ABSTRACT

The objective of the present work is to develop the biosensor based on gold nanoparticle to detect pathogenic microorganisms present in the environmental and clinical samples. In the present study, gold nanoparticle facilitated immuno-dot blot and electrochemical biosensors were developed. These biosensors can be mainly utilized to detect three types of microorganisms such as White Spot Syndrome Virus (WSSV), water polluting parasite Cryptosporidium parvum and mycobacterium tuberculosis present in the real world samples.

White Spot Syndrome (WSS) is a worldwide ailment of penaeid shrimp, a lethal and contagious disease in shrimp caused by the White Spot Syndrome Virus (WSSV), which require early detection and routine screening under field conditions. Although polymerase chain reaction (PCR) based detection of the causative viral nucleic acid has many advantages, including sensitivity, the need for sophisticated equipment and technical expertise is ruled out in its routine usage. In contrast, protein-based immuno detection methods are easier to perform, even by laymen. However, in order to enhance the efficiency of the immuno assay to detect WSSV, a sensitive alkaline phosphatase conjugated secondary antibody coupled gold nanoparticle based immuno-dot blot assay has been developed. Data resulted from the immuno-dot blot assay, it is ascertained that the gold nanoparticle based immuno-dot
blot assay can detect 1 ng/mL of purified WSSV visually, whereas in the conventional assay can detect up to 80 ng/mL of WSSV. It is concluded that gold nanoparticle based immuno-dot blot assay shows enhanced sensitivity by 80 fold when compared to than that of conventional method.

The performance of the immuno-dot blot assay was further improved by developing a novel dual labeled gold nanoparticle based immuno-dot blot assay to detect water polluting parasite like Cryptosporidium parvum. The zoonotic protozoan parasite Cryptosporidium parvum pose a significant threat to public health. Even a small number of C.parvum present in water and food causes a serious infection to the human beings. Hence, a suitable sensitive method is required to detect C.parvum at lower concentration. In this context, a sensitive immuno assay has been developed based on dual labeled (both anti-cysts monoclonal antibody and alkaline phosphatase conjugated) gold nanoparticle to detect C. parvum in the drinking water samples. Data resulted from the experiment indicated that the sensitivity of the immuno-dot blot assay is enhanced by 500 fold when compared to that of conventional method and visually be able to detect up to 10 oocysts/mL at a minimal processing period. The method reported in the present study proved to be more feasible for the routine screening of C. parvum presents in the water and food samples.

In addition to the above, the present work is further extended to develop a simple and sensitive gold nanoparticle based electrochemical immuno sensor to detect C. parvum in drinking water. The electrochemical biosensor was fabricated by immobilization of capture anti-cysts McAb on
gold nanoparticle treated amine functionalized ITO electrode. Subsequently *C. parvum* and dual labeled gold nanoparticle (ALP and anti–cysts McAb) were introduced through sandwich hybridization. The redox reactions of [Fe (CN)₆]³⁻/ [Fe (CN)₆]⁴⁻ on the electrode surface was analyzed by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) to assess the efficiency of immobilization process. After the sandwich immuno reaction, the dual labeled gold nanoparticle immobilized electrode was used to hydrolyze *para*- nitro phenyl phosphate (*p*-NPP) to produce the electroactive *para*- nitro phenol (*p*-NP), which can be measured by differential pulse voltammetry (DPV) analysis. The results obtained from the experiment indicated that the sensitivity of electrochemical immuno sensor was enhanced by gold nanoparticle with the limit of detection of 3 oocysts/mL at minimum processing period. It was also evidenced that the gold nanoparticle based electrochemical immuno sensor showed higher sensitivity, acceptable precision, reproducibility, stability and can be readily applied to monitor multi analytes present in the environment as compared to that of conventional immuno assays.

The present work was also extended to study the biomedical utility of gold nanoparticle in biosensor development. In this context, gold nanoparticle based electrochemical DNA biosensor was developed to detect the *Mycobacterium tuberculosis* in the clinical samples. The electrochemical DNA biosensor was fabricated using sandwich detection strategy involving two kinds of DNA probes specific to the *Mycobacterium* sp. genomic DNA. Both the enzyme alkaline phosphatase and detector probe were conjugated on
the gold nanoparticle and subsequently hybridized with target genomic DNA immobilized on capture probe modified SAM /ITO electrode. It is inferred from the results that, the newly developed dual labeled (Probe DNA and ALP) gold nanoparticle based electrochemical DNA biosensor can be utilized for the diagnosis of *mycobacterium* sp. in sputum samples and the detection limit of 1.25 ng/mL of genomic DNA was noticed. Consequently, the projected gold nanoparticle based electrochemical DNA sensor stands for high specificity, simple processing, cost competitive and reproducibility for the detection of *mycobacterium* sp in clinical samples.

It is concluded that the developed gold nanoparticle based immuno and electrochemical biosensor can be applied to detect pathogenic microorganism present in the environmental and clinical samples with superior sensitivity and specificity over the conventional method. It is also suggested that the gold nanoparticle can be used to enhance the performance of immuno and DNA biosensor to detect the pathogenic microorganisms at earlier stage.