO_2/GA/Lipase	New fabrication chemistry and standardization	Yes	-	100-500 mg/dL	100 mg/dL, 500 mg/dL
3	*Staphylococcus aureus*	Optical (Spectrophotometry)	Whole *Staphylococcus aureus* bacterial cells imprinted on polyacrylamide gel	Proof-of-concept	No	75 min.	-	5.763×10^6 CFU/ml for *Staphylococcus aureus*, 5.13×10^6 CFU/ml for *Salmonella typhi* and 11.42×10^6 CFU/ml for *Escherichia coli*
4	SARS-CoV-2	Electrochemical (Cyclic voltammetry)	GCE/PVA-MWCNTs-ssDNA/GA	Use of Novel ssDNA aptamer and standardization	Yes	30 min.	1×10^{-10} M, 1×10^{-15} M	1×10^{-8} M, 1×10^{-15} M

Table 6.1. Summary of the research work carried out in the thesis

Thus, continued Covid-19 pandemic has highlighted the need to develop rapid and cost effective detection kits. Herein, the development of a label free electrochemical oligonucleotide-chip for the detection of Nsp3 protein gene of SARS-CoV-2 has been constructed. Oxidized MWCNTs were functionalized with ssDNA oligonucleotide probe via ester linkages and was characterized by FT-IR analysis. Then, ssDNA-MWCNTs complex was dispersed into PVA polymer matrix. The dispersion was then drop casted onto screen printed carbon electrode followed by its crosslinking with glutaraldehyde. Electrochemical response of the DNA chip after 10 min of hybridization with its complementary strand was recorded in 0.5 NaCl and 50 mM $K_3[Fe(CN)_6]$ dissolved in 50 mM PB (pH=8). 20 µM methylene blue was used as a redox marker before its measurement in electrolyte. The DNA chip showed linearity from 1×10^{-10} to 1×10^{-15} M for its complementary oligonucleotide strand with detection limit of 1×10^{-8} M. The DNA chip showed high specificity and acceptable replicability. The oligonucleotide DNA-chip sensor is cost effective, skips amplification step, offers faster analysis time and on-site testing which is comparable to qPCR.

In conclusion, the research work of the thesis concentrated on the design of novel biosensors for the detection of organophosphorus pesticides, triglycerides, SARS-CoV-2 and *Staphylococcus aureus*. Considerable amount of time and efforts were dedicated to trouble shooting of the issues faced by current biosensors and conventional methods. The work proposes new fabrication chemistries and standardization of the electrochemical biosensors for triglyceride and SARS-CoV-2. Electrochemical biosensor for organophosphorus pesticide demonstrates the immobilization of the enzyme acetylcholine esterase without using any binder or polymer. The results of whole cell molecular imprinting used for the detection of *Staphylococcus aureus* proved that the technique is non-specific and unreliable in stark contrast to the literature published by other laboratories.