SYNTHESIS AND CHARACTERIZATION OF PROTEIN TEMPLATED MULTIFUNCTIONAL NOBLE METAL QUANTUM CLUSTERS FOR BIOMEDICAL APPLICATIONS

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By

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ABSTRACT

Noble metal quantum clusters (NMQC) are sub-nanometer core sized materials made up of several tens of atoms with distinct optical, electrical, and chemical properties compared to those of their larger counterparts viz. plasmonic nanoparticles. Synthesis of atomically precise noble metal quantum clusters using novel synthetic strategies is an emerging area of recent research. In the past decades, the synthesis of AuQCs was achieved using various templates like phosphine, thiols, peptides, amino acids, enzymes, dendrimers, DNA, and proteins. Among these, protein template-based synthesis of the cluster, in particular, Au, Ag and Cu with molecule like properties have exhibited considerable attention in different fundamental fields from chemistry and biology to biomaterials due to its excellent luminescence, outstanding biocompatibility, ultra small size, large stocks shift etc. The protein protected metal quantum clusters provide more attractive features such as one-pot and green synthetic method, good aqueous solubility, relatively low environmental impact, mild reaction conditions, excellent biocompatibility, and versatile surface chemistry. Thus protein stabilized QCs are superior over other conventional fluorescent probes such as semiconductor quantum dots, organic dyes and engineered fluorescent proteins, which face limitations like intrinsic toxicity in biological systems, high rate of photobleaching and relatively large size. The combination of unique optical and electronic properties of the metal quantum cluster together with the inherent biological properties of protein make NMQCs promising candidates for various biomedical applications like imaging, sensing, delivery, and therapeutics. In spite of the notable progress recently made in the utilization of these clusters in medicine, they have been suffering from low quantum yield, less pH stability, and face oxidative decomposition by reactive oxygen species, unstable at high temperature and the high cost of protein. We are addressing some of these challenges by introducing some new proteins for the synthesis of QCs. This thesis discusses the synthesis of three different protein stabilized NMQC, particularly, AuQC, AgQC, AuAgQC and CuQC, characterization, photophysical studies, surface modification and explores the application of these QC in biosensing, imaging, and delivery.

Surface functionalization of bovine serum albumin-stabilized gold quantum cluster (AuQC@BSA) by an enzyme, acetylcholinesterase (AChE) via non-covalent interaction provide the ability of selective detection of acetylcholine (ACh). ACh is a neurotransmitter and AChE is a specific enzyme for ACh degradation, found in central and peripheral nervous system. It is a biomarker for various neuropsychiatric and neuropsychological diseases such as Alzheimer's and Parkinson. Successful incorporation of AChE over Ach is proved by various spectroscopic techniques. Electrospray ionization mass spectrometric (ESI MS) analysis proves the retention of enzymatic activity of AChE after immobilization on the AuQC@BSA surface. The fluorescence quenching of AuQC@BSA-AChE occurred with increasing the concentration of ACh. The systematic fluorescence study shows that the hydrolyzed product of ACh, choline (Ch) is the reason for the fluorescence quenching. It is a positively charged quaternary ammonium salt generated in the medium which interact with negatively charged QC and in turn changes the environment of QC and quenches the fluorescence. The lowest value

detected by this method was found to be 10 nM and shows excellent selectivity. A paper-based sensor shows the possibility of naked eye detection for ACh.

Contrary to the protein derived from animals, plant proteins are the suitable candidates for the sustainable synthesis of QC owing to its low cost, natural abundance, high stability, and sustainability. Gluten, a complex protein derived from wheat, made up of hundreds of different proteins was used for the 'green' and 'one-pot' synthesis of AuQC. The major drawback of conventional protein stabilized QCs, oxidative decomposition by reactive oxygen species (ROS) is prevented by the synthesized AuQC@gluten, owing to the large complex nature of the protein. A 'turn-on' sensing of creatinine is possible by using AuQC@gluten with the assistance of picric acid (PA). PA acts as a fluorescence quencher and creatinine is recovered 60 % of quenched fluorescence. Creatinine is a biomarker for kidney diseases. The mechanism of interaction of QC with the analyte is systematically studied.

The low quantum yield of the QCs can reduce the sensitivity of sensors. AuAg Alloy QC shows enhanced stability and QY due to its synergetic interaction and the 'silver effect'. Gluten stabilized AgAuQC (AgAuQC@gluten) shows high stability in acidic to extremely basic pH and much higher QY than AuQC@gluten. The sensitive and selective detection of bilirubin is established using the synthesized AgAuQC@gluten through fluorescence quenching. Bilirubin is a biomarker for liver disorder. The spectroscopic investigation implies that the electron transfer and inner filter effect is the leading cause of fluorescence quenching.

Ratiometric sensing method is considered as a more sensitive method for the detection due to the monitoring of two emission wavelengths. The thesis also discusses the design of a novel strategy for the preparation of sustainable dual emitting nanohybrid for ratiometric detection of mercury Hg (II) ion. The dual emitting composite contains blue-green emitting graphene quantum dot (GQD) and red emitting AuQC, prepared using gluten as the raw material. The in-situ growth of AuQC in gluten functionalized GQD, produces a dual emitting composite (AuQC@GQD). A uniform distribution of AuQC@GQD on the surface of nanofibre by electrospinning technique is an added score in sensing. A rapid visual detection of Hg (II) using the electrospun mat shows the practicality of the developed sensor.

Fabrication of polymer-QC composite is not much explored due to its less stability in the preparation conditions. The melt mixing of polyurethane and CuQC@gluten at 80 ^{o}C provides a complete dispersion of CuQC in the polymer matrix. The release of Cu(II) ion from CuQC-PU matrix at physiological condition leads to the utilization of this composite towards the fabrication of the intrauterine device for contraceptive applications since the action of Cu (II) ion in the uterus prevents the fertilization process. The proposed method can overcome the drawbacks of conventional intrauterine devices (CuT), such as bleeding in the initial days due to burst release of Cu (II) ion.

The biocompatible protein stabilized AuQC can act as a scaffold as well as a tracking agent for drug delivery applications. Curcumin (CUR) is an anticancer

hydrophobic drug, the poor aqueous solubility and rapid degradation limits its use in cancer therapy. A 99.8 % encapsulation of CUR in gliadin stabilized AuQC (AuQC@gliadin) is possible by simple mixing. The bioavailability of CUR can be enhanced by this strategy. It prevents the rapid degradation of CUR at physiological and alkaline pH condition. Photophysical properties of CUR are enhanced in aqueous solution of AuQC@gliadin. Folic acid (FA) conjugated AuQC@gliadin-CUR shows the targeted release of CUR in the cancer cell, without affecting the normal cell. Thus the protein stabilized AuQCs not only act as an encapsulating agent but also endows high aqueous stability and intense luminescence so that it can be used to track the delivery of CUR into cells.