INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: "HUMAN SERUM ALBUMIN-STABILIZED GOLD NANOCLUSTERS AND THEIR APPLICATIONS FOR DETECTION OF BILIRUBIN IN SERUM SAMPLES"

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SHORT ABSTRACT

The present investigation is focused on the utilization of human serum albumin-stabilized gold nanocluster (HSA-AuNC) as biorecognition element for detection of bilirubin in optical and electrochemical transducer platforms. The natural affinity of bilirubin to HSA and some unique optical and electrochemical features of gold nanoclusters (AuNCs) were exploited to develop the detection techniques. Following a simple microwave-based technique AuNCs were synthesized in the protein matrix of HSA. Using transmission electron microscopy (TEM) and matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS), the size and the number of gold atom in these NCs were determined to be ~ 2.5 nm and 18, respectively. The formation of AuNCs in the protein matrix though caused some perturbation in the secondary structure of the protein, the binding of bilirubin to the HSA-AuNC assembly was not much altered as evident from the reasonably good binding constant of 0.55 x 10^6 L $mole^{-1}$. Zeta potential studies revealed that the formation of nanoclusters caused to increase the surface charge density of the HSA protein. The HSA-stabilized AuNC, when excited at $\lambda_{380\ nm}$, produced an intense fluorescence at $\lambda_{640\ nm}$. Interestingly, the binding of bilirubin with HSA-AuNC quenched the fluorescence in a concentration dependent manner. The fluorescence quenching phenomena, which obeyed a simple static quenching mechanism, was utilized for interference-free detection of bilirubin with minimum detection limit (DL) of 248 ± 12 nM at a signal-to-noise (S/N) ratio of 3. Additionally, a peroxidase-like catalytic activity of these nanoclusters was observed and exploited for the detection of bilirubin following a colorimetric approach. The detection involves a decrease in absorbance of bilirubin at λ_{440nm} in the reaction solution upon addition of H_2O_2 due to peroxidative catalytic effect of the AuNCs. The minimum DL for bilirubin as discerned from the analysis was 200 ± 19 nM. The HSA-stabilized AuNC was also found to act as electron transfer bridge during electrocatalytic oxidation of bilirubin on a fabricated electrode surface. The bioelectrode was fabricated by covalently immobilizing HSA-AuNC on silanized indium tin oxide (ITO) electrodes using N-ethyl-N'-(3-dimethylaminopropyl) carbodimide (EDC) and N-hydroxysuccinimide (NHS) ligand chemistry. The bioelectrode was characterized by using various advanced spectroscopic and electrochemical techniques. The AuNCs in the protein matrix acted as an electronic bridge by contacting a specific redox active moiety of the HSA-

attached bilirubin molecule and the polarized electrode (0.27 V vs. Ag/AgCl). The HSA-AuNC based bioelectrode showed linear response range of 0.2 µM to 7 µM for bilirubin with a DL of 86.32 nM at an S/N ratio of 3. The bioelectrode showed efficient electron transfer rate constant (Ks) 1.38 s^{-1}. The fabricated bioelectrode was also found to exclude interference caused by the commonly co-existing electroactive species in blood serum such as ascorbic acid, uric acid, lactic acid and glucose thus, can be used as biosensors for highly selective analysis of bilirubin in serum samples. The bioelectrode retained ~75 % of its initial activity at the end of 15 measurements while it retained ~80 % of its original response after storing for 7 days at 4 ºC. Finally, a microfluidic paper-based electrochemical device (µPED) was developed for the detection of bilirubin by using the HSA-AuNC as an electrochemical probe laying over an array of zinc oxide nanorods (ZnO-NRs) on a patterned paper surface. The µPED comprised of two layers of rectangular papers patterned with alkene ketene dimer (AKD) printing to produce circular detection zone with a loading capacity of 10 µl. A novel, stable conducting graphite ink was developed by using silk sericin as a binder. The counter electrode and reference electrode were placed on the top layer of paper while, working electrode was grafted on the bottom layer of the paper zone. The zinc oxide nanorods (ZnO-NRs) were grown in-situ over the working electrode via solvothermal method. Later, the HSA-AuNC was immobilized over ZnO-NRs following electrostatic interaction principle. The morphological characteristics of the working electrodes were analyzed by FESEM technique. The µPED containing HSA-AuNC/ZnO-NRs showed linear response range of 0.5 µM to 35 µM (R^2 = 0.994) of bilirubin with a detection limit of 57.3 nM at an S/N of 3. The results embodied in this thesis have established the HSA-stabilized AuNC as suitable bio-recognition system for detection of free bilirubin in serum sample in optical (fluorescence and colorimetric) as well as electrochemical platforms.