CHAPTER 8

SUMMARY AND CONCLUSION

The present investigation involves the synthesis and characterization of Ag@SiO$_2$, NIR emitting core shell Ag$_2$S@SiO$_2$ nanospheres, Si@SiO$_2$ core shell nanoparticle, antibody conjugated Ag@SiO$_2$, antibody conjugated core shell Ag$_2$S@SiO$_2$ nanospheres, DNA conjugated carbon dots, DNA conjugated silicon dots and DNA conjugated Si@SiO$_2$ core shell nanoparticle. Their application in the development of optical immunosensor and DNA biosensor to detect pathogenic microorganisms and heavy metal ions were also studied. The function and importance of core shell nanoparticle, quantum dots and its biological applications have been reviewed in Chapter 1. The experimental procedures adopted to synthesize core shell nanoparticle, preparation of antibody conjugated core shell nanoparticle and DNA conjugated core shell nanoparticle, development of immunoassay and fluorescent assay towards the fabrication for the development of optical biosensor are explained in Chapter 2.

A special prominence is laid on the role of core shell nanoparticle (Ag@SiO$_2$, Ag$_2$S@SiO$_2$ & Si@SiO$_2$) and quantum dots (Carbon dots & Silicon dots) in the development of biosensors for the detection of various pathogenic microorganisms like Escherichia coli (E. coli), water polluting parasite Cryptosporidium parvum and heavy metal ions mercury from environmental samples from Chapter 3 to Chapter 7.
The development of optical immunosensor based on dual labeled core shell (DLCS) Ag@SiO$_2$ nanoparticle for the sensitive detection of *E. coli* is discussed in Chapter 3. The principle for the development of dual labeled core shell Ag@SiO$_2$ nanoparticles based optical immunosensor for the detection of *E. coli* is as follows. Anti-*E. coli* McAb was immobilized on the glycidylsilane functionalized quartz glass plate and subsequently incubated with the target *E. coli*. Finally the dual labeled core shell Ag@SiO$_2$ nanoparticles were immobilized on the glass plate. The principle is based on sandwich form of the antibody immunoassay. The metal enhanced fluorescent dual labeled core shell Ag@SiO$_2$ nanoparticle was used to improve the efficiency of the immunoassay. The change in photoluminescence was measured to detect the target pathogen *E. coli* followed by treatment with DLCS nanoparticles. The silica (SiO$_2$) shell on the Ag nanoparticles surface acts as an anchorage platform for both the antibody and the dye.

The photoluminescence intensity of the immunoassay was increased, due to the presence of more number of dual labeled core shell Ag@SiO$_2$ nanoparticle in the assay. It was confirmed that the dual labeled core shell Ag@SiO$_2$ nanoparticle successfully enhanced the performance of the optical immunosensor by achieving higher sensitivity and specificity with detection limit of 5 CFU/mL with minimal assay period. Furthermore, the DLCS enhanced optical immunosensor also efficiently detected the presence of *E. coli* in real time water samples, which was comparably detected by the conventional PCR. Consequently, the designed dual labeled core shell Ag@Silica nanoparticle based optical immunosensor has high specificity, and easy operation, and is sensitive for screening and monitoring the *E. coli* in the environmental samples at an early stage.

Further, optical immunosensor developed using near infrared emitting core shell Ag$_2$S@silica nanospheres and their applications for the
sensitive detection of *C. parvum* in the environmental samples are discussed in Chapter 4. The principle of the anti-oocysts McAb immobilized NIR emitting core–shell Ag$_2$S@silica nanosphere based optical immunosensor is based on the sandwich form of the antibody immunoassay. Initially, anti-oocysts McAb was immobilized on the (3-glycidoxypropyl) methyldiethoxysilane functionalized quartz glass plate and subsequently incubated with the target *C. parvum*. The glass plates were then treated with anti-oocysts McAb immobilized core–shell Ag$_2$S@silica nanospheres and used to measure the photoluminescence (PL) spectra to detect the presence of the target pathogen.

The consecutive immobilization of GPTMS, primary antioocysts monoclonal antibody, oocysts and anti-oocysts McAb immobilized core–shell Ag$_2$S@silica nanospheres on the quartz glass were characterized by absorption and PL spectroscopy. The sensitivity of the assay was increased with an increase in the number of oocysts so that, increase in numbers of core shell Ag$_2$S@silica nanosphere available for the immuno reaction. Consequently, NIR emitting core–shell Ag$_2$S@silica nanosphere successfully enhanced the performance of the optical immunosensor by achieving higher sensitivity, specificity, especially the detection limit of 10 oocysts per mL. Moreover, the antioocysts McAb immobilized NIR emitting core–shell Ag$_2$S@silica nanospheres can be employed for the optical immunosensing of *C. parvum* in environmental samples. Accordingly, the designed method of enhanced optical immunosensor stands for fast, easy operation high sensitive and specificity assess for the detection of *C. parvum* in drinking water samples.

Chapter 5 investigates the biomedical application of carbon dots based optical DNA mercury sensor in the water samples. In this context, carbon dots based optical DNA mercury sensor was developed and utilized to detect the heavy metal Hg$^{2+}$ ions in the environmental samples. The principle
for the fluorescent sensor based on the MoS$_2$ nanosheet/DNA/carbon dots nanoassembly is as follows. DNA was conjugated on the surface of the carbon dots which act as the fluorescent probe (FRET donor). The fluorescent probe would be adsorbed on the surface of the MoS$_2$ nanosheets due to van der Waals force between nucleobases and the basal plane of MoS$_2$ nanosheets. The absorption of carbon dot onto MoS$_2$ leads to fluorescence quenching of carbon dots since MoS$_2$ nanosheets acts as a quencher (FRET acceptor) which can absorb the energy from fluorescent probe. The subsequent addition of Hg$^{2+}$ ions results in the formation of T-Hg$^{2+}$-T co-ordination complex and thus detaches the fluorescent probes from MoS$_2$ nanosheets. Consequently, the FRET effect between DNA functionalized Carbon dots and MoS$_2$ nanosheets were prevented and thus the fluorescence intensity of fluorescent probe increases. The sensitivity and selectivity of the proposed sensor was evaluated that the increase in concentration of Hg$^{2+}$ ions results in gradual enhancement of fluorescence intensities. The detection limit was found to be 1.02 nM which is superior to that of the reported fluorescent mercury sensors. It was further confirmed that the developed carbon dots based optical DNA mercury sensor stands for higher sensitivity and highly specific towards the detection of mercury ions in the environmental samples.

Further, the development of sensitive fluorescent mercury sensor based on the fluorescence quenching of MoS$_2$ nanosheet/DNA/Silicon dots nanoassembly for the efficient detection of Hg$^{2+}$ ions in the environmental samples is focused in Chapter 6. The principle for the fluorescent sensor based on the interaction between MoS$_2$ nanosheet/DNA/Silicon dots nanoassembly and Hg$^{2+}$ ions. Initially, fluorescent probe/ molecular recognition (FRET donor) was prepared by functionalization of silicon dots with DNA according to the 5' - terminal −SH reaction. In the absence of Hg$^{2+}$ ions, the fluorescent probe would be adsorbed on the surface of the MoS$_2$ nanosheets because of van der Waals force between nucleobases and the basal
plane of MoS\textsubscript{2} nanosheets. Under these conditions, the silicon dots and MoS\textsubscript{2} nanosheets (quencher/FRET acceptor) are close to each other, resulting in fluorescence quenching due to fluorescence resonance energy transfer.

On the contrary, when Hg\textsuperscript{2+} ions was present in the sensor assay solution, mercury mediated base pairs (T–Hg\textsuperscript{2+}–T) induce the detachment of fluorescent probe from the surface of the MoS\textsubscript{2} nanosheets. Thus, the fluorescent probes sustain its fluorescence intensity. From the obtained results, it is ascertained that addition of Hg\textsuperscript{2+} to the assay solution induced the detachment of fluorescent probe from the surface of the MoS\textsubscript{2} nanosheets and thus the fluorescent probes sustain its fluorescence intensity. The results obtained confirm the eminence sensitivity in the environmental monitoring of mercury as compared to that of customary method with lowest detection limit of 0.86 nM. Furthermore, the MoS\textsubscript{2} nanosheet/DNA/Silicon dots nanoassembly based fluorescent mercury sensor also efficiently detected the presence of Hg\textsuperscript{2+} ions in real time water samples, which was comparably detected by the conventional AAS (Atomic Absorbance Spectrometer). Consequently, the designed MoS\textsubscript{2} nanosheet/DNA/Silicon dots nanoassembly based fluorescent mercury sensor has high specificity, and easy operation, and is sensitive for screening and monitoring the mercury in the environmental samples at an early stage.

In continuation to Chapter 6, Chapter 7 investigates the DNA labeled Si@SiO\textsubscript{2} core shell nanoparticle facilitated fluorescent sensor to detect heavy metal Hg\textsuperscript{2+} ions in the environmental water samples. The developed optical mercury biosensor was fabricated using a “sandwich” detection strategy involving DNA labeled Si@SiO\textsubscript{2} core shell nanoparticles (fluorescent probe) and probe DNA. Probe DNA was immobilized on the 3-(Glycidoxypropyl) methyldiethoxysilane functionalized quartz glass plate and subsequently incubated with the Hg\textsuperscript{2+} ions and fluorescent probe (DNA
labeled Si@SiO$_2$ core shell nanoparticle). The detection protocol is based on the formation of coordination complex of thymine with Hg$^{2+}$ ion. In the absence of Hg$^{2+}$ ions, Probe DNA and fluorescent probe cannot able to hybridize with each other due to their strong electrostatic repulsion.

On the other hand, presence of Hg$^{2+}$ ions influence the DNA hybridization resulted from the formation of mercury mediated (Thymine–Hg$^{2+}$–Thymine) base pairs. Consequently, the acquaintance of fluorescent probe with DNA immobilized quartz glass plate resulted in the fluorescence enhancement and it was used to measure photoluminescence (PL) spectra to detect the presence of mercury. The sensitivity of the assay was increased due to the higher amount of Hg$^{2+}$ions available for acquaintance of fluorescent probe. It was affirmed and demonstrated that the DNA labeled Si@SiO$_2$ core shell nanoparticle based fluorescent mercury sensor has higher sensitivity and specificity with a detection limit of as low as 0.92 nM. Moreover, results obtained from the recover test of the developed fluorescent mercury sensor. Consequently, the projected DNA labeled Si@SiO$_2$ core shell nanoparticle facilitated fluorescent sensor assay stands for high sensitive, specificity, easier processing, cost competitive and higher recovery percentages for the detection of trace amount of Hg$^{2+}$ ions in real environmental samples.

It is concluded that the newly developed dual labeled Ag@SiO$_2$ core–shell nanoparticle, NIR emitting Ag$_2$S@silica core–shell nanospheres, DNA conjugated Carbon dots, DNA hybridized Silicon dots and DNA labeled Si@SiO$_2$ core–shell nanoparticle based optical biosensors in the present work possess the following advantages over the conventional methods such as high sensitivity, specificity, very less duration of the assay required, does not required any sophisticated instruments, simple processing, cost effective and more convenient for the routine screening of environmental water samples.
It is also concluded that the core–shell nanoparticle and quantum dots is used to enhance the performance of optical immunosensor and DNA biosensor to detect the pathogenic microorganisms and heavy metal Hg\(^{2+}\) ions. Further the present work can also be extended in the future towards the development of ready-made kits for easy evaluation and routine screening of pathogenic microorganisms and Hg\(^{2+}\) ions present in environmental water samples at earlier stage.