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Synthesis of mercaptopropionic acid stabilized CDS quantum dots for bioimaging in breast cancer

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ABSTRACT

Semiconductor inorganic nanocrystals or quantum dots (QDs) are nowadays extensively used for imaging and analysis of bio-molecules owing to their superior optical properties over conventional organic fluorophores. They have excellent potential for synthesizing molecular probes against various biological markers such as free antigens, cell surface markers/antigens, bacteria, viruses and tissues. Traditional synthesis protocols of the QDs generally lead to the formation of hydrophobic nanocrystals. For biological applications, post-synthesis modifications need to be introduced to render required hydrophilicity. However, such additional steps make the tiny QDs structures bulky, which is unwanted in subsequent in-vivo executions. The present work reports a simple method for the direct synthesis of hydrophilic carboxyl (–COOH) functionalized CdS QDs using mercaptopropionic acid as a sulfur source and stabilizer. This aqueous synthesis route avoids the requirement of extra surface modification steps. The size and surface morphology of the synthesized CdS QDs were studied by electron microscopy. The average diameter of the QDs has been found to be in the range of 2-3.5nm. Spectral studies confirmed the grafting of –COOH terminal on the synthesized nanocrystals. Band gap energy and the theoretical size of the particles were calculated and found in good agreement with the experimental analysis. Due to the size quantization effect, the estimated band gap energy (2.6eV) of the QDs was on a higher side than that reported (2.4eV) for the bulk material. The synthesized nanocrystals can be further conjugated with bio-molecules for high-throughput drug screening, clinical immunological assays and protein-protein interaction studies.

Keywords: CdS; quantum dots; mercaptopropionic acid; band gap energy; breast cancer.

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Introduction

Due to their unique physical, chemical, electrical and optical properties, the nanoparticles have attracted great research interest in the recent years **[1]**. The nanoparticles possess large surface area to volume ratio which can be used to transport/immobilize biological or chemical structures for various applications. Semiconducting nanocrystals or 'quantum dots' (QDs) offer superior optical properties over conventional organic fluorophores. QDs exhibit unique properties due to the quantum size effects arising from the confinement of electrons, holes and phonons in them. The confinement phenomenon leads to dramatic changes in the materials' optical and conductive properties **[2].** Quantum confinement of the charge carriers is correlated with the blue shift in that particular material's band gap energy and can be observed in the absorption spectra **[3].**QDs are usually comprised of group II-VI,III-V elements which make an important class of materials with wide range of applications**[4].** The semiconducting nanocrystals of CdS are one of the more prominently studied and reported QDs for various applications. In bulk form, the CdS crystalisa hexagonal wurtzite -type structure with band gap energy 'Eg' of 2.42 eV **[5].**

In absence of any surface passivation, most of the nanoparticle systems undergo aggregation leading to an impact on the optical properties. Thus, it is of paramount significance to develop methods for the surface passivation of nanoparticles. Organic stabilizers have been the most frequently reported capping agent for the above purpose **[6].** Apart from maintaining the desired size distribution, the capping with stabilizers provides specific functional groups that may be exploited for various biological labeling **[7].**

The synthesis of CdS nanoparticles has been reported by direct reaction of metals with sulfur powders under high temperature**[8]**, thermal decomposition of molecular precursors containing M–S bonds **[9],**through the application of H_2S as the sulfur source at higher temperature **[10]** and precipitation of metal ions with Na_2S **[11,12]**. The above synthesis routes involve complex process control; use of harmful reagents and long synthesis time **[13].** Despite of several available synthesis routes, the reproducible preparation of the CdS QDs with good mechanical and optical properties still remains a fascinating area of research. In this paper, we report simple one pot colloidal synthesis route for the CdS QDs with reproducible structural and optical properties. Mercapto propionic acid (MPA) has been used as sulfur source and it has been possible to directly achieve the water soluble carboxyl functionalized CdS QDs, which may further find applications in disease diagnosis.

Experimental

Apparatus

UV-visible spectroscopy (Varian Carry 5000 spectrophotometer) was performed in dual beam mode keeping the scan rate to 600nm/min. Photoluminescence (PL) spectroscopy was carried out on the Varian Carry eclipse fluorescence spectrophotometer. The absorption and PL spectra were taken in quartz cuvettes of 1cm path length under ambient conditions. Transmission electron microscopy (TEM) investigations were performed on Hitachi H-7100 microscope. Fourier transform infrared (FTIR) spectroscopy was carried out using thermo Nicolet FTIR. XRD studies were investigated on Miniflex-II, Rigaku, Japan.

Materials

$CdCl_2.5H_2O$ and mercapto propionic acid (MPA) were purchased from Sigma Aldrich. All other chemicals used were also of analytical grade from Sigma Aldrich / Merck. Deionized water with resistivity of 18MΩ cm^{-1}(ELGA, USA) was used for the preparation of aqueous solutions and buffers.

Methodology

For the synthesis of CdS QDs, 100 mL of 0.02 M $CdCl_2{\cdot}2.5H_2O$ was first taken in a 250 mL round bottom flask. 1 mL MPA was introduced under mild stirring condition. The pH of the solution was adjusted to 10 using 1.0 M NaOH. Thereafter, 300µL of 30% H_2O_2 was added drop-wise in the solution. The reaction mixture was refluxed at 100^oC. First sample was collected just at the start of refluxing. Further aliquots were pipetted out at regular time intervals to investigate the effect of reaction time on the size of the formed CdS QDs. The formed nanocrystals were centrifuged and washed thoroughly with ethanol to remove unreacted ions. These nanocrystals were then resuspended in deionised water. The powder sample for TEM and XRD investigations was prepared by drying a small volume of the CdS QDs under vacuum.

Results and discussion

Watersoluble CdS QDs have been synthesized using MPA as a stabilizer. Nanocrystals of the different sizes have been obtained during the course of the reaction in **Fig. 1**. The particles collected at the different times exhibited absorbance peaks at 360, 380, 395, 420 nm in **Fig. 2** with a respective PL at 540, 565, 595, and 620 nm in **Fig. 3a**. The size of the particles increases with increasing reaction time due to the progressive nucleation, which is spectroscopically underlined by red shifts in the wavelengths of maximum absorbance.

Band gap energy and nanocrystal size computations

Band gap energy is an important basic property of semiconductor compounds. Materials exhibiting band gaps in the range of 0.3- 3.8 eV are considered as semiconductors. For this, the material under examination is scanned through a range of wavelengths and the occurred optical excitations of electrons across the band gap are monitored. Band gap energy is correlated by the emergence of strong absorbance peak in the form of λ_{max}. Bulk CdS has a band gap value of 2.4 eV. We have calculated the band gap energy of herein synthesized CdS QDs to determine the size quantization effect with respect to the reaction time. Band gap energy of respective nanocrystals was calculated on the basis of absorbance readings.

Absorption factor 'f(λ_{ex}) or α'is used in estimating the fraction of the excitation light absorbed by the nanocrystal according to the equation 1; wherein 'A(λ_{ex})' denotes the observed absorbance data, 'c' is the concentration, 'l' is the optical path length and 'T(λ_{ex})'is the observed transmittance.

$$f(\lambda_{ex}) = 1 - 10^{-A(\lambda_{ex})} = 1 - 10^{-\varepsilon(\lambda_{ex})cl} = 1 - T(\lambda_{ex}) \quad (1)$$

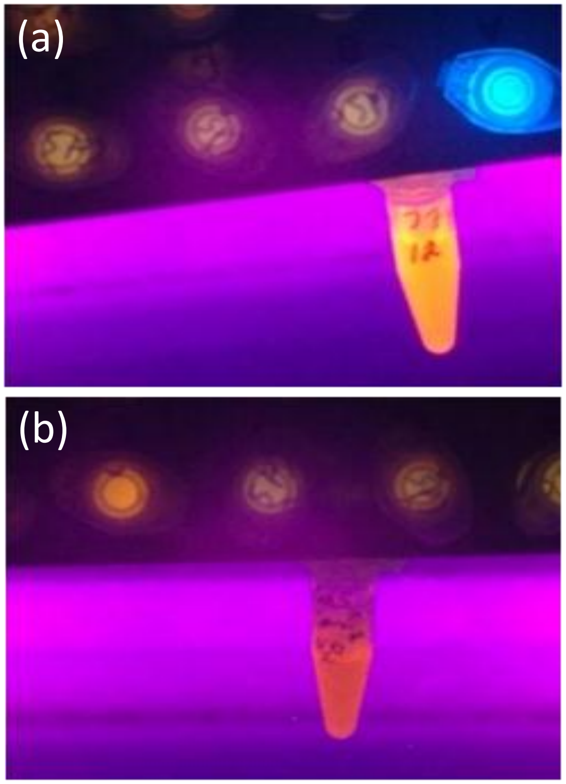

Fig. 1. Fluorescent images of (a-b) CdS nanocrystals observed under ultraviolet illuminator.

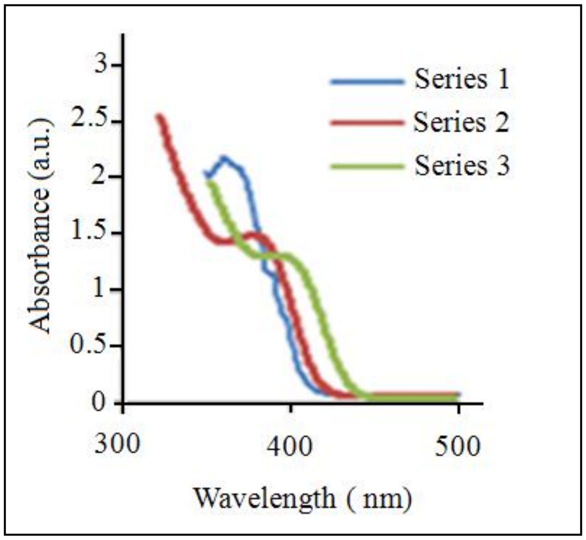

Fig. 2. UV-Visible spectra of different samples of synthesized QDs

The obtained values of 'α' were used to draw a plot between 'hν' and'$\frac{1}{\sqrt{h\upsilon\alpha}}$'. The band gap energy was then estimated by determining the energy at which the extrapolation of the baseline and absorption edge intersects each other. The observations revealed that the band gap energy of the different CdS QDs samples as 2.91, 2.83, 2.73 and 2.68 eV for λ_{max} 360, 380, 395 and 420 nm, respectively.

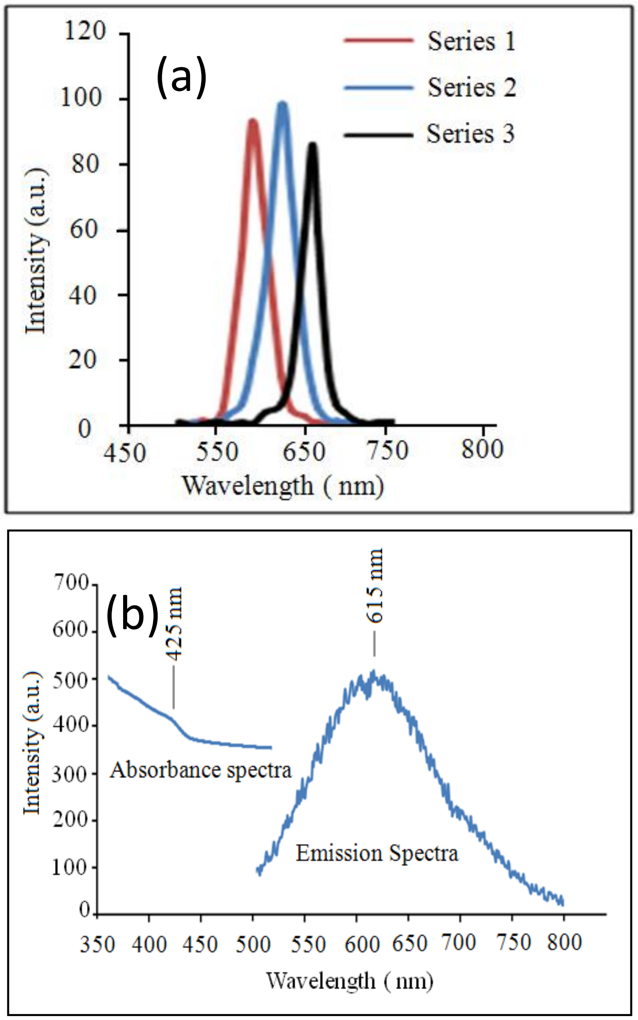

Fig. 3. (a) PL spectra of different samples of synthesized QDs, (b) Optical characteristic of QDs with excitation wavelength of 425 nm and emission wavelength of 615 nm.

The above band gap energy values have been further used in calculating the particle radius as given by equation (2)

$$E_g(QD) = E_{g,0} + \frac{\hbar^2\pi^2}{2m_{eh}R^2} \quad (2)$$

where 'E_g' is the above estimated band gap value of the CdS QDs and'$E_{g,0}$' is the band gap value of the bulk material. The term 'm_{eh}' can be estimated by solving the equation (3) taking in account the values of m_e (effective mass of electrons) and m_h(effective masses of holes)

$$m_{eh} = \frac{m_e m_h}{(m_e+m_h)} \quad (3)$$

The calculations have revealed the theoretical particle size of the synthesized CdS QDs to be 2.1, 2.3, 2.6 and 3.1nm for the 'E_g' values of 2.91, 2.83, 2.73 and 2.68 eV, respectively.

Photoluminescence studies

The samples excited by 360, 380 and 395 nm wavelengths showed emission peaks at 540, 565 and 595 nm, respectively in **Fig. 3a**. Likewise in the absorbance studies, the increasing size of the CdS QDs caused the red shift in emission peaks. Overall, the synthesized CdS QDs showed desired emission intensity and it has been possible to tune their emission wavelength. **Fig. 3b** elaborates the absorption and emission spectra of one particular sample of the CdS QDs. Typical properties of broad absorption and sharp emission are fairly evidenced.

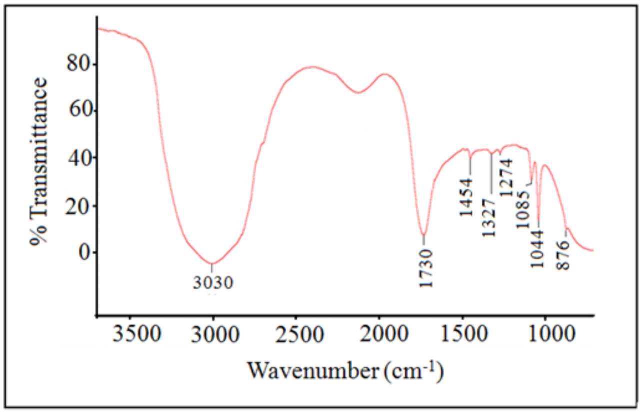

Fig. 4. FTIR spectra of QDs.

Functional group analysis (FTIR studies)

FTIR studies **Fig. 4** confirmed the capping of the CdS QDs by functional -COOH. The observed peak at 3030 cm^{-1} accounts for the -OH stretching of -COOH group and the C-H stretching of alkyl groups. The peak at 1730 cm^{-1} shows the stretching of carbonyl group. The S-C vibration is highlighted by the appearance of a peak at relatively lower wave number (876 cm^{-1}). C-O stretching is indicated by the occurrence of bands ranging from 1300 to 1000 cm^{-1}. Another peak at 1340 cm^{-1} suggests the involved C-H bending.

TEM and XRD studies

The results of TEM and XRD investigations are given in **Fig. 5a** and **5b**. The microscopic data reveals the formation of uniformly distributed spherical nanocrystals with average size of 3±0.5nm. The experimentally derived particle size is in good agreement with the theoretically calculations. XRD data further reveals that the CdS QDs encompasses within a zinc blend crystal structure. Three major peaks were observed at 26.6^0 (111), 44^0 (220) and 52.8^0(311) corresponding to the cubic phase of the CdS (JCPDS No. 10-454). Appearance of several weak peaks indicates the co-existence of some hexagonal CdS crystal. This may be attributed to small differences in the free energies of the cubic and hexagonal phases.

Conclusion

A simple aqueous synthesis route has been proposed for the synthesis of carboxyl functionalized hydrophilic CdS QDs. The product has good and tunable emission properties. The formed CdS nanocrystals prominently bear the zinc blende structure with their particle size lying well below the Bohr excitation radii. Hydrophilic nature and the availability of functional groups on the CdS QDs makes them potential labeling agent for the disease diagnosis. Efforts are on to synthesize the bio-nanoconjugates of CdS QDs with Estrogen receptor antibodies for the diagnosis of breast cancer.

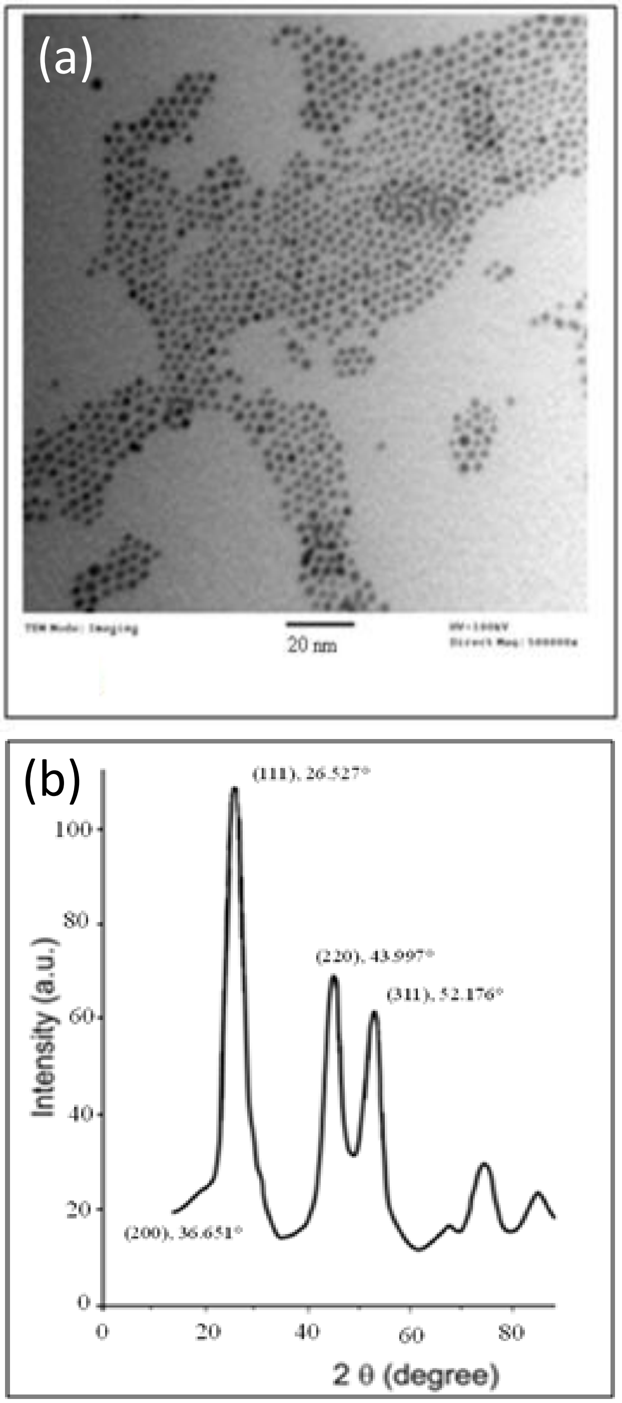

Fig. 5. (a) TEM micrograph and (b) XRD spectra of QDs.

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