2.1. Introduction

Quantum dots (QDs) are semiconductor nanocrystals that exhibit significant optical properties including bright luminescence, a broad excitation profile, narrow emission peaks, and exceptional photostability. By changing the size of QDs, absorption and emission spectra can be finely tuned. These nanostructures have been investigated for a wide variety of applications, such as optical probes for imaging, target labeling, temperature sensing, and sensitizers for solar cells (Clapp et al., 2004). Away from their essential applications, QDs also play an important role in the form of educational tool. In the field of quantum chemistry, QDs serve as an excellent instance because of their size-dependent optical properties. Particularly, QDs consist of cadmium (Cd) and selenium (Se) which absorb and emit the visible radiation and as a result, this phenomenon allows to observe visually size-dependent quantum phenomena of QDs. Moreover, the large Bohr radius of CdSe QDs which is around 5.6 nm also provide to study the quantum confinement effects of a broad range of sizes (Landry et al., 2013). Rapid application of nanotechnology and nanomaterials represents a promising vista for the development of anti-cancer therapeutics. A large number of nanoparticles including organic and inorganic materials have been developed for delivery systems in cancer therapy since last two decades. Synthesis of various kinds of nanomaterials such as quantum dots (QDs), carbon nanofiber (CNFs), carbon nanotubes (CNTs), and fullerenes and their biological applications including cancer therapy have been developed more increasingly (Madani et al., 2013; Ashfaq et al., 2014; García-Hevia et al., 2014; Shi et al., 2014). QDs are attractive fluorophores for biomedical imaging and also biological tagging.
(Nune et al., 2009). Quantum dots labelled with bevacizumab complexes has been used for *in vivo* imaging of tumors in xenograft model (Gazouli et al., 2014). Cadmium Selenide/Cadmium Telluride (CdSe/CdTe) QDs, when coated with lipids demonstrated extraordinarily high specificity for cancer cells (Shao et al., 2014). Small size of particles makes nanotechnology as a boon in medicine and industry. In the chemically synthesized metal nanoparticles, the toxic chemicals and strong reducing agents like sodium citrate which are involved in the synthesis process and byproducts formed during the synthesis play a major role in producing the cytotoxic effect. Capping agents or the stabilizing agents are reducing agents like sodium citrate which gives more negative surface charge to the nanoparticles. This negative surface charge also plays a pivotal role in the toxic effect of the chemically synthesized gold nanoparticles (Iravani et al., 2011).

Before time CdSe QDs synthesis are not appropriate for an undergraduate student laboratory setting, as they are both hazardous and complicated, utilizing highly toxic and air-sensitive reagents, for instance, dimethylcadmium and bis- (trimethylsilyl) selenium. As well, most early synthesis was conducted at high temperatures above 200°C and afterward these were improved by using safer chemicals, such as cadmium oxide and elemental Se (Landry et al., 2013). To initiate the reaction, these processes involve the heating Cd precursor solution to 225°C and then adding the Se precursor solution into the Cd solution. Previously from several years, reaction temperatures used in QD synthesis have been gradually decreased instantly. In recent times, some researchers Bartl and his co-workers profitably synthesized the high-quality CdSe QDs using the temperature below 200°C. However, the toxicity of the starting material is minimized by
employing cadmium acetate dihydrate, and lower concentrations of reactants in contrast to the conventional high-temperature trials (Landry et al., 2013). Furthermore, by using two precursor solutions mixing with a third growth solution including fatty amine ligands (octylamine or octadecyl amine), CdSe QDs was successfully synthesized at temperatures ranging from 50 to 130 °C under an inert gas atmosphere. The growth of CdSe QDs is slower at a lower temperature; however, the optical properties of the final products are equivalent after few hrs to several days of the reactions (Park et al., 2007). Motivated by this research, two robust methods were developed to synthesize CdSe QDs under ambient atmosphere. Further, an optimized reaction temperature of 165°C is used to finalize the synthesis of QDs and then the characterization of the QDs is carried out within a reasonable period of time. More importantly, the relatively low temperature is easy to achieve, safe and also allows the reaction to proceed at a more comfortable rate. Additionally, both methods utilize significantly reduced the concentration of each metal component. CdSe-derived nanoparticles with sizes below 10 nm exhibit a property known as quantum confinement. Quantum confinement results when the electrons in a material are confined to a very small volume. Quantum confinement is size dependent, meaning the properties of CdSe nanoparticles are tunable based on their size (Landry et al., 2013). A common type of CdSe nanoparticles is a CdSe QDs and the dissimilarity in their energy states is due to the electronic transitions which vary and depends on quantum dots size. It has been found that larger quantum dots have closer electronic states than smaller quantum dots. Thus, the energy required to excite an electron from HOMO to LUMO is lower than the same electronic transition in a smaller quantum dot.
Moreover, the quantum confinement effect could be also observed as a red shift in absorbance spectra for nanomaterials with larger diameters. CdSe QDs is widely utilized in a variety of applications including light emitting diodes, solar cells and fluorescent tagging (Neukirch et al., 2014). CdSe-based materials also have potential uses in biomedical imaging. Human tissue is permeable to far infra-red light. By injecting appropriately prepared CdSe nanoparticles into injured tissue, it may be possible to image the tissue in those injured areas. Nanocrystal materials are one of the most interesting branches of today’s nanoscience and nanotechnology. Semiconductor nanocrystals exhibit unique size and shape-dependent optical properties due to the quantum confinement effects and thus may find a wide range of applications in optoelectronic devices, photocatalysis, solar energy conversion and biological imaging (Roszek et al., 2005).

Nanoparticles are utilized in a various field of interest especially in both science and technology due to their unique features and their size characteristics. Small size nanoparticles create a potential risk for health and environment comparable to a larger size. Accordingly, size measurement of the nanoparticle is an important task for the application of particles to evaluate potential toxicity in the field of science and technology. Previously various methods have been employed especially non-imaging measuring methods including dynamic light scattering or small angle x-ray scattering which were suitable for the measurement size distributions of large ensembles of nanoparticles but not free from difficulties (Buhr et al., 2009). Therefore, direct imaging of small samples of individual particles is necessary and indispensable for traceable
calibration of particle size and form. Due to their high resolution and high imaging speed, SEM and TEM are the standard methods for direct imaging and dimensional measurements of micro- and nanostructures. Electron microscopes are scientific instruments that use a beam of energetic electrons to examine objects on a very fine scale. The process of this technique involves three steps (a) a high-energy electron beam irradiate on a very thin sample and this radiation is diffracted by the lattices of a crystalline or semi-crystalline material and it propagates along different directions, (b) forward-scattered electrons are employed for imaging and angular distribution analysis (contrasting SEM in which backscattered electrons are detected) and (c) the analysis of energy of emitted X-rays. While TEM typically achieves a higher lateral resolution than SEM due to electron energies well above 100 keV, purchase and operation costs exceed those of SEM several times. Therefore, a comparatively inexpensive and easy to use transmission electron microscope can be realized by using an ordinary SEM equipped with a suitable transmission electron detector. SEM in the transmission mode is thus a serious option to study nanoparticles with sizes down to about 10 nm.

The electronic structures of atoms, ions, molecules or crystals through exciting electrons from the ground to excited states (absorption) and relaxing from the excited to ground states (emission) are used for determination in UV-vis. It deals with the study of electronic transitions between orbitals or bands of atoms, ions or molecules in the gaseous, liquid and solid state. Small metallic nanoparticles are proven to have the property for absorption and scattering electromagnetic radiation. The metallic nanoparticles are also known to exhibit different characteristic colors. Hence, UV-vis
spectroscopy can be utilized to study the unique optical properties of nanoparticles (Wilson et al., 2009). This work concerns the experimental studies of the CdSe nanoparticles in terms of its production and the optical properties. UV analysis, TEM, XRD and SEM are taken into our consideration to investigate and analyze the obtained results. Optical characterization of QDs is usually provided by UV-vis, which offers the fast, nondestructive and contactless option. As already mentioned, the optical properties (fluorescence emission) of QDs can be fine-tuned by the QDs size, which is a key parameter that determines the spectral position and purity of photoluminescence. QDs size is generally calculated using conventional techniques like TEM, XRD, and SEM. In short, these allowed us to synthesize and characterize the nanomaterials. In this study, CdSe QDs nanoparticles were prepared by chemical methods and the particles size and optical properties were investigated by UV-vis, TEM, XRD, and SEM.
2.2. Materials and Methods

Chemicals and reagents

Cadmium oxide (CdO), oleic acid, selenium powder, trioctylphosphine, octadecene, Hoechst 33258, propidium iodide (PI), and 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) dye were purchased from Sigma Aldrich, USA. DMEM F-12 medium, Fetal bovine serum (FBS), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) dye, and antibiotic solution were purchased from Himedia, USA. All the reagents used in this study were of high purity grade.

Chemical synthesis of CdSe Quantum dots

CdSe QDs were synthesized using the standard protocol. In a typical procedure, 320 mg Se powder was added to 3 ml octadecene solution in round bottom flask onto a stirrer hot plate. Approximately, 0.3 ml trioctylphosphine oxide was added in Se solution to dissolve the selenium completely. The prepared stock solution was used as a precursor for the preparation of different sizes of QDs. Approximately, 312 mg of cadmium oxide (CdO) was added with 0.6 ml oleic acid and 10 ml octadecene in a round bottom flask and cadmium solution was heated. When the temperature reached 240°C, 1 ml of selenium solution was transferred to cadmium solution. The color of this CdSe (QDs1) 2nm, under the ultraviolet light was appeared blue in color. The procedure was repeated at 250°C temperature to produce different sizes of QDs (QDs2) which appeared green in color. As the temperature was increased to 270°C, the color of QDs (QDs3) of different sizes was turned to red in color.
Isolation of CdSe QDs nanoparticles

This procedure was used to separate CdSe nanoparticles from the octadecene solvent. In brief, octadecene CdSe QDs suspension was resuspended in absolute ethanol. Samples were washed with ethanol by three times and centrifuged at 3,000 rpm for 5 min followed by removal of ethanol layer until no suspension was further obtained. Different sizes of QDs thus were isolated and resuspended in n-hexane solvent.

Characterization of CdSe QDs

CdSe QDs nanoparticles were characterized by UV-Vis spectroscopy, TEM, XRD and SEM. UV-vis measurements were performed on a Shimadzu dual-beam spectrophotometer (model UV-1601 PC, Canada, USA) with a 1-cm quartz cell. Transmission electron microscopy was performed by drying a drop of a suspension of CdSe nanoparticles onto formvar-coated TEM copper grids followed by analysis on Tecnai\textsuperscript{TM} G\textsuperscript{2} Spirit BioTWIN, (FEI, USA) equipped with Gaton orius Tm CCD camera controller which was operated at an accelerating voltage of 80 kV. For TEM characterization, samples were prepared by using 2 μl of QDs sample dissolved in 8 μl of n-hexane solvent and further bath sonicated for 10 min. Approximately, 4 μl of the sample solution was put on formvar coating grids and grids were then dried for 2-3 h in desiccators under vacuum. Thereafter, formvar coating grids were put in a goniometer and electron micrographs were obtained. The crystallinity of the prepared QDs samples and the crystal size were determined from the XRD spectra through Rigaku Miniflex II diffractometer in the 2θ range of 10–80° using Cu Kα radiation of wavelength $\lambda = 1.5406$ Å. For SEM study, approximately 2 μl of QD sample was taken and dissolved into 8μl
of n-hexane solvent. Again, bath sonicated for 10 min and 4 μl of sample solution were taken on the aluminum stub for air dry. Further, the sample was kept back for coating the gold-palladium and subsequently analyzed for sample detector.
2.3 Results and Discussion

Characterization Study

CdSe Quantum dots were successfully synthesized at various temperatures which appeared diverse in color at a different temperature under ultraviolet light. The prepared different sizes of QDs displayed very prominent fluorescence as shown in Figure 2.1A. Results showed that QDs1 appeared blue in color at 240°C, QDs2 showed in green color at 250°C while QDs3 appeared red in color at 270°C under UV lamp. Previous studies have reported that change in color is directly correlated with sizes of QDs (Gui et al., 2011; Tamaki et al., 2014). Interestingly, our synthesized CdSe QDs also affirm this argument.

Characterization of CdSe QDs by UV-vis spectrometer

The optical characterizations of QDs were performed at room temperature. The absorbance of different sizes of CdSe QDs was estimated by Uv-vis. As shown in the absorption spectra of Figure 2.1B, a gradual shift was observed from shorter wavelength towards the longer wavelength with increased in particle size. A definite peak of smallest nanoparticles is confined to 500 nm. Another set of peaks were observed which shifted towards the longer wavelength as the particle size increased. This study showed that a blue shift in the first exciton peak position signal represented a decrease in CdSe core diameter while second and third exciton peak position showed an increase in CdSe core diameter.
Characterization of CdSe QDs by TEM

TEM was the first type of electron microscope to be developed and is patterned exactly on the light transmission microscope except that a focused beam of electrons is used instead of light to see through the specimen. TEM is typically used for high-resolution imaging of thin films of a solid sample for nanostructural and compositional analysis. The topographic information obtained by TEM in the vicinity of atomic resolution can be utilized for structural characterization and identification of various phases of nanomaterials (Wilson et al., 2009; Reimer et al., 2013).

To elucidate the particle size of QDs, TEM study was performed. The particles sizes were found in the range of 2-4 nm (Figure 2.1C). Interestingly, a similar study reported that the synthesized CdSe QDs, showed yellow to red fluorescence under UV light and the particles sizes were found in the range of 2.1-6.8 nm as confirmed by TEM (Surana et al., 2014).
Figure 2.1 Characterization of CdSe nanoparticles. (A) CdSe QDs of size 2 nm, 3 nm and 4 nm showing (a) blue (b) green and (c) red fluorescence under UV light (B) UV-vis absorption spectra of CdSe nanoparticles of (a) 2 nm (b) 3 nm and (c) 4 nm size (C) TEM images of CdSe QDs nanoparticles of (a) 2 nm (b) 3 nm and (c) 4 nm size.
Characterization of CdSe QDs by XRD

XRD is a unique method for crystallinity determination of nanomaterials. XRD is basically used for distinguishing between amorphous and crystalline material and quantification of the percent crystallinity of a sample. Hence, in this present study, the crystal structure of QDs CdSe was evaluated by X-Ray diffraction measurements, with Rigaku Miniflex II diffractometer in the 2θ range of 10-80° using Cu Kα radiation of wavelength λ = 1.5406 Å. XRD pattern of liquid nanoparticles was recorded as shown in Figure 2.2. The three broad and distinct peaks in every XRD pattern of Figure 2.2 A was found at 2θ= 26.1°, 40° and 49.9° corresponding to the crystal planes (111), (220) and (311), respectively. XRD pattern of Figure 2.2B was found at 2θ= 25°, 41.9 and 49.9° corresponding to the crystal planes (111), (220) and (311), respectively. Consequently, the XRD pattern of Figure 2.2 C was found at 2θ= 25.9°, 43° and 50° corresponding to the crystal planes (111), (220) and (311), respectively. The peaks exhibited significant line broadening, which shows particles formed are small in size (Wang et al., 2011). The size was calculated using Debye Scherrer’s formula (d=0.94λ/ β cosθ) and was found to be from 1.5 nm to 3.5 nm for the (111) crystal face which demonstrates that the product is a real quantum dots nanocrystal (Kalasad et al., 2009).
Figure 2.2 XRD patterns of CdSe nanoparticles. (A) CdSe QDs of size 1.5 nm (B) CdSe QDs of size 2.4 nm (C) CdSe QDs of size 3.5 nm
**Characterization of CdSe QDs by SEM**

SEM is a type of electron microscope that images a sample by scanning it with a high-energy beam of electrons in a raster scan pattern. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition, and other properties such as electrical conductivity (LEO 430). In this study, SEM displayed the topography structure of QDs having round in shape (Figure 2.3). An earlier study has also reported the similar pattern of TEM study of CdSe/CdS nanoparticles (Gupta et al., 2017).

![SEM images of CdSe QDs nanoparticles](image)

**Figure 2.3 Characterization of QDs by SEM.** SEM images of CdSe QDs nanoparticles of (a) 2 nm (b) 3 nm and (c) 4 nm size.
2.4. Conclusion

In this study, CdSe QDs nanoparticles were prepared by chemical methods and the particles size and optical properties were investigated by UV-vis, TEM, and XRD. These studies provide the good fluorescent property with the small size of quantum dots shift towards the blue region and larger particles size towards red region under UV-vis. TEM results showed that the synthesized CdSe QDs have different sizes viz. 2, 3, and 4 nm and showing blue, green and red color, respectively under UV light. These particles have strong luminescent emission intensity under excitation of UV light with the color emission depending upon the size of CdSe QDs. Furthermore, the XRD peak of CdSe QDs exhibited significant line broadening, which shows particles formed are small in size. The size of nanoparticles was calculated using the formula of Debye Scherrer (d=0.94λ/ β cosθ) and the particles size was found in the range from 1.5 nm to 3.5 nm for the (111) crystal face which confirmed that the particles were true quantum dots nanocrystal. In TEM study, all different sizes of particles have shown uniform size and SEM study displayed topography structure of QDs having round in shape. This study of QDs might be used for further cadmium-based industrial and biomedical purpose.