Fluorescence Imaging of *E. coli* using CdSe Quantum Dots

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**Abstract:** Rapid and reliable method for the selective detection of pathogenic bacteria is critical in environmental monitoring, disease control, food safety and for the diagnosis of infectious diseases. In this study, we have synthesized hydrophilic fluorescent CdSe Quantum dots (CDSe QDs) and characterized using UV-Vis, IR and fluorescence spectroscopy. Crystallinity of the synthesized Qdots were analysed using XRD. As synthesized CdSe QDs were used for the imaging of buccal epithelial cells and pathogenic bacteria. Selectivity of CdSe QDs to detect *E. coli* was improved by the functionalization of QDs with glycerol. Easy imaging steps and decreased imaging time with the current procedure shows the potential for biomedical imaging.

**Keywords:** Fluorescence imaging, CdSe quantum dots, pathogen detection, *E.coli* sensor

1. **Introduction:**

Infectious diseases due to the lack of sanitation, food contamination and hospital acquired diseases are major problem to the society. The threat of infectious disease is high in undeveloped countries and most of the rural areas in developed countries. Pathogenic bacteria in food industry causes 30,000 hospitalizations and over 1,000 death each year in certain countries[1]. Although many efficient technological progress have been made towards early detection of pathogens [2] assurance of the effective surveillance, treatment and control measures by effective healthcare services should be necessary. Introduction of innovative methods using nanomaterials have made it to move forward with providing fast and reliable results[3]. Nanomaterials exhibit excellent optical, electrical and mechanical properties[4]. One of the most inspiring applications of nanoparticles is imaging using fluorescent semiconducting quantum dots [5]. They have attracted wide attention especially in biomedical imaging due to their interesting optical and electronic properties[6,7]. They could also effectively improve the detection limit, real-time and label free analysis of proteins and enzymes and detection of lethal pathogens [8].

Cadmium selenide (CdSe) semiconducting quantum dots, which is an yellow-orange inorganic solid which belongs to a n-type semiconductor, commonly used for bioimaging [9]. Most of the quantum dots are synthesized in organic solvent at high temperature and results in the formation of hydrophobic quantum dots [10]. Hence it requires the conversion of hydrophobic quantum dot to hydrophilic phase[11]. Many methods are adopted for the phase transfer by modifying the surface of quantum dots by bifunctional surface compounds or with the help of surface active agents which have the ability to form a secondary shell around the hydrophobic particles[12]. These nanoparticles were made hydrophilic with the help of a capping agent like cetyltrimethylammonium bromide (CTAB) [13].

In this work, we have synthesised CdSe quantum dots by wet chemical method and made it hydrophilic by ligand exchange method. The CdSe quantum dots were thoroughly characterised using microscopic, spectroscopic and fluorescence imaging. The water-soluble CdSe quantum dots were used for tagging with bacterial cells and buccal epithelial cells.
2. Experimental

2.1 Chemicals and reagents

Cadmium oxide (CdO) (99.99%) selenium powder (99.99%), triocetyl phosphine (TOP), octadecene, oleic acid (OA) and cetyltrimethylammonium bromide (CTAB) were purchased from Sigma – Aldrich, India. Hexane and chloroform purchased from Finar Chemicals and used without further purification. All other chemicals were analytical grade and used as received. Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae used in this experiment were obtained from Amrita School of Biotechnology, Amritapuri campus. The bacterial strains were subcultured using Luria Bertani broth and incubated overnight at 37 °C.

2.2 Synthesis of CdSe nanoparticles

Synthesis of CdSe was performed as reported in the literature [11]. Briefly, a stock solution of Se precursor was prepared by combining 0.45 g of Se and 75 mL of 1-octadecene in a 100 mL beaker and to that 6 mL of trioctylphosphine was added and stirred on a hot plate at 450 rpm until complete dissolution takes place [14]. The stock solution was kept at room temperature in a sealed container. Cadmium precursor was made by adding 0.95 g of cadmium oxide to a 250 mL round bottom flask. To the same 9 mL oleic acid and 150 mL 1-octadecene were added. A thermometer was also inserted to record the temperature. The mixture was heated to 180 °C and 15 mL selenium precursor was added[14][7]. The temperature was maintained at 180 °C and samples were drawn using a micropipette at different time interval between 0 to 9 minutes to optimise the particle size [15].

2.3 Hydrophilization of CdSe quantum dots

The solution contains CdSe quantum dots was washed twice with acetone and centrifuged at 8000 rpm for 30 minute. The pellet was then redispersed in hexane. Water soluble QDs were obtained by dispersing 5 mL of CdSe QD solution in 5 mL chloroform and this was added to 10 mL 1M CTAB. Obtained solution was centrifuged at 8000 rpm for 30 minutes. The precipitate formed after the centrifugation was redispersed in water [16].

2.4 Characterization of Hydrophilised CdSe quantum dots

Spectroscopic analyses of the CdSe were carried out using UV-Vis spectrophotometer (Pharmaspec 1700, Shimadzu), Bio spectrometer (Eppendorf, Germany) and fluorescence spectrofluorophotometer (Shimadzu RF-6000). Chemical characterization was performed using FTIR spectrophotometer and X-Ray diffraction with Rigaku Miniflex 600 model.

2.5 Mammalian buccal epithelial and bacterial imaging by CdSe quantum dots (Qdots)

Saliva sample was collected in a sterile container followed by diluting it in 200 μL of 10 mM PBS buffer. The minimum required concentration of CdSe QDs to tag with Mammalian buccal epithelium cells was optimized by varying the Qdots added. Effect of pH and incubation time for the tagging of the cells were studied.

For bacterial tagging and imaging pure culture of bacterial strains such as E. coli, Staphylococcus aureus, Klebsiella pneumoniae were grown in LB media. 1 mL of each bacterial culture was centrifuged at 7000 rpm for 8 minutes and the pellet was redispersed in 1 mL PBS buffer. This was then mixed with CdSe quantum dots. 10 μL of the sample was then drop casted on a well cleaned glass slide and kept for drying at room temperature. Tagging efficiency of bacterial cell with CdSe QDs was tested by imaging with fluorescence microscope. Various parameters were optimized for the
bacterial cell tagging with CdSe quantum dots such as specific dilution, volume variation, incubation time and pH of the testing medium.

2.6 Functionalisation of CdSe quantum dots

CdSe quantum dots were functionalised using various reagents such as cysteamine, glutaraldehyde and glycerol to enhance specificity. 1 mL of cysteamine (0.1 M), glutaraldehyde (0.1 M) and glycerol (0.5M) were separately mixed with 1 mL of CdSe QDs and stirred at 600 rpm for 1 hour. The solution was centrifuged to remove the excess reagents. The modified CdSe QDs were incubated with bacterial cell and observed the tagging using fluorescence microscope.

3. Results and discussion

3.1 Characterization of CdSe Nanoparticle

The CdSe QDs obtained at different time intervals were shown in Figure 1A. The intensity of yellow colour increases with time. The photoluminescence spectra of the CdSe QDs withdrawn from the reaction mixture at different time intervals was taken. In all the cases, the fluorescence spectra showed well-resolved emission peak, signifying a sufficiently narrow size distribution of the QDs. It was found that the emission peak undergoes a red shift with time. The synthesized CdSe Qdots showed fluorescence emission at 509 nm [16] (Figure 1B).

![Figure 1: CdSe Qdots with different reaction times (A) and photoluminescence spectrum of CdSe QDs (B)](image-url)
3.2 Characterization of hydrophilised CdSe quantum dots

The absorption spectrum of hydrophilised quantum dots show a peak at 525 nm characteristic of CdSe quantum dots (Figure 2A). After excitation at this wavelength the colloid showed intense fluorescence at 541 nm [16] (Figure 2B).

![Figure 2: (A) UV-Vis spectra and (B) fluorescence spectra of hydrophilic CdSe Qdots](image)

FT-IR spectroscopy was carried out to identify the functional groups present on CdSe quantum dots (Figure 3) The peak near 3500-3400 cm\(^{-1}\) represents the presence of O-H functional groups present on the CdSe nanoparticle. C=O stretching vibrations of COOH groups are present near 1650 cm\(^{-1}\) and C-H stretching is observed near 2900 cm\(^{-1}\). The band between 1100 cm\(^{-1}\) represents the presence of C-O bending vibrations. Presence of the O-H, COOH functional groups increases the water solubility of CdSe nanoparticle [17].

![Figure 3. FT-IR spectra of hydrophilized CdSe QDs](image)
The X-ray diffraction patterns obtained for the CdSe quantum dots is presented in Figure 4. The diffraction patterns obtained were compared with JCPDS data for CdSe and assigned different planes for the peaks[18]. XRD pattern of the CdSe sample exhibits three broad peaks, which are at 2θ values 23.55°, 43°, 55.11°. By comparing the interplanar spacing values and 2θ different planes were assigned for these diffraction patterns are with miller indices of (111), (220) and (311) of CdSe nanostructures [19]. The broad and low intensity diffraction peaks indicate that the particles are of nanocrystalline.

![XRD spectrum of CdSe QDs](image)

Figure 4. XRD spectrum of CdSe QDs

3.3 Fluorescence imaging of mammalian buccal epithelial cells

![Fluorescence image of buccal epithelial cells](image)

Figure 5. (A) Bright field image and (B) fluorescence microscopic images of buccal epithelial cells tagged with CdSe quantum dots under 40x magnification.

Fluorescence image of buccal epithelial cells was performed using the synthesized hydrophilic CdSe quantum dots [20] and is depicted in Figure 5B and its bright field image is shown in Figure 5A. Images shows that the QDs selectively tags the epithelial cells and the fluorescence image of the
cells obtained [21]. In order to know the optimum amount of CdSe required for tagging the cells several experiments were performed and it is found that for 2 μL of epithelial cells 13 μL of the synthesized QDs are required. Experiments on the time of incubation lead to the conclusion that 20 minutes of incubation is required for efficient tagging to obtain the images.

3.4 Optimization of pH for fluorescent imaging

In order to obtain the optimum pH for QD tagging with bacterial cells, studies were carried out at different pH ranging from 1-10 [22]. Fluorescence studies show that the tagging was effective at pH 7. No tagging was observed at other pH conditions. Thus, the pH for the further investigations were fixed as 7. Figure 6 shows the representative images at pH 1, 5 and 7[14].

![Figure 6. Fluorescence images of the tagged bacterial cells at pH 1, 5 and 7](image)

3.5 Fluorescence imaging of bacterial cells

Three pathogenic bacteria *E. coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae* were selected in this work for fluorescence imaging[22]. Figure 7 shows the bright field image and the fluorescence imaged of CdSe tagged bacterial cells. It can be seen that the CdSe QDs non-specifically binds with the bacterial cells and give fluorescent images. It is reported that small sized water-soluble CdSe QDs could interact with the amine or carboxylic groups present on the bacterial surface with the carboxylic or amine groups present on the CdSe QDs [23]. After a series of experiments it is found that 25 μL of the as-prepared quantum dots were required for the effective tagging and imaging of bacterial cells. Different time of incubation was studied and 20 minutes of incubation was found to be optimum [23].
3.6 Functionalized CdSe Quantum dots for selective bacteria imaging

Eventhough the quantum dots were efficient in tagging with different bacterial cells and in imaging them. But the QDs were non specific. In order to improve the selectivity\[24\] of the quantum dots CdSe QDs were functiolized with cysteamine, gluteraldehyde and glycerol. The tagging efficiency of the functionalized particles were tested by imaging bacterial cells. Results obtained was consolidated in Table 1.
Table 1: Efficiency of functionalized CdSe for bacteria tagging

| Bacteria               | CdSe functionalized with |
|------------------------|--------------------------|
|                        | Cysteamine   | Glutaraldehyde | Glycerol |
| E. coli                | -            | -              | ✓        |
| Staphylococcus aureus  | -            | -              | *        |
| Klebsiella pneumonia   | -            | -              | -        |

✓ Tagging * Poor Tagging - Not tagging

It is understood from Table 1 that the glycerol modified quantum dots selectively binds with *E. coli* which is an important pathogen to be detected for food and water borne diseases. Figure 8 shows that the efficiency of glycerol functionalized CdSe Qdots for tagging *E. Coli*.

Figure 8. Fluorescent images of glycerol functionalized CdSe Qdots tagged, *E. coli* cells (A, B) and *Staphylococcus aureus* (C, D)
4. Conclusion

Hydrophilic fluorescent CdSe quantum dots capped with cetyltrimethylammonium bromide was synthesized by wet chemical method. The hydrophilic CdSe quantum dots show absorption at 525 nm and a strong fluorescence emission at 541 nm. The quantum dots were found to be efficient for fluorescence imaging of buccal epithelial cells and pathogenic bacteria. The selectivity of the Qdots towards E. coli was improvised by the functionalization of glycerol on CdSe quantum dots. Easy tagging procedure and less time for cell imaging using the CdSe and functionalized CdSe shows future applications in biomedical and environmental imaging.

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