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ORIGINAL PAPER

Manganese Doped Zinc Sulfide Quantum Dots for Detection of *Escherichia coli*

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Abstract A novel biocompatible chitosan passivated manganese doped zinc sulfide (Mn doped ZnS) nanophosphor has been synthesized through a simple aqueous precipitation reaction. Upon excitation with ultraviolet light, the quantum dots (QDs) emit an orange luminescence peaking at 590 nm, which is visible to the naked eye. These chitosan coated Mn doped ZnS QDs can have potential applications in bio-labeling, particularly in fluorescence-based imaging. One of the envisioned applications of these QDs is in improving the conventional, organic dye-reliant Fluorescence in situ Hybridization (FISH) technique, a widely used method for microbial detection. Here we demonstrate that the chitosan-capped Mn doped ZnS QDs are suitable for this purpose.

Keywords Quantum dots · Zinc sulphide · Doping · Chitosan · Bio-labeling

Introduction

In recent years, considerable interest has grown in nanometer dimension semiconductors due to quantum size effects. Nanocrystalline semiconductor particles exhibit novel properties due to large number of surface atoms and the three dimensional confinement of electrons. Altering the size of the particle alters the degree of confinement of the electrons and affects the electronic structure, in particular band edges, which render tunable optical gaps with particle size. QDs have been used for biological applications, mainly for biological detection and tagging [1–6]. They have several advantages over conventional organic dyes like narrow emission and broad absorption spectra, which can be excited with light of a single wavelength to emit different colors. They also show excellent photostability as compared to the fluorophores [7]. These inherent advantages of the QDs aid in the integration of nanotechnology and biotechnology, leading to major advances in medical diagnostics, molecular biology and cell biology.

One potential application of QDs is in labeling of oligonucleotide probes used for microbial detection via Fluorescence in situ Hybridization (FISH). A widely applied molecular technique, FISH allows the visualization and identification of microorganisms in their natural habitat or in diseased tissues [8]. A major limitation of FISH is its dependence on organic fluorophores whose susceptibility to light-induced degradation forces microbiologists and medical technologists to work under controlled lighting conditions (mostly in dark). The photostable QDs are ideal alternative to the organic dyes. However, before they can be applied in FISH, the QDs must first be proven to be biocompatible and amenable for uptake by the microorganisms.

Capping QDs with organic acids, such as mercaptoacetic acid (MAA) is a common approach to render the QDs biocompatible by making them soluble in aqueous biological buffers [9] and microorganisms have been shown to take up organic acid-capped CdS or CdSe QDs [10–12]. Numerous reports on the synthesis of manganese doped ZnS nanocrystals in aqueous media are available in the

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literature [13–18]. Warad et al. [19] has reported the synthesis of ZnS nanoparticles capped with sodium hexametaphosphate. In the present work, we describe the synthesis of biocompatible chitosan-capped Mn-doped ZnS QDs in an aqueous medium as well as the potential of these QDs for microbial detection through fluorescence labeling. Chitosan is a biodegradable and biocompatible polymer with polycationic properties in slightly acidic conditions. In dilute acids, the amines get protonated to form NH_3^+ and these protonated amines act as binding sites to form metal complexes, imparting a chelating property to them [20]. The large number of amines in the long chain chitosan acts as multiple binding sites and the whole polymer molecule effectively encapsulates the nanoparticles due to the presence of Zn atoms on the surface.

Materials and Methods

Synthesis and Characterization of QDs

Coprecipitation reaction was employed to synthesize Mn doped ZnS QDs in aqueous medium and surface passivation and stabilization was achieved using a biopolymer chitosan. 0.1 M zinc acetate and 0.01 M manganese acetate was mixed together with 0.1% chitosan and constantly stirred and heated at 80 °C. After cooling the mixture to room temperature, 0.1 M sodium sulfide was added drop wise in an ice bath with constant stirring. The stable solution was centrifuged at 4,000 rpm for 20 min. Excess chitosan in the colloid was removed by adding acetic acid and leaving the suspension to stand overnight. The suspension was then dialyzed to remove the excess unreacted ions. The dialyzed colloid was freeze dried to get fine powder of Mn doped ZnS QDs. These powders are readily redispersable in water. The dry powder was washed repeatedly with deionized water to remove excess chitosan not attached to the QDs. Samples were prepared with 5 mM concentration of zinc acetate and 50μM manganese acetate was used in the samples for doping. Sample characterization were carried out using UV-Visible Spectroscopy (Ocean optics), Transmission Electron Microscopy (JEOL JEM 2010), X-ray Diffraction (JEOL JDX-3530), Fourier Transform Infrared Spectroscopy (Perkin Elmer) and Photoluminescence spectroscopy (Perkin Elmer LS55 Fluorescence Spectrometer).

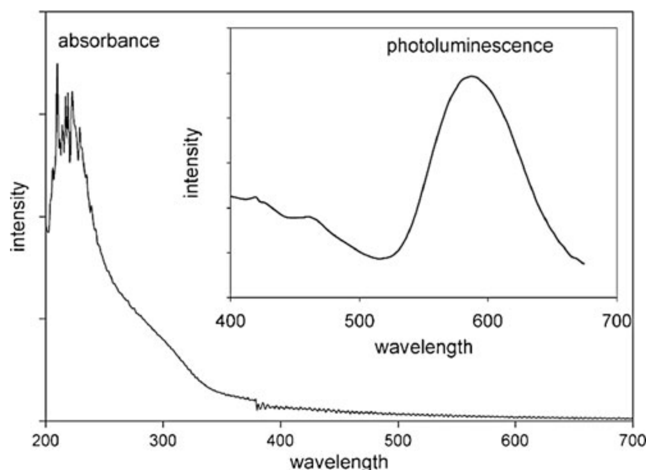

Fig. 1 Absorption and photoluminescence spectra of Mn doped ZnS. The photoluminescence measurement was taken at an excitation wavelength of 220 nm (peak absorption wavelength)

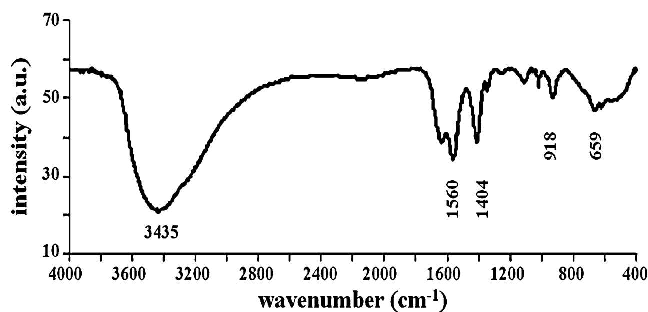

Fig. 2 FTIR spectra of repeatedly washed chitosan capped Mn doped ZnS QDs. Peaks at 1,560 and 659 cm^{-1} are indicative of NH_2 groups

Internal Labeling of *Escherichia coli* with QDs

Preparation of Microorganisms

Escherichia coli (*E. coli*) was cultivated in nutrient broth and incubated at 37 °C overnight. The cells were harvested, washed with phosphate buffered saline (PBS), and then fixed with paraformaldehyde. The fixed cells were suspended in 50% v/v ethanol-1X PBS and the cell density was adjusted to an absorbance of 0.9 at 420 nm. To further permeabilize the fixed cells, ethanol-PBS was first removed and then the cells were re-suspended in 5% Nonidet P40 Substitute. The suspension was incubated for up to 1 h at 37 °C. The permeabilized cells were washed twice with 1X PBS.

Translocation of QDs

The fixed and permeabilized cells were incubated with 1 mL of 5 mM chitosan-capped Mn doped ZnS QDs for 5 h at 65 °C. After incubation, the suspension was centrifuged (Hettich-Zentrifugen, Mikro22R, 4,000 rpm, 3 min). The supernatant was collected for absorbance measurement. The cells were washed with 1 mL 0.1 M acetate buffer containing 0.9 M NaCl. After centrifugation, the supernatant was collected. Finally the cells were lysed through ultrasonic processing. The cell lysate

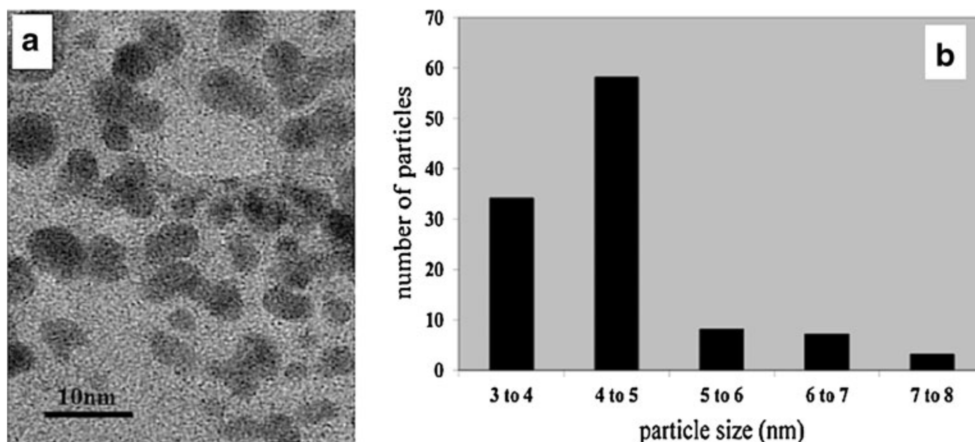

Fig. 3 **a** High resolution transmission electron microscope (HRTEM) image showing Mn doped ZnS QDs of size between 3 and 8 nm **b** particle size distribution of the Mn doped ZnS QDs (100 QDs sampled) showing dominance of QDs in the range of 4 to 5 nm

(1 mL) was separated from the debris by centrifugation and the lysate was collected for absorbance measurement. Blanks (i.e. cells only and QDs only) were prepared and used as reference. The absorbance of the QDs, post-incubation supernatant, post-washing supernatant, and cell lysate at 300 nm were measured by UV–vis spectrophotometer (Shimadzu UV 160A).

Imaging Bacteria with Mn Doped ZnS QDs

The fixed and permeabilized *E. coli* cells were incubated with 5 mM chitosan-capped Mn doped ZnS QDs in 0.1 M acetate buffer (pH 5) for 2 h at 65 °C. The cells were washed once with 0.1 M acetate buffer containing 0.9 M NaCl and once with milliQ water. The cells were transferred to microscope slides for observation under Olympus BH-2 RFCA epifluorescence microscope. Micrographs were acquired with Nikon Coolpix 4500 camera and the images were processed using the ImageJ software developed by the US National Institutes of Heath (http://imagej.nih.gov/ij/).

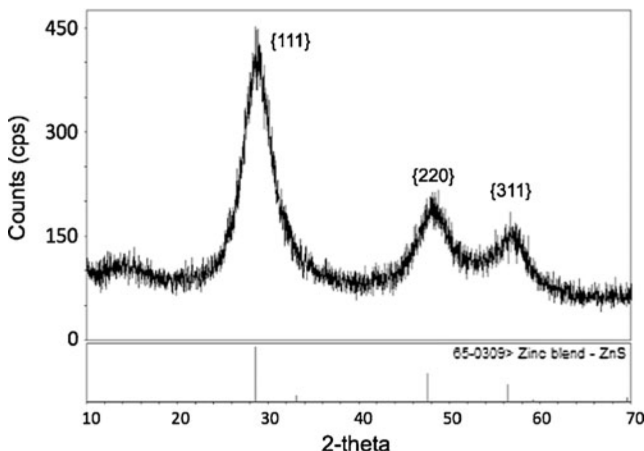

Fig. 4 XRD pattern of the Mn doped ZnS QDs which confirm that the QDs have the zinc blende structure. The standard peaks for the zinc blende structure (JCPDS card 65–0309) have also been included

Results and Discussion

Properties of Mn Doped ZnS QDs

One of the major disadvantages of organic fluorophores for bio-labeling applications is their narrow excitation band that overlaps with the emission band. Doped metal sulfide QDs in retrospect, being semiconductor materials, can be excited at any wavelength above the band gap and the emission wavelength depends on the position of the dopant ion in the semiconductor band structure. ZnS is a semiconducting material with a direct band gap of 3.6 eV and absorb light in the UV part of the electromagnetic spectrum. The optical absorption edge for the Mn doped ZnS QDs is found to be around 220 nm, which is wide as is characteristic for any semiconductor material. The typical photoluminescence (PL) spectra of the doped QDs when excited at a wavelength of 255 nm showed a strong peak at 590 nm and a smaller one at 424 nm (Fig. 1). The PL peak at 424 nm is characteristic of ZnS and the emission at 590 nm arises from the manganese sites within the band gap. The orange luminescence originating due to the presence of Mn^{2+} can be attributed to the recombination of a bound exciton or via hole trapping at an Mn^{2+} center.

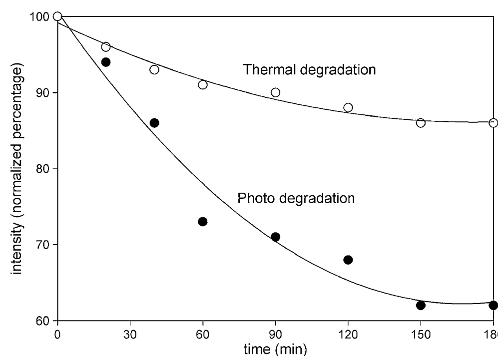

Fig. 5 Plot of the photoluminescence intensity at 590 nm upon exposure to UV light at 320 lux (photo degradation) and thermal treatment (thermal degradation) at 65 °C with time

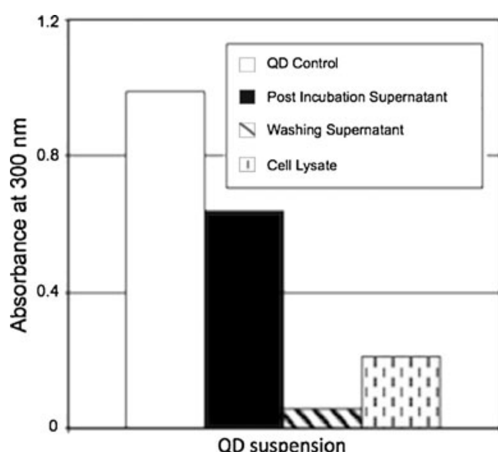

Fig. 6 Absorbance at 300-nm of post-incubation supernatant, washing supernatant, and cell lysate from *E. coli* cells incubated with chitosan-capped Mn doped ZnS QDs

Subsequent recombination with an electron in shallow traps results in Mn^{2+} going into an excited state (Mn^{2+*}). The strong orange emission at 590 nm results from radiative recombination at localized states of Mn within the ZnS band gap

Fourier Transform Infrared (FTIR) Spectroscopy was carried out to confirm the presence of chitosan, a polymer containing amine (NH_2) groups, on the QDs. Figure 2 shows a typical FTIR spectrum obtained from Mn doped ZnS QDs stabilized by chitosan. The spectrum shows a strong peak at 1,560 cm^{-1} and a weak one at 659 cm^{-1} indicating the presence of NH_2 groups. The detection of the amine groups even after careful and rigorous washing of the colloidal QDs suggests that chitosan is attached to the QDs and does indeed inhibit the growth of particle sizes during the precipitation reactions.

High Resolution Transmission Electron Microscopy analysis reveals that the Mn doped ZnS QDs are between 3 and 8 nm in diameter (Fig. 3a). The dominance of QDs in the size range of 4–5 nm can be noted from the particle size distribution shown in Fig. 3b. X-ray diffraction (XRD) studies show that the particle contains nanocrystallites having Zinc Blende structure conforming to the JCPDS file 65–0309. The peaks corresponding to planes {111}, {220} and {311} have been indexed which is in conformation to TEM observations. The XRD pattern is shown in Fig. 4. The QDs are polycrystalline and have an average crystallite size of approximately 2–3 nm. The Mn doped ZnS QDs are individually dispersed in the colloidal suspension and are small enough to pass through the pores in microbial cell walls. The orange luminescence from the Mn doped ZnS QDs upon excitation with UV light can be observed with naked eyes.

It is important for any good fluorophore to be both photo- as well as thermo-stable. As the translocation of the QDs on to the *E. coli* cells is carried at a temperature of 65 °C, it is important that the QDs do not lose their luminescence when maintained at that temperature. Further, as the imaging is done under UV light illumination, the QDs need to be UV photo-stable as well. To confirm the stability of the Mn doped ZnS QDs, we exposed them to continuous thermal (at 65 °C) and optical (UV light at 320 lux) excitations for 3 h and noted the decrease in luminescence. The results are shown in Fig. 5. Both the luminescence decays were fitted using a quadratic polynomial $f = y_o + ax + bx^2$. It can be observed from Fig. 5 that the thermal degradation curve saturates after 150 min at 88% of the initial luminescence intensity. The photodegradation curve also shows a similar trend and saturates at 62%.

Uptake of Mn Doped ZnS QDs by Escherichia coli

It has already been demonstrated that *E. coli* cells can take up organic acid-capped CdS or CdSe QDs [5–7]. Unlike the organic acids which produce water soluble QDs with a net negative charge, chitosan makes the particles positively

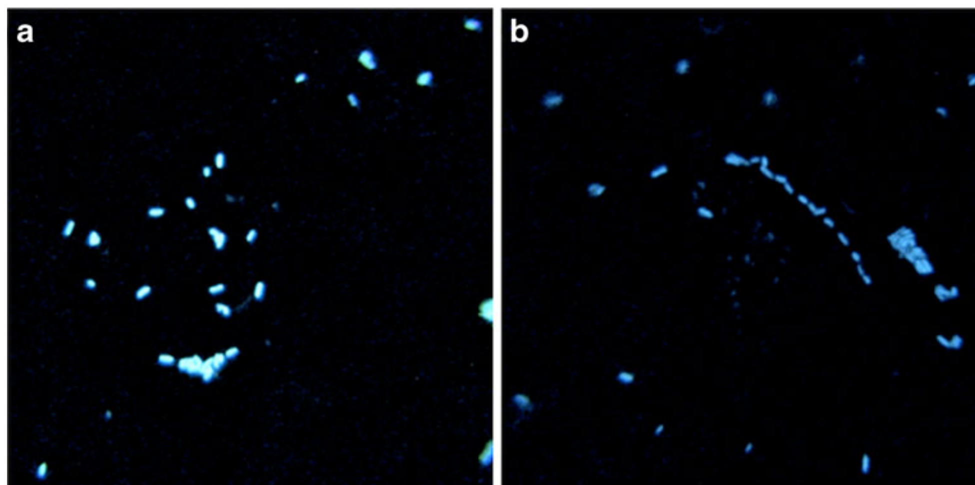

Fig. 7 Representative micrographs of **a** chitosan-capped Mn doped ZnS QD-labeled *E. coli* cells and **b** unlabeled (control) *E. coli* cells. The images were recorded under the U filter cube (excitation: UG1, 300–400 nm; emission: L420, >420 nm) of the Olympus BH-2 RFCA epifluorescence microscope and processed by applying similar background subtraction and level and window adjustment conditions using ImageJ software

charged due to the protonated amine groups which bind easily to the phosphoryl and carboxyl groups on the bacterial cell wall. Due to these interactions, there was an apprehension whether chitosan capped QDs could pass through the cell walls rather than be trapped on the cell surface. To determine if the chitosan-capped Qdots can go inside *E. coli*, permeabilized cells were incubated with the QDs and then the translocation of the QDs from the suspension into the cells was monitored by measuring the optical absorbance at 300 nm of the post-incubation supernatant, washing supernatant, and the cell lysate. The results are shown in Fig. 6.

The decrease in the absorbance at 300 nm of the post-incubation supernatant indicates that the cells were able to remove the QDs from the suspension. The QDs can either move inside the cells or just attach on the cell surface. To release the QDs bound on the surface, we washed the cells with NaCl solution, a strategy known to disrupt the interaction between chitosan and *E. coli* [21]. The washing supernatant showed an absorbance at 300 nm, indicating that some of the QDs were indeed attached on the cell surface. To find out if QDs were able to penetrate the cells, the cells were lysed through ultrasonic processing and the absorbance at 300 nm of the cell lysate was measured (corrected with the absorbance of lysate from control cells). The detection of QDs in the cell lysate indicates that QDs were able to get inside the cells.

Fluorescence Imaging of Microbial Cells with Mn doped ZnS QDs

The internalization of the Mn doped ZnS QDs by *E. coli* was further established by epifluorescence microscopy. A typical micrograph of the QD-labeled *E. coli* cells observed under the U filter set (excitation: UG1, 300–400 nm; emission: L420, >420 nm) is shown in Fig. 7a. The QD-labeled cells were found to emit stronger fluorescence signals compared with the control (unlabeled) cells (Fig. 7b).

The ability of the chitosan-capped Mn doped ZnS QDs to penetrate microbial cells and serve as internal fluorescence label, as well as the presence of amines groups that can serve as sites for bioconjugation make these QDs ideal candidates as tags for phylogenetic oligonucleotide probes for FISH. Further research is underway to determine the optimum conditions for the preparation of microorganisms for internal labeling using our fluorescent QDs.

Conclusions

Biocompatible QDs of manganese doped zinc sulfide have been successfully synthesized using a simple co-precipitation method carried out entirely in an aqueous medium. Surface passivation to ensure long time stability and luminosity of the QDs was achieved by capping them with the biopolymer chitosan. With sizes of 3 to 5 nm, the QDs are small enough to pass through the bacterial cell wall of surfactant-permeabilized microorganisms. The colloidal solution of the QDs has a wide optical absorption band starting from 250 nm into the UV region and show strong photoluminescence peak centered around 590 nm, making them suitable for fluorescence labeling. The uptake of the photostable QDs by the microorganisms, which was established by UV–vis spectroscopy and epifluorescence microscopy, suggest that the QDs are ideal alternative to the organic dyes used in FISH, which are highly susceptible to light-induced degradation.

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