DEVELOPMENT, CHARACTERIZATION & PROCESSING OF QUANTUM DOTS FOR IMAGING IN UV-VISIBLE RANGE

Project report submitted in fulfillment for the requirement of degree of

Bachelor of Technology
Department of Biotechnology/Bioinformatics

By

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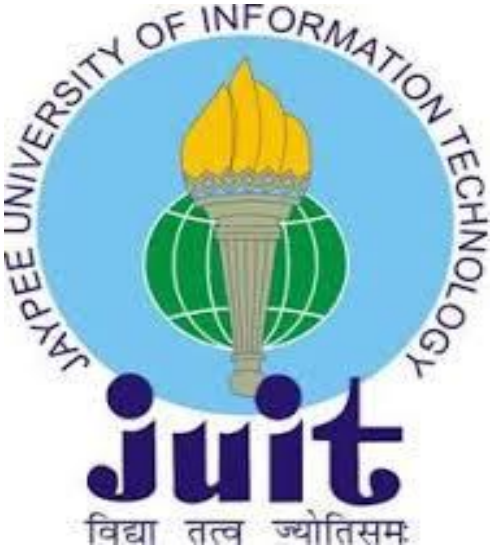

Department of Biotechnology/Bioinformatics
JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY
SOLAN, 173234, H.P., INDIA
MAY, 2016

DECLARATION

We hereby declare that the work reported in the B-Tech thesis entitled **"Development, Characterization & Processing of Quantum Dots for Imaging in UV-Visible range"** submitted at **Jaypee University of Information Technology, Waknaghat India,** is an authentic record of our work carried out under the supervision of **Dr**. **Ragini Raj Singh**. We have not submitted this work elsewhere for any other degree or diploma.

Archita Sharma **Ayushi Arora**

Date:

Department of Biotechnology/ Bioinformatics
Jaypee University of Information Technology, Waknaghat, Solan, India

CERTIFICATE

This is to certify that the thesis entitled **"Development, Characterization & Processing of Quantum Dots for Imaging in UV-Visible range"** submitted by **Ms. Archita Sharma** and **Ms. Ayushi Arora** at **Jaypee University of Information Technology, India** is a bonafide record of her original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

Dr. Ragini Raj Singh **Date:**
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Archita Sharma

Ayushi Arora

ABSTRACT

This thesis work preferentially concerned with the synthesis of quantum dots (QDs) by solution growth method at room temperature and their characterization. Excellent structural and optical and surface properties have been attained like high luminescence and stable structure. Prepared quantum dots structures and core/shell quantum dots structure are cadmium selenide (CdSe), Zinc sulfide (ZnS) and Cadmium selenide/ Zinc sulfide (CdSe/ZnS). The work has been systematically described in five different chapters as follows:

Chapter I - Contains a brief introduction of quantum dots, core/shell quantum dots and their applications especially in the field of biology.

Chapter II - Describes the synthesis of quantum dots, core/shell structures and encapsulated quantum dots and the experimental techniques used for the characterization of prepared materials (QDs) for structural, optical and surface properties.

Chapter III - Shows the development and analysis of CdSe, ZnS and CdSe/ZnS core/shell quantum dots (QDs), to study their structural properties, optical properties and surface nature. All the quantum dots have been prepared by wet chemical method using 2-mercaptoethanol as a stabilizer. We have employed UV-Vis Spectroscopy (Abs), photoluminescence spectroscopy (PL), Transmission Electron Microscopy (TEM), X-Ray Diffraction (XRD), Energy Dispersive X-Ray Diffraction spectroscopy (EDX), Fourier Transform Infrared Microscopy (FTIR), Fluorescence microscopy techniques to characterize ZnS, CdSe and CdSe/ZnS core/shell quantum dots (QDs).

Chapter IV- Deals with the biocompatibility and toxicity of polymer encapsulated quantum dots and cytotoxicity testing for in vivo purposes specifically.

Finally the overall conclusion of work is described in **chapter V**.

CONTENTS

LIST OF ABBREVATIONS

Abs	:	Absorbance
CdTe	:	Cadmium telluride
CdSe	:	Cadmium selenide
CdS	:	Cadmium sulfide
DOS	:	Density of states
EDX	:	Energy Dispersive X-Ray Spectroscopy
FM	:	Fluorescence Microscopy
FTIR	:	Fourier Transform Infrared Microscopy
MDCK	:	Madin-Darby Canine Kidney Epithelial Cells
MPE	:	2- mercaptoethanol
MTT	:	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PL	:	Photoluminescence
QDs	:	Quantum dots
TEM	:	Transmission Electron Microscopy
XRD	:	X-Ray Diffraction
ZnS	:	Zinc Sulfide
ZnSe	:	Zinc Selenide
ZnTe	:	Zinc Telluride
ZnO	:	Zinc oxide

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CHAPTER 1

INTRODUCTION

QUANTUM DOTS IN BIOLOGY:

Nano structured materials are of interest because they can bridge the gap between the bulk and molecular levels and leads to entirely new avenues for applications, especially in electronics, optoelectronics and biology (Salvatore A., Bruce P et al 2005). During the last two decades, a great deal of attention has been focused on the optoelectronic properties of nano structured semiconductors or quantum dots as many fundamental properties are size dependent in the nanometer range. A QDs is zero dimensional relative to the bulk, and the limited number of electrons results in discrete quantized energies in the density of states (DOS) for non aggregated zero dimensional structures.

The first discovery of QDs were by **Alexey Ekimov in 1981** in a glass matrix and then in colloidal solutions by **Louis E. Brus in 1985**. The term "quantum dot" was coined by **Mark Reed.**

Quantum confinement of both the electron and hole in all three dimensions will lead to an increase in the effective band gap of the material with decreasing crystallite size.
Semiconductor nano crystallites (QDs) having smaller radii than the bulk exciton Bohr radius constitute a class of materials which is an intermediate between molecular and bulk forms of matter. Consequently, both the optical absorption and emission of quantum dots shift to the blue (higher energies) as the size of the dots gets smaller. Although nano crystallites have not yet completed their evolution into bulk solids, structural studies indicate that they retain the bulk crystal structure and lattice parameter. Recent advances in the synthesis of nano crystallites have paved the way for numerous spectroscopic studies assigning the quantum dot electronic states and mapping out their evolution as a function of size.

Core-shell quantum dots exhibit novel properties making them attractive from both an experimental and a practical point of view. Overcoating of nano crystallites with higher band gap inorganic materials have been shown to improve the photoluminescence quantum yields

by passivating surface non-radiative recombination sites. Particles passivated with inorganic shell structures are more robust than organically passivated dots and have greater tolerance to processing conditions necessary for incorporation into solid state structures. Some examples of core-shell quantum dot structures include CdS on CdSe and CdSe on CdS, ZnS grown on CdS, ZnS on CdSe and the inverse structure.

The most unique property of the QDs is quantum confinement, which modifies the density of states (DOS) near the band edges. QDs lie between the discrete atomic and continuous bulk materials. Quantum confinement effects are observed when the size is sufficiently small.

Fig. 1: The wavelength of light emitted by quantum dots is tunable by changing the particle size. In this image, all of the quantum dot samples are excited by the same wavelength, but emit different visible wavelengths depending on particle size.
(Joshua James Angell 2011)

Among many properties that exhibit a dependence upon size in QDs, two are of particular importance. The first is a blue shift (increase) of band-gap energy when the nanoparticle diameters are below a particular value that depends on the type of semiconductor. This is called a quantum confinement effect. The second important property is

the observation of discrete, well separated energy states due to the small number of atoms in QDs compared to the bulk. This leads to the electronic states of each energy level exhibiting wave functions that are more atomic-like. Since the QDs solutions for Schrödinger wave equation are very similar to those for electrons bound to a nucleus, QDs are called artificial atom, and atomic-like sharp emission peaks are possible.

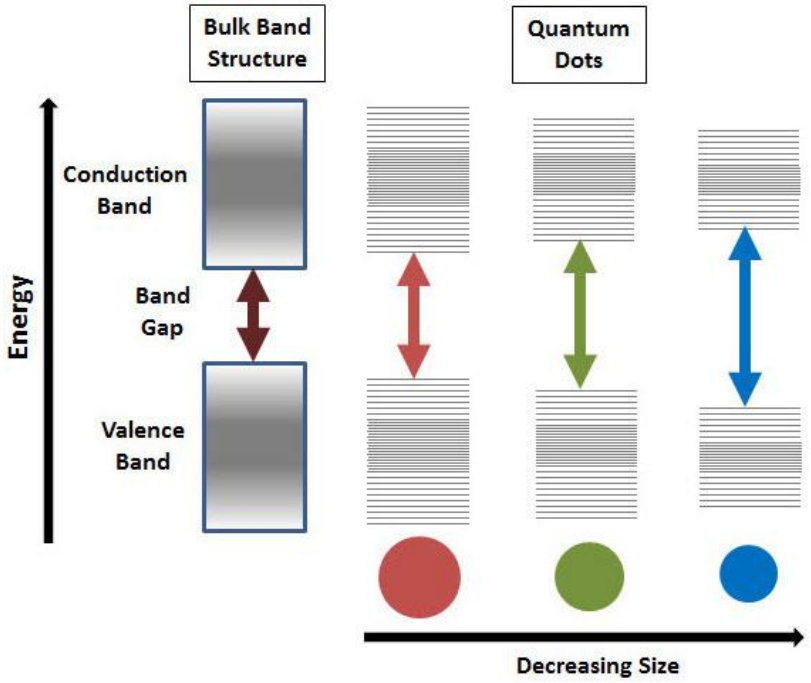


Fig.2: Energy band gap presentation from bulk to quantum dots.

(Jonathan Melville 2015)

Group II- IV Quantum Dots:

- **Cadmium selenide (CdSe)**
- Cadmium sulfide (CdS)
- Cadmium telluride (CdTe)
- Zinc oxide (ZnO)
- Zinc selenide (ZnSe)
- **Zinc sulfide (ZnS)**

Semiconductor Nanoparticles

Group 14 (old group IV) Si, Ge
III-V Materials: GaN, GaP, GaAs, InP, InAs
II-VI Materials: ZnO, ZnS, ZnSe, CdS, CdSe, CdTe

STRUCTURES OF QUANTUM DOTS:

a) **Cadmium selenide (CdSe):** It is a solid, binary compound of cadmium and selenium. CdSe is a semiconducting material, but has yet to find many applications in manufacturing.

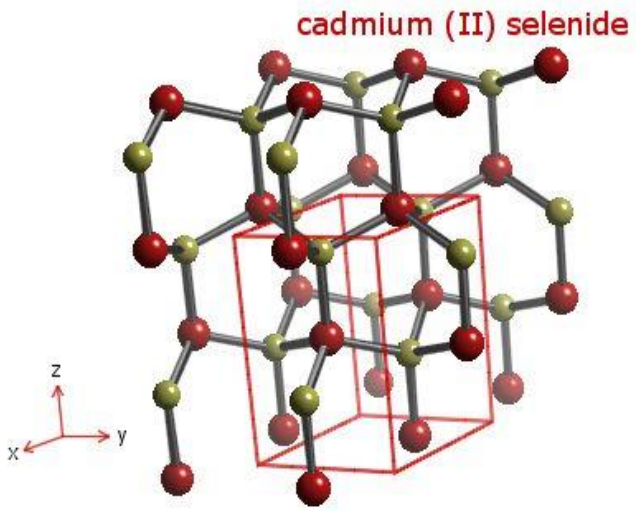


Fig.3: Crystal structure of cadmium Selenide.

b) **Zinc sulfide (ZnS):** It is a white coloured powder or crystal which is typically encountered in the more stable cubic form and known also as zinc blende or

sphalerite. The hexagonal form is also known as a synthetic material and as mineral wurtzite.

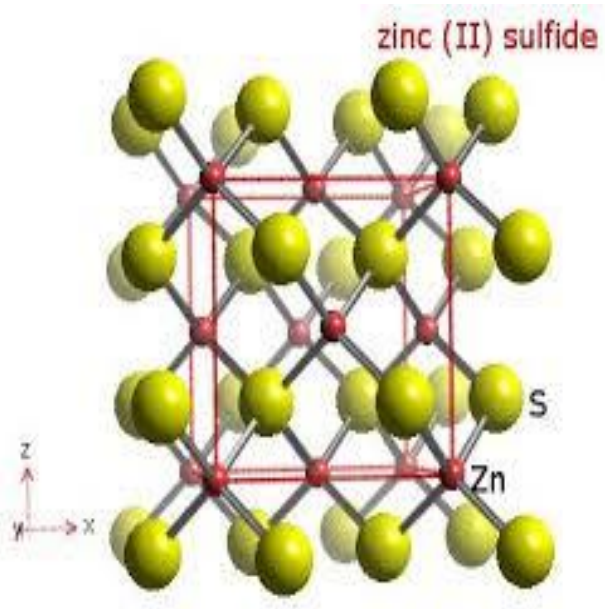
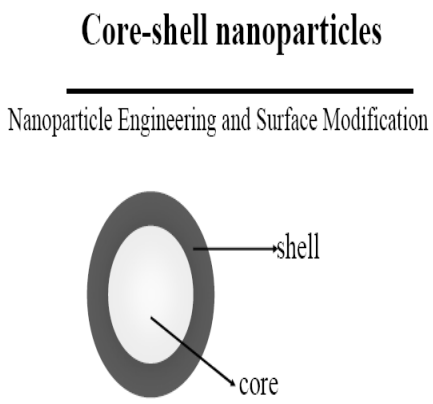


Fig.4: Crystal structure of Zinc sulphite.

CdSe/ZnS core/shell:



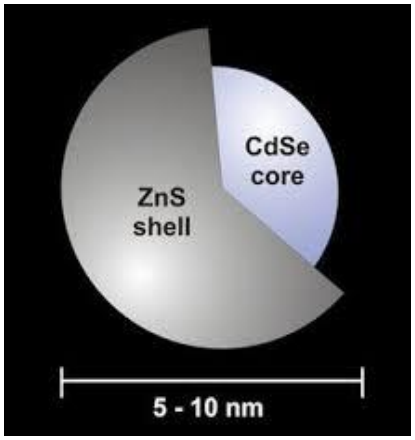


Fig.5: CdSe/ZnS Core/shell Structure

WHY CORE SHELL?

1. Chemical and colloidal stability:

-Nanoparticle degradation through chemical etching.

-Agglomeration caused by strong van der Waals attractive forces.

2. Tuning of physical properties:

-For example, the optical properties of metal nanoparticles are influenced by their environments.

-Controlled surface modification can alter these properties.

3. Control of interparticle interaction within assemblies:

-Collective properties of nanoparticle assemblies are influenced to a large extent by the separation between the particles.

-Coating the particles with a uniform shell of inert material could control the distance between the particles.

APPLICATIONS:

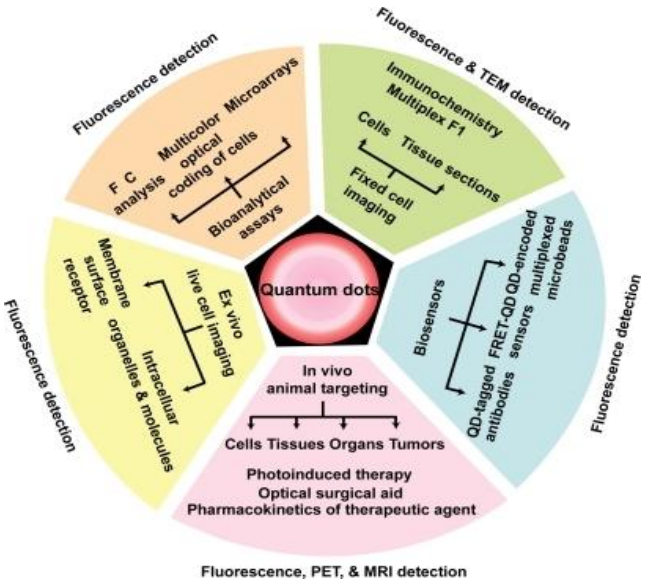


Fig 6: Application of quantum dots in various fields.

(N O' Farrell, A Houlton et all 2006)

Quantum dots are particularly significant for optical applications due to their high extinction coefficient. High-quality quantum dots are well suited for optical encoding and multiplexing applications due to their broad excitation profiles and narrow emission spectra (Bera D, Qian L et al 2010). The new generations of quantum dots have far-reaching potential for the study of intracellular processes at the single-molecule level, high-resolution cellular imaging, long-term in vivo observation of cell trafficking, tumor targeting, and diagnostics.

Cell labelling:

One of the broadest uses of fluorescent probes in biology is the labeling of cellular structures. Multicolor labeling of cells is a powerful technique for visualizing many of these structures simultaneously, such as cytoskeletal proteins or organelles, and to elucidate intracellular processes. QDs are excellent fluorescent probes for long-term multicolor cell labelling and due to their broad absorption profiles; they can be efficiently excited at any wavelength smaller than their initial band edge absorption.

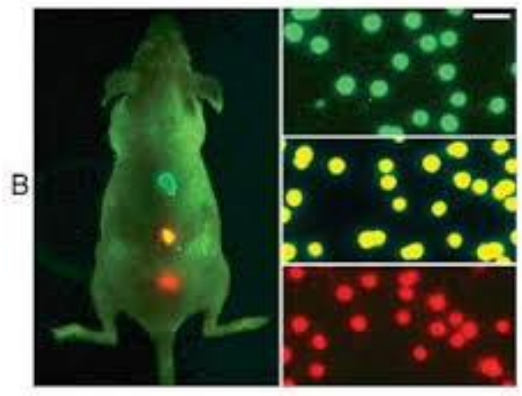


Fig 7: Labelling of cells in mice.
(Maksym W Yezhelyev 2006)

In-Vivo imaging:

The ability to visualize native processes occurring in living organisms is invaluable for clinical diagnostic applications, yet remains elusive in practice due to conventional imaging limitations and the availability of suitable fluorescence markers. Regarding the latter, many

organic dyes have very short lifetimes (~ 1 n), are susceptible to photodegradation, and show inadequate fluorescence brightness .Additionally, tissue auto fluorescence can exhibit similar spectroscopic characteristics making it difficult to resolve the desired signal from unwanted background. Due to their unique photo physical properties, QDs are promising fluorophores for *in* vivo fluorescence imaging and can overcome many of the usual limitations of dyes (Kim, Pudavar S, Bonoiu H et al 2007).

Diagnostics:

One medical area where QDs may have significant impact is in diagnostics and clinical assays. The unique properties of QDs have been investigated almost exclusively for two techniques that require the use of diagnostic fluorophores: immunolabeling and nucleic acid detection.

POLYMER ENCAPSULATION:

It is a process in which tiny particles or droplets are surrounded by a coating to give small capsules, of many useful properties. It can also be used to enclose solids, liquids, or gases inside a micrometric wall made of hard or soft soluble film, in order to reduce dosing frequency and prevent the degradation of pharmaceuticals. The coating materials generally used for coating are ethyl cellulose, polyvinyl alcohol, gelatin, sodium alginate etc.

CYTOTOXICITY TESTING:

Interest in taking advantage of the unique spectral properties of semiconductor quantum dots (QDs) has driven their widespread use in biological applications such as in vitro cellular labeling/imaging and sensing. Despite their demonstrated utility, concerns over the potential toxic effects of QD core materials on cellular proliferation and homeostasis have persisted, leaving in question the suitability of QDs as alternatives for more traditional fluorescent materials (e.g. organic dyes, fluorescent proteins) for in vitro cellular applications.

MOTIVATION:

Quantum dots are particularly significant for optical applications due to their high extinction coefficient. High-quality quantum dots are well suited for optical encoding and multiplexing applications due to their broad excitation profiles and narrow/symmetric emission spectra. The new generations of quantum dots have far-reaching potential for the study of intracellular processes at the single-molecule level, high-resolution cellular imaging, long-term in vivo observation of cell trafficking, tumor targeting, and diagnostics.

The use of luminescent colloidal quantum dots in biological investigations has increased dramatically over the past several years due to their unique size-dependent optical properties and recent advances in bio-functionalization.

OBJECTIVES:

1. To synthesize II-VI group (CdSe, ZnS) quantum dots and their core/shell structures (CdSe/ZnS).
2. Characterization of QDs and core/shell QDs including optical, structural, surface chemical and morphological studies using various techniques.
3. To develop methodology to make as prepared quantum dots hydrophilic, non-toxic, and bio-conjugatable via encapsulation with different polymers (PEG, PAA)
4. To test the biocompatibility and cytotoxicity of core/shell QDs along with their photoluminescence studies to make them available for biomedical applications.

CHAPTER 2

EXPERIMENTAL TECHNIQUES

SYNTHESIS PROCEDURE:

a) Cadmium Selenide (CdSe):

- Take 50 ml distilled water in a beaker.
- Then add 3M cadmium chloride ($CdCl_2$) into the beaker.
- Followed by this add 1.37 M TEA(Triethanolamine) buffer
- Maintain temperature of the beaker at 70 degrees.
- Add ammonia to set pH(=11) of the mixture.
- Add the sodium seleno sulphate which provides selenide ions.
- Stirr the mixture for 3 hours.
- Wash the mixture with distilled water.
- Centrifuge the mixture.
- Filter the mixture.
- Let dry the filtered material.

b) Zinc Sulphide (ZnS):

- Take 80 ml of distilled water in a beaker.
- Add 0.575gm zinc sulphate into it.
- Add 7ml of hydrazine hydrate into the mixture.
- Set the pH of beaker at 9.8.
- Add thiourea 0.213gm into the beaker.
- Stirr the mixture for 3 hours.
- Filter the mixture and let dry the filtered material.

c) CdSe/ZnS core/shell:

- CdSe QDs by wet chemical route at 35±1^{o}C temperature have been synthesized.
- ZnS shell layer was grown on CdSe core QDs using two aqueous solutions of Zn^{2+} and S^{2-} ions which were obtained by dissolving the compounds zinc sulphate dehydrate.
- Ammonium sulphate by mixing in 50 ml of distilled water. Ammonia was added to it until the formation of clear metallic complexes has been achieved at pH 9.5.
- After this, 2.5 ml of prepared CdSe solution was added.
- Then thiourea was added in 50 ml of prepared solution.
- After that appropriate amount of 2-mercaptoethanol solution was added in solution for passivation then continuously stirred for 5 hours.
- When the reaction was completed CdSe/ZnS core/shell nanoparticles were centrifuged, washed and dispersed in distilled water before storage.

d) Polymer Encapsulation:

- A 0.5 gm of quantum dot sample was first mixed with 20ml of distilled water.
- To the mixture added double the amount of polymer PEG (Merck 400).
- The sample was kept for 48hrs in the shaker.

CHARACTERIZATION TECHNIQUES:

a) ABSORBANCE: It is common logarithm of the ratio of incident to transmitted radiant power through a material. It is dimensionless. It is an increasing function of path length which approaches to zero as the path length

approaches to zero. The extent to which a sample absorbs light depends strongly on the wavelength of light and for this reason, spectrophotometry is performed using monochromatic light. In analyzing a new sample first the sample's absorbance spectrum is determined. The absorbance spectrum shows how the absorbance of light depends upon the wavelength of the light. The spectrum is a plot of absorbance v/s wavelength and is characterized by the wavelength (λ_{max}) at which the absorbance is the greatest.

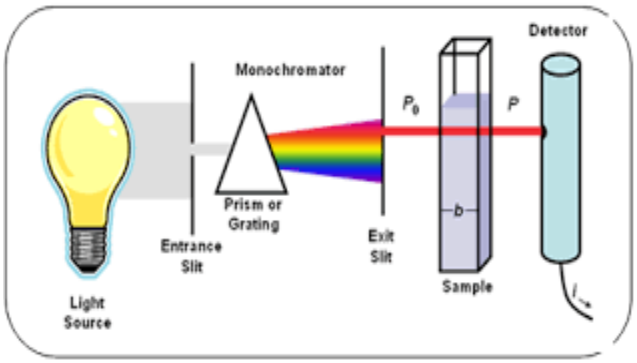


Fig 8: UV- Vis Spectrophotometer Instrumentation.

b) PHOTOLUMINESCENCE: It is light emission from any form of matter after the absorption of photons. It is one of many forms of luminescence and is initiated by photoexcitation. Time periods between absorption and emission may vary: ranging from short femto second-regime for emission involving free-carrier plasma in inorganic semiconductors up to milliseconds for phosphorescent processes in molecular systems; and under special circumstances delay of emission may even span to minutes or hours.

c) X-RAY DIFFRACTION: The XRD Shimadzu-6000 system consists of the equipment main unit and a data processing unit which controls it. The equipment main unit consists of the X-ray generator unit, goniometer unit and CPU which controls them. The generator unit and goniometer unit are controlled by the data

processing unit. In X-ray diffractometer, X-rays emitted from the X-ray tube undergo the limitation of the divergence slit and hit the sample loaded in the center of the Goniometer. The X-rays diffracted from the sample coverage on the receiving slit which is located in the position symmetrical with respect to the X-ray focus of the X-ray tube, viewed from the sample. These X-rays are captured by the scintillation detector, after elimination of noise components, are counted by the pulse height analyzer (Dabbousi B, Rodriguez J et al 1997). The distance between the atomic planes where X-rays are diffracted can be obtained from the Bragg condition given as:

$$2d\sin\theta = n\lambda \qquad (\ldots i)$$

Where θ=peak angle position of diffracted X-rays and λ is the wavelength of X-rays.

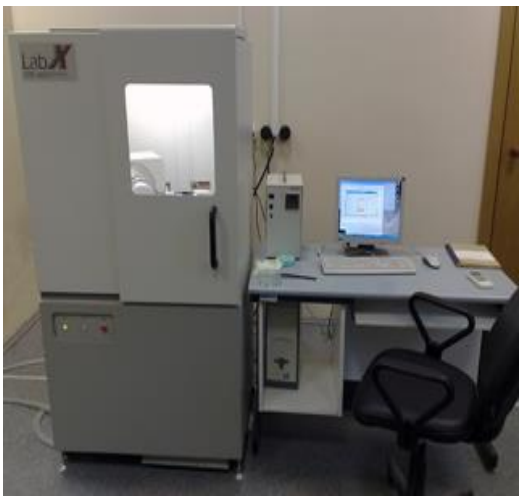


Fig. 9: Shimadzu (XRD 6000) X-Ray diffractrometer employed for XRD experiments.

d) TRANSMISSION ELECTRON MICROSCOPE (TEM): Transmission electron microscopy is used to study the microstructure of the samples with a magnification of $3x10^5$ to 10^6 and a resolution of 0.1 nm. It constitutes of (1) two or three condenser lenses to focus the electron beam on the sample, (2) an objective lens to form the diffraction in the back focal plane and the image of the sample in the image plane (3)

some intermediate lenses to magnify the image or the diffraction pattern on the screen.

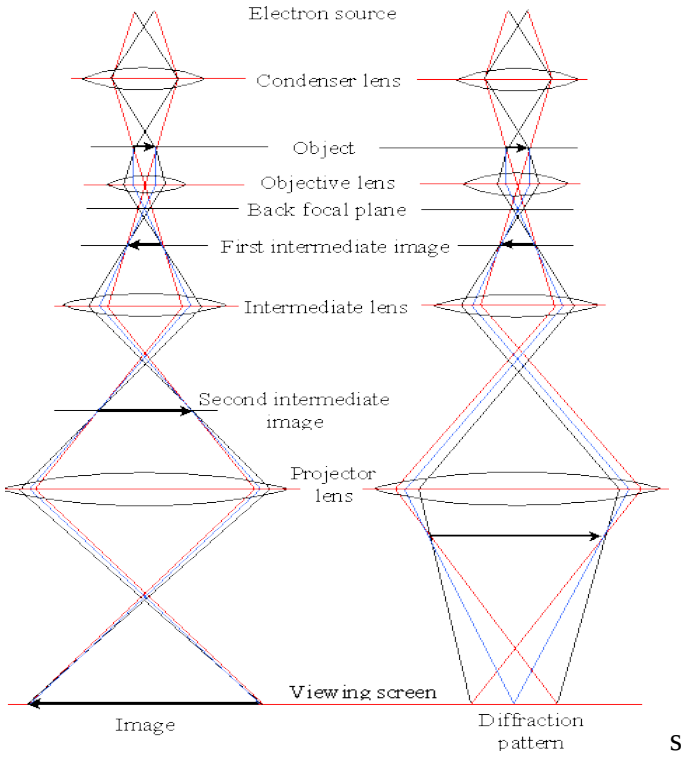


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Fig.10: Transmission Electron Microscopy.

e) **ENERGY DISPERSIVE X-RAY SPECTROSCOPY:** It is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray emission spectrum. To stimulate the emission of characteristic X-rays from a specimen, a high-energy beam of charged particles such as electrons or protons or a beam of X-rays, is focused into the sample being studied. The incident beam may excite an electron in an inner shell, ejecting it from the shell while creating an electron hole where the electron was. An electron from an outer, higher-energy shell then fills the hole, and the difference in energy between the higher-energy shell and the lower energy shell

may be released in the form of an X-ray. The number and energy of the X-rays emitted from a specimen can be measured by an energy-dispersive spectrometer.

f) **FLOUROSCENCE MICROSCOPY:** It is an optical microscope that uses fluorescence and phosphorescence instead of or in addition to reflection and absorption to study properties of organic or inorganic substances. The "fluorescence microscope" refers to any microscope that uses fluorescence to generate an image, whether it is a more simple set up like an epifluorescence microscope(Xu, C., Zipfel, W. et al 1996) or a more complicated design such as a confocal microscope, which uses optical sectioning to get better resolution of the fluorescent image.

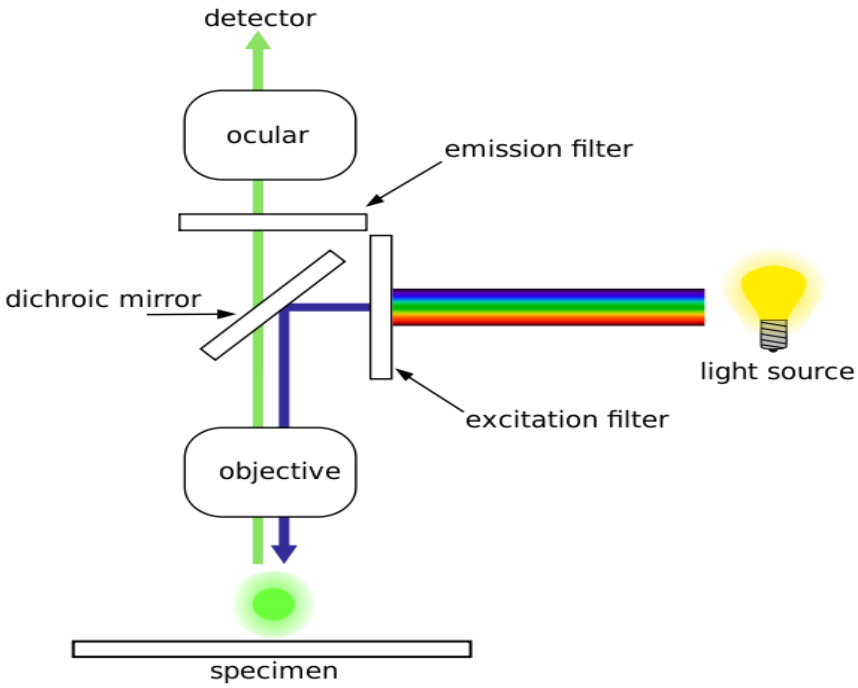


Fig.11: Fluorescence Microscopy

g) **FOURIER TRANSFORM INFRARED MICROSCOPY (FTIR):** It is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over

a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range.

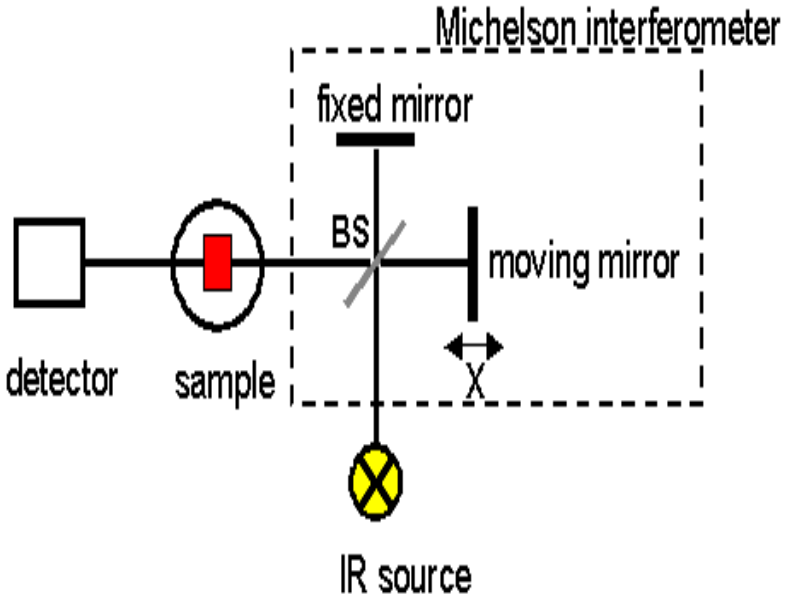
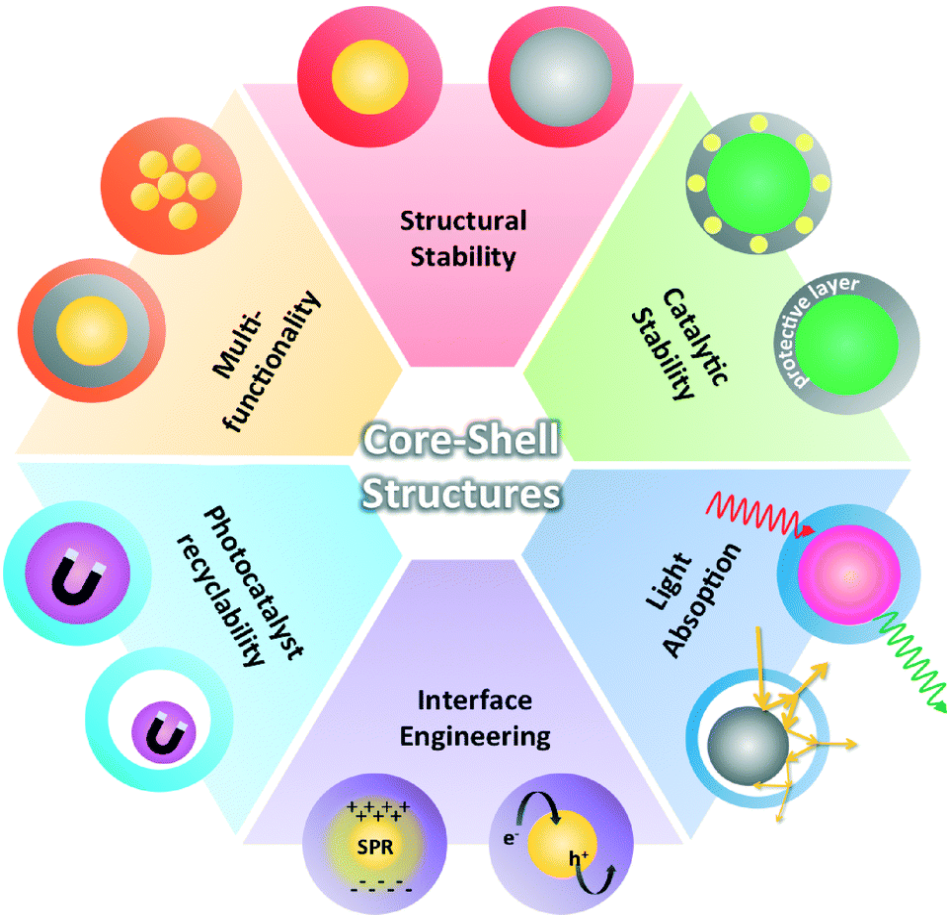


Fig. 12: FTIR Instrumentation

CHAPTER 3

DEVELOPMENT OF CdSe AND CdSe/ZnS QUANTUM DOTS AND THEIR CHARACTERIZATION



INTRODUCTION:

A QD is a nano crystal made of semiconductor materials that is small enough to exhibit quantum mechanical properties. Specifically, its excitons are confined in all three spatial dimensions. The electronic properties of these materials are intermediate between those of bulk semiconductors and of discrete molecules.

Researchers have studied applications for quantum dots in transistors, solar cells, LEDs, and diode lasers. They have also investigated quantum dots as agents for medical imaging and as possible qubits in quantum computing.

Quantum confinement of both the electron and hole in all three dimensions leads to an increase in the effective band gap of the material with decreasing crystallite size. Consequently, both the optical absorption and emission of quantum dots shift to the blue (higher energies) as the size of the dots gets smaller. Although nano crystallites have not yet completed their evolution into bulk solids, structural studies indicate that they retain the bulk crystal structure and lattice parameter. Recent advances in the synthesis of highly monodisperse nano crystallites have paved the way for numerous spectroscopic studies assigning the quantum dot electronic states and mapping out their evolution as a function of size (Terenziani, Katan K, Badaeva C et al 2008).

The dependence on size arises from (1) changes of the surface-to-volume ratio with size, and from (2) quantum confinement effects. Nevertheless, QDs exhibit different colour of emission with change in size.

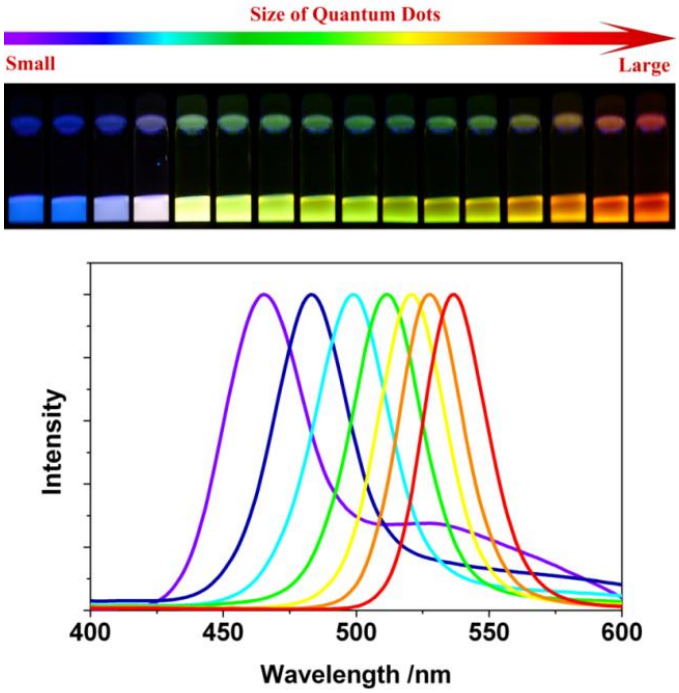


Fig.13: Representation of Quantum confinement by the means of PL. (Debasis Bera , Lei Qian et all 2010)

RESULTS AND DISCUSSION:

PHOTOLUMINISCENSE:

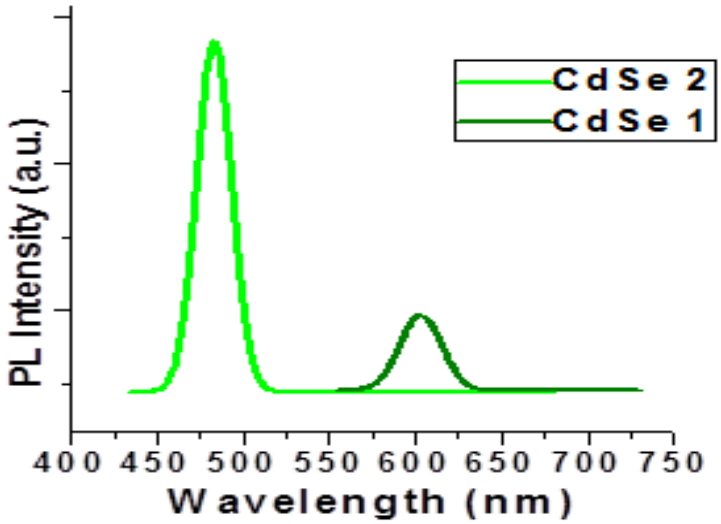


Fig.14: PL emission spectra of two different sized CdSe QDs

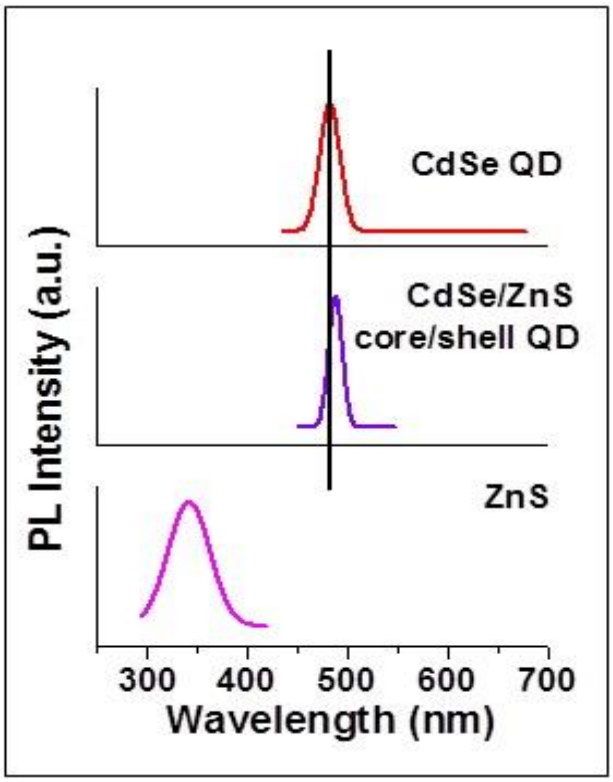


Fig. 15: PL emission spectra of CdSe 1, CdSe1/ZnS and ZnS

The luminescence properties of 2-MPE capped CdSe1(2 part concentration of cappant) and CdSe2(1 part concentration of cappant) QDs shown in figure14and 15. PL spectra were recorded at an excitation wavelength of 400 nm. It is well known that surface defect states play important roles in the luminescence properties of nanoparticles, which act as radiative or nonradiative centers in nanoparticles. The PL spectrum of the CdSe1 and CdSe2 QDs was dominated by very strong emission peak at around 480 nm, 590 nm respectively. The fluorescence enhancement may be attributed to a much higher concentration of radiative recombination centers. The PL intensity of 2-MPE capped particles is dramatically enhanced. It is observed that the photoluminescence efficiency of capped CdSe QDs with higher concentration of 2-mercaptoethanol show high intensity due to passivation of surfaces.

Emission spectra for as prepared CdSe1 QDs , CdSe1/ZnS and ZnS QDs have been recorded (figure 14) and have been presented. The PL spectrum of the CdSe1 , CdSe1/ZnS and ZnS

QDs was dominated by very strong emission peak at around 480 nm, 485 nm and 340 nm respectively. As shown in the figure there is a small red shift in the CdSe1 and CdSe1/ZnS structure, this is due to the increase in the size of QD as a result of overcoating of ZnS on already prepared CdSe1. Not shown in the figure presently but there is a sharp increment in the PL intensity when there is formation of shell on the core. This structure along with the enhanced luminescence properties gives the CdSe QDs a layer that is biocompatible and these quantum dots can be used in biological applications.

ABSORBANCE:

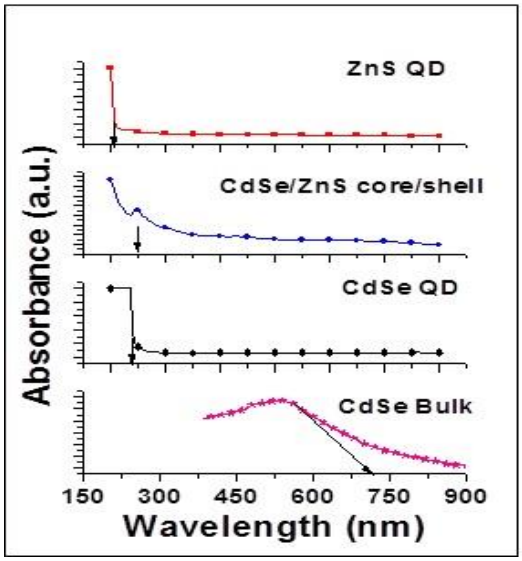


Fig.16: Optical absorbance spectra ZnS, CdSe1/ZnS, CdSe QD and CdSe bulk. The arrow is indicating the absorption edge in each case

CdSe/ZnS is type I core/shell structure: Band gap of the shell (ZnS) is larger than that of core (CdSe). The conduction and valance band offset are such that the conduction band of shell is higher than that of core, while the valance band of the shell has lower energy than that of the core. This leads to an effective confinement of electron and hole in the core material. The optical band gap of CdSe, ZnS and CdSe/ZnS has been determined from the absorption spectra. The UV–visible absorption spectra of ZnS, CdSe, and CdSe/ZnS QDs have been

presented in figure 13. The absorption edges occur at 240 nm (ZnS), 270 nm (CdSe), 310 nm (CdSe/ZnS) and 700 nm for bulk CdSe QDs.

As the size decreases, the absorption of the surface states becomes more intensive and the absorption peak shifts toward blue. This indicates that the contents of the surface states increase as the size of the particles is decreased. As the surface/volume ratio increases, the size decreases and ions at the surface increase rapidly. Thus the surface states (dangling bonds, defect sites, or traps) increase rapidly via this surface reconstruction. The absorption edge of CdSe/ZnS core/shell show a blue shift as compared to bulk CdSe. The absorption edge of the optical energy band gap of CdSe, ZnS and CdSe/ZnS nanostructures have been calculated using the formula

$$E_{eV} = hc / \lambda \qquad (..ii)$$

where h = Planck's constant and E = energy band gap of the semiconducting nanoparticles. It has been noticed that the values of the band gaps are higher than that of bulk values of CdSe (1.7 eV) and ZnS (3.9 eV) indicating the strong quantum confinement. The band gap energies gradually increase from 1.7 eV to 4.6 eV in case of CdSe and from 4.23 to 5.2 eV for ZnS.

X- RAY DIFFRACTION:

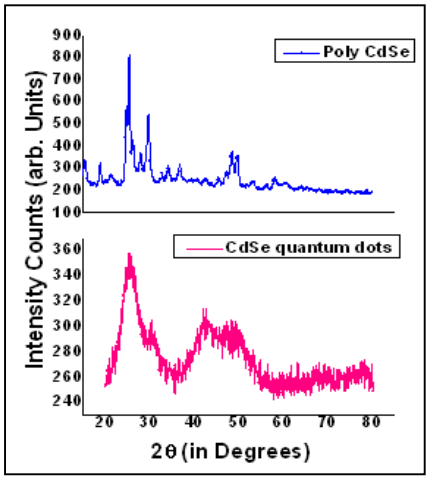


Fig.17: XRD spectra of bulk CdSe and CdSe QDs

X-ray diffraction spectra of CdSe poly and 2-mercaptoethanol capped CdSe quantum dots have been shown in figure 17. It has been reported that CdSe exists in face centered cubic (fcc) crystalline phase. The prominent peaks (111), (200), (220), (311) were shows in figure 14 indexed to the cubic structure. The successive broadening in XRD peaks show that the particle size substantially reduces with addition of the stabilizer. It means cappent appropriately cap CdSe QDs which arrest the size. The crystallite size (*D*) of the QDs have been calculated using Scherer's formula

$$D = k\lambda / \beta \cos\theta \qquad (\ldots \text{iii})$$

Where λ is the wavelength of the x-rays used, β the full width at half maximum of the preferred XRD peak, and θ the Bragg angle. The average crystallite size obtained for CdSe QDs is 2 nm, which was obtained from the width of the (111) peak.

TRANSMISSION ELECTRON MICROSCOPY:

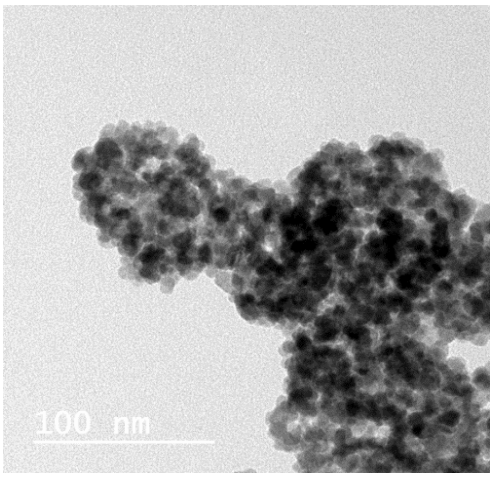


Fig.18: TEM Image of CdSe nanoparticles

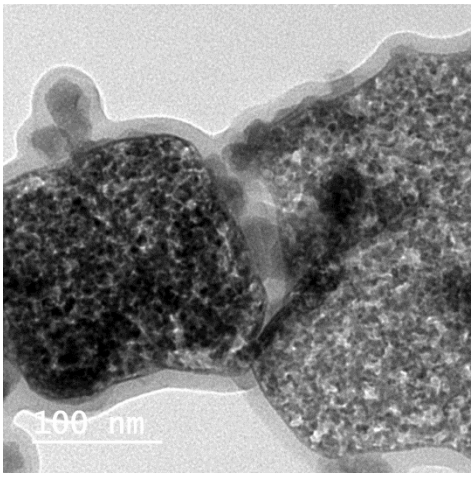


Fig.19: TEM of ZnS nanoparticles

The particle size of CdSe and ZnS QDs have been determined by TEM to make sure the effectiveness of stabilizer and the result is presented in figure15 and 16. TEM image (figure 18 and 19) of the CdSe and ZnS QDs shows highly monodispersed nanoparticles with average sizes of 4 nm and 1 nm approximately . It is also clear from the figure that there is no agglomeration of QDs and we can obtain either the fine solution or even powder of uniformly

distributed nanoparticles for various applications. The particle sizes obtained from TEM are slightly greater than the size obtained from XRD analysis. The size obtained by XRD and TEM has the correlation that the XRD size is usually equals or smaller than the size obtained by TEM.

ENERGY DISPERSIVE X-RAY SPECTROSCOPY:

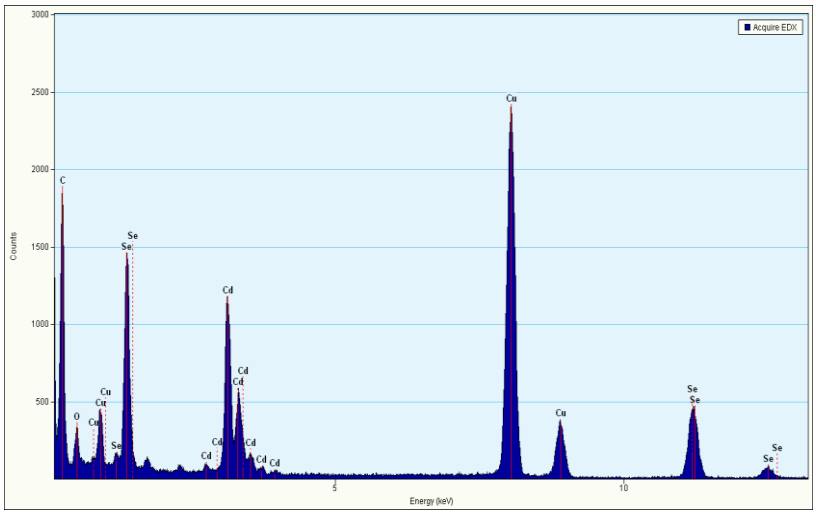


Fig.20: EDX spectra CdSe QDs

We have employed our sample of CdSe quantum dot for elemental analysis and the results obtained revealed that the prepared samples are of high stiochiometry. The obtained ratio of Cd: Se is 1:1. There is no trace of the presence of any other element or impurity in the system.

ELEMENT	WEIGHT %	ATOMIC %
C(K)	24.16	64.41
O(K)	2.05	4.11
Cu(K)	40.59	20.45
Se(K)	13.04	5.28
Cd(L)	20.13	5.73

Tab.1: Percentage of elements in quantum dots.

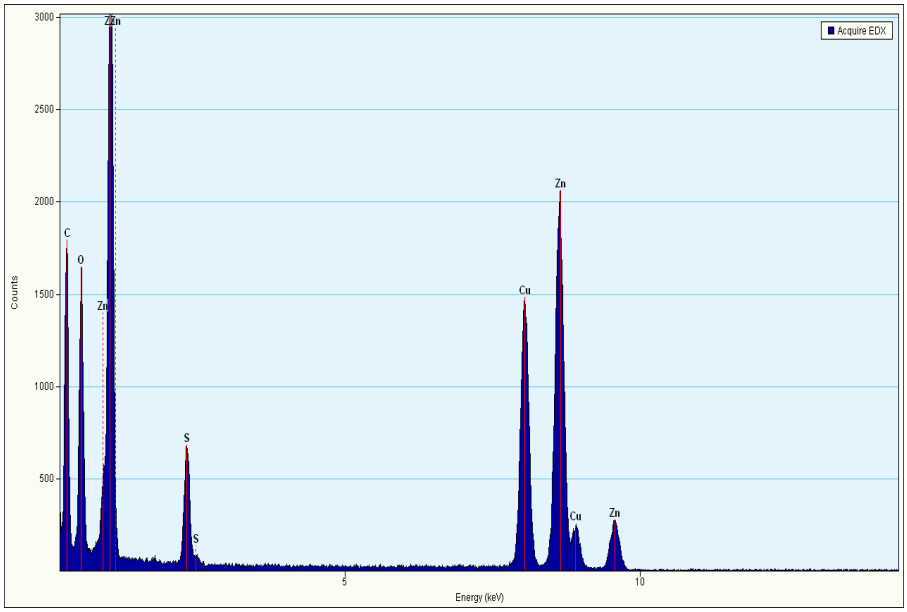


Fig.21: EDX spectra of ZnS QDs

We have employed our sample of ZnS quantum dot for elemental analysis and the results obtained revealed that the prepared samples are of stiochiometry. The obtained ratio of Zn: S is 1:0.8. There is no trace of the presence of any other element or impurity in the system.

FLOUROSCENCE MICROSCOPE:

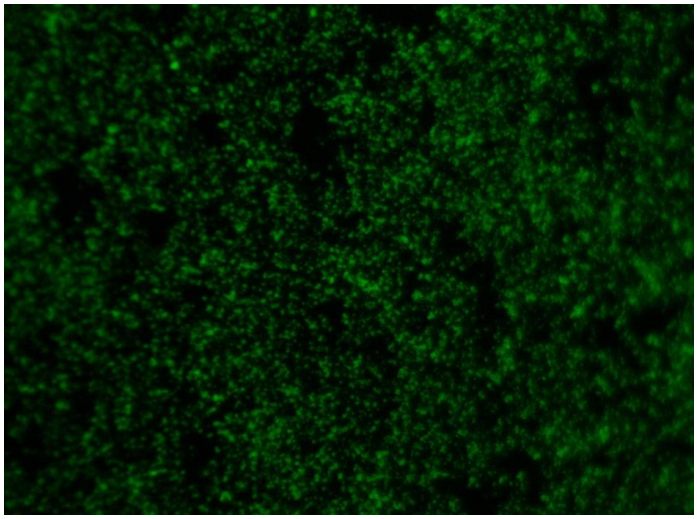
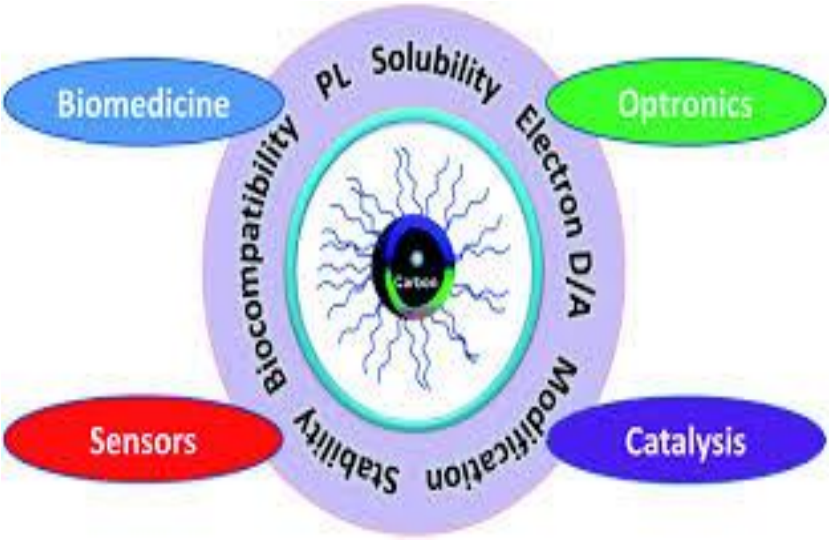


Fig.22: Fluorescence microscopic image of QDs

We have obtained highly luminescent CdSe and ZnS quantum dots as shown above in figure 19. As shown in figure, there is no agglomeration in quantum dots and the quantum dots are of very high brightness.

CHAPTER 4

BIOCOMPATIBILITY AND TOXICITY



POLYMER ENCAPSULATION:

Quantum dots cannot be used directly because their metallic toxicity, non-dissolubility, photo-luminescence instability prevent the direct utility of QDs in biological media. Such hybrid materials can provide solubility and robust colloidal and optical stability in water. At the same time, polymers can carry ionic or reactive functional groups for incorporation into the end-use application of QDs, such as receptor targeting and cell attachment. For more extensive and effective biological applications, QDs have been encapsulated or surface modified to prevent aggregation and make them biocompatible. The various strategies have been used to make them water-soluble such as surface functionalization with water-soluble ligands and encapsulation within block-copolymer micelles.

The strategy of using amphiphilic polymers is generally superior to the surface modification, because (a) there is no direct interaction with the QD surface atoms and therefore can preserve the original quantum efficiency to a highest extent (b) the presence of hydrophobic polymer domains around QDs may strengthen the hydrophobic interaction to form more steady structures and consequently more stable water-soluble QDs (c) these amphiphilic polymers can be tailor-made to have good stability in aqueous media and introduce other functional moieties on the surface of QDs.

Polymer micelles have been extensively studied for solubilization of hydrophobic drugs and bioactive agents due to their unique properties including the nano-scaled size, high water-solubility, high structural stability, high carrying capacity of hydrophobic agents, and easiness in introducing functional moieties on the outer shell. Especially, poly(ethylene glycol)-poly(D,L-lactide) diblock copolymer micelles are the most frequently used system because of their biocompatibility and biodegradability.

Luminescent CdSe/ZnS QDs, with emission in the red region of the spectrum, were synthesized and encapsulated in polyethylene glycol (PEG) to prepare water-soluble, biocompatible QDs. The resultant encapsulated QDs were characterized using various analytical techniques such as UV-Vis measurement, fluorescence spectroscopy, transmission electron microscopy (TEM). The encapsulated QDs are spherical having a diameter in the range of 20–150 nm. The encapsulated QDs are highly luminescent and have high potential for applications in biomedical imaging and detection.

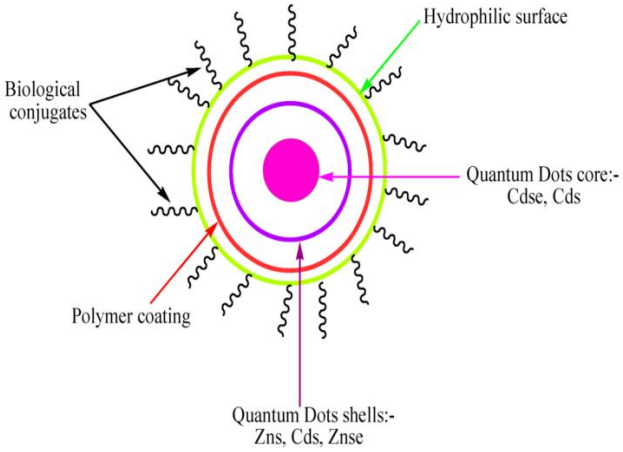


Fig. 23: Polymer Encapsulation

RESULTS AND DISCCUSSION:

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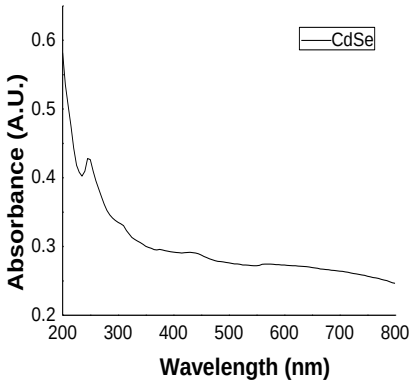


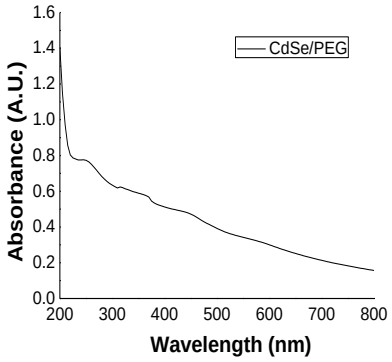
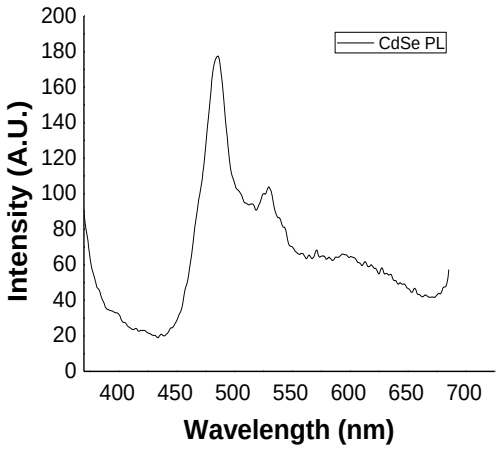


Fig. 24: Abs of CdSe QD

Fig. 25: Abs of encapsulated QD

The absorbance is observed in both the graphs at 250 nm. This shows that there is no position shift in the spectrum which is an utmost requirement to us. The consequence of polymer encapsulation is that there is no change in the size of particular quantum dots and hence no changes in bad gap which results in no wavelength change and thus no change in optical properties.

PHOTOLUMINESCENCE:



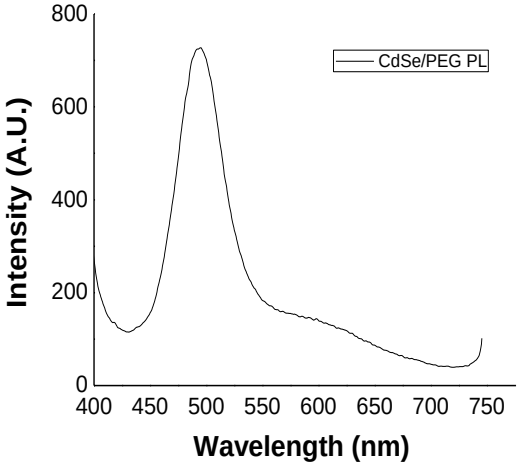


Fig. 26: PL of CdSe QD

Fig. 27: PL of encapsulated QD

With polymer encapsulation there is an increase in the intensity as shown in the figure 27. The peaks observed in the figure 26 are generally due to surface defects which are intensity/ luminescence killer. With polymer encapsulation these surface defects are eliminated and thus, results in increase in the intensity.

TRANSMISSION ELECTRON MICROSCOPY:

Fig. 28: TEM of CdSe QD

Fig. 29: TEM of CdSe/PEG

The particle size quantum dots have been determined by TEM. The particle size is generally in the range of 1.82 nm- 2nm respectively. With polymer encapsulation, there is no agglomeration and thus stabilized polymer encapsulated quantum dot structure.

FLUORESCENCE MICROSCOPY:

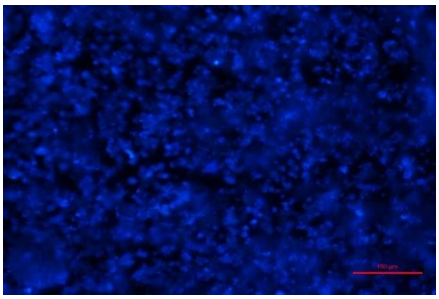


Fig. 30: Fluorescence Microscopy CdSe QD

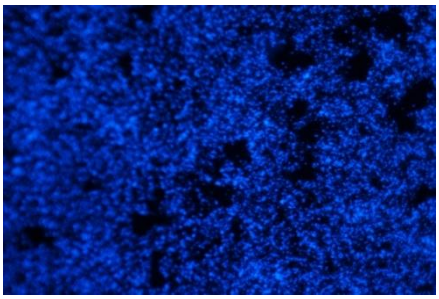


Fig. 31: Fluorescence Microscopy of CdSe/PEG

The fluorescence microscopy image (figure 31) tells us that with decrease in agglomeration, there is an increase in an intensity/ luminescence.

FOURIER TRANSFORM INFRARED MICROSCOPY:

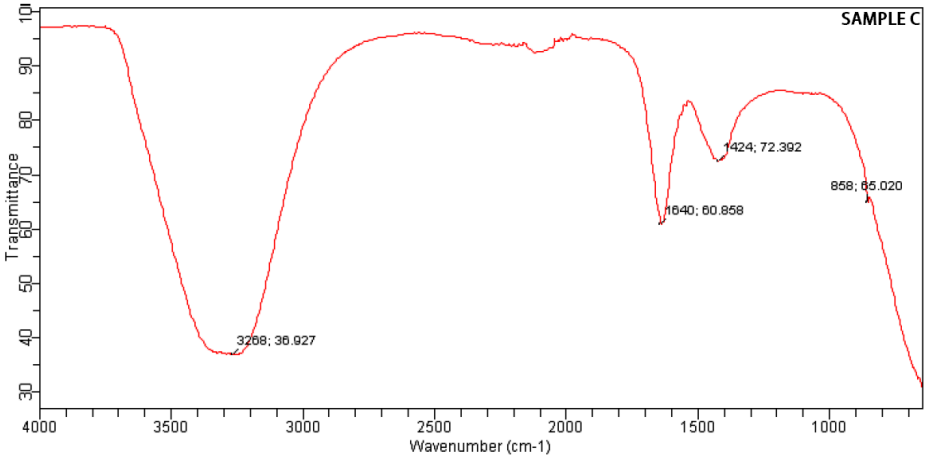


Fig. 32: FTIR of CdSe QD

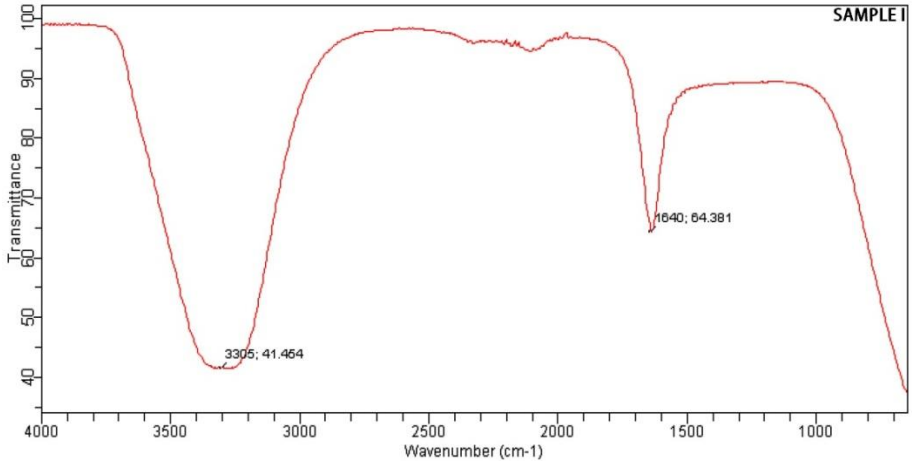


Fig.33: FTIR of CdSe/PEG

From the above results we came to know that polymer encapsulated QDs are of biocompatible nature and there is removal of surface defects which otherwise would result in decreasing the stability of QDs. When QDs were encapsulated with polymer (PEG) only ammonia(1640) and hydroxyl(1400) functional groups were present which are biocompatible having easy attachment to the biomolecules

CYTOTOXICITY TESTING:

Owing to certain advantages, QDs have attracted considerable attention in the field of biology and medicine over the past two decades. Certainly, cellular imaging is one of important usages in biology and medicine, and it is an important method of cellular analysis, especially analysis of biological procession in cell. However, two important properties could influence on the usage of QD's in cellular imaging, that is, cytotoxicity and fluorescence stability, especially fluorescence stability in cell.

More and more people have paid attention to the cytotoxicity of QDs and their toxicity for organism. Up to now, various studies have shown that the toxicity of QDs is affected by many factors, including physico-chemical property of the particles themselves (such as particle size, stability, dispersion, surface charge, surface modification groups, oxidation state), the concentration of quantum dots, the receptor cells (in vivo) coming from different species, and culture (exposure) time.

The fact that CdSe/ZnS core-shell-shell structured QDs are nearly nontoxic to cells further confirmed the role of released cadmium ions on cytotoxicity, and the effective protection of the ZnS shell. However, intracellular level of Cd^{2+} ions cannot be the only reason since the comparison with $CdCl_2$-treated cells suggests there are other factors scontributed to the cytotoxicity of QDs.

The synthesized CdSe quantum dots, CdSe/ZnS core/shell and PEG coated core/shell containing unreacted precursors ions and stabilizers were purified by dialysis. The solutions were dialyzed against Tris-HCl buffer (pH = 10.0) for 48 hours with frequent changing of the buffer. The solution was continuously stirred to maintain well mixed conditions and mass transfer through the membrane. Dialysis was performed using dialysis tube with 12–14 kDa

molecular weight cut off. The dialyzed samples were collected and diluted to dose concentration.

RESULTS AND DISCUSSION:

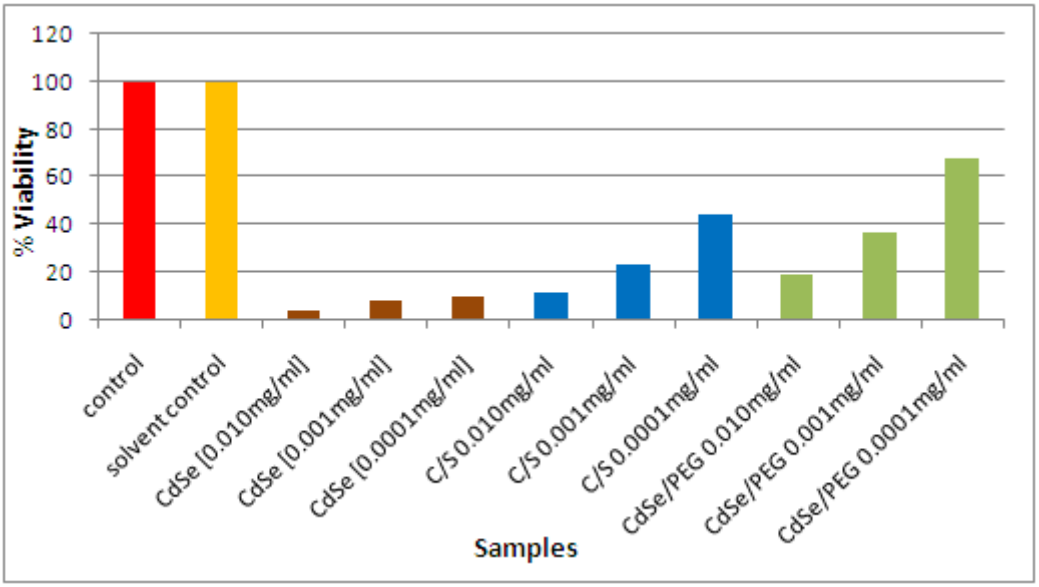


Fig.34: Cytotoxicity Testing

Cytotoxicity testing of bare CdSe , CdSe/ZnS and CdSe encapsulated by polymers were done by MTT assay using MDCK cell lines. Phenol purchased from sigma was used as positive control. Cells without samples were used as negative controls. The CdSe and CdSe/Polymer were prepared at concentration 0.010mg/ml in double distilled water. Further concentrations were prepared by dilution. Different dilutions of Samples, negative control and positive control in triplicate were placed on MDCK cells. Incubate for 24 hours after that remove QDs then add DMSO then again incubates.

We studied cytotoxicity of bare CdSe QDs and their core shell, polymer encapsulated structures. Graph shows that cell viability decreases as concentration increases. Different concentrations of all samples were used and it was observed that cell shows better results comparable to negative control when exposed to core/Shell and polymer encapsulated structures.

CHAPTER 5

SUMMARY

SUMMARY

The CdSe, ZnS and CdSe/ZnS QDs whose emission spans most of the visible spectrum have been described which have been synthesized using wet chemical growth method. Their structural as well as optical properties have been investigated by XRD, UV-Vis spectroscopy, photo luminescence spectroscopy, EDX and TEM. XRD results indicated that lower synthesis temperature resulted in lower grain size. The ZnS shell appears on CdSe QDs properly covered and creates dislocations or other defects to accommodate the mismatch in lattice constants between ZnS and CdSe. The influence of surface passivation on the optical properties has also been evaluated. The UV-Visible spectra show a large blue shift attributing the enhanced optical properties, this size dependent blue shift in absorption edge is attributed to the quantum size effect. PL measurements show effectiveness of capping and core/shell structure formation as there is clear vindication of drastically enhanced luminescence. In summary, highly luminescent, nearly monodispersed CdSe, ZnS and CdSe/ZnS QDs has been prepared. The particle sizes of CdSe, ZnS and CdSe/ZnS QDs as determined from XRD and TEM images were in good agreement. Fluoroscence microscopy images shows the efficacy of our synthesis method and is an evidence that this technique is very much capable for producing high luminescence CdSe and CdSe/ZnS QDs. Core/shell semiconductor QDs that were prepared by our method are low-cost, safe, and environmental friendly. Color tunable CdSe, ZnS and CdSe/ZnS QDs were created which are monodispersed possessing high stability and; narrow PL spectra that indicate superior monochromaticity. This method can also be applicable to synthesize the other tunable, monochromatic core/shell semiconductor QDs for the application in the field of nano-bio-technology.

To increase the stability and biocompatibility of QDs, we opted the method of polymer encapsulation and cytotoxicity testing. From the results derived above, that is, PL, FTIR and cytotoxicity testing the biocompatibility and stability has been increased considerably with

respect to core/shell quantum dots and quantum dots respectively. Therefore, it has been concluded that these encapsulated QDs can be used for in vivo imaging and other biological tests which was the main goal of our work.

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