ABSTRACT

In this research work, we report a highly sensitive and specific electrochemical method for simultaneous detection of multiple pathogen targets using quantum dots (QDs). Nanoscale architecture of DNA-linked particles are attractive for electrochemical detection of DNA hybridization. Moreover, QDs have the potential to simplify the performance of a multiplex analysis. Therefore, the main objective of this work is to achieve a multiple simultaneous detection of npcRNA sequences of three enteric pathogens (*Vibrio cholerae, Salmonella* sp., *Shigella* sp.) by using QDs as the electrochemical nanomaterial label. In this work, QDs (PbS, CdS, ZnS) were synthesized and characterized by 4 methods, comparison under UV and ambient light, photoluminescence study, UV-vis absorption and electrochemical detection. With the standard of 0.01 ppm concentration of PbS, CdS and ZnS which gave a response of 0.27 ± 0.04 μA, 0.25 ± 0.04 μA and 0.27 ± 0.02 μA, respectively, ratio study was done. The results of the ratio study done indicated corresponding results with the all the ratios as well as the standard, which indicated no cross interferences of each QDs signal response. The QDs of PbS, CdS and ZnS were each conjugated with probes synthesized from novel npcRNA of different species of enteric pathogens (*Vibrio cholerae, Salmonella* sp., *Shigella* sp.) respectively. The detection of QDs was done by Square Wave Anodic Stripping Voltammetry (SWASV) method and by using Screen Printed Carbon Electrode (SPCE) as the platform. The sandwich assay type detection was done, whereby single pathogens were first detected separately. This was followed by the simultaneous detection of the multiple pathogens. When dissolved with acid, each of the quantum dots gave out a very distinct peak for each of the target. Hence, this allows for further quantification of the DNA concentration of target detected, since the peak height equals to concentration of target. The peak of the each of the QDs, PbS , CdS and ZnS, were detected at peak position of 0.5 V, 0.75 V and 1.1 V and the

peak current obtained for PbS, CdS and ZnS with target DNA (*Vibrio cholerae, Salmonella* sp., *Shigella* sp.) were 1.07 ± 0.06 µA, 1.87 ± 0.04 µA and 1.15 ± 0.18 µA, respectively. Calibration curve of the multiplex sandwich assay was done with the range of 1000 fM to 0.05 fM and the limit of detection obtained was in the attomolar range of 50 aM for PbS (*Vibrio cholerae*), 54 aM for CdS (*Salmonella* sp.) and 47 aM ZnS (*Shigella* sp.). Real sample detection was also done whereby, PCR products of the pathogens were obtained. Calibration curve of the real sample multiplex detection was done from 1000 fM to 0.05 fM and the limit of detection was 50 aM for both PbS (*Vibrio cholerae*) and CdS (*Salmonella* sp.) and 54 aM for ZnS (*Shigella* sp.), which was also in the attomolar range. Hence, we have developed an ultrasensitive, highly specific and effective multiplex enteric pathogens detection system using QDs as a label.

Keywords: Quantum dots, enteric pathogens, Square Wave Anodic Stripping Voltammetry, npcRNA, electrochemical detection