Synthesis, optical and morphological characterization of CdSe/ZnSe quantum dots for cytotoxicity studies

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A treatise submitted in fulfilment of the requirements for the Master of Science in Nanoscience-Chemistry to be awarded at the Nelson Mandela Metropolitan University

December 2013
Dedication

This treatise is dedicated to my mother,
Nomqweno Princess Nkaule,
who taught me to value education and put first things first,
also to my loving son,
Thembelihle Unqobile Nkaule,
who has persevered my study period,
the Almighty Lord,
who deserves glory and is worthy to be saved.
Acknowledgements

My most fervent appreciation is extended to my supervisor Prof. Thomas Gerber for his overwhelming support, Co-supervisor Dr Martin Onani from the tough lessons I obtained from knowing him, Dr Paul Mushonga for his assistance, Prof Farouk Ameer and Dr Amar Fadhal for his overwhelming support. You have been my pillar of strength, my inspiration and my exemplar. Thus, nurturing my personal growth which has made the completion of this thesis possible. Professor Emmanuel Iwouha for his guidance and intellectual insight.

My gratitude is extended to the Department of Science and Technology (DST) for the financial support of this project. I am also indebted to Dr Subelia Botha, Mr Yunus Kippie, Mr Rajan Sharma for assistance with the HRTEM, EDS, SAD and TGA. My deepest appreciation to the Sensor Lab group, Dr Waryo Testfyo, Christopher Edozie and Dr Agbaugbau for all their support.

My boundless appreciation goes to all fellow Nanoscience delegates, who have carved their memory with a mere greeting or a smile. Also, the Nanoscience platform director, Prof Knoesen and Nanoscience platform administrative staff, Mrs Valencia Jamalie. I wish to thank Dr Ntevheleni Thovhongi and Nicole Sibuyi for your patience, assistance and guidance for biological studies. Special thanks to Mr Andile Mantyi, Bongani Makhoba and Timothy Lesch for assisting with chemistry technical aspects of research in the Chemistry department.

To my mom, thank you for your unwavering support and constant motivation in driving the completion of this thesis; son, siblings and step-father, for your love and support. I will always greatly cherish you and my accomplishments would be meaningless without you.

Love you always.
Abstract

Colon cancer (CC) ranks high in morbidity and mortality amongst the most frequent occurring cancers worldwide. Mortality rates are mostly caused by mis-diagnosis and the poor efficacy of treatment. The aim of this study was to enhance our insights of quantum dots, for early detection and targeted drug delivery, thereby reducing toxicity to normal cells and reducing side effects that are caused by previous colon cancer medicine.

The synthesis, characterization and cytotoxicity studies of CdSe/ZnSe quantum dots (QDs), nanocrystals are reported. Toxicological properties of the Cd$^{2+}$ core are reduced by capping quantum dots with ZnSe, varying chain length and functional group ligands. Fluorescence wavelength and their size is improved by varying Cd$^{2+}$ source and varying nanocrystal synthesis growth temperature. CdSe/ZnSe quantum dots are characterized with FT-IR to elucidate their structure. High-resolution transmission electron microscopy (HRTEM), X-Ray Diffraction (EDX), Photoluminescence spectroscopy (PL) and Ultraviolet-visible spectroscopy (UV-Vis) are used to measure their size and composition.

Ligand exchange reactions are conducted with the use of 3-Mercaptopropanoic acid (3-MPA) to facilitate biocompatibility and stability of CdSe/ZnSe QDs. Temperature stability of various ligand capped and stabilized CdSe/ZnSe QDs are measured by using thermogravimetric analysis (TGA). Caco-2 cell line is cultured from colon cancer, and cytotoxic studies are conducted to test for cell viability of various capped 3-Mercaptopropanoic acid (3-MPA) CdSe/ZnSe quantum dots at various concentrations.

Myristic acid capped CdSe/ZnSe quantum dots produce high fluoresenting mono-disperse quantum dots. The capping material, synthesis temperature and Cd$^{2+}$ source of CdSe/ZnSe QDs affect fluorescence wavelength and thermal stability of quantum dots. Fluorescence wavelength is improved by using CdCl$_2$.7H$_2$O source of Cd$^{2+}$. Cytotoxicity was found to be dependent on the
concentration and the capping material of quantum dots. CdSe/ZnSe quantum dots toxicity is adjusted and reduced by varying the length, size and type of the capping ligand on the surface of quantum dots.
Declaration

I Anati Nomxolisi Nkaule, student number 212462725, hereby declare that the treatise for Master of Science in Nanoscience-Chemistry to be awarded is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

Anati Nomxolisi Nkaule  
December, 2013

Signed

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### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tr>
<td>Ab</td>
<td>Antibody</td>
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<tr>
<td>α-CDs</td>
<td>Alpha-cyclodextrins</td>
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<tr>
<td>CDCI$_3$</td>
<td>Deuterated chloroform</td>
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<td>CdSe</td>
<td>Cadmium selenide</td>
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<td>ODE</td>
<td>1-Octadecene</td>
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<td>PL</td>
<td>Photoluminescence</td>
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<td>QD</td>
<td>Quantum dot</td>
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<tr>
<td>SXRD</td>
<td>Synchrotron X-ray diffraction</td>
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<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
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<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TOP</td>
<td>Tri-n-octylphosphine</td>
</tr>
<tr>
<td>TOPO</td>
<td>Tri-n-octylphosphine oxide</td>
</tr>
<tr>
<td>TPPM</td>
<td>Two pulse phase modulated</td>
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<tr>
<td>VB</td>
<td>Valence band</td>
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<tr>
<td>WZ</td>
<td>Wurtzite</td>
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<tr>
<td>XPS</td>
<td>X-ray photo electron spectroscopy</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
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<tr>
<td>MTT</td>
<td>3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide</td>
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<tr>
<td>UV-Vis</td>
<td>Ultraviolet–visible spectroscopy</td>
</tr>
<tr>
<td>C-H</td>
<td>carbon-hydrogen bond</td>
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<tr>
<td>cm</td>
<td>centimeter</td>
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<td>cm$^{-1}$</td>
<td>wavenumber</td>
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<tr>
<td>COO</td>
<td>carboxylate</td>
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Conference and oral contributions


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Chapter

Introduction and overview of the study
1.1 Motivation

The research reported herein is motivated by the urgent need to find alternative colon cancer early diagnosis and targeted drug delivery medication. Colon cancer is one of the most invasive malignancy that lead to mortality around the world. Colon cancer is not only elusive, but also immune to preventative measures (Wagner et al., 2006).

One of the causes of colon cancer is the transformation of normal colonic epithelium that progresses in multiple steps to adenomatous polyp, which suddenly form an invasive cancer over years. Figure 1.1 (below), shows various stages of polyp development, before and after removal of polyp. This transformation is accompanied by a number of recently elucidated genetic alterations. Initially, malignant phenotype is generated from mutations in two classes of tumour suppressor genes, proto-oncogenes and genes, are thought to have an advantage for proliferation to cells (Akhtar-Zaidi & Corradin, 2012). Other various factors that lead to the development of cancer are growth in population and cell mutation (Wheeler et al., 2013).

Throughout the world, research to cure cancer is one of the a key priorities for governments, non-governmental organizations, activist organizations and citizens across the entire world because of the increase in population that is affected by cancer and a number of death cases that continue to take place (Iwaniak, Coetzee & Cooper, 2012). The world’s medicine demand is projected to double by 2050, particularly due to progressive, advanced occurring diseases that are challenging to treat (Wagner et al., 2006). There is a tremendous belief that advancing technology will lead to new types of diseases due to the potential risks of carcinogenic chemicals that are used, which become extremely toxic to living organisms (Carley & Porter, 2011).

Furthermore, side-effects caused by the treatment have been reported. The typical form is cis-diammine dichloro platnum(II) (Rozenceweig, Hoff & Slavikand, 1977), also known as cisplatin compound, is one of the reported testicular drug with a variety of side-effects and limitations. Limitations of these conventional drugs are drug resistance (Florea & Basselberg, 2011), nausea, vomiting, neurotoxicity and nephrotoxicity due to the action of thiol groups on biomolecules, such as glutathione with cisplatin (Dong et al., 2008). Also, there is a risk, associated with surgical removal of the polyp. Surgical removal leads to formation of scars, pain and loss of life due to surgical side effects.
Figure 1.1: Shows (a) the structure of the colon with colon cancer, (b) stages of polyp development, (c) before removal, removal and after removal of polyp, and (d) post colon cancer surgical removal treatment.

Quantum dots (QDs) show extra-ordinary unique properties than conventional imaging and therapeutic tools, such as unique optical and electrical properties of different character than those of the corresponding bulk material due to their nano-scale dimensions (Wise, 2000). The apparent curiosity in its properties is stimulated by the ability of photons to be emitted under excitation which is visible to the human eye. The wavelength of photon emission is dependent on both the type of material which the core is made up of and the size of the quantum dots (Parak, Ellegrino & Plank, 2005). Quantum dots are able to be tuned during the synthesis process by changing various parameters to give nanoparticles that emit the desired colour of light (Bera et al., 2010).

Success in large volumes of QDs (e.g. one kilogram) will yield sufficient actual quantum dots for industrial scale applications. New forms of quantum dots with additional properties are sought to continuously improve biological applicability of QDs without being toxic to the biological cells (Chan & Bhatia, 2004).
1.2 Challenges and limitations

Toxicity of Cd\(^{2+}\) based quantum dots present plausible and incompletely assessed risks (Lovric, 2005). Therefore, a thin margin exists between safe applicable limits and cytotoxic unacceptable limits. Quantum dots that are composed of Cd\(^{2+}\) are known to be carcinogenic to humans. Cd\(^{2+}\) toxicity is associated with kidney and liver injury, osteoporosis, osteomalacia, skeletal deformations and neurological diseases (Rzgalinski, 2009). Therefore, cadmium is classified as a hazardous waste, and needs to be treated as such (Rzgalinski & Strobl, 2009).

A number of publications have demonstrated that particle size of quantum dots affects toxicity at the intracellular and animal level. Zhang (2007) claims that 2.2 nm CdTe QDs are more toxic than larger 5.2 nm particles to biological cells. In order to use quantum dots for biological imaging, toxicological considerations must be made. Quantum dots that are based on heavy metal chalcogenide compounds, such as CdSe and CdTe, can leach heavy Cd\(^{2+}\) metals into the body tissues, thereby limiting their intended function. To resolve this limiting problem, a non-heavy metal shell, such as ZnS, ZnTe or ZnSe are used as a protecting barrier (Rzgalinski & Strobl, 2009; Swalec, 2011).

Biological tests have proved that conjugated quantum dots go to cells containing their target molecule, without attaching to non-targeted cells (Chan & Nie, 1998). Biological tests have also proved that QDs used in biological tests can still be present in the body environment, four months later. If dots are going to remain in the body for this long, toxicological effects of QDs exposure must be known. There is a need for research to be performed on the disposal of quantum dots after they have been used (Rzgalinski & Strobl, 2009).

Dose parameters of QDs for use in humans are not set, though it is known that QDs can remain in the body for extended time period (Rzgalinski & Strobl, 2009). But since there are no set standards in the production methods of quantum dots, a specific standards dosage would be difficult to define. QDs need to be fully characterized before such standards can be set (Rzgalinski & Strobl, 2009; Swalec, 2011).
1.3 Quantum dots (QDs)

1.3.1 What are quantum dots

Quantum dots are light-emitting semiconductive nanocrystalline particles that have even artificial atoms, unique characteristics and various functions. Quantum dots are typically made up of elements belonging to the periodic groups of II-VI, III-V, or IV-VI (Murray, 2000; Norris, 1995 & Swalec, 2011).

Hanson (2007) identified QDs as artificial structured materials that have a capacity to load electrons. Hardman (2006) describes them as having a narrow spectrum shape and an ability to be excellent tagging alternative systems than conventional fluorescent dyes. Furthermore, Hardman (2006) describes their unique, special physicochemical properties as being different from other naturally occurring biogenic and anthropogenic family of nanoparticles. The unique properties of QDs are inherently associated their 2 to 10 nm small size and discrete composition of atoms with several hundred to a few thousands of atoms. Atoms behave differently at a small length-scale, and this give them novel electronic and optical properties that are attributed to a phenomenon known as quantum confinement (Dabbousi, 1997).

Alivisatos (2004) illustrates quantum dots as systems that have captivated biomedical field research in the last decade, due to their unique size-tunable emission spectra, optical properties, improved brightness, superior photo-stability, and simultaneous excitation of multiple fluorescence colours. Gao (2002) describes QDs as being successful biological systems when compared to organic dyes and fluorescent proteins because of their ability to act as biological conjugates. Quantum dots properties allow them to be used as various imaging tools, fixed cell labeling, imaging of live cell dynamics, in-situ tissue profiling, fluorescence detection, sensing and in-vivo animal imaging (Nie, 2007).
1.4 Discovery of quantum dots

Two independent groups in Russia were the first to discover QDs. QDs were isolated in 1981 by Alexei I. Ekimov in a glass matrix. Again, in the United States they were found by Louis E. Brus & Alexander Efros within colloidal solutions. The term “quantum dot” was later adopted by the American physicist Mark A. Reed to portray the fact that they are perceived to be a special class of semi-conductors that have confined three spatial dimension excitons, with small physical size of the particles (Reed, 1998). This confinement makes them possess properties that range between those of bulk semi-conductors and discrete molecules (Norris, 1995 & Murray, 2000).

1.4.1 Importance of researching quantum dots (QDs)

Olivier (2010) describes that in 2010, the global market for QDs was worth an estimated US $ 67 million in revenues. The study estimates the market to increase over the next 5 years at a compound annual growth rate (CAGR) of 59.3%, reaching almost US $ 670 million by the year 2015, with a tenfold increase. Optoelectronics represent one of the greatest successful market sectors. This area was launched in 2010 and is expected to increase at a 128.4% compound annual growth rate (CAGR) to reach a value of US $ 310 million in 2015.

The biomedical sector was valued at US $ 48 million in 2010. This sector is expected to increase at a 30% compound annual growth rate (CAGR) to reach a value of US $ 179 million in 2011 (Olivier, 2011). Figure 1.2 shows the global market revenue for QDs in promising market sectors, 2009–2015 (millions) (Olivier, 2011).
1.4.2 Advantage of quantum dots over fluorescent dyes

Chan & Nie (1998) describe quantum dots as being much more stable against photo-bleaching than fluorescent dyes. Furthermore, they emphasize that they are much brighter. The fluorescence intensity of a single CdSe QD is equivalent to that of ~20 rhodamine molecules. Overall, quantum dots are considered to be more photostable than alternative fluorescent dyes, more especially for biological use (Reiss, 2003).

As compared to quantum dots, fluorescent dyes exhibit both narrow excitation ranges, accompanied by a wide and symmetrical fluorescent spectrum that trails into the red region. This property exhibition makes analysis difficult during the use of multiple fluorescent dyes because the tail overlaps with other peaks, artificially increasing or decreasing the fluorescent intensity. The quantum dots produce ideal properties and characteristics for use in multi-tests, that are a narrow and symmetrical fluorescence spectrum (Bruchez, 1998).
1.5 Properties of quantum dots

1.5.1 Fluorescent properties

Fluorescent colour of a quantum dot is tied directly to its size, and size is a direct function of growth. Purity of color or monodispersity is therefore also dependent of size and growth (Qu & Peng, 2002), and can be measured by the full-width half-maximum of the fluorescent spectrum. Full-width half-maximum is independent of the peak fluorescent wavelength. QD nanocrystals have a higher full-width half maximum than that of single particles, meaning that not all the dots in a sample are the same size and color (Qu & Peng, 2002).

Fluorescent intensity can be measured by the photoluminescent quantum yield of the quantum dots, which varies between synthesis methods (Qu & Peng, 2002). Brightness of a dot can be improved by adding another II-VI layer, such as ZnS (Talapin, 2001). The same could be achieved by exchanging the capping ligands for primary amines (Talapin, 2001). The quantum yield (QY) of quantum dots varies greatly with the synthesis method. Quantum dots have been reported with QY at a percentage of fifteen for green, six for red, fifty and all the way up to eighty percent (Bruchez, 1998; Qu & Peng, 2002; Talapin, 2001 & Peng, 2002) stated that even a spectacularly low fluorescence of approximately 5% is sufficient for use in biological tests.

1.5.2 Absorption Properties

Various combinations of Group II-VI have different characteristic absorption spectrum shapes (Murray, Norris & Bawendi, 1993). CdSe quantum dots have a spectrum that has three bumps as the absorption asymptotes (Swalec, 2011). CdS quantum dots have a spectrum that has two bumps mean while CdTe quantum dots have an absorption spectrum that has one bump. Furthermore, sharp absorption spectrums suggest highly monodisperse samples (Murray, Norris & Bawendi, 1993).

1.5.3 Dimension properties

Nose et al (2006) state that “The size and shape of quantum dots is directly dependent upon growth time, and depends at least in part upon the amines added”. To grow QDs, heat must be applied to the solution. Swalec (2011) along with Murray Norris and Bawendi (1993) discovered
that size and size distribution are also dependent upon growth temperature (Murray, Norris & Bawendi, 1993).

Increased overall growth rate leads to fast formation of quantum rods, while quantum dots will form if it is slow. Pure TOPO growth was so fast that quantum rods always formed. If an impurity, such as hexyl-phosphonic acid (HPA), was added, growth slowed enough that quantum rods would form. And just like quantum dots, the growth rate and so size of quantum rods can be controlled by time and other variables (Peng, 2000; Chen, 2002).

Quantum rods were proved to be very similar to quantum dots in their fluorescence property. Also their orientation and alignment properties that dots do not have (Peng, 2000; Chen, 2002). As the size of a quantum dot increases, so does the fluorescent peak wavelength, meaning the dots shift from blue to red as their size increases (Idowu, Lamprecht & Nyokong, 2008).

Quantum dots that fluorescence in the orange and red regions are difficult to make. Qu & Peng (2002) found that stearic acid is a good capping material for growing both orange and red-fluorescing dots. These dots could also be grown at high temperatures, which is necessary for the growth of quantum dots. For QDs, dots that fluoresce in the red range, it was found that primary amines are essential as capping agents, the fluorescent quantum yield in these regions is naturally low (Qu & Peng, 2002).

1.6 Composition materials for quantum dots

The composition material of quantum dots are semi-conducting materials. As it applies to LEDs, the importance for radiative recombination of electrons and holes to generate light means that only bandgap materials that are direct can be utilized to produce fluorescent quantum dots. Overall composition of quantum dots are ordinarily made from group III-V and group II-VI semiconductors, such as the following: CdSe, CdS, InP, and ZnS.

The band-gap of the quantum dot composition material is very important due to it’s influence to its properties. Various composition materials are utilized to make quantum dots in order to modify or adjust required properties that are suitable for the application. The first ever produced quantum dots were made primarily from group II-VI semi-conductors, such as cadmium and zinc chalcogenides. Table 1.1 shows the important parameters of bulk semiconductors commonly used for quantum dots.
### Table 1.1: Important parameters of bulk semiconductors commonly used for quantum dots

<table>
<thead>
<tr>
<th>Material</th>
<th>Structure [300K]</th>
<th>Type</th>
<th>Energy gap [eV]</th>
<th>Lattice parameter [Å]</th>
<th>Density [kg m(^{-3})]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnS</td>
<td>Zinc blende</td>
<td>II-IV</td>
<td>3.61</td>
<td>5.41</td>
<td>4090</td>
</tr>
<tr>
<td>ZnSe</td>
<td>Zinc blende</td>
<td>II-IV</td>
<td>2.69</td>
<td>5.668</td>
<td>5266</td>
</tr>
<tr>
<td>ZnTe</td>
<td>Zinc blende</td>
<td>II-IV</td>
<td>2.39</td>
<td>6.104</td>
<td>5636</td>
</tr>
<tr>
<td>CdS</td>
<td>Wurtzite</td>
<td>II-IV</td>
<td>2.49</td>
<td>4.136</td>
<td>4820</td>
</tr>
<tr>
<td>CdSe</td>
<td>Wurtzite</td>
<td>II-IV</td>
<td>1.74</td>
<td>4.3</td>
<td>5870</td>
</tr>
<tr>
<td>CdTe</td>
<td>Zinc blende</td>
<td>II-IV</td>
<td>1.43</td>
<td>6.096</td>
<td>6095</td>
</tr>
<tr>
<td>GaN</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>3.44</td>
<td>3.55</td>
<td>4138</td>
</tr>
<tr>
<td>GaP</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>2.27</td>
<td>5.45</td>
<td>5318</td>
</tr>
<tr>
<td>GaAs</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>1.42</td>
<td>5.653</td>
<td>5614</td>
</tr>
<tr>
<td>GaSb</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>0.75</td>
<td>6.096</td>
<td>5870</td>
</tr>
<tr>
<td>InN</td>
<td>Wurtzite</td>
<td>III-V</td>
<td>0.8</td>
<td>3.545</td>
<td>6810</td>
</tr>
<tr>
<td>InP</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>1.35</td>
<td>5.869</td>
<td>4787</td>
</tr>
<tr>
<td>InAs</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>0.35</td>
<td>6.058</td>
<td>5667</td>
</tr>
<tr>
<td>InAs</td>
<td>Zinc blende</td>
<td>III-V</td>
<td>0.23</td>
<td>6.479</td>
<td>5774</td>
</tr>
<tr>
<td>PbS</td>
<td>Rocksalt</td>
<td>IV-VI</td>
<td>0.41</td>
<td>5.936</td>
<td>7597</td>
</tr>
<tr>
<td>PbSe</td>
<td>Rocksalt</td>
<td>IV-VI</td>
<td>0.28</td>
<td>6.117</td>
<td>8260</td>
</tr>
<tr>
<td>PbTe</td>
<td>Rocksalt</td>
<td>IV-VI</td>
<td>0.31</td>
<td>6.462</td>
<td>8219</td>
</tr>
</tbody>
</table>

The majority of group II-VI and group III-V semiconductor materials, crystallize in both their hexagonal wurtzite or cubic zinc-blende form as described in Figure 1.4 below. For ZnSe and CdTe materials, there is very little difference in energy between the zinc-blende and wurtzite structures, and so they can manifest into wurtzite-zincblende polytypism form (Reiss, 2003).
Depending on the controlled synthesis parameters or conditions, these nanocrystals may crystallize in either structure form or both forms may co-exists in the same nanoparticle. Pb chalcogenides are known to crystallize into forming a rock-salt structure, also CdSe quantum dots have been observed to crystallize into this rock-salt structure, considerably if the diameter exceeds 11 nanometer. Figure 1.4 illustrates the crystal structures of quantum dots.

![Figure 1.4: Illustrates the crystal structure of quantum dots (A): Wurtzite and (B): Zinc-blende crystal structures.](image)

### 1.6.1. The quantum dot core

The most synthesized QDs are composed of the core inner material which is an assembly of group II-VI elements. The most popular used composition elements of the core, are cadmium and zinc. The most commonly used group VI column elements in from the periodic table are selenium, tellurium, and sulfur. Prabably, the most studied combination is CdSe, along with CdTe close behind. ZnS is also dominant in research (Reiss, 2003).

Chan & Nie (1998) contends that the group II-VI core has a tendency to flash like a fire-fly, with very high fluorescence. Therefore, quantum dots are coated with an outer layer so as to make the fluorescence constant. The outer layer for coating quantum dots is either a different II-VI compound, or a buffer, also known as a stabilizer or capper. Thioglycolic acid (TGA, also called mercaptoacetic acid), trioctylphosphine (TOP) and trioctylphosphine oxide (TOPO) are commonly used stabilizers (Talapin, 2001). This second layer prevents or reduces the dots from flashing like a fire-fly and adjusts the properties of the dots, such as either increasing fluorescence or making them water soluble (Chan & Nie, 1998).
1.6.2. Core-Shell for quantum dots (QDs)

Production of Core-Shell quantum dots is motivated by their nanometer diameter size. This gives them a very high surface-to-volume ratio property for approximately 80% of the atoms that reside on the surface. There is a significant effect on optical and structural properties of the particles due to this high surface-to-volume ratio. Additionally, Reiss & Peter (2009) contend that “surface defects, such as dangling bonds, are surface-related trap states that act as non-radiative recombination sites which degrade the fluorescence quantum yield of quantum dots”.

The organic ligands that surround colloidal quantum dots possess some extent for chemical reactivity surface reduction, but do not provide sufficient protection from the surrounding environment or complete passivation of surface defects (Reiss, Peter, Myriam & Li, 1999). To improve surface reactivity, a secondary semi-conductive shell layer can be epitaxially grown surrounding to surround the core particle. The coating of the core process or outcome improves the quantum yield to as much as up to ten times, and also leads to increased stability against photo-oxidation, uv-illumination and environmental attack (Grabolle, Markus, Ziegler, Merkulov & Nann, 2008).

![Illustration of CdSe quantum dot prior and after coating with ZnS.](image)

1.6.3 Capping agents and conjugation of quantum dots (QDs)

One of the significant advantages of capping quantum dots with either a buffer or group II-VI layers include reducing the toxicity of the core (Rzigalinski & Strobl, 2009), improving or increasing their quantum yield (Reiss, 2003) and modifying them to be water soluble (Bruchez, 1998).
Nose (2006) tested different capping amines for CdSe quantum dot, so as to evaluate their various fluorescence properties. The end result was a shifting fluorescent blue peak as the chain length of capping amines increased. Additionally, the correlating results proved that as the chain length increases, the particle size reduces (Nose, 2006). Amine chain length is non-dependent on fluorescent intensity, meaning that the size of a peak fluorescent wavelength is not proportional to the class of amines (Nose, 2006).

To be specific, bulky molecules have a slower diffusion rate which proves that quantum dots that are capped with bulky molecules tend to grow slowly, resulting to smaller diameter size. The particle size depends on both the molecular mass and the stereo-chemical shape of the amines, which affect the diffusion rate. Additionally, Nose (2006) concluded that the fluorescent intensity depends on pressure (kPa), and also that dissociative properties become weaker as the intensity increases. In other words, changing the type of used capping amines adjusts the fluorescence color and intensity of the quantum dots.

Quantum dots can be conjugated to other molecules. This greatly expands the uses and applications of quantum dots. Molecules that have been successfully conjugated include antibodies and other proteins and peptides. Tests have shown that conjugation did not cause the QDs to aggregate, nor did the ‘optical properties’ of the dots change (Chan & Nie, 1998; Chen, 2007). There are several ways to conjugate quantum dots. One is to cap the dots with avidin and utilize the ‘avidin-biotin system’ to connect the dots to an antibody (Goldman, 2002). Goldman (2002) were able to successfully conjugate the quantum dots, and show that the dots were functional in immunoassays.

Another conjugation method is through coordinating the carboxylic group of the thiol with the amine group on BSA. This method was used to conjugate CdTe dots capped with TGA, L-cys, or 3-mercaptopropionic acid to bovine serum albumin. This also proved to be a successful conjugation method, and though the conjugated QDs were observed to have slightly decreased absorption intensity, the emission intensity was increased. It was also found that conjugating BSA to quantum dots decreased the fluorescence of the bovine serum albumin (BSA), but not that of the QDs (Idowu, Lamprecht & Nyokong 2008). Figure 1.6 shows the conjugation of a quantum dot.
1.7 How do quantum dots (QDS) function?

1.7.1 Semiconductive properties

Quantum dots work by using their semi-conductive properties, resulting in quantum dots electronic properties. The process whereby both the conduction bands and valence bands overlap in both metals and other conductors, with exclusion of a significant energy barrier for elevating electrons from the valence to the conduction band result to a semi-conductive material (Meystre & Sargent, 2007).

There is a large energy barrier for elevating electrons from the valence to the conduction band inside insulators, this eliminates conduction. There is intermediate conduction between conductors and insulators within semiconductors as presented by the figure below. Figure 1.7 is an illustration of the conduction for metals, semiconductors, and insulators energy band. Band-gaps (Eg) for metals, semiconductors, and insulators are greater than 4 eV, ranging between 0.5 and 3.5 eV, below 0.1 eV, respectively (Ashcroft & Mermin, 1976).
Quantum confinement properties

Quantum dots also function by possessing a tunable band-gap due to a concept called quantum confinement. To understand quantum confinement, we need to look at how energy bands work in atoms and work our way up to the bulk scale. Atoms have degenerate, discrete energies at which electrons can reside, allowing more than one electron to reside in a single energy level. When atoms are brought together, their electron clouds start to interact and the degenerate states split into different energy levels (Fox, 2010).

Once the number of atoms interacting reaches the bulk level, the states are split into so many energy levels that the states can be considered continuous because the spacing between energy levels is infinite. Furthermore, Fox (2010) contends that the excitons are confined to a space smaller than the exciton Bohr radius, or the spatial separation between the electron and the hole left behind when it jumps the band-gap, less states become available. This continues until excitons are confined in all three dimensions, at which point the energy levels become discrete (Figure 1.8).
Fox (2010) states that at this scale, quantum dots act similarly to large molecules; adding or subtracting single orbitals can shift the energy levels in the material, changing the band-gap and making their emission tunable. This occurs when all three dimensions of a particle are smaller than the exciton Bohr radius (Figure 1.9).
size and its resultant band-gap, based on the material being used and its band-gap in the bulk form in Figure 1.10 and Equation (1.1).

Figure 1.10: A quantum dot exhibits bandgap tunability.

In the equation, $E_{g}^{QD}$ is the theoretical band-gap of the quantum dot, $E_{gbulk}$ is the band-gap of the bulk material, $h$ is Planck’s constant, $r$ is the radius of the nanoparticle, $m_o$ is the electron mass, $m_e^*$ is the effective mass of the electron for the material, $m_h^*$ is the effective mass of the hole for the material, $e$ is the charge of the electron, $\varepsilon_o$ is the permittivity of free space, and $\varepsilon$ is the permittivity of the material.

$$E_{g}^{QD} = E_{gbulk} + \frac{1}{(8m_o r)} \left( \frac{1}{m_e^*} + \frac{1}{m_h^*} \right) - \frac{1.8e^2}{4\pi\varepsilon_o r} \quad (1.1)$$

The first term is based on the properties of the bulk material, the second term is based on the particle-in-a-box confinement of the exciton, and the third term is based on the Coulombic attraction between the electron and the hole. While it is not a perfect fit to experimental values, what we can see from this equation is that the band-gap, and therefore the wavelength of light emitted, changes significantly with small changes in particle size (Dissanyake, 1995).

1.7.3 Fluorescence properties

Quantum dots also function by utilizing their fluorescence properties. When an incoming photon of sufficient energy, greater than the band-gap of the material, is absorbed by the material, an electron is excited from the valence band to the conduction band, forming a hole in the valence band. When the electron relaxes back down to the valence band, recombining with the hole left behind by its absence, a photon is emitted, with energy proportional to the band-gap of the
material (Figure 1.11). This mechanism is why a quantum dot can absorb all wavelengths of light greater than its band-gap and down-convert it to a specific wavelength (Ekimov & Efros, 1990).

![Mechanism of excitation and emission due to radiative recombination of an electron and hole.](image)

**Figure 1.11: Mechanism of excitation and emission due to radiative recombination of an electron and hole.**

### 1.8 Synthesis and characterization techniques

#### 1.8.1 History for the synthesis of quantum dots (QDs)

The history of quantum dot synthesis reaches back to glass blowers inadvertently nucleating quantum dots of cadmium and zinc species in glasses. Glass workers added cadmium and zinc sulfides and selenides to the melt to create glasses with rich yellow, orange, and red hues, producing very small concentrations of quantum dots. More recently in the 1980s, this process was controlled more directly, but still required extremely high temperatures and control was very limited (Hines & Guyot-Sionnest, 1996).

Once molecular beam epitaxy became popular in research institutions, it was used to deposit very thin layers of semiconductor materials, creating quantum wells which exhibit quantum confinement in one dimension but not the other two. Moreover, Hines & Guyot-Sionnest (1996) argues that by depositing semiconductors on substrates with a large degree of lattice mismatch, it
was found that the layer would bead up into droplets, forming quantum dots. However, this approach limited size dispersions to greater than 10%.

Another direction was sought for quantum dot synthesis, especially focused on size control. In this method, quantum dots were synthesized within micelles, limiting their growth to the size of the micelle. While this method did not require high temperature, organic solvents, or complicated equipment, the size distribution was poor and the concentration was limited, as well as the quantum dots exhibiting poor crystallinity and a large degree of defects (Gerion, 2001).

The major breakthrough that made quantum dot synthesis easier and more controllable was the advent of nucleation and growth techniques to synthesize quantum dots in high temperature organic solvents. In nucleation and growth processes to make quantum dots, ionic sources of the constituent materials are needed, such as Cd$^{2+}$. These methods utilized the pyrolysis of organometallic precursors to produce monodisperse (less than 5% size dispersion) quantum dots made of cadmium chalcogenides (Hines & Guyot-Sionnest, 1996).

Figure 1.12: Nucleation mechanism of quantum dots crystals.

In this nucleation and growth process, an excess of organometallic precursors, such as dimethylcadmium and selenium-trioctylphosphine (Se-TOP) were injected into a hot solution of coordinating solvent, such as a mixture of trioctylphosphine and trioctylphosphine oxide (TOP/TOPO) at over 280°C, supersaturating the solution. During the first few seconds following the injection, particles nucleate homogeneously depleting the reactants, followed by particle growth, Ostwald ripening, and eventually saturation of the solution (Figure 1.12). This procedure was the first to result in quantum dots with sufficiently high quantum yield, 10% and 20% range,
coordinated with organic ligands stabilizing the colloid, as well as producing mono-disperse nanoparticles (Hines & Guyot-Sionnest, 1996).

Since the development of a nucleation and growth technique for generating quantum dots, almost all newer techniques have developed from it, changing solvents and precursors and increasing the quantum yield and mono-dispersity, as well as introducing greater control in the process. In 2002, a major development was made towards using “green chemistry” to generate quantum dots. The pyrolysis of organometallic precursors produces quantum dots of high quality, except that the precursors are not air-stable but are pyrophoric and extremely toxic. Additionally, the reaction was not very tunable, so there was less balance between nucleation and growth controlled (Hardikar & Matijevic, 2000).

A non-coordinating organic solvent octadecene (ODE) in the presence of the surfactant oleic acid and cadmium oxide as a Cd$^{2+}$ source, and a solution of elemental sulfur and ODE as the sulfur source is a “green” method that was developed by the Peng group. Not only were the precursors air-stable and less toxic than organometallic precursors, but the reaction could be tuned by varying the concentration of oleic acid.

**Figure 1.14:** Absorbance of quantum dots produced using the CdO/ODE/OA method, showing tunable reactivity of the precursors through adjustment of the ligand concentration
1.8.2 Fabrication of quantum dots

QDs can be fabricated by using various methodologies, that are metal organic chemical vapor deposition (MOCVD), molecular beam epitaxy (MBE), colloidal synthesis and electron beam lithography (EBL). Each one of these methodologies had both advantages and disadvantages for various kinds of applications. Synthetic techniques can be grouped into two major categories: either top-down approach or bottom-up approach techniques, with each category comprising of different sub-groups as illustrated below, in Figure 1.15 Schematic of different methods for synthesizing quantum dots (Gerion & Pinaud, 2001).

Figure 1.13: Nucleation and growth of nanoparticles in a solution of hot organic solvents.
1.8.2.1 Electron beam lithography

Electron beam lithography technique is used to create extremely, perfect fine patterns that are used in the electronics industry for manufacturing advanced integrated circuits. This takes place by scanning a beam of electrons in a rastered and patterned manner across a surface that is covered with an electron sensitive film. This is followed by removing either the exposed or non-exposed areas of the resist. The aim of the electron beam lithography technique is to create tiny structures in the resist that can be later transferred to the substrate material by the etching process (Huffaker et al., 2000).

Elarde et al., (2005) describes electron beam lithography as a technique that consists of etching pillars which are located within electron well hetero-structures. This process utilizes the electric potential to deplete either the electron or hole ‘gas’ in the heterostructure, thus forming three-dimensional confinement for the electrons/holes. The quantum well hetero-structures provide confinement in one direction, whereas the pillars provide confinement in the other two directions. This method yields smooth confinement boundaries, provides good control of the size and shape and forms regular and uniform array of well-defined QDs.

Furthermore Elarde et al., (2005) argues that the disadvantages of this method are that it is slow and has a high tendency of contamination; it also does not yield large quantities of QDs and any
synthesized QDs are bound to the substrate and as such cannot be removed. The fabrication process allowed for control of the position and emission wavelength of the QD thus enabling its incorporation into a semiconductor laser diode (Figure 1.16)

![Image](image_url)

Figure 1.16: The colour micrograph of QDs synthesized by electron beam lithography and etching technique.

1.8.2.2 Bottom–up technique

The most common method to produce QDs is through bottom up approach. This can be done either with chemical vapor deposition or colloidal synthesis. In vapor phase deposition, layering a desired material that doesn't match properly with the lattice of the substrate results in high strain at the interface, causing the layer to start nucleating into small QDs. This technique creates QDs in dense arrays that self-assemble in an orderly manner, but the uniformity of their size distribution is a major concern, as it is impossible to control their formation as strictly as the top down technique. In colloidal synthesis, molecular or ionic precursors to the QDs are allowed to react together in solution to produce QD materials as colloids (Qian & Bera, 2008).

1.8.2.3 Vapor phase deposition / self organized QDs

QD layers are being investigated for their application in storage devices (Nadarajah et al., 2008), infrared detectors (Schittenhelm et al., 1998), semiconductor lasers (Fafard et al., 1999 and quantum computation (Bonadeo et al., 1998).
Coherent optical control of the quantum state of a single quantum dot carrier confinement can be attained in these devices by embedding a smaller band-gap material within a larger band-gap material. Such confinement can be easily obtained in one dimension by the growth of very thin planar films by methods as chemical vapor deposition (CVD) or molecular beam epitaxy (MBE) (Borovitskaya et al., 2002; Shur et al., 2002). With these methods the layer thickness can be controlled efficiently on the Ångstrom scale and is very efficient and cost effective when compared to the electron beam lithography.

One of the methods of forming self assembled QDs is the Stranski-Krastanov (S-K) growth method, in which one semiconductor is deposited on another semiconductor layer with a large lattice mismatch between them. Epitaxial growth initiates in a layer-by-layer fashion and the lattice mismatch strain is minimized by the formation of several nanometer sized 3-D islands by a thin wetting excited States in InAs Self-Assembled Quantum Dots. These islands are highly crystalline and mostly of pyramidal shape of 100-300 Å in the base dimensions and 20-80 Å height (Ribeiro, Garcia & Petroff, 1997).

Charging Dynamics in InAs Sel-assembled quantum dots. These islands grow coherently on the substrate without generating misfit dislocations until a certain critical strain energy density corresponding to a critical size, is exceeded (Eaglesham et al., 1990). The strain of the substrate is relieved beyond this critical size by the formation of dislocations near the edges of the islands (Nozik et al., 2010).

Even though tension provides a successful mechanism for increased generation of dislocation-free 3D islands, there are still many other needs that should be fulfilled in order to utilize these islands as QDs (Schittenhelm et al., 1998). As the electronic features of QDs are directly proportional to the size and shape, it becomes necessary to understand the influence of growth parameters like temperature or total coverage on physical properties. The major set-backs of this technique are the mono-dispersity size of QDs produced typically about ±10% and the lack of control over the positioning of individual QDs (Ribeiro, Garcia & Petroff, 1997).

This size distribution gets augmented with the possible distribution in strain, composition and structural shape, giving rise to large non-uniformity of the properties of QDs and inhomogeneous broadening of the optical spectral features thus degrading the performance of QD devices (Schmidt, 1996; Ribeiro, Garcia & Petroff, 1997). Some of the semiconductor systems in which this kind of self-assembled growth has been achieved are Ge/Si (Eaglesham, and Cerullo, 1990), CdSe/ZnSe (Xin et al., 2000), InP/InGaP (Carlsson et al., 1994), InP/AlGaAs (Hanna et al., 1997).
InGaN/AlN (Yamaguchi et al., 1992), PbSe/PbEuTe (Springholz et al., 1998) and GaSb/GaAs (Hatimi et al., 1995).

In a report by Petroff (2001) the growth of InxGa1–xAs QDs within a GaAs substrate is described. As shown by the atomic force micrograph images in (Figure 1.17) the growth process for this system can be controlled to produce new QD shapes, dimensions, and lattices. The growth pattern changes from a random nucleation of islands in Figure 1.17 (a) into a periodic one by simple control of the QD nucleation on the surface.

Figure 1.17: AFM image of epitaxially grown islands on the surface of GaAs substrate (a) random nucleation of InAs islands, (b) Random distribution of InxGa1–xAs ring-shaped islands, (c) of InAs islands of a 2D lattice on a GaAs substrate.

1.8.2.4 Solvothermal synthesis

The solvothermal method is similar to hydrothermal synthesis except that organic solvents are substituted with water. The chemical reaction takes place in a stainless steel vessels or autoclaves. The synthesis process involves using a solvent under moderate to high pressures that range between 1 atm and 10,000 atm, and temperature range of 100°C and 1000°C. These conditions facilitates the interaction of precursors during QDs synthesis. This synthesis technique can be used to synthesize nanoparticles with a variety of morphologies such as spheres (3D), rods (2D), or wires (1D).
The solvothermal technique combines the advantages of both the sol-gel (Oliveira, Schnitzler & Zarbin, 2003), hydrothermal routes (Andersson et al., 2002) solvothermal synthesis enables for precise control over the size, shape, particle-distribution, and crystallinity of the nanoparticles. These characteristics can be modified by simply varying certain synthesis parameters, including reaction temperature, reaction time, type of solvent, type of surfactant and type of precursor. Figure 1.18 shows the schematic diagram of solvothermal synthesis set-up: (1) stainless steel autoclave (2) precursor solution (3) Teflon liner (4) stainless steel lid (5) spring.

Figure 1.18: Schematic diagram of solvothermal synthesis set-up: (1) stainless steel autoclave (2) precursor solution (3) Teflon liner (4) stainless steel lid (5) spring.

A recent study for solvothermal QDs synthesis, elucidated the degree to which this method is essential for controlling the size of the II–VI and III–V QDs (Chen et al., 2002). Synthesis of QDs typically needs a proper chosen source material soluble in a selected solvent and a surfactant that fabricates/caps/stabilizes, the QDs with simultaneous arrest of its growth. Many QDs applications are dependent on size, shape control, and solvothermal synthesis is a key technique for obtaining this control. QDs can be synthesized in various shapes including spheres, rods, tetrapods, and teardrops by simply controlling the temperature, concentration or reaction time (Chen et al., 2002).

Additionally, even a shell of one composition material (e.g. ZnS) can be synthesized with a core coverage of another nanocrystal (e.g., CdS) (Thongtem, Pilapong, & Thongtem, 2009). The core can be used as a seed to grow larger particles by adjusting the concentration after the initial
growth. QDs that can be used in biosensors require hydrophilic surface moieties to be compatible with biomolecules. Hydrothermally synthesized QDs are an excellent option in biotechnological applications because due to the presence hydrophilic surfaces (e.g., hydroxyl groups). The outcome of hydrophilic surfaces is the reduction of quantum yield, which is one of the necessary properties for their functionality. However, solvothermal methods can be used to synthesize QDs, which can be followed by the addition of hydrophilic surfactants.

1.8.2.5. Colloidal synthesis

Quantum dots have been modified by making use of expensive, sophisticated molecular beam epitaxy (MBE) and/ chemical vapor deposition (CVD) equipment. However this is changing with the introduction of colloidal QDs, synthesized by less expensive wet-chemical processes. Large bulk quantities of QDs can be generated in the colloidal “bottom-up” technique. In this wet chemical technique nanocrystal particles are made atom-by-atom, i.e. from molecules to clusters to particles (Yin, 2005).

Reduced or non-complete coordination of the surface inorganic atoms in the nanocrystal particle leads to the production of non-fully coordinated and highly reactive atomic “dangling bonds” on the surface atoms of the QDs, which leads to particle agglomeration. There are various ways to overcome this problem, one of them is passivating (e.g., capping) the exposed atoms with protective organic groups. The outermost layer of the encapsulating-capping agent helps to inhibit particle agglomeration, protecting the nanocrystal particles from the surrounding chemical environment and in many instances even providing a means of chemical bonding to other organic, inorganic or biological materials (Yan & Cui, 2010).

Yin (2005) argues that the capping agent or modifying agent is usually the solvent in which the synthesis of nanocrystal takes place and it consists of a Lewis base compound. The modifying agent consists of a material that is able to coordinate donor-type to the surface of the nanocrystal and may include mono- or multi-dentate ligands of the phosphines (triocetylphosphine, triphenolphosphine, t-butylphosphine), phosphine oxides (triocetylphosphine oxide), alkyl phosphonic acids, alkyl-amine (hexadecylamine, octylamine), aryl-amines, pyridines, long chain fatty acids, and thiophenones. If the capping agent provides has a chemical linkages to other inorganic or organic materials, a functional group is directed away from the surface of QDs and is present to bond or react with other present molecules, such as primary, secondary amines, alcohols, carboxylic acids, azides, or hydroxyl groups.
The most important issues related to the synthesis of high-quality semiconductor nanoparticles are particle uniformity, size distribution, quantum efficiencies, long-term chemical stability, and long-term photostability. For the synthesis of QDs, a seed molecular cluster is placed in a solvent in the presence of other precursors to initiate particle growth. The molecular cluster used as a seed mostly consists of the same elements as those required in the subsequent QD, or sometimes different elements that are not required in the final QDs, but which facilitate the seeding process. Some precursors may not be present at the beginning of the reaction process: however as the reaction proceeds and the temperature increased, additional amounts of precursors are periodically added to the reaction either drop-wise as a solution or as a solid (Lucey, 2005).

Li (2005) describes the production of nanocrystals from the precursor has to be conducted out under controlled conditions to ensure that the mono-distribution of the cluster compound is maintained throughout nanoparticle growth in order to obtain a monodisperse population of nanoparticles. As at this stage some clusters grow at the expense of others due to Ostwald ripening and it leads to nanoparticles of variant size. A schematic set-up of a colloidal synthetic reaction is shown in (Figure 1.19).

![Figure 1.19: Reaction set-up for colloidal synthesis of QDs](image)

The organometallic precursor route for the synthesis of CdSe QDs can solve this problem quite well. It involves high-temperature decomposition of an organometallic precursor such as
dimethylcadmium (Cd(CH₃)₂) in a coordinating solvent consisting of a mixture of trioctylphoshine (TOP) and trioctylphoshine oxide (TOPO) (Murray, Norris, and Bawendi, 1993). TOP acts as a solvent of the Cd²⁺ precursor and serves as the source of surface ligands that generate QDs. CdSe QDs with a narrow size distribution can be formed by separating the nucleation and growth stage. Even though this route generates high-quality QDs, it still possesses some challenges and limitations. For instance, (Cd(CH₃)₂) is extremely hazardous, pyrophoric, air-sensitive and is an expensive material. (Cd(CH₃)₂ may also be explosive during the reaction, when gas is released and this route needs the use of a complicated glove-box methodology. Peng’s research group previously, proposed the replacement of (Cd(CH₃)₂) with other Cd²⁺ source compounds such as CdO and cadmium acetate (Qu and Peng, 2001).

All discussed synthetic techniques were conducted, using the coordinating solvent TOPO and TOP. A technique that excludes the use of TOP is desired because, it is less expensive, less toxic and more environmentally friendly. Boatman et al., have proposed route that does not use TOP to synthesize CdSe QDs. A non-coordinating solvent 1-octadecene (ODE) and a surface-binding ligand, oleic acid are used during the synthesis of CdSe QDs (Boatman and Lisensky, 2005) (see Figure 1.20).

Figure 1.20: Colloidal suspensions of CdSe QDs with increasing size from left (1.8 nm) to right (4.0-nm). Bottom: Samples viewed in ambient light, Top: The same samples viewed under long-wave ultraviolet illumination. Adapted from (Boatman and Lisensky, 2005).
Sun et al., (2009) reported a method in which they used common vegetable oils as coordination solvents in the synthesis of high quality CdSe nanocrystals synthesized CdSe QDs using N, N-dimethyl-oleoyl amide instead of TOP and oleic acid as primary capping ligands and benzophenone as secondary ligands. Temperature dependence of optical properties and size tunability CdSe quantum dots via non-TOP synthesis (Wang et al., 2009). Schematic of the various possible modifications of QDs have been mentioned in (Figure 1.21).

![Surface modification and functionalization of QD's](image)

**Figure 1.21: Modification of surface of QDs.**

This introduction concludes all aspects of colloidal synthesis for QDs synthesis and also, there are still areas in which detailed study is not fully achieved. For instance no systematic investigation has been done on the effect of the modification/capping/fabricating materials with various chain lengths and functional groups on the surface of CdSe/ZnSe QDs, effect of temperature on various capped CdSe/ZnSe QDs, effect of Cd²⁺ source for growth of CdSe/ZnSe QDs and the effect of a capping layer on prevention of toxicity to caco-2 cell line.

### 1.9 Applications of quantum dots

Quantum dots find use in many applications that need strong, stable fluorescence with tunable emission. Quantum dots are primarily applied in biological studies and photovoltaics.
1.9.1 Imaging biological applications of QDs

One of the primary areas of commercialized research for quantum dots is in biological diagnostics and targetted drug delivery. Quantum dots size is approximately the same as a the size of the protein, making it easy for them to enter cells in a similar approach. Most fluorescent dyes are organic molecules such as xanthenes or rhodamine, and fluorescein. There are a couple of key issues with organic dyes that can be remediated with quantum dots. There is a strong relationship between the absorbance and fluorescence of organic dyes with molecular structures as shown in table 2.3. (below).

Various molecular structures and weights require excitation and detection at specific wavelengths. Organic fluorophores degradation occurs over time during excitation, referred to as photobleaching. Quantum dots exhibit photobrightening than photobleaching, with excitation process taking place for extended periods of time, allowing for long term imaging periods. Most quantum dots are only stable in organic solvents as prepared. To modify them to be soluble, quantum dots are usually encapsulated in a polymer shell or a micelle to make them soluble in aqueous solvents.

Another way is by attaching them to biotags or polymer. However, after all of the coatings and functionalization, the hydrodynamic diameter of a quantum dot can often be much larger than its core diameter, limiting the effectiveness of having such a small particle. The quantum dots are used in biological application for cytotoxicity studies, diagnostics and drug delivery.
<table>
<thead>
<tr>
<th></th>
<th>ORGANIC DYE</th>
<th>QUANTUM DOT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption spectra</td>
<td>Discrete bands FWHM 35 to 100 nm</td>
<td>Broad with steady increase toward UV wavelengths</td>
</tr>
<tr>
<td>Molar absorption</td>
<td>104 to 105</td>
<td>105 to 106</td>
</tr>
<tr>
<td>coefficient</td>
<td>Assymmetric FWHM 35 to100 nm</td>
<td>Symmetric Gaussian FWHM 30 to 90 nm</td>
</tr>
<tr>
<td>Emission spectra</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quantum yield</td>
<td>50% to 100%</td>
<td>10% to 80%</td>
</tr>
<tr>
<td>Fluorescence lifetime</td>
<td>1 to 10 ns</td>
<td>10 to 100 ns</td>
</tr>
<tr>
<td>Binding</td>
<td>Via functional groups following established protocols</td>
<td>Via ligand chemistry; few protocols available</td>
</tr>
<tr>
<td></td>
<td>Often several dyes bind to a single biomolecule</td>
<td>Several biomolecules bind to a single quantum dot</td>
</tr>
<tr>
<td>Size</td>
<td>~0.5 nm; small molecule</td>
<td>6 to 60 nm (hydrodynamic diameter); colloid</td>
</tr>
<tr>
<td>Photochemical</td>
<td>Sufficient for most applications</td>
<td>High Orders of magnitude higher than organic dyes</td>
</tr>
<tr>
<td>stability</td>
<td>Can be insufficient for high-light flux and long term imaging</td>
<td>Possible photobrightening</td>
</tr>
<tr>
<td>Toxicity</td>
<td>Very low to high, depending on molecule</td>
<td>Little known yet</td>
</tr>
<tr>
<td></td>
<td>Must prevent heavy metal leakage</td>
<td>Potential nanotoxicity</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Good, owing to defined molecular structure and established</td>
<td>Limited by complex structure and surface chemistry</td>
</tr>
<tr>
<td></td>
<td>characterization</td>
<td>Limited data available</td>
</tr>
</tbody>
</table>
1.9.2 Biological application of QDs

Cytotoxicity of QDs has been observed in a large number of in vitro studies affecting cell growth and viability. The extent of cytotoxicity has been found to be dependent upon a number of factors including size, capping materials, colour, dose of QDs, surface chemistry, coating bioactivity and processing parameters (Medintz et al., 2005).

Even if not inducing significant alterations in cell physiology, QDs can produce subtle alterations of function which may affect the quality of data derived from their use. A number of mechanisms have been postulated to be responsible for QDs cytotoxicity. These include desorption of free Cd\(^{2+}\) (QDs core degradation), free radical formation, and interaction of QDs with intracellular components. Examination of QD toxicity in a hepatocyte culture model showed that exposure of core CdSe to an oxidative environment causes decomposition and desorption of Cd ions. Such exposure during synthesis and processing played an important role in subsequent toxicity.

Addition of a silica (SiO\(_2\)) and ZnS shell can reduce oxidation, but is unable to eliminate it, particularly under concomitant exposure to UV light. The addition of ligand shells has also been observed to reduce Cd desorption, but again is unable to eliminate it under oxidative conditions, and ligand addition brings its own attendant problems as will be discussed. The generation of free radicals, particularly reactive oxygen species has also been seen to contribute to toxicity. Nicking of DNA was seen both in DNA incubated with QDs in the dark, and under UV exposure.

This was attributed to photo-generated and surface generated free radical exposure. CdSe core QDs induced apoptosis in a number of apoptotic pathways, and downregulation of survival signalling molecules. The composition of the core, and also the colour of the QD (a reflection of core size) appear to influence toxicity. These studies also observed that addition of a ZnS shell was beneficial, and that free radical generation was reduced. However, DNA nicking was the result of incubation of CdSe/ZnS QDs with a biotin ligand. The dependency of generation of free radicals on Cd\(^{2+}\) desorption is unclear, but is possible because Cd\(^{2+}\) has been shown to generate free radicals. A similar reduction in free radical generation as Cd\(^{2+}\) desorption is seen on addition of a ZnS shell. In addition to the effects of the QDs core, ligands added to render the probe biologically active may have toxic effects on cells. Mercaptopropionic acid (MPA) and mercaptoacetic acid (MAA), which are commonly used for solubilisation, have both been shown to be mildly cytotoxic.
Cysteamine and TOPO have been shown to have the ability to damage DNA in the absence of the QD core. PEGylated QDs have been shown to have reduced cytotoxicity, but modification of these to produce PEG-amine for biological activity renders them cytotoxic once again. Unfortunately, interpretation of information on cytotoxicity is difficult as a result of differences in cellular handling of QDs and the possible contribution of unexpected factors to toxicity.

The reduced cytotoxicity seen with QD-PEG compared with unmodified QDs has been found to be related to reduced uptake of these modified QDs, and not necessarily to an inherently reduced toxicity. The way in which QDs are handled by cells after uptake is also variable, and different intracellular fates are likely to contribute to different toxicity. Handling has been shown to be affected by size, colour and coating, and different handling has even been observed between QDs with the same coating but different emission wavelengths.

It is extremely difficult to estimate the true extent of QD cytotoxicity with this accumulated limited data, as in which factors contribute, effects they may have. Groups III–V QDs may provide a more stable alternative to groups II–VI QDs due to the presence of a covalent, rather than an ionic, bond, and have been proved to have lower cytotoxicity. However these QDs are difficult to prepare over a short space of time, and tend to produce lower quantum efficiency (Figure 1.22).

![Cytotoxicity mechanism caused by biological application of quantum dots.](image)

Figure 1.22: Cytotoxicity mechanism caused by biological application of quantum dots.
1.10 Toxicity of Cd$^{2+}$ based quantum dots

In order to use quantum dots for biological imaging, toxicological considerations must be made. Quantum dots are based on heavy metal chalcogenide compounds, such as CdSe and CdTe, can leach heavy Cd$^{2+}$ metals into the tissue, thereby limiting their functionality. To remediate this problem, a non-heavy metal shell, such as ZnS, or ZnSe are used as a barrier. There are no standards in the production of quantum dots because a standard dose would be difficult to define. Also, quantum dots need to be standardized and fully characterized before such standards can be set (Rzigalinski & Strobl, 2009; Swalec, 2011).

Tests have shown that conjugated quantum dots go to cells containing their target molecule, and not to cells that do not (Chan and Nie 1998). Tests have also shown that quantum dots used in biological tests can still be present in the body four months later (Rzigalinski and Strobl, 2009). If QDs are going to remain in the body this long, toxic effects of such exposure must be known. Research also needs to be performed on the disposal of quantum dots after they have been used. The cadmium would classify them as hazardous waste, and they need to be treated as such. Quantum dots often contain cadmium, a chemical that is known to be toxic to several major organ systems in humans (Rzigalinski and Strobl, 2009).

Rzigalinski and Strobl (2009) point out that reaction function and effects tend to differ greatly between bulk amounts and the nano-level, and that, even so, the effects of cadmium at the nano-scale have not be studied. However, it is known that coating a cadmium-containing core with something like ZnS will reduce the toxicity of the core.

Dosing parameters of quantum dots used in immunoassays are not set, though it is known that quantum dots remain in the body for some time. But since there are no standards in the production of quantum dots, a standard dose would be difficult to define. Quantum dots need to be standardized and fully characterized before such standards can be set (Rzigalinski & Strobl 2009; Swalec, 2011).

Tests have shown that conjugated quantum dots go to cells containing their target molecule, without attaching to cells that do not (Chan & Nie, 1998).
1.11 Treatise objectives

The aim of this thesis is to introduce some of the concepts related to sampling for synthesis process improvement of quantum dots, so to improve the chemical, physical properties and biological of quantum dots. Specific guidance related to determining a useful sample size is given to a wider clinical care audience so that it can be applied in the improvement of projects in health systems.

Parameters are adjusted during the synthesis of 3-MPA CdSe/ZnSe quantum dots by varying synthesis reaction process temperature and studying the properties. Chemical compounds that are sources of cadmium (Cd\(^{2+}\)) are varied to study the reaction dynamic effects during synthesis, quantum efficiency, size distribution and cytotoxicity of the quantum dots. CdSe/ZnSe QDs are capped with various agents to study the effect of chain length and functional groups which are palmetic acid, oleic acid, mystiric acid, octylamine, oleyamine, α-cyclodextrins and 3-mercaptopropanoic acid (3-MPA).

Synthesized capped CdSe/ZnSe QDs results are optically studied by using photoluminescence spectroscopy (PL) and UV-vis spectroscopy. The structure of the capping agents is studied by using Fourier transform electron microscopy and mass spectroscopy. The chemical or elemental composition is studied by using energy-dispersive X-ray Spectroscopy (EDX). Morphological characterization of CdSe/ZnSe quantum dots is studied to investigate the size and monodispersity of CdSe/ZnSe quantum dots by using transmission electron microscope (TEM).

The stability of 3-MPA-CdSe Quantum dots and other various capped CdSe/ZnSe Quantum Dots is studied to investigate both safety an sustainance at various temperatures for by using thermal gravimetric analysis (TGA). Cell-culture is conducted to study cytotoxicity properties of various capped CdSe/ZnSe QDs, along with 3-MPA ligand to facilitate solubility for biological studies.

1.12 Overview of the treatise

The study is arranged in six chapters. Below is an overview of the structural arrangement of the contents of the study.

In Chapter 1, we briefly introduced the emergent need for the study of colon cancer medication, causes of colon cancer and non-effective conventional medication. Overview for the study of CdSe/ZnSe QDs is discussed in detail, along with the thesis objectives.
Chapter 2 generally provides the conceptual framework of quantum dots and a theoretical discussion of synthesis type, advantages, uses and toxicology of quantum dots (QDs). Biological toxicity and diagnostics application of quantum dots are intensively described with illustrations. Various synthesis techniques with their advantages and disadvantages are discussed in depth to justify the synthesis technique that is used in the study of this thesis.

Chapter 3 focuses on the materials, instrumentation and the experiments conducted for the study. Optical and morphological characterization techniques and methods are described in detail in Chapter 4, Chapter 5 discusses application of synthesized quantum dots in cytotoxicity investigations. Chapter 6 concludes the study and recommends future work.
Chapter 2

Experimental design and methodology
2.1 Materials instrumentation and experimental

This chapter briefly describes a general overview that is related to various chemicals, experimental procedures and instrumentation that was employed during the synthesis and characterization of the CdSe-ZnSe QDs, in preparation for the treatise. A detailed experimental procedure is given in the relevant chapters.

2.1.1 Materials

Table 2.1 lists the chemicals that were used in this study, along with the chemical supplier and their purity. All used chemicals were of analytical grade and were used as purchased without any further modification and purification. Cadmium chloride (CdCl₂), cadmium oxide, palmitic acid, oleic acid, alpha-cyclodextrin, trioctylphosphine, selenium powder, 1-octadecene (ODE, 90%), trioctylphosphine (TOP), sodium chloride pellets, 3-mercaptopropionic acid (MPA), oleyamine, octylamine or zinc undecylate were purchased from Sigma Aldrich. Solvents that were used during synthesis and characterization included hexane, acetone, deionized water, methanol and chloroform. The media, used for biological studies and cell culture is perbenzoic acid, (2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt (WST), Dulbecco's Modified Eagle Medium (DMEM) and Phosphate Buffered Saline (PBS).

**Table 2.1 (a) : List of chemicals**

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
<th>Supplier</th>
<th>Purity %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium oxide</td>
<td>CdO</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>1-octadecene</td>
<td>C₁₈H₃₆</td>
<td>Sigma- Aldrich</td>
<td>90.0</td>
</tr>
<tr>
<td>Selenium</td>
<td>Se</td>
<td>Sigma- Aldrich</td>
<td>99.9</td>
</tr>
<tr>
<td>Anhydrous methanol</td>
<td>CH₃OH</td>
<td>Sigma- Aldrich</td>
<td>95.0</td>
</tr>
<tr>
<td>Acetone</td>
<td>CH₃COOH</td>
<td>Sigma- Aldrich</td>
<td>95.0</td>
</tr>
<tr>
<td>Toluene</td>
<td>C₆H₂CH₃</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>Butanol</td>
<td>C₄H₈OH</td>
<td>Sigma- Aldrich</td>
<td>99.4</td>
</tr>
<tr>
<td>Deuterated chloroform</td>
<td>CDCl₃</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>Chemical Name</td>
<td>Chemical Formula</td>
<td>Supplier</td>
<td>Purity</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------</td>
<td>----------------</td>
<td>----------</td>
</tr>
<tr>
<td>Oleyamine</td>
<td>C_{18}H_{37}N</td>
<td>Sigma- Aldrich</td>
<td>90.0</td>
</tr>
<tr>
<td>Cadmium chloride hydrate</td>
<td>CdCl₂·7H₂O</td>
<td>Sigma- Aldrich</td>
<td>99.9</td>
</tr>
<tr>
<td>Cadmium oxide</td>
<td>CdO</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>Tri-n-octylphoshine</td>
<td>OP(C₈H₁₇)₃</td>
<td>Sigma- Aldrich</td>
<td>90.0</td>
</tr>
<tr>
<td>Alpha-cyclodextrin</td>
<td>C₃₆H₆₀O₃</td>
<td>Sigma- Aldrich</td>
<td>90.0</td>
</tr>
<tr>
<td>Octylamine</td>
<td>C₈H₁₉N</td>
<td>Sigma- Aldrich</td>
<td>90.0</td>
</tr>
<tr>
<td>Mystiric acid</td>
<td>C₁₄H₂₈O₂</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>3-Mercaptoanoic acid</td>
<td>C₃H₆O₂S</td>
<td>Sigma- Aldrich</td>
<td>99.5</td>
</tr>
<tr>
<td>2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium sodium salt or WST</td>
<td>C₁₉H₁₁I₅N₃NaO₈S₂</td>
<td>Invitrogen</td>
<td>99.5</td>
</tr>
<tr>
<td>Phosphate Buffered Saline</td>
<td>PBS</td>
<td>Invitrogen</td>
<td>99.5</td>
</tr>
</tbody>
</table>
Figure 2.1: List of figures for equipment
2.2 Instrumentation

Various types of instruments were used for optical, morphological and characterization of the synthesized CdSe/ZnSe Quantum Dots nanocrystals. For this project, optical characterization techniques included photoluminescence spectroscopy and ultraviolet spectroscopy. Morphological characterization instrumentation included transmission electron microscopy (TEM), X-ray diffraction (XRD), scanning electron microscope (SEM) and Energy Dispersive Spectroscopy Systems (EDS/EDX. Temperature stability study techniques involved the use of thermogravimetric analysis (TGA). Structural elucidation methods included Fourier transform infrared spectroscopy (FTIR).

2.2.1 Photoluminuscent spectroscopy

As defined in chapter 1, the fluorescence technique is referred to as the external stimulus radiation emission that comes from an excited fluorophore due to the formation of a fluorophore of the same multiplicity spin. Figure 2.2 below shows the fluorescence process.

![Figure 2.2: The fluorescence process illustrated by the Jamblonski diagram](image-url)
The photoluminescence spectra (PL) data for fluorescence was obtained from the HORIBA Nanolog FL3-22-TRIAx. The detector range spanned between 200 and 850 nm with a R928 standard signal detector photomultiplier tube (PMT). The absorbances for the standard for hexane were initially measured with the same excitation wavelength as the CdSe/ZnSe core and CdSe/ZnSe core-shell QDs in hexane. The photoluminescent spectra of the aliquots were measured and recorded. The fluorescence intensity was calculated from the obtained spectrum.

2.2.2 Ultraviolet spectroscopy (UV-Vis)

2.2.3 Transmission electron microscope (TEM)

Characterization studies were conducted with a transmission electron microscope (TEM) on a field emission TECHNAI F20 TEM. The samples were primarily effectively dissolved in chloroform, and sonicated for 30 minutes to facilitate solubility. The chloroform CdSe/ZnSe quantum dots nanocrystals mixture was drop-coated on ultra-thin carbon copper grids and allowed to dry at room temperature. The mixture was sputtered with a high energy electron beam through a very thin sample grid. The image was obtained from a fluorescent screen after focusing with electromagnetic lenses. The resultant inference pattern resulted from the transmitted and
diffracted beams, imaging an atomic scale resolution of crystal lattice fringes, line defects, planar, interfaces and grain boundaries.

2.2.4 Scanning electron microscope (SEM)

The scanning electron microscope (SEM) is a kind of electron microscope that produces sample surface images by scanning the surface in a raster scan pattern with high-energy electron beam. As presented by the schematic diagram below in Figure 2.4. The sonicated mixture of chloroform and CdSe/ZnSe quantum dots specimen was placed in a sample chamber. The beam was turned on from the FEI brand, Quanta 400 FEG model SEM at a very large voltage scale, from 500 Volts to 30 kVolts by steps of 1 Volt.

After ejection and supply of electrons, from the electron gun which is located at the top of the system, electrons were supplied at a constant energy through the column. The generation of an image on the CRT monitor of computer was the result of the formation of the secondary electrons from the interaction of CdSe/ZnSe QDs sample atoms. This sample interaction lead to the formation of back-scattered electrons and X-rays that entailed information regarding the topography, composition, electrical conductivity and other properties of the CdSe/ZnSe QDs sample. A compositional contrast produced by back-scattered electron images in the scanning electron microscope (SEM) is a result of the different atomic elemental number and their distribution.

Figure 2.4: The use of the Scanning Electron Microscope with EDS attachment.
2.2.5 Energy dispersive spectroscopy systems (EDS/EDX)

The EDS of a solid-state detector system with scanning electron microscope is illustrated in Figure 2.4. X-ray photons from the CdSe/ZnSe QDs specimen passed through a thin window, isolating the environment of the specimen chamber from the detector, into a cooled, reverse-bias p-i-n (p-type, intrinsic, n-type) CdSe/ZnSe nanocrystal. Each individual X-ray photon absorption of led to the ejection of a photoelectron, that gave up most of the energy to form electron-hole pairs. The photoelectron was swept away by the applied bias, forming a charge pulse. This charge pulse converted to a voltage pulse by a charge-to-voltage converter. The signal was amplified by a linear amplifier and then finally passed to a computer X-ray analyzer that displayed data by voltage as a histogram.

2.2.6 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is a method which is performed by heating a solid sample with a known temperature profile, followed by measurement of the change in mass as the temperature profile progressed. The data for TGA was presented as a plot of mass vs. temperature. The temperature stability of the CdSe/ZnSe QDs was examined to observe biocompatibility and biothermal stability of quantum dots within the body, during high temperature UV-illumination process. The results were examined to locate the on-set temperature points where there is an increase or decrease in the slope of the plot and change in material composition.
2.2.7 Fourier transform infrared spectroscopy (FT-IR)

The nature of the compositional changes that occurred in CdSe/ZnSe QDs was further explored by using Fourier transform infrared spectroscopy (FT-IR). The FT-IR was used to identify chemical species present in the synthesized CdSe/ZnSe QDs at various stages in processing and at various capping ligands. For FT-IR measurement, the absorption spectrum CdSe/ZnSe QDs was taken over a large range of IR wavelengths. A range of 4000 cm\(^{-1}\) to 450 cm\(^{-1}\) was used in our measurements. The information concerning the content of the sample material was obtained by examining the spectral location and the intensity of the observed absorption peaks.

An FT-IR absorption spectra is a result of transitions between vibrational energy states within a molecule. The motion of a diatomic molecule or two atoms is a proper presentation for molecular vibrational states that can arise from a range of molecular structures. The FT-IR spectrum uses a principle of varying vibrational modes of each molecule within a structure, thereby identifying that molecule (Ai et al., 2013). An absorption spectra of the quantum dots was compared to a known reference spectrum, enabling the identification of the present QDs molecules within a sample.
Chapter 3

Chemical and biological experimental section
3.1 Preparation and synthesis of precursors

This synthetic method of CdSe QDs takes place in a few steps over a three hour period, with pre-preparation of Cd, Zn and Se precursors. The experiment was set-up to allow argon gas and vacuum to be purged through the capillary tubes, to the round-bottom flasks which contained the reaction mixtures. Also, N$_2$ gas was purged before and during reactions to prevent oxidation. The vessels were all greased, ends fitted and sealed with a rubber septa. Heating was achieved by using a heating mantle, connected to a variable auto-transformer.

The magnetic stirrer was then placed in a round-bottom flask that was attached to a condenser. The magnetic stirrer was turned on to approximately 400 rpm. The condenser was connected in a way as to retain the product as sufficient as possible. Large batches of CdSe and CdSe/ZnSe QDs had to be synthesized to reduce the batch to batch variability between ZnSe coatings that would be introduced if multiple smaller volume CdSe batches were used. To generate an increased volume of CdSe and CdSe/ZnSe QDs, the procedure discussed in this study was modified to scale-up to larger volumes. The scaling up did not affect the ability to coat the CdSe QDs, except that it produced lower concentrations than smaller batches.

Quantum dots were synthesized at various temperatures measured with a thermocouple that was inserted into the round bottom flask. A silicon oil bath was used for heating the reaction mixtures. The chemical reaction took place in the following protocol:

3.2 Preparation of cadmium oxide precursor (CdO) solution

A mass of 0.110 g of CdO was reacted with 1mL of oleic acid (OA) and 10 ml of 0.90% l-octadecene (ODE) in a 3-neck round bottom flask. The reaction mixture was initially reacted at 120 °C under vacuum while stirring for 10 min to remove moisture. This reaction mixture was heated in a heating mantle connected to a temperature controller.

The reaction mixture was then reacted in the presence of argon gas at 120 °C. The reaction temperature was then increased to 225 °C until the colour of CdO precursor turned from dark-orange to a clear CdO precursor.

\[
\text{CdO} + \text{C}_{18}\text{H}_{34}\text{O}_2 + \text{ODE} + ( \Delta t_1=225^\circ \text{C}, t_2= 120 ^\circ \text{C} ) \rightarrow (\text{Ar}, \text{Vacuum}) \rightarrow \text{CdO prec.} \quad (2.1)
\]
CdCl$_2$•7H$_2$O + ODE + (Δt$_1$=225 ⁰C, t$_2$= 120 ⁰C) → (Ar, Vacuum) → CdCl$_2$ prec. \hspace{1cm} (2.2)

Se + TOP + ODE + (Δt$_1$= 120 ⁰C + Ar, Vacuum) → Se-TOP prec. \hspace{1cm} (2.3)

C$_{22}$H$_{38}$O$_4$Zn + TOP + ODE+ (Δt$_1$=90 ⁰C + Ar, Vacuum ) → C$_{22}$H$_{38}$O$_4$Zn prec. \hspace{1cm} (2.4)

### 3.3 Preparation of cadmium chloride precursor (CdCl$_2$) solution

A mass of 0.20 g of cadmium chloride hydrate crystals (CdCl$_2$•7H$_2$O) were dissolved in 1mL of oleic acid and 10 mL of 0.90%, 1-octadecene (ODE) in a 3-neck round-bottom flask. The reaction mixture was heated, degassed and gassed with argon as in Section 2.3.1. CdCl$_2$•7H$_2$O dissolved faster than CdO to form a clear solution. The reaction temperature was then increased to 225 ⁰C, until the the CdCl$_2$ precursor colour turned from a white mixture to a clear CdCl$_2$ precursor solution. The reaction temperature was then reduced from 225 ⁰C to 120 ⁰C in order to prepare it for the next reaction step.

### 3.4 Preparation of TOP-Se precursor

The general procedure for chemical reaction was adaped from literature with a number of adjustments. A mass of 0.268 g of selenium powder was dissolved in 2 mL of 90%, tri-octylphosphine (TOP) to form TOP-Se mixture. The TOP-Se mixture was heated to 90 ⁰C, using a 2-neck flask after adding 10 mL of 1-octadecene (ODE) solvent. The TOP-Se reaction mixture was initially subjected to vacuum for 10 min to remove moisture, while stirring. The reaction mixture was then reacted in the presence of argon gas, until the black selenium reaction mixture turned to a clear selenium precursor.

### 3.5 Preparation of Zinc undercylate precursor

A mass of 0.4643 g, 0.001 mol for (0.1 M) zinc undercylate white powder, was dissolved in a 2-neck round-bottom flask with 90% of TOP and 9 mL of ODE, using a syringe. Excess moisture was then removed by using vacuum at 90ºC with sufficient stirring. The reaction was reacted under argon gas. The white zinc undercylate powder dissolved to form a clear Zn precursor solution.
3.6 Synthesis of CdSe Core QDs from CdO and CdCl₂ source

A cadmium oleate solution was prepared by dissolving 0.11 g of CdO powder in oleic acid and ODE, at approximately 225 °C in a 250 mL three-neck round-bottom flask. The heated reaction mixture was heated in a the heating mantle that was controlled by a variac. The temperature of the cadmium oleate solution was then lowered to approximately 120 °C and allowed to stabilize for adding TOP-Se precursor. 2 mL of the TOP-Se precursor was swiftly injected and the reaction time was monitored. The RB flask was removed from the heating mantle, after the desired reaction time was reached and quenched in a silicon bath at room temperature for the solution to reach room temperature.

CdCl₂ precursor was synthesized as in section 3.3.2. A volume of 2 mL of prepared TOPSe precursor was added to the prepared CdCl₂ to form a CdSe chlorate core. The two precursors were reacted under argon gas at 120 °C with stirring for 10 min. Exactly, after 10 min orange CdSe-core mixture was formed. The CdSe-chlorate core aliquots were prepared by dissolving 1.2 mL of sample into a vial, filled with 2 mL of pure analytical grade hexane.

3.6.1 Synthesis of CdSe/ZnSe Core-shell Quantum Dots at 90°C and 120°C

The ZnSe shell was grown by adding 4mL of Zn precursor to the CdSe-core at 90 °C in the presence of argon gas with sufficient stirring to form a homogeneous mono-layer of zinc around the CdSe-core for 10 min. Exactly, 1.27 mL of selenium precursor was added to the CdSe-core-Zn mixture by using a syringe. 0.1 mL CdSe/ZnSe sample were collected between 3 min to 30 min time interval. The samples were characterized with photoluminous spectroscopy.

The reaction was repeated by growing by adding 4 mL of Zn precursor to the CdSe-core at 120 °C in the presence of argon gas, with sufficient stirring to form a homogeneous mono-layer of zinc around the CdSe-core for 10 minutes. A volume of 1.27 mL of selenium precursor was added to the CdSe-core-Zn mixture by using a syringe. A volume of 0.1 mL CdSe/ZnSe sample were collected between 3 min to 30 min time intervals. The samples were characterized with photoluminous spectroscopy.
3.7 The effect of varying the capping material for CdSe/ZnSe QDs

3.7.1 Synthesis of palmetric acid capped-CdSe/ZnSe QDs

The CdSe core was initially prepared from the cadmium(II) palmetate precursor by reacting exactly 0.110 g of CdO with 0.354 g of palmetric acid in 10 mL of 0.90% and 1-octadecene (ODE), using a 3-neck round-bottom flask. The heated reaction mixture was initially reacted at 225 °C under vacuum, and was then reduced to 120 °C in the presence of argon gas while stirring for 10 minutes. This reaction mixture was heated in a heating mantle, connected to a temperature controller. The aliquot for the core was collected after 10 min in hexane solvent. Selenium precursor and zinc precursor were added to form the ZnSe shell. Aliquots were collected at time intervals in hexane. After an hour the reaction mixture turned from light-yellow to deep-yellowish orange.

3.7.2 Synthesis of mystiric acid capped-CdSe/ZnSe QDs

CdSe core was prepared from cadmium(II) mystirate precursor that was synthesized as by reacting 0.110 g of CdO with 0.1956 g of mystiric acid in 10mL of 0.90%, 1-octadecene (ODE). The heated reaction mixture was initially reacted at 225 °C in the presence of vacuum, then reduced to 120 °C under argon gas. This reaction mixture was heated in a heating mantle, connected to a temperature controller for 10 min. The aliquot for the core was collected after 10 min in hexane solvent. Selenium precursor and zinc precursor were added as in previous experiments to form the ZnSe shell. Aliquots were collected at time intervals in hexane. After an hour, the reaction mixture turned from light-yellow to light-yellowish orange crystalline mixture.

3.7.3 Synthesis of oleyamine capped-CdSe/ZnSe QDs

The CdSe core was initially prepared from cadmium(II) oleyamine precursor that was synthesized by weighing 0.110 g of CdO, dissolved with 1 mL of oleyamine and 10 mL of 0.90%, 1-octadecene (ODE) in a 3-neck round-bottom flask. 2 mL of (TOP) was added to facilitate solubility of CdO. The heated reaction mixture was initially reacted at 225 °C in the presence of vacuum, then reduced to 120 °C in the presence of argon gas while stirring for 10 min. This reaction mixture temperature was monitored for 10 min, prior collection of the aliquot for the core. Selenium precursor and zinc precursor were added to form the ZnSe shell. Aliquots were
collected at time intervals in hexane. After an hour, the reaction mixture turned from light-red to deep-maroon.

The synthesis of oleyamine capped CdSe/ZnSe quantum dots was repeated, except that CdO precursor was replaced by CdCl$_2$ precursor. 0.20 g of CdCl$_2$$\cdot$7H$_2$O crystals were dissolved in 10 mL of ODE and 1 mL TOP for 30 min, in the presence of argon gas and an initial temperature of 220 °C. The clear solution temperature was then reduced to 90 °C to add 2 mL of prepared selenium mixture. This reaction mixture temperature was monitored for 10 min, prior collection of the aliquot for the core. 4 mL of zinc precursor was added to form the ZnSe shell. Aliquots were collected at time intervals in hexane. After an hour the reaction mixture turned from light-red to fluorescing bright red quantum dots.

3.7.4 Synthesis of octylamine capped-CdSe/ZnSe QDs

The octylamine capped quantum dots were synthesized by dissolving 0.110 g CdO in a volume of 10 mL of ODE and 1 mL of octylamine at 225 °C. A volume of 2 mL, TOP was added to facilitate solubility of CdO. The reaction was repeated by dissolving a mass of 0.251 grams for CdCl$_2$ hydrate crystals in 1 mL of octylamine and 10 mL of ODE solvent. The reaction mixture was initially heated at 225 °C. The temperature was then reduced to 120 °C to add selenium precursor. The CdSe core was formed after 10 min. Zinc precursor was added to form the ZnSe shell. Aliquots were collected at time intervals in hexane. After an hour the reaction mixture turned from light-red to deep-red. The reaction for synthesizing octylamine capped-CdSe/ZnSe QDs from CdO and CdCl$_2$ precursors was repeated by growing the quantum dot crystals at 90 °C to monitor rate of crystal growth. Aliquots were collected in hexane for analysis.

3.7.5 The synthesis of alpha-cyclodextrin capped-CdSe/ZnSe QDs

The $\alpha$-cyclodextrin capped quantum dots were synthesized by dissolving 0.240 g of CdCl$_2$.7H$_2$O and 0.1 g of $\alpha$-cyclodextrin powder in TOP co-ordinating solvent. 9 mL of ODE solvent was added to the $\alpha$-cyclodextrin mixture and 1 ml of oleic acid. The reaction mixture was heated at an initial temperature of 225 °C, in the presence of argon gas, after degassing. The reaction mixture temperature was then reduced to 120 °C for the next CdSe growth reaction stage. The CdSe core was formed after 10 min. Zinc precursor was added to form the ZnSe shell. Aliquots were
collected at time intervals in hexane. After an hour the reaction mixture turned from light-red to deep-red.

### 3.7.6 The synthesis of stearic acid capped-CdSe/ZnSe QDs from CdCl₂ precursor

The stearic acid capped quantum dots were synthesized by dissolving 0.240 g of CdCl₂.7H₂O in 1 ml of stearic acid and a TOP co-ordinating solvent. 9 mL of ODE solvent was added to the stearic acid mixture. After degassing, the reaction mixture was heated at an initial temperature of 225 °C. The reaction mixture temperature was then reduced to 120 °C for the next CdSe growth reaction stage. The core growth stage was performed as depicted in Section 2.4.1. After an hour the reaction mixture turned to a reddish-brown color.

### 3.8 Purification and precipitation of CdSe/ZnSe QDs

Purification of all previously synthesized QDs was achieved by suspending quantum dots in 15% of hexane to elute all the impurities to the bottom of the plastic tube. QDs were immediately emptied from a vial tube to a glass beaker. The solid waste material was safely discarded from the vial tubes. After adding 99.5+% acetone (anti-solvent), centrifugation was employed at 3000-4000 rpm for 5 min.

The QDs separated from the acetone solvent and pellets that settled at the bottom of the plastic tube were formed. The clear and cloudy supernatant with precursor contents that were in QDs was seperated acetone was carefully removed and decanted by using a transfer pipette to make sure not to disturb the quantum dot layer in the bottom of the plastic tube vial. The QDs in plastic vials were then covered with an aluminium foil and placed in cool temperatures to prepare them for the next ligand exchange stage. Figure 3.5 shows ligand exchange reactions for surface modification of CdSe/ZnSe.

If preferably, fluorescence measurements can be determined when selective precipitation occurs, generating a cloudy supernatant of quantum dots. Small amount of QDs will be left in the supernatant, and larger quantum dots will be the first to precipitate. If this takes place, the alternative is to transfer the cloudy supernatant into a separate centrifuge tube with up-scaling acetone and extending centrifugation time, until precipitated.
3.8.1 The ligand exchange reaction for various capped-CdSe/ZnSe QDs

0.15 mg of precipitated and pelleted QDs ligands were exchanged with 3-MPA ligands to make them biologically compatible with biological cells, dissolving them in 0.5 mL of chloroform. A beaker was used to react 5 ml of 3-mercaptopropionic acid (0.44 mL, 5 mmol) in 5 mL of deionised water. In a separate beaker, 5 g of NaOH pellets were dissolved in 5 ml of deionised water. The 3-MPA reaction mixture was mixed with the NaOH mixture to adjust the pH to 11.2. The exact volume of 0.5 mL of chloroform, containing QDs was reacted with 1 mL of the pH adjusted, 1mL of 3-MPA solution. The reaction mixture was rotated for 4 hrs, with the use of a vortex. The process achieved the formation of 3-MPA-capped CdSe/ZnSe QDs phase-transfer process.

3.9 Biological experimental section

3.9.1 Cell culture and trypsinization of caco-2-cells

Cytotoxicity studies were carried out by initially, culturing tissue cells in a pre-warmed cell growth DMEM tissue culture flask medium with 1% of antibodies and 10% of fetal bovine serum. Date of culture and cell line name were indicated by labelling the flask, prior incubation in a humidified incubator at 37 °C and 5% of CO₂. As described in Figure 3.2, the growing caco-2-cells were observed and monitored daily under a Nikon inverted light microscope to keep track of their confluency. After the cells reached a confluency of 80%, 5 mL of PBS was used to wash the caco-2-cells and the cell culture medium was decanted.

![Image of growing cultured caco-2 cells](image-url)

Figure 3.2: Growing cultured caco-2 cells.
A mass percent of 80% grown confluent caco-2-cells were incubated at 37 °C after pre-warmth and 3 mL sterile trypsin was added in order to inactivate the trysin. The process was followed by the trypsin mixture into a 15 mL sterile tube, using a sterile pipette. Cells were recovered and centrifuged at 300 g in room temperature and a time period of 3 min. The supernatant was decanted as depicted in Figure 3.3, after centrifugation with re-suspension of cells in 5 mL of Phosphate Buffered Saline (PBS) and 3 min centrifugation of 300 x g, at room temperature. The cells were pelleted and the pellets were then re-suspended in a complete medium.

Figure 3.3: Caco-2 cells treatment with CdSe/ZnSe QDs.
Chapter 4

Results and discussions
4.1. Introduction

QDs have potential of revolutionizing nanopharmaceuticals as targetted drug delivery systems and as early diagnostics tools. The concern of toxicology in quantum dots, calls for alternatives for fabrication or modification of thier surfaces in order to reduce this property. The size-tunable fluorescence property and ease of functionalization for tissue targeting property of cadmium-containing nanocrystals, show great promise for diagnosis of cancer and targeted drug delivery. Although, information regarding pharmacology and toxicology of quantum dots (QDs) needs much further development. This makes it difficult to assess the risks associated with this new nanotechnology. Additionally, nanotechnology poses yet another risk for toxic cadmium, which will now enter the biological realm in it’s nano-dimension.

We report the experimental procedure and characterization for cadmium selenide with zinc selenide shell. Their biocompatibility and cytotoxicity characteristics are assessed by testing for cytotoxicity studies. Data for results are obtained, analyzed and discussed. Finally, the cytotoxicity studies are conducted with cell viability results in chapter 5. Toxicity of cadmium-containing QDs on cultured cells is discussed and future perspectives of the cancer disease treatment are projected. This chapter reports their toxicity when conjugated to various bulky ligands.

Lastly, the conclusion chapter identifies critical gaps in the knowledge of cadmium quantum dot toxicity and possible gaps that need to be assessed to enable CdSe/ZnSe QDs nanotechnology to transit safely from bench to bedside. This chapter reports the results obtained from the experiments. Numerous characteristics of CdSe/ZnSe QDs are discussed with results below, including the effect of temperature on growth rate, time, size, absorbance and fluorescence intensity, the effect of capping on growth rate and toxicity, and biocompatibility.

The effect of these parameters is evaluated in order to determine which is the best method for growing CdSe/ZnSe quantum dots for biological use or cytotoxicity studies. Comparison of parameters is included to determine the best results.
4.1.1 Optical and morphological characterization of CdSe/ZnSe QDs

Data of aliquots were collected at time intervals and studied by using the nanolog instrument in order to determine the growth rate and wavelength of the QDs. The UV-vis spectroscopic results were obtained from a UV-vis spectrometer to investigate the absorbance and emission wavelengths of the QDs. The batch results were identified by FTIR studies, to investigate the presence of functional groups and bonds that are attached on the surface of the CdSe/ZnSe QDs. Batch results of aliquots were identified on the same day on which the quantum dots were grown. Quantum dots were excited at a wavelength of 380 nm.

The fluorescence spectra were taken from 450 to 700 nm with the highest slit width of 2 nm, unless stated otherwise. Initially, fluorescence spectrums gave unsatisfactory results that led to the repetition of the experiments. The first experiments were mainly focused on studying the proper range for growth of the CdSe core. Absorption and fluorescence spectra, aliquots were compared to a blank sample of analytical grade hexane, as the dots were hexane solvent. Samples were diluted with hexane to obtain proper readings.

The ‘OriginPro 8’ computer program was used to obtain graphs for absorption and fluorescence spectra. Preferably, narrow, symmetrical curves with good range of high peak intensities are expected for fluorescence spectrums. The experiment aims to obtain the colours which range from green to red.

4.1 Organometallic synthesis of CdSe/ZnSe QDs

Initially the CdSe core was grown for 30 min without adding the shell in order to monitor the crystal growth. The PL-spec was obtained in Figure 4.1, below. There is a consistent crystal growth between 3 min and 5 min. There is a reasonable, significant crystal growth shift between 5-10 minutes that is caused by the time gap for aliquot sample collection time. Towards the end of the 30 min of crystal growth there is slow growth and the nanocrystals are less monodisperse. Self-aggregation of the nanocrystals occurs towards the end of the reaction, caused by the reduced reaction vessel volume as the amount of the nanocrystals increase. Another parameter that needs to be focussed on is the volume of the reaction vessel. To facilitate uniform nanocrystal size dispersion, a vessel with a larger volume is necessary.
Various capping materials were constructed around the CdSe/ZnSe QDs to monitor improvement in shell growth, fluorescence, chain length, functional group and monodispersity of nanocrystals from temperatures of 90 °C to 120 °C, and from various Cd\(^{2+}\) sources. The construction of a shell of material around the CdS\(_{e}/\)ZnSe QDs core-shell, improved the fluorescence properties as well as it made the QDs more stable. Another advantage of core-shell structure is that the nature of the shell can be chosen to be more biocompatible than the materials from the core, as cytotoxicity of these constructs seem to be a major drawback. A cloudy reddish-white QDs batch was formed after the addition of the ZnSe shell around the core. The solubility in water of the isolated QDs decreases greatly indicating that the capping materials without terminal, –COOH groups are not biocompatible as MPA is (Gerion et al., 2001).
Figure 4.2: Oleic acid capped-CdSe/ZnSe-Qds.

Figure 4.2 shows capped oleic acid-CdSe/ZnSe QDs that were successfully synthesized as discussed in the previous chapter. During the initial synthesis process, the TOP, ODE and oleic acid were used to dissolve the source of Cd\textsuperscript{2+} (CdO/CdCl\textsubscript{2},7H\textsubscript{2}O). The TOP acted as a co-ordinating solvent. The binding of co-ordinating phosphonic oxide solvent to semiconductor nanocrystals, facilitated capping layer exchange reaction after addition of TOP. Electrophilic-nucleophilic attack reaction occurred between the acidic alkyl chain and the TOP. The reaction took place via the electrophilic substitution reaction as illustrated in Figure 4.3. To analyze this binding process for wurtzite, CdSe quantum dots are used as seeds in the growth of highly luminescent CdSe/ZnSe QDs.

Figure 4.3: Micellar solubilization reaction to remove phosphonic group from trioctylphosphine.
The reaction below shows the electrophilic substitution reaction between the trioctylphoshine and the acidic group of oleic acid, on the surface of the CdSe/ZnSe QDs. As soon as the selenium precursor was added to the Cd precursor, the crystallization process initiated itself. The light yellow colour was observed after adding the Se precursor at 90 °C, meanwhile adding the Se precursor at 120°C resulted to a more intense orange colour. The CdSe core sample was taken after 10 min in hexane solvent for optical analysis. After taking the CdSe core sample, zinc precursor was added. The addition of the zinc precursor resulted to the formation of the cloudy yellowish orange mixture. As time progressed, the crystals continued to grow and the colour of the reaction mixture became intense, fluorescing to yellow-orange. The chemical reaction is shown in Figure 4.4.

Figure 4.4: Synthesis of CdO-precursor with oleic acid.
4.2 Preparation and ligand exchange reactions

The oleic acid consists is a long chain that consists of 18 carbons, with a double bond that exists between the 9th and 10th carbons. The oleic acid carbonyl group is attached to the quantum dots through the O-bond with the CH3 terminal end groups that prevent the oleic acid capped QDs from dissolving in DMSO and PBS for biological studies. The H-bonds within the chain and the terminal groups of the chain, inhibit solubility of these fabricated QDs. The H-bonds lead to hydrophobic bonds, preventing solubility in water and other biological related medium. Due to this solubility problem, ligand exchange reactions were conducted. The terminal hydrophobic bonds were inverted to –COOH groups through ligand exchange reactions in all various capped QDs.

Various prepared CdSe/ZnSe QDs were capped with various ligands, namely oleic acid, palmetic acid, mystiric acid, cyclodextrins, octylamine and oleyamine. These mentioned capping ligands make them dispersable in the non-polar solvents, chloroform and hexane. MPA ligands were attached to the CdSe/ZnSe QDs in order to facilitate solubility and biocompatibility for cytotoxicity studies. Phase transfer reactions are essential to modify the CdSe/ZnSe quantum dots in order to make them biocompatible and soluble in aqueous media.

Similar reactions were conducted with mercaptoacetic acid by Bharali et al. Various ligands have been used for surface fabrication of QDs (i.e. thioglycolic acid, dihydrolipoic acid and mercaptosuccinic acid). For the preparation of this thesis, 3-mercaptopropanoic acid for surface modification was used to form CdSe/ZnSe-MPA QDs. Both carboxylic and thiol moieties are utilized to anchor the surface of the quantum dots in order to bioconjugate them to biological molecules. In the Figure 4.4, oleic acid layer is exchanged with 3-MPA by introducing a thiol functional group that forms a bond with the QDs crystal surface. The terminal hydrophilic carboxylic acid groups are readily available for conjugation to biomolecules of interest. The schematic presentation of phase transfer ligand exchange process occurred as illustrated in Figure 4.5.
The hydrophobic surfaces were successfully converted to hydrophilic surfaces through ligand exchange reactions and adding chloroform to purify and precipitate QDs pellets. Addition of chloroform lead to dispersion of the QDs. 3-MPA is less dense that the QDs in chloroform, therefore the QDs in chloroform settle at the bottom of the vial tube. After 4 hours of vortex mixing and reaction with MPA, QDs leave the chloroform phase and invert to the MPA phase. The pH for the PBS medium was adjusted to 11, above the value of $pK_a$(MPA) $= 10.8$ value. Deprotonation reaction of MPA thiol group took place, generating a thiolate anion (-S$^-$) with high affinity to CdSe/ZnSe QDs surface than in it’s protonated state. The reaction sequence consits of transformation of the QDs-chloroform solution into the deprotonated ligand, containing PBS.

In order to confirm that 3-mercaptopropanoic acid molecules were conjugated to the CdSe/ZnSe QDs surface, the Fourier transform infrared spectroscopy (FT-IR) was used to investigate the changes in the chemical bonds presented by the oleic acid capped samples Figure 4.6 to 3-MPA capped samples.
As seen in Figure 4.6, the MPA stabilized CdSe/ZnSe QDs now exhibit a symmetric and assymmetric stretching vibrations of COO$^-$ at 1568 and 1402 cm$^{-1}$ which indicates that the acid is surface chemisorbed via it's oxygen atom (Joet, 2005). Additionally, the characteristic sorption peak of COO$^-$ that was due to the acidic groups from oleic acid have disappeared. Previously, the QDs surface was capped with oleic acid. The FT-IR spectra in Figure 4.7 illustrates the transformation of oleic acid bonds to that of the 3-MPA bonds.
Figure 4.7: FT-IR spectra for 3-MPA-capped CdSe/ZnSe QDS.

FT-IR studies in Figure 4.7 validate that CdSe/ZnSe QDs are capped with functional –COOH groups. The observed peak at 3273 cm\(^{-1}\) accounts for the -OH stretching of -COOH group and the C-H stretching of alkyl groups. The peak at 1640 cm\(^{-1}\) illustrates the stretching of the carbonyl group. The S-C vibration is emphasized by the appearance of a peak at relatively lower wave number at 876 cm\(^{-1}\), towards the edge of the spectrum. C-O stretching is proved by the occurrence of bands that range from 1300 to 1000 cm\(^{-1}\). Furthermore, peak at 1340 cm\(^{-1}\) suggests the composition of alkyl C-H vibrations.

Ligand exchange reactions are explained by reaction energy transfer theory, that is a powerful tool for enabling understanding of the optical properties for hybrid materials. According to Rolinsk et al. (1999), the Förster resonance energy transfer (FRET) event adjusts and changes both the photoluminescence efficiency and the emission colors that are given by the optically active, donor and acceptor. The quenching of emission and the acceptor can be proved by energy transfer and can be proved with decreasing or quenching both emission for the donor, and increase in emission for the acceptor. This is represented by energy transfer with decreasing or quenching of the emission. The excited state of the life time for the donor is decreased.

It is possible to understand the Förster resonance energy transfer, properly. Förster resonance energy transfer is a non-radiative process that leads to an excited donor (D) state through transfer of energy to an acceptor proximal ground-state (A), using a long-range of dipole–dipole interactions (Rolinsk et al., 1999).
4.3 The effect of a capping ligand on CdSe/ZnSe quantum dots

Various capping materials were used to functionalize the surface of the CdSe/ZnSe QDs. This surface fabrication was carried out in order to study the chemical, physical and biological properties of CdSe/ZnSe QDs. The QDs were functionalized with various chain lengths and functional groups as listed in the table 4.1, below:

Table 4.1: Capping materials for CdSe/ZnSe quantum dots.

<table>
<thead>
<tr>
<th>Chain length</th>
<th>Capping-CdSe/ZnSe compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amines with various chain lengths</td>
<td></td>
</tr>
<tr>
<td>C = 8</td>
<td>Octylamine-CdSe/ZnSe QDs</td>
</tr>
<tr>
<td>C=17</td>
<td>Oleyamine-CdSe/ZnSe QDs</td>
</tr>
<tr>
<td>Sugars</td>
<td>6-ring structure</td>
</tr>
<tr>
<td>α- Cyclodextrins-CdSe/ZnSe QDs</td>
<td></td>
</tr>
<tr>
<td>Acids with various chain length</td>
<td></td>
</tr>
<tr>
<td>C=20</td>
<td>Oleic acid-CdSe/ZnSe QDs</td>
</tr>
<tr>
<td>C=16</td>
<td>Palmetic acid-CdSe/ZnSe QDs</td>
</tr>
<tr>
<td>C=17</td>
<td>Mystiric acid-CdSe/ZnSe QDs</td>
</tr>
<tr>
<td>C=18</td>
<td>Stearic acid-CdSe/ZnSe QDs</td>
</tr>
</tbody>
</table>

4.3.1 Oleyamine capped CdSe/ZnSe QDs

Data of oleyamine capped CdSe/ZnSe QDs aliquots were collected at various time intervals and studied by using the nanolog instrument in order to obtain the growth rate and wavelength of the QDs. Also, UV-vis results were obtained to investigate absorbance and emission wavelengths of the QDs. The batch results were identified by FT-IR studies which showed the presence of functional groups and bonds that are attached on the surface of the CdSe/ZnSe QDs. CdSe core shows crystal growth of between 3 min and 30 min, while Figure 4.8 shows the growth of the CdSe core after 10 min and the growth of the ZnSe shell around the core at time intervals. The minute intervals are indicated on the PL-spectra.
Oleyamine capped CdSe/ZnSe QDs were synthesized. The synthesized batch mixture turned deep-red, after 65 minutes. The highly luminous CdSe/ZnSe QDs showed a maximum wavelength of 599 nm. There is a significant shift in wavelength after 10 min for ZnSe shell growth. There is no clear separation of the peaks, showing that the crystals are not monodisperse. Both the PL-spec and the HRTEM results show crystal self-aggregation, during the shell growth phase.

The HRTEM micrograph illustrates the lattice fringes of the oleyamine capped QDs. There is a continuous formation of the nanocrystals, this shows the epitaxial nature of the ZnSe shell layer. The HRTEM image correlates with that of the SAD image in terms of the diameter of the quantum dots. The diameter of the quantum dots is 10 nm and acceptable for biological application. For biological application there is a need for QDs to have a diameter that is greater than 5 nm but less than 20 nm.
Figure 4.9: The photoluminescent spectrum of oleyamine capped CdSe/ZnSe QDs.

The surface area diffraction micrograph shows that the QDs consists of various elemental compositions. Various elemental compositions scatter light differently. The central part shows the composition of the core, followed by various of layers around the central core. The ring pattern given in Figure 3.10 illustrates that the overall self-assembly is composed of local ordered domains. Each domain has a superlattice structure. The crystal domains grow independently of each other and join together to produce a thin circular pattern.

Figure 4.10: (a) HRTEM micrograph and (b) Surface area diffraction (SAD) micrograph for oleyamine capped QDs.
The FT-IR spectrum gave the characteristic peaks for oleyamine peaks. The oleylamine displays typical modes of amine groups. The peak at 1566 cm$^{-1}$ is due to the NH$_2$ scissoring mode and the peak at about 3338 to 3275 cm$^{-1}$ is assigned to the $\nu$(N–H) stretching mode. There is no evidence for presence of oleylamine coordination with superficial Cd$^{2+}$ atoms of the quantum dots, since the typical peak at 1566 cm$^{-1}$, caused by the NH$_2$ scissoring mode, just appears as a small shoulder of the 1566 cm$^{-1}$ peak. Within the region of 3300 cm$^{-1}$ there is a non-withstanding, characteristic of $\nu$(N–H) stretching mode due to the appearance of the broad poorly defined signal (Bersuker, 1984). However, this result is not conclusive. A significant number of oleylamine molecules could be bonded to a limited number of Cd$^{2+}$ superficial ions.

![Figure 4.11: FT-IR spectra of oleyamine capped CdSe/ZnSe QDs.](image)

**4.3.2 Octylamine capped CdSe/ZnSe QDs**

The PL spectra of octylamine capped CdSe/ZnSe QDs aliquots were collected in order to compare the properties of C-8 octylamine to the previously discussed C-17 oleyamine. The influence of the capping material was studied by using PL-spectrum.
Figure 4.12: The photoluminescent spectrum and the structure of octylamine capped CdSe/ZnSe QDs.

Exactly after adding the Zn precursor, there is a significant peak shift for the wavelength from 515 to 528 nm, due to the variation in precursor composition that are causing crystal growth. Other wavelength peaks appear at 529, 533 and 536 nm. There is about 2 % loss of fluorescence intensity after adding the Zn precursor. This can be accounted by the reduction in fluorescence of the fire-fly like, flashing core. The intensity slightly decreases as wavelength (nm) increases.

Figure 4.13: HRTEM micrograph of the octylamine capped CdSe/ZnSe QDs shows the presence of nanocrystals with varying size diameter for on sample and Surface area
Diffraction (SAD) micrograph for octylamine capped QDs shows the core and multiple layers around the core.

Figure 4.13 for HRTEM shows evidence of different particle size for octylamine capped QDs that exist within one sample. There exists particle size of 2 to 5 nm range. Again, the HRTEM micrograph illustrates that sample has a very narrow nanocrystal size distribution. The SAD image shows the size diameter of the nanocrystal and different molecules (i.e. the core and the surrounding layers).

![Octylamine capped CdSe/ZnSe QDs](image)

**Figure 4.14: The FT-IR spectra for octylamine-CdSe/ZnSe QDs.**

The octylamine displays usual modes for amine groups. The vibrational peak at 1539 cm\(^{-1}\) is due to the NH\(_2\) scissoring mode, while the peak at about 3000 cm\(^{-1}\) is assigned to the -N–H stretching mode. There is no evidence for the presence of octylamine coordinated with superficial cadmium atoms of the NCs or QDs, since the typical peak at 1539 cm\(^{-1}\) (due to the NH\(_2\) scissoring mode) just appears as a small shoulder in that range of 1539 cm\(^{-1}\) peak. Notwithstanding, in the region of 3000 cm\(^{-1}\) (characteristic of the -N–H stretching mode) a poorly broad defined signal appears.

Additionally, this result is not evident because, in that same region, the signal corresponding to dimers of oleic acid can be detected. Maybe the quantity of octylamine molecules bonded to CdSe/ZnSe superficial ions is low. However, the presence of both octylamine ligands bonded to Cd\(^{2+}\)/Zn\(^{2+}\) ions is compatible with the present FT-IR spectrum.
4.3.3 Alpha-cyclodextrins capped CdSe/ZnSe QDs

Herein, α-CD sugar molecules were used to cap the surface of the CdSe/ZnSe QDs. The objective of capping with α-CD is to generate stable semiconductor CdSe/ZnSe QDs. The α-cyclodextrin dosage plays an important role in the quality of the CD-CdSe/ZnSe QDs, as shown in the PL-spectra. The PL-spectra in figure 4.16 shows that increasing α-CD dosage, increases fluorescent intensity. The cavities of cyclodextrins are hydrophobic, leading to inclusion of compounds within the cage structure (Martin, 2003). The EDS/EDX micrograph show evidence of the presence of TOP due to the presence of the phosphine and oxygen groups within the sample. TOPO/TOP could be included by cyclodextrins, due to the interaction of the hydrocarbon tail of the previously hydrophobic cavity of the latter.

The α-CD QDs are pulled within the water molecules because of their hydrophilic outer cavity. Phase transfer reactions to aqueous phase are highly dependant on concentration of the TOP or TOPO. Low concentration of CdSe/ZnSe QDs form few surface bound ligands. The higher the concentration of the QDs, the better the capping ligands attach and form alpha-cyclodextrin CdSe/ZnSe QDs complexes. These complexes are not completely hydrophilic, as a result the 3-MPA was used as a stabilizer before biological application.

Figure 4.15 : The formation of Cyclodextrin-CdSe/ZnSe quantum dots complex.
Figure 4.16: (left) The PL-spectra for cyclodextrin capped quantum dots and (right) The HRTEM image for Cd(II) cyclodextrin complex.

The PL-spectra shows that the wavelength increases as time increases. This is a clear indication of crystal growth. After 10 min of growing a ZnSe shell there is a strong increase in the intensity of the cyclodextrin capped CdSe/ZnSe QDs. The use of cyclodextrin capped QDs improves both fluorescence wavelength and intensity over 60 minutes time interval. The HRTEM images of the \( \alpha \)-CD QDs show that the diameter of the nanocrystals are almost identical, indicating monodisperse molecules in either chloroform or hexane. Phase transfer was successfully achieved from high dosages of CdSe/ZnSe QDs. \( \alpha \)-CD complexes were unstable in water.

Figure 4.17: The EDX/EDS spectrograph for cyclodextrin capped quantum dots and the SAD image for Cd(II)cyclodextrin complex.
The EDS/EDX shows clear evidence that the phosphine and oxygen atom, coming from TOP, are present in the α-CD QDs complex due to the appearance of the elements in the micrograph. The copper element could be appearing due to the copper grids that were used to embed the sample. Chlorine is the result of impurities that could have been present in the starting materials.

Figure 4.18: FT-IR spectra of alpha-cyclodextrin capped CdSe/ZnSe QDs.

The FT-IR spectra depicts the α-CD capped CdSe/ZnSe QDs. The spectral features and position of the peaks show numerous vibrational modes, confirming the presence of cyclodextrins on the surface of quantum dots. Firstly, the strong band at 1157 cm\(^{-1}\) correlates with the asymmetric glycoside vibration, \(v_a\) (C-O-C); vibrational bands occurring at 3400 cm\(^{-1}\) occur due to O-H vibrations.

Figure 4.18 shows the FT-IR bands are occurring in the finger print region and clearly indicates that the α-CD caged molecules have attached to the surface of the QDs. Moreover, the FT-IR spectrum of the α-CD capped CdSe/ZnSe QDs show subtle variations from cyclodextrins which can be due to the quality of the TOP. The shift that appears at 1030 cm\(^{-1}\) of the \(-\text{C-C-}\) or \(-\text{C-O-}\) stretching vibration of the alpha-cyclodextrin cadmium selenide/zinc selenide complex and the usual peak for the symmetric stretch of CH\(_2\) in the alkyl chain of TOP, 2852 cm\(^{-1}\) indicate inclusion-type interaction for α-CD capped CdSe/ZnSe QDs complex (figure 4.18).
4.3.3.1 The Cd(II) oleate quantum dots or oleic acid capped CdSe/ZnSe QDs

Various chain lengths of acidic groups were used for capping the surface of the CdSe/ZnSe QDs. Both are C-18 fluorescence spectrums for oleic acid capped QDs, illustrated in Figure 4.19 (a) show the trend one would expect from the presence of a fluorescing material. The fluorescence intensity remains constant, throughout the core growth process. Figure 4.19 (a) graph also shows that the CdSe core dots grow slowly in size over time, the peak fluorescence wavelength is increasing from the green region towards the red, even when the intensity remains constant. All crystals show limited overall size distribution of around 550 nm. After adding the ZnSe shell, a big wavelength shift is noticed, until 15 minutes where the crystals begin to grow within each other (a clear sign of self-aggregation) that is also shown in figure 4.19 (b).

![Figure 4.19 (a): The PL-spectra for oleic acid capped quantum dots and (b) the HRTEM image for Cd(II) oleate complex.](image)

There are several characteristics of QDs that can be analyzed. The noticable ones are the rate of growth and the size of the particles. For CdSe/ZnSe QDs, fluorescent wavelength is a function of size. As the CdSe/ZnSe QDs grow, their size also increase, along with the peak fluorescent wavelength. Therefore size of the CdSe/ZnSe QDs can be directly analyzed by measuring the wavelength. The HRTEM image for cadmium oleate QDs correlate with the *pl-spec*, they show that after some time the QDs aggregate. The EDS/EDX scheme show that there is still presence of the TOP within the sample. The trioctyolphosphine does not leave the sample, after capping as
expected. The TOP is supposed to act as a coordinating solvent, not a final integral part of the QDs due to its toxicity to biological cells.

To confirm the conjugation of oleic acid molecules to the CdSe/ZnSe QDs, Fourier transform infrared (FT-IR) spectroscopy was used to determine the alterations in the chemical bonds. Oleic acid capped CdSe/ZnSe QDs exhibited symmetric and dissymmetric stretching vibrations of COO\(^{-}\) at 1568 cm\(^{-1}\) and 1402 cm\(^{-1}\), as illustrated in Figure 4.21.
Figure 4.21, depicts FT-IR spectra of the oleic acid capped QDs, nanocrystals with adsorbed oleic acid show the stretching of carbonyl bond in the adsorbed oleic acid is observed at 2995 cm\(^{-1}\). The broad peak is a result of the \(-\text{OH}\) functional group, from the \(-\text{COOH}\) has disappeared due to the availability of the bond between the QDs and the carbonyl bond. The adsorption of the carboxyl of oleic acid on CdSe/ZnSe QDs surfaces causes a significant red shift of the stretching of the carbonyl. It is reasonable that the adsorption weakens the stretching of the carbonyl groups. Moreover, a peak at 1641 cm\(^{-1}\) is also observed in the FTIR of the CdSe/ZnSe nanocrystals with adsorbed oleic acid. The peak refers to the oleic acid molecules that are physically blended in the sample.
4.3.4 Stearic acid capped CdSe/ZnSe QDs

The reported stearic acid capped QDs show abnormal growth. There is an initial blue shift in wavelength after adding the ZnSe shell. Red shift only occurs after 30 min of crystal growth. The observed fluorescence peak is non-symmetrical and there is non-uniform size distribution of the nanocrystals. Stearic acid is a poor capping agent due to the production of low quality quantum dots (figure 4.22).

Figure 4.22: The PL-spec for stearic acid capped CdSe/ZnSe QDs.

Figure 4.23: FT-IR spectra of stearic acid capped CdSe/ZnSe QDs.
The bonded –OH stretch to the surface of the CdSe/ZnSe QDs appears at 2344 cm\(^{-1}\). The assymetric and symmetric CH\(_3\) bend can be accounted for the frequency at 1465 and 1397 cm\(^{-1}\). It can be concluded that not all the stearic acid ligands have attached to the surface of the quantum dots due to the presence of the broad O-H stretching at 3461 cm\(^{-1}\). The methylene assymetric C-H stretching appears at 2948 cm\(^{-1}\). At 2918 cm\(^{-1}\), there is evidence of the long C=O stretching frequency. Moreover, the carboxylate assymetric and symmetric COO- stretching are appearing at 1539 and 1465 cm\(^{-1}\). At 720 cm\(^{-1}\) there is an oscillation that is caused by the presence of the 5\(^{th}\) carbon, within the chain.

![Rate of CdSe core growth in time](image)

**Figure 4.24:** A plot of the wavelength (nm) vs time (min) for CdSe core growth.

A plot of the emission wavelength (nm) obtained from the PL-spectrum against time (minutes) initially showed gradual slope which is a signal of the initial stage of the crystallization process. There is continuous nanocrystal growth due to the presence of the starting material. The nucleation stage was initiated after 1 min of crystal growth.

Towards the end of the reaction, there is slow crystal growth due to reduced vessel size container which leads to non-uniform size distribution of the nanocrystals and nanoparticle surface defects. Consumption of starting materials simultaneously takes place as the produced mass of nanocrystal increase. It can be concluded that the concentration of the starting materials plays a huge role in growth of the quantum dots and fluorescence intensity for growth of CdSe/ZnSe QDs.
Figure 4.25: The plot of wavelength (nm) Vs time(min) for various capped CdSe/ZnSe core-shell growth.

A plot of wavelength (nm) against time (min) for various capped CdSe/ZnSe core-shell QDs in figure 4.25 show a sharp increase in slope. Mystiric acid capped CdSe/ZnSe QDs increase in wavelength (nm) as time (min) increases, a clear signal of rapid crystal growth. Oleyamine capped CdSe/ZnSe QDs nucleation phase occurs faster begiine the highest fluorescence wavelength than all the other capped QDs but the growth rate is not as rapid as the mystiric acic capped CdSe/ZnSe QDs. CdSe/ZnSe stearate QDs have low fluorescence wavelength and there is retarded crystal growth. Meaning stearic acid ligands produce low quality quantum dots.

4.4 Effect of temperature on CdSe/ZnSe quantum dots synthesis

Temperatures were varied during the synthesis of CdSe/ZnSe QDs to measure and compare the quality of quantum dots synthesized at lower temperatures, and again at higher temperatures. Octylamine was used as a capping agent on the CdSe/ZnSe QDs. The procedure was repeated for synthesis as discussed in Chapter 3, from disolving CdO precursor in octylamine solvent, ODE
and TOP (co-ordinating solvent). Again, octylamine capped CdSe/ZnSe QDs were grown from CdCl₂·H₂O precursor. The PL-spectra were obtained in both cases CdSe core on Figure 4.26 (left), were crystal growth of the core nanocrystal occured after 10 min. The samples were again collectected for core-shell between 10 min and 50 min, shown in Figure 4.26 (right). Growth of the CdSe core occured after 10 min. The minute intervals are indicated on the PL-spectra.

Figure 4.26: The PL (left) spectra and UV-vis (right) showsthe effect of temperature on synthesis of octylamine capped-CdSe/ZnSe QDs at 120 ⁰C for both core and core-shell growth.

Figure 4.27: The PL (left) spectra and UV-vis (right) showsthe effect of temperature on synthesis of octylamine capped-CdSe/ZnSe QDs at 90 ⁰C for both core and core-shell growth.
Figure 4.28: The effect of temperature on synthesis of oleic acid capped-CdSe/ZnSe QDs at (left) 120 °C and at (right) 90 °C for both core and core-shell growth.

Figure 4.29: HRTEM shows non-uniform oleic acid capped-CdSe/ZnSe QDs and the effect of synthesis at high temperature.
The CdSe/ZnSe QDs batches, synthesized at 90 °C are uniformly distributed in size than those synthesized at 120 °C. QDs that were synthesized at high temperatures, grew within themselves during the nucleation and crystallization phase. Ostwald ripening is observed at high temperatures. Batches form a precipitate at high temperatures and are unable to continue, growing farther in size. This is indicated by Figure 4.29. For this reason, it was determined that the higher temperatures for synthesizing QDs are not always the best. It is good to optimise the temperature, although the higher temperatures can yield unequal distributed large crystals as seen in the PL spectr and UV-vis spectra for QDs synthesized at higher temperatures. Again, the longer synthesis time and lower temperatures obtained larger crystals and achieved best QDs batches.

The fluorescence (FWHM) of samples that were generated at higher temperatures tend to be lower than that of the batches generated at lower temperatures, primarily due to the rapid increase of crystal growth with high temperatures. Also, after the reaction was stopped there was sudden cooling (large effect of temperature change) fast cooling rate. For nucleation and growth processes, the rate at which the growth is stopped (cooling rate) determines the distribution of nanocrystal particle sizes in the reaction mixture. When the sample is quenched very quickly, the FWHM tends to be both smaller and broader than when the sample is cooled more slowly. Due to the interdependence of fluorescence wavelength to the nanocrystal particle size, as dictated by quantum confinement, a broad FWHM signifies a broad distribution of nanoparticle sizes in the reaction mixture.

Varying temperature and time for the growth of both the core and the shell, were carefully monitored. Preliminary experiments showed that the time and temperature relationship had a relatively significant effect on the fluorescence wavelength of the quantum dots after capping. This relationship is caused by the red shift from Ostwald ripening, during the heating process. The heating process for the CdSe solution with a heating mantle required a slow rate of heating at room temperature, at which the required time changed even when the same settings were used. The fluorescence curves show that while the ZnSe coating procedure increased the fluorescence significantly over uncoated samples for both coated samples, when longer heating times were used, there was a greater red shift in the fluorescence due to greater CdSe core growth.
4.5 Effect of Cd\(^{2+}\) source on CdSe/ZnSe for synthesis of quantum dots

The Cd\(^{2+}\) source was varied, as discussed previously in order to investigate the rate of growth, crystal size and crystal size distribution. Figure 4.31 shows the PL spectra that compare the source of QDs. The CdO source produces QDs with unequal size distribution as wavelength increases. The intensity of the QDs that are synthesized from CdO decreases as wavelength increases. The sizes of the crystals increase in size as time increases. The wavelength varies between 480 nm-520 nm for CdO source QDs.
The CdCl₂ source produces crystals that are well and equally distributed in size as the wavelength, time increases. In other words, the crystals are mono-disperse. The intensity of the QDs is constant as compared to those of QDs, synthesized from the CdO as a source. The wavelength varies between 440 to 585 nm for CdO source QDs. Variation in Cd²⁺ source leads to variation in
wavelength, size distribution and size range. Furthermore, from the results it is evident that the intensity is greatly affected by the source of Cd$^{2+}$. The variations in this parameter could be attributed to the rate of solubility of the starting materials. The CdO molecules are less soluble than the CdCl$_2$ molecules, meaning less availability for effective reaction to take place. There is a correlation between the HRTEM results and the previous PL-spectra results, for mystiric acid capped QDs.

![Graph showing PL-spectra and HRTEM results for palmitic acid capped QDs from CdO source.](image)

**Figure 4.33:** (left) PL-spectra and (right), HRTEM for palmitic acid capped from CdO source.

![SEM images for palmitic acid capped QDs from CdO source.](image)

**Figure 4.34:** SEM images for palmitic acid capped QDs from CdO source.

The HRTEM and SEM images show that the growth of the core-shell after 10 minutes leads to the formation of 2 nm hexagon crystal structures. The crystallization process after growth of the ZnSe shell around the 2 nm CdSe core-shell results in the crystal size of 20 nm for varying sizes, as
depicted by the Figure 4.33 and Figure 4.34. These dots were fabricated by using palmitic acid as a capping agent/buffer. They were also made using CdO precursor and TOP as a co-ordinating solvent, along with the ODE as a heat transfer solvent. The zinc undercylate precursor was allowed to grow for 10 min to produce an initial uniform or homogenous layer of Zinc. The homogenous layer was produced to prevent the CdSe layer from mixing with ZnSe grown shell, by allowing the Zn$^{2+}$ layer to grow for 10 min prior adding the selenium precursor. After adding the Se precursor to the CdO precursor, CdSe core particle growth occured at a faster rate. The aliquot that was obtained after 50 min, and this showed a cloudy-white sediment that was followed by stopping the reaction.

Figure 4.35: (left), PL-spectra and ( right), HRTEM for synthesis of palmitic acid QDs from CdCl$_2$.7H$_2$O source.

Figure 4.36: Scanning electron microscope image of palmitic acid capped QDs from CdCl$_2$.7H$_2$O source.
The HRTEM, SEM and selected area electron diffraction images are given in Figure 4.35 and 4.36. CdSe core of QDs have mixed spherical and hexagonal nanocrystal diameter of 2 nm. Figure 4.37 shows the SAD image where three circular ring patterns diffract light differently.

The central illuminating core is diffracting light differently from the second circular zinc blende structure ring. The circles with index <311>, <220> and <111> confirm lattice crystalline planes that correlate to the standard, calculated d-spacing values of 0.1772, 0.3404 and 0.2069. EDX data further illustrates CdSe/ZnSe QDs core that encompasses within a zinc blend crystal structure and a variety of impurities that might be a result of contamination during the synthesis process.

Figure 4.37. (a), shows the HRTEM image for CdSe/ZnSe core-shell after 10 min and (b), shows the SAED pattern for CdSe core to CdSe/ZnSe core-shell QDs.
The effect of varying Cd\textsuperscript{2+} source plays a role in CdSe/ZnSe QDS as illustrated in Figure 4.37. The table below evidently shows correlation of the pl spectrum and HRTEM, by showing that CdCl\textsubscript{2} source of Cd\textsuperscript{2+} is produces quantum dots with high fluorescence wavelength than those synthesized from a CdO source. This shows that the CdCl\textsubscript{2} molecules are readily available for the reaction to take place than the CdO molecules. CdO is less soluble in TOP and ODE than the CdCl\textsubscript{2} starting material. The amount of TOP had to be increased by 50\% to dissolve CdO starting material. A volume of 1 ml TOP was used to dissolve CdCl\textsubscript{2} starting material. It can be concluded that the CdCl\textsubscript{2} source is suitable for biological application due to the lesser presence of TOP in the QDs.
Figure 4.39: Plot of wavelength (nm) vs Time(min) for varying Cd^{2+} source.

Figure 4.40: Thermogravimetric analysis (TGA) to determine thermal stability
TGA spectrum was obtained in the presence of water, to assess the life-time thermal stability of the QDs within the body temperature range of 20 °C to 37 °C. TGA shows that the QDs are stable over a long temperature range. The octylamine capped QDs are stable up to 300 °C with (about 15% of weight loss), initial deformation of the amine group followed by the (about 100% weight) chain loss of between 250-400 °C. The α-cyclodextrins capped QDs show stability up until 300 °C, meanwhile the 3 MPA-stabilized QDs sustain temperatures for a longer temperature range. This shows that the MPA QDs are still bio-thermally stable at even higher temperatures.
Chapter 5

Cytotoxicity study of CdSe/ZnSe quantum dots
5.1 Introduction

In the previous section, the various capped CdSe/ZnSe QDs were synthesized, optically and morphologically characterized with elucidation of structures. The CdSe/ZnSe QDs surfaces were then modified by attaching 3-MPA ligands on their surfaces to facilitate their solubility. The CdSe/ZnSe Quantum Dots are capped to facilitate their solubility, bio-compatibility and bio-availability for use in cytotoxicity studies. Once quantum dots are applied to caco-2-cells and other cell types, the cells undergo cell proliferation and viability in different ways.

The major objective for altering CdSe/ZnSe QDs is to adjust their physico-chemical properties for safe biological use. An in-vitro toxicity assay uses a technique of assessing cell metabolism (i.e. MTT and/WST 1 assay or trypan blue) to test and measure viability and cytotoxicity of drugs, QDs or florescent dyes. The WST 1 assay is used in this study in order to measure the reduction of enzymes, (i.e. within the yellow 2-(4-Iodo phenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium sodium salt of succinate dehydrogenase) for mitochondria activity of live cells. Cytotoxicity studies for various capped MPA-CdSe/ZnSe QDs that were previously synthesized and characterized were biologically tested.

5.1.1 Cytotoxicity study of various capped MPA-CdSe/ZnSe QDs

The cytotoxicity of CdSe/ZnSe QDS was evaluated by using the WST 1 assay. This step was conducted by initially conducting cell culture and trypsinization of caco-2-cells, as discussed in chapter 4 of the experimental section. Having various capped CdSe/ZnSe QDs, various viability results are expected at different concentrations. The QDs were not bio-conjugated to a targeting peptide, therefore the cells are not expected to kill the cells. The cells are expected to remain being alive or to sustain the QDs in order to prove that they are safe to be used as both therapeutics and imaging. The cells are cytotoxic to the cells only when they are bio-conjugated with a cell targeting molecule.

5.1.2 Caco-2-cells treatment with MPA-CdSe/ZnSe QDs

Treatment with MPA-(oleic acid-CdSe/ZnSe QDs), MPA-(palmitic acid-CdSe/ZnSe QDs), MPA-(mystiric acid-CdSe/ZnSe QDs), MPA-(α-cyclodextrins-CdSe/ZnSe QDs), MPA-
(octylamine-CdSe/ZnSe QDs), and MPA-(oleylamine-CdSe/ZnSe QDs) were done in triplicate on cultured cells after 24 hours. Readings were taken after shaking the plates in a rotating shaker for 10 minutes at 560 nm, using a BMG LabTech Polar Star Omegar microplate reader.

Results from the microplate reader were tabulated as a average absorbance of each drug dose concentration. Three wells were seeded without treatment with quantum dots and WST 1 to be used as a reference of treated wells. Results of triplicate wells were expressed as average ±SD. The calculations for cell viability (%) vs concentration (mg/ml), according to the appendix in the results chapter of this treatise were conducted and expressed in a cell viability graph Figure 5.1.

![Figure 5.1: Cytotoxicity studies to investigate cell viability of various capped MPA-CdSe/ZnSe QDs in caco-2 cell line.](image)

Figure 5.1 shows the cytotoxicity of various capped MPA-CdSe/ZnSe QDs. The results were obtained from concentrations of 10.5, 17.7, 28.49, and 63 ng/ml in order to compare the effects of capping agents. The black bar represents the well with untreated cells at 100%
viability, showing that 100% of cells are still alive. Comparing the treated plates at various concentrations, it is clear that oleyamine capped, mystiric acid and oleic acid capped CdSe/ZnSe QDs are friendly to biological cells at 10.5 ng/mL, because the cells continued to grow even after treatment with QDs. Palmitic acid capped quantum dots are toxic than other capped QDs due to 5% of cell death after treatment, even at 10 ng/ml concentration (figure 5.1 and figure 5.2). The results show that α-CD-CdSe/ZnSe QDs are friendly and less toxic to the cells, as published by Martin Del Valle (2003).

![Investigation of CdSe/ZnSe QDs toxicity on Caco-2 cells at various treatment concentrations](image)

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**Figure 5.2:** The plot of cell viability (%) against QDs treatment concentration (ng/mL).
Chapter 6

Conclusion and future research work
6.1 Conclusion

The experimental results from the EDS/EDX demonstrate that the binding strength of the CdSe-TOP bond exceeds that of the CdSe-OA bond due to the presence of the phosphine group (Chapter 4, EDX spectrum). Ligand exchange with excess oleic acid do not induce desorption of phosphonic species, whereas ligand exchange with alpha-cyclodextrins and MPA exchanged QDs with excess phosphonic acid. FTIR shows that the after ligand exchange with MPA, the latter quantitatively replaces the oleic acid with a 1:1 stoichiometry.

Both the stoichiometry of the alpha-cyclodextrins/phosphonic acid exchange interaction and the ratio between the Cd surface excess and the ligand density indicate that phosphonic acids bind as hydrogen phosphonates to the CdSe surface. As with the CdSe synthesis, the formation of ZnSe as a shell on CdSe quantum dots was achieved through a colloidal process under N₂ gas flow. Nanocrystal growth is dependent on the capping material. The mystiric acid capped CdSe/ZnSe QDs grow rapidly in size, after adding the precursors. Oleyamine capped CdSe/ZnSe QDs have the highest and broad fluorescence wavelength 542 nm to 600 nm, than all the other capped QDs but the growth rate is not as rapid as the mystiric acid capped CdSe/ZnSe QDs.

CdSe/ZnSe stearate QDs have low fluorescence wavelength and there is retarded crystal growth, meaning stearic acid ligands produce low quality quantum dots. It can be concluded from the results, that rapid crystal growth of the quantum dots does not mean fluorescence of the quantum dots will increase. Rate of crystal growth is not a function of fluorescence wavelength for CdSe/ZnSe quantum dots. CD-QDs have the lowest fluorescence than all the other capped QDs but consistent crystal growth when compared to the poor quality stearic acid capped CdSe/ZnSe QDs.

Experiments showed that the time and temperature relationship have a significant effect on the fluorescence wavelength of the quantum dots, even after introducing the capping material. This relationship is caused by the red shifting and the Ostwald ripening effect, during the heating process. The effect of the capping material has an effect on thermal stability of the CdSe/ZnSe QDS, decreasing chances of the toxic Cd²⁺ eruption of the core to the biological cells. Thermogravimetric analysis gives a clear evidence that the CdSe/ZnSe capping materials are responsible for temperature stability over long temperature range. The MPA-CdSe/ZnSe stabilized quantum dots sustained long high temperature range without
degradation. This shows that MPA capped QDs are not only compatible, but are also bio-compatible at even higher temperatures. The octylamine capped QDs and cyclodextrins capped QDs also sustained over above 400 °C, though they later on slowly degraded than the MPA stabilized QDs (refer to chapter 4, TGA spectrum).

The MPA-QDs has good and emission tunable properties. The formed CdSe/ZnSe QDS 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 CdSe/ZnSe QDs makes them excellent potential labeling and therapeutic agent for the colon cancer disease diagnosis. Varying Cd²⁺ source and temperature has an effect on shape, fluorescence wavelength and intensity of CdSe/ZnSe QDs.

Synthesized CdSe/ZnSe quantum dots are stable, even at temperatures. Cytotoxicity tests show that the synthesized CdSe/ZnSe QDs are friendly to the cells. A maximum of 5% cell death for octylamine at high concentration of 63 ng/ml takes place. Both the capping material and the concentration affect the toxicity of the CdSe/ZnSe QDS. Cell viability was best obtained from oleic acid-QDs that were 17.7 ng/ml and 28 ng/ml concentration. The capping material has an effect on size growth, fluorescence wavelength and size distribution of CdSe/ZnSe quantum dots.

**6.2 Future research work**

A simple organomettalics synthesis route has been proposed for the synthesis of 3-MPA functionalized hydrophilic CdSe/ZnSe quantum dots. Efforts are to synthesize the bio-nanoconjugates of CdSe/ZnSe QDs with caco-2 cells receptor antibodies for both therapy and diagnosis of colon cancer. Caco-2 cell death would then be the objective of the study. CdSe/ZnSe QDs which do not induce cancer toxicity will also be conjugated with a colon cancer drug to investigate therapeutic effects.
Chapter 7

References
7.1 References


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